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Comparative Genetic Diversity and Genetic Structure of Three Chinese Silkworm Species *Bombyx mori* L. (Lepidoptera: Bombycidae), *Antheraea pernyi* Guérin-Meneville and *Samia cynthia ricini* Donovan (Lepidoptera: Saturniidae)

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ABSTRACT - The genetic diversity and genetic structure of three Chinese silkworm species *Bombyx mori* L., *Antheraea pernyi* Guérin-Meneville and *Samia cynthia ricini* Donovan were comparatively assessed based on RAPD markers. At the species level, *A. pernyi* and *B. mori* showed high levels of genetic diversity, whereas *S. cynthia ricini* showed low level of genetic diversity. However, at the strain level, *A. pernyi* had relatively highest genetic diversity and *B. mori* had lowest genetic diversity. Analysis of molecular variance (AMOVA) suggested that 60% and 72% of genetic variation resided within strains in *A. pernyi* and *S. cynthia ricini*, respectively, whereas only 16% of genetic variation occurred within strains in *B. mori*. In UPGMA dendrogram, individuals of *A. pernyi* and *B. mori* formed the strain-specific genetic clades, whereas those of *S. cynthia ricini* were distributed in a mixed way. The implications of these results for the conservation and utilization in breeding programs of three silkworm species are discussed.

KEY WORDS: RAPD, genetic variation, AMOVA, dendrogram

Most silkmoths belong to two families of Lepidoptera, Bombycidae and Saturniidae, which secrete several varieties of silk fibers. They include the domesticated silkworm (*Bombyx mori* L.) and the wild silkworms, the Chinese oak silkworm (*Antheraea pernyi* Guérin-Meneville), the Indian tropical tasar silkworm (*Antheraea mylitta* Drury), the Japanese oak silkworm (*Antheraea yamamai* Guérin-Meneville), and the eri silkworm (*Samia cynthia ricini* Donovan). Silk production based on these moths, especially *B. mori*, *A. pernyi* and *S. cynthia ricini*, plays an economically important role in the world. Developing nations like China, India, Brazil, and some of the Latin American countries have taken up sericulture to provide employment to the people in rural area, since it involves intensive labor and is key to locally improve life quality.

The domesticated silkworm *B. mori* has been used for silk production by Chinese farmers for about 5000 years (Xiang *et al* 2005). It was progressively spread to Korea, Japan, India, Brazil, and the rest of the world. Today, it is one of the most economically important, beneficial insect exploited in many developing countries. In the long history of domestication, more than 3000 strains have been developed and maintained. Broadly, four geographical races are identified: Chinese, Japanese, European and Tropical. Usually, the species needs 20-26 days to complete its larval development. Through intense selection, *B. mori* has become a truly domesticated insect whose survival and reproduction strictly depends on humans.

The Chinese oak silkworm *A. pernyi* is the most well-known species among wild silkmoths, and is commercially cultivated for silk production mainly in China, India and Korea. According to historic records, domesticated Chinese oak silkworm originated in the Province of Shandong, China about 400 years ago (Liu *et al* 2010). It is currently used mostly as a source of insect food (larva, pupa and moth) and for cosmetics. There are more than one hundred strains in China, which are divided into four races based on the larval skin color: yellow, blue, white, and yellow-cyan. In 2005, about seven million kilograms of cocoons (pupae) were produced in China, accounting for 90% of world production. The worms usually take 45-50 days to complete their larval development. This species is semi-domestic, having its larval development in the field, while the egg, pupal and adult stages in the rearing room.

The eri silkworm *S. cynthia ricini* known for its white or brick-red eri silk is widely distributed in India, China, Japan,

and Brazil. Its exploitation for sericulture was initiated in the Brahmaputra valley, Northeastern India (Vijayan *et al* 2006). In 1950s, this silkworm species was successfully introduced into China for silk production. The hatched larvae are reared in rearing-houses, and require 30-32 days to complete their larval development. Its cocoons cannot be reeled, as they are made up of uneven fibers. Usually, after moth emergence, cocoons are used for producing spun-yarn. In the present day, more than forty strains have been developed and maintained in China. These strains are classified into different races based on the larval color (yellow, blue, white), blood color and cocoon shape.

In order to better understand the genetic basis of the three silkworm species, it is necessary to assess the pattern of genetic variation within and between strains. Furthermore, the large number of strains within one species has been the focus of study on the role of genetic diversity during and after domestication (Goldsmith *et al* 2005). Recently, many studies have been carried out to assess the genetic diversity of silkmoths (Gui *et al* 2001, Zuo *et al* 2001, Hou *et al* 2005, Kar *et al* 2005, Li *et al* 2005, Vijayan *et al* 2006, Li *et al* 2007, Mirhoseini *et al* 2007); however, the information on genetic diversity within and between strains of the three economically important silkworm species is also scanty.

DNA markers are considered to be the most common means for measuring genetic diversity between individuals or within and between related species or populations because of their abundant polymorphism and the fact that they are independent of environmental conditions (reviewed by Behura 2006). A suitable tool is the RAPD technique (Williams *et al* 1990), which can provide a virtually unlimited number of neutral DNA markers and can be used to estimate population genetic parameters (Lynch & Milligan 1994). It has proved to be an appropriate method for obtaining genetic markers for many different kinds of organisms including insects, demanding no prior information about genomic organization (Kim & Sappington 2004). Due to these advantages, RAPD has been widely used to investigate genetic diversity and genetic structure of insects (reviewed by Behura 2006, Hiragi *et al* 2009, Souza *et al* 2009). Two major drawbacks of RAPD markers are their lack of reproducibility and the loss of complete genotypic information due to the fact that most RAPD bands are dominantly inherited (Lynch & Milligan 1994). Nevertheless, properly performed RAPD analysis is a useful and reliable tool for studying the genetic diversity and genetic structure (Perrson *et al* 2002). Analysis of molecular variance (AMOVA), which is not influenced by the dominance of the used markers, can be used to determine the partitioning of RAPD variation between and within populations (Huff *et al* 1993).

In the present study, the genetic diversity and genetic structure of the three silkworm species in China were estimated simultaneously using RAPD markers. The aims to this study were to assess the genetic diversity of the three silkworm species in a parallel study and to investigate the molecular variation within and among strains from the three silkworm species. This information will provide effective and efficient measures for germplasm conservation and utilization of the three silkworms. This is the first attempt to compare levels of genetic structure among the three silkworm species.

Material and Methods

Silkworm materials. A total of 40 samples of *A. pernyi* (16 from four strains), *S. cynthia ricini* (12 from three strains) and *B. mori* (12 from three strains) were collected for the study (Table 1). These strains are reared extensively

Table 1 Characteristics of silkworm strains used in this study.

Species / strain	Strain ID	Race[1]	Voltinism	Source
Antheraea pernyi				
He41	Ap1-4	Yellow	Monovoltine	Henan, China
Siqing	Ap5-8	Yellow-cyan	Monovoltine	Liaoning, China
Qinghuang	Ap9-12	Yellow-cyan	Bivoltine	Liaoning, China
Xinghuang	Ap13-16	Yellow	Bivoltine	Shandong, China
Samia cynthia ricini				
Shenlan	Sc1-4	Blue	Polyvoltine	Guangxi, China
Chuhuahuang	Sc5-8	Yellow	Polyvoltine	Guangxi, China
Gao 1	Sc9-12	White	Polyvoltine	Guangxi, China
Bombyx mori				
Dazao	Bm1-4	Tropical	Bivoltine	Guangdong, China
C108	Bm5-8	Chinese	Bivoltine	Chongqing, China
7532	Bm9-12	Japanese	Bivoltine	Fukuoka, Japan

[1]The strains of *A. pernyi* and *S. cynthia ricini* are grouped into different races based on the larval skin color, whereas those of *B. mori* are based on the geographical origin.

at different zones in China to produce the cocoon for silk production. They represent a high degree of divergence with respect to geographic origin and morphological, qualitative, quantitative characters. Of the four *A. pernyi* strains, three named Siqing, Qinghuang and Xinghuang were kindly provided by the Sericultural Institute of Liaoning Province, Fengcheng, Liaoning Province, China, and one named He 41 was obtained from the Sericultural Institute of Henan Province, Nanyang, Henan Province, China. Strains of *S. cynthia ricini* were obtained from the Sericultural Research Institute, Chinese Academy of Agricultural Sciences, Zhenjing, Jiangsu Province, China, while *B. mori* strains were obtained from the Institute of Sericulture and System Biology, Southwest University, Chongqing, China. Four individuals were randomly selected from each strain of three silkworm species.

DNA preparation and RAPD analysis. Total genomic DNA of individual insects was extracted by the use of proteinase-K and phenol-chloroform method using about 500 mg of a pupal macerate produced in pre-chilled mortar and pestle with liquid nitrogen. The pupal macerate was transferred to a 5-ml polypropylene tube and incubated at 50ºC overnight in the presence of proteinase-K (75 µg ml^{-1}) by adding 1.5 ml of lysis buffer (10 mM Tris-HCl pH 8.0, 100 mM EDTA, 0.5% SDS). After incubation, an equal volume of phenol-chloroform (1:1) was added to the incubation mixture and mixed thoroughly. The supernatant was collected and the DNA precipitated with two volumes of ethanol after centrifugation. The precipitated DNA was washed with 70% ethanol, air dried, and resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The RNA present in the extracts was removed through the treatment with RNAase A (10 mg ml^{-1}) at 37ºC for 1h. The DNA was then re-extracted following the steps early described. Quantification of the DNA was performed by electrophoresis on a 0.8% agarose gel stained with ethidium bromide.

A total of 40 decamer primers obtained from Operon Kit-L and -Y (Operon technologies Inc, USA) were used to screen the suitable primers. Of these, thirteen were selected based on the clarity and consistency of the resulting banding patterns (Table 2). RAPD-PCR was performed in 25 µl of a reaction mixture containing 30 ng DNA, 2.0 µl of 10×Buffer, 2.0 µl of dNTP mix (10 mM), 2 mM $MgCl_2$, 1.0 µl of primer (0.5 µM) and 1.0 U of *Taq* DNA polymerase (Promega Biotech Co., Ltd, USA). Amplication was carried out in a GeneAmp PCR System 9700 Thermal Cycler (PerkinElmer Inc. USA). The PCR schedule followed was 94ºC for 60 s followed by 35 cycles of 94 ºC for 30 s, 40ºC for 60 s, 72ºC for 90 s and a final extension of 10 min at 72ºC. A negative control without genomic DNA was performed for each series of PCR amplification to ensure no contamination. The PCR products were separated on a 1.4% agarose gel and identified by ethidium bromide staining. The experiment was performed twice and reproducible bands were scored and used for analysis. Weak bands were not considered for analysis.

Data analysis. RAPD markers were scored in a binary form as presence (1) and absence (0) of amplified bands for each sample. In accordance to the dominance relationship usually found in RAPD-PCR loci, the statistical analysis followed four general principles previously described (Apostol *et al* 1996): (1) RAPD loci segregate as dominant Mendelian markers, (2) genotypic frequencies are in accordance with Hardy-Weinberg equilibrium, (3) recessive alleles are identical and originate from the same mutational events, (4) dominant alleles also originate from the same mutational events.

The program DCFA 1.1 (Zhang & Ge 2002) was used to process the RAPD data for program POPGENE 1.31 (Yeh *et al* 1997) and WINAMOVA (Excoffier *et al* 1992). To estimate genetic variation with RAPDs, the percentage

Table 2 List of RAPD primers used and polymorphism generated among three silkworms.

Primer	Primer sequence (5'-3')	Number of bands				Percentage polymorphic loci (%)		
		Total	Ap	Sc	Bm	Ap	Sc	Bm
OPL06	GAGGGAAGAG	14	11	7	8	90.91	42.86	87.50
OPL09	TGCGAGAGTC	11	4	4	6	50	50	66.67
OPL10	TGGGAGATGG	18	14	8	11	85.71	12.50	81.82
OPL11	ACGATGAGCC	14	9	6	9	66.67	50	77.78
OPL12	GGGCGGTACT	9	5	4	7	80	25	42.86
OPL19	GAGTGGTGAC	13	11	10	10	90.91	50	50
OPL20	TGGTGGACCA	14	6	7	8	50	14.29	50
OPY04	GGCTGCAATG	10	5	7	6	80	57.14	33.33
OPY06	AAGGCTCACC	8	6	6	7	66.67	0	57.14
OPY10	CAAACGTGGG	11	8	6	8	62.50	16.67	62.50
OPY13	GGGTCTCGGT	10	8	4	4	100	75	50
OPY15	AGTCGCCCTT	7	6	3	3	100	66.67	66.67
OPY18	GTGGAGTCAG	8	5	7	4	60	57.14	50

of polymorphic bands (PPB), Nei's gene diversity (h) and Shannon's index of diversity (I) were calculated with POPGENE. The coefficient for gene differentiation (*G*st) and gene flow (*Nm*) were calculated by POPGENE on the basis of gene frequencies.

Pair wise genetic distance matrices were generated using Nei's coefficient of similarity (Nei & Li 1979). A dendrogram was generated from the above matrix using unweighted pair group method with arithmetical averages (UPGMA; Sneath & Sokal 1973) on TREECONW 1.3b software program (Van de Peer & De Wachter 1997). The UPGMA method does not assume rate constancy of evolution among the tested strains.

A drawback of RAPD markers is that dominance precludes the use of *F* statistic-based analysis of population structure. So, analysis of molecular variance (AMOVA; Excoffier *et al* 1992), which is not influenced by the dominance of the used markers, was preformed to describe the distribution of variance among and within strains for each silkworm species. The statistical program has been used effectively to obtain an assessment of genetic variation in many organisms using dominant RAPD markers (eg. Moya *et al* 2001, Gallusser *et al* 2004, Zhang *et al* 2007, Zhao *et al* 2008).

Results

RAPD profiles. Of the 40 primers tested, 13 of them producing a clear pattern of bands were selected for further use with all samples. For the selected primers, the same bands were always amplified in subsequent PCR analysis. These 13 primers generated 147 bands (Table 2), of which only two were shared, showing 98.6% polymorphism among the 40 samples analyzed. Most bands were in the range of ~200-2000 bp. The number of bands produced by individual primers varied from 7 (OPY-15) to 18 (OPL-10), with an average of 11.3 bands per primer. The extent of polymorphism within a species also varied. The range of polymorphism in *A. pernyi*, *S. cynthia ricini* and *B. mori* was 50-100%, 0-75% and 33.3-87.5%, respectively.

Strain-specific bands were detected in all three strains of *B. mori* with relatively high frequencies (three bands in Dazao, four in C108 and four in 7532), showing that RAPD divergence among *B. mori* strains was mainly attributed to allele fixation. However, no strain-specific bands were detected in the strains of *A. pernyi* and *S. cynthia ricini*, except for Siqing, a strain of *A. pernyi*, in which one strain-specific band was detected. The results suggested that RAPD divergence among strains of *A. pernyi* and *S. cynthia ricini* was mainly attributed to differences in the frequency rather than to allele fixation.

Genetic diversity at the species level. In the three silkworms, *A. pernyi* showed the highest genetic diversity at the species level. As for the 16 *A. pernyi* samples, 13 RAPD primers produced 98 scored bands, of which 77 were polymorphic (PPB 78.57%). Average number of alleles observed and average number of effective alleles was 1.7778 and 1.4613, respectively. Nei's gene diversity (h) was 0.2666 and Shannon's information index (I) was 0.3983 (Table 3).

Samia cynthia ricini showed the lowest genetic diversity at the species level. From the 12 samples of *S. cynthia ricini*, a total of 79 bands were amplified by 13 primers, and only 30 bands were polymorphic (PPB 37.97%). Average number of alleles observed and average number of effective alleles was 1.3875 and 1.2486, respectively. Nei's gene diversity (h) was 0.1407 and Shannon's information index (I) was 0.2079 (Table 3).

Bombyx mori also showed high levels of genetic diversity at the species level. From the 12 samples of *B. mori*, 13 RAPD primers generated 91 bands, of which 56 were polymorphic (PPB 61.54%). Average number of alleles observed and average number of effective alleles was 1.6264 and 1.4747, respectively. Nei's gene diversity (h) was 0.2654 and Shannon's information index (I) was 0.3835 (Table 3).

Genetic diversity at the strain level. At the strain level, all four strains of *A. pernyi* also showed the highest genetic diversity compared to those of *S. cynthia ricini* and *B. mori*. Among the four strains, 13 RAPD primers amplified 72-87 bands, of which 28-39 were polymorphic (PPB 28.28-39.39%). The mean percentage polymorphic loci PPB% was 35.35%. Average number of alleles observed and average number of effective alleles was 1.3535 and 1.2341, respectively. The mean Nei's gene diversity (h) was 0.1336 (0.1101-0.1520) and the mean Shannon's information index (I) was 0.1976 (0.1617-0.2208).

All three strains of *S. cynthia ricini* showed moderate levels of genetic diversity at the strain level. Among the three strains, a total of 76-79 bands were amplified by 13 primers, of which 15-20 were polymorphic (PPB 18.75-25%). The mean percentage polymorphic loci PPB% was 20.83%. Average number of alleles observed and average number of effective alleles was 1.2083 and 1.1556, respectively. The mean Nei's gene diversity (h) was 0.0858 (0.0738-0.1041) and the mean Shannon's information index (I) was 0.1244 (0.1079-0.1506).

All three strains of *B. mori* showed the lowest genetic diversity at the strain level among the three silkworms. Among the three strains, 13 RAPD primers amplified 61-66 bands, of which 6-12 bands were polymorphic (PPB 6.59-13.19%). The mean percentage polymorphic loci PPB% was only 10.99%. Average number of alleles observed and average number of effective alleles was 1.1099 and 1.0845, respectively. The mean Nei's gene diversity (h) was 0.0458 (0.0281-0.0562) and the mean Shannon's information index (I) was 0.0663 (0.0405-0.0806).

Genetic structure. In *A. pernyi* and *S. cynthia ricini*, AMOVA showed that a large proportion of genetic variation (60.23% and 72.51%, respectively) resided among individuals within strains, where a small part (39.77% and 27.49%, respectively) resided among strains, while a much larger proportion of genetic variation (84.08%) occurred among strains and a relatively smaller proportion of genetic variation (15.92%) occurred among *B. mori* specimens within strains (Table 4). The P values for all analyses of variance were highly significant.

The coefficient of gene differentiation *G*st calculated

Table 3 RAPD diversity in three silkworm species and their strains studied.

Strain	Size	Number of bands	Number of polymorphic loci	Percentage polymorphic loci (%)	Observed number of alleles (*A*a)	Effective number of alleles (*A*e)	Nei's gene diversity (*h*)	Shannon's information index (*I*)
Antheraea pernyi								
He41	4	85	36	36.36	1.3636	1.2301	0.1324	0.1974
Siqing	4	83	39	39.39	1.3939	1.2338	0.1398	0.2104
Qinghuang	4	87	37	37.37	1.3737	1.2766	0.1520	0.2208
Xinghuang	4	72	28	28.28	1.2828	1.1957	0.1101	0.1617
Mean	4	81.8	35	35.35	1.3535	1.2341	0.1336	0.1976
Species	16	98	77	78.57	1.7778	1.4613	0.2666	0.3983
SD					0.4179	0.379	0.1939	0.2676
Samia cynthia ricini								
Shenlan	4	79	20	25	1.2500	1.1895	0.1041	0.1506
Chuhuahuang	4	76	15	18.75	1.1875	1.1444	0.0795	0.1147
Gao 1	4	79	15	18.75	1.1875	1.1329	0.0738	0.1079
Mean	4	78	16.7	20.83	1.2083	1.1556	0.0858	0.1244
Species	12	79	30	37.97	1.3875	1.2486	0.1407	0.2079
SD					0.4903	0.3731	0.1994	0.2847
Bombyx mori								
Dazao	4	61	12	13.19	1.1319	1.0960	0.0533	0.0777
C108	4	65	6	6.59	1.0659	1.0518	0.0281	0.0405
7532	4	66	12	13.19	1.1319	1.1056	0.0562	0.0806
Mean	4	64	10	10.99	1.1099	1.0845	0.0458	0.0663
Species	12	91	56	61.54	1.6264	1.4747	0.2654	0.3835
SD					0.4864	0.3956	0.2141	0.3050

Table 4 Analysis of molecular variance (AMOVA) in three silkworm species.

Source of variance	Degrees of freedom (d.f.)	Sum of squares (SSD)	Expected mean squares (MSD)	Variance component	Percentage (%)	P value
Antheraea pernyi						
Among strains	3	0.922	0.307	0.056	39.77%	< 0.001
Within strains	12	1.013	0.084	0.084	60.23%	< 0.001
Samia cynthia ricini						
Among strains	2	0.177	0.089	0.013	27.49%	< 0.001
Within strains	9	0.317	0.034	0.035	72.51%	< 0.001
Bombyx mori						
Among strains	2	0.999	0.500	0.119	84.08%	< 0.001
Within strains	9	0.203	0.023	0.023	15.92%	< 0.001

by POPGENE showed similar results (Table 5). In the three species, the *Nm* (estimate of gene flow) values calculated by POPGENE were all smaller than 1 (0.1045-0.7813).

Cluster analysis. The mean genetic distance among individuals within strains of *A. pernyi*, *S. cynthia ricini* and *B. mori* was 0.169 (0.111-0.237), 0.070 (0.044-0.113) and 0.045 (0.008-0.081), while it was 0.280 (0.200-0.366), 0.097 (0.059-0.143) and 0.284 (0.206-0.356) among strains. When all 40 individuals were taken for analysis without considering any strain status, in the dendrogram generated by UPGMA of the Nei's genetic distance matrices for all the samples, three clusters were clearly formed separating the three silkworm species from each other (Fig 1). In the dendrogram, samples from strains of *A. pernyi* and *B. mori* formed strain-specific genetic clades. The three *B. mori* strain-specific clades were supported by bootstrap values of 100%, and the four *A. pernyi* strain-specific clades were supported by bootstrap values of 59-85%. However, 12 individuals from three strains of *S. cynthia ricini* did not form strain-specific genetic clades, showing an erratic distribution.

Discussion

RAPD markers. In the present study, RAPD markers generated from 13 random primers revealed sufficient polymorphism to investigate and compare the genetic diversity of three silkworm species. Several strains with a high degree of divergence were sampled for each silkworm species. Theoretically, the number of molecular marker loci is more important than sample size (Nei 1978). In many studies, the strategy that involves fewer samples and a larger number of molecular marker loci has been used in animals including insects (eg. Georgiadis *et al* 1990, Nguyen *et al* 2007). In this study, 79-98 RAPD loci were analyzed for each of the three silkworms; although the sample size was not large, the results obtained are reliable as they were reproducible (Georgiadis *et al* 1990). The reliability of our data is shown as it compares to the data reported for strains of *B. mori* by others (Yu *et al* 2000).

Analysis of the RAPD profile showed that the percentage of polymorphic loci (PPB%) of *S. cynthia ricini* was 37.97% (30 out of 79 bands), suggesting a low genetic diversity, which also agreed with those previously observed based on RAPD markers (36.63%) (Zuo *et al* 2001) and ISSR markers (37.66%) (Xu *et al* 2005). The calculated Nei's gene diversity (h) for *A. pernyi* also agreed with the value (0.2692) estimated from 68 different strains (unpublished data). The large set of markers obtained in this study, as well as the low cost and easiness of use, confirm the applicability of the RAPD technique in studying silkworm population genetics. Therefore, this efficient genetic fingerprinting technique would be useful for characterizing the large number of strains

Table 5 Partition of genetic diversity within and between strains.

Species	Total gene diversity (*Ht*)	Gene diversity within strain (*Hs*)	Coefficient of gene differentiation (*Gst*)	Gene flow (*Nm*)
Antheraea pernyi	0.2666	0.1336	0.4989	0.5023
SD	0.0376	0.0153		
Samia cynthia ricini	0.1407	0.0858	0.3902	0.7813
SD	0.0398	0.0019		
Bombyx mori	0.2654	0.0458	0.8272	0.1045
SD	0.0458	0.0086		

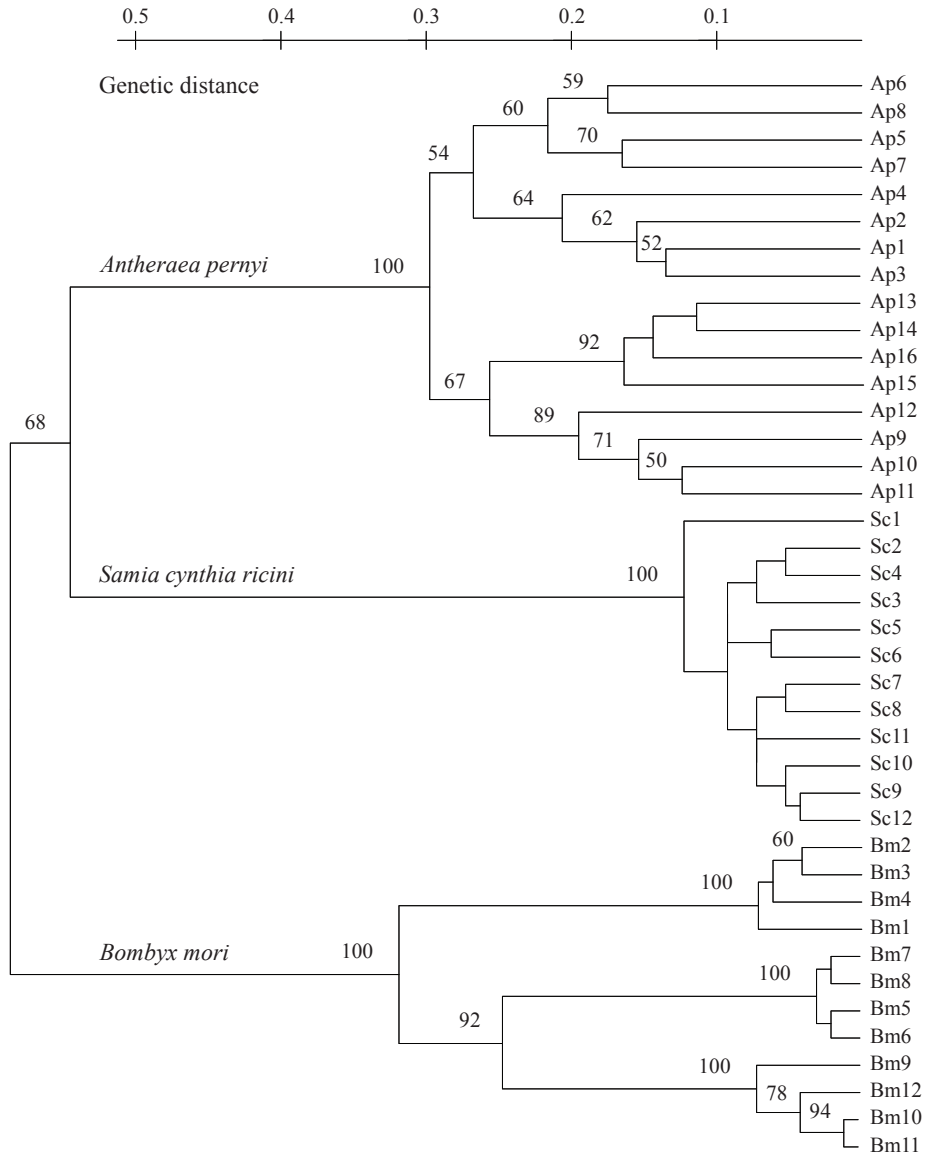

Fig 1 UPGMA dendrogram based on Nei's genetic distance obtained for *Antheraea pernyi*, *Samia cynthia ricini* and *Bombyx mori* using RAPD markers. Numbers on the branch indicate bootstrap support out of 100 replications.

of the three silkworm species kept in germplasm centers.

Genetic diversity. A PPB around 50% is regarded as high genetic diversity (Zhao *et al* 2006). Therefore, the results reported here revealed high genetic diversity at the species level for *A. pernyi* (PPB = 78.57%; h = 0.2666; I = 0.3983) and *B. mori* (PPB = 61.54%; h = 0.2654; I = 0.3835), whereas low genetic diversity for the Chinese populations of *S. cynthia ricini* (PPB = 37.97%; h = 0.1407; I = 0.2079).

A high genetic diversity for Indian populations of *S. cynthia ricini* at the species level (PPB = 64.13%; h = 0.3334; I = 0.3475), as well as at the population level (PPB = 14.43-31.52%; h = 0.051-0.109; I = 0.076-0.162) was assessed based on ISSR markers (Vijayan *et al* 2006). Compared to the data found in Indian populations, the genetic diversity of Chinese populations observed in this study (PPB = 37.97%; h = 0.1407; I = 0.2079) was very low at the species level. However, the genetic diversity of *S. cynthia ricini* at the strain level (PPB = 20.83%; h = 0.0858; I = 0.1244) was well within the range calculated by Vijayan *et al* (2006). UPGMA analysis of Indian populations showed that the individuals grouped according to their population affinity, and none changed its population cluster, which was different from that found in Chinese populations (Fig 1).

Among the Indian populations, the gene differentiation (G_{st}) was 0.657 (0.3563-0.6971) and 87% of inter-population genetic variability occurred. For the Chinese populations, the gene differentiation (G_{st}) was 0.390 and only 27.5% of inter-population genetic variability occurred. According to the genetic diversity theory, the maximum genetic diversity of a domesticated species would be presented in the region where it human exploitation was initiated. Eri silkworm was introduced from India into China for wild silk production in 1950s. Therefore, Chinese populations of *S. cynthia*

ricini might be derived from a common ancestor population that originated from India, and they were divergent only for a short period of time. Moreover, analysis of genetic diversity of Chinese populations showed that selection and inbreeding of lines from the ancestor population caused genetic divergence, as previously observed (Andrea *et al* 2000, Pradeep *et al* 2005).

The genetic diversity for Iranian native *B. mori* strains is assessed based on AFLP markers by Mirhoseini *et al* (2007). The dendrogram clustered all individuals belonging to the same strain in the same clade, as found in this study. AFLP markers revealed a relatively high Shannon's information index (I = 0.502) for Iranian native *B. mori* strains, when compared with the data revealed by RAPD markers in this study (I = 0.384), although the observed PPB% was very similar between them. The mean *G*st value of seven Iranian strains was found to be 0.51, which was lower than the data observed in this study (0.83). The differences may be due to different strains sampled and different marker systems.

Genetic structure. Genetic structure analysis is essential to understand the evolutionary processes such as gene flow, natural selection, and genetic drift taking place in a population. Gene flow plays a critical role in differentiation among populations. Generally, $Nm > 1$ is sufficient to overcome the effects of genetic drift, and that $Nm < 1$ indicates that genetic differentiation is likely to occur between populations due to the effects of genetic drift (Allendorf 1983). The gene flow (0.1045-0.7813) among the strains of each of the three silkworm species was smaller than one, suggesting that there is no general mixing of the strains and high genetic differentiation of them. Genetic drift caused by artificial selection must have played an important role in yielding the current populations of the three silkworms.

The AMOVA analysis showed that a higher genetic variation within rather than between populations of *A. pernyi* and the Chinese populations of *S. cynthia ricini*, as found in *Antheraea mylitta* Hübner (Kar *et al* 2005). This is a pattern frequently observed in wild plants (Zhang *et al* 2007, Zhao *et al* 2008, Tahan *et al* 2009) and wild insects (Moya *et al* 2001, Gallusser *et al* 2004). However, only 16% of genetic variation occurred within strains and 84% among strains of *B. mori* in China, as observed in Indian populations of *S. cynthia ricini* (Vijayan *et al* 2006). This pattern is also found in many domesticated animals (Huang *et al* 1996, Hao *et al* 2000) and cultivated plants (Wang *et al* 2008). *Antheraea pernyi* and *A. mylitta*, which are reared in the field, had higher levels of genetic diversity at the strain level than *B. mori*, which is reared under controlled conditions. The higher genetic variability present in strains of the former two species could be regarded as an adaptive strategy for increasing the population fitness in a spatiotemporally heterogeneous and uncertain environment, and that the broad-niche species are characterized by high heterozygosity as compared with narrow-niche species (Hedrick 1986).

A value of gene differentiation (G_{st}) >0.25 is generally regarded as the threshold quantities beyond which significant population differentiation occurs (Slatkin 1987). All the three silkworms revealed significant population differentiation. However, UPGMA dendrogram showed that the individuals from strains of *A. pernyi* and *B. mori* formed strain-specific genetic clades, whereas those from Chinese populations of *S. cynthia ricini* did not. These results showed that *B. mori* strains exhibit a high inbreeding status, and that the present *A. pernyi* populations are at the threshold of differentiation. The tested *S. cynthia ricini* populations are in the verge of genetic differentiation, as they have accumulated about >25% genetic variability among them. Further genetic drift in *A. pernyi* populations have already resulted in divergent strains, while the Chinese populations of *S. cynthia ricini* may result in the formation of a single strain. Domestication over long period of time and the selection pressure under man-made rearing conditions might have resulted in this genetic diversification of the populations of the wild silkworms (Kar *et al* 2005).

Implications for conservation of germplasms and breeding programs. Knowledge of genetic variation within and between strains provides essential information in the formulation of appropriate management strategies directed towards their conservation and the establishment of effective and efficient breeding programs (Milligan *et al* 1994). Cross-breeding strategies have been extensively used as a means of harnessing heterosis in the silkworm. Selection of genetically pure and divergent parental strains is critical to the success of a hybridization program in silkworm. It has been shown that the parental homozygosity, also known as genetic purity of a strain, has a distinct influence on the degree of manifestation of heterosis (Nagaraju & Goldsmith 2002). Low homozygosity often results in low degree of manifestation of F_1 heterosis. The degree of manifestation of F_1 heterosis in *A. pernyi* and *S. cynthia ricini* is about 10-20%, which is less than that observed in *B. mori* (20-40%).

The genetic diversity and genetic structure unraveled in this study supports that the lower degree of manifestation of F_1 heterosis in *A. pernyi* and *S. cynthia ricini* is mainly caused by lower homozygosity and close relationship of strains, respectively. Artificial selection has been widely used in the breeding programs for silk moths. Continuous selection and inbreeding can increase the frequency of homozygotes and make homozygous effect stronger (Whitlock 2002). Thus, continuous selection and inbreeding can be used to improve the parental homozygosity for *A. pernyi*, while introduction of novel germplasm of *S. cynthia ricini* from India into China must be a good way to enhance the genetic diversity of Chinese populations.

The genetic diversity revealed in this study is of much use in formulating strategies to maintain the genetic diversity present in these silkworm species in China. For *B. mori*, the low genetic variation (ca. 16%) within the individuals of a population points to the fact that the populations are homogenous in nature, whereas the higher genetic variability (ca. 84%) among the populations indicates that the populations have already differentiated into separate genetic pools. Hence, these different gene pools should be conserved separately and maintained without any inter-mixing. For *A. pernyi* and *S. cynthia ricini*, however, the higher genetic variation (>60%) within the individuals of a population points to the fact that the populations are heterogeneous, whereas the low genetic variability (<40%) among the populations indicate that the populations have not already differentiated

into separate genetic pools. Unlike for what was suggested for *B. mori*, we must raise more individuals in a population and fewer core populations to conserve the most genetic diversity for *A. pernyi* and *S. cynthia ricini*. To address the selection of core populations and effective population size, different kinds of marker systems, including morphological, physiological, biochemical, and molecular can be used.

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