Linkage map construction for silkworm (*Bombyx mori* L.) based on amplified fragment length polymorphism markers

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Abstract

The domesticated silkworm, *Bombyx mori*, is of high commercial importance as a silk producer and is also widely used for implementation of basic and applied research. It is important to understand its genome organization using molecular markers for genetic studies and for breeding purposes. In this study, a genetic linkage map using 204 amplified fragment length polymorphism (AFLP) markers was developed. Twenty *Pst*I/*Taq*I primer combinations were used to genotype 78 progenies from an F_2 population of the P107×Khorasan Lemon cross. Each primer combination generated an average of 10.2 AFLP markers qualified for linkage mapping. All the 204 AFLP markers were assigned to 12 linkage groups at the Logarithm of Odds (LOD) threshold of 2. The number of markers in the linkage groups ranged from 2 to 53. There were seven major linkage groups with 13-53 markers and five small linkage groups with 2-6 markers. The 12 linkage groups varied in length from 12.3 to 938.4 cM and the total length of linkage map was 4262 cM, giving an average marker resolution of 20.89 cM. This study presents the preliminary step for further marker-assisted research on silkworm, including Quantitative Trait Loci (QTL) and introgression analyses.

Keywords: Silkworm; *Bombyx mori*; Linkage map; AFLP markers

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INTRODUCTION

Silkworm, *Bombyx mori* L., domesticated for silk production is an agriculturally important insect and comprises a large number of geographical races and inbred lines that show substantial variation in their qualitative and quantitative traits (Mirhoseini *et al*., 2007). Currently, it is the major economic resource for nearly 30 million families in countries such as China, India, Vietnam, and Thailand (Miao *et al*., 2005). With the establishment of stable transformation (Tamura *et al*., 2000; Yamao *et al*., 1999), the silkworm has shown the potential to produce pharmaceutically important proteins in high yield (Tomita *et al*., 2003); opening up new applications for sericulture in medical, agricultural, and industrial fields (Yamamoto *et al*., 2006). Analysis of the silkworm genome began a few years ago because of its importance for breeding and genetic studies, for isolating valuable genes and promoters and for comparative genomics (Goldsmith *et al*., 2005). Mita *et al*. (2003) first initiated intensive sequencing of the silkworm genome using expressed sequence tags (ESTs). Recently, this group (Mita *et al*., 2004) and a second group (Xia *et al.,* 2004) reported the results of whole-genome shotgun sequencing and provided public access to the assembled silkworm genome data (http://www.dna.affrc.go.jp/genome/; Wang *et al.* 2005; http:// silkworm. genomics.org.cn/). Genetic linkage mapping has proven to be a powerful tool in genetic studies of many organisms. A complete linkage map is necessary to efficiently carry out molecular-based analyses such as molecular marker-

assisted selection, QTL mapping of agronomically important traits, prediction of heterosis and comprehensive investigations of genomic evolution between lineages (Tan *et al.*, 2001). Genetic linkage mapping of the silkworm (*B. mori* L.) as an important insect using molecular markers is essential for genetic studies and for breeding purposes.

Presently, genome studies in *B. mori* have generated genetic linkage maps based on morphological markers (Doira *et al*., 1992) and molecular markers including the Restriction Fragment Length Polymorphism (RFLP) (Nguu *et al*., 2005; Shi *et al*., 1995; Goldsmith, 1991), Random Amplified Polymorphic DNA (RAPD) (Li *et al.*, 2000; Yasukochi, 1998; Promboon *et al*., 1995), Selective Amplification of DNA Fragments (SADF) and RAPD (He *et al*., 2001), amplified fragment length polymerphism (AFLP) (Sima *et al*., 2006; Lu *et al*., 2004; Tan *et al*., 2001), the microsatellite (Miao *et al*., 2005) and Single Nucleotide Polymorphism (SNP) (Yamamoto *et al.*, 2006). The AFLP technique (Vos *et al.,* 1995; Zabeau and Vos, 1992) has demonstrated to be a convenient and reliable tool to generate highly polymorphic molecular markers that greatly facilitate building linkage maps (Qi *et al*., 1998; Waugh *et al*., 1997; Becker *et al*., 1995). AFLP markers do allow one to construct linkage maps with wide genome coverage without engaging in extensive sequencing or marker development programmes. The AFLP technique is also faster than individual codominant marker types because a single polymerase chain reaction (PCR) can derive multiple loci simultaneously (Erickson *et al*., 2004). Because of these features, AFLP has been widely employed for genetic mapping in various organisms.

Since the AFLP technique enables the generation of many polymorphic markers in a single PCR, it can be used to generate high-resolution genetic maps. This study reports a high-resolution AFLP-based genetic linkage map of silkworm (*B. mori* L.). Development of the linkage map lays an important foundation for future genomics research on the silkworm and provides valuable tools for determining the genetic basis of economically important traits, such as silk production and resistance to diseases.

MATERIALS AND METHODS

Insect materials and crosses: One F_2 segregating family that had resulted from mating between a Japanese inbred line (P107) as female parent and an Iranian native strain (Khorasan Lemon) as male parent was used in the study. These two inbred lines ands strain exhibit high phenotype diversity for economically important characters such as whole cocoon weight, cocoon shell weight and cocoon shell percentage suggesting that considerable polymorphism exists at the DNA level (Dalirsefat and Mirhoseini, 2007). Indeed, the highest and the least quantities of mentioned traits corresponded to P107 and Khorasan Lemon, respectively. These inbred lines and strains have undergone a high degree of inbreeding and are relatively homozygous. A number of 78 individuals (39 females and 39 males) from the F_2 population were used to construct a genetic linkage map. The parents and the F_1 progeny were used to establish the segregation pattern of the molecular markers. The crossing experiments were established in the Iran Silkworm Research Center (ISRC) located in Rasht, center of Guilan province.

AFLP analysis: Genomic DNAs were isolated individually from all the parents, F_1 and F_2 populations at the moth stage by using the phenol/chloroform method (Suzuki *et al*., 1972) and as modified by Nagaraja and Nagaraju (1995). DNAs were quantified using a known standard (λDNA) (DNA Lambda, Roche, Germany) on agarose gels.

All individuals were subjected to genotyping with AFLP markers according to Vos *et al*. (1995) with some modifications. Briefly, genomic DNA was double digested with *Pst*I and *Taq*I restriction enzymes which can produce polymorphic DNA fragments in the silkworm (Mirhoseini *et al*., 2007; Tan *et al*., 2001) The DNA fragments were ligated with *Pst*I and *Taq*I adaptors, generating template DNA for PCR amplification. Two primers were designed on the basis of the adaptor sequences and restriction site sequences for use in Polymerase Chain Reaction (PCR) amplification. Selective nucleotide sequences were added to the 3'-end of each primer. PCR amplification was conducted in two steps: a pre-amplification and a selective amplification. For the selective amplification, a total of 81 primer combinations obtained from two sets of *Pst*I and *Taq*I selective primers (Table 1) were screened. Among them, 20 primer pairs that produced fragments with clear dominant inheritance patterns and were reproducible were used for linkage analysis. Polymorphism screening of AFLP products was conducted on a 6% polyacrylamide gel using a SequiGen

Table 1. Adapters and primers used in AFLP analysis.

Name		Sequence
Adapters *Pst*I	*Pst* top strand	5′-GACGTGACGGCCGTCATGCA
	Pst bottom strand	5′-TGACGGCCGTCACG
Adapters *Taq*I	*Taq* top strand	5′-GACGATGAGTCCTGAG
	Taq bottom strand	5′-CGCTCAGGACTCAT
Primers *Pst*I [a]	P01	5′-GACGGCCGTCATGCAG
	P21	5′-GACGGCCGTCATG CAG**TA**
	P22	5′-GACGGCCGTCATGCAG **AT**
	P23	5′-GACGGCCGTCATGCAG **TC**
	P24	5′-GACGGCCGTCATGCAG **AC**
	P31	5′-GACGGCCGTCATGCAG **AAC**
	P32	5′-GACGGCCGTCATGCAG **AGA**
	P33	5′-GACGGCCGTCATGCAG **ATG**
	P34	5′-GACGGCCGTCATGCAG **AAG**
	P35	5′-GACGGCCGTCATGCAG **TAT**
Primers *Taq*I	T01	5′-GATGAGTCCTGAGCGA
	T21	5′-GATGAGTCCTGAGCGA **TA**
	T22	5′-GATGAGTCCTGAGCGA **AT**
	T23	5′-GATGAGTCCTGAGCGA **TC**
	T24	5′-GATGAGTCCTGAGCGA **TG**
	T31	5′-GATGAGTCCTGAGCGA **AAT**
	T32	5′-GATGAGTCCTGAGCGA **ACA**
	T33	5′-GATGAGTCCTGAGCGA **AAG**
	T34	5′-GATGAGTCCTGAGCGA **AGC**
	T35	5′-GATGAGTCCTGAGCGA **TAC**

[a]Selective nucleotides are shown as bold letters.

38×30 cm gel apparatus (BioRad Laboratories Inc., Hercules, CA, USA). Bands were detected by the silver staining procedure (Promega, Technical manual No.023) and gel images were scanned and saved as jpeg files for scoring and further analysis.

Linkage analysis and map construction: Using genotype information of 81 AFLP primer combinations, 20 primer combinations which produced clearly readable and polymorphic fragments among parents were employed to analyze linkage mapping. The AFLP fragments were scored based on 0 and 1 and then converted to A, B, C and D letters according to Map manager QTX (Manly *et al*., 2001) instruction manual. The data were analysed by using the Kosambi's map function (Kosambi, 1944) of Map manager QTX (Manly *et al*., 2001) to develop a linkage map for population. By genotyping 78 F_2 progenies using 204 polymorphic bands, a genotypic data matrix in a dimension of 78×204 was constructed and used for linkage mapping. Recombination rates among markers were first evaluated and were then converted to the map distance based on centiMorgan using Kosambi's map function (Kosambi, 1944).

RESULTS

Among the 81 AFLP primer combinations screened, approximately one-third of the primer combinations (28) produced polymorphic fragments between the P107 inbred line and Khorasan Lemon native strain. Twenty pairs of AFLP primer combinations for segregation analysis of the F_2 populations based on reproducibility and the degree of polymorphism were selected. Only the polymorphic fragments that segregated in a dominant manner and could be scored unambiguously were used for linkage map construction. An example of AFLP gel electrophoresis and polymorphism screening corresponding to the TP13 primer (Ptat-Ttac) is shown in Figure 1.

Twenty *Pst*I/*Taq*I primer combinations produced a total of 845 clearly detected bands of which 204 qualified polymorphic fragments showing good agreement of 3:1 segregation were analyzed for linkage mapping. The frequency of polymorphic AFLP markers derived from the clearly detected bands of the P107×Khorasan Lemon cross was 24.14% (Table 2). This frequency was close to that (25.7%) obtained in the Dazao×C_{100} silkworm cross (Lu *et al*., 2004) as well as 27.2% in the eastern oyster, *Crassostrea virginica* Gmelin, (Yu and Guo, 2003) but significantly higher than that

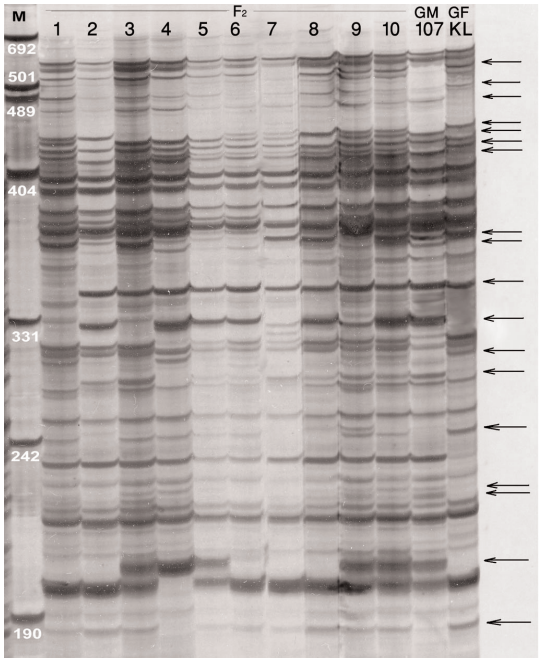

Figure 1. An example of AFLP gel electrophoresis and polymorphism screening correspond to TP13 primer (Ptat- Ttac). GM-107 and GF-KL represent female and male parents, respectively. M stands for standard molecular size marker. Polymorphic bands are shown by arrows.

Table 2. The observed polymorphisms of twenty *Taq*I and *Pst*I primer combinations used in the parents and F_2 population.

Primer name (Primer combination)[a]	Total number of bands	Number of polymorphic bands in parents	Number of polymorphic bands in parents separately		Rate of observed polymorphism (%)
			P107	Khorasan Lemon	
TP1(T34-P22)	43	15	6	9	34.88
TP2(T34-P31)	44	8	3	5	18.18
TP3(T32-P22)	60	14	4	10	23.33
TP4(T31-P22)	41	8	6	2	19.51
TP5(T33-P32)	38	7	4	3	18.42
TP6(T33-P24)	46	9	4	5	19.56
TP7(T32-P34)	42	12	8	4	28.57
TP8(T24-P31)	50	8	5	3	16.00
TP9(T23-P31)	40	8	5	3	20.00
TP10(T22-P31)	50	11	8	3	22.00
TP11(T32-P23)	45	11	5	6	24.44
TP12(T24-P35)	45	7	5	2	15.55
TP13(T35-P35)	53	12	8	4	22.64
TP14(T21-P35)	45	10	3	7	22.22
TP15(T34-P35)	32	11	4	7	34.37
TP16(T33-P35)	42	16	6	10	38.09
TP17(T32-P21)	35	9	2	7	25.71
TP18(T33-P21)	33	9	4	5	27.27
TP19(T34-P33)	32	11	5	6	34.37
TP20(T32-P33)	29	8	5	3	27.58
Total	845	204	100	104	492.69
Average	42.25	10.2	5	5.2	24.63

[a]Primer combinations and sequences shown in Table 1.

(14.0%) in the Proctor×Nudinka cross reported in barley (Castiglioni *et al*., 1998) and also 11.2% in the Guppy fish, *Poecilia reticulata* (Shen *et al*., 2007). However it was dramatically lower than (60.7%) that in the no. 782×od100 cross for the silkworm (Tan *et al*., 2001).

Aproximatly, 104 fragments (51%) of 204 polymorphic fragments were detected in the male parent, Khorasan Lemon strain, and 100 fragments (49%) were observed in the female parent, the P107 inbred line. On average, each primer combination generated 10.2 polymorphic fragments that could be used for linkage mapping. The number of polymorphic bands produced using the 20 primer combinations ranged from 7 bands (18.42% and 15.55%) corresponding to TP5 and TP12, to 16 bands (38.09%) for TP16. The different levels of polymorphism observed for each primer combination are illustrated in Table 2.

The linkage map generated from the P107× Khorasan Lemon cross contained 204 AFLP markers that were assigned to 12 linkage groups at the LOD threshold of 2 (Fig. 2). The largest and the smallest linkage groups belonged to LG10 with 53 markers and LG11 with 2 markers covering 938.4 cM and 12.3 cM of silkworm genome, respectively. The average distance between markers was 20.89 cM. Seven major linkage groups consisting of LG1, LG2, LG3, LG8, LG9, LG10 and LG12 contained 13-53 markers whereas five small linkage groups including LG4, LG5, LG6, LG7 and LG11 had 2-6 markers.

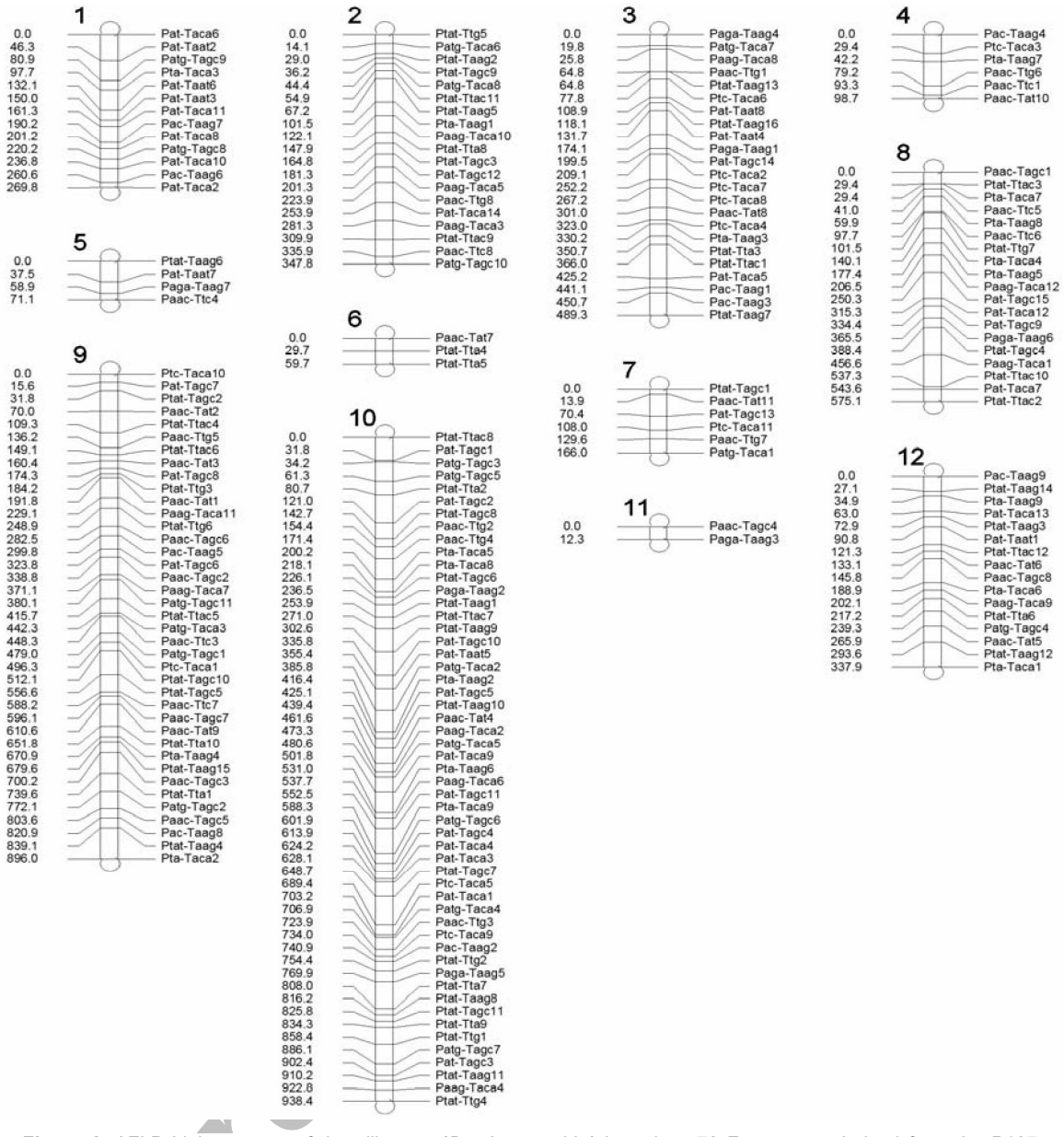

Figure 2. AFLP Linkage map of the silkworm (*Bombyx mori* L.) based on 78 F_2 progeny derived from the P107× Khorasan Lemon cross. The numbers on the left side of each linkage group are genetic distances in Kosambi centiMorgans. AFLP markers are designated by the *Pst*I and *Taq*I primer names on the right side of each linkage group.

DISCUSSION

In present study, we developed an AFLP-based linkage map for the silkworm (*B. mori*) was developed. In the early nineteenth century, the silkworm developed into a model for scientific discovery in microbiology, physiology, and genetics at a period when enormous pattern alterations had effected our perception of biology (Willis *et al*., 1995). Consequently the availability of molecular linkage maps is very valuable in the improvement of research in such disciplines. The map generated in this study consists of 204 AFLP markers. The current map has a total length of 4262 cM and an average marker resolution of 20.89 cM.

Different molecular marker techniques which have their advantages and disadvantages are currently being used to construct genetic linkage maps. Simple sequence Repeats (SSRs) are highly prized as molecular markers due to their codominance and high levels of polymorphism, but a significant effort is required to develop SSR-based maps. The SNP-based genetic markers have attracted significant attention when cre-

Table 3. AFLP markers generated by the same primer combinations mapped to similar positions in the linkage groups of silkworm.

Linkage group	AFLP markers
LG1	[Pat-Taat3 and Pat-Taat6]
LG3	[Ptc-Taca2, Ptc-Taca7 and Ptc-Taca8] [Pac-Taag1 and Pac-Taag3]
LG6	[Ptat-Tta4 and Ptat-Tta5]
LG9	[Ptat-Tagc5 and Ptat-Tagc10]
LG10	[Patg-Tagc3 and Patg-Tagc35] [Paac-Ttg2 and Paac-Ttg4] [Pta-Taca5 and Pta-Taca8] [Pat-Taca3 and Pat-Taca4]

ating dense genetic linkage maps. SNPs are the most abundant class of polymorphisms and they also provide gene-based markers that may prove useful in identifying candidate genes of interest to be associated with quantitative trait loci (Rafalski, 2002). However the main disadvantage of SNPs is the small number of alleles typically present. AFLP markers are easy to use and reveal large sets of genetic loci, but their transferability between detection platforms (for instance, polyacrylamide gel electrophoresis, gel-based sequencers, and capillary sequencers) can sometimes be difficult and cumbersome (Papa *et al*., 2005). AFLP not only has higher reproducibility, resolution, and sensitivity at the whole genome level compared to other techniques, but it also has the capability to amplify between 50 and 100 fragments simultaneously (Vos *et al*., 1995).

In this study, polymorphic AFLP fragments with a clear dominant inheritance pattern were employed to construct a linkage map; that is, the suitable fragments must show complete dominance expression in one parent and complete recessive expression in the other, and all F1 individuals must be heterozygous. Several studies have demonstrated segregation of some AFLP fragments in a codominant manner (Yin *et al*., 2002; Piepho and Koch, 2000; Castiglioni *et al*., 1999). However, it is extremely difficult to identify codominantly segregating fragments from the polyacrylamide amongst several hundred AFLP fragments suggesting the lack of interest in employing the codominant AFLP markers (Zhong *et al*., 2004).

A total of 24.14% of clearly readable and qualified AFLP bands were polymorphic between the P107 inbred line and Khorasan Lemon native strain of the silkworm. A higher level (61%) of polymorphic AFLP marker has been reported by Tan *et al*. (2001) in a single backcross (no. 782 and od100) family of silkworm (Table 2). To explain this approach, they accounted for several factors: First, employing two distinct silkworm strains as in the present study, P107 and Khorasan Lemon are two examples of distinct silkworm inbred lines and strains. The former is from the Japanese bivoltine system and the latter is from the Iranian native monovoltine system. Second, detecting high levels of polymorphisms by the AFLP technique (Wan *et al*., 1999; Huys *et al*., 1996; Latorra and Schanfield, 1996; Mackill *et al*., 1996) and lastly the fact that a large fraction of the silkworm genome consists of families of transposable elements such as *Bm1*, *BMC1* (a member of the *LINE1* a family of transposable elements), *mariner*, *mariner*-like elements (*Bmmar1*), long terminal repeat transposons (LTRs), non-long terminal transposons (nonLTRs) and others (Shimizu *et al.,* 2000; Wang *et al*., 2000; Tomita *et al*., 1997; Robertson and Asplund, 1996; Xiong *et al*., 1993; Xiong and Eickbush, 1993; Herrer and Wang, 1991; Ueda *et al*., 1986). A relatively high level (24.14%) of polymorphic AFLP markers in this study compared to the results obtained in barley (Castiglioni *et al.,* 1998) and Guppy fish (Shen *et al*., 2007) may be due to the previously mentioned three factors.

Among the 20 pairs of AFLP primer combinations applied in this study, an average of 10.2 polymorphic AFLP markers per primer combination for linkage mapping was recognized. This rate was close to that (7.1) of barley (Castiglioni *et al*., 1998) and significantly higher than those obtained by AFLP linkage map studies in tef (*Eragrostis tef* (Zucc.) Trotter) (Bai *et al*., 1999) and red flour beetle (Zhong *et al*., 2004) which produced on average 4.5 and 4.8 polymorphic fragments per primer combination, respectively. However it was considerably lower than two other AFLP linkage and QTL mapping studies on silkworm with 35.7 (Tan *et al*., 2001) and 36.4 (Lu *et al*., 2004) fragments per primer. This may be due to the degree of differences between parental lines and strains and primer combinations.

In this study the total recombination distance over 12 linkage groups was 4262 cM which was longer than previous estimates in silkworm, i.e., 1800 cM for the dense RAPD map (Yasukochi, 1998), 3676.7 cM for the AFLP map in a single backcross family (Lu *et al*., 2004), 3431.9 cM for the SSR linkage map (Miao *et al*., 2005), 1868.10 cM and 2677.50 cM for the AFLP maps in two F_2 subgroups (Sima *et al*., 2006) and 1305 cM for SNP-based linkage map (Yamamoto *et al*.,

2006). However it was shorter than the total length of AFLP linkage map (6512 cM) reported in silkworm (Tan *et al*., 2001). Miao *et al*. (2005) have suggested that although many conditions influence map length, including differences in mating strategy and strains used, the distribution of markers is a possible causative aspect and increased marker density should converge on a more realistic map length value. It has been demonstrated theoretically that, with additional markers typed, the map length may increase when marker density is not saturated or may decrease when marker density is in a saturation state (Tan *et al*., 2001). For instance, Causse *et al*. (1994) have constructed a rice map with 762 markers covering 4026.3 cM, whereas Harushima *et al*. (1998) have obtained a 2275 marker genetic map of rice covering 1521.6 cM. This may explain why the length of the AFLP map of this study is larger than that of the silkworm linkage map studies mentioned above except for Tan *et al.*'s (2001) AFLP map. Considering that the estimated genome size of *B. mori* is 530 Mbp (Gage, 1974), the average physical distance per recombination distance is about 124 kb/cM. It seems that the AFLP markers do not exhibit significant clustering near centromeres or the distal region of chromosomes, suggesting that they provide good coverage of the genome (Zhong *et al*., 2006) (Fig. 2).

Several publications reported that AFLP markers generated from *Eco*RI/*Mse*I restriction enzymes tend to cluster around centromere regions (Haanstra *et al*., 1999; Vuylsteke *et al*., 1999; Young *et al*., 1999; Qi *et al*., 1998). In the present study, strict clustering of AFLP markers generated from *Pst*I/*Taq*I restriction enzymes was not observed. It is important to note that in this investigation, some AFLP markers generated by the same primer combinations have been mapped to similar positions (Table. 3). It has also been reported that some *Eco*RI/*Mse*I based AFLP markers generated by the same primer combinations are mapped to similar positions in the linkage groups of red flour beetle (*T. castaneum*). It may be that these AFLP primer combinations have amplified gene clusters or repeated sequences that are physically linked (Zhong *et al*., 2004).

The AFLP map of this research consisted of 12 linkage groups whereas the haploid genome of the silkworm has 28 chromosomes. As reported in previous studies, it may be due to nonequivalence between the number of linkage groups and the number of chromosomes (He, 1998; Young *et al*., 1998; Promboon *et al*., 1995). In the RFLP based linkage map by Goldsmith (1991), 15 linkage groups were reported. However by using morphological (Doira, 1992), RAPD (Yasukochi 1998), RFLP (Nguu *et al*., 2005), AFLP (Sima *et al*., 2006) and SNP (Yamamoto *et al*., 2006) markers, 28 linkage groups and by using SSR markers (Miao *et al.*, 2005), 29 linkage groups have been recognized in silkworm. It has also been indicated that the large number of chromosomes in the haploid silkworm genome (n=28), typical of lepidoptera, makes it difficult to construct maps without missing some chromosomes (Yasukochi, 1998).

In summary, 204 AFLP markers were employed to construct a linkage map for *B. mori*, with an average marker resolution of 10.2 cM. Since AFLP amplification is highly reproducible, the development of an AFLP linkage map provides an invaluable tool for studying silkworm genetics, such as identification of strain-specific markers for tracking allele frequency changes and QTL analysis for economically important traits.

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References

Bai G, Tefera H, Ayele M, Nguyen HT (1999). A genetic linkage map of tef (*Eragrostis tef* (Zucc.) Trotter) based on amplified fragment length polymorphism. *Theor Appl Genet*. 99: 599-604.

Becker J, Vos P, Kuiper M, Salamini F, Heun M (1995). Combined mapping of AFLP and RFLP markers in barley. *Mol Gen Genet*. 249: 65-73.

Castiglioni P, Pozzi C, Heun M, Terzi V, Mu¨ller KJ, Rohde W, Salamini F (1998). An AFLP-Based Procedure for the Efficient Mapping of Mutations and DNA Probes in Barley. *Genetics* 149: 2039-2056.

Causse MA, Fulton TM, Cho YG, Ahn SN, Chumwongse J, Wu K, Xiao J, Yu Z, Ronald PC, Hanington SE, Second G, McCouch SR, Tanksley SD (1994). Saturated molecular map of the rice genome based on an interspecific backcross population. *Genetics* 138: 1251-1274.

Dalirsefat SB, Mirhoseini SZ (2007). Assessing Genetic diversity

in Iranian native silkworm (*Bombyx mori* L.) strains and Japanese commercial lines using AFLP markers. *Iranian J Biotechnol*. 5: 25-33.

Doira H, Fujii H, Kawaguchi Y, Kihara H, Banno Y (1992). Important Genetic Resources. In: *Genetic stocks and mutations of Bombyx mori*. Isseido Press, Fukuoka, Japan. PP. 73.

Erickson DL, Fenster BC, StenØien HK, Price D (2004). Quantitative trait locus analyses and the study of evolutionary process. *Mol Ecol*. 13: 2505-2522.

Gage LP (1974). The *Bombyx mori* genome: analysis by DNA reassociation kinetics. *Chromosoma* 45: 27-42.

Goldsmith MR (1991). The *Bombyx mori* genome-mapping project. *Sericologia* 31: 145-155.

Goldsmith MR, Shimada T, Abe H (2005). The genetics and genomics of the silkworm, *Bombyx mori*. *Annu Rev Entomol*. 50: 71-100.

Haanstra JPW, Wye C, Verbakel H, Meijer-Dekens F, Van DBP, Odinot P, van Heusden AW, Tanksley S, Lindhout P, Peleman J (1999). An integrated high-density RFLP-AFLP map of tomato based on two *Lycopersicon esculentum* 3 L. pennellii F2 populations. *Theor Appl Genet*. 99: 254-271.

Harushima Y, Yano M, Shomura A, Sato M, Shimano T, Kuboki Y, Yamamoto T, Lin SY, Antonio BA, Parco A, Kajiya H, Huang N, Yamamoto K, Nagamura Y, Kurata N, Khush GS, Sasaki T (1998). A high-density rice genetic linkage map with 2275 markers using a single F2 population. *Genetics* 148: 479-494.

He NJ (1998). Construction of the molecular linkage map of the silkworm (*Bombyx mori* L.). *Ph.D. Thesis*, Southwest Agricultural University, Chongqing, China.

He LJ, Lu C, Li B, Zhou ZY, Xiang ZH (2001). The construction of linkage map of *Bombyx mori* by combination of SADF and RAPD. *Acta Entomologia Sinica* (in Chinese) 44: 476-482.

Herrer RJ, Wang J (1991). Evidence for a relationship between the *Bombyx mori* middle repetitive Bm1 sequence family and U1 snRNA. *Genetica* 84: 31-37.

Huys G, Coopman R, Janssen P, Kersters K (1996). High-resolution genotypic analysis of the genus Aeromonas by AFLP fingerprinting. *Int J Syst Bacteriol*. 46: 572-580.

Kosambi DD (1944). The estimation of map distance from recombination values. *Ann Eugen*. 12: 172-175.

Latorra D, Schanfield MS (1996). Analysis of human specificity in AFLP systems APOB, PAH, and D1S80. *Forensic Sci Int*. 83: 15-25.

Li B, Lu C, Zhou ZY (2000). The Construction of RAPD linkage map of *Bombyx mori*. *Acta Genetica Sinica* (in Chinese) 27: 127-132.

Lu C, Li B, Zhao A, Xiang Z (2004). QTL mapping of economically important traits in Silkworm (*Bombyx mori*). *Sci China C Life Sci*. 47: 477-484.

Mackill DJ, Zhang Z, Redona ED, Colowit PM (1996). Level of polymorphism and genetic mapping of AFLP markers in rice. *Genome* 39: 969-977.

Manly KF, Cudmore JR, Meer JM (2001). Map Manager QTX: cross-platform software for genetic mapping. *Mamm Genome*. 12: 930-932.

Miao XX, Xu SJ, Lia MH, Lia MW, Huang JH, Dai FY, Marino SW, Mills DR, Zeng P, Mita K, Jia SH, Zhang Y, Liu WB, Xiang H, Guo QH, Xu AY, Kong XY, Lin HX, Shi YZ, Lu G, Zhang X, Huang W, Yasukochi Y, Sugasaki T, Shimada T, Nagaraju J, Xiang ZH, Wang SY, Goldsmith MR, Lu C, Zhao GP, Huang YP (2005). Simple sequence repeat-based consensus linkage map of *Bombyx mori*. *Proc Natl Acad Sci USA*. 102: 16303-16308.

Mirhoseini SZ, Dalirsefat SB, Pour Khairandish M (2007). Genetic Characterization of Iranian Native *Bombyx mori* Strains by using Amplified Fragment Length Polymorphism Markers. *J Econ Entomol*. 100: 939-945.

Mita K, Morimyo M, Okano K, Koike Y, Nohata J, *et al*. (2003). The construction of an EST database for *Bombyx mori* and its application. *Proc Natl Acad Soc USA*. 100: 14121-14126.

Mita K, Kasahara M, Sasaki S, Nagayasu Y, Yamada T, *et al*. (2004). The genome sequence of silkworm, *Bombyx mori*. *DNA Res*. 11: 27-35.

Nagaraja GM and Nagaraju J (1995) Genome fingerprinting in silkworm, *Bombyx mori*, using random arbitrary primers. *Electrophoresis* 16: 1633-1638.

Nguu EK, Kadono-Okada K, Mase K, Kosegawa E, Hara W (2005). Molecular linkage map for the silkworm, *Bombyx mori*, based on restriction fragment length polymorphism of cDNA clones. *J Insect Biotechnol Sericol*. 74: 5-13.

Papa R, Troggio M, Ajmone-Marsan P, Nonnis Marzano F (2005). An improved protocol for the production of AFLP™ markers in complex genomes by means of capillary electrophoresis. *J Anim Breed Genet*. 122: 62-68.

Piepho HP, Koch G (2000). Codominant analysis of banding data from a dominant marker system by normal mixtures. *Genetics* 155: 1459-1468.

Promboon A, Shimada T, Fujiwara H and Kobayashi M (1995) Linkage map of RAPDs in the silkworm, *Bombyx mori*. *Genet Res*. 66: 1-7.

Qi X, Stam P, Lindhout P (1998). Use of the locus specific AFLP markers to construct a high density molecular map in barley. *Theor Appl Genet*. 96: 376-384.

Rafalski A (2002). Applications of single nucleotide polymorphisms in crop genetics. *Curr Opin Plant Biol*. 5: 94-100.

Robertson HM, Asplund ML (1996). Bmmar1: a basal lineage of the mariner family of transposable elements in the silkworm moth, *Bombyx mori*. *Insect Biochem Mol Biol*. 26: 945-954.

Shen X, Yang G, Liu Y, Liao M, Wang X, Zhu M, Song W, Zou G, Wei Q, Wang D, Chen D (2007). Construction of genetic linkage maps of guppy (*Poecilia reticulata*) based on AFLP and microsatellite DNA markers. *Agriculture* 271: 178-187.

Shi J, Heckel DG, Goldsmith MR (1995). A genetic linkage map for the domesticated silkworm, *Bombyx mori,* based on restriction fragment length polymorphisms. *Genet Res*. 66: 109-126.

Shimizu K, Kamba M, Sonobe H, Kanda T, Klinakis AG, Savakis C, Tamura T (2000). Extra chromosomal transposition of the transposable element minos occurs in embryos of the silkworm *Bombyx mori*. *Insect Mol Biol*. 9: 277-281.

Sima YH, Li B, Chen DX, Sun DB, Zhao AC, Zhang L, Lu C, He SM, Xiang ZH (2006). Construction and analysis of an AFLP molecular linkage map of the silkworm (*Bombyx mori*). *Chin J Agricu Biotechnol*. 3: 25-31.

Suzuki Y, Gage LP, Brown DD (1972). The genes for fibroin in *Bombyx mori*. *J Mol Biol*. 70: 637-649.

Tamura T, Thibert C, Royer C, Kanda T, Eappen A, Kamba M, Kômoto N, Thomas JL, Mauchamp B, Chavancy G, Shirk P, Fraser M, Prudhomme JC, Couble P (2000). Germline transformation of the silkworm, *Bombyx mori* L. using a piggyBac transposon-derived vector. *Nat Biotechnol*. 18: 81-84.

Tan YD, Wan CL, Zhu YF, Lu C, Xiang Z, Deng HW (2001). An amplified fragment length polymorphism map of the silkworm. *Genetics* 157: 1277-1284.

Tan YD, Ma RL (1998) Estimates of lengths of genome and chromosomes of rice using molecular markers. *J Biomath*. (Chinese) 13: 1022-1027.

Tomita M, Munetsuna H, Sato T, Adachi T, Hino R, Hayashi M, Shimizu K, Nakamura N, Tamura T, Yoshizato K (2003). Transgenic silkworms produce recombinant human type III procollagen in cocoons. *Nat Biotechnol*. 21: 52-56.

Tomita S, Sohn BH and Tamura T (1997). Cloning and characterization of a mariner-like element in the silkworm, *Bombyx mori*. *Genes Genet Syst*. 72: 219-228.

Ueda H, Mizuno S, Shimura K (1986). Transposable genetic element found in the 5'-flanking region of the fibroin H-chain gene in a genomic clone from the silkworm *Bombyx mori*. *J Mol Biol*. 190: 319-327.

Vos P, Hogers R, Bleeker M, Reijans M, Lee TVD, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res*. 23: 4407-4414.

Vuylsteke M, Mank R, Antonise R, Bastiaans E, Senior ML, Stuber CW, Melchinger AE, Luebberstedt T, Xia XC, Stam P, Zabeau M, Kuiper M (1999). Two high-density AFLP linkage maps of Zea mays L.: analysis of distribution of AFLP markers. *Theor Appl Genet*. 99: 921-935.

Wan CL, Zhu YF, Tan YD and Lu C (1999). Application of AFLP markers to detection of genetic polymorphic loci in the silkworm (*Bombyx mori*, L.). *Biotechnology* (Chinese) 9: 4-9.

Wang W, Swevers L and Iatrou K (2000). Mariner (Mos1) transposase and genomic integration of foreign gene sequences in *Bombyx mori* cells. *Insect Mol Biol*. 9: 145-155.

Wang J, Xia Q, He X, Dai M, Ruan J, *et al*. (2005). SilkDB: a knowledge base for silkworm biology and genomics. *Nucleic Acids Res*. 33: 399-402.

Waugh R, Bonar N, Baird E, Thomas B, Graner A, Thos WTB, Powell W (1997). Homology of AFLP products in three mapping populations of barley. *Mol Gen Genet*. 255: 311-321.

Willis JH, Wilkins AS, Goldsmith MR (1995). A brief history of Lepidoptera as model systems. In *Molecular Model Systems in the Lepidoptera*. ed. MR Goldsmith and AS Wilkins, Cambridge/ New York: Cambridge Univ. Press. PP. 1-20.

Xia Q, Zhou Z, Lu C, Cheng D, Dai F, *et al*. (2004). A draft sequence for the genome of the domesticated silkworm (*Bombyx mori*). *Science* 306: 1937-1940.

Xiong Y, Eickbush TH (1993). Dong, a non-long terminal repeat (non-LTR) retrotransposable element from *Bombyx mori*. *Nucleic Acids Res*. 21: 1318.

Xiong Y, Burke WD, Eickbush TH (1993). Pao, a highly divergent retrotransposable element from *Bombyx mori* containing long terminal repeats with tandem copies of the putative R region. *Nucleic Acids Res*. 21: 2117-2123.

Yamamoto K, Narukawa J, Kadono-Okuda K, Nohata J, Sasanuma M, Suetsugu Y, Banno Y, Fujii H, Goldsmith MR, Mita K (2006). Construction of a single nucleotide polymorphism linkage map for the Silkworm, *Bombyx mori*, based on bacterial artificial chromosome end sequences. *Genetics* 173: 151-161.

Yamao M, Katayama N, Nakazawa H, Yamakawa M, Hayashi Y, Hara S, Kamei K, Mori H (1999). Gene targeting in the silkworm by use of a baculovirus. *Genes Dev*. 13: 511-516.

Yasukochi Y (1998). A dense genetic map of the silkworm, *Bombyx mori*, covering all chromosomes based on 1018 molecular markers. *Genetics* 150: 1513-1525.

Yin T, Zhang X, Huang M, Wang M, Zhuge Q, Tu S, Zhu L, Wu R (2002). Molecular linkage maps of the Populus genome. *Genome* 45: 541-555.

Young WP, Wheeler PA, Coryell VH, Keim P, Thorgaard GH (1998). A detailed linkage map of rainbow trout produced using doubled haploids. *Genetics* 148: 839-850.

Young WP, Schupp JM, Keim P (1999). DNA methylation and AFLP marker distribution in the soybean genome. *Theor Appl Genet*. 99: 785-790.

Yu Z, Guo X (2003). Genetic linkage map of the Eastern Oyster *Crassostrea virginica* Gmelin. *Biol Bull*. 204: 327-338.

Zabeau M, Vos P (1992). Selective restriction fragment amplification: A general method for DNA fingerprinting, European Patent Application no. 92402629.7, publication no. 0 534 858 Al.

Zhong D, Menge DM, Temu EA, Chen H, Yan G (2006). Amplified fragment length polymorphism mapping of quantitative trait loci for malaria parasite susceptibility in the yellow fever mosquito *Aedes aegypti*. *Genetics* 173: 1337-1345.

Zhong D, Pai A, Yan G (2004). AFLP-based genetic linkage map for the red flour beetle, *Tribolium castaneum*. *J Hered*. 95: 53-61.