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Construction of genetic linkage maps of guppy (*Poecilia reticulata*) based on AFLP and microsatellite DNA markers

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Abstract

Genetic linkage maps of guppy (*Poecilia reticulata*) were constructed using microsatellite DNA and AFLP markers and a pseudo-testcross mapping strategy. $(AC)_n$ microsatellite DNA markers were developed and used to genotype sixty-one full-sib progenies (F_1) and their two parents. Of 293 microsatellite DNA markers, 101 segregated at 1:1 or 1:1:1:1 ratios. In addition, 336 AFLP markers segregated also in F_1 progenies at 1:1 ratio, which were produced using 91 primer combinations. All these markers were mapped with two linkage maps produced, one each parent. Female map included 135 markers in 22 linkage groups, covering a total of 1267.7 cM in length. Male map included 172 markers in 20 linkage groups, covering a total of 1771.2 cM in length.

Keywords: *Poecilia reticulata*; Linkage map; AFLP; Microsatellite DNA

1. Introduction

Guppy fish (*Poecilia reticulata*) belongs to class Actinopterygii (ray-finned fish), order Cyprinodontiformes, family Poeciliidae and genus *Poecilia*. It mainly inhabits the coastal streams and rivers of Venezuela, Guyana, Surinam and several of the Lesser Antilles including Trinidad and Tobago (Haskins and Haskins, 1951; Yamamoto, 1975). Since 1950s, breeders have domesticated, artificially selected and genetically improved wild guppy for traits such as brilliant color, longer fin and larger body size. At present, different guppy strains have been created 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). Guppy has become popular among aquarists and hobbyists and played an important role in the freshwater ornamental fish industry worldwide. Guppy is also a model fish for studying the genetic bases of heterosis, aging and inbreed depletion crucial for aquaculture because of its relatively short life cycle, easiness of breeding and reproduction, availability of commercial strains homologous for the traits such as body color and

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tail shape, and very importantly the availability of evaluation methods of heterosis, aging and inbreed depletion (Nakajima and Fujio, 1993; Fujio et al., 1995; Shikano et al., 1997).

Genetic linkage maps have become powerful tools of research in many organisms (Dib et al., 1996). A complete linkage map is necessary for efficiently carrying out molecular based analyses, e.g. location of quantitative trait locus and map-based cloning (Lander and Botstein, 1989). Genetic linkage maps have been developed for almost all major and important cultured fish, e.g., tilapia, rainbow trout, catfish, Atlantic salmon, medaka and Arctic char (Young et al., 1998; Sakamoto et al., 2000; Gilbey et al., 2004; Woram et al., 2004; Lee et al., 2005). For guppy, a preliminary linkage map has been constructed (Winge, 1927), which included 18 genes on sex chromosomes. In addition, Khoo et al. (2003) constructed RAPD marker linkage map and Watanabe et al. (2005, 2004) constructed microsatellite DNA and AFLP marker linkage map of guppy, respectively. However, these previous maps were restricted by the number of markers appropriate for their applications.

In the present study, two parent-specific genetic linkage maps of guppy were constructed by analyzing the segregation of AFLP and microsatellite DNA markers in 61 F_1 progenies. Our objectives were to: (1) estimate the genome lengths of guppy; and (2) construct moderately dense linkage maps.

2. Materials and methods

2.1. Mapping population

In Chinese ornamental fish market, the dealers used to self-cross relatively pure commercial strains, which will cause the inbreeding depression in a few generations. In order to recover the vigor, the dealers used to cross the females of a strain with the males of the other, producing morphologically similar but genetically different fish for sale. Fortunately, this facilitates our mapping trial; the number of loci segregating in the offspring of a cross between the relatively pure strains is less than that in the offspring of a cross between the lines purchased from the market.

Two parental lines used in this study were purchased from the local ornamental fish market, Qingdao, China. Both female and male individuals of one line (red line) had pink body and red tail while both female and male individuals of the other line (black line) had black (rare part) and grey (front part) body and red tail. These parental lines are homozygous for desired morphological traits as were observed in their self-crossing offspring, but heterozygous in genetic background to some extent as was determined in microsatellite DNA marker screening (of 221 markers amplified robust bands, 128 were polymorphic in at least one parent). The first filial generation was obtained by making a cross between a female individual of red line and a male individual of black line, with F_1 progenies reared for more than 50 days to sexual maturation. All F_1 progenies were similar to black line in body and tail color. A total of 61 progenies (31 female and 30 male) were used for constructing parent-specific linkage maps.

2.2. DNA extraction

DNA was extracted from muscle tissue following the method described by Dinesh et al. (1993) with minor modifications. Approximately, 100 mg of tissue was cut into small pieces which were then placed in 1 ml STE buffer (10 mM Tris–HCl, pH 8.0; 50 mM EDTA, pH 8.0; 200 mM NaCl and 0.5% SDS) and digested with 0.5 mg/ml proteinase K at 55 °C overnight (>8 h). The resulting solution was extracted with phenol and chloroform. DNA was precipitated with ethanol and dissolved in TE buffer. The quality and concentration of DNA were assessed by agarose gel electrophoresis.

2.3. AFLP analysis

AFLP analysis was carried out essentially as described by Vos et al. (1995). DNA was digested with *Eco*R I and *Mse* I before ligation to restriction site-specific adaptors. Pre-amplification was carried out using adaptor-specific primers with one selective base overhang each primer (*Eco*R I adaptor primer with A and *Mse* I adaptor primer with C). The pre-amplification product was diluted (20-fold) and used for selective amplification. Selective amplification was carried out with diluted pre-amplification product and primers with three selective bases overhang each primer. In total, 91 primer combinations were selected for AFLP analysis. The products of selective amplification were separated on polyacrylamide gel with the bands visualized by silver staining.

Mse I and *Eco*R I selective primers were named with capital letters and numbers, respectively (Table 1). AFLP marker was named with the name of *Mse* I selective primer and that of *Eco*R I selective primer followed by letter f (fragment) and the size of the fragment in base pairs (Zhang et al., 2006), e.g., A8f169 referred to the 169 bp fragment generated by *Mse* I primer A (CTA) and *Eco*R I primer 8 (AGC).

Table 1
The number of polymorphic loci detected with AFLP analysis

	E_1ACC	E_2AGG	E_3ACA	E_4ACT	E_5ACG	E_6AGA	E_7AGT	E_8AGC	Total
M_ACTA	5	6	–	9	10	5	3	7	45
M_BCAA	4	7	3	8	6	3	5	8	44
M_CCGA	5	3	9	11	6	5	7	5	51
M_DCTT	8	12	3	5	7	5	4	7	51
M_ECTG	5	8	6	7	7	7	8	9	57
M_FCTC	11	7	8	9	–	2	–	7	44
M_GCAT	5	9	8	5	8	7	3	7	52
M_HCAG	7	4	6	5	7	5	4	6	44
M_ICAC	5	6	8	32	5	–	7	3	37
M_JCGT	7	8	5	36	6	7	4	3	46
M_KCGG	8	9	3	6	7	8	2	7	50
M_LCGC	5	6	8	–	3	6	5	6	39
Total	75	85	67	76	72	60	52	75	560

2.4. Microsatellite DNA analysis

A genomic DNA library enriched with CA/GT-microsatellite DNA containing short fragments was constructed using DNA extracted from a guppy individual using FIASCO (Fast Isolation by AFLP of Sequences Containing Repeats) method described by Zane et al. (2002). A biotinylated $(CA)_{12}$ oligonucleotide was used as probe. DNA fragments containing microsatellite DNA were ligated with pMD18-T vector (TaKaRa, Dalian, China) and electroporated into *E. coli* JM109. Each colony was subjected to 3 individual PCR screening reactions. In the first reaction, universal forward and reverse sequencing primers (RV-M and M13-47) were used. In the second reaction, one universal forward primer was used in combination with the $(CA)_{12}$ oligonucleotide probe and in the third the other universal reverse primer was used in combination with the $(CA)_{12}$ oligonucleotide probe. The

Table 2
A portion of mapped microsatellite DNA primers developed in this study[a]

Locus	Accession no.	Primers (5′–3′)		Temperature (°C)
		Forward	Reverse	
G103	EF508706	GTTGGCGTCTGTGGTTT	TCAGTTATCTTTGGGGAG	56
G118	EF508710	TGCCAGCATAGAAACAA	CAGAAAACCCACGACTT	57
G121	EF508707	AACTATGTCTCACCGTCAA	TATCCGTTTCTCAGGCT	53
G126	EF508708	CCATCGTGGGAAAACCG	GGCACTCCAACATAAAGG	55
G143	EF508709	ATTTCCACTTGCTCCTG	AACCATAACCAAAACCTAA	56
G188	EF508711	ATCTACCTCAACCGCATCA	TATCTTGGGGCACATCG	55
G226	EF508712	CGCAGGCTTTTCGTTCA	AACCTTCCCTTTTCTGTG	56
G235	EF508713	AGAAATACAAACCCATCACC	CAATAAAAGAGGGAAGGA	58
G237	EF508714	AGAGCGTGGACGGTGGA	AAGCGGGCTAAGTATGG	62
G251	EF508715	TGGCAGGATGTGGGAGA	TGCCGTATTTTCTGACCAC	58
G260	EF508716	TCGCTTTCGTCCATTTG	ACTGCGGGGTTCTTTGA	55
G269	EF508717	TCCTTCAAGCAGTTCCG	CAGACCGCACAGAGCAA	58
G276	EF508718	ACAGCGAGGAACAGCAT	GGGGACAGTTTGGAAGA	58
G303	EF508719	GTATTTGTTCTTGGGCTCT	GCTCAATGTGACGCTCTAA	58
G318	EF508720	GGCTTTGTGCTCTGTGA	TTCCTCCATTCCATACCTT	55
G341	EF508721	AGGCAGAGCGAGGTGTT	AGAAGGGAAGGGAGGGA	62
G373	EF508722	CAACTGCCCCATTCACA	AACTCAACGAACCCCAC	57
G375	EF508723	CTTCGGCTTTCAGGAGT	TACGCTGTCCCAGTCAC	60
G376	EF508724	TACAGCATCTGGCATCC	GGCACTCGCACTATTTCTA	57
G380	EF508725	CATCCATTTGTGCCAGGTC	AGGGTGGGCTTGGTTTA	55
G387	EF508726	GTTCCAGATAGAGCGATGC	TTGTGCCTTCCTGTTGC	55
G392	EF508727	TTGTGGTTGAAACGGAG	TGTGGCGAAATGTAAGAG	55
G501	EF508728	ACTTACACTCACTGGCTCA	AACTTTACCACCATTCACA	55
G507	EF508729	AGGGTGGGTGTCAGGAA	AGCCCGAGCGAGGTATG	55
G508	EF508730	CGGATGTGAGAAACTGAG	TGTTATGCTGCTGGGTG	55

[a] The other portion of microsatellite DNA primers included those developed ourselves (Sheng et al., 2007) and those published previously by other authors.

PCR amplification reaction was carried out in a volume of 25 μl containing 10 mM Tris–HCl, pH8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM dNTPs (each), 200 μM primers (each direction), 1 U *Taq* DNA polymerase and about 1 μl overnight bacterial culture. Thermal cycling condition was as follows: denaturation at 94 °C for 5 min, followed by

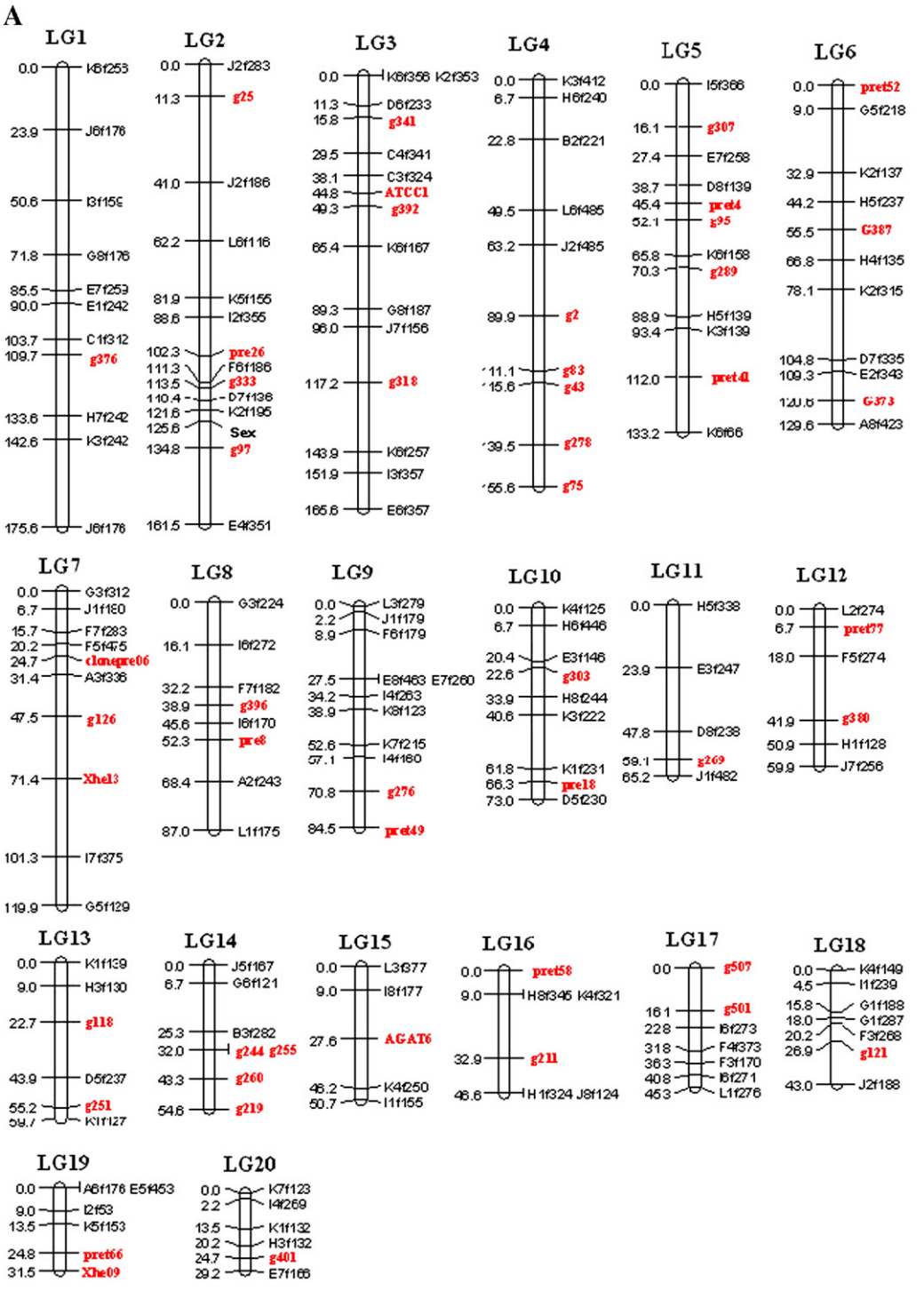

Fig. 1. Male (A) and female (B) parents linkage maps of guppy (*Poecilia reticulata*). Markers are shown on the right of the linkage group bar. The distances between loci are given on the left in Kosambi cM.

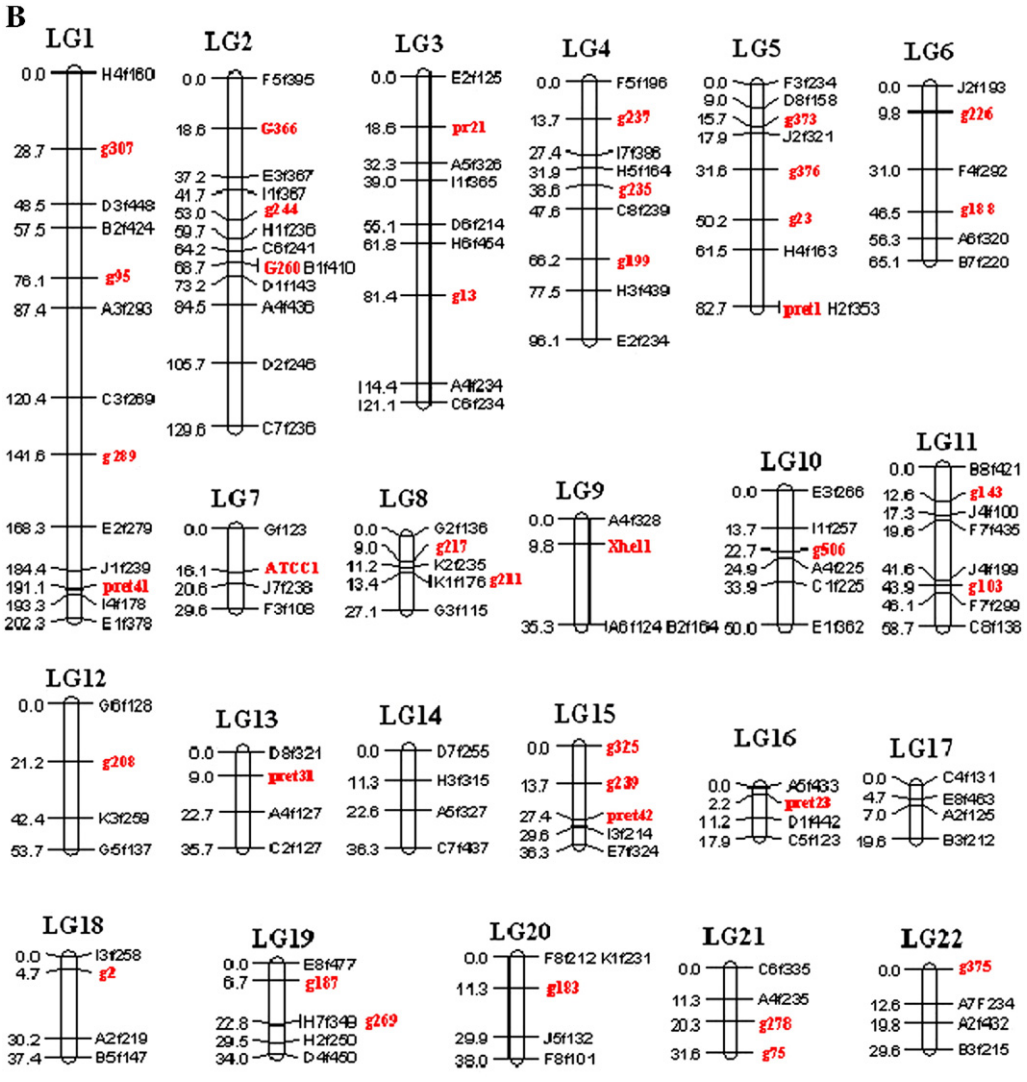

Fig. 1 (*continued*).

30 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 1 min and an extra extension at 72 °C for 10 min. Those clones generating different products in the first screening reaction and in either the second or the third one were sequenced with an ABI3700 sequencer using both universal sequencing primers. Microsatellite DNA primers were designed using Primer Premier 5 (http:// www.premierbiosoft.com/primerdesign/). A collection of 89 guppy microsatellite DNA markers published previously by Paterson and Crispo (2005), Olendorf et al. (2004), Becher et al. (2002) and Watanabe et al. (2003, 2004), five of Gila topminnow (Parker et al., 1998) and 10 of green swordtail (Yue and Orban, 2004) were tried also in this study. Microsatellite DNA amplification was carried out in a volume of 25 μl containing 10 mM Tris–HCl, pH8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM dNTPs (each), 200 μM primers (each direction), 1 U *Taq* DNA polymerase and about 50 ng DNA as template. Thermal cycling condition was as follows: denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at the appropriate temperature of each primer pair (listed in Table 2 or being published previously) for 1 min, and extension at 72 °C for 1 min followed by an extra extension at 72 °C for 10 min. The PCR product was separated on a 6% denaturing polyacrylamide gel and visualized by silver staining.

2.5. Segregation analysis

Segregating markers were judged for deviations from the expected 1:1 (of AFLP marker and microsatellite DNA marker polymorphic in one parent) or 1:1:1:1 (of

microsatellite DNA marker polymorphic in both parents) phenotypic ratios with χ^2 testing. Those markers segregating at the expected ratios were used to construct linkage maps. Their segregation type was scored as for backcross population with H representing the presence of a band and A representing the absence of a band. Two data sets were obtained for the maternal and paternal parents, respectively (Zhang et al., 2006). Each data set was first entered into the mapping software MapMaker/Exp v. 3.0 (Lander et al., 1987) following F_2 backcross model, then duplicated by changing A to H and H to A. The reciprocal data sets detected linkage of markers by creating two identical linkage groups (Li et al., 2005).

2.6. Linkage analysis

Markers were organized into linkage groups with the GROUP command at the minimum LOD of 3.5 and the maximum distance between two loci of 40 cM. Preliminary order of markers in each linkage group with less than nine markers was established with successive COMPARE, ORDER and MAP command. Marker order on the linkage group with more than 9 markers was established using the THREE POINT, ORDER and MAP commands to obtain the order of markers with unique placement, followed by the TRY command to find the most likely placement of the remaining markers. Once the framework linkage groups were established, the relatively less stringent criteria (LOD=2.5 and θ=0.40) were applied to test whether there were any additional markers or distorted markers that could be mapped. Map distance in CentiMorgans was calculated with Kosambi's mapping function. Linkage map was drawn using the "MapChart" Package (Voorrips, 2002; Li et al., 2005).

2.7. Map length and coverage

Map length and map coverage were calculated based on mapped markers. Two approaches were used to estimate map length: Ge_1, the genome was estimated by adding $2s$ (s is the average space of the linkage map) to the length of each group to account for chromosome ends (Fishman et al., 2001); and Ge_2, the genome was calculated by multiplying the length of each linkage group by $(m+1)/(m-1)$, where m is the number of markers in each group. The estimated map length is the sum of revised length of all linkage groups (Chakravarti et al., 1991). The average of the two estimates was used as the estimated genome length (Ge). The observed map length was calculated as the length of the map (Gof) and the total length (Goa) considering all the markers on the map, the triplets and doublets. The observed genome coverages, Gof and Goa, were calculated as Gof/Ge and Goa/Ge, respectively (Zhang et al., 2006).

3. Results

3.1. Marker polymorphism and segregation

Ninety-one AFLP primer combinations produced about 5000 AFLP bands. On average, each primer combination produced 50–60 bands with the size between 50 and 500 bp. In total, 560 bands were found to be polymorphic (Table 1), of them, 336 (173 in female and 163 in male) segregated at 1:1 ratio, 53 segregated at 3:1 ratio, 151 (81 in female and 70 in male) deviated significantly from 1:1 ratio and 20 deviated from 3:1 ratio ($P<0.05$).

In total, we isolated 2074 positive plasmids from microsatellite DNA enriched genomic library of guppy. The library was enriched for simple dinucleotide (CA) repeats. Two hundred and eighty-seven desirable clones were sequenced, of them, 262 contained microsatellite DNA motif. These sequences containing microsatellite DNA were newly isolated; no significant match was found in BlastN searching against the sequences deposited in GenBank. From these sequences, 189 with desirable flanking sequences were used for designing microsatellite DNA marker primers.

One hundred and eighty-nine pairs of primers and 104 pairs published previously were screened for segregation in mapping population. In total, 221 microsatellite DNA markers amplified robust bands, of them, 128 were polymorphic and therefore informative in one or both parents. Thirty-seven in female and 55 in male segregated at 1:1 ratio, 9 segregated at 1:1:1:1 and no loci segregated at 1:2:1 ratio. Among loci suitable for mapping, higher rate of polymorphism was seen in male (58%) than in female parent (42%). Twenty-seven microsatellite DNA deviating from expected 1:1 or 1:1:1:1 ratios ($P<0.05$) were excluded.

3.2. Genetic linkage map

Two linkage maps were constructed: one for male parent and the other for female parent. The male parent map consisted of 227 markers, of these, 172 (122 AFLP, 49 microsatellite DNA and the sex determinant) were assigned to 20 linkage groups, which covered 1771.2 cM in length with an average interval of 11.7 cM. The length of the linkage groups ranged from 31.5 to 175.6 cM, the number of markers per group varied from 4 to 14 (Fig. 1A). The remaining 16 distributed as four triplets and two doublets (Fig. 2A). The female parent map

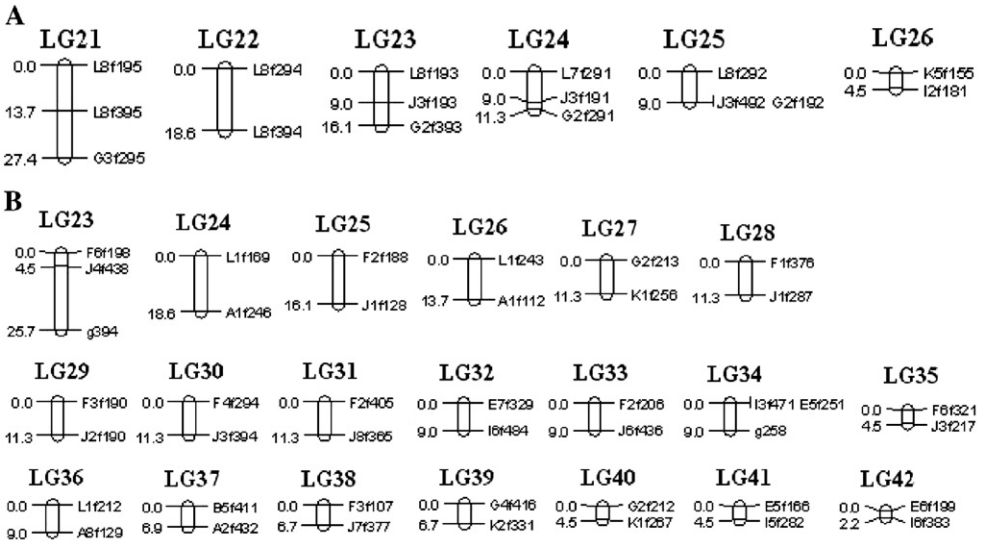

Fig. 2. Linkage triplets and doublets of male (A) and female (B) guppy parents (*Poecilia reticulata*). Markers are shown on the right. The distances between loci are given on the left in Kosambi cM.

consisted of 219 markers, of them 135 (97 AFLP and 38 microsatellite DNA) were assigned to 22 linkage groups, which covered 1267.7 cM in length with an average interval of 11.2 cM, each contained a minimum of four markers (Fig. 1B). The remaining 42 markers distributed as two triplets and eighteen doublets (Fig. 2B). The length of the individual linkage group varied from 19.6 to 202.3 cM. Although the majority of map intervals were less than 15 cM, some large gaps remained in two maps.

Forty markers (29 AFLP and 11 microsatellite DNA) did not show linkage to any other marker and were not assigned to any of the linkage groups. Thirty-eight markers (26 AFLP and 12 microsatellite DNA) were eliminated because they showed uncertain linkage or spanned very large map distances.

3.3. Genome estimation and map coverage

Map lengths were estimated as 2240.39 cM (Ge_1) and 2224.41 cM (Ge_2) with the average of 2232.21 cM for the male and 1761.34 cM and 1738.02 cM with the average of 1749.68 cM for female. On the basis of the expected genome lengths, genome coverage of male and female maps was 79.3% and 72.5%, respectively. When considering all the triplets and doublets, the map coverage increased to 84.5% for the male and 84.0% for the female.

3.4. Mapping of sex marker

The sex of the mapping progenies was treated as a marker for linkage analysis in the female and male (Li et al., 2003). Of 61 progenies, 30 were female and 31 were male. The sex marker was the only phenotypic trait placed on the map. In the male parent map, sex was mapped onto linkage group 2, which contains 13 markers including four microsatellite DNA and nine AFLP. Four microsatellite DNA markers separated from the sex locus at the distance of 114.3 cM, 23.3 cM, 12.1 cM and 9.2 cM, respectively (Fig. 1A).

4. Discussion

4.1. Molecular markers

A fine linkage map requires a large number of molecular markers. AFLP markers have the potential to efficiently and rapidly construct high-resolution maps and to identify and isolate those closely linked with desirable traits. However, AFLP markers are difficult to transfer among labs and populations, which limit the extension of AFLP map application (Wang et al., 2004). Microsatellite DNA markers are better for linkage mapping than AFLP because of their high polymorphism, heterozygosity, co-dominance and wide transportability across different mapping populations (Rafalski et al., 1996). In recent years, several microsatellite DNA markers have been developed for guppy (Becher et al., 2002; Watanabe et al., 2003, 2004; Paterson and Crispo, 2005) with their cross-species amplifications tried as well (Parker et al., 1998; Yue and Orban, 2004). However, the number of microsatellite DNA markers of guppy is still very limited. Here, we reported the development of polymorphic microsatellite

DNA markers of guppy. The procedure used for library enrichment was very efficient with nearly 91.2% of colonies yielded microsatellite sequences. Such an efficiency of library enrichment means that it can no longer be considered that the sequencing of microsatellite DNA requires a large investment of time and effort. The development of these microsatellite DNA markers will certainly facilitate the exploration of the genetic basis of diverse biological phenomena, promoting the breeding of guppy and the management of its genetic resources.

4.2. Linkage map

Linkage maps, especially high density ones, facilitate a number of important biological investigations. They are necessary for efficient mapping of quantitative trait loci (QTL), complement of marker-assisted selection (MAS) and comparative genome mapping (Lander and Botstein, 1989). Based on female and male segregating data, two sex-specific linkage maps were constructed. The estimate of genome length was 2232.41 cM and 1749.68 cM for male and female, respectively. The guppy maps here are obviously incomplete as were indicated by the presence of large gaps. Despite the presence of gaps, the maps provided reasonably good coverage of the guppy genome, 84.5% for male map and 84.0% for female map when all linked markers are considered. Therefore, the maps provided a basic framework for gene and QTL mapping in guppy.

Theoretically, the two separate maps could be merged into a composite one by using markers (AFLP markers at 3:1 ratio and microsatellite DNA markers at 1:1:1:1 ratio) that are heterozygous in both parents (Zhang et al., 2006), but such a merge can be poorly performed with dominant markers like AFLPs (Li et al., 2003). In this study, the markers segregating at 3:1 ratio also showed poor linkage with those segregating at 1:1 ratio (data not shown). Currently, because only nine common microsatellite markers were placed in both maps, it is difficult to merge two maps.

4.3. Marker distribution

The distributions of AFLP and microsatellite DNA markers are mostly random on guppy male and female maps (Fig. 1). In published literatures, AFLP markers distribute randomly in some species (Remington et al., 1999; Cervera et al., 2001), but cluster in others (Waldbieser et al., 2001; Sakamoto et al., 2000). In fish, highly clustered AFLP or microsatellite DNA markers were observed in medaka, rainbow trout, tilapia and channel catfish (Naruse et al., 2000; Young et al., 1998; Agresti et al., 2000; Liu et al., 2003). The reasons for high level of marker clustering are not known yet (Watanabe et al., 2005). Some potential causes are proposed, which include great variation at specific restriction sites and existence of repeat sequences in a genomic region. Markers tend to cluster around regions where recombination is suppressed. Usually, these regions are centromeres and telomeres (Tanksley et al., 1992; Rouppe van der Voort et al., 1997).

4.4. Segregation distortion

In this study, segregation distortion ratio of AFLP markers is about 30.53% when considering all polymorphic bands. Segregation distortion ratio is 30.04% (male) and 31.88% (female) when considering 1:1 segregation pattern in either male parent or female parent. Microsatellite DNA markers polymorphic in both parents were tested against a 1:1:1:1 ($d.f.=3$) segregation ratio and against a 1:1 ($d.f.=1$) segregation when polymorphic in only one parent (Zhang et al., 2006). Twenty-one percent of the segregating microsatellite DNA markers were distorted. The level of segregation distortion observed in this study is higher than in other studies, e.g., 16% in catfish (Liu et al., 2003) and 13.3% in rainbow trout (Young et al., 1998). Segregation distortion is a problem often encountered in mapping populations (Jiang et al., 2000). Several factors could cause segregation distortion, e.g., the amplification of the same size fragment from several different genomic regions (Faris et al., 1998), the distortion of the transmission between genetically divergent genomes (Fishman et al., 2001), and sampling in finite mapping populations and preferential fertilization and zygotic selection (Rick, 1969).

4.5. Sex-linked marker

The guppy has 23 pairs of chromosomes, of which 22 are autosomal and one is sex determining. Males are heterogametic, thus the sex determination mechanism is basically a XX–XY type (Winge, 1922; Winge and Ditlevsen, 1947). The guppy is also unique among other teleosts in that almost all the genes determining color patterns (not including body color) are sex-linked or sex limited (Winge, 1922, 1927). Khoo et al. (2003) and Watanabe et al. (2005) located some molecular markers on the Y chromosome, which were adjacent to a sex-determining region, SdR. In this study, the sex locus was mapped only on the male parent map, together with nine AFLP markers and four microsatellite DNA markers. In

contrast, there were no markers linked to sex on the female parent map. The confirmation of sex-linked markers on the paternal, but not the maternal map agreed with the XX–XY sex determination mechanism of guppy. With the same strategy, sex was mapped on the male map of Pacific oyster (Li et al., 2003) and on the female map of kuruma prawn, zhikong scallop and Pacific white shrimp (Li et al., 2005, 2003; Zhang et al., 2006).

In commercial fish, experiments which need many generations are time consuming, but there are many themes which require several generations to obtain genetic information, such as QTL analysis. Guppy, having a short generation interval was suggested as an important model fish for studying genetics and breeding in aquaculture (Nakajima and Taniguchi, 2001). Genetic mapping is a step towards marker-assisted selection. This study has shown that linkage maps can be constructed rapidly for the guppy using molecular markers. At present, we are also trying to develop microsatellite DNA markers and construct an integrated and nearly saturated genetic map combining microsatellite DNA and AFLP markers. It will serve as a tool for the advancement of guppy genetics including the mapping of economical traits, e.g. various phenotypic characteristics, stress response and growth rate. These studies will also be important for understanding the genetic basis of the economic traits of economically important fish.

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