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# Identification of quantitative trait locus (QTL) linked to dorsal fin length from preliminary linkage map of molly fish, *Poecilia* sp.

Bun Poh Keong [a,*], Siti Shapor Siraj [a], Siti Khalijah Daud [b], Jothi Malar Panandam [c], Arina Nadia Abdul Rahman [a]

[a] Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang Selangor, Malaysia
[b] Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang Selangor, Malaysia
[c] Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang Selangor, Malaysia

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ABSTRACT

A preliminary linkage map was constructed by applying backcross and testcross strategy using microsatellite (SSR) markers developed for *Xiphophorus* and *Poecilia reticulata* in ornamental fish, molly *Poecilia* sp. The linkage map having 18 SSR loci consisted of four linkage groups that spanned a map size of 516.1 cM. Association between genotypes and phenotypes was tested in a random fashion and QTL for dorsal fin length was found to be linked to locus Msb069 on linkage group 2. Coincidentally, locus Msb069 was also reported as putative homologue primer pairs containing SSRs repeat motif which encoded hSMP-1, a sex determining locus. Dorsal fin length particularly in males of *Poecilia latipinna* is an important feature during courtship display. Therefore, we speculate that both dorsal fin length and putative hSMP-1 gene formed a close proximity to male sexual characteristics.



## 1. Introduction

Mollies belong to genus *Poecilia*, subgenus *Mollienesia* (*sensu* Miller, 1975) and are among the ornamental fish species cultured for trading in this country. According to Ptacek and Breden (1998), this fish species could be divided into two main complexes based on morphological and behavioural differences, namely *Poecilia sphenops* and *Poecilia latipinna*. Fish in the former complex have short dorsal fins while the latter have long sail-like dorsal fins. Dorsal fin length is an important feature in molly fish particularly in males of *P. latipinna*. Males of *P. latipinna* are well known for their distinctive enlarged dorsal fin that resembles a fan and is an important feature during courtship display (Ptacek, 2002). Courtship display is a mating behaviour performed only by males of *P. latipinna* and such attempt is aimed to attract the attention of potential mates. Apart from that, the dorsal fin is one of the morphological features that determined its trading price and quality. Other fish traits such as melanophore pattern, caudal fin type and body colour are also major price determinants for trading. Further to this, most of these traits are quantitatively inherited. Nevertheless, not much attention has been paid to discover the genetic architecture of quantitatively inherited traits. Although fishes represent almost half of the vertebrate species on Earth (Nelson, 2006), studies on quantitative trait loci (QTL) have been conducted on fewer than 30 cultured fish species to date (Wang et al., 2011).

Hence, the objectives of this study were i) to construct a preliminary linkage map and ii) to test the association between economically important traits and molecular markers using the preliminary linkage map constructed for molly fish, *Poecilia* sp.

## 2. Materials and methods

### 2.1. Mapping cross

Parental stocks used in this study originated from the USA but had been locally cultured for 15 years at Ulu Tiram, Johore Malaysia. The first generation of hybrids was generated from *P. latipinna* and *P. sphenops*. Backcross hybrids were produced by crossing six $F_1$ female hybrids with recurrent male (n = 6; $BC_1$-A hybrids derived from 1 pedigree) and a male $F_1$ hybrid with recurrent female (n = 50; $BC_1$-B hybrids derived from 1 pedigree). Testcross hybrids were generated by crossing a male $F_1$ hybrid with a genetically unrelated female *P. sphenops* (n = 21; $F_2$-1 hybrids derived from 1 pedigree). All 77 progenies derived from the two backcrosses and the testcross were genotyped and pooled in the analysis. All breeding trials and maintenance

*Abbreviations:* μl, Microlitre; BC, Backcross; bp, Base pairs; cM, Centimorgan; DFL, Dorsal fin length; $F_1$, First filial generation; $F_2$, Second filial generation; LG, Linkage group; LOD, Logarithm of odds; min, Minute; n, Number; °C, Degree celsius; P, Probability; QTL, Quantitative Trait Loci; $R^2$, Coefficient of determination; sp., Species; SSRs, Microsatellites; USA, United States of America.

* Corresponding author at: Department of Biology, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900, Tanjong Malim, Perak, Malaysia. Tel.: +60 15 4811 7328.
*E-mail address:* bunpoh@yahoo.com (B.P. Keong).

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of fingerlings were carried out in the Aquaculture Research Centre, Universiti Putra Malaysia, Puchong since mid October 2009.

### 2.2. Genotyping

DNA was extracted from freshly sampled fin clip following a method described by Qiagen Dneasy blood and tissue kit with minor adjustments on centrifuge duration and speed (Catalog no. 69504). Two sets of microsatellite (SSRs) markers were used. The first set comprised of 123 SSRs primer pairs developed for *Xiphophorus* by Walter et al. (2004) and was downloaded from (Xiphophorus Microsatellite Loci Database, http://www.xiphophorus.txstate.edu/research/xiphbase/microsat.html) while the second set comprised 19 SSR primer pairs designed by Watanabe et al. (2003) for *Poecilia reticulata*. These selected SSR primer pairs were able to cross-amplify on two different but closely related species and were successfully positioned in their respective linkage maps. The PCR reaction volumes were scaled down to 10 μl and thermal cycling conditions were according to description by Promega product information (Catalog#M8295). The programming profile was one pre-denaturing at 95 °C for 2 min, 35 cycles of 1 min at 95 °C, 1 min at 55 °C, 1 min at 72 °C and a final extension of 5 min at 72 °C.

### 2.3. Phenotyping of traits

Four main phenotypic traits were evaluated, namely dorsal fin length, melanophore pattern, caudal fin type (round and lyretail) and body colour type (coloured and black). Dorsal fin length was measured from the anterior to the posterior base of dorsal fin to the nearest mm using vernier callipers. Traits of melanophore pattern, caudal fin and body colour type were examined and recorded as nominal data (1 for presence and 0 for absence).

### 2.4. Linkage and QTL analysis

Linkage map was constructed using MapManager QTX (Manly et al., 2001) to establish a 'framework' locus and map orders for linkage group (LG) following Kosambi's (1944) mapping function. This programme started by choosing two tightly linked (the pair with the highest LOD scores) loci to establish a linkage map. Subsequent loci were added in the order of increasing recombination frequency. All estimations of genotype–phenotype in QTL mapping were performed using Qgene 4.0 software (Joehanes and Nelson, 2008) at LOD score between 2.0 and 3.0. Simple interval mapping (SIM) was used to test association of genotype–phenotype for dorsal fin length. For nominal data with two categories such as in phenotypic traits of melanophore pattern, caudal fin type and body colour type, test analyses were performed using MIM-GLZ (Multiple IM-Generalised linear model).

## 3. Results

### 3.1. Assessment of polymorphism, map statistics and putative homologue gene sequences

A total of 142 (123 *Xiphophorus* SSR + 19 *Poecilia* SSR) primer pairs were screened and 95 (84 + 11) showed cross-species amplification. Among the 95 SSRs, only 29 SSR loci were polymorphic when genotyped on both parents and $F_1$ hybrid. Polymorphic SSR loci amplified alleles between product size range of 100 bp to 300 bp. Among the 29 SSR loci, only 18 were informative and showed linkage between them. These 18 SSR loci were distributed into four linkage groups covering a map size of 516.1 cM (Fig. 1).

Data comparison was performed on the first set of primer pairs with information from Walter et al. (2004). Seven out of 123 SSR primer pairs were identified as putative homologue primer pairs of known genes (Table 1). No gene homology was found for the second set of SSR primer pairs used in this study.

### 3.2. QTL analysis

A QTL for dorsal fin length was detected on linkage group 2 between marker loci Msd021 and Msb068 (with peak at marker locus Msb069), which spans a chromosome distance of 66.3 cM. The additive effect for this QTL was negative, indicating increasing allelic effect from *P. latipinna*. The phenotypic variation explained (PVE) based on $R^2$ value was 20.8% which is above the widely accepted threshold ($R^2 = 10\%$) (Table 2, Fig. 2). No QTL was identified for melanophore pattern, caudal fin type and body colour type.

## 4. Discussion and conclusions

The ability of SSRs to cross-amplify enables markers from genetic linkage map of one species to be superimposed on another closely

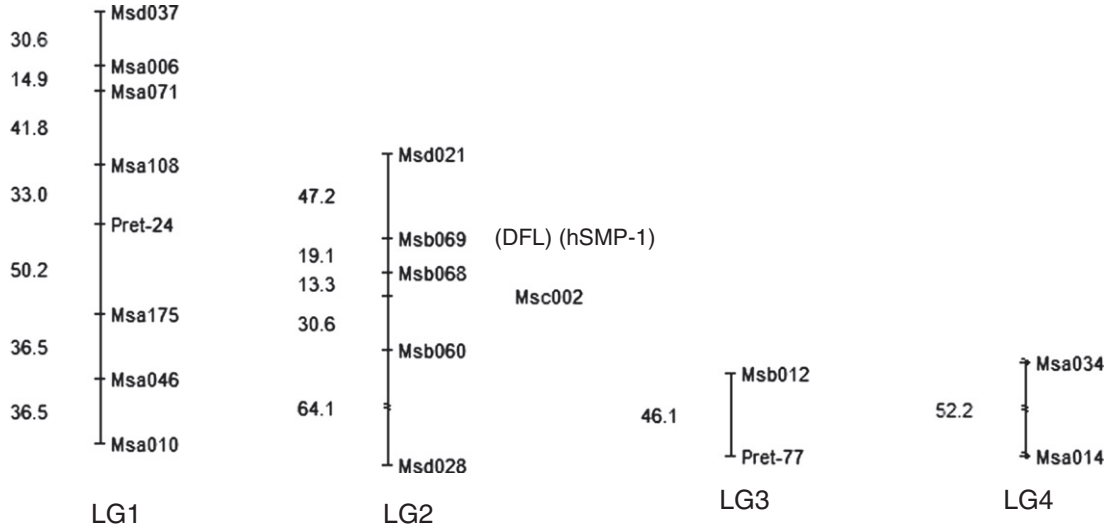


**Fig. 1.** Genetic linkage map of molly *Poecilia* sp., based on SSR loci developed by Walter et al. (2004) and Watanabe et al. (2003) derived from two backcrosses and a testcross progenies generated by crossing *P. latipinna* and *P. sphenops*. Numbers on the left indicate Kosambi's map distance (cM) and anchored primer pairs on the right. LG represents linkage group. Codes inside parentheses were quantitative trait loci of 'DFL' dorsal fin length detected in this study and putative homologue containing SSRs of 'hSMP-1' human sperm membrane protein.