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Linkage Analysis and Mapping of Three Sex-Linked Color Pattern Genes in the Guppy, *Poecilia reticulata*

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ABSTRACT—Three phenotypic color pattern genes of the guppy (*Poecilia reticulata*), i.e., black caudal-peduncle (*Bcp*), red tail (*Rdt*) and variegated tail patterning (*Var*), were genetically analyzed and mapped. Crosses between the Tuxedo (TUX) and Green Variegated (GV) guppy strains commercially cultured in Singapore were used to determine the gene control of these color patterns. F_1 progenies were produced by single-pair reciprocal crossing between TUX and GV, 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 color phenotypes and sex, and tested by chi-square analyses. The *Bcp*, *Rdt* and *Var* color pattern genes, located at different loci on the X- and Y-chromosomes, showed single gene inheritance and dominant expression in both sexes. Their corresponding recessive alleles, Bcp^+, Rdt^+ and Var^+, do not produce any color patterns. Genotypes of Tuxedo males are proposed to be $X_{Bcp,Rdt,Var^+}Y_{Bcp,Rdt,Var^+}$ (type I), $X_{Bcp^+,Rdt,Var^+}Y_{Bcp,Rdt,Var^+}$ (type II) and $X_{Bcp,Rdt,Var^+}Y_{Bcp^+,Rdt,Var^+}$ (type III) while females are $X_{Bcp,Rdt,Var^+}X_{Bcp,Rdt,Var^+}$. Green Variegated males and females have the $X_{Bcp^+,Rdt^+,Var}Y_{Bcp^+,Rdt^+,Var}$ and $X_{Bcp^+,Rdt^+,Var}X_{Bcp^+,Rdt^+,Var}$ genotypes, respectively. Close linkages of 3.1, 2.3 and 2.2 map units were estimated for the sex-determining region (SdR)–*Rdt*, *Rdt*–*Bcp*, and SdR–*Var* gene pairs, respectively, while *Bcp* was approximately 5.1 map units from the SdR. The phenotypic map order of the guppy Y-chromosome is inferred to be *Var*–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). The guppy shows distinct sexual dimorphism whereby males are smaller than females and their anal fin is modified into a copulatory organ, the gonopodium. Complex polymorphic spots and patches of colors on the body and fins are also expressed by sexually mature males while females are devoid of bright color patterns, being olive-brown with hyaline fins (Haskins and Haskins, 1951). The guppy was introduced into Singapore and other parts of South-East Asia in the late 1930s as a biological control for mosquitoes (Herre, 1940).

The guppy is popular among commercial guppy breeders and hobbyists who have 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, culture of fancy guppy strains began in the early 1950s. About 30–40 different strains are reared in monoculture guppy farms (Fernando and Phang, 1985). The guppy is one of the top 10 most popularly farmed ornamental fish in Singapore which exported US$48 million worth of ornamental fish in 1997 (Cheong, 1998).

The guppy is unique in that almost all the genes involved in color pigmentation and patterning are sex-linked. It has 23 pairs of chromosomes, 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 shown to have Y-linked inheritance of genes (Schmidt, 1920). Kirpichnikov (1981) documented 17 Y-linked genes that are passed only 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. Some of these color pattern genes, e.g., *Maculatus* (*Ma*), *Armatus* (*Ar*) and *Pauper* (*Pa*), influence sex determination in wild-type guppies (Schmidt, 1920; Winge, 1922a, b, 1927, 1934; Winge and Ditlevsen, 1947). These genes are usually found close to or within a short sex-determining region (designated as SdR) on the Y-chromosome, and are presumably linked tightly to a gene for maleness (Winge, 1927, 1934; Winge and Ditlevsen, 1947; Kirpichnikov, 1981). The SdR may also represent a dominant factor for male-determination and possibly has a recessive female-determining region at a simi-

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lar position on the X-chromosome. Genes for background body coloration, e.g., blond (*b*), gold (*g*), albino (*a*) and blue (*bl*) are, however, autosomally inherited and recessive to their wild-type alleles (Haskins and Druzba, 1938; Goodrich *et al.*, 1944, 1947; Dzwillo, 1959; Kirpichnikov, 1981).

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

This paper presents the genetic linkage analyses of three sex-linked color pattern genes: black caudal-peduncle (*Bcp*), red tail (*Rdt*) and variegated tail (*Var*) (Khoo *et al.*, 1999a, b), their interactions with each other and the SdR, and their relationships to the blue tail (*Blt*), green tail (*Grt*) and snakeskin body-snakeskin tail (*Ssb-Sst*) traits that were investigated in our earlier studies (Fernando and Phang, 1989; Phang *et al.*, 1989a, b, 1990; Phang and Fernando, 1991). Map distances of these genes from the sex-determining region and from each other were determined from recombination rates. We report the mapping of these gene loci of domesticated guppies onto the phenotypic map of wild-type guppy sex chromosomes that was originally constructed by Winge (1927, 1934) and later revised by Winge and Ditlevsen (1947), Yamamoto (1975), Kirpichnikov (1981) and Purdom (1993).

MATERIALS AND METHODS

Source of the fish

Three- to four-week old fry of the Tuxedo (TUX) and Green Variegated (GV) guppy strains were obtained from highly inbred and well-established TUX and GV stocks of the Chin Lam Brothers Tropical Fish Farm and Swee Hing & Brothers Aquarium Co., respectively, in Singapore. Tuxedo and Green Variegated are the commercial names given to these strains by guppy breeders. Male and female juveniles, distinguishable by the expression of their color patterns due

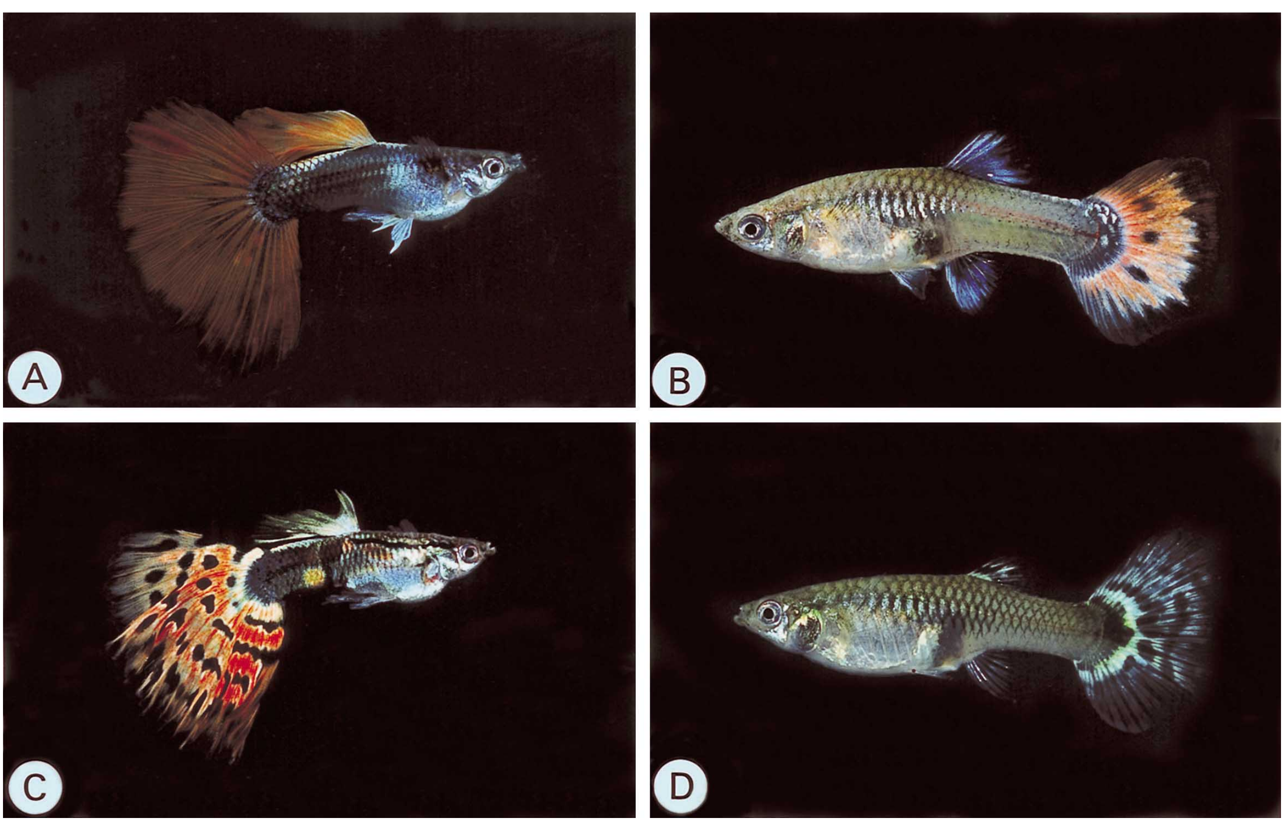

Fig. 1. (**A**) Adult male guppy of the Tuxedo (TUX) strain showing a black caudal-peduncle and red tail. (**B**) Adult female guppy of the TUX strain with grey caudal-peduncle and faint red tinges on an opaque greyish-white tail. (**C**) Adult male guppy of the Green Variegated (GV) strain displaying an orange tail with yellow streaks, and numerous black spots and patterns of different shapes and sizes. (**D**) Adult female guppy of the GV strain with wild-type female olive-brown background body coloration and faint greyish-brown variegated patterns on a yellowish tail.

to sexual dimorphism, were cultured separately according to Khoo *et al.* (1999a, b) for another three to four weeks before being used for reciprocal crosses between the TUX and GV strains. This was to ensure that juvenile males were fully mature (as indicated by a well-developed gonopodium) and females had not been previously inseminated. Under laboratory conditions, domesticated guppies reach sexual maturation at six to eight weeks of age.

Description of the strains

Adult males of the Tuxedo (TUX) strain have black or dark grey

Table 1. Mating results of crosses between Tuxedo (TUX) males and Green Variegated (GV) 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*, *Bcp* and *Var* genes were not considered in chi-square analyses. (Phenotypes: TUX=Tuxedo with black caudal-peduncle and red tail [grey caudal-peduncle and faint red tinges on an opaque greyish-white tail in TUX females]; RT=red tail without black caudal-peduncle; BCP=black caudal-peduncle without red tail; VAR=tail with variegated patterns; TUXVAR=Tuxedo with variegated tail patterns; RTVAR=red tail with variegated patterns; BCPVAR=black caudal-peduncle with variegated tail patterns. Genes: *Bcp* =black caudal-peduncle gene; Bcp^+= absence of black caudal-peduncle gene; *Rdt* =red tail gene; Rdt^+=absence of red tail gene; *Var*=variegated tail pattern gene; Var^+=absence of variegated tail pattern gene).

A. TUX ♂♂ × GV ♀♀ (Parental Cross)

TUX ♂♂ type	Mating pair designation	No. of F_1 broods	Observed numbers for each F_1 phenotypic class (expected numbers): ♂♂ TUXVAR	♂♂ RTVAR	♀♀ TUXVAR	♀♀ RTVAR	Expected 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 Homogeneity	Putative parental genotypes: ♂♂ TUX	♀♀ GV
I	TG5	2	10 (12)		14 (12)		1:1	0.666	0.376					
	TG6	2	7 (6)		5 (6)		1:1	0.334	0.084					
	TG9	3	26 (21.5)		17 (21.5)		1:1	1.884	1.488					
	TG15	2	6 (7.5)		9 (7.5)		1:1	0.600	0.266	5.236	0.004	5.232	X_{Bcp,Rdt,Var^+}	$X_{Bcp^+,Rdt^+,Var}$
	TG18	3	27 (28.5)		30 (28.5)		1:1	0.158	0.070	(*df*=9)	(*df*=1)	(*df*=8)	Y_{Bcp,Rdt,Var^+}	$X_{Bcp^+,Rdt^+,Var}$
	TG19	4	40 (38.5)		37 (38.5)		1:1	0.116	0.052					
	TG21	2	6 (7)		8 (7)		1:1	0.286	0.072					
	TG23	2	5 (4)		3 (4)		1:1	0.500	0.126					
	TG25	2	5 (6.5)		8 (6.5)		1:1	0.692	0.308					
	Pooled:	22	132 (131.5)		131 (131.5)		1:1	0.004	0.000					
II	TG7	3	20 (26)			32 (26)	1:1	2.770	2.326	–	–	–	X_{Bcp^+,Rdt,Var^+} Y_{Bcp,Rdt,Var^+}	$X_{Bcp^+,Rdt^+,Var}$ $X_{Bcp^+,Rdt^+,Var}$
III	TG1	4		21 (21.5)	22 (21.5)		1:1	0.024	0.000	0.310	0.158	0.152	X_{Bcp,Rdt,Var^+}	$X_{Bcp^+,Rdt^+,Var}$
	TG20	2		6 (7)	8 (7)		1:1	0.286	0.072	(*df*=2)	(*df*=1)	(*df*=1)	Y_{Bcp^+,Rdt,Var^+}	$X_{Bcp^+,Rdt^+,Var}$
	Pooled:	6		27 (28.5)	30 (28.5)		1:1	0.158	0.070					

df : degrees of freedom

B. F_1 ♂♂ × F_1 ♀♀ (Full-sib F_1 Cross)

TUX ♂♂ type	Mating pair designation	No. of F_2 broods (No. of F_1 pairs)	Observed numbers of each F_2 phenotypic class (expected numbers): ♂♂ TUX	♂♂ TUXVAR	♂♂ RTVAR	♂♂ BCPVAR	♂♂ RT	♂♂ VAR	♀♀ TUXVAR	♀♀ RTVAR	♀♀ VAR	♀♀ TUX	Expected F_2 ratio	Chi-square Goodness-of-fit Test (*df*=3): χ^2	χ^2_{adj}	Total χ^2	Pooled χ^2	χ^2 for Homogeneity	Putative F_1 genotypes [phenotypes]: ♂♂	♀♀
I	TG5	4 (2)	14 (14.5)	16 (14.5)					13 (14.5)		15 (14.5)	1§	1:1:1:1	0.344	0.138					
	TG6	12 (3)	31 (36.5)	41 (36.5)				2§	37 (36.5)		37 (36.5)		1:1:1:1	1.398	1.123					
	TG9	4 (3)	8 (7.5)	7 (7.5)					9 (7.5)		6 (7.5)		1:1:1:1	0.666	0.266					
	TG15	3 (3)	5 (6.5)	11 (6.5)					4 (6.5)		6 (6.5)		1:1:1:1	4.461	3.231	10.981	1.597	9.384	$X_{Bcp^+,Rdt^+,Var}$	X_{Bcp,Rdt,Var^+}
	TG18	3 (3)	8 (8)	8 (8)					9 (8)		7 (8)		1:1:1:1	0.250	0.124	(*df*=27)	(*df*=3)	(*df*=24)	Y_{Bcp,Rdt,Var^+}	$X_{Bcp^+,Rdt^+,Var}$
	TG19	8 (3)	17 (21.5)	21 (21.5)					24 (21.5)		24 (21.5)	2§	1:1:1:1	1.536	1.116				[TUXVAR]	[TUXVAR]
	TG21	4 (3)	17 (14.5)	15 (14.5)				1§	13 (14.5)		13 (14.5)		1:1:1:1	0.758	0.414					
	TG23	7 (2)	17 (19)	20 (19)		1§			21 (19)		18 (19)		1:1:1:1	0.528	0.262					
	TG25	5 (2)	12 (12.25)	10 (12.25)				1§	15 (12.25)		12 (12.25)	1§	1:1:1:1	1.040	0.673					
	Pooled:	50 (24)	129 (140.75)	149 (140.75)		1§		4§	145 (140.75)		140 (140.75)	4§	1:1:1:1	1.597	1.426					
II	TG7	9 (3)	25 (25.75)	26 (25.75)	1§			2§	7§	27 (25.75)	25 (25.75)	2§	1:1:1:1	0.107	0.028	–	–	–	$X_{Bcp^+,Rdt^+,Var}$ Y_{Bcp,Rdt,Var^+} [TUXVAR]	X_{Bcp^+,Rdt,Var^+} $X_{Bcp^+,Rdt^+,Var}$ [RTVAR]
III	TG1	2 (2)	6 (7.5)		7 (7.5)				6 (7.5)		11 (7.5)		1:1:1:1	2.266	1.466	2.932	1.584	1.348	$X_{Bcp^+,Rdt^+,Var}$	X_{Bcp,Rdt,Var^+}
	TG20	9 (2)	18 (16.5)	3§	14 (16.5)		1§		16 (16.5)		18 (16.5)		1:1:1:1	0.666	0.364	(*df*=6)	(*df*=3)	(*df*=3)	Y_{Bcp^+,Rdt,Var^+}	$X_{Bcp^+,Rdt^+,Var}$
	Pooled:	11 (4)	24 (24)	3§	21 (24)		1§		22 (24)		29 (24)		1:1:1:1	1.584	1.208				[RTVAR]	[TUXVAR]

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

pigmentation on the caudal-peduncle region, and a caudal fin that ranges from blood-red to orange-red in color (Fig. 1A). TUX females show wild-type olive-brown body coloration and grey caudal-peduncle with red tinges of varying intensity on an opaque greyish-white tail (Fig. 1B). The TUX strain has been shown to carry the black caudal-peduncle (*Bcp*) and red tail (*Rdt*) color pattern genes by Fernando and Phang (1990) and Khoo *et al.* (1999b).

Adult Green Variegated (GV) males display wild-type male body coloration which consists of polymorphic patches of various colors that are overlaid by a green metallic sheen. GV males also have a bright orange caudal fin with a mosaic pattern of black spots of different shapes and sizes, and some yellow streaks (Fig. 1C). GV females display wild-type female body coloration and greyish-brown variegated patterns on a yellowish translucent tail (Fig. 1D). The variegated tail patterning of the GV strain is due to the *Var* color pattern gene (Khoo *et al.*, 1999a).

Reciprocal crosses

To establish the mode of inheritance and linkage of the black caudal-peduncle, red tail and variegated tail color patterns, single-pair reciprocal crosses were made between six-week old mature virgin fish of the TUX and GV strains. 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 produce the F_2 generation. The following notations were used: TUX♂♂ × GV♀♀ (Table 1A) and GV♂♂ × TUX♀♀ (Table 2A) for parental crosses, and F_1♂♂ × F_1♀♀ (Tables 1B and 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). F_1 and F_2 offspring were segregated according to color phenotype and sex. Their color patterns were des-

Table 2. Mating results of crosses between Green Variegated (GV) 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*, *Bcp* and *Var* genes were not considered in chi-square analyses. (Phenotypes: TUX=Tuxedo with black caudal-peduncle and red tail [grey caudal-peduncle and faint red tinges on an opaque greyish-white tail in TUX females]; VAR=tail with variegated patterns; TUXVAR=Tuxedo with variegated tail patterns; RTVAR=red tail with variegated patterns; BCPVAR=black caudal-peduncle with variegated tail patterns. Genes: *Bcp*=black caudal-peduncle gene; Bcp^+= absence of black caudal-peduncle gene; *Rdt*=red tail gene; Rdt^+=absence of red tail gene; *Var*=variegated tail pattern gene; Var^+=absence of variegated tail pattern gene).

A. GV ♂♂ × TUX♀♀ (Parental Cross)

Mating pair designation	No. of F_1 broods	Observed numbers for each F_1 phenotypic class (expected numbers) ♂♂ TUXVAR	♂♂ RTVAR	♂♂ VAR	♀♀ TUXVAR	♀♀ RTVAR	♀♀ VAR	Expected ratio of ♂:♀ (*df*)	Chi-square Goodness-of-fit Test χ^2	χ^2_{adj}	Total χ^2	Pooled χ^2	χ^2 for Homogeneity	Putative parental genotypes ♂♂ GV	♀♀ TUX
GT1	4	30 (29)			28 (29)			1:1 (1)	0.068	0.018					
GT2	3	7 (9.5)			12 (9.5)			1:1 (1)	1.316	0.842					
GT3	3	12 (13)			14 (13)			1:1 (1)	0.154	0.038	3.864 (*df*=7)	0.080 (*df*=1)	3.784 (*df*=6)	$X_{Bcp^+,Rdt^+,Var}$ $Y_{Bcp^+,Rdt^+,Var}$	X_{Bcp,Rdt,Var^+} X_{Bcp,Rdt,Var^+}
GT5	1	6 (4.5)			3 (4.5)			1:1 (1)	1.000	0.444					
GT8	2	13 (12.5)			12 (12.5)			1:1 (1)	0.040	0.000					
GT9	3	18 (21)			24 (21)			1:1 (1)	0.858	0.596					
GT11	2	12 (10.5)			9 (10.5)			1:1 (1)	0.428	0.190					
Pooled:	18	98 (100)			102 (100)			1:1 (1)	0.080	0.046					
GT7	4	25 (18.5)		18 (18.5)	15 (18.5)		16 (18.5)	1:1:1:1 (3)	3.298	2.648	–	–	–	$X_{Bcp^+,Rdt^+,Var}$ $Y_{Bcp^+,Rdt^+,Var}$	X_{Bcp,Rdt,Var^+} X_{Bcp^+,Rdt^+,Var^+}
GT4	2	10 (9)	9 (9)		10 (9)	7 (9)		1:1:1:1 (3)	0.666	0.334	2.266 (*df*=6)	1.857 (*df*=3)	0.409 (*df*=3)	$X_{Bcp^+,Rdt^+,Var}$ $Y_{Bcp^+,Rdt^+,Var}$	X_{Bcp,Rdt,Var^+} X_{Bcp^+,Rdt,Var^+}
GT16	2	7 (5)	5 (5)		5 (5)	3 (5)		1:1:1:1 (3)	1.600	1.000					
Pooled:	4	17 (14)	14 (14)		15 (14)	10 (14)		1:1:1:1 (3)	1.857	1.357					

df : degrees of freedom

B. F_1 ♂♂ × F_1 ♀♀ (Full-sib F_1 Cross)

Mating designation	No. of F_2 broods (No. of F_1 pairs)	Observed numbers for each F_2 phenotypic class (expected numbers) ♂♂ TUXVAR	♂♂ VAR	♂♂ TUX	♂♂ BCPVAR	♀♀ TUXVAR	♀♀ TUX	Expected F_2 ratio	Chi-square Goodness-of-fit Test (*df*=3) χ^2	χ^2_{adj}	Total χ^2	Pooled χ^2	χ^2 for Homogeneity	Putative F_1 genotypes [phenotypes] ♂♂	♀♀
GT1	14 (2)	26 (29.5)	24 (29.5)		2[#]	33 (29.5)	35 (29.5)	1:1:1:1	2.880	2.304					
GT2	5 (3)	7 (8.75)	7 (8.75)		1[#]	10 (8.75)	11 (8.75)	1:1:1:1	1.458	0.772					
GT3	9 (3)	18 (15.75)	14 (15.75)	1[#]		15 (15.75)	16 (15.75)	1:1:1:1	0.555	0.301	6.664 (*df*=18)	2.505 (*df*=3)	4.159 (*df*=15)	X_{Bcp,Rdt,Var^+} $Y_{Bcp^+,Rdt^+,Var}$ [TUXVAR]	$X_{Bcp^+,Rdt^+,Var}$ X_{Bcp,Rdt,Var^+} [TUXVAR]
GT5	12 (3)	21 (18.25)	16 (18.25)			18 (18.25)	18 (18.25)	1:1:1:1	0.697	0.451					
GT8[e]	0 (2)	0	0			0	0	1:1:1:1	–	–					
GT9	2 (3)	5 (3.75)	3 (3.75)			4 (3.75)	3 (3.75)	1:1:1:1	0.734	0.201					
GT11	22 (3)	24 (25.75)	25 (25.75)	6[#]	2[#]	28 (25.75)	26 (25.75)	1:1:1:1	0.340	0.184					
Pooled:	64 (19)	101 (101.75)	89 (101.75)	7[#]	5[#]	108 (101.75)	109 (101.75)	1:1:1:1	2.505	2.249					

df : degrees of freedom
[e] : exceptional case where full-sib F_1 crosses did not produce any F_2 progenies
[#] : recombinant data (not used for chi-square analyses)

ignated as TUX (black caudal-peduncle and red tail typical of the Tuxedo strain), TUXVAR (Tuxedo with variegated tail patterning), RTVAR (red tail with variegated patterns), BCPVAR (black caudal-peduncle with variegated tail patterns), RT (red tail) and VAR (variegated tail with a mosaic pattern of large black spots and patches). To facilitate description of the crosses, Tuxedo male parents of TUX ♂♂ × GV ♀♀ were typed using Roman numerals (I, II, III, IV and V) according to their putative genotypes following segregation and scoring of F_1 and F_2 progenies (Khoo *et al*., 1999b).

Statistical and linkage 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 the observed and expected numbers in each phenotypic class 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. Data was pooled when the χ^2 test for homogeneity indicated that there were no significant differences among the phenotypic frequencies, the observations were sufficiently uniform and the families homogeneous. 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). Individuals with exceptional coloration due to crossing-over of the black caudal-peduncle (*Bcp*), red tail (*Rdt*) and variegated tail (*Var*) genes between the X- and Y-chromosomes were not considered during chi-square analyses (Winge, 1922b, 1923, 1927, 1934; Nayudu, 1979; Phang *et al*., 1989a, b, 1990; Phang and Fernando, 1991; Khoo *et al*., 1999a, b).

Crossover fractions and map distances of *Bcp*, *Rdt* and *Var* relative to each other and the sex-determining region (SdR) were calculated according to Strickberger (1990), Phang *et al.* (1990), Phang and Fernando (1991), Purdom (1993) and Khoo *et al.* (1999a, b). Winge's (1922b, 1927, 1934) "zig-zag line diagram" method was used to test all possible linkage combinations between *Bcp*, *Rdt*, *Var* and the SdR, and to map these gene loci onto the sex chromosomes. Double recombinants were noted but excluded from all estimations of map distances (Winge, 1927, 1934; Purdom, 1993, Khoo *et al*., 1999b).

RESULTS

Segregation and recombination in F_1 and F_2 offspring of TUX × GV

Nine mating pairs of TUX♂♂ × GV♀♀ produced a total of 132 male and 131 female F_1 offspring (Table 1A). F_1 males had the black caudal-peduncle and red caudal fin of their TUX male parents but also displayed black spots and patches on the tail fin (Fig. 2A). F_1 females had a grey caudal-peduncle and an opaque greyish-white tail with red tinges and black spots (Fig. 2B). Phenotypically classed as Tuxedo with variegated tail patterning (TUXVAR), F_1 males and females inherited the black caudal-peduncle and red tail traits from their TUX male parents (type I) while variegated tail patterning was from the GV female parents (Fig. 3). Table 1A and Fig. 3 show two other crosses in which the TUX male parents were heterozygous for black caudal-peduncle. To facilitate describing these crosses and their offspring, the TUX males were labelled as types II and III. Types IV and V males (heterozygous for red tail) were not observed in this study although they were found among crosses between the Tuxedo strain and wild-type guppies (Khoo *et al*., 1999b). For mating pair TG7 (type II), there were three F_1 broods of 20 TUXVAR males and 32 females with variegated patterns on their reddish tails but without a black caudal-peduncle (RTVAR) (Figs. 2, 3, Table 1A). RTVAR males (27) and TUXVAR females (30) were produced by the cross between type III TUX males and GV females (mating pairs TG1 and TG20) (Figs. 2 and 3, Table 1A). For all three types (I, II and III) of TUX male parents, the number of F_1 males to females was consistent with the expected ratio of 1:1 (Table 1A).

The F_2 generation for type I TUX male parents comprised 129 TUX and 149 TUXVAR males, and 145 TUXVAR and 140 VAR females (Figs. 2A, 2B), with observed numbers conforming to the expected 1:1:1:1 phenotypic ratio (Table 1B, Fig. 3). Four F_2 phenotypes of 25 TUX and 26 TUXVAR males, and 27 RTVAR and 25 VAR females were obtained from three single-pair full-sib F_1 crosses of type II (Figs. 2A, 2B). These also agreed with the 1:1:1:1 ratio (Table 1B, Fig. 3). Mating pairs TG1 and TG20 (type III) gave four F_2 phenotypes of 24 TUX and 21 RTVAR males, and 22 TUXVAR and 29 VAR females (Figs. 2A, 2B) that concurred with the ratio of 1:1:1:1 (Table 1B, Fig. 3). Homogeneity χ^2 tests for types I and III TUX males showed that the F_1 and F_2 progenies did not form heterogeneous populations and were uniform (Tables 1A, 1B). F_1 and F_2 results also indicated that homozygous Tuxedo male and Green Variegated female parents had the $X_{Bcp,Rdt,Var}{}^{+}Y_{Bcp,Rdt,Var}{}^{+}$ (type I) and $X_{Bcp^+,Rdt^+,Var}X_{Bcp^+,Rdt^+,Var}$ genotypes, respectively (Table 1, Fig. 3). Conversely, genotypes of Tuxedo males heterozygous for black caudal-peduncle were $X_{Bcp^+,Rdt,Var}{}^{+}Y_{Bcp,Rdt,Var}{}^{+}$ (type II) and $X_{Bcp,Rdt,Var}{}^{+}Y_{Bcp^+,Rdt,Var}{}^{+}$ (type III). Fig. 3 shows the segregation and mechanism of inheritance of the *Bcp*, *Rdt* and *Var* genes.

Crossing-over between the *Bcp*, *Rdt* and *Var* color pattern genes, and the sex-determining region (SdR) from the Y- to the X-chromosome and vice versa in the F_1 parents produced F_2 offspring that did not conform to the expected phenotypic classes for TUX male parents of types I, II and III (Table 1B, Figs. 2 and 3). F_2 recombinants produced by type I were a BCPVAR and four VAR males, and four TUX females (Figs. 2A, 2B, Table 1B). Recombination frequency calculated from the percentage of VAR males out of the total number of F_2 males (4/283×100%) was 1.413% for the SdR–*Rdt* region (Table 1B). The four TUX females of 289 F_2 females gave a crossover rate of 1.384% between the *Var* locus and SdR. Occurrence of the VAR male and TUX female F_2 recombinants suggested that the Y-chromosome may have a gene map order of *Var*–SdR–*Rdt*–*Bcp* as these individuals could not be produced using Winge's (1922b, 1927, 1934) "zig-zag line diagram" method if the order had been either SdR–*Var*–*Rdt*–*Bcp* or SdR–*Rdt*–*Bcp*–*Var* (Figs. 3, 4). The BCPVAR recombinant male was not used to calculate map distance as it could be produced by single crossing-over that occurred simultaneously at SdR–*Rdt* in the F_1 male parent and *Rdt*–*Bcp* in the F_1 female parent. This individual could also result from double recombination between the SdR and *Bcp* whereby *Rdt* on the Y-chromosome of the F_1 male parent crossed over to the X. In the latter event, *Rdt* appears to lie between the SdR and *Bcp*, and has an estimated double crossover fre-

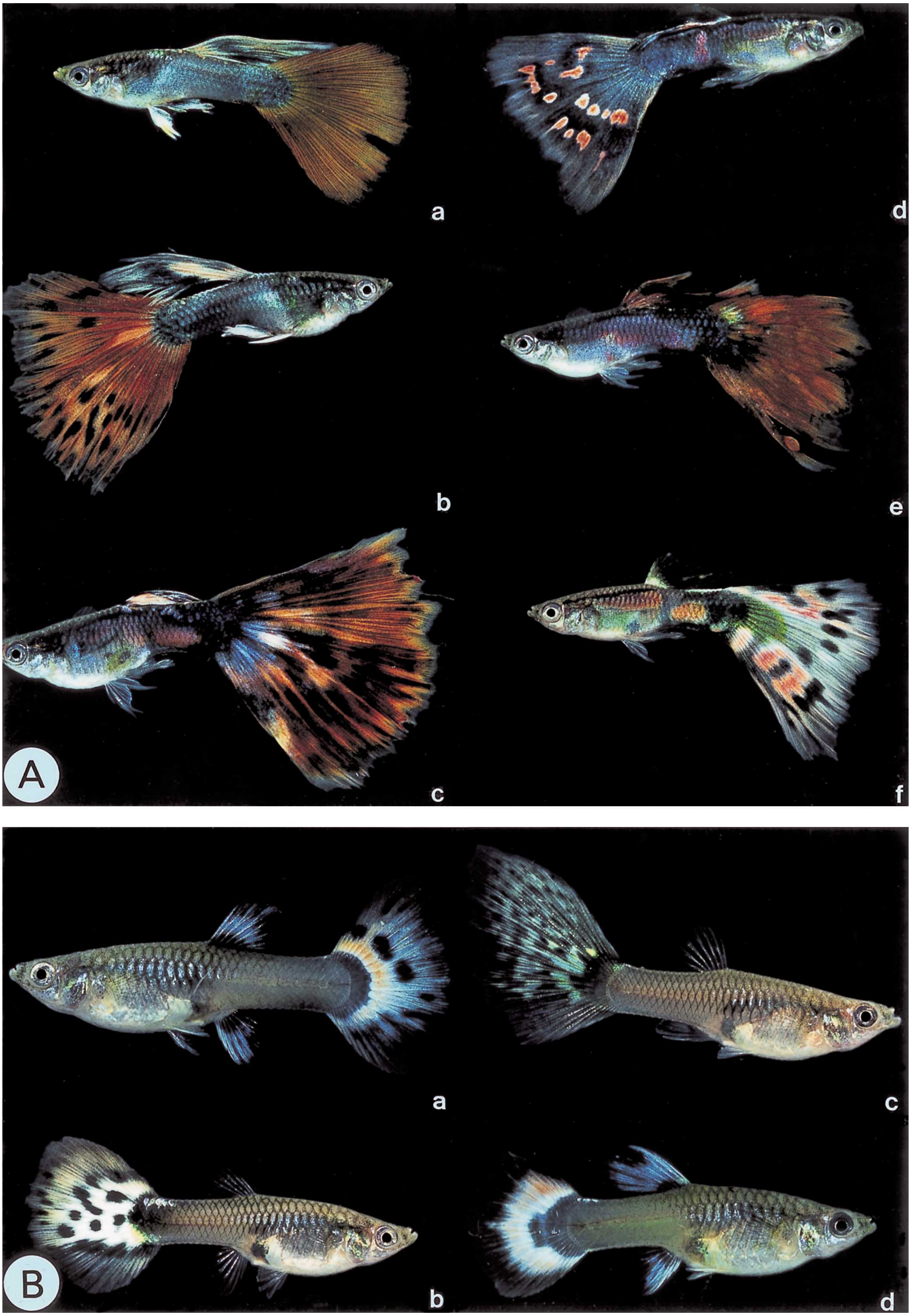

Fig. 2. Progenies (F_1, F_2 and recombinants) from Tuxedo ♂♂× Green Variegated ♀♀ and Green Variegated ♂♂×Tuxedo ♀♀ crosses had the following phenotypic color patterns for (**A**) males: (**a**) Tuxedo (TUX), (**b**) Tuxedo with variegated tail patterning (TUXVAR), (**c**) red tail with variegated patterns (RTVAR), (**d**) black caudal-peduncle with variegated tail patterns (BCPVAR), (**e**) red tail (RT) and (**f**) variegated tail with a mosaic pattern of large black spots and patches (VAR), and (**B**) females: (**a**) TUXVAR, (**b**) RTVAR, (**c**) VAR and (**d**) TUX.

	♂♂ Tuxedo		♀♀ Green Variegated		♂♂ Green Variegated		♀♀ Tuxedo	
P I	$X_{Bcp,Rdt,Var^+}Y_{Bcp,Rdt,Var^+}$	×	$X_{Bcp^+,Rdt^+,Var}X_{Bcp^+,Rdt^+,Var}$		$X_{Bcp^+,Rdt^+,Var}Y_{Bcp^+,Rdt^+,Var}$	×	$X_{Bcp,Rdt,Var^+}X_{Bcp,Rdt,Var^+}$	
II	$X_{Bcp^+,Rdt,Var^+}Y_{Bcp,Rdt,Var^+}$						$X_{Bcp,Rdt,Var^+}X_{Bcp^+,Rdt^+,Var^+}$	7
III	$X_{Bcp,Rdt,Var^+}Y_{Bcp^+,Rdt,Var^+}$						$X_{Bcp,Rdt,Var^+}X_{Bcp^+,Rdt,Var^+}$	416

	♂♂		♀♀		♂♂		♀♀	
F₁ I	$X_{Bcp^+,Rdt^+,Var}Y_{Bcp,Rdt,Var^+}$ TUXVAR	×	$X_{Bcp,Rdt,Var^+}X_{Bcp^+,Rdt^+,Var}$ TUXVAR		$X_{Bcp,Rdt,Var^+}Y_{Bcp^+,Rdt^+,Var}$ TUXVAR	×	$X_{Bcp^+,Rdt^+,Var}X_{Bcp,Rdt,Var^+}$ TUXVAR	
II	$X_{Bcp^+,Rdt^+,Var}Y_{Bcp,Rdt,Var^+}$ TUXVAR	×	$X_{Bcp^+,Rdt,Var^+}X_{Bcp^+,Rdt^+,Var}$ RTVAR		$X_{Bcp,Rdt,Var^+}Y_{Bcp^+,Rdt^+,Var}$ TUXVAR $X_{Bcp^+,Rdt^+,Var^+}Y_{Bcp^+,Rdt^+,Var}$ VAR		$X_{Bcp^+,Rdt^+,Var}X_{Bcp,Rdt,Var^+}$ TUXVAR $X_{Bcp^+,Rdt^+,Var}X_{Bcp^+,Rdt^+,Var^+}$ VAR	7
III	$X_{Bcp^+,Rdt^+Var}Y_{Bcp^+,Rdt,Var^+}$ RTVAR	×	$X_{Bcp,Rdt,Var^+}X_{Bcp^+,Rdt^+,Var}$ TUXVAR		$X_{Bcp,Rdt,Var^+}Y_{Bcp^+,Rdt^+,Var}$ TUXVAR $X_{Bcp^+,Rdt,Var^+}Y_{Bcp^+,Rdt^+,Var}$ RTVAR		$X_{Bcp^+,Rdt^+,Var}X_{Bcp,Rdt,Var^+}$ TUXVAR $X_{Bcp^+,Rdt^+,Var}X_{Bcp^+,Rdt,Var^+}$ RTVAR	416

	♂♂	♀♀		♂♂	♀♀
F₂ I	$X_{Bcp,Rdt,Var^+}Y_{Bcp,Rdt,Var^+}$ TUX $X_{Bcp^+,Rdt^+,Var}Y_{Bcp,Rdt,Var^+}$ TUXVAR	$X_{Bcp^+,Rdt^+,Var}X_{Bcp,Rdt,Var^+}$ TUXVAR $X_{Bcp^+,Rdt^+,Var}X_{Bcp^+,Rdt^+,Var}$ VAR		$X_{Bcp,Rdt,Var^+}Y_{Bcp^+,Rdt^+,Var}$ TUXVAR $X_{Bcp^+,Rdt^+,Var}Y_{Bcp^+,Rdt^+,Var}$ VAR	$X_{Bcp,Rdt,Var^+}X_{Bcp^+,Rdt^+,Var}$ TUXVAR $X_{Bcp,Rdt,Var^+}X_{Bcp,Rdt,Var^+}$ TUX
II	$X_{Bcp^+,Rdt,Var^+}Y_{Bcp,Rdt,Var^+}$ TUX $X_{Bcp^+,Rdt^+,Var}Y_{Bcp,Rdt,Var^+}$ TUXVAR	$X_{Bcp^+,Rdt^+,Var}X_{Bcp^+,Rdt,Var^+}$ RTVAR $X_{Bcp^+,Rdt^+,Var}X_{Bcp^+,Rdt^+,Var}$ VAR			
III	$X_{Bcp,Rdt,Var^+}Y_{Bcp^+,Rdt,Var^+}$ TUX $X_{Bcp^+,Rdt^+,Var}Y_{Bcp^+,Rdt,Var^+}$ RTVAR	$X_{Bcp^+,Rdt^+,Var}X_{Bcp,Rdt,Var^+}$ TUXVAR $X_{Bcp^+,Rdt^+,Var}X_{Bcp^+,Rdt^+,Var}$ VAR			

F₂ phenotypic ratio:-

TUX ♂♂ × GV ♀♀

I 1 TUX ♂ : 1 TUXVAR ♂ : 1 TUXVAR ♀ : 1 VAR ♀

II 1 TUX ♂ : 1 TUXVAR ♂ : 1 RTVAR ♀ : 1 VAR ♀

III 1 TUX ♂ : 1 RTVAR ♂ : 1 TUXVAR ♀ : 1 VAR ♀

GV ♂♂ × TUX ♀♀

1 TUXVAR ♂ : 1 VAR ♂ : 1 TUXVAR ♀ : 1 TUX ♀

F₂ Recombinants

	♂♂	♀♀		♂♂	♀♀
I	$X_{Bcp^+,Rdt^+,Var}Y_{Bcp^+,Rdt^+,Var^+}$ VAR $X_{Bcp^+,Rdt^+,Var}Y_{Bcp,Rdt^+,Var^+}$ BCPVAR♦,♣	$X_{Bcp^+,Rdt^+,Var^+}X_{Bcp,Rdt,Var^+}$ TUX	**R**	$X_{Bcp,Rdt,Var^+}Y_{Bcp^+,Rdt^+,Var^+}$ TUX $X_{Bcp^+,Rdt^+,Var}Y_{Bcp,Rdt^+,Var}$ $X_{Bcp,Rdt^+,Var}Y_{Bcp^+,Rdt^+,Var}$ BCPVAR #	–
II	$X_{Bcp^+,Rdt^+,Var}Y_{Bcp^+,Rdt^+,Var^+}$ VAR $X_{Bcp^+,Rdt^+,Var}Y_{Bcp^+,Rdt,Var^+}$ RTVAR	$X_{Bcp,Rdt,Var}X_{Bcp^+,Rdt^+,Var}$ $X_{Bcp,Rdt,Var}X_{Bcp^+,Rdt,Var^+}$ $X_{Bcp,Rdt^+,Var}X_{Bcp^+,Rdt,Var^+}$ TUXVAR♦ $X_{Bcp,Rdt,Var^+}X_{Bcp^+,Rdt,Var^+}$ $X_{Bcp,Rdt^+,Var^+}X_{Bcp^+,Rdt,Var^+}$ TUX♣			
III	$X_{Bcp^+,Rdt,Var^+}Y_{Bcp^+,Rdt,Var^+}$ $X_{Bcp^+,Rdt^+,Var^+}Y_{Bcp^+,Rdt,Var^+}$ RT♦ $X_{Bcp,Rdt,Var}Y_{Bcp^+,Rdt,Var^+}$ $X_{Bcp,Rdt^+,Var}Y_{Bcp^+,Rdt,Var^+}$ $X_{Bcp,Rdt,Var^+}Y_{Bcp^+,Rdt,Var}$ TUXVAR♦	–			

I : crosses where the Tuxedo ♂♂ parents were homozygous for *Bcp* and *Rdt*. II, III : crosses where the Tuxedo ♂♂ parents were heterozygous for *Bcp*. 7 : mating pair GT7 where the Tuxedo ♀ parent was heterozygous for *Bcp* and *Rdt*. 416 : mating pairs GT4 and GT16 where the Tuxedo ♀♀ parents were heterozygous for *Bcp*. ♦ : recombinants with several possible genotypes due to single crossovers between different loci in the F_1 ♂♂ and/or ♀♀ parents. ♣ : recombinant(s) possibly produced by double crossing-over in the F_1 ♂♂ parents. # : BCPVAR ♂♂ recombinants with two possible genotypes due to crossovers between *Bcp* and *Rdt* in the F_1 ♂♂ and ♀♀ parents.

Fig. 3. Schematic diagram of the proposed genetic models showing segregation of the *Bcp*, *Rdt* and *Var* colour pattern genes, and genotypes of the parents (**P**), F_1 and F_2 progenies, and recombinants (**R**) in reciprocal crosses between the Tuxedo and Green Variegated guppy strains.

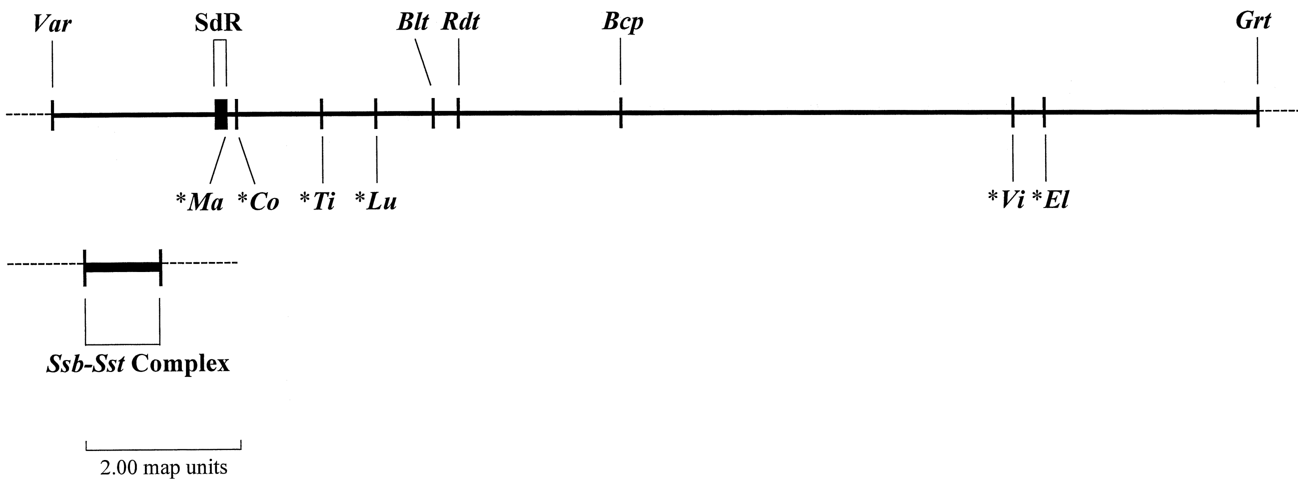

Fig. 4. Genetic map of the Y-chromosome of the guppy, *Poecilia reticulata*, showing the positions of the black caudal-peduncle (*Bcp*), red tail (*Rdt*) and variegated tail (*Var*) loci relative to the sex-determining region (SdR). Map distances of *Bcp*, *Rdt* and *Var* from the SdR are based on recombination frequencies estimated from Tables 1 & 2 and Khoo *et al.* (1999a, b). Gene order for blue tail (*Blt*), green tail (*Grt*) and the snakeskin body-snakeskin tail complex (*Ssb-Sst* complex) were reanalysed from the crossover data of Phang *et al.* (1989a, b, 1990) and Phang and Fernando (1991). The allele positions of Winge's (1927, 1934) color pattern genes of wild-type guppies (*): *Maculatus* (*Ma*), *Coccineus* (*Co*), *Tigrinus* (*Ti*), *Luteus* (*Lu*), *Vitellinus* (*Vi*) and *Elongatus* (*El*) were according to Kirpichnikov's (1981) and Purdom's (1993) revisions. The loci of color pattern genes of the domesticated guppy (*Bcp*, *Blt*, *Grt*, *Rdt*, *Ssb-Sst* complex and *Var*) are inferred to be located at similar positions on the X-chromosome. The size of the SdR is not according to scale as the number of male-determining genes within that region is not known.

quency of 0.353%.

A recombination of 3.704% was estimated for SdR–*Rdt* from two VAR males out of 54 F_2 males for type II (Fig. 2A, Table 1B). These VAR males further support a gene order of *Var*–SdR–*Rdt*–*Bcp* (Figs. 3, 4). From a solitary RTVAR male (Fig. 2A), 1.852 map units was estimated to separate *Bcp* from *Rdt* (Table 1B). TUXVAR F_2 recombinant females of mating pair TG7 (type II TUX male) were not used to calculate map distances as crossing-over could have taken place at SdR–*Rdt*, *Rdt*–*Bcp* or SdR–*Var* (Figs. 2B, 3). Double recombination at either *Var*–*Rdt* or *Var*–*Bcp* might have produced F_2 TUX females where the SdR crossed over to the X-chromosome from the Y during meiosis in the F_1 male parents. These events generate several possible genotypes for a particular phenotype, hence making it impossible to determine the actual region of crossover. Map distance was also not estimated from the F_2 RT male recombinant of a type III TUX male (mating pair TG20) as crossing-over could occur at *Var*–*Rdt* or *Rdt*–*Bcp* in the F_1 female parent to produce this individual (Fig. 2A, Table 1B). Combining the results of this study (SdR–*Rdt* =2.559±1.620 map units) with those of Khoo *et al.* (1999b), the mean genetic map distance between SdR and Rdt was estimated to be 3.055±1.687 map units (Fig. 4).

Segregation and recombination in F_1 and F_2 offspring of GV × TUX

Seven mating pairs (GT1, GT2, GT3, GT5, GT8, GT9 and GT11) of the reciprocal cross, GV♂♂ × TUX♀♀, gave 18 F_1 broods of 98 males and 102 females. All the F_1 offspring possessed black caudal-peduncle and red tail with variegated patterns typical of the TUXVAR phenotype described earlier (Figs. 2A, 2B). The observed numbers of F_1 male to female offspring agreed with the expected ratio of 1:1 (Table 2A, Fig. 3). Except for seven TUX and five BCPVAR males resulting from crossing-over in the F_1 parents, F_2 progenies segregated into 101 TUXVAR and 89 VAR males, and 108 TUXVAR and 109 TUX females according to the 1:1:1:1 phenotypic ratio (Table 2B, Figs. 2, 3). No F_2 data was obtained for mating pair GT8, as the two F_1♂♂× F_1♀♀ pairs did not produce any F_2 offspring (Table 2B). The TUX female parent of mating pair GT7 was heterozygous for both *Bcp* and *Rdt* as it produced 25 TUXVAR and 18 VAR males, and 15 TUXVAR and 16 VAR females that conformed to the expected F_1 ratio of 1:1:1:1 (Table 2A, Figs. 2, 3). Similarly, GT4 and GT16 gave 17 TUXVAR and 14 RTVAR male, and 15 TUXVAR and 10 RTVAR female F_1 progenies, indicating that these two TUX female parents were heterozygous for the *Bcp* gene. As shown by the homogeneity χ^2 values, the families were uniform and homogeneous (Table 2). Results for F_1 and F_2 progenies also showed that the Green Variegated male and Tuxedo female parents used in this study were homozygous (genotypes: $X_{Bcp^+,Rdt^+,Var}Y_{Bcp^+,Rdt^+,Var}$ and $X_{Bcp,Rdt,Var^+}X_{Bcp,Rdt,Var^+}$, respectively) (Table 2, Fig. 3). Putative genotypes of heterozygous Tuxedo females were $X_{Bcp,Rdt,Var^+}X_{Bcp^+,Rdt^+,Var^+}$ for mating pair GT7, and $X_{Bcp,Rdt,Var^+}X_{Bcp^+,Rdt,Var^+}$ for GT4 and GT16. The segregation of *Bcp*, *Rdt* and *Var*, and their mode of inheritance are illustrated in Fig. 3.

Seven F_2 males from the full-sib cross of GV♂♂×TUX♀♀ exhibited normal Tuxedo color patterns, i.e., a black caudal-peduncle and red tail without variegated tail patterning (Fig. 2A, Table 2B). The variegated tail (*Var*) pattern gene, in this instance, would have crossed-over from the Y-chromosome to the X in the F_1 male parents to produce the TUX phenotype. Since there were seven TUX males out of 202 F_2 male individuals, 3.465% recombination occurred between SdR and *Var* (Table 2B). Five BCPVAR F_2 males were also produced by this cross, giving a distance of 2.475 map units between the *Bcp* and *Rdt* loci (Fig. 2A, Table 2B). From the F_2 results of TUX♂♂×GV♀♀ and GV♂♂×TUX♂♂ (Tables 1, 2), map distances for *Bcp*–*Rdt* and SdR–*Var* were averaged to be 2.164±0.441 and 2.425±1.471 units, respectively. In conjunction with our previous analyses involving crosses between wild-type guppies with the Green Variegated (Khoo *et al*., 1999a) and Tuxedo (Khoo *et al*., 1999b) strains, the *Var* locus appears to lie 2.174±1.301 map units from the SdR while *Rdt* and *Bcp* are 2.330±1.416 map units apart (Fig. 4).

DISCUSSION

Inheritance of the *Bcp*, *Rdt* and *Var* color pattern genes

Observations for all parental (TUX♂♂×GV♀♀ and GV♂♂×TUX♀♀, Tables 1A, 2A) and full-sib (F_1♂♂×F_2♀♀, Tables 1B, 2B) crosses, initiated to determine the inheritance of the black caudal-peduncle (*Bcp*), red tail (*Rdt*) and variegated tail (*Var*) color patterns in domesticated guppy (*Poecilia reticulata*) strains, demonstrate that these color patterns are simple sex-linked traits controlled by single genes (Khoo *et al*., 1999a, b). In addition, our studies show that *Bcp*, *Rdt* and *Var* are dominantly expressed in both males and females, albeit the colors are more distinct and definitive in the males due to the presence of androgens (Figs. 1, 2, Tables 1, 2). These results confirm the preliminary findings of Fernando and Phang (1989, 1990) and Phang *et al.* (1990), and further support those of Khoo *et al*., (1999a, b). Each of the three color pattern genes has two alleles: *Bcp* which is dominant over Bcp^{+}, *Rdt* dominant over Rdt^{+} and *Var* over Var^{+}. Recessive alleles of these loci do not give rise to any color patterns. Tuxedo guppies used in this study are therefore proposed to have the $X_{Bcp,Rdt,Var^{+}}Y_{Bcp,Rdt,Var^{+}}$ (type I), $X_{Bcp^{+},Rdt,Var^{+}}Y_{Bcp,Rdt,Var^{+}}$ (type II) and $X_{Bcp,Rdt,Var^{+}}Y_{Bcp^{+},Rdt,Var^{+}}$ (type III) genotypes for males, and $X_{Bcp,Rdt,Var^{+}}X_{Bcp,Rdt,Var^{+}}$, $X_{Bcp,Rdt,Var^{+}}X_{Bcp^{+},Rdt^{+},Var^{+}}$ and $X_{Bcp,Rdt,Var^{+}}X_{Bcp^{+},Rdt,Var^{+}}$ for females (Tables 1, 2, Fig. 3). Genotypes of Green Variegated males and females are $X_{Bcp^{+},Rdt^{+},Var}Y_{Bcp^{+},Rdt^{+},Var}$ and $X_{Bcp^{+},Rdt^{+},Var}X_{Bcp^{+},Rdt^{+},Var}$, respectively.

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

This study proves that the *Bcp*, *Rdt* and *Var* genes are able to cross over from the X-chromosome to the Y and vice versa since male and female recombinants of the TUX, VAR, RT, TUXVAR, RTVAR and BCPVAR phenotypes were observed at the F_2 level of TUX♂♂×GV♀♀ (for types I, II and III TUX male parents) and GV♂♂×TUX♀♀ (Tables 1, 2, Figs. 2, 3). Alleles of color genes migrating between the X- and Y-chromosomes were initially documented by Winge (1922a, b, 1923) in wild-type guppies. 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 equal in size and indistinguishable by ordinary cytological methods. As a result of this homology between the sex chromosomes, genes are able to crossover along almost the whole length of their chromatids. Only a small segment on the Y-chromosome, the sex-determining region (SdR) which is presumed to contain male-determining genes, is known to be non-homologous and different from the X.

Double recombination may have given rise to a BCPVAR F_2 male (mating pair TG23) and two TUX F_2 females (mating pair TG7) (Table 1B, Fig. 3). Very high double crossover rates of 0.353% and 3.279% were obtained from these offspring, respectively. The TUX F_2 females may possibly be produced by a form of "sex-reversal" as a result of the instability of the genetic mechanism of sex-determination in the guppy (Kirpichnikov, 1981). This takes place when the SdR on the Y-chromosome undergoes double crossing-over with a segment that contains recessive female-determining genes on the X-chromosome (Winge, 1927, 1934; Winge and Ditlevsen, 1947; Kirpichnikov, 1981). Double crossover values were not included in our estimations of map distances because the number of crosses were limited and brood sizes were small (Khoo *et al*., 1999b). Moreover, Winge (1927, 1934) concluded that double crossing-over was unlikely to occur in the guppy as he could find only a few single crossovers among the thousands of guppies he crossed and examined. Also, recombination frequencies of up to 10% have been recorded only between the *Doppelschwert* (Double sword, *Ds*) and *Pigmentierte caudalis* (Caudal pigment, *Cp*) genes in wild-type guppies (Winge, 1927, 1934; Winge and Ditlevsen, 1947; Dzwillo, 1959; Nayudu, 1975, 1979; Kirpichnikov, 1981).

Based on our earlier studies (Khoo *et al*., 1999a, b), *Bcp*, *Rdt* and *Var* are inferred to be located within homologous regions on the X- and Y-chromosomes, and are about 5.147, 3.055±1.992 and 2.174±1.301 map units, respectively, from the SdR (Tables 1, 2, Fig. 4). The mean percentage recombination that involved *Rdt* and *Bcp* alone was 2.330±1.416. As expected, the map distance between SdR and *Bcp* (5.147 map units) is almost equal to the sum of distances (3.055+2.330=5.385 map units) for SdR–*Rdt* and *Rdt*–*Bcp* (Fig. 4) (Khoo *et al*., 1999b). Recombination rates 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 indicate that *Rdt* is closer to SdR than *Bcp* as there was less recombination between *Rdt* and SdR than between *Bcp* and SdR (Tables 1, 2). This further verifies the findings of Khoo *et al.* (1999b). It is therefore evident that the *Bcp* and *Rdt* loci are arranged in the following sequence, SdR–*Rdt*–*Bcp* (Fig. 4), as proposed earlier by Khoo *et al.* (1999b).

The locus for variegated tail patterning, *Var*, is shown in this study and by Khoo *et al.* (1999a) to be 2.174±1.301 map units from the SdR (Fig. 4). Testing all possible linkage combinations for *Bcp*, *Rdt*, *Var* and the SdR using the "zig-zag line diagram" method of Winge (1922b, 1927, 1934), we have found that the map order of these genes is most likely *Var*–SdR–*Rdt*–*Bcp* (Fig. 4). Recombinant individuals observed in this study could not be produced if the order had been SdR–*Var*–*Rdt*–*Bcp*, SdR–*Rdt*–*Bcp*–*Var* or SdR–*Rdt*–*Var*–*Bcp*. Furthermore, any arrangement in which *Bcp* lies closer to the SdR than *Rdt* has been ruled out by this study and Khoo *et al*. (1999b). Khoo *et al.* (1999b) also noted that the SdR–*Bcp*–*Rdt* sequence was possible in only one exceptional case in which a segment of the Y-chromosome containing at least one of these three color genes and the SdR may have undergone translocation or pericentric inversion. Using the "zig-zag line diagram" method, we have reanalyzed and assigned other phenotypic markers such as blue tail (*Blt*), green tail (*Grt*) and the snakeskin body-snakeskin tail complex (*Ssb*–*Sst* complex) (Phang *et al*., 1989a, b, 1990; Phang and Fernando, 1991) onto the preliminary genetic map (encompassing *Var*–SdR–*Rdt*–*Bcp*) of the guppy Y-chromosome (Fig. 4). Six loci of Winge's (1927, 1934) color pattern genes of wild-type guppies: *Maculatus* (*Ma*), *Coccineus* (*Co*), *Tigrinus* (*Ti*), *Luteus* (*Lu*), *Vitellinus* (*Vi*) and *Elongatus* (*El*), were also ordered onto this map based on Kirpichnikov's (1981) and Purdom's (1993) revisions.

Recently, the use of molecular techniques such as Arbitrarily Primed Polymerase Chain Reaction or Random Amplified Polymorphic DNA (AP-PCR/RAPD) fingerprinting (Welsh and McClelland, 1990; Williams *et al*., 1990) has proved to be a quick and reliable method for generating a large number of genetic markers for the construction of linkage maps of the zebrafish, *Danio rerio* (Johnson *et al*., 1994; Postlethwait *et al*., 1994), medaka, *Oryzias latipes* (Wada *et al*., 1995) and swordtail-platyfish hybrid of the genus *Xiphophorus* (Kazianis *et al*., 1996). In our on-going effort to identify and link genetic markers to the X- and Y-chromosomes of the guppy, Foo *et al*. (1995) showed that AP-PCR/RAPD markers were inherited in Mendelian fashion at the F_1 level. We have also found several AP-PCR/RAPD markers that could differentiate domesticated stocks and wild-type guppies (Chen, 1999). One of these, a 444 bp fragment amplified using a 10-mer primer OPJ-4 (Operon Technologies, USA), was absent in guppies with variegated tail patterning, while another 10-mer primer OPJ-7 amplified an 800 bp fragment in 80% of guppies with variegated tails (Chen, 1999). In view of applying these phenotypic and molecular markers in future studies to map the guppy sex chromosomes, we hypothesize the length of the guppy genome to be between 1,800 centiMorgans (cM) estimated by isozyme polymorphisms for *Xiphophorus* (Morizot *et al*., 1991) and 3,000 cM (1.7×10^9 bp or 600 kb/cM) by AP-PCR/RAPD for zebrafish (Postlethwait *et al*., 1994). Following the assignation of AP-PCR/RAPD markers to the 24 chromosomes of *Xiphophorus* (Kazianis *et al*., 1996), the genome size of *Xiphophorus* has been revised to about 2,700 cM (S. Kazianis, personal communication).

In conclusion, the black caudal-peduncle (*Bcp*), red tail (*Rdt*) and variegated tail (*Var*) color pattern genes of the domesticated guppy are (1) single genes located at three different loci, (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. Map distances for sex-determining region (SdR)–*Rdt*, *Rdt*–*Bcp*, SdR–*Bcp* and SdR–*Var* are approximately 3.1, 2.3, 5.1 and 2.2 map units, respectively, with a gene order of *Var*–SdR–*Rdt*–*Bcp*. To date, the number of sex-linked color genes reported for the guppy far outnumber the autosomal ones described (Winge, 1927, 1934; Winge and Ditlevsen, 1947; Dzwillo, 1959; Nayudu, 1975, 1979; Kirpichnikov, 1981; Fernando and Phang, 1989; Phang *et al*., 1989a, b, 1990; Phang and Fernando, 1991; Purdom, 1993; Khoo *et al*., 1999a, b). As such, additional genetic data is necessary to verify the preliminary genetic map shown in Fig. 4 through a larger sample size from more crosses and backcrosses. Consequently, this will allow the construction of a denser and more saturated map of the X- and Y-chromosomes using phenotypic and molecular genetic markers established by previous workers and those identified from on-going analyses of domesticated guppy strains.

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