# Genetic Basis of the Variegated Tail Pattern in the Guppy, Poecilia reticulata

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

Source: Zoological Science, 16(3) : 431-437

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.16.431

ZOOLOGICAL SCIENCE **16**: 431–437 (1999)


# Genetic Basis of the Variegated Tail Pattern in 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**—Variegated patterns on the caudal fin are a common and popular trait in guppy strains commercially cultured in Singapore. Gene control of this highly variable mosaic pattern of black spots and patches of different shapes and sizes on a brightly colored tail fin was elucidated by reciprocal crosses between the Green Variegated (GV) strain and wild-type (WT) stock. $F_1$ progenies were produced by single-pair crossing between GV and WT, while the $F_2$ generation was obtained from full-sib mating between $F_1$ males and $F_1$ females. Data for the $F_1$ and $F_2$ generations were segregated according to phenotypes and sex, and tested by chi-square analyses. Inheritance of variegated tail patterns appears to be determined by a single locus on the X- and Y-chromosomes. Genotypes of males and females of the GV strain are proposed to be $X_{Var}Y_{Var}$ and $X_{Var}X_{Var}$, respectively. The allele for variegated tail patterning, *Var*, is dominant over that of the wild-type, $Var^+$, which does not exhibit these patterns. Recombination frequency between the *Var* locus and sex-determining region (SdR) in male guppies was estimated to be about 1.9% (map distance ≈1.9 map units). The segregation and mode of inheritance of the *Var* gene are illustrated by genetic models.

## INTRODUCTION

The wild-type guppy, *Poecilia reticulata* Peters, is a poecilid native to freshwater streams, ponds and brackish-water swamps of Trinidad, Barbados, Venezuela, Guyana and north-eastern Brazil (Haskins and Haskins, 1951). This live-bearer shows striking sexual dimorphism. Wild-type males are smaller than females and their anal fin is modified into a copulatory organ called the gonopodium. Adult males exhibit complex polymorphic spots and patches varying in size, shape and color on the body and fins while the females are devoid of any color patterns, being a uniform olive-brown with hyaline fins (Haskins and Haskins, 1951; Endler, 1983). The wild-type guppy was introduced into Singapore and other parts of South-East Asia in the late 1930s for mosquito control (Herre, 1940). From these introductions, wild populations are now found in most streams, drains, canals and fish ponds.

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 (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 currently reared in monoculture farms (Fernando and Phang, 1985). The guppy plays an important role in the freshwater ornamental fish industry of Singapore whose total exports of ornamental fish in 1995 amounted to US$50 million (Ng, 1996).

The guppy is unique among other teleosts in that almost all the genes encoding for 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. The males are heterogametic (XY) but the females are homogametic (XX) (Winge, 1922a, b; Winge and Ditlevsen, 1947). Expression of color patterns in domesticated strains has been found to be determined by dominant sex-linked genes (Dzwillo, 1959; Nayudu, 1975, 1979; Fernando and Phang, 1989; Phang *et al.*, 1989a, b, 1990; Phang and Fernando, 1991). The color patterns of these strains 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 (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. In contrast, genes responsible for background body coloration such as blond (*bb*), gold (*gg*), albino (*aa*) and blue (*rr*) are autosomally inherited and recessive to their wild-

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

type alleles (Blacher, 1927; Haskins and Druzba, 1938; Goodrich *et al.*, 1944, 1947).

Color patterns on the caudal fin of domesticated guppy strains take the form of single bright colors, snakeskin-like reticulations and variegated mosaic patterns of two or more colors. Recent farm surveys show the popularity of reticulated snakeskin and variegated patterns (approximately 15 and 13 strains, respectively) among guppy strains that are commercially cultured for export. The snakeskin pattern has been found to be caused by two genes, *Sst* and *Ssb* (Phang *et al.*, 1989a, b, 1990; Phang and Fernando, 1991), while red, blue and green tail colors are attributed to the *Rdt*, *Blt* and *Grt* genes, respectively (Fernando and Phang, 1989; Phang *et al.*, 1991). The term "variegated" is usually applied in the guppy to an exceedingly variable mosaic pattern of black spots and patches of different shapes and sizes on a brightly colored tail fin. No two male or female offspring, including those of the same parents, have been observed to possess similar variegated patterns. With the exception of the *Variabilis* gene (*Va*) in wild-type guppies, described by Winge (1927) as having considerable phenotypic variation in the number, size and position of red side patches and black specks as well as a labyrinthine caudal fin pattern, there are no other reports on genes that control highly variable body and tail fin patterns in the guppy. Therefore, the main objective of this study was to investigate the genetic basis of the variegated tail pattern and its mode of inheritance in domesticated guppy strains.

## MATERIALS AND METHODS

### Source of the fish

Three- to four-week old fry of the Green Variegated (GV) guppy strain were obtained from Swee Hing & Brothers Aquarium Co. in Singapore. Wild-type (WT) guppies were collected from an isolated hill-stream near the Bukit Timah nature reserve in Singapore. Juvenile GV 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 is recognised by the formation of the gonopodium from the anal fin. Males, when spotted, were immediately removed and reared separately from females as virgin females were necessary for the reciprocal crosses.

### Description of the fish

Adult males and females of the GV strain have a total length of 3–4 cm and 5–6 cm, respectively. Adult GV males have a green metallic sheen overlying the wild-type male body coloration that comprise small spots or patches of various colors. Only GV males have an orange tail with some yellow streaks, and numerous black spots and patches of different shapes and sizes (Fig. 1A). GV females show wild-type female olive-brown body coloration and faint greyish-brown variegated patterns on a yellowish tail (Fig. 1B). Wild-type guppies are smaller than the domesticated GV strain. Adult WT males are 2–2.5 cm in length while the 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 totally lack any color patterns (Fig. 1D).

### Reciprocal crosses

Inheritance of the variegated tail pattern was elucidated by single-pair reciprocal crosses between the GV 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: GV ♂♂× WT ♀♀ (Table 1A) and WT ♂♂ × GV ♀♀ (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). $F_1$ and $F_2$ progenies were segregated and scored according to phenotypes and sex. All progenies displaying variegated tail patterns were designated as the VAR phenotype and those without such patterns, WT phenotype.

### Statistical analyses

Observed phenotypic distributions were tested for goodness-of-fit with predicted proportions using the chi-square ($\chi^2$) 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 $\chi^2$ to improve the approximation to the $\chi^2$ distribution, as shown by the $\chi^2_{adj}$ values. The $\chi^2$ 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 $\chi^2$ values had to be summed and $\chi^2_{adj}$ values were not additive (Sokal and Rohlf, 1981; Strickberger, 1990). Following Winge (1922b, 1923, 1927, 1934), Nayudu (1979), Phang *et al.* (1989a, b, 1990), and Phang and Fernando (1991), individuals with exceptional coloration due to crossing-over of the *Var* gene between the X- and Y-chromosomes were not considered in chi-square analyses. Recombination frequency and map distance between the *Var* locus and sex-determining region (designated as SdR), proposed by Schmidt (1920) and Winge (1922a, b, 1927, 1934) to be found on the Y-chromosome, were estimated according to Strickberger (1990), Phang *et al.* (1990), Phang and Fernando (1991), and Purdom (1993).

## RESULTS AND DISCUSSION

### Cross between GV males and WT females

Four mating pairs of GV♂♂ × WT♀♀ (PG1, PG4, PG5 and PG10) gave a total of 47 male and 63 female $F_1$ offspring in 13 broods, with an expected ratio of 1:1 (Table 1A). $F_1$ male progenies showed variegated tail patterns (VAR phenotype) and wild-type male body coloration (WT) (Fig. 1E), while $F_1$ females had faint greyish-brown variegated patterns on the tail and wild-type olive-brown background body coloration (Fig. 1F). In this typical cross, $F_1$ males and females could have inherited the variegated pattern only from the GV male parent. Table 1A also shows one mating pair, PG3, which gave almost exclusively all male $F_1$ offspring (18 males and one female). This is due to the instability of the genetic mechanism of sex determination in the guppy since a large number of weak additive male and female sex genes present in the autosomes and X-chromosome may alter the balance of sex (Nayudu, 1979; Kirpichnikov, 1981). The collective effect of these genes may be more pronounced than that of the sex factors on the sex chromosomes, thus producing more male offspring and vice versa. Another mating, PG9, produced only WT males (Fig. 1E) and VAR females (Table 1A). This is at-

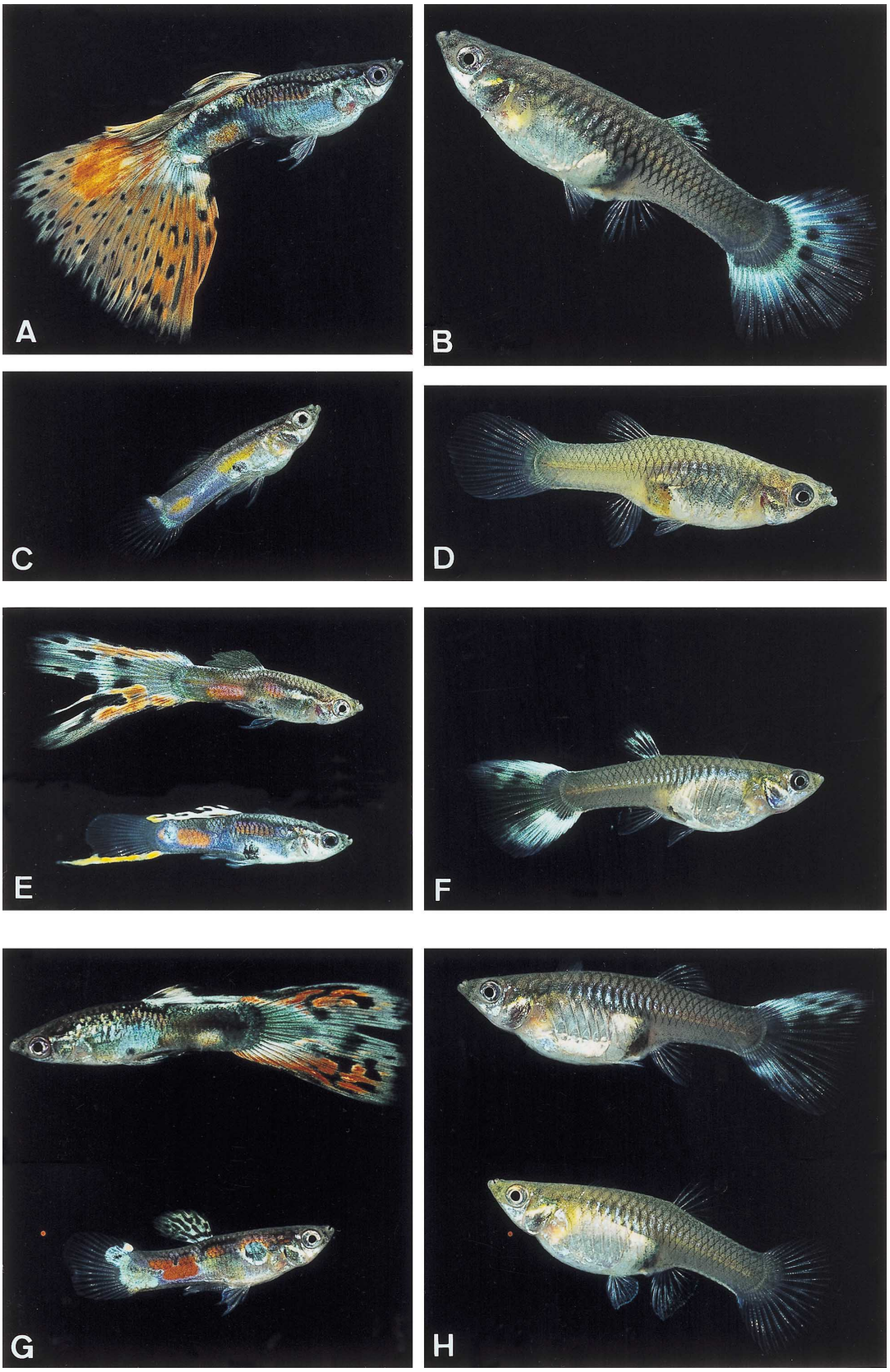


**Fig. 1.** (A) Adult male guppy of the Green Variegated strain. (B) Adult female guppy of the Green Variegated strain. (C) Adult male wild-type guppy. (D) Adult female wild-type guppy. (E) $F_1$ males showing typical variegated tail patterning (top) and wild-type tail without any patterns (bottom) from mating pair PG9. (F) $F_1$ female with variegated tail patterning. (G) $F_2$ males of the VAR (top) and WT (bottom) phenotypes. (H) $F_2$ females of the VAR (top) and WT (bottom) phenotypes.

**Table 1.** Mating results of crosses between Green Variegated (GV) male and wild-type (WT) female guppies 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 ($\chi^2_{adj}$) after application of Yates' correction for continuity, $\chi^2$ 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 *Var* gene were not considered in chi-square analyses. (Phenotypes: VAR = tail with variegated patterns, WT = wild-type tail without variegated patterns. Genes: *Var* = variegated tail pattern gene, $Var^+$ = absence of variegated tail pattern gene).

**A.** GV♂♂ × WT♀♀ (Parental Cross)

| Mating pair designation | No. of $F_1$ broods | Observed numbers for each $F_1$ phenotypic class (expected numbers) ♂♂ VAR | ♂♂ WT | ♀♀ VAR | Expected $F_1$ ratio of ♂ : ♀ | Chi-square Goodness-of-fit Test (*df*=1) $\chi^2$ | $\chi^2_{adj}$ | Total $\chi^2$ | Pooled $\chi^2$ | $\chi^2$ for Homogeneity | Putative parental genotypes ♂♂ GV | ♀♀ WT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PG1 | 5 | 22 (24) | | 26 (24) | 1:1 | 0.334 | 0.188 | | | | | |
| PG4 | 4 | 13 (15.5) | | 18 (15.5) | 1:1 | 0.806 | 0.516 | 2.812 | 2.328 | 0.484 | $X_{Var}Y_{Var}$ | $X_{Var}{}^+X_{Var}{}^+$ |
| PG5 | 2 | 5 (7) | | 9 (7) | 1:1 | 1.142 | 0.642 | (*df*=4) | (*df*=1) | (*df*=3) | | |
| PG10 | 2 | 7 (8.5) | | 10 (8.5) | 1:1 | 0.530 | 0.236 | | | | | |
| Pooled: | 13 | 47 (55) | | 63 (55) | 1:1 | 2.328 | 2.046 | | | | | |
| PG3[e] | 4 | 18 (9.5) | | 1 (9.5) | 1:1 | 15.210** | 13.474** | — | — | — | $X_{Var}Y_{Var}$ | $X_{Var}{}^+X_{Var}{}^+$ |
| PG9 | 1 | | 4 (3.5) | 3 (3.5) | 1:1 | 0.142 | 0.000 | — | — | — | $X_{Var}Y_{Var}{}^+$ | $X_{Var}{}^+X_{Var}{}^+$ |

** : significantly different at 1% level, *df* : degrees of freedom
[e] : exceptional case with a high number of $F_1$ males

**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) ♂♂ VAR | ♂♂ WT | ♀♀ VAR | ♀♀ WT | Expected $F_2$ ratio (*df*) | Chi-square Goodness-of-fit Test $\chi^2$ | $\chi^2_{adj}$ | Total $\chi^2$ | Pooled $\chi^2$ | $\chi^2$ for Homogeneity | Putative $F_1$ genotypes [phenotypes] ♂♂ | ♀♀ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PG1 | 10 (4) | 84 (80) | 5[§] | 44 (40) | 32 (40) | 2:1:1 (2) | 2.200 | 1.865 | | | | | |
| PG4 | 5 (3) | 45 (34.5) | 2[§] | 12 (17.25) | 12 (17.25) | 2:1:1 (2) | 6.392* | 5.515 | 9.903 | 3.128 | 6.775 | $X_{Var}{}^+Y_{Var}$ [VAR] | $X_{Var}X_{Var}{}^+$ [VAR] |
| PG5 | 10 (4) | 96 (96.5) | 1[§] | 47 (48.25) | 50 (48.25) | 2:1:1 (2) | 0.098 | 0.044 | (*df*=8) | (*df*=2) | (*df*=6) | | |
| PG10 | 6 (2) | 27 (23.5) | | 11 (11.75) | 9 (11.75) | 2:1:1 (2) | 1.213 | 0.819 | | | | | |
| Pooled: | 31 (13) | 252 (234.5) | 8[§] | 114 (117.25) | 103 (117.25) | 2:1:1 (2) | 3.128 | 2.908 | | | | | |
| PG3[e] | 0 (1) | 0 | | 0 | 0 | 2:1:1 (2) | — | — | — | — | — | $X_{Var}{}^+Y_{Var}$ [VAR] | $X_{Var}X_{Var}{}^+$ [VAR] |
| PG9 | 10 (3) | 59 (59.75) | 59 (59.75) | 63 (59.75) | 58 (59.75) | 1:1:1:1 (3) | 0.246 | 0.155 | — | — | — | $X_{Var}{}^+Y_{Var}{}^+$ [WT] | $X_{Var}X_{Var}{}^+$ [VAR] |

* : significantly different at 5% level, *df* : degrees of freedom
[e] : exceptional case where the only possible full-sib $F_1$ cross did not yield any $F_2$ progenies
[§] : recombinant males with wild-type (WT) coloration (data not used in chi-square analyses)

**Table 2.** Mating results of crosses between wild-type (WT) male and Green Variegated (GV) female guppies 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 ($\chi^2_{adj}$) after application of Yates' correction for continuity, $\chi^2$ test for homogeneity, probable genotypes and recombinants for the (**A**) $F_1$ generation of single-pair parental crosses, and (**B**) $F_2$ generation of single-pair crosses between full-sib $F_1$ males and $F_1$ females. Recombinants ([#]) due to crossing-over of the *Var* gene were not considered in chi-square analyses. (Phenotypes: VAR = tail with variegated patterns, WT = wild-type tail without variegated patterns. Genes: *Var* = variegated tail pattern gene, $Var^+$ = absence of variegated tail pattern gene).

**A.** WT♂♂ × GV♀♀ (Parental Cross)

| Mating pair designation | No. of $F_1$ broods | Observed numbers for each $F_1$ phenotypic class (expected numbers) ♂♂ VAR | ♀♀ VAR | Expected $F_1$ ratio of ♂ : ♀ | Chi-square Goodness-of-fit Test (*df*=1) $\chi^2$ | $\chi^2_{adj}$ | Total $\chi^2$ | Pooled $\chi^2$ | $\chi^2$ for Homogeneity | Putative parental genotypes ♂♂ WT | ♀♀ GV |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PW1 | 3 | 33 (30.5) | 28 (30.5) | 1:1 | 0.410 | 0.262 | | | | | |
| PW2 | 3 | 18 (23.5) | 29 (23.5) | 1:1 | 2.574 | 2.128 | | | | | |
| PW3 | 4 | 34 (30.5) | 27 (30.5) | 1:1 | 0.804 | 0.590 | 7.202 | 0.110 | 7.092 | $X_{Var}{}^+Y_{Var}{}^+$ | $X_{Var}X_{Var}$ |
| PW4 | 2 | 5 (3.5) | 2 (3.5) | 1:1 | 1.286 | 0.572 | (*df*=8) | (*df*=1) | (*df*=7) | | |
| PW5 | 3 | 20 (24.5) | 29 (24.5) | 1:1 | 1.654 | 1.306 | | | | | |
| PW6 | 2 | 17 (16) | 15 (16) | 1:1 | 0.126 | 0.032 | | | | | |
| PW7 | 3 | 6 (5.5) | 5 (5.5) | 1:1 | 0.090 | 0.000 | | | | | |
| PW8 | 3 | 29 (31) | 33 (31) | 1:1 | 0.258 | 0.146 | | | | | |
| Pooled: | 23 | 162 (165) | 168 (165) | 1:1 | 0.110 | 0.076 | | | | | |

*df* : degrees of freedom

**Table 2.** (continuation)
**B.** $F_1♂♂ \times F_1♀♀$ (Full-sib $F_1$ Cross)

| Mating pair desig-nation | No. of $F_2$ broods (No. of $F_1$ pairs) | Observed numbers for each $F_2$ phenotypic class (expected numbers) ♂♂ VAR | ♂♂ WT | ♀♀ VAR | ♀♀ WT | Expect-ed $F_2$ ratio | Chi-square Goodness-of-fit Test (*df* =2) $\chi^2$ | $\chi^2_{adj}$ | Total $\chi^2$ | Pooled $\chi^2$ | $\chi^2$ for Homo-geneity | Putative $F_1$ genotypes [phenotypes] ♂♂ | ♀♀ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PW1 | 7 (2) | 23 (28.25) | 25 (28.25) | 65 (56.5) | 1# | 1:1:2 | 2.629 | 2.200 | | | | | |
| PW2 | 7 (2) | 15 (16) | 15 (16) | 34 (32) | | 1:1:2 | 0.251 | 0.102 | | | | | |
| PW3 | 10 (3) | 19 (20.75) | 21 (20.75) | 43 (41.5) | | 1:1:2 | 0.205 | 0.102 | 9.397 | 1.512 | 7.885 | $X_{Var}Y_{Var}^+$ | $X_{Var}^+X_{Var}$ |
| PW4 | 10 (2) | 57 (55.75) | 57 (55.75) | 109 (111.5) | | 1:1:2 | 0.112 | 0.056 | (*df*=16) | (*df*=2) | (*df*=14) | [VAR] | [VAR] |
| PW5 | 6 (2) | 14 (13.25) | 13 (13.25) | 26 (26.5) | 1# | 1:1:2 | 0.056 | 0.010 | | | | | |
| PW6 | 5 (2) | 20 (19.5) | 15 (19.5) | 43 (39) | 1# | 1:1:2 | 1.461 | 1.135 | | | | | |
| PW7 | 6 (3) | 18 (18.5) | 21 (18.5) | 35 (37) | | 1:1:2 | 0.460 | 0.277 | | | | | |
| PW8 | 4 (2) | 15 (13.5) | 7 (13.5) | 32 (27) | | 1:1:2 | 4.223 | 3.491 | | | | | |
| Pooled: | 55 (18) | 181 (185.5) | 174 (185.5) | 387 (371) | 3# | 1:1:2 | 1.512 | 1.386 | | | | | |

*df* : degrees of freedom
# : recombinant females with wild-type (WT) coloration (data not used in chi-square analyses)

tributed to the possibility that the GV male parent was heterozygous for the *Var* gene and possessed the recessive allele, $Var^+$, on the Y-chromosome.

The $F_2$ generation comprised a total of 252 VAR males, 114 VAR females and 103 WT females (Fig. 1G, H) with the observed numbers agreeing with the expected 2:1:1 ratio (Table 1B). A slight exception occurred in mating PG4 where a higher number of VAR males caused significant difference at the 5% level but not at 1% (Table 1B). This was rectified when Yates' (1934) correction for continuity was applied. Three mating pairs (PG1, PG4 and PG5) gave eight exceptional males with WT body and tail coloration among the $F_2$ individuals (Table 1B). We speculate that the lack of variegated tail patterning in these males was due to crossing-over of the *Var* gene from the Y- to the X-chromosome in the $F_1$ VAR male parents. Since only the *Var* locus and sex-determining region (SdR) are considered in this study, the recombination frequency between *Var* and SdR is estimated to be 3.077%, i.e., eight WT male recombinants of a total 260 $F_2$ males (Table 1B). For mating pair PG9, the $F_2$ generation consisted of VAR males, WT males, VAR females and WT females (Fig. 1G, H) in a 1:1:1:1 expected ratio (Table 1B). In addition, homogeneity $\chi^2$ values showed that the pooled $F_1$ and $F_2$ generations of PG1, PG4, PG5 and PG10 were uniform and did not form heterogeneous populations (Table 1A, 1B). $\chi^2$ tests were not carried out for PG3 as the solitary $F_1$ female did not produce any $F_2$ progenies (Table 1B). The $F_1$ and $F_2$ results gave evidence that the putative genotype typical of GV males is $X_{Var}Y_{Var}$ while that of the heterozygous male of mating pair PG9 is $X_{Var}Y_{Var}^+$. A genetic model illustrating the segregation of the dominant *Var* gene is presented in Fig. 2.

### Cross between WT males and GV females

Eight matings (PW1–PW8) of the reciprocal cross, $WT♂♂ \times GV♀♀$, gave 23 $F_1$ broods consisting of 162 males and 168 females, all with variegated tail patterns (VAR phenotype) albeit fainter and less distinct in the females (Fig. 1E, F). The observed number of male to female offspring conformed to the expected 1:1 ratio (Table 2A). With the exception of three recombinant females with WT phenotype, the $F_2$ progeny of this cross segregated into 181 VAR males, 174 WT males and 387 VAR females (Fig. 1G, H) according to the 1:1:2 hypothetical ratio (Table 2B). The $F_1$ and $F_2$ progenies also formed homogeneous populations after their respective observations were pooled (Table 2A, 2B). The occurrence of three $F_2$ females for mating pairs PW1, PW5 and PW6 which had hyaline tails instead of the expected faint greyish-brown variegated patterns on a yellowish tail could be caused by crossing-over of the *Var* gene from the X- to the Y-chromosome in the $F_1$ VAR male parents (Table 2B). Since there were three WT female recombinants of a total of 390 $F_2$ females, the crossover frequency between the *Var* locus and SdR in this case is 0.769% (Table 2B). The $F_1$ and $F_2$ results therefore confirmed that the GV female parents used in this study were homozygous at the *Var* locus and possessed the genotype $X_{Var}X_{Var}$. The genetic model for the segregation of *Var* in this reciprocal cross is shown in Fig. 2.

### The *Var* gene

Results of all parental ($GV♂♂ \times WT♀♀$ and $WT♂♂ \times GV♀♀$, Tables 1A, 2A) and full-sib ($F_1♂♂ \times F_1♀♀$, Tables 1B, 2B) crosses indicate that the highly variable variegated tail pattern of the Green Variegated guppy strain is a simple sex-linked trait controlled by a single gene with two alleles, *Var* dominant for variegated tail pattern over $Var^+$ recessive for wild-type which do not exhibit these patterns (Fig. 2). This is the first study that reports the expression of the variegated tail pattern gene, *Var*, was dominant in both sexes. Our results also show that the *Var* allele is able to cross over from the Y- to the X-chromosome and vice versa as male and female recombinants of the WT phenotype were obtained from the $F_2$ progenies of $GV♂♂ \times WT♀♀$ and $WT♂♂ \times GV♀♀$, respectively (Tables 1B, 2B). 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 (1923, 1927) and Dzwillo (1959). This confirms that the sex chromosomes of the guppy share undifferentiated regions of homology along their chromatids in which pairing and cross-

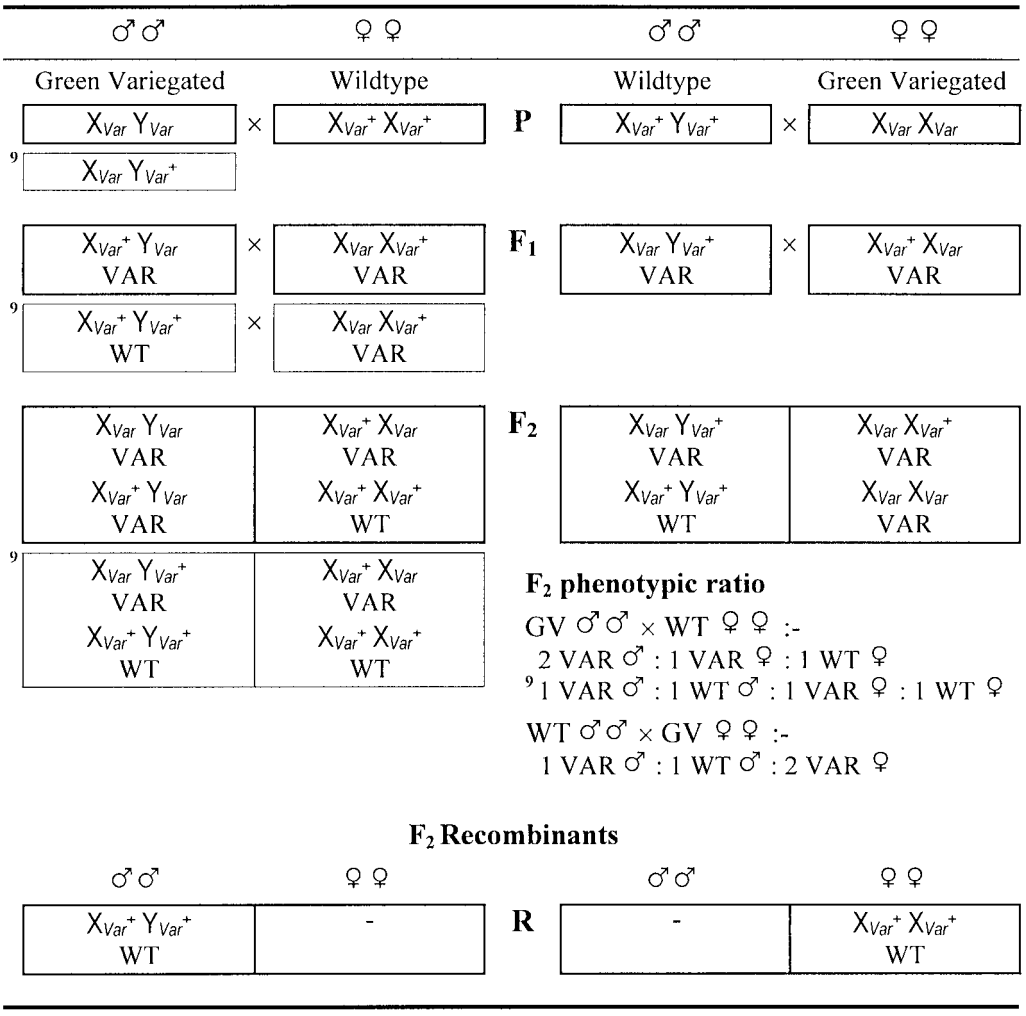


**Fig. 2.** Schematic diagram of the proposed genetic models illustrating the segregation of the *Var* tail color pattern gene, phenotypes and genotypes of the parents (**P**), $F_1$ and $F_2$ progenies, and recombinants (**R**) that occur in reciprocal crosses between the Green Variegated (GV) and wild-type (WT) guppies. Mating pair PG9 in which the GV ♂♂ parent was heterozygous for the *Var* gene and its $F_1$ and $F_2$ progenies (indicated as[9]) are shown within thin borders to distinguish them from the typical GV ♂♂ × WT ♀♀ cross.

ing-over can occur (Winge, 1923, 1934; Winge and Ditlevsen, 1938; Dzwillo, 1959; Nayudu, 1975, 1979; Kirpichnikov, 1981).

A mean crossover frequency of 1.923 (±1.632)% was obtained (Tables 1B, 2B) between the *Var* locus and sex-determining region (SdR) in the Y-chromosome of male guppies. The phenotypic map distance was thus approximately 1.923 (±1.632) map units. Winge (1927), Winge and Ditlevsen (1947), and Kirpichnikov (1981) proposed that genes found within or close to the SdR are either alleles of one of the same locus (allelomorphic) or belong to the so-called supergene family, i.e., a group of closely located and tightly linked loci with completely suppressed crossing-over. Our results indicate that *Var* is most likely found in a homologous region about 1.923 map units from the SdR (Tables 1B, 2B). This suggests that *Var* is situated close to the SdR since crossing-over between other genes, e.g., *Vitellinus* (*Vi*) and *Elongatus* (*El*), and *Doppelschwert* (*Ds*) and *Pigmentierte Caudalis* (*Cp*) occur with a frequency of up to 10% (Winge, 1927, 1934; Winge and Ditlevsen, 1947; Dzwillo, 1959; Kirpichnikov, 1981; Purdom, 1993). As initiated by Winge (1927, 1934), and later Winge and Ditlevsen (1947), Dzwillo (1959), Nayudu (1975, 1979), Kirpichnikov (1981), Phang *et al.* (1989a, b, 1990), Phang and Fernando (1991), and Purdom (1993), the recombinant data for *Var* and SdR reported here should contribute to the mapping of additional color pattern genes onto the sex chromosomes of the guppy.

In conclusion, the variegated tail pattern gene, *Var*, of domesticated selected guppy strains (1) shows dominant expression, and is (2) both X- and Y-linked, (3) fully capable of crossing-over from the Y- to the X-chromosome and vice versa, and (4) located about 1.9 map units from the sex-determining region. The variegated tail pattern trait may thus prove valuable as a genetic marker in linkage analyses and investigations of color patterns in the guppy.

## ACKNOWLEDGEMENTS

This project was funded by research grants from the European Commission (CI1*-CT94–0021) and National University of Singapore (RP3930327, RP3972366 and RP3981303) to V.P.E. Phang (P.I.), W.-K. Chan and T.M. Lim.

## REFERENCES

Blacher LJ (1927) Materials for the genetics of *Lebistes reticulatus* Peters. Trans Lab Exp Biol Zoopark Moscow 3: 139–152

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

Endler JA (1983) Natural and sexual selection on color patterns in poeciliid fishes. Env Biol Fishes 9: 173-190

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

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

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

Ng PS (1996) Singapore Facts and Pictures 1996. Ministry of Information and the Arts, Singapore, 158 p

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

Yates F (1934) Contingency tables involving small numbers and the $\chi^2$ test. J R Stat Soc (suppl) 1: 217–235

(Received December 7, 1998 / Accepted March 11, 1999)