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Microsatellite Analysis of Silkworm Strains (*Bombyx mori*) of Japan Origin Preserved in Korea

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Abstract

In order to understand the diversity and genetic relationships of silkworm strains preserved in Korea, we genotyped 78 *Bombyx mori* strains (Bombycidae: Lepidoptera) originating from Japan, using eight polymorphic microsatellite loci. We obtained per-locus allele numbers ranging from 5 to 16 (with an average value of 9.1), per-locus observed heterozygosity ranging from 0.13 to 1.00, and per-locus polymorphic information content ranging from 0.36 to 0.77, indicating that some loci are highly variable. Phylogenetic analysis with the eight concatenated microsatellite loci showed no clustering based on known strain characteristics and origin. Nineteen strain-specific apomorphic alleles, which discriminated 16 of the 78 silkworm strains, were obtained from eight loci. These strain-specific alleles can thus be utilized for routine discrimination of strains from Japan, without any further typing of other loci. Homozygotes were also observed at some loci (27 of 118 genotypes), which can also be used to discriminate several strains by typing a few loci. These results showed that eight microsatellite loci described herein were sufficiently variable to discriminate among the 78 silkworm strains we examined, and may be useful for future investigations of this economically important species.

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Introduction

The domesticated silkworm is the foundation of the silk industry in many countries, including China, India, and Brazil. More than 1000 inbred lines of the domesticated silkworm *Bombyx mori* are maintained worldwide. In Korea, 340 silkworm strains are preserved by the National Academy of Agricultural Science (NAAS; http://www.genebank.go.kr/), and approximately 78 strains originate from Japan. These strains do not have particular larval skin markings, but some strains show quail marking. Generally, the larvae of the Japanese strains, which are nearly bivoltine, are strong and resistant to adverse environments, but the larval period is comparatively long. Cocoons of these strains are peanut-shaped and frequently white in color (although some are green or yellow), and have a slightly short and thick filament (Lim *et al*., 1996; Table 1).

Microsatellites are simple sequence repeats (SSR) of one to six bases that are abundant in both coding and non-coding regions of all eukaryotic nuclear and some prokaryotic genomes (Tautz and Renz, 1984). Due to the allelic hyper-variability of these markers, conservation in flanking sequence, and co-dominant mode of

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Table 1. General information for the Japan-origin silkworm strains utilized in this study

Strain number	Strain	Origin	Voltinism	Moltinism	Egg color	Blood color	Cocoon color/shape
1	N6	Japan	2	4	B	W	W/Peanut
2	N9	Japan	2	4	B	W	W/Peanut
3	N12	Japan	2	4	B	Y	F/Peanut
4	N13	Japan	2	4	B	W	W/Peanut
5	N15	Japan	2	4	B	W	W/Peanut
6	N18	Japan	2	4	B	W	W/Peanut
7	N19	Japan	2	4	B	W	W/Peanut
8	N24	Japan	2	4	B	Y	Y/Peanut
9	N26	Japan	2	4	B	W	WPeanut
10	N27	Japan	2	4	B	W	W/Peanut
11	N28	Japan	2	4	B	W	W/Long peanut
12	N29	Japan	2	4	B	W	W/ Peanut
13	N32	Japan	2	4	B	W	W/ Peanut
14	N39	Japan	2	4	B	W	W/ Peanut
15	N43	Japan	2	4	W	W	W/ Peanut
16	N44	Japan	2	4	B	W	W/ Peanut
17	N50	Japan	2	4	B	W	W/ Peanut
21	N65	Japan	2	4	B	W	W/ Peanut
22	N69	Japan	2	4	W	W	W/Oval
23	N71	Japan	2	4	W	W	W/Oval
25	N76	Japan	2	4	B	W	W/Oval
26	N80	Japan	2	4	B	W	W/Oval
27	Mudung	Japan	2	4	B	W	W/Oval
28	Moran	Japan	2	4	B	W	W/Long peanut
30	Myohyang	Japan	2	4	B	W	W/ Peanut
31	Bakdu	Japan	2	4	B	W	W/Oval
32	BN	Japan	2	4	B	W	W/Oval
33	Biabkjam	Japan	2	4	B	W	W/ Peanut
34	BakanEB kwainggi	Japan	2	4	B	W	W/Long oval
35	Bukak	Japan	2	4	B	W	W/Long peanut
36	Sulak	Japan	2	4	B	W	W/Oval
37	34	Japan	2	4	B	W	W/Oval
38	SK-1	Japan	2	4	W	W	W/ Peanut
39	4051	Japan	2	4	B	W	W/Oval
42	Woosuk	Japan	2	4	B	W	W/Long peanut
43	Il9ho	Japan	2	4	B	W	W/ Peanut
44	Il83ho	Japan	2	4	B	W	W/ Spindle
47	RHS	Japan	2	4	B	W	W/Peanut
48	W109	Japan	2	4	B	W	W/Peanut

Table 1. Continued

Strain number	Strain	Origin	Voltinism	Moltinism	Egg color	Blood color	Cocoon color/shape
49	W il2	Japan	2	4	B	W	W/Long peanut
51	Jam109	Japan	2	4	B	W	W/Long peanut
52	Jam115	Japan	2	4	B	W	W/Peanut
53	J95	Japan	2	4	B	W	W/Peanut
54	JIN	Japan	2	4	B	W	W/Long peanut
56	Hwangyu	Japan	2	4	B	W	W/Peanut
161	Chunwa	Japan	2	4	B	W	LYG/Peanut
169	N30	Japan	2	4	B	Y	Y/Peanut
172	Suwon 10ho	Japan	2	4	B	W	W/Peanut
173	11	Japan	2	4	B	W	W/Peanut
174	Il 111	Japan	2	4	B	Y	Y/Oval
175	JF	Japan	2	4	B	W	W/Oval
176	Jam 107	Japan	2	4	B	W	W/Peanut
185	MD	Japan	2	4	B	W	W/Peanut
190	N20	Japan	2	4	B	W	W/Peanut
194	Kicho N27	Japan	2	4	B	W	W/Peanut
197	Kumkwangju	Japan	2	4	B	Y	Y/Long peanut
198	Hansang 3ho	Japan	2	4	B	W	W/Peanut
211	J137	Japan	2	4	B	W	W/Long peanut
232	Suwonjam 101	Japan	2	4	B	W	W/Peanut
234	Jam 105	Japan	unknown	4	B	W	W/Long peanut
240	Il 119	Japan	2	4	B	W	W/Peanut
249	Jam 117	Japan	2	4	B	W	W/Long oval
256	JP 1	Japan	2	4	B	W	W/Oval
276	Jam 121	Japan	2	4	B	W	W/Peanut
277	Jam 127	Japan	2	4	B	W	W/Peanut
278	Jam 129	Japan	2	4	B	W	W/Peanut
279	Jam 131	Japan	2	4	B	W	W/Peanut
280	Jam 135	Japan	2	4	B	W	W/Peanut
287	NB 18	Japan	2	4	B	W	W/Peanut
304	Jam 113	Japan	2	4	B	W	-
327	zepere or T(T:3) zeprre.tecter	Japan	unknown	4	W	W	W/Oval
328	X pe+	Japan	unknown	4	W	W	W/ Peanut
329	spil	Japan	unknown	4	B	W	LPG/Spindle
330	Artificial diet line	Japan	unknown	4	B	W	W/Oval
331	Ine	Japan	unknown	4	W	W	W/Short peanut
332	Xze	Japan	unknown	4	B	W	W/ Peanut
337	Jam 303	Japan	2	4	B	W	W/ Peanut
339	Jam 147	Japan	2	4	B	W	W/ Peanut

B, black; W, white; Y, yellow; LYG, light yellow green; LPG, light pale green, and -, no rigid cocoon shape.

Table 2. Information of the 8 microsatellite loci analyzed in 78 Japan-origin silkworm strains

Primer name		Primer sequence (5-3)[a]	Motifs[b]	Tem. (°C)[c]	Expected size (bp)	Size range (bp)	GenBank no.	References
K02	F	attgtaaccgatttgagaga	$(ga)_{23}$	46	107-122	105-121	DE376976	Unpublished
	R	attcgcacaataagttcact						
D49370	F	agcgcacccttatgacgat	$(cta)_2$-$(cta)_5$	48	220	202-235	D49370	Kawabata et al. (1995)
	R	gaaagtggaagtcgtgtact						
D90454	F	tgcgatgtctacatggtgg	$(ggc)_6$	54	183	181-184	D90454	Itoh et al. (1991)
	R	gtctctcgatagcttcct						
Bmsat127	F	aggcttagtgacgagccgt	$(tttga)_4$	50	193	180-193	DQ383507	Reddy et al. (1999)
	R	ggtgccaatcattcttatt						
D49948	F	acgcagacgagaagctcac	$(ca)_3$-$(ca)_5$	48	200	206-240	D49948	Ohta et al. (1995)
	R	actgcaccgtatatgcga						
Bmsat129	F	agttacccaggttgtctt	$(at)_{27}$	50	198	170-208	DQ383509	Reddy et al. (1999)
	R	cgacactggttctcatac						
X17219	F	gcaagccaccagttagatatgg	$(at)_9$	68	214	210-220	X17219	Michaille et al. (1990)
	R	cacgtacgttgcgttcaccg						
AF005384	F	caagatgtccaagagtg	$(gt)_2$-$(gtt)_4$-$(gc)_2$	46	225	177-181	AF005384	Unpublished
	R	ccggtgagagggacctt						

[a]Forward (F) and reverse (R) primer sequences for PCR. The forward primer was labeled with a fluorescent dye for genotyping.
[b]Sequences inside parenthesis indicate the motif sequence of the microsatellite DNA and subscripted numbers indicate number of repeats. originally found at the microsatellite locus.
[c]Tem., annealing temperature for PCR.

inheritance and recent advances in PCR technology, microsatellites are useful markers in several fields of sciences where detection of fine-scale genetic structure is required (Weber and May, 1989; Meglécz *et al*., 2007; Li *et al*., 2002).

Numerous microsatellite loci have been identified and described in the silkworm (e.g., Dharma Prasad *et al*., 2005; Li *et al*., 2005). Previously, Kim *et al*. (2010) genotyped 54 silkworm strains preserved in Korea using nine polymorphic microsatellite loci, and described the importance of these markers for strain discrimination. Subsequently, Kim *et al*. (2012) genotyped 85 silkworm strains originating from China (but preserved in Korea) using eight polymorphic microsatellite loci .

In this study, we selected eight microsatellite markers that were used in previous studies (Kim *et al*., 2010; 2012), and genotyped 78 silkworm strains of Japanese origin that were preserved in Korea. The goal of this investigation was to determine the utility of the markers in detecting DNA polymorphism, and to assess their potential for use in strain discrimination.

Materials and Methods

Silkworm strains

Seventy-eight *B. mori* silkworm strains originating from Japan were analyzed for this study. The voltanism, moltinism, egg color, blood color, and cocoon color/shape are presented in Table 1. These strains are under preservation at NAAS, Republic of Korea.

Genomic DNA extraction, PCR amplification, and genotyping

Approximately 100 eggs of each strain were crushed in a glass grinder in liquid nitrogen, and genomic DNA was extracted using the DNA Extraction Kit, in accordance with the manufacturer's instruction (Qiagen, USA). Eight microsatellite loci were successfully amplified using primers previously designed using

published and unpublished *B. mori* genomic DNA sequences (Table 2). In order to verify the presence of simple sequence repeats in the microsatellite loci, PCR amplicons of each locus from one or two strains were cloned and sequenced. Characteristics of the eight microsatellite loci, including the primer sequences, are presented in Table 2.

PCR was carried out in a 25 μL reaction volume containing ~30 ng of genomic DNA, 200 nM of each reverse and forward primer, 200 μM of each dNTP, 2.5 μL of 10× PCR buffer [50 mM KCl, 10 mM Tris-HCl (pH 8.8), 150 nM KCl, 1.5 mM $MgCl_2$], and 1 unit of FR-*Taq* DNA polymerase (Biomedic, Korea), using an ABI 2720 Thermal Cycler (Applied Biosystems, USA). The PCR cycling conditions were as follows: initial denaturation at 95°C for 3 min, followed by 30 cycles of 94°C for 30 s, annealing at 46°C~68°C for 30 s, extension at 72°C for 1 min, and a final extension step at 72°C for 6 min. Forward primers were labeled with 6-FAM fluorescent dye (Yue *et al*., 2000) for subsequent peak detection using fluorescence-based DNA electrophoresis. To verify successful DNA amplification, samples were electrophoresed through a 1.4% agarose gel for 1 h. For size analysis, 0.2 μL of PCR product was mixed with 9.8 μL of Hi-Di Formamide (Applied Biosystems) and 0.2 μL of LIZ-500 size standard (Applied Biosystems). The samples were then denatured at 95°C for 5 min, stored on ice, and analyzed using an ABI 3730xl DNA Analyzer (capillary sequencer, Applied Biosystems, USA). GeneMapper® version 4.0 (Applied Biosystems) was used to determine allele sizes. To verify the accuracy of size determination, electrophoresis was carried out a minimum of three times, generally using two independent PCR products.

Analysis of variation and phylogenetic tree construction

Observed heterozygosity (H_o; Weir, 1996), expected heterozygosity (E_o; Nei, 1987), Polymorphic Information Content (PIC; Bostein *et al*., 1980), and the allelic and genotypic frequencies at each locus were calculated using PowerMarker ver. 3.25 (Liu and Muse, 2005). Allelic PIC was calculated using the following formula: $PIC = 1- \sum (P_i)^2$, where P_i is the proportion of the strain carrying i^{th} allele, calculated for each microsatellite locus (Bostein *et al*., 1980). The relationships among silkworm strains were inferred using Neighbor-Joining (NJ) analysis of the distance matrix based on the shared allelic methods (Jin and Charkaborty, 1993); these analyses were also carried out using PowerMarker ver. 3.25 (Liu and Muse, 2005).

Results and Discussion

Characteristics of alleles

Fragment analysis of the 78 silkworm stains in eight microsatellite loci were largely successful; however, analysis of one strain at locus D49370, five strains at locus Bmsat127, two strains at locus D49948, and four strains at locus Bmsat129, one strain at locus X17219, and one strain at locus AF005384 were not successful, either due to amplification failure or multiple amplification. Thus, 76 of the 78 strains were genotyped on average at each locus (Table 3). It is likely that the unsuccessful amplification was due to null alleles, in which a mutation occurs within the primer region, resulting in a failure to amplify the complete product, or reduction of the allele number to a single allele (Kwok *et al*., 1990).

We detected 73 alleles at eight loci, and the average allele number at each locus was 9.1, ranging in number from five (locus D90454) to 16 (locus Bmsat129) (Table 4). Although the dinucleotide repeat locus Bmsat129 (Reddy *et al*., 1999) provided the highest allele number, the dinucleotide repeat loci K02 and X17219 (Unpublished, GenBank accession number DE376976; Michaille *et al*., 1990) provided only seven alleles . Thus, the allele number at each locus is unlikely to have a direct relationship with the length of the repeat motif. Previous analysis of silkworm strains from China showed that the loci Bmsat129 and AF005384 exhibited the highest allele numbers, which were as high as 14 (Kim *et al*., 2012); however, the strains we examined from Japan only exhibited eight alleles at the locus AF005384, thus indicating somewhat different alleles frequencies between the strains from China and Japan. The trinucleotide repeat locus D90454 (Itoh *et al*., 1991) exhibited the lowest number of alleles, only five, which is consistent with the previous study of silkworm strains from China (Kim *et al*., 2012).

Heterozygosity

In accordance with the allelic diversity, the number of genotypes was generally proportional to the allele number (Table 4). For

Table 3. Genotypes of 78 Japan-origin silkworm strains at each microsatellite locus

Strain number	Strain	Microsatellite locus (bp)															
		K02		D49370		D90454		Bmsat127		D49948		Bmsat129		X17219		AF005384	
1	N6	104	104	202	251	180	180	182	190	199	265	171	177	215	223	174	218
2	N9	110	110	215	257	177	180	190	190	199	265	169	169	-	-	174	174
3	N12	115	115	257	257	180	180	184	190	199	265	171	171	215	223	174	218
4	N13	115	115	215	257	180	180	184	190	199	239	169	169	217	223	174	218
5	N15	110	115	215	257	180	180	184	190	199	265	169	169	215	223	174	218
6	N18	115	115	257	257	180	180	184	190	199	199	169	169	215	223	174	218
7	N19	104	115	251	251	177	180	184	184	199	199	169	195	217	223	188	246
8	N24	104	104	251	251	180	180	160	188	199	199	171	175	205	215	188	246
9	N26	110	110	251	251	180	180	190	190	199	243	169	169	215	223	218	218
10	N27	104	115	251	251	183	183	192	192	199	265	171	177	215	223	174	218
11	N28	127	127	257	257	180	180	190	190	199	265	169	169	217	221	174	186
12	N29	104	115	257	257	180	180	182	190	199	199	169	169	217	223	174	218
13	N32	115	115	191	250	180	180	190	190	199	265	171	175	205	217	174	186
14	N39	115	115	215	251	180	180	190	190	199	265	171	177	215	223	174	218
15	N43	115	115	257	257	180	180	182	184	199	265	169	169	217	223	174	218
16	N44	115	115	192	257	180	180	190	190	199	243	171	177	217	223	174	188
17	N50	104	104	257	257	180	180	190	190	199	265	171	177	217	219	188	188
21	N65	104	104	202	257	177	180	190	190	199	265	-	-	215	223	174	218
22	N69	115	115	215	251	177	180	190	190	199	243	-	-	215	223	174	218
23	N71	104	104	202	251	180	180	190	190	199	265	169	187	205	217	174	188
25	N76	104	104	251	251	177	180	190	190	199	265	171	175	215	223	174	174
26	N80	104	115	215	257	177	180	190	190	199	265	165	169	215	221	218	218
27	Mudung	115	115	202	251	177	180	-	-	201	265	173	177	215	223	174	218
28	Moran	115	115	215	257	177	180	190	190	201	243	163	169	217	221	174	218
30	Myohyang	110	110	257	257	177	180	182	190	199	265	163	169	215	221	218	218
31	Bakdu	104	104	202	251	177	180	-	-	199	199	171	177	215	223	188	246
32	BN	120	120	251	257	180	180	190	190	199	199	-	-	217	223	246	268
33	Biabkjam	104	104	202	215	177	180	190	190	199	199	169	169	215	223	174	218
34	BakanEB kwainggi	104	104	215	257	177	180	190	190	199	199	171	171	215	221	218	218
35	Bukak	115	115	215	257	177	180	190	190	199	265	-	-	205	223	172	172
36	Sulak	104	104	251	251	177	180	190	190	199	265	171	177	207	219	188	246
37	34	104	104	202	257	180	180	190	190	199	239	169	177	205	223	174	218
38	SK-1	113	113	202	257	177	180	190	190	199	265	153	153	217	223	188	188
39	4051	104	104	202	251	177	180	190	190	191	199	169	169	215	219	188	246
42	Woosuk	110	110	215	257	177	180	190	190	199	221	171	177	217	223	188	188
43	Il9ho	110	110	202	251	177	180	190	190	191	199	169	169	215	223	188	268
44	Il83ho	104	104	202	257	177	180	160	188	199	199	171	177	215	219	218	218
47	RHS	115	115	202	251	177	180	190	190	199	265	153	171	215	219	188	268
48	W109	115	115	215	251	177	180	190	190	196	265	151	171	215	219	174	218

Table 3. Continued

Strain number	Strain	Microsatellite locus (bp)															
		K02		D49370		D90454		Bmsat127		D49948		Bmsat129		X17219		AF005384	
49	W il2	104	104	257	257	177	180	-	-	199	199	153	177	217	223	172	218
51	Jam109	115	115	202	251	177	180	190	190	199	265	171	177	217	223	174	218
52	Jam115	115	115	202	251	177	180	190	190	199	209	171	177	217	223	174	218
53	J95	104	104	257	257	177	180	190	190	199	265	169	171	215	223	174	218
54	JIN	104	110	202	257	177	180	190	190	-	-	169	169	215	221	174	218
56	Hwangyu	115	115	257	257	177	180	188	188	199	199	153	169	217	223	188	246
161	Chunwa	115	120	251	257	177	180	190	190	199	199	171	175	215	219	174	218
169	N30	115	115	215	251	177	180	-	-	191	199	171	177	207	217	174	218
172	Suwon 10ho	115	115	251	251	177	180	190	190	199	265	171	175	215	223	174	218
173	11	104	104	215	257	180	180	190	190	199	221	169	193	215	223	188	188
174	Il 111	104	104	251	251	177	180	190	190	199	199	177	179	215	223	-	-
175	JF	104	104	199	251	177	180	190	190	199	199	171	175	215	221	188	188
176	Jam 107	115	115	215	250	177	180	190	190	-	-	169	169	217	223	174	218
185	MD	115	115	251	257	177	180	190	190	199	265	169	171	217	223	174	218
190	N20	106	127	215	257	180	180	160	190	199	265	153	153	215	223	218	218
194	Kicho N27	104	104	257	257	174	180	190	190	199	199	169	169	215	219	186	246
197	Kumkwangju	113	113	215	257	180	180	228	228	199	265	169	169	217	223	218	218
198	Hansang 3ho	115	115	215	251	177	180	190	190	199	265	171	177	215	223	174	218
211	J137	104	115	202	224	177	180	190	190	199	243	169	169	215	223	188	218
232	Suwonjam 101	115	115	214	251	180	180	190	190	199	265	169	169	217	223	174	174
234	Jam 105	115	115	251	251	180	180	182	190	199	199	169	169	215	223	174	218
240	Il 119	115	115	-	-	180	180	190	190	199	239	171	177	215	223	174	174
249	Jam 117	115	115	202	251	177	180	182	190	199	265	171	177	217	223	174	218
256	JP 1	115	115	202	257	177	180	-	-	199	199	173	173	205	217	188	188
276	Jam 121	115	115	251	251	180	180	190	190	191	199	171	177	215	219	174	218
277	Jam 127	115	115	251	251	180	180	190	190	199	265	171	177	217	223	174	218
278	Jam 129	115	115	251	251	180	180	184	190	199	265	171	177	217	223	174	218
279	Jam 131	115	115	251	251	180	180	190	190	199	265	171	177	217	223	174	218
280	Jam 135	104	104	251	251	180	180	190	190	199	199	171	177	217	223	218	218
287	NB 18	106	115	251	251	177	180	190	190	199	239	171	175	215	223	174	218
304	Jam 113	115	115	257	257	180	180	190	190	199	199	169	169	217	223	174	218
327	Zepere*	104	104	257	257	171	180	184	190	199	235	187	187	205	223	218	255
328	X pe+	104	104	191	257	177	177	184	190	199	209	173	187	205	215	218	218
329	Spil	104	104	251	251	177	177	184	192	199	266	167	173	207	217	218	218
330	Artificial diet line	110	110	251	251	180	180	182	190	199	199	183	185	215	223	188	246
331	Lne	104	104	257	257	180	180	190	190	191	199	173	187	215	223	246	255
332	X^{ze}	104	104	251	251	180	180	186	186	199	265	173	173	215	217	218	255
337	Jam 303	115	115	251	251	180	180	190	190	199	265	171	177	217	223	174	218
339	Jam 147	115	115	251	251	180	180	190	190	199	266	171	177	217	223	174	218

* Zepere is also named as T (T:3) zeprre. Tecter.

Table 4. Summary statistics of the 8 microsatellite loci

Locus	Sample size	Availability[a]	Major allele frequency[b]	Genotype number	Allele number	*He*[c]	*Ho*[d]	PIC[e]
K02	78	1.00	0.47	12	7	0.64	0.13	0.57
D49370	77	0.99	0.39	15	10	0.71	0.56	0.67
D90454	78	1.00	0.70	6	5	0.44	0.53	0.36
Bmsat127	73	0.94	0.78	12	8	0.38	0.25	0.37
D49948	76	0.97	0.60	13	12	0.58	0.73	0.54
Bmsat129	74	0.95	0.32	23	16	0.79	0.65	0.77
X17219	77	0.99	0.34	12	7	0.76	1.00	0.72
AF005384	77	0.99	0.39	15	8	0.72	0.73	0.68
Mean	76	0.98	0.50	13.5	9.1	0.63	0.57	0.58

[a]Availability is defined as 1-*Obs*/*n*, where *Obs* is the number of observations and n is the number of individuals sampled (85 individuals).
[b]Major allele frequency indicate the sum of allele frequency with the most higher frequency.
[c]Expected heterozygosity (*He*) is defined as the probability that two randomly chosen alleles from the strains are different.
[d]Observed heterozygosity is simple the proportion of heterozygous individuals in the strains.
[e]PIC, polymorphic information contents.

example, the locus D90454, which exhibited only five alleles, resulted in only six genotypes in 78 strains, whereas the locus Bmsat129, which exhibited 16 alleles, resulted in 23 genotypes in 74 strains (Table 3). The frequency of the most common allele at each locus ranged from 0.32 (Bmsat129) to 0.78 (Bmsat127) (Table 4). Thus, some loci exhibited particular alleles at very high frequencies, but others did not. For example, allele 180 found at locus D90454 occurred in 75 of the 78 strains, either as a homozygote or heterozygote, but allele 177 was observed along with allele 180 in 42 strains, and mostly as a heterozygote,. The remaining four alleles, 171, 174, 177, and 183 were found along with allele 180 in only one strain, and either as a heterozygote or homozygote (Table 3). This pattern is identical to that observed in the strains from China, where the same loci were genotypes (Kim *et al*., 2012).

The expected (H_e) and the observed heterozygosity (H_o) over all microsatellite loci ranged from 0.38 to 0.79 and from 0.13 to 1.00, respectively (Table 4). The loci D90454, D49948, X17219, Bmsat129, and AF005384 exhibited somewhat higher or equivalent estimates of H_o versus H_e, but the remaining loci K02 (0.64 vs. 0.13), D49370 (0.71 vs. 0.56), Bmsat127 (0.38 vs. 0.25), and Bmsat129 (0.79 vs. 0.65) exhibited substantially lower estimates of H_o, potentially indicating inbreeding in these strains. Silkworm strains from China showed a similar pattern, in which the loci D90454, D49948, Bmsat129, and X17219 exhibited somewhat higher or equivalent estimates of H_o versus H_e. However, the remaining loci K02 (0.37 vs. 0.07), D49370 (0.56 vs. 0.45), Bmsat127 (0.35 vs. 0.17), and AF005384 (0.84 vs. 0.56) exhibited substantially lower estimates of H_o. Strains from Japan generally showed higher H_o than the strains from China, indicating that the latter has higher genetic diversity than the former . The PIC value of the Japanese strains we examined ranged from 0.36 to 0.77, with an average of 0.58 per locus (Table 4). The locus Bmsat129, which exhibited the high number of alleles, also exhibited the highest PIC value of 0.77, and K02, D49370, D49948, Bmsat129, X17219, and AF005384 all exhibited PIC values higher than 0.50, indicating the high power of these loci to discriminate among strains. The previous analysis of strains from China reported PIC values ranging from 0.34 to 0.82, with an average of 0.54 per locus (Kim *et al*., 2012). Furthermore, Kim *et al*. (2010) reported that 54 silkworm strains originating from several countries exhibited a mean PIC value of 0.47 over all loci (nine loci, including the eight loci used in this study). The PIC values at each locus ranged from 0.06 to 0.86, which is slightly higher than the values we calculated for the strains from Japan.

Relationships among silkworm strains

Phylogenetic analysis failed to detect any clear grouping among strains based on known characteristics, such as voltinism, moltinism, egg color, blood color, and cocoon color/shape (Fig. 1). For example, JP1, Hwangyu, N19, W il2, BN, Woosuk, SK-1, and 11 formed a

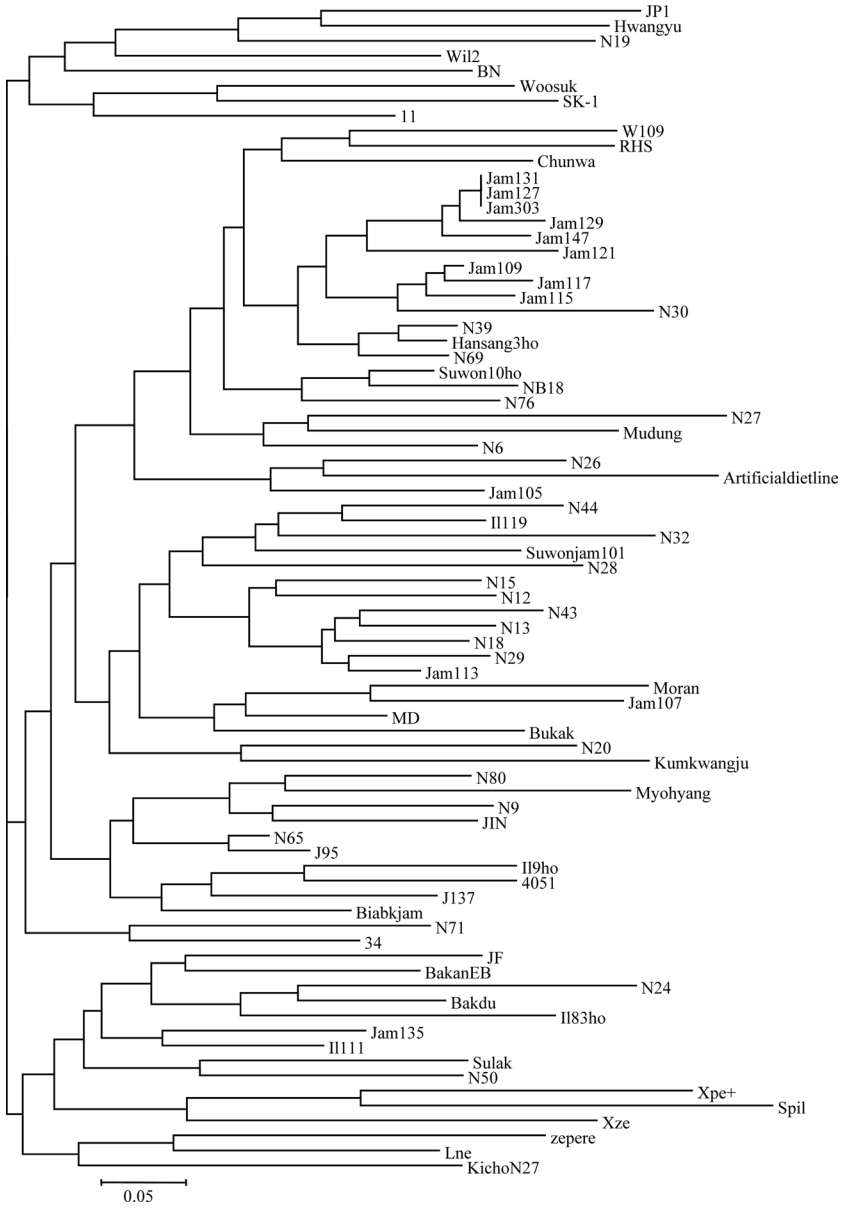

Fig. 1. Phylogenetic tree constructed using Neighbor-Joining analysis that illustrates the genetic relationships among 78 silkworm strains originating from Japan. The genetic distances between strains were calculated based on the shared allelic methods (Jin and Chakraborty, 1994) using the PowerMarker v3.25 program. The scale bar indicates the branch length.

clade in the NJ tree; although these strains share characteristics such as bi-voltinism, tetra-moltinism, white-colored blood, and white-colored cocoon, the egg color is either brown (most strains) or white (SK-1). Further, the cocoon shape is either peanut shaped (N19, SK-1, Hwangyu, and 11), long and peanut-shaped (Woosuk and W il2), or oval (JP1), indicating no known character-based grouping. Similar examples can be found in many other branches of the tree. Instead, the clustering pattern indicates that the microsatellite loci typed in this study may reflect genetic differences among strains that can be utilized for the discrimination of silkworm strains. Previous studies of silkworm strains originating from China also support this notion (Kim *et al*., 2010, 2012).

Strain discrimination

The eight microsatellite loci exhibited the presence of strain-specific alleles (Table 3). Locus D49370 provided the alleles 192, 199, 214, and 224, which were unique to strains N44 (no. 16), JF (no.

Table 5. Genotype frequency in each microsatellite marker among 78 silkworm strains

Locus	Allele 1	Allele 2	Number of strains	Frequency
K02	104	104	25	0.321
	104	110	1	0.013
	104	115	5	0.064
	106	115	1	0.013
	106	127	1	0.013
	110	110	6	0.077
	110	115	1	0.013
	113	113	2	0.026
	115	115	33	0.423
	115	120	1	0.013
	120	120	1	0.013
	127	127	1	0.013
D49370	191	250	1	0.013
	191	257	1	0.013
	192	257	1	0.013
	199	251	1	0.013
	202	215	1	0.013
	202	224	1	0.013
	202	251	10	0.130
	202	257	6	0.078
	214	251	1	0.013
	215	250	1	0.013
	215	251	5	0.065
	215	257	11	0.143
	251	251	20	0.260
	251	257	3	0.039
	257	257	14	0.182
D90454	171	180	1	0.013
	174	180	1	0.013
	177	177	2	0.026
	177	180	39	0.500
	180	180	34	0.436
	183	183	1	0.013
Bmsat127	160	188	2	0.027
	160	190	1	0.014
	182	184	1	0.014
	182	190	6	0.082
	184	184	1	0.014
	184	190	7	0.096
	184	192	1	0.014
	186	186	1	0.014
	188	188	1	0.014
	190	190	50	0.685
	192	192	1	0.014
	228	228	1	0.014

Table 5. Continued

Locus	Allele 1	Allele 2	Number of strains	Frequency
D49948	191	199	5	0.066
	196	265	1	0.013
	199	199	20	0.263
	199	209	2	0.026
	199	221	2	0.026
	199	235	1	0.013
	199	239	4	0.053
	199	243	4	0.053
	199	265	33	0.434
	199	266	2	0.026
	201	243	1	0.013
	201	265	1	0.013
Bmsat129	151	171	1	0.014
	153	153	2	0.027
	153	169	1	0.014
	153	171	1	0.014
	153	177	1	0.014
	163	169	2	0.027
	165	169	1	0.014
	167	173	1	0.014
	169	169	19	0.257
	169	171	2	0.027
	169	177	1	0.014
	169	187	1	0.014
	169	193	1	0.014
	169	195	1	0.014
	171	171	2	0.027
	171	175	7	0.095
	171	177	22	0.297
	173	173	2	0.027
	173	177	1	0.014
	173	187	2	0.027
	177	179	1	0.014
	183	185	1	0.014
	187	187	1	0.014
X17219	205	215	2	0.026
	205	217	3	0.039
	205	223	3	0.039
	207	217	2	0.026
	207	219	1	0.013
	215	217	1	0.013
	215	219	7	0.091
	215	221	5	0.065
	215	223	26	0.338
	217	219	1	0.013
	217	221	2	0.026
	217	223	24	0.312

Table 5. Continued

Locus	Allele 1	Allele 2	Number of strains	Frequency
	172	172	1	0.013
	172	218	1	0.013
	174	174	4	0.052
	174	186	2	0.026
	174	188	2	0.026
	174	218	36	0.468
	186	246	1	0.013
AF005384	188	188	6	0.078
	188	218	1	0.013
	188	246	7	0.091
	188	268	2	0.026
	218	218	10	0.130
	218	255	2	0.026
	246	255	1	0.013
	246	268	1	0.013

175), Suwonjam (no. 232), and J137 (no. 211), respectively (Table 3). Similarly, the locus D90454 exhibited alleles 171, 174, and 183, which were unique to strains zepere (no. 327), Kicho N27 (no. 194), and N27 (no. 10), respectively. The locus Bmsat127 exhibited alleles 186 and 228, unique to strains Xze (no. 332) and Kumkwangju (no. 197), respectively. Locus D49948 exhibited alleles 196 and 235, unique to strains W109 (no. 48) and Zepere (no. 327), respectively. Locus Bmsat129 exhibited alleles 151, 165, 167, 183, 193, 195, 179, and 185, unique to strains W109 (no. 48), N80 (no. 26), Spil (no. 329), Artificial diet line (no. 330), 11 (no. 173), N19 (no. 7), Il 111 (no. 174), and Artificial diet line (no. 330), respectively (Table 3). In total, 19 apomorphic alleles were observed, which discriminated 16 of the 78 silkworm strains. These strain-specific alleles can thus be utilized for the discrimination of *B. mori* strains without further typing of other loci.

Genotyping results showed that a substantial number of strains possessed homozygotic alleles (Table 5). At locus K02, 25 strains were homozygous for allele 104, six strains were homozygous for allele 110, two strains were homozygous for allele 113, 33 strains were homozygous for allele 115, one strain was homozygous for allele 120, and one strain was homozygous for allele 127 (Table 5). Similarly, 34 strains were homozygous at two alleles for locus D49370, 37 strains were homozygous at three alleles at locus D90454, 55 strains were homozygous at six alleles at locus Bmsat127, 20 strains were homozygous at one allele at locus D49948, 26 strains were homozygous at five alleles at locus Bmsat129, and 21 strains were homozygous at four alleles at locus AF005384 (Table 5). Consequently, 27 of the 118 genotypes were homozygous at one or more of the eight loci. Because we used ~100 eggs for the extraction of DNA from each *B. mori* strain, no hidden alleles are expected within the strains. Therefore, this particular combination of microsatellite loci effectively discriminated among silkworm strains from Japan. For other strains, typing of all eight loci is required for the discrimination of strains originating from Japan.

Previously, 85 silkworm strains originating from China were genotyped using the same microsatellite loci, and exhibited 22 strain-specific apomorphic alleles, which discriminated 19 of the 85 strains. Furthermore, a substantial number of homozygous strains were observed. The previous and current results support the continued use of these microsatellite loci for the discrimination of silkworm strains originating from China and Japan that are under preservation in Korea. Nevertheless, the isolation of additional microsatellite loci is needed to discriminate among the >300 silkworm strains that are currently preserved in Korea.

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