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DNA fingerprints of *Bombyx mori* L.

Testing of genotypic variability of parthenogenetic strains

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A comparative analysis of the total cellular DNA in certain parthenogenetic specimens of the silkworm (*Bombyx mori*), produced from the females of the parthenogenetic strains by two types of parthenogenesis, has been performed through the application of the DNA fingerprinting method based on M13 phage DNA as a hybridization probe. It has been shown that parental specimens and their genetically identical off-springs produced through ameiotic parthenogenesis have identical patterns of hybridization with the hypervariable DNA fragments. The off-springs produced through the meiotic type of parthenogenesis have individual-specific patterns of hybridization, revealing a high level of polymorphism of individual genotypes. The results obtained testify to the effectiveness and reliability of this promising method for identification of genotypic variability, marking and genomic characterization of parthenogenetic clones in the silkworm.

DNA fingerprinting; M13 minisatellite; *Bombyx mori*; Parthenogenetic strain

1. INTRODUCTION

The study of genotypic differences in organisms is of great importance for both fundamental and applied research. One of the promising methods in such studies is DNA fingerprinting, which is based on the application of minisatellite DNAs as highly effective probes of polymorphism revealing hypervariable genome loci [1]. The application of this method for assaying the silkworm genome is of considerable interest and is justified by the fact that the genetics of this species has been well studied and that the preparation of genetically identified clones and lines has been performed. Earlier a variant of DNA fingerprinting based on the application of the M13 phage DNA as a hybridization probe (2–4) was used by the authors for DNA typing a number of commercial breeds of silkworm that were not of genetically pure lines [5]. In the course of this experimental work, the level of individual polymorphism in species of different strains was evaluated, the most characteristic polymorphic loci revealed, the peculiarities of polymorphic DNA fragment distribution in the series specimens-family-breed observed and a formalized approach to passportization of the silkworm strains suggested. Certain difficulties in interpreting the data obtained arose due to the fact that the DNA of total eggs of strains and families were assayed, which presented a complex blend of individual genomes.

Here we report on a comparative analysis of the total cellular DNA in certain parthenogenetic specimens of the silkworm produced from genetically identical females of a parthenogenetic strain by two types of parthenogenesis, by applying the DNA fingerprinting method with M13 phage DNA as a hybridization probe.

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2. MATERIALS AND METHODS

The work was carried out on the DNA samples isolated from the muscular tissue of individual larvae of the silkworm. Parthenogenetic strain N: 9 [6] of the ameiotic type was chosen for the study. Its parental specimens, as well as off-spring produced by both ameiotic and meiotic parthenogenesis, were analyzed.

The DNA isolation, treatment by *Msp*I restriction endonuclease, electrophoretic fractionation of the DNA hydrolyzate, DNA transfer out of the gel on to nitrocellulose filters, preparation of ^{32}P-labelled M13 phage DNA and hybridization was carried out as described earlier [4,5].

3. RESULTS

In analyzing the distribution of hypervariable fragments in *Msp*I hydrolyzate of cellular DNA for fourteen parental specimens produced via ameiotic parthenogenesis (females) identical hybridization spectra have been observed. Fig. 1a shows the typical patterns for the three representatives of the given samples (lanes 1–3). The most characteristic hybridizing fragments (A,B,C,D) were designated by the authors previously as

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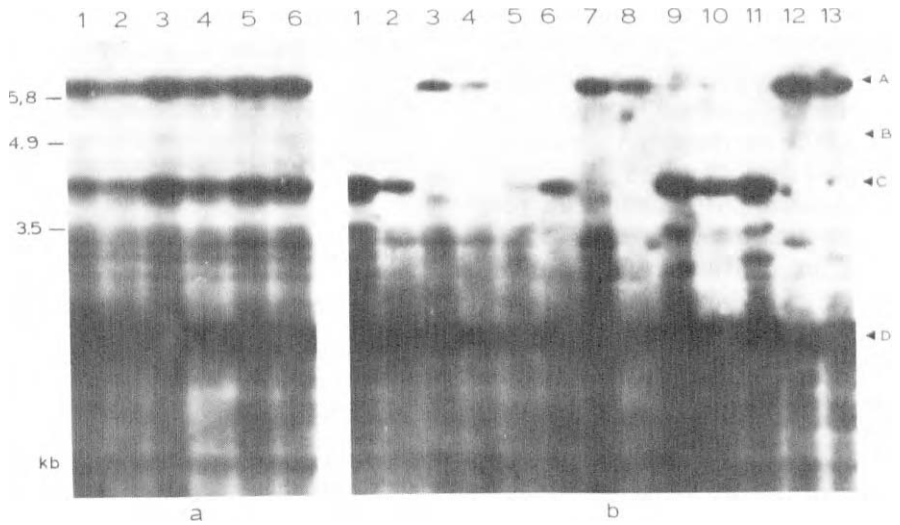

Fig. 1. Hypervariable sequences detected by M13 phage DNA in *Msp*I hydrolyzate of silkworm DNA. (a) Lanes 1–3, parental specimens of a parthenogenetic strain produced through ameiotic-type parthenogenesis; lanes 4–6: off-spring (female) produced through ameiotic-type parthenogenesis; (b) lanes 1–10: off-spring (male) produced through meiotic-type parthogenesis. 5–7 μg samples of DNA were digested with *Msp*I, electrophoresed through a 20-cm long 0.8% agarose gel and transferred by blotting to a nitrocellulose membrane filter. ^{32}P-labelled M13 phage DNA was used as a hybridization probe. The hybridization solution contained 4 × SSC, 0.1% SDS, 10 × Denhardt's solution. Salmon DNA was not added to the hybridization mixture. Incubation went on for 16–20 h at 60°C. After the hybridization the filters were washed in 1 × SSC, 0.1% SDS at 60°C, and exposed to X-ray film.

population markers, which were typical of the silkworm [5]. The peculiarity of this clone is that the B fragment reveals itself as a minor band, which is poorly detectable in the hybridization pictures. The hybridizing fragments in the range over 6 kb are missing in the spectra, which, under the conditions used, is characteristic of silkworm DNA of a number of strains [5]. On the other hand, in the individual DNAs of the parthenogenetic strain there is a large number of hybridizing *Msp*I fragments in the lowmolecular weight zone.

In reproducing this clone through the same ameiotic type of parthenogenesis there arise specimens (females) that are genetically identical to both the parents and each other. This has been shown on the DNAs from 12 off-spring and characteristic results are presented in lanes 4–6 of Fig. 1a.

In analyzing DNAs from 13 individuals of the parthenogenetic strain produced through meiotic parthenogenesis (males) individual-specific hybridization patterns were obtained which differed from eachother in the number and character of the distribution of the hypervariable fragments (Fig. 1b). Unlike the females that were produced through ameiotic parthenogenesis the typical B fragment was not present in the hybridization spectra, while the A and C fragments occurred in some specimens only; the typical D fragment was present in the DNAs of both parent and off-spring. Certain differences were detected in a number of bands located between the C and D fragments, thus yielding enhanced levels of polymorphism in the fingerprints.

It should be emphasized that DNA fingerprinting, under the conditions used, reveals an incomparably greater number of genotypic variants compared to that revealed via traditional methods based on protein polymorphism and variability of individual enzymes and their various forms in the silkworm [8].

4. DISCUSSION

A peculiar feature of the parthenogenetic strain, N: 9, is that the diploid nucleus of the non-fertilized egg, induced to development by heating [6], does not undergo reduction division as is usually the case, and the development of the egg proceeds with the maternal nucleus fully retained. Consequently, parthenogenetic individuals represent genetically identical females that are exact replicas of the mother (P ♀ ZW,Aa,Bb → Fp ♀ ZW,Aa,Bb). In producing the off-spring through meiotic parthenogenesis [7] an unfertilized nucleus of the egg undergoes both reduction and equational division of maturation, resulting in the emergence of the haploid pronucleus. Two identical nuclei appear as a result of the mitotic division, and fuse to yield a diploid nucleus. The egg harboring the diploid nucleus undergoes further development. Such a cytogenetic mechanism results in the occurrence of dissimilar individuals (P ♀ ZW,Aa → Fp ♂ ZZ,AA ♂ ZZ,aa) of a male sex only, because the eggs of WW,AA and WW,aa genotypes perish. At the same time the number of off-spring types would increase in proportion to the square or the heterozygous allele pairs, whereas the specimens would differ from each other in combination with different alleles (AA, Aa, aa).

The current paper presents an analysis of the charac-

ter of the distribution of the hypervariable fragments in the individuals produced through ameiotic and meiotic parthenogenesis that has for the first time been made by applying the DNA fingerprinting method. It has been shown that the specimens of the parthenogenetic strain heterozygous for many alleles, though genetically identical and reproduced via ameiotic parthenogenesis, reveal identical spectra of hybridization with hypervariable DNA sequences in two successive parthenogenetic generations. This is indicative of high reproducibility of the method that adequately detects a genotype. In producing off-spring from the females of a parthenogenetic strain through parthenogenesis of meiotic type (males) splitting by alleles takes place. Accordingly high individual polymorphism of hybridization spectra is observed. A sufficiently large difference detected in the DNA spectra between closely related mothers and sons, that arose only on the basis of the mother's genotype, shows a high resolution level for the DNA fingerprinting method for the study of genotypic variability. Accuracy of DNA fingerprinting is sufficiently high to permit a thorough genomic analysis of the silkworm to be made. Its application is deemed to be promising for passportization of strains and parthenogenetic strains of the silkworm in selection works.

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