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DNA fingerprinting in silkworm *Bombyx mori* using banded krait minor satellite DNA-derived probe

The genomic DNA from thirteen different ecotypes and inbred lines of silkworm, *Bombyx mori*, were analyzed by digesting with *Bst*NI and *Hin*fI restriction enzymes followed by hybridization with banded krait minor satellite DNA (Bkm)–2(8) minisatellite probe. The DNA fingerprinting revealed 9–31 discrete intense bands, some of which were ecotype/inbred line-specific. Individual specific DNA fingerprints in two representative genotypes and their F_1 hybrid offspring were also obtained. Individuals of a given parental line showed very similar profiles and the hybrid offspring showed the combined profile of both parents. The presence of bands specific to diapausing and nondiapausing strains and to particular genotypes indicate their potential use for marker-assisted breeding and varietal identification.

1 Introduction

The various ecotypes and synthetic inbred lines of silkworm, *Bombyx mori*, display large differences in their quantitative and qualitative attributes that ultimately control silk yield [1, 2]. The study of genetic variability in this moth is therefore of considerable interest not only to calculate genetic distance and analyze pedigree relationships but also to generate molecular markers linked to various economic traits. DNA fingerprinting with an M13 phage DNA probe [3] has been done previously to reveal genetic variability in ameiotic and meiotic parthenogenetic clones of silkworm [4, 5]. However, genome analysis of diverse ecotypes and inbred lines of silkworm has not been attempted so far. In the present study, we report the potential of a minisatellite probe, banded krait minor satellite DNA (Bkm)-2(8), [6–8] for the characterization of different silkworm genomes. This probe reveals a high level of restriction fragment length polymorphism (RFLP) in a variety of eukaryotes [8–11] and has been used as a multilocus probe in DNA fingerprinting in humans, crocodilians, *etc.* [12–14]. We demonstrate here the successful use of this probe in generating DNA fingerprints in all thirteen genotypes studied. Using the two representative diverse genotypes, Nistari and NB_1, we also show that the genotype-specific fingerprints are inherited by their F_1 hybrid offspring.

2 Materials and methods

2.1 Silkworm genotypes

Six diapausing (Hu_{204}, KA, NB_1, NB_7, NB_4D_2 and NB_{18}) and seven nondiapausing (C. nichi, Gungnong, Moria, Nistari, Pure Mysore, Diazo and Sarupat) silkworm genotypes [15] were selected to check the effectiveness of the Bkm-2(8) probe in generating DNA fingerprints. These genotypes differ in the following characteristics – larval duration, cocoon weight, cocoon shell weight, susceptibility to diseases, silk filament length, cocoon shell ratio and voltinism (refers to number of life cycles in a year) [15]. Of the thirteen genotypes, NB_1 and Nistari represent the most divergence and were therefore selected to obtain initial results on their individual specificity of DNA fingerprints as well as on the inheritance pattern of fingerprints of their F_1 hybrid offspring. NB_1, an inbred line derived from a Japanese hybrid, having the potential to yield high quality cocoons and better-quality silk under optimum eco-climatic and nutritive conditions. However, it is susceptible to diseases under tropical agroclimatic conditions. On the other hand, the Indian ecotype, Nistari, is nondiapausing, disease-resistant and highly acclimatized to the agroclimatic conditions of the tropics. However, it spins cocoons of low shell weight and silk of poor quality [15].

2.2 DNA isolation, restriction digestion and Southern hybridization

High molecular weight (HMW) genomic DNA was isolated from pooled posterior silk glands of fifth instar (day 4) larvae of each of the thirteen genotypes mentioned above. Two stocks of Nistari, Nistari (H) and Nistari (L), are maintained in our laboratory. For pooled DNA samples, Nistari (L) was used. To obtain initial results on intrapopulation genetic variability, sex specificity, and mode of inheritance, HMW genomic DNA was extracted from six individual moths (three males and three females) from each of NB_1 and Nistari (H) as well as four F_1 hybrid individuals (two males and two females) resulting from a cross between Nistari (H) female and NB_1 male. The procedure of Sprague *et al.* [16] was followed for isolation of DNA. 10–12 μg of DNA per sample was digested to completion with *Bst*NI or *Hin*fI restriction enzymes according to the recommendation of the supplier (New England Biolabs). Digested samples were run in 30 cm long, 5 mm thick, horizontal, 1% agarose gels at 60 V for 16–18 h in TPE buffer (15 mM Tris-HCl, 18 mM NaH_2PO_4, 0.5 mM EDTA, pH 7.8). Gel-fractionated DNA samples were transferred onto Hybond-N membranes (Amersham,

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Nonstandard abbreviation: Bkm, banded krait minor satellite DNA

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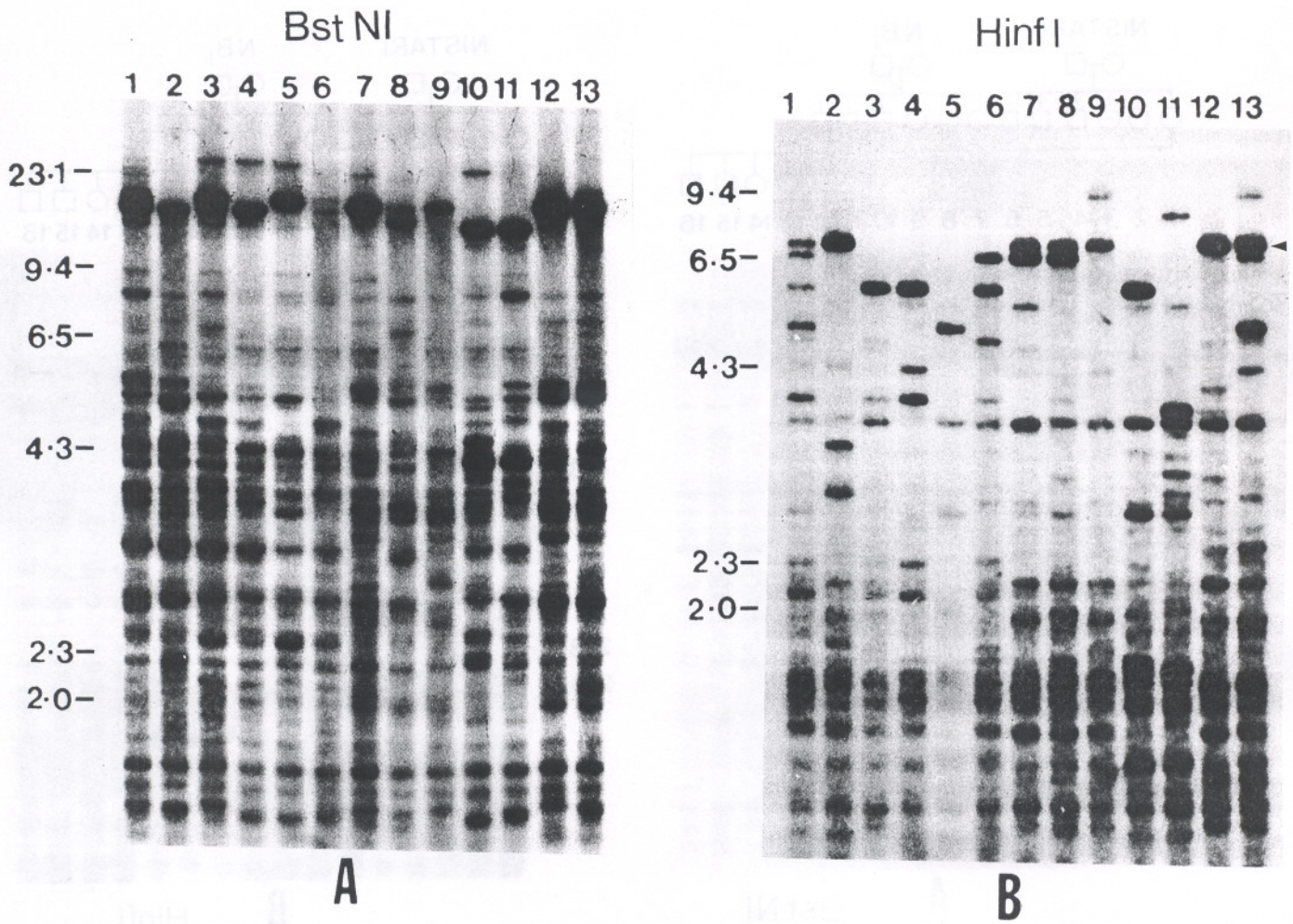

Figure 1. Bkm-2(8) hybridization pattern of (A) *Bst*NI and (B) *Hin*fI digested DNA from thirteen silkworm genotypes. Lanes (1)–(13) are pooled DNA samples of Hu_{204}, C. nichi, Gungnong, Moria, Diazo, Sarupat, KA, NB_1, NB_7, Nistari, Pure Mysore, NB_4D_2 and NB_{18}, respectively. Each lane contains 10–12 μg of DNA pooled from 12 individuals. *Hin*fI profiles (B) are unique to each genotype. The arrow indicates the band shared by all diapausing genotypes (lanes 1, 7, 8, 9, 12 and 13) and one non-diapausing genotype, C. nichi (lane 2). Note its absence in all the other non-diapausing genotypes (lanes 3, 4, 5, 6, 10 and 11). Numbers on the left indicate DNA fragment sizes in kb.

UK) at a pressure of 30 mm Hg using a vaccum blotting apparatus. The membranes were baked at 80°C for 2 h. The subclone Bkm-2(8) containing a 545 bp DNA sequence consisting of 66 copies of GATA repeats interspersed with variable number of dinucleotide TA repeats in several locations was used as probe [6–8]. A single-stranded probe [17] was prepared to a specific activity of 0.7–3.0 × 10^8 cpm/μg, using alpha [^{32}P]-dATP (specific activity, 3000 Ci/mmol; Jonaki, BARC, India). The blots were then washed in 7% SDS and 0.5 M sodium phosphate buffer, pH 7.5, at 60°C for 2–3 h and then hybridized with 1–2 × 10^6 cpm/mL of probe in the same but fresh buffer at 60°C for 14–16 h. The blots were then washed in 2 × SSC (1 x SSC is 0.15 M NaCl,, 0.015 M sodium citrate, pH 7.2) containing 0.1% SDS for 15 min, once at room temperature and thrice at 60°C. The membranes were then exposed to X-ray films for 1–3 days.

2.3 Analysis of fingerprints

Autoradiographs were examined visually to score the number of hybridized bands. All bands showing similar molecular weights were considered to be identical. Each lane was scored for the presence or absence of a particular fragment.

3 Results

3.1 Bkm-2(8) reveals genotype-specific DNA fingerprint patterns

The various silkworm genotypes, as mentioned earlier, are ecotypes and highly inbred lines and, therefore, DNA fingerprinting with pooled DNA samples was carried out. Figures 1A and 1B show the DNA profiles of these genotypes after *Bst*NI and *Hin*fI digestion, respectively. In *Bst*NI digested samples (Fig. 1A), the bands varied in number from 22 (NB_7, lane 9) to 31 (Gungnong, lane 3). However, the average number of bands (26.5) in diapausing (lane 1, 7, 8, 9, 12 and 13) and non-diapausing (lanes 2, 3, 4, 5, 6, 10 and 11) ecotypes remained the same. For *Hin*fI digestion (Fig. 1B), the number of bands varied from 9 (Diazo, lane 5) to 25 (NB_{18}, lane 13). The average number of bands in diapausing (lanes 1, 7, 8, 9, 12 and 13) and nondiapausing (lanes 2, 3, 4, 5, 6, 10 and 11) genotypes were 20.5 and 15.4, respectively. Although *Bst*NI DNA fingerprints (Fig. 1A) could differentiate between different genotypes, the *Hin*fI profiles (Fig. 1B) were found to be more useful for distinguishing diapausing (lanes 1, 7, 8, 9, 12 and 13) and nondiapausing (lanes 3, 4, 5, 6, 10 and 11) genotypes.

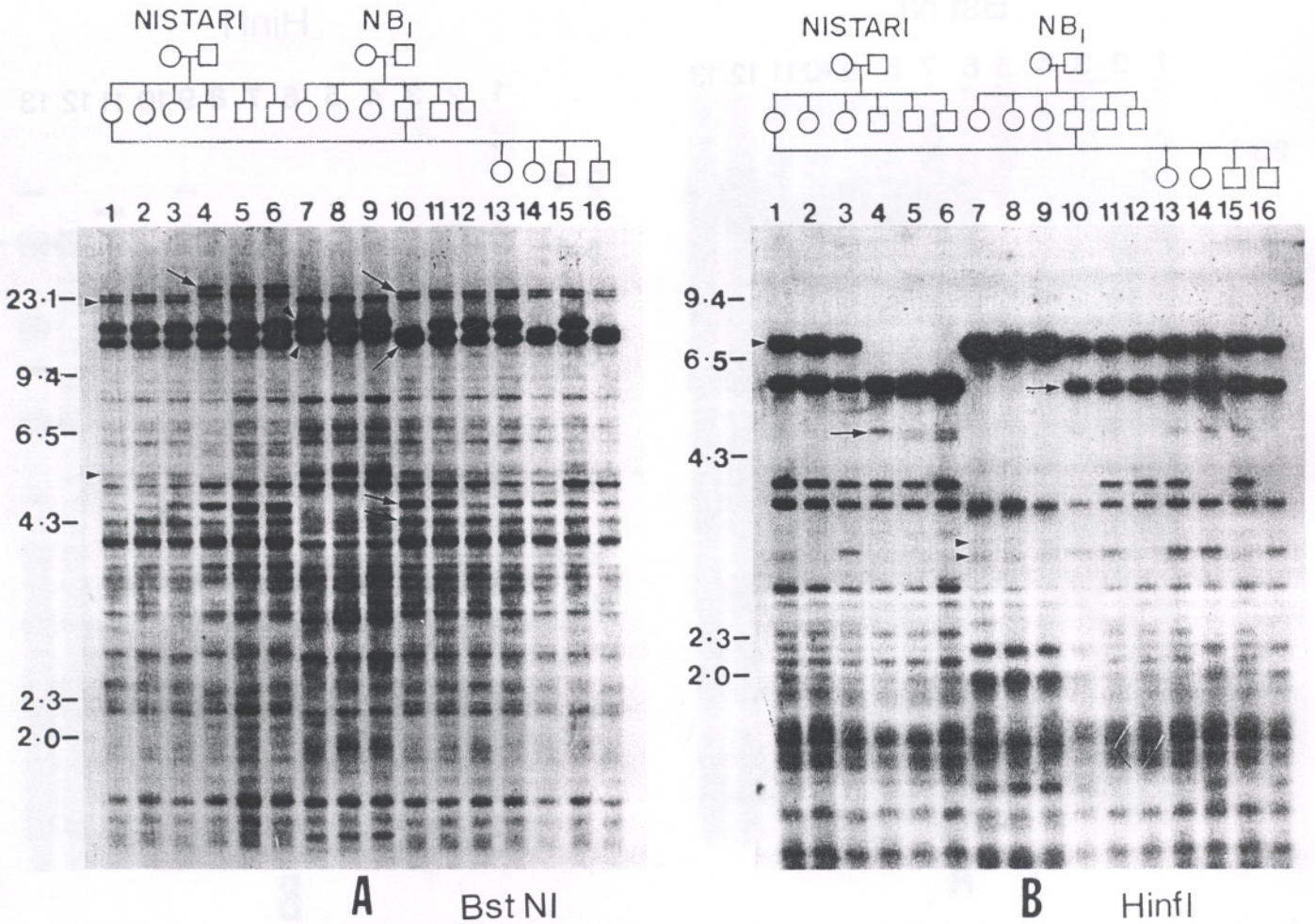

Figure 2. Bkm-2(8) hybridization patterns of (A) *Bst*NI and (B) *Hin*fI digested DNA from male and female individuals of Nistari and NB_1 genotypes and their F_1 offspring. Lanes 1–6 are Nistari; 7–12, NB_1; 13–16, F_1 hybrid individuals. The squares and circles represent males and females, respectively. Arrow and arrowheads indicate polymorphic bands observed in males and females, respectively.

This is because the bands unique to specific genotypes are of relatively low molecular weight (<10 kbp) in *Hin*fI profiles than that of *Bst*NI (>10 kbp) and are therefore better resolved in the former. A band of 6.9 kbp was observed only in the diapausing genotypes (Fig. 1B, lanes 1, 7, 8, 9, 12 and 13) and one nondiapausing genotype, C. Nichi (Fig. 1B, lane 2). Each of the thirteen genotypes exhibit a unique DNA profile after digestion with *Hin*fI. For example, the nondiapausing genotype Pure Mysore (Fig. 1B, lane 11), showed four genotype-specific bands (M_r 8, 3.7, 3.5 and 2.9 kbp) whereas NB1, a diapausing genotype, showed only one genotype-specific band (2 kbp).

3.2 Inheritance of DNA fingerprint pattern in F1 hybrid offspring

Comparison of DNA fingerprints of individuals of Nistari and NB_1 genotypes showed that, within each genotype, DNA fingerprint patterns are virtually identical (Fig. 2A and 2B). But polymorphic bands between sexes of the same genotype were observed in both the genotypes (Fig. 2A and B). However, sex-specific inheritance pattern was absent in F_1 hybrid offspring, resulting from a cross of Nistari female and NB_1 male, after digestion with either of the enzymes (Fig. 2A and Fig. 2B).

4 Discussion

The silkworm comprises a large number of ecotypes and synthetic inbred lines that show a high degree of divergence with respect to geographic origin, morphological, quantitative and qualitative traits [1, 2]. It is therefore important to study genetic diversity of various silkworm genotypes for the purpose of strain improvement. We have presented here our initial results on genome analysis in silkworm using multilocus DNA fingerprinting with Bkm-2(8) probe. DNA fingerprints obtained for the thirteen genotypes indicate that this probe can be used to determine genetic relatedness among them. The clear proof for this can be seen in the hybridization profiles of two sister lines, NB_4D_2 and NB_{18} (Fig. 1A and 1B, lanes 12 and 13) which are derived from common parentage [18]. These two genotypes show homology for most of the bands, indicating their high degree of genetic relatedness. Work is in progress to generate subsets of data that would help to test for congruence and establishing genetic relatedness among various genotypes. The observation that there are bands specific to diapausing and nondiapausing strains suggest that the possible linkage of these markers with the traits of economic interest could be explored for their possible use in "marker assisted breeding" since these two sets of strains differ in

their quantitative and qualitative characters. Only one genotype, C. Nichi, which is a nondiapausing type, also showed a band of 6.9 kb (Fig. 1B, lane 2) which is seen in all the diapausing genotypes. This result is not surprising since C. Nichi was diapausing when it was brought to India from Japan almost 75 years ago and has changed towards nondiapausing characters in the course of continuous breeding under tropical conditions. Many genotype-specific bands seen in *Bst*NI (Fig. 1A) and *Hin*fI (Fig. 1B) profiles could be used as potential markers for varietal identification and germplasm screening.

The comparison of individual fingerprints of Nistari and NB_1 indicated that individuals of Nistari tend to show more similar profiles than those of NB_1. The Nistari genotype was introduced in India almost 500 years ago and has a long history of inbreeding [19]. Our results therefore might suggest a higher level of homozygosity in Nistari. However, the homozygosity cannot be determined at this time because of lack of data on a large sample size. The polymorphic bands observed between the sexes of both Nistari and NB_1 genotypes need further analysis using data on reciprocal and backcrosses that would help us to understand sex-specific polymorphism.

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