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Mendelian Inheritance of Microsatellite Markers in Southeast Asian River Catfish, *Mystus nemurus*

[1]HOH BOON PENG, [1]SITI SHAPOR SIRAJ, [1]TAN SOON GUAN & [2]KHATIJAH YUSOFF
[1]*Department of Biology Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[2]*Department of Microbiology Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
email: hohpeng@yahoo.com

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ABSTRAK

Lapan primer polimorfik yang telah direka, Mnc434a, Mnc65b, Mnc441, MnBp5-1-2b, MnBp5-1-30b, MnBp5-2-2b, MnBp8-4-43a, dan MnBp8-4-43b, digunakan untuk menguji corak pengasingan dalam uji kaji famili Mystus nemurus. *Induk yang dipilih secara rawak dari populasi Terengganu digunakan untuk menghasilkan populasi F_1. DNA telah diekstrak daripada sampel tisu induk, manakala DNA progeni telah diekstrak daripada keseluruhan ikan yang berumur satu bulan. Analisis Chi-kuasa dua telah dijalankan untuk memastikan kesignifikanan keputusan yang didapati. Keputusan menunjukkan bahawa kelapan-lapan lokus mikrosatelit yang diuji terasing hampir dengan nisbah pewarisan Mendel, yang berguna untuk menghasilkan satu peta genetik bagi spesies ini.*

ABSTRACT

Eight previously designed polymorphic microsatellite loci, namely, Mnc434a, Mnc65b, Mnc441, MnBp5-1-02b, MnBp5-1-30b, MnBp5-2-02b, MnBp8-4-43a, and MnBp8-4-43b, were used to examine their modes of segregation in Mystus nemurus *family study. Randomly selected broodstocks from Terengganu population were used to generate F_1 population. DNA was isolated from tissue samples of the parents, whilst the whole one-month-old F_1 progeny was used to isolate the DNA. Chi-square analysis was performed to test the significance of the results obtained. It was shown that all the eight microsatellite loci examined segregated close to Mendelian inheritance ratios, which would be useful in generating a genetic linkage map for this species.*

INTRODUCTION

The Southeast Asian River Catfish (*Mystus nemurus*), locally known as "ikan baung," is an important food fish cultured in Southeast Asian countries, especially in Malaysia and Thailand. Therefore, it is of interest to obtain genetic information on the local species in order to construct an informative genetic linkage map for efficient use in any future breeding program. However, knowledge of Mendelian ratios must be available before any linkage map can be constructed. Mendelian inheritance deals with hereditary transmission of genes or genetic markers from one generation to the next. One key principle is segregation in which the two alleles in an individual separate during the formation of gametes so that each gamete is equally likely to contain either member of the pair. Earlier studies had been done based on various types of markers, particularly on the dominant markers (Siraj *et al.* 1998; Chong *et al.* 1999; Leesa-Nga *et al.* 2000; Hoh *et al.* 2003; Usmani *et al.* 2003). Nonetheless, very limited information was available for these markers to characterize the species and to construct a genetic linkage map. Therefore, there is a need for a more powerful marker.

Microsatellite, a single locus codominant, and highly polymorphic marker, has been the marker of choice in past studies. This marker has been found in all eukaryotic organisms studied to date and is widely applied in the construction of genetic linkage maps in various organisms.

In this study, eight polymorphic microsatellite markers, which were isolated by using the

enrichment techniques-Random Amplified Hybridizing Microsatellites (RAHMS); Hoh *et al.* 2004), and 5' anchored Polymerase Chain Reaction (PCR) (unpublished), were examined for their modes of segregation in a fish family, which contained 100 offsprings. The results obtained would determine the usefulness of these loci in future studies of genetic linkage mapping in this species.

MATERIALS AND METHODS

Samples

A family of *M. nemurus* with a total of 100 F_1 progenies was bred from a wild population in Terengganu. Broodstocks with body weight ranging from 550g to 650g were randomly selected. Each female was injected twice with ovaprim (Syndel International Cooperation, Canada) at 0.1ml kg^{-1} body weight for the first injection, and 0.4ml kg^{-1} body weight for the second injection after eight hours interval, while each male was injected once with ovaprim at 0.4ml kg^{-1} body weight. The males and females were kept separately in one-tonne capacity fiberglass tanks prior to stripping. After eight hours, eggs from female and sperm from male were stripped into a porcelain bowl for dry fertilization and spread into 2 one tonne-fibre tanks. Hatching occurred around 24 to 48 hours after fertilization. Cultured *Artemia* was fed to the progenies twice a day for two weeks and then changed to red worms (commercially available) for the following weeks. F_1 progenies were harvested at the end of the fourth week and samples of tissue from the parents were collected. DNA extraction was done using QiaAmp DNA minikit (Qiagen, USA) according to the manufacturer's instruction.

Microsatellite Analysis

Eight primer pairs, specifically designed for this species using the 5' anchored PCR method and Random Amplified Hybridizing Microsatellites (RAHMs) technique, were screened against both the parents and the F_1 progenies (Table 1). Amplifications were performed using the MJ Research PTC200 thermal cycler (USA). The reaction mixture contained about 30ng DNA template, 1mM of each nucleotide, 1 unit of *Taq* polymerase (Promega, USA), 1 X PCR reaction buffer, 1mM Mg^{2+}, and 10pmol of each microsatellite primer pairs in a total volume of 10μl. The amplification conditions included 4 min of initial denaturation at 94°C, then followed by 35 cycles of 30s denaturation at 94°C, 30s of appropriate annealing temperature (as shown in Table 1), 30s extension at 72°C. A final extension of 5 min at 72°C was also included. Amplicons were examined against 20bp ladder (BMA, USA) on either 4% metaphor gels and then later validated with 8% non-denaturing polyacrylamide gels, and then photographed using Alpha Imager gel documentation system (Siber Hegner, Germany) after ethidium bromide staining.

Data Analysis

Segregation of microsatellite loci were expected to be in a 1:1 ratio for markers heterozygous in either one of the parents; 1:2:1 and 1:1:1:1 ratios for multiallelic segregating among two parents. Goodness of fit to the Mendelian inheritance ratio was determined by χ^2 analysis (Chong *et al.* 1999). The null hypothesis of this study was that the alleles segregating do not deviate from Mendelian inheritance ratio at a significance level of P= 0.05.

RESULTS

All the eight markers examined showed variations between the parents. Six primer pairs segregated according to Mendelian expectations at a significance level of P=0.05. Four of these loci were expected to show Mendelian ratio of 1:1; while three were expected to show a ratio of 1:2:1. The last one, which had three alleles in the parents were expected to segregate in the ratio of 1:1:1:1 (Table 2). Locus MnBp5-1-30b was observed to have a segregation ratio of 2.125:1 (*Fig. 1*); while MnBp5-1-05b segregated in the ratio of 1:1.19:2.25:1.81, slightly deviated from the expected ratio.

DISCUSSION AND CONCLUSIONS

There have been very few reports on the study on Mendelian inheritance of microsatellite markers (Usmani 2002; Chan 2003). Therefore, information on the Mendelian inheritance ratio available is limited. Besides, examination of these studies showed relatively low variation on their family sample (Usmani 2002; Chan 2003).

The codominant nature of microsatellite markers enables the identification of both homozygous and heterozygous at a particular loci. Genotypes and phenotypes of parents and offsprings could be determined directly. Consequently this feature makes them very

TABLE 1
Markers tested for Mendelian inheritance ratio, annealing temperature and the accession numbers

No	Locus	Primer sequence	Repeat motif	Annealing temp (°C)	GenBank accession
1.	Mnc434a	F: TCAGCATGCGACTAAAACA			
		R: TGGTTTTTCAGCAGTATTGG	$(TAT)_3N_5(CA)_{10}$	55	AF346466
2.	Mnc65b	F: CCTGGTTTTTCAGCAGTATT			
		R: GGATCAGCATGCAACTAAA	$(GT)_{10}N_4(ATA)_3$	55	AF346467
3.	Mnc441	F: CAGGTGGAACATTTTGGAT			
		R: TTTAGAGCTATTCCCTTGGA	$(AAAT)_4AAT$		
			$(TGG)_3$		55 AF346470
4.	MnBp5-1-02b	F: TCAAAGTGAGGAGATGGA			
		R: TTTTGTCACTACAGAGCTGCAT	$(TG)_{10}$	60	AY205992
5.	MnBp5-2-02b	F: ACACCAAAGAGATGACCATT			
		R: TCTCTGTGAAACGCTTCTTT	$(GA)_{12}N_5(GA)_5$		
			$(CA)_{10}$		
			$(A)_{11}N_5(A)_8(GA)_5$	55	AF205994
6.	MnBp5-1-30b	F: TTTGGCTACTAGAGACTGACTT			
		R: GGATTATTAGGCAAAACGTG	$(TG)_4$	55	AY852259
7.	MnBp8-4-43a	F: GTTATTTTCGTTGTTGTTG			
		R: GACCGAAGAACATAAACTAT	$(GTT)_5(GT)_2$	55	AY806612
8.	MnBp8-4-43b	F: CACGTGTGTAAGATAAATAG			
		R: GCACTGAGAAATGTGAGAAA	$(AAGG)_5(GA)_{12}GAAAA(GAAA)_2$		55 AY806612

TABLE 2
The observed and expected microsatellites Mendelian inheritance ratios among F_1 progenies and their χ^2 values

Locus	Parent (♂x♀)	Obs. F_1 genotype	Exp. ratio	χ^2	Probability, P
Mnc434a	DD X CD	45:55	CD:DD 50:50	1.00	0.317
Mnc65b	EE X DE	48:52	DE:EE 50:50	0.16	0.689
Mnc441	CG X CG	27:49:24	CC:CG:GG 25:50:25	0.54	0.763
MnBp5-2-02b	JK X JK	30:50:20	JJ:JK:KK 25:50:25	2.00	0.368
MnBp8-4-43a	AA X AB	44:56	AA:AB 50:50	1.44	0.230
MnBp8-4-43b	NP X NP	21:52:27	NN:NP:PP 25:50:25	0.88	0.644
MnBp5-1-30b	GG X GH	68:32	GG:GH 50:50	12.96	0.000*
MnBp5-1-02b	FI X GI	17:19:35:29	FG:FI:GI:II 25:25:25:25	10.16	0.017*

*significant deviation at P<0.05.

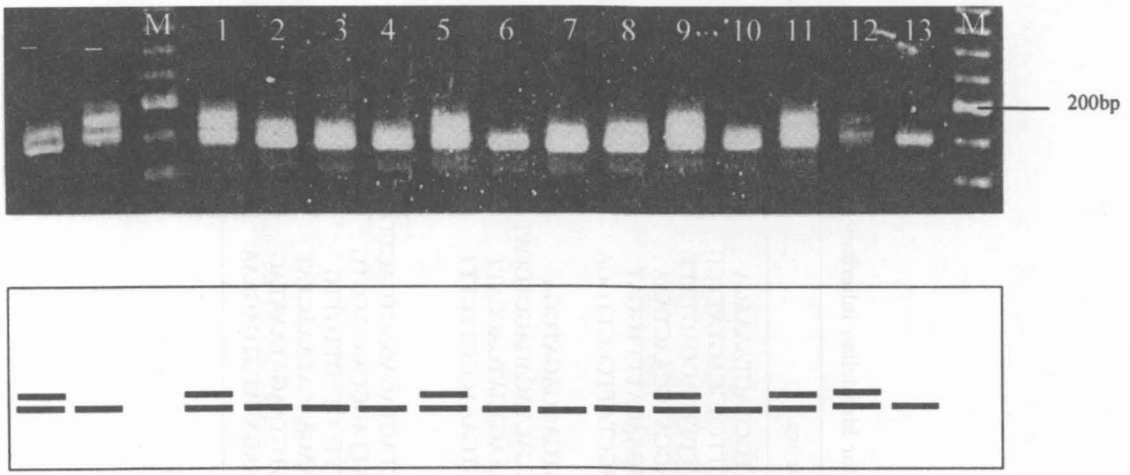

Fig. 1: Profile of primer MnBp5-1-30b for family study. Lane 1, Female; Lane2, Male; Lane 3- 13, progenies F_1; Lane M, 20bp ladder

attractive markers. In contrast, the genotypes of parents studied previously could only be inferred by phenotypic ratio observed in the progenies. While it was demonstrated that the RAPD and AFLP studied showed inheritance in a dominant Mendelian fashion (Chong *et al.* 1999), direct determination of heterozygosity is only possible using backcross.

The unusual segregation ratio shown by MnBp5-1-30b was probably due to the meiotic drive, in which two alleles did not show Mendelian segregation from the heterozygous genotype. This could be due to biological mechanisms that would favor the maintenance of a large number of alleles in the natural populations, which is not uncommon in mammals, insects, fungi and other organisms (Hartl 2000).

Locus MnBp8-4-43a and MnBp8-4-43b are syntenic. They are physically close to each other as these markers are isolated from the same insert. χ^2 analysis showed that there was significant linkage between these loci (χ^2= 11.742, P=0.03). Though it is not suitable to use both of

these loci together in the population studies, the linkage of these two loci may have provided us with valuable information for the Quantitative Trait Loci (QTL) and evolutionary studies. Nonetheless, not all syntenic loci are linked. Findings are often influenced by the sample sizes, interference of the other loci, and different recombination rates among the individuals. There are advantages of studying Mendelian inheritance of a species. First, the information obtained will be used for the construction of a genetic linkage map. Secondly, this study ensures the presence of bands that are inherited in the Mendelian fashion, as this is important for monitoring the effectiveness of selective programs and QTLs (Quantitative Trait Loci). Thirdly, it could be used for the application of pedigree tracing of broodstocks in the breeding programs conducted in any hatchery. Thus, microsatellite markers have proven to be markers of choice for genetic characterization, population structure, and molecular breeding programs.

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