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GENERAL GENETICS

Length Polymorphism of Integrated Copies of *R1* and *R2* Retrotransposons in the German Cockroach (*Blattella germanica*) as a Potential Marker for Population and Phylogenetic Studies

A. S. Kagramanova[a], A. L. Korolev[a], C. Schal[b], and D. V. Mukha[a]

[a] *Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow, 119991 Russia; fax: (495) 135-12-89; e-mail: myxa@vigg.ru*

[b] *Department of Entomology and W.M. Keck Center for Behavioral Biology, North Carolina State University, Raleigh, NC 27695, United States*

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Abstract—Using polymerase chain reaction technique with primers flanking target sites of retrotransposons *R1* and *R2*, integrated copies of these transposable elements were amplified in various cockroach species (Blattodea). It was shown that each species has a unique pattern of "5'-truncated copies" with the definite set of amplified fragments of different lengths. Intraspecies polymorphism was revealed in analysis of German cockroach specimens obtained upon individual mating. This is the first report providing results of identifying, cloning, and sequencing extended fragments (5'-truncated copies) of *Blattella germanica R1* and *R2* retrotransposons. It may be assumed that patterns of 5'-truncated copies of *R1* and *R2* elements can be used as markers in population and phylogenetic studies. Moreover, cloned and sequenced fragments will be employed in our further studies for screening of the German cockroach genomic library in order to detect full-length copies in this class transposable elements.

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INTRODUCTION

Transposable genetic elements were detected in virtually all examined eukaryotes, a proportion of genomic DNA reaching several tens percent in these elements.

Clusters of ribosomal genes in practically all arthropods, specifically, in insects described so far, contain *R1* and *R2* transposable elements. Both elements represent two different families, lacking long terminal repeats (non-LTR) of retrotransposons, and have unique sites of integration in the sequence of gene 28S of ribosomal RNA (28S rRNA) [1].

Genes of 28S rRNA contain one transposable element (*R1* or *R2*) or both retrotransposons [2, 3]. In *Drosophila melanogaster*, 17–67% of 28S genes contain *R1* elements and 2–28%, *R2* elements, depending on the line. The overall percent of 28S genes containing *R1* or *R2* elements was shown to vary within the range 32–77% [3]. Insertions of both elements lead to inactivation of 28S genes and significantly diminish the level of their transcription [4, 5].

It was found that upon transposition of non-LTR retrotransposons, in particular, *R1* and *R2* elements, their 5' end is often truncated mainly due to a failure of reverse transcriptase, for an unknown reason, to completely read the RNA template of the transposable element. Therefore, 28S genes contain both the integrated full-length functional copies of these elements and the so-called 5'-truncated copies of different lengths, which are predominant but defective. In addition, integration of a DNA copy of the transposable element into the genome is followed by the emergence of nonextended deletions and/or duplications [6].

Unique 5'-junctions allow the translocation dynamics of *R1* and *R2* elements to be traced in *D. melanogaster* populations. For both types of retrotransposons, the patterns of 5'-junctions were determined in various geographical isolates of *Drosophila*, and the described patterns were shown to differ from one another [3]. This result convincingly demonstrates that *R1* and *R2* elements are actively transposed at the species level. Recent studies of isofemale lines of *Drosophila simulans* obtained from the same population [1] also revealed differences between patterns of 5'-junctions in various lines, which suggests that the transposition dynamics of *R1* and *R2* elements is sufficiently high to be noticeable at the level of one population. Meanwhile, the rate of retrotransposition of new copies of these elements into 28S genes is rather low per generation in the *D. melanogaster* population: 12.5×10^{-4} and 0.7×10^{-4} for *R1* and *R2*, respectively [6]. Consequently, patterns of 5'-truncated copies can be successfully used as a marker to characterize populations, especially, in animals with slow alternation of generations.

Phylogenetic analysis of *R1* and *R2* retrotransposons in *Drosophila* showed that the characteristic feature of this type transposable elements is vertical

transfer [7, 8]. Moreover, phylogenetic analysis of *R1* and *R2* retrotransposons in evolutionarily distant taxa of arthropods suggests the horizontal transfer and allows one to determine an approximate time of the origin of *R1* and *R2* elements as 600 Myr [9, 10]. Differences between nucleotide sequences in the 3'-region of *R1* retroelements in *Drosophila* reflect phylogenetic distances between species to which they belong [11].

From our point of view, molecular–genetic study of new, previously unknown species, i.e., those that are not conventional models, is essential for understanding the diversity of genetic processes underlying functional activity and evolutionary variability of transposable elements. As a main subject in this study, we chose a species that has not been examined so far in this respect, *Blattella germanica* (German cockroach), a representative of the most ancient order of insects. The extended fragments of *R1* and *R2* retrotransposons in *B. germanica* were detected, cloned, and sequenced for the first time. We assume that patterns of 5'-truncated copies of *R1* and *R2* elements can be used as markers in population and phylogenetic studies.

MATERIALS AND METHODS

Total DNA of *B. germanica* was isolated by adding sodium perchlorate according to a method described in [12] with some modifications. Insects were lysed in 600 µl of a solution containing 400 mM mTris-HCl, pH 8.0; 60 mM EDTA, pH 8.0; 150 mM NaCl; 1% SDS; proteinase K (700 µg/ml) and incubated at 60°C for 3 h. We added 0.3 vols. of 5 M sodium perchlorate solution to the cell lysate, and the mixture was subjected to vigorous agitation. Next, a 1.5 volume of chloroform was added, the mixture was mixed well for 5–7 min and centrifuged for 5 min at 14 000 rpm. The upper DNA-containing phase was gently transferred to a clean tube, without touching the intermediate layer, and DNA was precipitated using 2 vol. of 96% ethyl alcohol. The DNA precipitate was washed with 70% ethanol, air-dried, and dissolved in 50–100 µl of distilled autoclaved water.

Amplification of DNA fragments 3 to 4 kb was conducted in a volume of 20 µl by means of a set of reagents GenePak™ PCR Core (laboratory Izogen). We used the following amplification regime: preliminary heating at 94°C for 5 min; 30 cycles: 5 min at 95°C, 2 min at 53°C, 3 min at 72°C; final elongation for 7 min at 72°C.

For amplification of DNA fragments with the length higher than 4 kb, we used PCR optimized to conduct amplification of extended DNA fragments (Long-PCR) in a volume of 50 µl with the use of a Long-PCR Enzyme Mix (Fermentas).

Long-PCR conditions: preliminary heating at 94°C for 30 s; 10 cycles: 30 s at 95°C, 30 s at 55°C, 7 min at 68°C; 20 cycles: 30 s at 95°C, 30 s at 55°C, 7 min at 68°C with additional 10 s in each cycle; final elongation for 7 min at 68°C.

PCR was run in a Primus automated thermal cycler (MWG-Biotech). Primers flanking the integration site of *R1* elements were 28S_R1_first, 5'-cgcgcatgaatggattaacg-3' (forward) and 28S_R1, 5'-tgccagactagagtcaagct-3' (reverse) [13]. Primers flanking the integration site of *R2* elements were 28S_R2_first, 5'-gtgctgacgcaatgtgatttc-3' (forward) [7] and 28S_R2, 5'-cgttaatccattcatgcgcg-3' (reverse). The PCR product 5 to 10 µl was fractionated in 1% agarose gel with TAE buffer, pH 8.0 or in 3% polyacrylamide gel in TBE buffer with subsequent visualization of the result of electrophoresis after staining gels with ethidium bromide.

DNA amplification fragments of German cockroach *R1* and *R2* elements were cloned using a plasmid vector pGEM-T-Easy Vector (Promega) as recommended by the manufacturer.

Sequence analysis of cloned DNA fragments was conducted by the Sanger method in an ABI PRISM 310 sequencer with a set of reagents Big Dye Termination KIT V. 3.0 (PE Applied Biosystems, Foster City CA) according to the protocol of the supplier.

We used computer database and Internet resources in the NCBI server (http://www.ncbi.nlm.nih.gov) and GeneBee (http://www.genebee.msu.ru). Programs of comparative analysis of nucleotide sequences Chromas Pro and BLAST were used to search for the sequences identical to sequences recorded in the files of the international database.

RESULTS AND DISCUSSION

Identification of Integrated Copies of R1 and R2 Elements in Representatives of the Order Blattodea

Using polymerase chain reaction with two pairs of primers flanking (respectively) integration sites of retrotransposons *R1* [13] and *R2*, we amplified integrated copies of these transposable elements in divergent insect species: (1) *B. germanica*; (2) *Supella longipalpa*; (3) *Periplaneta australasiae*; (4) *Periplaneta brunea;* (5) *Periplaneta fuliginosa*; (6) *Parcoblatta lata;* (7) *Cryptocerus punctulatus*; (8) *D. melanogaster*; and (9) *Adalia bipunctata.* The first seven species are representatives of the order Blattodea, including the main subject in this study, the German cockroach *B. germanica*, whereas the latter two species are taxons evolutionarily distant from this order: *D. melanogaster* (order Diptera) and *A. bipunctata* (order Coleoptera). As seen in Figs. 1a and 1b, each species has the individual set of 5'-truncated copies of *R1* and *R2* elements with sizes varying within the wide range from approximately 250 bp to about 2.7–3.0 kb and from approximately 500 bp to about 1.8 kb, respectively; note that some species differed from others in that they manifested major and minor variants (lanes *2, 3, 4, 5*, and *8*). The common property of all species is

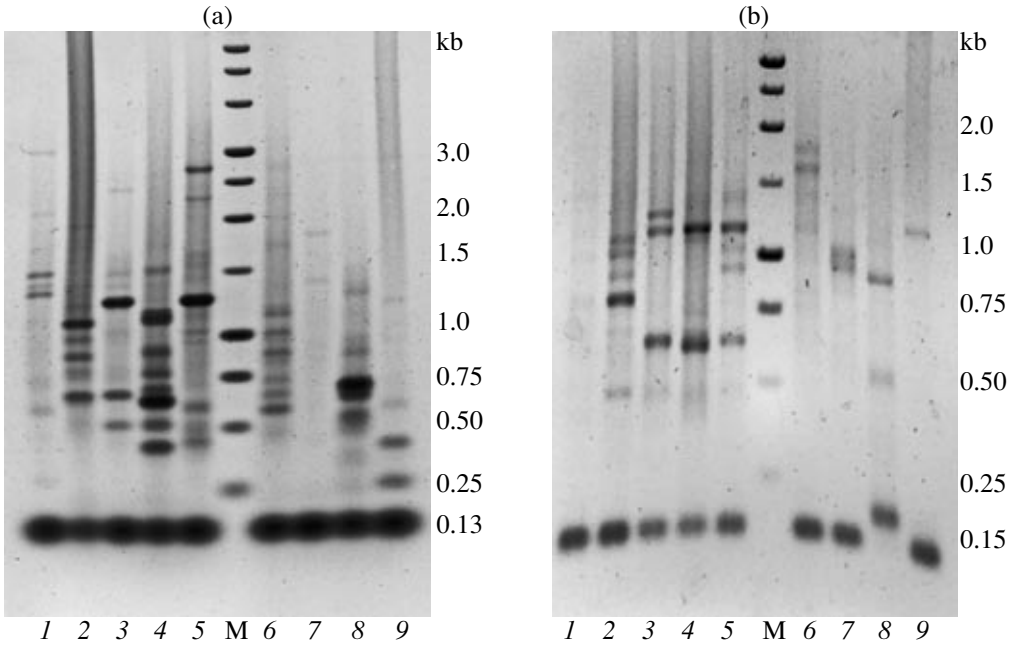

Fig. 1. Result of gel electrophoresis of amplification products from 5'-truncated copies of integrated copies of *R1* (a) and *R2* (b) retrotransposons in divergent insect species. *1*, *B. germanica, 2*, *S. longipalpa*, *3*, *P. australasiae*, *4*, *P. brunea*; *5*, *P. fuliginosa*, *6, P. lata*; *7*, *C. punctulatus; 8*, *D. melanogaster, 9*, *A. bipunctata.* M, marker DNA.

the justified presence of 130- and 150-bp bands (for *R1* and *R2* transposable elements, respectively), i.e., fragments of 28S rDNA lacking insertion.

Previous works demonstrated that differences between nucleotide sequences in the 3'-region of *R1* retroelements in *Drosophila* reflect phylogenetic distances between species to which they belong [11]. Possibly, a comparative analysis of nucleotide sequences of *R1* and *R2* elements can be used in phylogenetic studies of other insect species, in particular, representatives of the order Blattodea, whose phylogenetic relationships remain poorly studied.

In addition, we identified intraspecies polymorphism in patterns of 5'-truncated copies of *R1* elements.

Figure 2 presents the result of electrophoresis in polyacrylamide gel of DNA amplification products for individuals of the first generation (females and males) and their parents (*P*♀, female; *P*♂, male). The cluster of *B. germanica* ribosomal genes is known to locate in the X chromosome; sex regulation: X0, male; XX, female. As seen in Fig. 2, males of the first generation inherit one of X chromosomes from the female parent, whereas females of the first generation inherit the X chromosomes from both parents. Note that there is a difference in the intensity of staining a 0.5-kb band between the male parent (marked by the asterisk in Fig. 2) and his daughters: the relatively low amount of this fragment was observed in females of the first generation. Apparently, this is conditioned by properties of the multiplex PCR reaction. Upon simultaneous amplification of fragments varying in length within a wide range, nonspecific underamplification of definite DNA regions may be observed.

Three identified variants of 5'-undertranscript patterns of *R1* elements in males of the fourth generation

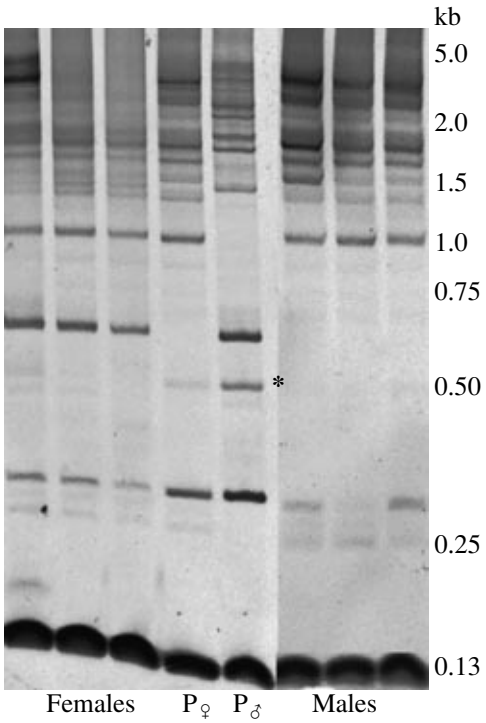

Fig. 2. Inheritance of 5'-truncated copies in *B. germanica* males and females of the first generation obtained upon individual mating. *P*♀, female parent, *P*♂, male parent.

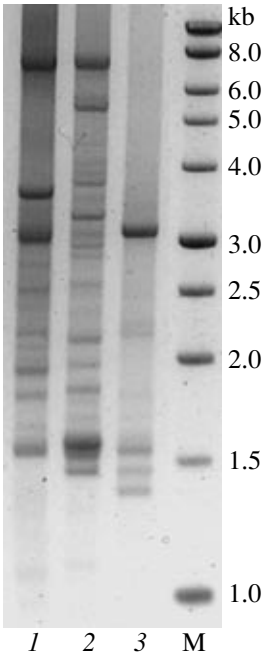

Fig. 3. Intraspecies polymorphism of patterns of 5'-truncated copies in *B. germanica* males of the fourth generation obtained upon individual mating. Lanes *1, 2, 3* appearing after gel electrophoresis of DNA amplification products obtained with primers 28S_R1_first/28S_R1 from three males, respectively. M, marker DNA.

obtained in another individual mating are shown in Fig. 3. The patterns presented apparently correspond to three variants of X chromosomes inherited by these males from the original parents. Since the PCR optimized for amplification of extended DNA fragments was used in this case, we succeeded in detecting copies that were more than 3 kb in size (including those of 7 kb). Amplified fragments of about 7 kb seem to correspond to full-length functional copies of *R1* elements. By comparing patterns with each other, we found that they do not exhibit any resemblance in the size of 5'-truncated copies; each pattern was unique. Probably, the 5'-truncated copies showed stable Mendelian inheritance here. This assumption is likely to be true at a low frequency of both retrotransposition and recombination of transposable elements within the rDNA cluster. It is well may be that each individual population has an individual pattern 5'-truncated copies, which can be used as markers in population studies.

In previous studies on *D. simulans* populations (the cluster of ribosomal genes in *D. simulans* is located, unlike that of *D. melanogaster,* only on the X chromosome [14]), various variants of X chromosomes were also detected in this organism. It was shown that each X chromosome had a unique set of 5'-truncated copies of *R1* elements and that no recombination occurred between these variants.

Analysis of Nucleotide Sequences in Cloned Copies of 5'-Truncated copies of R1 and R2 Retrotransposons of the German Cockroach

Several variants of PCR products with sizes varying from approximately 800 bp to about 3.0 kb obtained using primers 28S_R1_first/28S_R1 and 28S_R2_first/28S_R2 were cloned. We chose seven variants of *R1* clones and six different-sized clones of *R2* transposable elements

(a)

Amino acids: identical 36% (49/134), positive, 58% (79/134)

```
Rt-Bg  2359 WNIALEPILEKLQRLEDLTEVVAFADDILLIVGGRSRAVIEQKATNIISQLHAWCTEVKL 2180
            WNI ++ +L   QRLE L  +  +ADD+LL++ G +R+ +E+K   ++S + AW  EV +
Rt-Da  15   WNILMDVLL---QRLEPLGALSRYADDLLLLIEGNARSELERKGEELMSIVGAWGVEVGV 71

Rt-Bg  2179 SLAPHKTTYMLLRGNLQRDPVIRLGERSLKRGKVTKYLGIHIDEGMRFHDHI-----RLT 2015
             ++  KT  MLL+G L+R P++R    SL     T+YLGI + E + F  HI     RLT
Rt-Da  72   CVSTSKTAIMLLKGILRRPPLVRFAGASLPYNASTRYLGITVGERLSFLPHITGLRDRT 131

Rt-Bg  2014 SAKAKLAMNRIIGI 1973
                 A++R++ +
Rt-Da   132 GVVG--ALSRVLRV 143
```

(b)

Amino acids: identical 50% (46/91), positive, 71% (65/91)

```
Rt-Bg:  692  DVTVRHEDSDYLERARREKIDKYQALLPSLIAQFGATGGEVLPIVVGTRGAMPKLTMEAL
        871  DVTVR+E   YL+  R EKI KY+ +L SL     +   EV+PIV+G+RGA+P+ T +AL
Rt-Nv:  1175 DVTVRYEHRTYLDEGRTEKIGKYRQILSSLRRDLHSNAEEVIPIVIGSRGAIPRETRKAL 1234

Rt-Bg:  872  AQLGITGRGRLKTISLMSLRSSIEIYHGFLD 964
             ++LGI G+    TISL++LRSS+EI + F+D
Rt-Nv:  1235 SKLGI-GKSDWLTISLIALRSSLEIVNAFMD 1264
```

Fig. 4. Comparative analysis with BLAST (blastx) program of the highly conserved domain of reverse transcriptase in: (a) retrotransposons *R1* of *B. germanica* and *D. ambigua*; (b) retrotransposons *R2* in *B. germanica* and *N. vitripennis.* Rt-Bg, Rt-Da, Rt-Nv, highly conserved domains of reverse transcriptase in *B. germanica*, *D. ambigua*, and *N. vitripennis.*

for further analysis and determined their complete sequence. A comparative analysis of the obtained nucleotide sequences and the database of GenBank failed to reveal marked resemblance between them. However, when sequences of amino acids obtained through translation *in silico* of the examined nucleotide sequences were compared, appreciable homology of the fragments with the domain of reverse transcriptase (Rt) of *R1* and *R2* elements in other insects was detected. As an example, in Fig. 4a we present data obtained after comparing amino acids of reverse transcriptase of *R1* and *R2* retrotransposons in *B. germanica* and *Drosophila ambigua* (AAA21257) showing the high percentage of positive amino acids (58%), 36% being identical in all of these amino acids. Figure 4b presents a comparison of the aminoacids of reverse transcriptase of *R2*-retrotransposons in *B. germanica* and *Nasonia* vitripennis (AF090145) with 71% of positive aminoacids, of which 50% are identical. The Rt domain was reported to be a most highly conserved region of this type mobile retrotransposons [2].

Cloned and sequenced fragments of two families (*R1* and *R2*) of retrotransposons belonging to the class of non-LTR elements will be employed in screening the *B. germanica* genomic library for further structure–function analysis of full-length copies in this class of transposable elements.

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REFERENCES

1. Perez-Gonsalez, C.E. and Eickbush, T.H., Dynamics of *R1* and *R2* Elements in the rDNA Locus of *Drosophila simulans, Genetics,* 2001, vol. 158, no. 4, pp. 1557–1567.
2. Jakubczak, J., Xiong, Y., and Eickbush, T.H., Type 1 (*R1*) and Type 2 (*R2*) Ribosomal DNA Insertions of *Drosophila melanogaster* Are Retrotransposable Elements Closely Related to Those of *Bombyx mori, J. Mol. Biol.*, 1990, vol. 212, no. 1, pp. 37–52.
3. Jakubczak, J., Zenni, M.K., Woodruff, R.C., and Eickbush, T.H., Turnover of *R1* (Type I) and *R2* (Type II) Retrotransposable Elements in the Ribosomal DNA of *Drosophila melanogaster, Genetics,* 1992, vol. 131, no. 1, pp. 129–142.
4. Long, E.O. and Dawid, I.B., Expression of Ribosomal DNA Insertions in *Drosophila melanogaster, Annu. Rev. Biochem.*, 1979, vol. 49, no. 3, pp. 727–764.
5. Kidd, S.J. and Glover, D.M., *Drosophila melanogaster* Ribosomal DNA Containing Type II Insertions Is Variably Transcribed in Different Strains and Tissues, *J. Mol. Biol.*, 1981, vol. 151, no. 4, pp. 645–662.
6. Perez-Gonsalez, C.E. and Eicbush, T.H., Rates of *R1* and *R2* Retrotransposition and Elimination from the rDNA Locus of *Drosophila melanogaster, Genetics*, 2002, vol. 162, no. 2, pp. 799–811.
7. Lathe, W.C. and Eickbush, T.H., A Single Lineage of *R2* Retrotransposable Elements Is an Active, Evolutionary Stable Component of the *Drosophila* rDNA Locus, *Mol. Biol. Evol.*, 1997, vol. 14, no. 12, pp. 1232–1241.
8. Gentile, K., Burke, W.D., and Eicbush, T.H., Multiple Lineages of *R1* Retrotransposable Elements Can Coexist in the rDNA Loci of *Drosophila, Mol. Biol. Evol.*, 2001, vol. 18, no. 2, pp. 235–245.
9. Malik, H.S., Burke, W.D., and Eickbush, T.H., The Age and Evolution of Non-LTR Retrotransposable Elements, *Mol. Biol. Evol.*, 1999, vol. 16, no. 6, pp. 793–805.
10. Burke, W.D., Malik, H.S., Lathe, W.C., and Eickbush, T.H., Are Retrotransposons Long-Term Hitchhikers?, *Nature*, 1998, vol. 392, no. 6672, pp. 141–142.
11. Lathe, W.C., Burke, W.D., Eickbush, D.G., and Eickbush, T.H., Evolutionary Stability of the *R1* Retrotransposable Element in the Genus *Drosophila, Mol. Biol. Evol.*, 1995, vol. 12, no. 6, pp. 1094–1105.
12. Zinov'eva, N.A., Gladyr', E.A., Ernst, L.K., and Brem, G., *Vvedenie v molekulyarnuyu gennuyu diagnostiku sel'skokhozyaistvennykh zhivotnykh* (Introduction to Molecular Gene Diagnosis in Farm Animals), Dubrovitsy: VIZh, 2002.
13. Burke, W.D., Eickbush, D.G., Xiong, Y., et al., Sequence Relationship of Retrotransposable Elements *R1* and *R2* within and between Divergent Insect Species, *Mol. Biol. Evol.*, 1993, vol. 10, no. 1, pp. 163–185.
14. Lohe, A.R. and Roberts, P.A., An Unusual Y Chromosome of *Drosophila simulans* Carrying Amplified rDNA Spacer without rDNA Genes, *Genetics*, 1990, vol. 125, no. 2, pp. 399–406.