Available online at www.sciencedirect.com
ScienceDirect
Biochemical Systematics and Ecology 34 (2006) 868–874
biochemical systematics and ecology
www.elsevier.com/locate/biochemsyseco

A genetic diversity study of silkworm using cleaved amplified polymorphic sequence (CAPS) markers

Jianhua Huang [a,b], Muwang Li [a,b], Yong Zhang [a,b], Wenbin Liu [a,b], Minghui Li [a,b], Xuexia Miao [a], Yongping Huang [a,*]

[a] *Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 300 Feng Lin Road, Shanghai 200032, PR China*
[b] *Graduate School of the Chinese Academy of Sciences, Beijing 100039, PR China*

Received 13 August 2005; accepted 29 June 2006

Abstract

The silkworm, *Bombyx mori* is a beneficial insect of great economic importance in China for its silk production. In this study, we obtained 11 cleaved amplified polymorphic sequence (CAPS) markers and one PCR polymorphism marker from the genes of the silkworm, *B. mori*. A backcross progeny analysis showed that all these molecular markers were segregated in a Mendelian fashion and that polymorphisms were co-dominant. These markers were used to investigate the genetic diversity among 29 strains of *B. mori* from China, Japan and Europe. Cluster analysis, based on the genetic similarities calculated from CAPS data, grouped these strains roughly according to their geographical origin. One group contained silkworm strains from Europe and some of the Japanese strains were interspersed into the Chinese groups, whereas other Japanese strains clustered together.

Keywords: Silkworm; *Bombyx mori*; CAPS markers; Polymorphism; Genetic diversity; Geographical; Restriction endonuclease

1. Introduction

The silkworm, *Bombyx mori* is of great economic importance in China. About >20 million families in this country raise the silkworm as their economic resource. In addition, the silkworm is one of the best studied lepidopteran insects, including many destructive species for agriculture and forestry, because of the large number of mutants that have been identified (Goldsmith, 1995; Nagaraju and Goldsmith, 2002). This species was domesticated from the wild silkworm, *Bombyx mandarina*, in China at least 5000 years ago (Xiang, 1995) and then it subsequently spread throughout Asia (e.g. Korea, Japan, and India), and into Europe (France and Italy). At present, silkworm strains have been classified into four main geographical origins: Chinese, Japanese, European and tropical origin (Xia et al., 1998). It is difficult to assume their phylogeny and genetic diversity from their phenotypic traits, though the phenotypic traits of silkworm strains have been noted widely (Tazima, 1964; Doira, 1978; Doria, 1992). In order to better understand the origin and

* Corresponding author. Tel.: +86 21 54924046; fax: +86 21 54924047.
E-mail address: yphuang@sibs.ac.cn (Y. Huang).

0305-1978/$ - see front matter © 2006 Published by Elsevier Ltd.
doi:10.1016/j.bse.2006.06.011

the genetic relationships in silkworm, *B. mori*, it seems ideal to use some kind of valuable markers to study the silkworm strains within different geographical regions.

In recent years, DNA-based molecular markers such as random amplified polymorphic DNA (RAPD) (Nagaraja and Nagaraju, 1995; Xia et al., 1998; Lu et al., 2002), amplified fragment length polymorphism (AFLP) (Lu et al., 2001), restriction fragment length polymorphism (RFLP) (Sethuraman et al., 2002), simple sequence repeats (SSR) (Shen et al., 2004; Li et al., 2005) and inter simple sequence repeats (ISSR) (Chatterjee and Mohandas, 2003) have been widely adopted in silkworm genetic diversity studies. In general, these studies provided the positive information to enhance the understanding of silkworm phylogeny. However, until now no attempts have been made to isolate and characterize molecular markers from identified gene sequences, which showed much slower evolution speed than those DNA-based molecular markers.

Cleaved amplified polymorphic sequence (CAPS) markers are based on PCR amplification of known genes and cDNA sequences. PCR products are digested by restriction enzymes, generating a simple type of data coded as heterozygote or homozygote (Konieczny and Ausubel, 1993). CAPS markers have been used to study genetic diversity in some species of plants such as *Arabidopsis thaliana* (Hardtke et al., 1996; Barth et al., 2002), *Camellia sinensis* (Kaundun and Matsumoto, 2003), and *Cryptomeria japonica* (Tsumura et al., 1999). In this report, CAPS markers from silkworm gene sequences were developed for determining the phylogeny and genetic diversity of 29 strains of domesticated silkworm strains from different geographical origins.

2. Materials and methods

2.1. Animal material

Two Chinese silkworm strains, C108 as female and Dazao as male, and their F1 generation, were used to select the CAPS markers. A single pair mating backcross between female C108 × F1 (C108 × Dazao) was raised to analyze the genetic mode of inheritance of these markers. Both the parental silkworms and the collection of 29 silkworm strains were obtained from the Sericultural Research Institute, Chinese Academy of Agricultural Sciences (CASS), Zhengjiang, Jiangsu Province, China. This collection represents major genetic strains of silkworms in cultures of Chinese, Japanese and European geographical origins.

2.2. Primer design

Eleven primer pairs based on known silkworm genes in GenBank (Table 1) were designed with the software Primer Premier 5. All primers were synthesized in Bioasia Company (Shanghai, China).

2.3. DNA isolation and CAPS marker search

DNA was isolated from whole silk glands using a slightly modified phenol–chloroform extraction (Yasukochi, 1998). The chloroform–isoamyl extraction step was repeated twice in order to purify the DNA.

CAPS reactions were modified slightly using the protocol of Konieczny and Ausubel (1993). Each PCR reaction mix (30 μl) for the CAPS marker search used 15 ng DNA in 1 × PCR buffer, 0.2 μM dNTP each, 10 pM forward and reverse primers each, and 1 unit Taq DNA polymerase (Dingguo, Beijing, China). Each amplified reaction (3 μl) for Dazao, C108 and their F1 generation was digested with restriction enzymes according to manufacturer's instructions. Forty different restriction endonucleases (Takara, Tokyo, Japan) were tested, namely, *Alw*I, *Aat*II, *Afa*I, *Alu*I, *Apa*I, *Bam*HI, *Bcl*I, *Bgl*I, *Bgl*II, *Cfr*13I, *Dra*I, *Dde*I, *Eco*RI, *Eco*RV, *Fok*I, *Hha*I, *Hin*dIII, *Hae*III, *Hin*cII, *Kpn*I, *Mbo*I, *Mlu*I, *Msp*I, *Msc*I, *Nde*I, *Nsi*I, *Nco*I, *Nhe*I, *Pst*I, *Pvu*II, *Psh*BI, *Pvu*I, *Sal*I, *Sca*I, *Sac*II, *Ssp*I, *Sac*I, *Taq*I, *Xba*I and *Xho*I, and the enzyme digestion products were separated on 2% TAE agarose gels for CAPS marker assay.

2.4. Genetic analysis

For genetic analysis using CAPS markers, 11 gene–enzyme combinations and one PCR marker were used (Table 2). Each silkworm strain DNA pool for genetic analysis was constructed from the silk glands of 20 individuals.