UNIVERSITI PUTRA MALAYSIA

PLOIDY DETECTION, GENETIC DIVERSITY, AND QUANTITATIVE TRAIT LOCI MAPPING IN MOLLY FISH (*Poecilia* spp.)

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By

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PLOIDY DETECTION, GENETIC DIVERSITY, AND QUANTITATIVE TRAIT LOCI MAPPING IN MOLLY FISH (*Poecilia* spp.)

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Chairperson: Professor Siti Shapor Siraj, PhD

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A study was conducted to construct a preliminary linkage map for molly, *Poecilia* and to test association between markers and QTL traits. In linkage map construction, three major steps were involved. First is the development of mapping population. Background genetic information of the parental species such as occurrence of triploidy and genetic variability were crucial before any attempt to develop a mapping population. Triploid detection was carried out in this study by using highly polymorphic microsatellite (SSRs) primer pairs. Ten SSRs primer pairs could clearly differentiate among one, two and three alleles which could be used to infer different ploidy levels. The presence of two alleles indicates a diploid at that particular locus and evidence of three alleles could be inferred as triploid. If such association was direct, the occurrence of triploidy in molly fish sampled in this study was extremely low and negligible. The genetic diversity

among the four species of live-bearer fishes namely *Xiphophorus maculatus, Xiphophorus helleri, Poecilia reticulata* and *Poecilia latipinna* were revealed using 15 polymorphic SSRs primer pairs. A total of 131 samples which comprised of these four species of live-bearers were collected in the middle of October 2009 from Aquatic International, Subang Jaya, Malaysia. Results showed that the clustering pattern of both *Xiphophorus* and *Poecilia* as revealed by these molecular markers seems to be in accordance with their taxonomy. In *P. latipinna,* the occurrence of inbreeding or outbreeding was absent as indicated by the level of heterozygosity. Majority of alleles were homozygous within *P. latipinna* and this seems to resemble the genome characteristic of a purebred fish. Purebred fish is an ideal parental material in any mapping population.

A mapping population developed through backcross and testcross strategy was successfully attempted. A parallel study on the mode of inheritance of background body colour had also been initiated by evaluating body colour of progenies derived from crosses between two different colour variants of molly, [*P. latipinna* (non-black) and *P. sphenops* (black)]. Non-black body colour was found to be completely dominant over black and was not sex-linked. Multiple genes interaction which acted nonadditively was also found to be influencing this phenotypic trait. However, such interaction effect was restricted in crosses generated between genetically related fish.

The second step in linkage map construction is identification of polymorphism using molecular markers. A total of 142 SSRs primer pairs which includes those that were

developed for *Xiphophorus maculatus* (123 primer pairs) and *Poecilia reticulata* (19 primer pairs) were screened and 95 primer pairs were able to be amplified on the DNA of molly, *Poecilia*. However, only 29 SSRs primer pairs were polymorphic when genotyped on 77 progenies derived from two backcrosses (n = 56) and a testcross (n = 21). The fish samples used for the development of mapping population were collected from Aquatic International Subang Jaya, Malaysia.

The final step is linkage analysis of polymorphic markers. Among the 29 polymorphic SSRs primer pairs identified, only 18 were informative and could be used to construct the linkage map. The constructed linkage map consists of four linkage groups covering a map size of 516.1 cM. One potential QTL, dorsal fin length was successfully associated between marker regions of Msd021, Msb069 and Msb068 on linkage group 2.

The major drawback found in this study was low percentage of polymorphic SSRs primer pairs. Such condition not only greatly restricted the number of markers that could be positioned on the linkage map but also minimizes the number of linkage group that could be generated. Map enrichment effort using both amplified fragment length polymorphism (AFLP) markers and development of new SSRs primer pairs for this species are strongly suggested.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGESANAN PLOIDI, KEPELBAGAIAN GENETIK, DAN PEMETAAN LOKUS TRAIT KUANTITATIF IKAN MOLLY (*Poecilia* spp.)

Oleh

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Satu kajian dijalankan untuk membina rantaian peta awalan molly, *Poecilia* dan mengenalpasti sama ada terdapat gabungan antara penanda dengan ciri QTL. Pembinaan pemetaan jaringan melibatkan tiga langkah utama. Pertama ialah pembangunan populasi pemetaan. Kajian asas genetik tentang spesies induk seperti kewujudan 'triploid' dan variasi genetik adalah penting sebelum percubaan untuk membangunkan populasi pemetaan. Pengesanan 'triploid' dalam kajian ini dijalankan dengan menggunakan penanda mikrosatelit (SSRs) yang polimofik. Sepuluh penanda SSRs ini dapat membezakan antara satu, dua dan tiga alel boleh digunakan untuk menghasilkan tahap 'ploidy' yang berbeza. Kemunculan dua alel menunjukkan 'diploid' di lokus yang tertentu dan bukti tiga alel boleh digunakan untuk membuat kesimpulan sebagai

'triploid'. Jika hubungan ini boleh digunakan secara terus, kewujudan 'triploid' dalam ikan molly yang di sampel dalam kajian ini adalah berada pada tahap yang rendah dan boleh diabaikan. Kepelbagaian genetik antara keempat-empat ikan 'life-bearer' iaitu *Xiphophorus maculatus, Xiphophorus helleri, Poecilia reticulata* dan *Poecilia latipinna* didedahkan dengan 15 penanda SSRs yang polimofik. Sejumlah 131 sampel yang terdiri daripada empat spesies 'live-bearer' telah dikutip pada pertengahan Oktober 2009 dari Akuatik Antarabangsa, Subang Jaya, Malaysia. Keputusan menunjukkan corak kelompok untuk kedua-dua *Xiphophorus* dan *Poecilia* seolah-olah menyerupai pengelompokan berdasarkan taksonominya. *P. latipinna*, tidak menunjukkan keadaan pembiakbakaan dalaman ataupun luaran sepertimana yang ditunjukkan oleh tahap heterozigositi. Kebanyakkan alel dalam *P. latipinna* adalah homozigot dan ini menyerupai ciri-ciri genom ikan ras tulen. Ikan ras tulen merupakan induk yang paling sesuai dalam membangunkan populasi pemetaan.

Populasi pemetaan yang dibangunkan melalui strategi pembiakbakaan kacukan balik dan kacukan uji telah berjaya dilaksanakan. Kajian selari pada mod pewarisan warna belakang latar badan telah dilaksanakan dengan menilai warna badan anak ikan yang dihasilkan melalui kacukan antara dua ikan molly yang mempunyai variasi warna badan yang berlainan, [*P. latipinna* (bukan hitam) dan *P. sphenops* (hitam)]. Bukan hitam didapati adalah dominan kepada hitam dan pewarisan ini tidak berkaitan seks. Interaksi pelbagai gen secara 'nonadditive' juga didapati mempengaruhi ciri fenotip ini. Namun begitu, kesan interaksi ini hanya boleh dikesan di antara kacukan yang mempunyai pertalian genetik.

Langkah kedua dalam pembinaan pemetaan genetik adalah mengenalpasti penanda yang polimorfik. Sejumlah 142 penanda SSRs yang dibangunkan untuk *Xiphophorus maculatus* (123 penanda) dan *Poecilia reticulata* (19 penanda) telah diperiksa dan hanya 95 yang menunjukan amplifikasi pada DNA molly, *Poecilia*. Namun begitu, hanya 29 penanda SSRs yang polimofik semasa digenotip pada 77 progeni yang terhasil daripada kacukan balik (n = 56) dan kacukan uji (n = 21). Sampel ikan yang digunakan dalam pembangunan populasi pemetaan dikutip daripada Akuatik Antarabangsa, Subang Jaya, Malaysia.

Langkah akhir ialah analisis jaringan penanda yang polimorfik. Antara 29 penanda SSRs yang polimofik, hanya 18 yang berinformasi untuk digunakan dalam membina peta jaringan genetik. Peta jaringan genetik yang dibina terdiri daripada empat kumpulan jaringan yang merangkumi jarak peta 516.1 cM. Satu QTL yang berpotensi, panjang sirip dorsal berjaya dikaitkan dengan penanda Msd021, Msb069 dan Msb068 pada peta jaringan nombor 2.

Kelemahan utama yang dikesan daripada kajian ini adalah peratusan yang rendah pada penanda SSRs yang polimofik. Keadaan ini bukan sahaja menghadkan bilangan penanda yang boleh dipetakan malah menghadkan bilangan peta jaringan yang boleh dijana. Usaha untuk menghasilkan peta jaringan yang padat dengan menggunakan penanda 'amplified fragment length polymorphism (AFLP)' dan pembangunan penanda SSRs yang baru untuk spesies ini adalah disyorkan.

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I certify that a Thesis Examination Committee has met on 6th September 2012 to conduct the final examination of Remmy Keong Bun Poh on his thesis entitled Ploidy Detection, Genetic Diversity, and Quantitative Trait Loci Mapping in Molly Fish (*Poecilia* spp.) in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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REMMY KEONG BUN POH

Date: 6 September 2012

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