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MOLECULAR GENETICS

Nuclear rDNA Variability in Laboratory Strains of the German Cockroach *Blattella germanica* L. (Blattellidae)

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Abstract—The polymorphism of nuclear ribosomal DNA has been studied in male German cockroaches. The RFLP analysis has been used to identify seven types of *Hin*dIII fragments, the variation of which may serve as the basis for the differentiation of laboratory strains with respect to some population indices, including the average number of fragments per animal (*N*), the proportion of polymorphic loci (*P*), and the average pairwise similarity (APS). The interpopulation variation (F_{ST}) calculated for nine haplotypes is 53.85%.

INTRODUCTION

The German cockroach *Blattella germanica* L. (Dictioptera: Blattellidae) is one of the most widespread synanthropic species of cockroach. The increased insecticide resistance of this species initiated genetic studies. It has been found that the diploid chromosome set of the German cockroach consists of 11 pairs of autosomes and one (males X0) or two (females XX) sex chromosomes [1, 2]. The cluster of ribosomal genes or ribosomal DNA (rDNA) has been located to the X chromosome by cytological methods. The mutation load has been estimated at 0.02–0.04% of mutations per animal. Thus far, population studies on German cockroaches have been focused on the numbers, density, growth rate, age structure, capacity for migration, and some other biological parameters [3–5].

The population genetic structure of this cosmopolitan species remains largely unknown. Only a few studies on this subject have been published. For example, Cloarec *et al.* [6] studied genetic variation of 41 protein loci of *B. germanica* and did not find considerable differences between the populations from two remote cities, although the differences between the local urban populations were statistically significant. Jobet *et al.* [7] studied the coevolution of *B. germanica* and one of its nematode parasites with the use of RAPD markers. These authors reported the estimates of interpopulation variation in natural populations of the German cockroach that also indicated an absence of variation at large geographical scale.

The cluster of nuclear ribosomal genes is a widely used marker of population variation in animals. Eukaryotic rDNA is a unique structural and functional genomic formation in which genetic elements drastically differing in evolutionary conservatism are combined [8]. For example, rRNA genes are the most conserved; only a few single substitutions or a complete absence of variable sites within a species are found in their nucleotide sequences. The nontranscribed spacer (NTS) is the most variable region of eukaryotic rDNA; one genome may contain several structural variants of NTS. These characteristics of the eukaryotic rDNA cluster permit its effective use in evolutionary and population molecular genetic studies [8–10].

Blattella germanica is a suitable model object for studying variation of the ribosomal gene cluster of individual X chromosomes, because its rDNA is located in the X chromosome, of which males have only one [1, 2]. Our earlier studies on rDNA of male *B. germanica* demonstrated that this species had a marked intraspecies *Hin*dIII polymorphism of the ribosomal gene cluster [11–13] revealed by a species-specific rDNA probe [13]. In this study, we used the same probe to estimate the variation of nuclear rDNA in three laboratory strains of *B. germanica* and the prospects of the use of this probe for studying natural populations.

MATERIALS AND METHODS

Samples of 27 animals (males) each were kindly provided by the Department of Entomology of North Carolina State University (United States). Strain Black marked with autosomal mutation *Bl* (*Black body*) [14] has been maintained under laboratory conditions for

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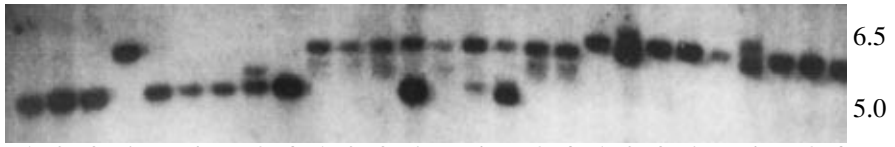
(a)



(b)

rDNA fragment	rDNA haplotypes								
	J_1	J_2	J_3	P_1	P_2	P_3	P_4	B_1	B_2
A									—
B								—	—
C		—		—	—	—	—		—
D			—	—					
E				—			—		
F	—		—		—	—			
G					—				

Fig. 1. (a) Results of the blot hybridization analysis of the *Hin*dIII restriction fragments of total DNAs from individual (*1–9*) male German cockroaches belonging to three laboratory strains (Black, Jacksonville, and Prestage P-6) according to the frequencies of patterns in each strain. (b) A diagram of the polymorphism of the ribosomal gene cluster.

30 years. Strains Jacksonville and Prestage P-6 are of natural origin (North Carolina) and have been maintained under laboratory conditions for five years. Strain Jacksonville originated from cockroaches caught in a residential building, and strain Prestage P-6, from those caught in a production area of a stockbreeding enterprise.

Earlier, we described the methods for isolating total DNA of individual males and identifying *Hin*dIII genomic by Southern blot hybridization [11–13, 15] with the use of a *B. germanica* species-specific probe containing the 3' terminal region of the 18S rRNA gene (188 bp), the 5.8S rRNA gene (142 bp), the 5' terminal and the central regions of the 28S rRNA gene (1282 bp), and internal transcribed spacers ITS1 (661 bp) and ITS2 (464 bp) [15]. The total length of the fragment cloned in plasmid pUC19 was 2737 bp.

The autoradiographs obtained for each sample were used to compile a binary matrix of characters where the presence or the absence of an rRNA hybridization fragment of the given size was denoted as 1 or 0, respectively. This matrix was used for statistical treatment of the results. For each strain, we calculated the average number of fragments per animal (*N*), the proportion of polymorphic loci at a 95% confidence (P_{95}), and the average intrastrain pairwise similarity (APS). The significance of differences between strains in *N* and *APS* values was calculated using the permutation test [16]. The estimated parameters were considered significant at $P < 0.05$. The sample differentiation was estimated by the subdivision index F_{ST} [17].

The POPGENE 3.1 software was used for the statistical treatment [18].

RESULTS

Figure 1 shows the results of the blot hybridization analysis of the *Hin*dIII restriction fragments of the total DNAs of individual male German cockroaches according to their frequencies in the three strains (Fig. 1a) and a diagram of the fragments and pattern variants (Fig. 1b). We detected a total of seven fragments (A–G) with lengths varying from 5.0 to 6.5 kb (Fig. 1a). The strains studied had both common and strain-specific fragments. Fragment C was the only one common for all of the three samples; however, its frequency in the Prestage P-6 strain (1.000) considerably exceeded those in the Jacksonville and Black strains (0.111 and 0.222, respectively) (Table 1). Two more fragments, D and F, were common for the Prestage P-6 and Jacksonville strains. Their frequencies were, respectively, 0.556 and 0.333 in the Prestage P-6 strain and 0.111 and 0.889 in the Jacksonville strain. There were also fragments specific for strains Black (A and B) and Prestage P-6 (E and G). However, only fragment B marked all cockroaches in the respective strain (Black). We did not find specific fragments in strain Jacksonville; however, it differed in a very high frequency of fragment F (0.889).

Table 2 shows indices characterizing intrastrain variation: the average number of rDNA fragments per animal (*N*), the proportion of polymorphic loci (*P*), and the average pairwise similarity within a strain (APS).

Table 1. Frequency distribution of rDNA fragments in *B. germanica* strains studied

rDNA fragment	Jacksonville	Prestage P-6	Black
A	–	–	0.222 ± 0.080
B	–	–	1.000
C	0.111 ± 0.060	1.000	0.222 ± 0.080
D	0.111 ± 0.060	0.556 ± 0.096	–
E	–	0.667 ± 0.091	–
F	0.889 ± 0.060	0.333 ± 0.091	–
G	–	0.222 ± 0.080	–

Table 2. Genetic diversity of rDNA estimates in three strains of *B. germanica*

Strain	Average number of fragments per animal, $N \pm SE$	Proportion of polymorphic loci, P	Average pairwise similarity within strains, APS ± SE
Jacksonville	1.111 ± 0.062	0.429	0.735 ± 0.019
Prestage P-6	2.778 ± 0.082	0.571	0.673 ± 0.085
Black	1.444 ± 0.163	0.286	0.821 ± 0.058

Table 3. Significance of differences between laboratory strains with respect to average pairwise similarity (APS) within samples (above the diagonal) and average number of fragments per animal (N; below the diagonal)

Samples	Jacksonville	Prestage P-6	Black
Jacksonville	–	0.0452	0.0296
Prestage P-6	0.0000	–	0.0000
Black	0.0498	0.0000	–

Note: The number of permutations is 1023.

Table 4. Frequencies of rDNA haplotypes in three laboratory strains of the German cockroach *B. germanica*

Haplotype	Jacksonville	Prestage P-6	Black
J_1	0.778 ± 0.080	–	–
J_2	0.111 ± 0.060	–	–
J_3	0.111 ± 0.060	–	–
P_1	–	0.556 ± 0.096	–
P_2	–	0.222 ± 0.080	–
P_3	–	0.111 ± 0.060	–
P_4	–	0.111 ± 0.060	–
B_1	–	–	0.778 ± 0.080
B_2	–	–	0.222 ± 0.080

The Prestage P-6 and Black strains were the most and the least heterogeneous, respectively, among the three strains studied. The proportion of polymorphic loci in the Black strain was almost two times smaller than in the Prestage P-6 and Jacksonville strains (0.286, 0.571, and 0.429, respectively). The Black and Prestage P-6 strains differed from each other with respect to the N and APS indices (Table 2). The Jacksonville and Prestage P-6 strains differed from each other only in the average number of fragments per animal (N = 1.111 and 2.778, respectively). The differences between the strains with respect to N and APS were significant ($P < 0.05$) (Table 3).

Since the ribosomal gene cluster of *B. germanica* is located in the X chromosome, and males, in contrast to females, have only one X chromosome [1, 2], individual rDNA spectra of male cockroaches may be called haplotypes. In Fig. 1b and Table 4, the haplotypes detected in the three strains are designated by letters J, P, and B (the first letters in the names of the strains) with numerical subscripts. We identified a total of nine haplotypes, two, three, and four of which were found in strains Black, Jacksonville, and Prestage P-6, respectively. Each strain was characterized by a strictly specific set of haplotypes, with only one of them prevailing in each particular strain (Table 4); these were B_1 (77.8%), J_1 (77.8%), and P_1 (55.6%) in the Black, Jacksonville, and Prestage P-6 strains, respectively.

The differentiation of populations estimated by the F_{ST} index was 0.539.

DISCUSSION

We studied rDNA variation in three laboratory strains of the German cockroach with the use of an rDNA probe specific for *B. germanica.* The probe was a relatively long rDNA fragment comprising both gene nucleotide sequences and spacers. Earlier, we analyzed the *Hin*dIII variation of rDNA of *B. germanica* with the use of a species-specific and a universal probes containing the nucleotide sequences of 18S- and 28S-like rDNAs of the protozoan *Tetrahimena pyriformis* [11, 13]. Most of the variation detected in this region may have been related to the variation in the numbers and/or types of subrepeats of the NTS. Additional studies are required to clarify the specific cause of the variability of German cockroach rDNA revealed by the tested probe.

Our data indicate that the strains studied are characterized by a high similarity of rDNA structures within strains (APS > 0.673) and a small number (from two to four) of strain-specific haplotypes that allow the identification of samples. In addition, the comparison of the indices of intrastrain similarity, the average number of fragments per animal, and the proportion of polymorphic loci have confirmed the distinct differentiation of the strains studied.

The observed variation pattern can be explained by the origin and history of the strains studied. For exam-

ple, the Black strain, which has been artificially maintained under laboratory conditions for 30 years, may have lost most of the original variation because of gene drift. The founder effect also cannot be excluded, because the frequency of the *Bl* mutation marking this strain is extremely low in natural populations [19]. The higher polymorphism of the Prestage P-6 and Jacksonville strains may be explained by the considerably shorter duration of their maintenance under laboratory conditions (five years) and the larger number of founders taken from natural populations. The observed difference between the Prestage P-6 and Jacksonville strains with respect to polymorphism may be related to both the difference in genetic structure between the original natural populations and the effect of gene drift, which may have enhanced their differentiation.

Thus, the molecular probe used in this study has proved to be effective for revealing rDNA variation in three laboratory strains, which makes it a promising tool for studying the organization of natural populations of *B. germanica.*

The intraspecies structure of the German cockroach remains an open question notwithstanding the sanitary and hygienic importance of this species, as well as its biological specificity (the old phylogenetic age, evolutionary conservatism, low genetic load, and high resistance to insecticides). Only two studies published to date dealt with natural populations of *B. germanica*. Several dozens German cockroach populations in the cities of Rennes and Séte (France) located almost 900 km apart were the objects of one of them [6]. Forty-one isoenzyme loci, eight of which were polymorphic, served as markers. In the other work, the coevolution of *B. germanica* and its nematode parasite *Blatticola blattae* was studied wit the use of RAPD markers [7]. The main result of these studies presented in the form of the subdivision index F_{ST} demonstrated that differentiation of neighboring local populations and the absence of differentiation between cockroach populations of the two remote cities. For example, the F_{ST} values for eight allozyme loci in *B. germanica* from the city populations varied from 0.059 to 0.874, whereas this parameter for RAPD markers was estimated at 0.229. The subdivision index obtained in our study (0.539) indicates a considerable differentiation of laboratory strains compared to natural populations of *B. germanica.* This agrees with the theory and factual data on the populations of *Drosophila,* the insect most comprehensively studied in this respect. The maintenance of *Drosophila* populations with relatively small effective sizes for several generations usually leads to substantial differentiation as a result of gene drift [20].

We are planning to study the variability of the ribosomal gene cluster in various natural populations of *B. germanica* to determine the patterns of micro- and macrogeographical differentiations of this widespread species.

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