有用昆虫の可動因子 (1)

誌名	The Japanese journal of genetics
ISSN	0021504X
著者	木村, 澄 奥村, 隆史 蜷木, 理
巻/号	68巻1号
掲載ページ	p. 63-71
発行年月	1993年2月

Jpn. J. Genet. (1993) **68**, *pp.* 63–71

Transposable elements in commercially useful insects: I. Southern hybridization study of silkworms and honeybees using *Drosophila* probes

Kiyoshi KIMURA[1,3,*], Takashi OKUMURA[1], Osamu NINAKI[2], Margaret G. KIDWELL[3] and Koichi SUZUKI[4]

[1]*Apiculture Laboratory, Department of Animal Genetics, National Institute of Animal Industry, Tsukuba Norin Danchi P.O. Box 5, Ibaraki 305, Japan*
[2]*Matsumoto Branch, National Institute of Sericulture and Entomological Science, Matsumoto, Nagano 356, Japan*
[3]*Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721, USA*
[4]*Laboratory of Applied Entomology, Faculty of Agriculture, Iwate University, Morioka 060, Japan*

(*Received 29 January 1993*)

ABSTRACT

As a first step in surveying transposable elements in silkworms and honeybees, hybridization analyses were carried out using 16 known families of *Drosophila* transposable elements as probes. *jockey* and *G* were the only transposable elements that hybridized with genomic DNA of either honeybees or silkworms under the conditions of this study. *jockey* hybridized with genomic DNA of both European honeybees (*Apis mellifera*) and silkworms (*Bombyx mori* and *Antheraea yamamai*) and showed significant bands in Southern blots. Banding patterns were highly polymorphic. *jockey* did not, however, hybridize with any strains of the Asian honeybee (*A. cerana*). *G* elements showed a faint signal with the Asian honeybee, but not with any other insects tested. The results suggest that, even though it has some limitations, this approach can be used in practice as a first preliminary step in surveys for the presence of transposable elements in organisms which do not have good genetic information.

1. INTRODUCTION

Recent developments in biotechnology offer several approaches for the genetic manipulation of silkworms, honeybees and other commercially important insects. In particular, direct DNA transformation provides a promising method for improvement of these insects. However, although several attempts to transform silkworms and honeybees have been reported (Miline et al., 1986; Tamura et al., 1990), a major problem in developing a transformation system to introduce DNA into these insect genomes is the absence of a suitable vector, such as the *P* element of *Drosophila*.

* Corresponding author

Transposable elements have been identified in a broad range of organisms and some of them are already being used as transformation vectors (Berg and Howe, 1989). Consequently it is reasonable to assume that transposable elements can also be developed as good vectors for silkworms and honeybees.

Only a few transposable elements have previously been reported in silkworms (e.g., Spoerel et al., 1986; Ueda et al.,1986), and none have been reported in honeybees. Therefore, as a first step in the identification of transposable elements suitable for transformation, we surveyed silkworm and honeybee genomes by Southern blot analyses using *D. melanogaster* transposable elements as probes under conditions of low stringency hybridization.

2. MATERIALS AND METHODS

Silkworm strains

Bombyx mori: Seven commercially-used strains were surveyed: e21, e30, N48, C440, N52, N789 and N43. All strains were provided by the Silkworm Stock Center in Matsumoto, Japan. Strain e21 was used as a standard in this experiment.

Antheraea yamamai: A wild strain from Morioka, Japan, was used. This strain has been maintained for 2 years in the laboratory of one of us (K.S.) at Iwate University. *A. yamamai* is a native Japanese silkworm species and is often used for special silk production in Japan.

Honeybee strains

Apis mellifera: A wild strain of *A. mellifera* was kindly provided by the Carl Hayden Bee Research Center in Tucson, Arizona.

Apis cerana japonica: A wild strain captured at Iwate Prefecture, Japan, was kindly provided by Mr. Seita Fujiwara, Morioka, Japan. Other local species of *Apis cerana* were obtained from three regions of Asia (Nepal, Thailand and Malaysia). These were gifts of Dr. Masami Sasaki (Tamagawa University Honeybee Research Center in Tokyo, Japan).

Plasmids

Sixteen transposable elements from *D. melanogaster* were used in this study (see Table 1). All vector plasmids, except *pogo*, were from the laboratory collection of one of us (MGK). *pogo* was the gift of Dr. Kevin O'Hare. Plasmid DNAs were purified by chromatography on pZ523 columns (5′-3′ Inc., Paoli, Pennsylvania) (Zervos et al., 1988).

DNA extraction and labeling

Genomic DNAs were isolated from larvae and/or adults following standard procedures for *Drosophila* with a little modification (Daniels and Strausbaugh,

1986).

Southern blotting procedures

Specific DNA fragments to be used as probes were isolated using DEAE membranes (NA-45 paper, Schleicher & Schuell) and labeled with digoxigenin by random primed method using the "Genius kit" (Bohringer and Mannheim).

Genomic hybridization was performed by the method recommended by the manufacturer (Schleicher & Schuell) for Nytran membranes.

Genomic DNA samples were digested with appropriate restriction enzymes and electrophorased on 0.9% agarose gels with a TBE buffer.

Hybridization was performed by the methods recommended for the "Genius kit", by the manufacturer (Bohringer and Mannheim) with modification to reduce the stringency. (Washings after hybridization were performed at 42°C with high salt solution (1 M NaCl, 0.1% SDS), instead of the recommended conditions (low salt solution (0.1 M NaCl, 0.1% SDS) at 65°C).

Table 1. Summary of the results of hybridization experiments

Transposable element	Reference	*A. mellifera*	*A. cerana*	*B. mori*	*A. yamamai*
Class I[1)]					
copia (pcDm5002)[2)]	Dunsmuir et al. (1980)	−	−	−	−
297 (pcDm4006)	Rubin et al. (1981)	−	−	−	−
412 (pcDm2042)	Rubin et al. (1981)	−	−	−	−
mdg-1	Ilyin et al. (1980a)	−	−	−	−
mdg-3	Ilyin et al. (1980b)	−	−	−	−
gypsy (pGYPSY)	Modolell et al. (1983)	−	−	−	−
F (pA22.7)	Karess and Rubin (1982)	−	−	−	−
I (pI407)	Bucheton et al. (1984)	−	−	−	−
D (p3.8BTE)	Pittler and Davis (1987)	−	−	−	−
G (pG3A)	Di Nocera et al. (1986)	−	+	−	−
jockey (pJpN)	Mizrokhi et al. (1988)	++	−	++	++
Class II					
P (pπ25.7BWC)	O'Hare and Rubin (1983)	−	−	−	−
hobo (pH 101)	Balckman et al. (1987)	−	−	−	−
pogo	Tudor et al. (1992)	−	−	−	−
HB 1	Potter (1982)	−	−	−	−
FB (pFB3-BS4.1)	Potter et al. (1980)	−	−	−	−

[1)] Transposable elements are classified according to Finnegan (1989).

[2)] Names of the plasmids are shown in parentheses if available.

[3)] —: negative at low and normal stringencies; +: positive at low stringency; ++ :positve at normal stringency.

3. RESULTS

We first looked for the presence of sequences homologous to the 16 transposable elements shown in Table 1 in one strain from each of *B. mori* (e21), *A. yamamai*,

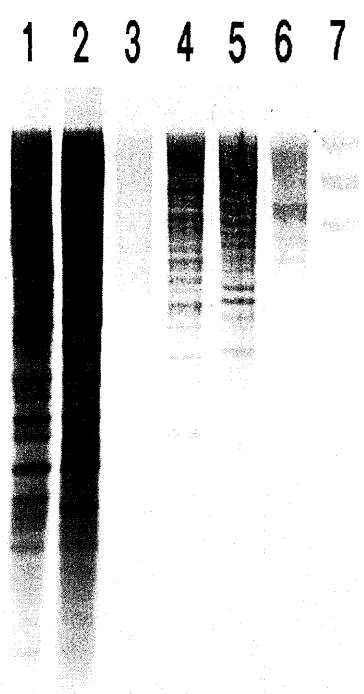

Fig. 1. Southern blot analysis of insect genomic DNAs probed with the *D. melanogaster jockey* element. Genomic DNAs were digested by the restriction enzyme *Bam*HI. Hybridization was performed under conditions of low stringency. Lanes are as follows: 1. *D. melanogaster* Harwich; 2. *D. melanogaster* Canton S; 3. *A. cerana japonica*; 4. *A. mellifera*; 5. *B. mori* e21; 6. *A. yamamai*; 7. λ/*Hin*dIII size marker. Under these conditions the λ/*Hin*dIII marker can be seen on the blot. For more details see text.

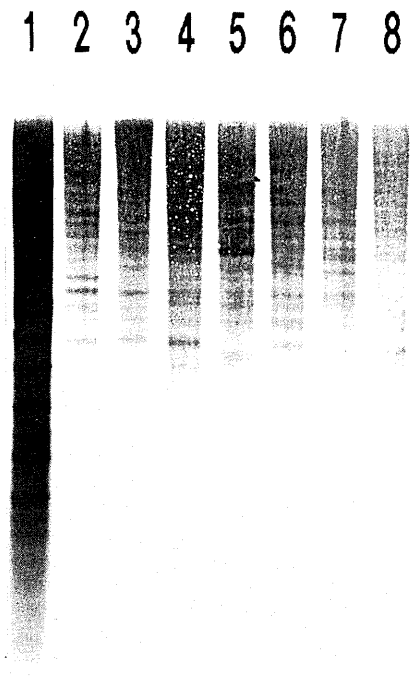

Fig. 2. Southern blot analysis of genomic DNAs of several strains of silkworms probed with the *D. melanogaster jockey* element. Genomic DNAs were digested by the restriction enzyme *Bam*HI. Hybridization was performed under conditions of low stringency. Lanes are as follows: 1. *D. melanogaster* Canton S; 2. *B. mori* e21; 3. *B. mori* e30; 4. *B. mori* N48; 5. *B. mori* C440; 6. *B. mori* N52; 7. *B. mori* N789; 8. *B. mori* N43.

A. mellifera, and *A. cerana* by means of Southern analysis. The results are summarized in Table 1.

No elements except *jockey* and *G* elements hybridized to either honeybees or silkworm genomic DNA under the conditions used in this experiment. The *jockey* element probe showed relatively strong hybridization bands with both *A. mellifera* and *B. mori* (Fig. 1). From the density of the *Drosophila* control bands (lanes 1 and 2) and those of the λ/*Hin*dIII markers (lane 7), the low stringency of the conditions can be seen. Neither *A. cerana japonica* nor *A. cerana* strains from other countries showed any signal under these conditions (data not shown).

Hybridization bands to the *Drosophila jockey* element were also seen for *A. mellifera* and *B. mori* when higher stringency conditions were applied (data not shown). Southern banding patterns showed a high degree of polymorphism among *B. mori* strains (Fig. 2) suggesting that the *jockey* element may be transposing in this species. This explanation seems more likely than one involving a restriction enzyme polymorphism because there is so much variability.

Using the *G* element probe, faint bands are seen with *A. cerana japonica* (Fig. 3), but not with other species. Bands can be seen in the *B. mori* and *A. mellifera* lanes. However, these are at the higher molecular weight than the 22 kb

Fig. 3. Southern blot analysis of insect genomic DNAs probed with the *D. melanogaster G* element. Genomic DNAs were digested by the restriction enzyme *Hin*dIII. Hybridization was performed under conditions of low stringency. Lanes are as follows: 1. *D. melanogaster* Harwich; 2. *D. melanogaster* Canton S; 3. *A. cerana japonica*; 4. *A. mellifera*; 5. *B. mori*.

fragment (the largest fragment of λ/*Hin*dIII marker) and these are single bands. Therefore, it is quite unlikely that these bands are *G* element-like sequences. Neither *B. mori* strains nor *A. yamamai* showed any bands at all with the *G* element probe (data not shown).

4. DISCUSSION

It is a common practice to screen for non-mobile gene sequences using homologous sequences from other, related, organism as probes. In a similar way transposable element sequences can be used to detect the presence of homologous sequences. This approach was previously used by de Frutos et al. (1992) in an intergeneric survey of transposable elements in the obscura group of *Drosophila* using *D. melanogaster* elements as probes. We have extended this approach to screen for transposable elements in insects from different families than *Drosophila* and demonstrated its feasibility at the inter-order level.

There are several merits to this kind of approach. First, the method is quick, easy and inexpensive, provided that suitable transposable element probes from other species are available. Second, no mutant strains or genetic information about the target species are required for this approach. Many transposable elements in well-studied species, such as *Drosophila*, have been identified as insertional mutants in previously identified genes. Even though many mutations have been reported in honeybees, no established mutant strains of honeybees can be available now. That has severely restricted the types of study that can be made.

Apart from its advantages, this method also has some limitations. For example, it is dependent on the use of previously identified element sequences and, therefore, cannot be used to find new elements. Further, if the transposable elements in two species of organisms shares only a part of their sequence, the method of Southern blotting may not be powerful enough to pick up identifiable signals. In a similar way, complete absence of an element family cannot be concluded on the basis of negative results. Absence of visible bands may merely reflect the hybridization conditions in a particular experiment. Obviously, the use of alternative techniques should be tried in further surveys. An alternative approach using PCR may also be considered. However, it is often tricky to make suitable primers for target organisms. Also this method shares some of the drawbacks of the Southern hybridization method in being dependent on the existence of previously discovered elements.

Over all, our approach appears to be adequate as the first step of a survey and, when followed by other approaches, such as PCR, together with cloning and sequencing, should provide a start to the successful identification of additional transposable elements.

Eukaryotic transposable elements may be divided into two classes based on

their transposition mechanism (Finnegan, 1989). Class I elements transpose via RNA intermediates and Class II elements transpose directly from DNA to DNA. Our final goal is to find transposable elements which can be used as transformation vectors. Most of transposable element used as transformation vector in other species belong to Class II elements (e.g. *P* element of *Drosophila*, and the *Ac*/*Ds* elements of maize). Both *jockey* and the *G* element belong to the Class I group. It is therefore uncertain whether they can be adapted for use as efficient transformation vectors. However, even if these element can not be used as transformation vectors, they may still be useful as genetic markers or for other purposes. Genetic markers would be particularly valuable in honeybees because at present there are only a few cloned genes or other markers.

The *jockey* and *G* elements are similar to *LINE* elements of various organisms in their structural organization and coding potential (Priimagi et al., 1988). Transposable elements of this group are known to share a high degree of sequence homology, particularly *jockey* and the *F* element. However, it should be noted that the *LINE* elements, *I*, *D* and *F*, did not show any signal when used as probes to genomic DNA of either honeybees or silkworms in our experiments.

Evidence for horizontal transfer of many different types of transposable elements has been accumulating recently (for review see Kidwell (1992)). There is previous evidence that the *jockey* element may have been horizontally transferred between *Drosophila funebris* and *Drosophila melanogaster* (Mizrokhi and Mazo, 1990). Based on the absence of *jockey*-like sequences in *A. cerana* and their presence in *A. mellifera*, it is possible that *jockey* homologous sequences may also have been transmitted from another organism to *A. mellifera*, but not to its sibling species, *A. cerana*. In order to test this hypothesis, *jockey*-like sequence will be screened in other *Apis* species.

In summary, although these results illustrate the limitations of our approach, they also show that surveys employing Southern blot hybridization can be a satisfactory method for preliminary studies of transposable elements in evolution of these element as well as for more general studies of the evolution of these elements.

We thank Dr. K. O'Hare for the generous gift of *pogo* plasmids. We also wish to thank Drs. Mark Q. Benedict and Jonathan B. Clark for helpful comments on earlier drafts of the manuscript.

REFERENCES

Berg, D. E. and Howe, M. (eds.) (1989). *Mobile DNA*, American Society of Microbiology Publications, Washington DC.

Blackman, R. K., Grimaila, R., Macy, M., Koehler, D. and Gelbart, W. M. (1987). Mobilization of *hobo* elements residing within the decapentaplegic gene complex. suggestion of a new hybrid dysgenesis system in *Drosophila melanogaster*. *Cell* **49**, 497–505.

Daniels, S. B. and Strausbaugh, L. D. (1986). The distribution of *P* element sequences in *Drosophila*: the *willistoni* and *saltans* species groups. *J. Mol. Evol.* **23**, 123–148.

de Frutos, R., Peterson, K. P. and Kidwell, M. G. (1992). Distribution of *Drosophila melanogaster* transposable element sequences in species of the *obscura* group. *Chromosoma* **101**, 293–300.

Di Nocera, P. P. and Dawid, I. B. (1983). Interdigitated arrangement of two oligo(A)-terminated DNA sequences in *Drosophila*. *Nucleic Acids Res.* **11**, 5475–5482.

Dunsmuir, P., Brorein, W. J. Jr., Simon, M. A. and Rubin, G. A. (1980). Insertion of the *Drosophila* transposable element *copia* generates a 5 base pair duplication. *Cell* **21**, 575–579.

Finnegan, D. J.(1989). Eukaryotic transposable elements and genome evolution. *Trends Genet.* **5**, 103–107.

Ilyin, Y. V., Chmeliauskite, V. G., Ananiev, E. V., Lyubomirskaya, N. V., Kulguskin, V. V., Bayev, A. Jr. and Georgiev, G. P. (1980a). Mobile dispersed genetic element mdg1 of *Drosophila melanogaster*: structural organization. *Nucleic Acids Res.* **8**, 5333–5346.

Ilyin, Y. V., Chmeliauskite, V. G., Ananiev, E. V. and Georgiev, G. P. (1980b). Isolation and characterization of a new family of mobile dispersed genetic elements, *mdg3*, in *Drosophila melanogaster*. *Chromosoma* **81**, 27–53.

Karess, R. E. and Rubin, G. M. (1982). A small tandem duplication is responsible for the unstable *white-ivory* mutation in *Drosophila*. *Cell* **30**, 63–69.

Kidwell, M. G. (1992). Horizontal transfer. *Curr. Opin. Genet. Develop.* **2**, 868–873.

Miline Jr., C. P., Phillips, J. P. and Krell, P. J. (1988). Microinjection of early honeybee embryos. *J. Apic. Res.* **27**, 84–89.

Mizrohki, L. J., Georgieva, S. G. and Ilyin, Y. L.(1988). *jockey*, a mobile *Drosophila* element similar to mammalian LINEs, is transcribed from the internal promoter by RNA polymerase II. *Cell* **54**, 685–691.

Mizrohki, L. J. and Mazo, A. M. (1990). Evidence for horizontal transmission of the mobile element *jockey* between distant *Drosophila* species. *Proc. Natl. Acad. Sci. USA* **87**, 9216–9220.

Modollel, J., Bender, W. and Meselson, M. (1983). *Drosophila melanogaster* mutations suppressible by the suppresser of *hairy-wing* are insertions of a 7.3-kilobase mobile element. *Proc. Natl. Acad. Sci. USA* **80**, 1678–1682.

O'Hare, K. and Rubin, G. M. (1983). Structures of *P* transposable elements and their sites of insertion and excision in the *Drosophila melanogaster* genome. *Cell* **34**, 25–35.

Pittler, S. J. and Davis R. L. (1987). A new family of the poly-deoxyadenylated class of *Drosophila* transposable element identified by a representative member at the *dunce* locus. *Mol. Gen. Genet.* **208**, 325–328.

Potter, S. S. (1982). DNA sequence of a foldback transposable element in Drosophila. *Nature* **297**, 201–204

Potter, S., Truett, M., Phillips, M. and Mather, A. (1980). Eucaryotic transposable genetic elements with inverted terminal repeats. *Cell* **20**, 639–647.

Priimagi, A. F., Mizrokhi, L. J. and Ilyin, Y. V. (1988). The *Drosophila* mobile element *jockey* belongs to LINES and contains coding sequences homologous to some retroviral proteins. *Gene* **70**, 253–262.

Rubin, G. M., Brorein, W. J. Jr., Dunsmuir, P., Flavell, A. J., Levis, R., Strobel, E., Toole, J. J. and Young, E. (1981). *Copia*-like transposable elements in the *Drosophila* genome. *Cold Spring Harbor Symp. Quant. Biol.* **45**, 619–628.

Spoerel, N., Nguyen, H. T. and Kaftos F. C. (1986). Gene regulation and evolution in the *Chorion* locus of *Bombyx mori*: structural and developmental characterization of four egg shell genes and their flanking DNA regions. *J. Mol. Biol.* **190**, 23–35.

Tamura, T., Kanda, T., Takiya, S., Okano, K. and Maekawa, H. (1990). Transient expression of chimeric CAT genes injected into early embryos of the domesticated silkworm *Bombyx mori*. *Jpn. J. Genet.* **65**, 401–410.

Tudor, M., Lobocka, M., Goodell, M., Pettitt, J. and O'Hare, K. (1992). The *pogo* transposable

element family of *Drosophila melanogaster*. *Mol. Gen. Genet.* **232**, 126–134.

Ueda, H., Mizuno, S. and Shimura, K. (1986). Transposable element found in the 5'-flanking region of the fibroin H-chain Gene in a genomic clone from the silkworm *Bombyx mori*. *J. Mol. Biol.* **190**, 319–327.

Zervos, P. H., Morris, L. M. and Hellwig, R. J. (1988). A novel method for rapid isolation of plasmid DNA. *BioTechniques* **6**, 238–242.