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SHORT NOTE

Analysis of intraspecies polymorphism in the ribosomal DNA cluster of the cockroach *Blattella germanica*

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Abstract

***Hin*dIII restriction digests of the rDNA repeat unit of the German cockroach, *Blattella germanica*, reveal significant intraspecies sequence polymorphism. This variability is probably caused by structural differences within the nontranscribed spacer regions (NTS) of the ribosomal repeat unit. *Hin*dIII rDNA fragment polymorphisms in three cockroach strains show that individuals from different populations may have different *Hin*dIII rDNA patterns, whereas individuals within populations exhibit relatively similar rDNA patterns. We suggest that *Hin*dIII restriction fragment polymorphisms within cockroach ribosomal DNA will be a valuable tool for measuring population-level parameters within and between natural cockroach populations.**

Keywords: *Blattella*, ribosomal DNA structure, polymorphism.

Introduction

Eukaryotic ribosomal RNA genes (rDNA) are arranged in tandemly repeated clusters, with each cluster containing the genes for 18S-, 5.8S-, and 28S-like ribosomal RNAs. The number of repeat unit copies varies greatly across eukaryotic genomes and within populations (Long & Dawid, 1980). Repeat units are generally homogeneous within individual genomes due to concerted evolution (Dover, 1982; Arnheim, 1983). However, both inter- and intraspecies sequence variation within ribosomal repeats has been used extensively in molecular systematic and population genetic studies (Baldwin *et al*., 1995; Schaal *et al*., 1987; Hillis & Dixon, 1991; Wesson *et al*., 1992; Suzuki *et al*., 1994; Whiting *et al*., 1997).

The rDNA repeats may be located within one or a few chromosomes and are associated with the formation of the nucleolar organizer (NO). The NO of the German cockroach, *Blattella germanica*, is located within the X chromosome (Ross, 1988). The basic organization of the rDNA has been conserved in most eukaryotic systems. The genes are separated by several spacers, namely the NTS (nontranscribed spacer), and ITS1 and ITS2 (internal transcribed spacers). The NTS separates neighbouring repeat units, ITS1 is located between the 18S- and 5.8S-like coding regions, and ITS2 lies between the 5.8S- and 28S-like genes. The entire unit is transcribed by RNA polymerase I as a single 45S precursor molecule. The maturation of ribosomal RNAs occurs by an ordered cleavage and removal of the spacer sequences (Gerbi, 1985).

The nuceotide sequence and length of various structural elements of rDNA units are differentially conserved over evolutionary time, with the most variable being the NTS (Hillis & Dixon, 1991). In many eukaryotic species it has been shown that the high variability of the NTS is due to short subrepeats that differ in both their nucleotide composition and position within NTS (Rogers & Bendich, 1987).

The NTS region also contains transcription regulatory sequences, and therefore the NTS could experience selection associated with maintenance of gene expression (Clegg, 1990; Echeverria *et al*., 1994). These regulatory elements are often included in the subrepeats, and it has been shown that not only the composition of subrepeats, but also their number and methylation level are important for transcription (Sardana *et al*., 1993). The variability of ribosomal NTS has been related to such important biological characteristics as developmental rates and level of metabolism in *Drosophila* (Cluster *et al*., 1987) or grain yield in maize (Rocheford *et al*., 1990).

In this study, we investigate and describe levels of intraspecies polymorphism in the rDNA repeat unit of the cockroach *Blattella germanica*. We show that *Hin*dIII restriction enzyme digestion may be used to reveal variability due to structural differences within the NTS. Furthermore, *Hin*dIII rDNA

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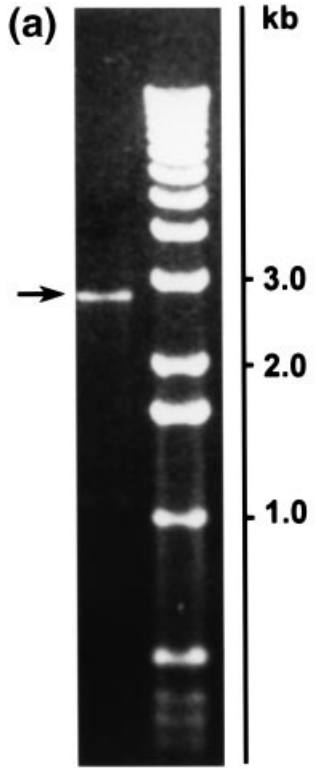

(b)

CTACTAGATGGTTCGATTAGTCTTTCGCCCCTATACCCAGCTCTGACGAT
CGATTTGCACGTCAGACTCGCTGCGGACCTCCATCAGGGTTTCCCCTGAC
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CTCTGGGTGCGCCTGCGGCCGAGGCCGAGGCGCCCCGGGAGTGCGGGCCC
GGACACTCGCCGCGGAGGGCGAGGCCAGACGGCCATCCTCCCAACAAACA
TCGGCAACGCCACGAGTGACGCGCCTTTGCCATGCTTTCACTGCGCCTTT
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CCCCAAGGCAACTTCGGGCGCCCTGCGTGGACAAGAATGAGTCTCCCCCT
TTCGCCTATTCGGATCTGTCCCGTTTACCCTTGAACGGTTTCACGTACTG
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cttcatcgacccacgagccgagtgatccaccgttcagggttgtaATGATA
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CGCGCCACAAGGCGGTTACCAGACGCAGCACGTGAAGCATGCAGACCCTT
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CGCACACAAAAGCGCCGTACCCATAAAAAGGGCAACCTTCTCCGCCGAAA
CGGACGAAAGAGTTGCCTAAGTAAAAAACAATGTGCTCAAAAGCCCTTCC
AGTAAAAACAGAATTCGCCCGCGCGCGCGCGTCCACCAACGAACGACACA
CACGGGGAATCTCTCTCTCCTCCACAAAACTTCCATAGTGGCAACGATAA
ATTCC*ACGTTAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTGTTAC*
GACTTTTACTTCCTCTAAATAATCAAGTTTGGGCATCTTCCCGGTGGAAC
GTGGGAGCAAGCCCCCGCGCCCGCCGGTCCGAAGACCTCACTAAATCATT
CAATCGGTAGTAGCGACGGGCGGTGTGTACAAACGGCAGGGAC

Figure 1. Amplification and sequence analysis of the cloned *B. germanica* rDNA fragment. (a) Electrophoresis in 1% agarose: lane 1, products of the amplification; lane 2, 1 kb DNA ladder. Arrow indicates the amplified DNA fragment used for cloning. (b) Nucleotide sequence of the cloned fragment. Main structural elements of the analysed *B. germanica* rDNA fragment are indicated using various fonts: 5′ fragment of the 28S rDNA – capital bold; 3′ fragment of the 18S rDNA – capital bold italic; 5.8S rDNA – lower case bold; ITS1 and ITS2 – roman capital. Sequences corresponding to the primers used for amplification of the cloned fragment are underlined. The positions of the *Hin*dIII sites are indicated by double underline.

polymorphism is compared in individual insects from three cockroach populations differing in age of laboratory culture.

Results and discussion

Cloning and sequencing of the B. germanica *rDNA fragment*

DAMS-18 and DAMS-28 primers amplify a 2.7-kb fragment, spanning large portions of the 18S and 28S genes and the entire intergenic regions of the rDNA repeat unit of *B. germanica* from extracted total genomic DNA (see Experimental procedures). The amplified fragment (Fig. 1a) was cloned and sequenced (Fig. 1b). The identification of structural elements with the amplified rDNA repeat unit was carried out by alignment with rDNA sequences of closely related taxa from the EMBL and GenBank databases. FASTA analysis of this sequence showed the highest

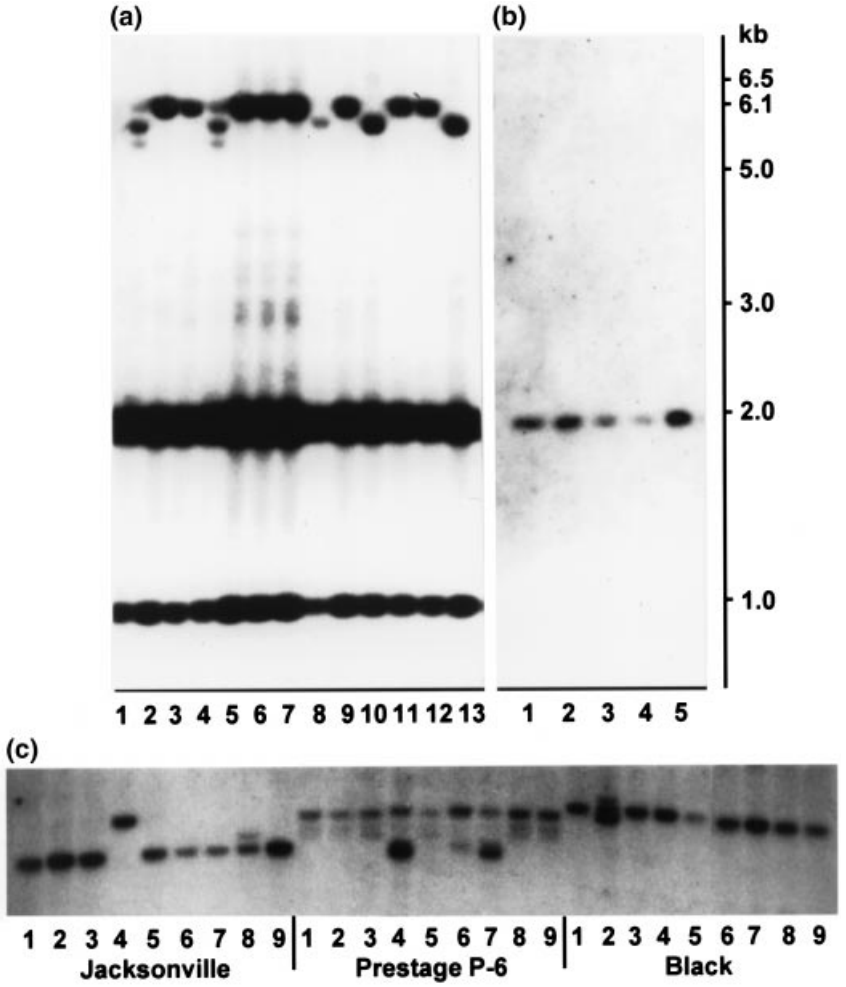

Figure 2. Southern analysis of *Hin*dIII digested total DNA of individual cockroaches. The blot was probed with (a, c) – cloned *B. germanica* rDNA fragment (Figure 1b); (b) – cloned 18S-like fragment *T. pyriformis* rDNA. (a) Lanes 1–13: DNAs of random individual cockroaches from different laboratory strains. (b) DNAs of the cockroaches used in (a); lanes 1–5 in (b) correspond to lanes 1, 2, 3, 8, 10, respectively, in (a). (c) The fragment of the Southern blot of individual *B. germanica* total DNA (lanes 1–9) from three different strains (Jacksonville, Prestage P-6, and Black); only polymorphic hybridization zones are displayed.

similarity values for comparisons with a part of the 28S rDNA of the honey-bee *Apis mellifera* (GenBank #X89529), and with a part of the 18S rDNA of the beetle *Tenebrio molitor* (GenBank #X07801). This high similarity to insect rDNAs increases our confidence that the amplified product could not belong to a parasite or symbiont of *B. germanica.* The entire cloned fragment was used as a probe for *B. germanica* rDNA analysis by Southern blot-hybridization techniques.

Hin*dIII restriction polymorphism of the* B. germanica *rDNA cluster*

As a first step towards using rDNA in cockroach population genetics, we analysed *Hin*dIII restriction fragment polymorphism among randomly drawn individuals from different laboratory and field-collected strains of *B. germanica.* Only adult males were used in the analysis. Individually isolated cockroach DNA samples were digested using *Hin*dIII restriction enzyme and probed by blot-hybridization using the cloned 2.7 kb *B. germanica* rDNA amplification fragment (Fig. 2a). Several hybridization zones were revealed. Two zones, approximately 1 and 2 kb in length, were consistently found across all samples (Fig. 2a), and several longer zones, 5–6.5 kb in length, were polymorphic within colonies. Individual cockroaches can be distinguished by the number (from one to three) of these longer polymorphic zones. This suggests that the rDNAs of different individuals within a population are not identical, but probably contain diverse types of ribosomal repeats.

Which rDNA structural elements are variable with respect to the *Hin*dIII restriction sites? The position of the *Hin*dIII sites throughout the cloned *B. germanica* rDNA fragment are indicated in Fig. 1(b). The fragment between these two *Hin*dIII sites is 953 bp (Fig. 1b, Fig. 3a) and it corresponds to the ~1 kb-sized zone in the Southern blot hybridization (Fig. 2a). *Hin*dIII restricted DNA samples used for the first blot-hybridization procedure, but that exhibited variable rDNA patterns, were used in a second hybridization using the 18S-like molecular probe (Sidorenko *et al.*, 1997). A single similar-sized hybridization zone coinciding with the 2 kb zone in Fig. 2a was revealed over all examined cockroach individuals (Fig. 2b).

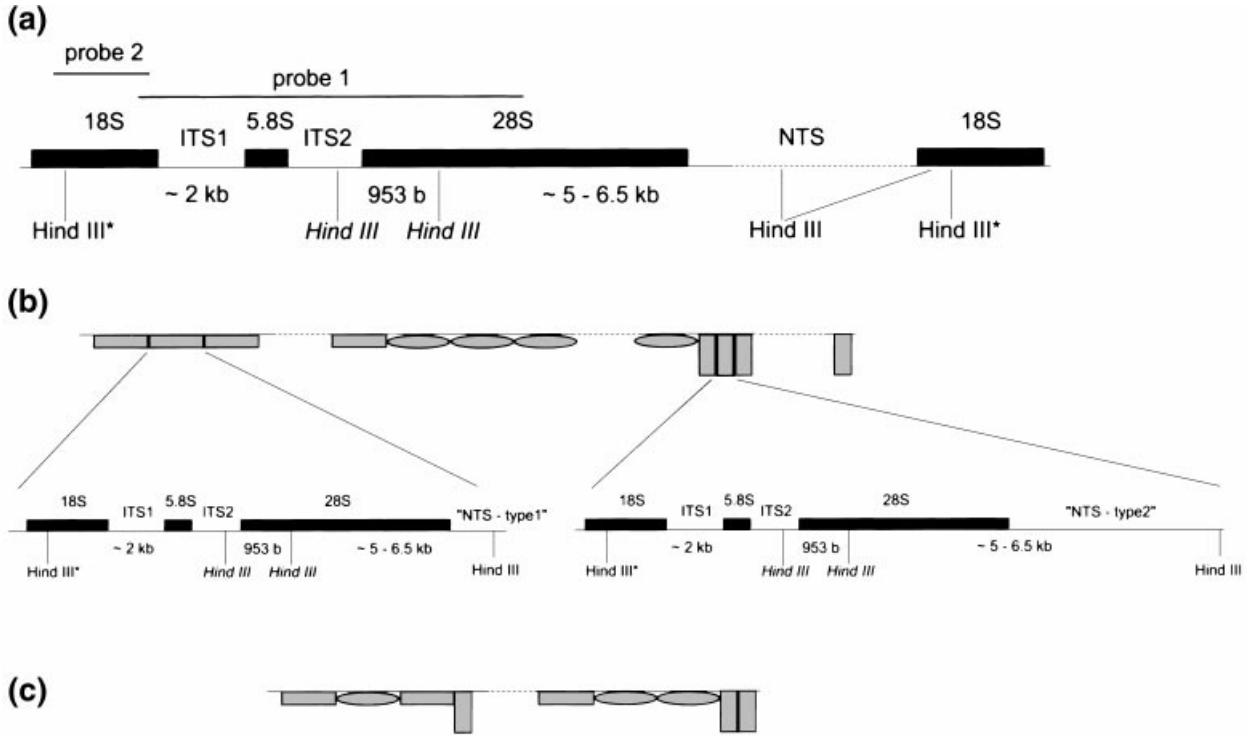

Figure 3. Organization of the *B. germanica* rDNA structure. (A) *HindIII* – the position of *Hin*dIII sites defined by sequencing (Figure 1b), HindIII* – the position of HindIII site within 18S-like rDNA, HindIII – variable *Hin*dIII sites. Probe 1 – the position of the cloned *B. germanica* rDNA fragment (Figure 1b). Probe 2 – the position of the *B. germanica* rDNA sequence, which is homologous to the cloned 18S-like fragment of the *T. pyriformis* rDNA. (b) and (c) – two models of the *B. germanica* rDNA repeats organization: according to the first (b), the rDNA repeats carrying similar nontranscribed spacers are located tandemly; according to the second model (c), the rDNA repeats carrying different nontranscribed spacers are distributed randomly.

The *Hin*dIII site, indicated by an asterisk in Fig. 3(a), may be located either within the 18S-like rDNA or in the NTS. It is noteworthy that the size of the fragment revealed by hybridization with 18S-homologous probe is about 2 kb. The size of the ITS1, 5.8S and of a part of the ITS2 upstream of the *Hin*dIII site is 1217 b (see Fig. 1b and Fig. 3a). Thus, the size of the fragment located between *Hin*dIII* and the end of the 18S-like rDNA (Fig. 3a) must be < 800 bp. It is as yet unknown whether there are any eukaryotic 18S-like rDNAs shorter than 1 kb – the average size of this molecule, where known, exceeds 1.5 kb. Consequently, the *Hin*dIII* restriction site must be located within the 18S-like cockroach rDNA. The location of the next *Hin*dIII site(s) within the rDNA repeats is variable. The site(s) can not be located within the 28S-like cockroach rDNA because the sizes of the respective hybridization zones (Fig. 2a) are much larger (5.0–6.5 kb) than any known repeat unit. If these sites were located in the 28S gene, it would be necessary to estimate the size of the *B. germanica* 28S-like rDNA between 6.0 and 7.5 kb. It seems most reasonable to conclude, then, that the position of these *Hin*dIII sites must fall within the NTS or the 18S-like rDNA, between the beginning of the gene and the *Hin*dIII* site (Fig. 3a).

It is known that the structural elements of the rDNA repeats, such as 18S-, 5.8S- and 28S-like genes, include the most evolutionarily conserved DNAs, whereas the NTS are the most variable parts (Rogers & Bendich, 1987). It may be proposed that the differences of the lengths of the polymorphic fragments (Fig. 2a) are accounted for by differences in the NTS structure, and namely by the lengths of rDNA sequences between the end of 28S rDNA and the *Hin*dIII site (see Fig. 3a). Based on this reasoning, two modes of *B. germanica* rDNA organization appear plausible. According to the first model, similar rDNA repeats are located tandemly as represented schematically in Fig. 3(b). The second model proposes that different rDNA repeats are distributed randomly (Fig. 3c). The choice between these two models will become possible upon further investigation of the arrangement rDNA types in progeny from crosses of parents carrying differing rDNA repeats.

Hin*dIII restriction rDNA polymorphism in different strains of* B. germanica

We examined the *Hin*dIII restriction rDNA polymorphism of three *B. germanica* strains differing in the age of the laboratory culture. We analysed one ‘old’ strain (marked

by the mutation 'Black') (Ross & Cochran, 1966), which had been maintained in the laboratory for over 30 years, and two relatively young laboratory strains collected in the field 3 and 11 years ago ('Prestage P-6' and 'Jacksonville', respectively). Thirty individuals from each strain were surveyed for *Hin*dIII restriction fragment polymorphisms. Several diagnostic *Hin*dIII rDNA patterns were found; they are represented in Fig. 2(c) according to the frequency of occurrence in each strain. Comparison of rDNA variation among different laboratory strains shows that 'Black', the oldest of the three strains, is more homogeneous than the other two. Three patterns were revealed within the Jacksonville strain: 78% N1, 11% N2, 11% N3 (lanes 1, 4, 8 in 'Jacksonville'); three patterns within Prestage P-6: 67% N1, 22% N2, 11% N3 (lanes 1, 6, 7 in 'Prestage P-6'); and two within Black: 89% N1, 11% N2 (lanes 1, 2 in 'Black'). In general, we characterized patterns according to the number of hybridization zones within each lane (individual) without regard to specific hybridization intesity. The intensity of hybridization did vary across the individuals and strains (compare lanes 5 and 6 in 'Jacksonville' or lanes 1 and 5 in 'Prestage P-6'). Variation in hybridization intensity could indicate differences in the quantity of specific rDNA repeats (Fig. 3b) among individuals within a strain.

Increased homogeneity in the 'Black' strain may be due to a comparatively smaller founding population size ('founder effect'), random fixation of one or a few rDNA 'types' over time, or positive selection for a dominant rDNA type. Further study of laboratory cultures of known age, and comparisons to variation found in natural populations, will be necessary to test specific population genetic mechanisms affecting observed rDNA variation.

Experimental procedures

General laboratory procedures

Isolation of plasmid DNA, restriction endonuclease digestion, ligation, agarose gel electrophoresis, and Southern blot-hybridization were performed as described in Sambrook *et al.* (1989). Total cockroach DNA was isolated from whole individuals by homogenization in extraction buffer (Sambrook *et al.*, 1989) followed by phenol-chloroform extraction and ethanol precipitation following standard protocols (Sambrook *et al.*, 1989).

Polymerase chain reaction

PCR amplifications for overlapping fragments within the *B. germanica* rDNA repeat unit, were carried out using Taq DNA Polymerase (Promega) and the PTC-100 Thermal Cycler (MJ Research Inc., USA). We used the following primers: DAMS18 – gtccctgccgtttgtacaca; DAMS28 – ctactagatggttcgattagtc the description of which will follow in a separate article. Each reaction contained 0.1 μg DNA template, 1.5 mM $MgCl_2$, 1 mM each dNTP, and 0.2 pmol each prime. The PCR regimen was as follows: initial template denaturation at 95°C for 5 min, followed by thirty cycles of 94°C for 1 min, 55°C for 2 min, and 72°C for 3 min, and a final 7 min elongation cycle at 72°C.

DNA sequencing

The PCR product, cloned into pUC19, was sequenced using the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq FS DNA Polymerase (Perkin Elmer Inc., USA) according to the accompanying protocol. Sequences were obtained using an ABI Prism 377 automated sequencer (Applied Biosystems, Inc., USA).

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