RAPD Technique in Silkworm (*Bombyx mori*) : Strain Differentiation and Identification

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Abstract

Fifty 10-mer primers of a total 244 screened gave positive responses to DNAs of five morphologically different strains of silkworm. Of the sum of 381 bands, 335 were clearly polymorphic. Some of the DNA fragments were strain specific and some could differentiate the multivoltine from the bivoltine strains or vice versa. The genetic similarity among the strains and their phylogenetic relationships were discussed.

1. Introduction

Since the discovery in 1990 of the random amplified polymorphic DNA or RAPD trchnique [1], it has been extensively used for several purposes, for examples, individual or strain identification [2],[3],[4]; genetic variation of populations [5],[6],[7]; linkage gene map and the phylogenetic relationship [8],[9],[10]. The RAPD technique was first introduced to construct a first linkage map of RAPD in the silkworm by Promboon *et al.* [11].

Silkworm is a domesticated insect having been cultured for a period of over 5000 years [12]. Consequently numbers of strains have been conserved and improved. The longest period of the life cycle is the larval stage and often requires strain identification to avoid contamination. Differentiation usually employs larval markings though this is not always possible. Farmers in Thailand generally rear two races of worms, the commercial bivoltine variety and the local multivoltine strains. For breeders, a number of conspecific lines are often kept for breeding purposes. This superimposes the maintaining load especially the multivoltine race. The feasibility study on the possibility of using the RAPD technique to determine the genetic diversification, strain identification and tracing the ancestry of individual strains is particularly important.

2. Materials and Methods

2.1 Silkworm

The silkworm employed here comprised five strains which could be distinguished morphologically. These were three strains of multivoltine type, two of which were the indigenous Thai (NangLueng and NangLai) and a strain (S_5) evolved in the Genetic Laboratory, Kasetsart University. The other two were improved bivoltine strains, namely KU105 and KU106. Both are of commercial value and have Chinese (KU 106) and Japanese (KU105) origins. All strains had been inbred in the laboratory for more than 25 generations. Three individual worms represented each strain.

2.2 RAPD analysis

Genomic DNA was derived from the posterior silk glands of the healthy fifth instar larvae at the third or fourth feeding day

depending on the race. The DNA extraction procedure followed that of the *Drosophila* method by Zyskind and Bernstein [13].

The PCR reaction mixture of 15 µl contained; 150 ng of genomic DNA; 0.2 mM each of dATP, dCTP, dGTP and dTTP (Amresco, Ohio); 24.75 pg of 10-mer primer (Operon Technologies, Alamedo, CA); and 0.375 unit of Taq polymerase (Perkin-Elmer, Cetus). 100 µl of mineral oil was overlaid on the mixture to prevent evaporation. Amplification conditions were set according to Williams *et al.* [1] on a thermal cycler (Astec-PC70, Tokyo). The resulting amplified DNA products were analyzed on 2 percent agarose gel employing 1 kb ladder (Life Technologies, Gaithersburg, MD) as a molecular weight standard. The gels were then stained with ethidium bromide and photographed with polaroid 665 film under UV light.

2.3 Data analysis

The RAPD patterns of individual strains were compared to determine the sizes and presence (scored as 1) or absence (scored as 0) of DNA fragments: The genetic similarities among the strains were calculated pairwisely by the formula $M = N_{ab}/N_T$ [8],[14]. N_{ab} is the total number of matches (both present or absent) in the strains, a and b. N_T is the total number of DNA fragments scored. M is equal to 1 when the two strains have an identical fragment pattern, and equal to 0 when there were no common bands between the two. The dendrogram was constructed by the PAUP version 3.1 computer package by Swofford (1990).

3. Result

Of the 244 oligonucleotide primers, 50 yielded series of amplified polymorphic DNA fragments of varying length. The number of fragments per primer ranged from the maximum of 18 discrete bands in OPW-19 to the minimum of one in OPK-12, OPK-16, OPY-7 and OPY-10. The average number of bands per primer was 7.62±4.65. Among the total 381 bands produced, most appeared sharp but had intensity variation. The sizes of amplified DNA fragments were in a range of >100 bp to <2000 bp. Very few fragments exceeded 2000 bp. Forty six (12%) of the total bands, common to all the five strains, were regarded as monomorphic. Some of the 335 polymorphic bands were unique and could be used to discriminate the bi- from the multivoltine varieties (Figure 1 and Table 1). Furthermore, some were strain specific (Table 2).

Table 1. Primers with conserved DNA fragment sizes which can be used to delineate the multivoltine and bilvoltine strains of silkworm.

Multi from Bi
OPL-02: 336-459-613-808-905 OPL-18: 485-514-789 OPN-05: 455-1,018-1,639 OPX-06: 362-553-640 OPY-18: 485-514-789
Bi from Multi
OPL-17: 652-903-1,484 OPN-05: 984-1,130-1,583

Note: Primers with less than 3 DNA markers were not included.

Table 2. Strain specific DNA fragment markers analysed from the total scored bands yielded by 50 decamer primers.

Strains	Primers and DNA fragment markers (bp)
NangLuen g	OPL-06: 476-681-915 OPM-02: 598-934-1,173 OPM-12: 982-1,283-1,459 OPY-20: 298-658-730
S_5	OPL-05: 498-1,383 OPK-19: 298-475
NangLai	OPK-19: 370-514-586-622-874 OPX-19: 682-850-1,327 OPN-10: 287-514-544 OPY-14: 344-514-874
KU106	OPU-18: 1,155-1,361-1,876 OPU-19: 640-672-1,293
KU105	OPL-12: 561-778-995

Note: where posible primers which gave less than 3 specific fragments were not included.

The genetic similarity (M value), among the strains used in the present work indicated the close similarity between KU105 and KU106 (M=0.775). Both were bivoltine in nature and

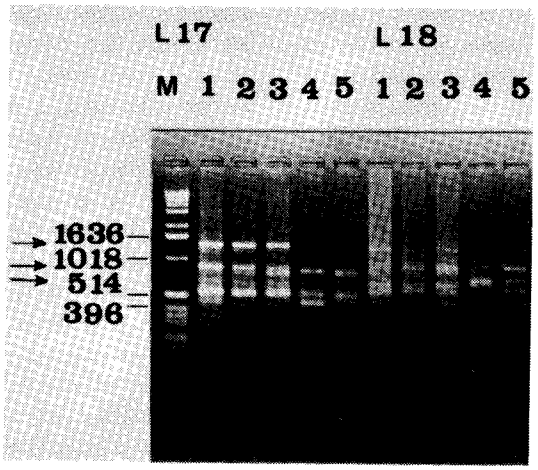

Figure 1. DNA fingerprints (after PCR with primer OPL-17 and OPL-18) of five strains of silkworm. Arrows indicate the bands which can differentiate the multivotine from the bivoltine (OPL-17) and vice versa (OPL-18). Lane 1-NangLueng, 2-S_5, 3-NangLai, 4-KU106, 6-KU105 and M represents markers.

made good pairing in the F_1 egg production. Within the three strains of the multivoltine variety, NangLueng was relatively closer to S_5 (M=0.668) than to NangLai (M=0.592). The multivoltine and bivoltine groups had a rather remote relationship since the genetic similarities of any pairwise comparisons went below 50 percent. The exceptionally close similarity of S_5 to KU106 and KU105 (M=0.546 and 0.537 respectively) was noted. On the contrary, NangLai and KU105 were the most distantly related strains with the M as low as 0.363. Most results of genetic similarity conformed well with the dendrogram profile, constructed by the PAUP program (Figure 2). Accordingly, KU105 and KU106 shared the common ancester while between the multi- and bivoltine groups, S5 was the linker. NangLueng existed as the least similar to the bivoltine race.

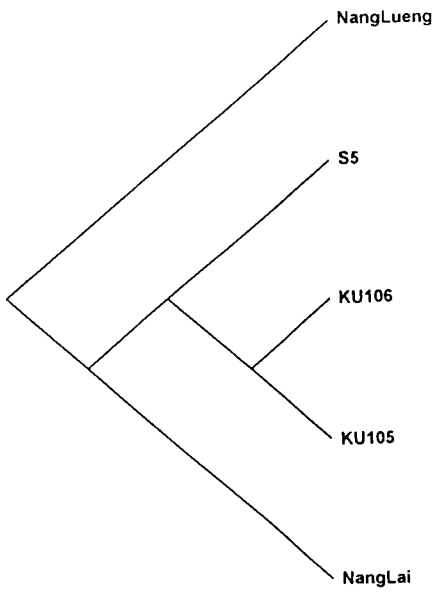

Figure 2. The dendrogram based on The PAUP computer package. [Note : S_5 is the linker between the groups of multivoltines (NangLueng, S_5 and NangLai) and bivoltine (KU106 and KU105)]

4. Discussion

It was apparent in this study that RAPD technique was sensitive enough to detect differences between strains of silkworm in which differentiation is not always possible

morphologically. Of the arbitarily chosen three multivoltine strains used in the present work, besides the lemon body color in larvae of NangLueng and the zebra stripes in NangLai, all other characteristics were indistinguishable. Moreover, other larval markings were not present in the multivoltine lines in Thailand. This caused problems in maintaining them as inbred lines when numbers of strains have to be reared simultaneously. Therefore, the possible use of conserved fragments to distinguish the multivoltine from the bivoltine strains and vice versa, and to recognise certain strains by specific DNA markers poses a particularly useful tool for the future patent of those silkworm strains having commercial value. Such RAPD fingerprints have been successfully applied to identify individuals at the levels of species and populations in several organisms for examples; nematodes [15], mosquitoes [3],[8], and fish [16].

The genetic similarity among the five strains of silkworm in the present study agreed completely with the strain origins. KU106 and KU105 were derived from the bivoltine root and showed high similarity in their genetic constituents. Equally was the multivoltine group as justified by the M value resulting from comparisons between any pair of the Thai local strains. The relatively high M values between the Thai and the Chinese variety (KU106) reflected a close relationship between them while a rather remote relation appeared between the Thai and the Japanese variety (KU105). In general, Chinese strains bearing economically useful charactersistics resembled the multivoltine strains. The genetical similarity information obtained from the RAPD analysis could help breeders to minimise the number of multivoltine strains to be kept for future breeding stocks.

The genetic similarity (GM) and the derived dendrogram (DD) corresponded well with each other even though the basis of calculation differed as discussed in detail by Black [14]. S_5 was a linker between the Thai local race and the introduced bivoltine race. The underlying reason lay in it's derivation. The strain has evolved from a mixed population of 5 (lines) multi- and 2 (lines) bivoltine parents (composite parents) and consecutive selection was performed thereafter for more than 8 years. From the morphology and the feeding behavior we believe that NangLai is also an improved strain through a very long period of selection. On the other hand, NangLueng is an indigenous strain and hence essentially expresses the most remote relationship to the bivoltine race according to the RAPD dataset. The only difference between the GM and DD lay on the least genetic similarity of NangLai and the bivoltine race whereas in the DD it was NangLeng. The phylogenetic tree obtained in the present study suggested the possible use of the RAPD technique in a strain evolutionary study providing that a lot of RAPD data has been accumulated. Thus, Kambhampati *et al.*[8] could not deduce the ancestral relationship between the two subgroups of *Aedes* mosquitoe using just 2 arbitrary random primers and neither did Black [14] in the leafhopper.

5. Acknowledgements

A part of this work was supported by the Institute of Research and Development of Kasetsart University. We are grateful to Dr. Toru Shimada, University of Tokyo, for his helpful suggestions and for providing us with some materials to make this work possible.

6. References

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