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Electrophoretic Karyotype of Intracellular Yeast-like Symbiotes in Rice Planthoppers and Anobiid Beetles

HIROAKI NODA AND NOBUYUKI KAWAHARA[1]

National Institute of Sericultural and Entomological Science, Owashi, Tsukuba, Ibaraki 305, Japan

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Chromosomal DNA molecules of intracellular yeast-like symbiotes (YLS) of three species of rice planthoppers and two species of anobiid beetles have been separated by pulsed-field gel electrophoresis. Probable chromosome numbers of *Nilaparvata lugens*, *Sogatella furcifera*, and *Laodelphax striatellus*, were 4, 4, and 5, respectively, and tentative genome sizes were 17.3, 17.6, and 20.1 Mbp, respectively, based upon migration of individual chromosome-sized DNA relative to the size standards of *Schizosaccharomyces pombe*, *Hansenula wingei*, and *Saccharomyces cerevisiae* chromosomes. Chromosome numbers of *Lasioderma serricorne* and *Stegobium paniceum* were 11 and 15, respectively, and total genome sizes were 20.9 and 15.1 Mbp, respectively. The chromosomes carrying ribosomal RNA genes were identified by Southern blot analysis. Chromosomal organization and the genome size of YLS were similar to those of nonsymbiotic yeasts and fungi. © 1995 Academic Press, Inc.

KEY WORDS: planthopper; anobiid beetle; *Nilaparvata lugens; Sogatella furcifera; Laodelphax striatellus; Lasioderma serricorne; Stegobium paniceum;* yeast-like symbiote; pulsed-field gel electrophoresis; CHEF; electrophoretic karyotype; chromosomal DNA.

INTRODUCTION

Many microorganisms live harmlessly within the cells of a wide range of insects. These intracellular symbiotes have been broadly separated into three large morphological groups or forms of microbial life: bacteria, rickettsiae, and yeasts (Steinhaus, 1949). Yeast-like symbiotes (YLS) are sometimes found in Homoptera and Coleoptera. Well-known examples of intracellular YLS are those of anobiid beetles (Steinhaus, 1949; Buchner, 1965; Jurzitza, 1979), scale insects (Steinhaus, 1949; Buchner, 1965; Tremblay, 1989), and planthoppers (Nasu and Suenaga, 1958; Noda, 1977).

YLS of the anobiid beetles live in the protrusions located at the beginning of the midgut (Jurzitza, 1979). The symbiotes are smeared on the surface of the eggs when eggs are oviposited through the pouches near the top of the ovipositor. Newly hatched weevils ingest the symbiotes on the surface of the egg shell. The YLS of the anobiid beetles, *Lasioderma serricorne* and *Stegobium paniceum*, were cultured on an agar plate (Jurzitza, 1979). YLS of the planthoppers, in contrast, inhabit the fat body and are transmitted transovarially (Noda, 1977). The symbiotes are confined in eggs and have no stage when they are outside the insect's body. Reliable methods of culturing YLS of the planthoppers have not been established, but the YLS of rice planthoppers were isolated by Percoll density gradient centrifugation (Noda and Omura, 1992).

Studies on genetic organization are important in clarifying the origin of YLS and deducing the relationship between the symbiotes and host insects. An improved understanding of genetic systems and the availability of intact genomic DNA by recombinant DNA technique together with the recently developed polymerase chain reaction (PCR) method (Saiki *et al.*, 1988) widen the range of genetic studies to organisms for whom genetic information is only limited. Recent advancement in pulsed-field gel electrophoresis (PFGE) (Schwartz and Cantor, 1984; Chu *et al.*, 1986) also enables the separation of chromosome-sized DNA of eukaryotic microorganisms. Electrophoretic karyotype has been studied in many eukaryotic microorganisms by PFGE (Mills and McCluskey, 1990; Pfeifer and Khachatourians, 1993).

In this paper we describe the separation of chromosomal DNA of YLS of three species of rice planthoppers and two species of anobiid beetles by PFGE. Ribosomal RNA genes are also mapped on the separated chromosomal DNA bands of YLS. This paper provides the first estimate of the number and size of chromosomes in the yeast-like symbiotes of insects.

MATERIALS AND METHODS

Insects and Symbiotes

The three species of rice planthoppers used were reared on rice seedlings: the brown planthopper

[1] Present address: Central Research Institute, Mitsui Toatsu Chemical, Inc., Togo, Mobara, Chiba 297, Japan.

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Nilaparvata lugens, the white-backed planthopper *Sogatella furcifera,* and the small brown planthopper *Laodelphax striatellus.* Anobiid beetles, the cigarette beetle *Lasioderma serricorne,* and the drugstore beetle *Stegobium paniceum* were provided by H. Fujii of National Food Research Institute and were reared on wheat bran.

YLS of the rice planthoppers were purified as described by Noda and Omura (1992) using Percoll buoyant density gradient centrifugation. Yeast-like symbiotes of the anobiid beetles were isolated from midgut ceca of adults (Jurzitza, 1979; Shen and Dowd, 1989). The alimentary tract was aseptically dissected and homogenized with a physiological salt solution, and the homogenate was spread on YPD agar (0.5% yeast extract, 1% peptone, 2% glucose, and 1.5% agar) or PPGA (potato extract from 20% weight per final volume, 0.5% peptone, 0.5% glucose, 0.3% Na_2HPO_4–$12H_2O$, 0.05% KH_2PO_4, and 1.5% agar). Colonies of anobiid yeasts were recognized about 1 week later at 26°C. YLS of *S. paniceum* was the more slow growing of the two. For the collection of yeast cells, YLS were incubated in YPD liquid medium at 28°C and were centrifuged at 600*g* for 5 min.

Preparation of Intact Chromosomal DNA

The purified or cultured YLS were pelleted by low centrifugation in a microfuge tube and the cells were resuspended with an equal volume of 50 mM EDTA, pH 8.0. One-third volume of Lyticase (Sigma) solution (0.2% in 0.01 M sodium phosphate buffer, pH 7.5, containing 50% glycerol) was added and incubated at 37°C for 25 min. Three volumes of 1% melted agarose (low-melt preparative-grade agarose, Bio-Rad) solution was added to the spheroplast solution, and the agarose solution was put into a mold chamber and allowed to cool at 4°C. The agarose sample plugs were removed from the mold and were washed with 0.5 M EDTA, followed by incubation in LET buffer (0.45 M EDTA, pH 8.0, 0.01 M Tris, pH 7.5, 7.5% 2-mercaptoethanol) at 37°C overnight. After washing in 50 mM EDTA, pH 8.0, the agarose plugs were treated with 0.2% proteinase K in NDS buffer (0.45 M EDTA, pH 8.0, 0.01 M Tris, pH 7.5, 1% lauroylsarcosine) at 50°C overnight. The plugs were stored in 50 mM EDTA at 4°C. DNA size standards of *Schizosaccharomyces pombe, Hansenula wingei,* and *Saccharomyces cerevisiae* were purchased from Bio-Rad (Richmond, CA). The chromosomal sizes of *S. pombe* were 5.7, 4.6, and 3.5 Mbp; those of *H. wingei* were 3.13, 2.70, 2.35, 1.81, 1.66, 1.37, and 1.05 Mbp; and those of *S. cerevisiae* were 2200, 1600, 1125, 1020, 945, 825, 785, 750, 680, 610, 565, 450, 365, 285, and 225 kbp.

Contour-Clamped Homogeneous Field (CHEF) Conditions

Chromosome separation was performed using an alternating-field gel electrophoresis system which employs CHEF, specifically the CHEF-DR II electrophoresis system (Bio-Rad). One percent, 0.8%, or 0.6% agarose (high-strength analytical grade, Bio-Rad) solutions in 0.5× TBE buffer (45 mM Tris, 45 mM boric acid, 1 mM EDTA, pH 8.0) were prepared and 150 ml of the melted agarose was poured into a casting stand (127 × 139 mm) and allowed to cool. DNA samples cut from the agarose plugs were put into the gel wells and were sealed with 0.6–0.8% agarose (low-melt preparative grade) in 0.5× TBE buffer. The agarose gel and electrophoresis buffer, 0.5× TBE, were precooled at 4°C. Temperature of the buffer in the electrophoresis apparatus was maintained at about 9–10°C by a cooling unit (Taiyo Coolnit CL-15, TAITEC Corp., Koshigaya). One percent agarose gel was used for the separation of small-sized chromosomes, and 0.8 and 0.6% was used for the large-sized ones. The voltage, switching intervals, and total times of electrophoresis are described in the Results and figure legends. Following electrophoresis the gels were stained with ethidium bromide (0.5 μg/ml) for 15 min and destained in distilled water for 2 hr.

Transfer and Hybridization Conditions

The agarose gels were incubated with 0.25 M HCl for 15 min and washed with distilled water. They were then incubated twice in 0.5 M NaOH–1.5 M NaCl for 30 and 15 min to denature the DNA and neutralized twice with 0.5 M Tris–HCl (pH 7.5)–1.5 M NaCl for 30 and 15 min. The DNA was transferred to a nylon membrane (Hybond-N^+, Amersham) by capillary action with 20× SSC (3 M NaCl, 0.3 M sodium citrate) overnight. The filter was rinsed with 5× SSC (0.75 M NaCl, 0.075 M sodium citrate), dried, and irradiated with 120 mJ/cm^2 of uv by a uv cross-linker (Spectrolinker XL-1500, Spectronics Corporation, New York) to link the DNA to the membrane.

The chromosome bands which carry ribosomal RNA genes were detected using enhanced chemiluminescence (ECL) direct nucleic acid labeling and detection systems (Amersham). The blotted membrane was preincubated with hybridization buffer (5% blocking agent, 0.5 M NaCl) and incubated with DNA probe in the hybridization buffer at 42°C overnight. The membrane was washed twice with primary wash buffer (6 M urea, 0.4% SDS, 0.5× SSC) for 20 min each time at 42°C and then washed twice with secondary wash buffer (2× SSC) for 5 min each time at room temperature. After incubation with ECL detection reagent for 1 min, the membrane was exposed to Hyperfilm-ECL (Amersham) in the dark.

Preparation of rDNA Probes

Small nuclear ribosomal RNA genes (18S rRNA genes, 18S rDNA) were amplified by PCR. Template DNAs were extracted from the purified YLS of *N. lugens* and cultured YLS of *L. serricorne* and *S. pani-*

ceum. Cells were suspended in 50 mM EDTA, pH 8.0, and were digested with Lyticase as described above. The spheroplasts of the symbiotes were pelleted by low-speed centrifugation and resuspended in 50 mM Tris buffer (pH 7.2) containing 20 mM EDTA. Sodium dodecyl sulfate (SDS) was added to the suspension at a final concentration of 1% and the suspension was incubated at 65°C for 30 min. One-third volume of 5 M potassium acetate was added and the suspension was kept on ice for 60 min. After centrifugation at 10,000 rpm for 10 min, ethanol was added to the supernatant to precipitate nucleic acids. The nucleic acids were treated with 20 μg/ml RNase A in TE buffer (10 mM Tris–HCl, pH 8.0, 1 mM EDTA) at 37°C for 15 min and the DNA was precipitated with propanol (Rose *et al.*, 1990).

A pair of primers, NS1 5'GTAGTCATATGCTTGTCTC3' and NS8 5'TCCGCAGGTTCACCTACGGA, which amplifies nearly the entire size of 18S rDNA, 1769 bp, of *S. cerevisiae* (White *et al.*, 1990), was synthesized with a DNA synthesizer (Applied Biosystems, Model 392) and used for amplification of rDNA of YLS of the anobiid beetles. Another pair of primers NS5 5'AACTTAAAGGAATTGACGGAAG and NS8, which amplifies about 610 bp at the 3'-end of 18S rDNA, was also used for amplification of rDNA and YLS of *N. lugens*. Amplifications were performed in 50 μl of 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl, and 0.001% (w/v) gelatin with 60 μM of each dNTP, 20 pmol of each primer, 100 ng template DNA, and 2.5 U *Taq* DNA polymerase (Perkin Elmer Cetus, Norwalk). The temperature regimen for 30 cycles was 30 sec at 95°C, 30 sec at 55°C, and 2 min at 72°C. PCR-amplified DNAs used here have been sequenced and proved to correspond to a part of the 18S rDNA (Noda *et al.*, 1995).

The amplified DNAs were diluted to the concentration of 10 ng/μl and denatured in boiling water for 5 min. The DNA was labeled with ECL labeling reagent according to the manufacturer's recommendation and immediately used or stored in 50% glycerol at −20°C.

RESULTS

More than two samples were prepared and each YLS species was examined more than 10 times under various CHEF conditions. A CHEF condition for separation of *S. cerevisiae* chromosomes (60-sec pulse, 15 hr, 200 V/90-sec pulse, 9 hr, 200 V, 1% agarose gel, 10°C) was first used for each symbiote in order to examine the smaller chromosomes. To determine larger chromosomes, agarose gel concentration was lowered from 1 to 0.8% and the voltage from 200 to 50 or 40 V, and the switching intervals of the pulse were raised. To detect much larger sized chromosomes, e.g., more than 10 Mbp, the samples were electrophoresed under a condition of 160-min pulse, 9 days, 30 V, 0.6% agarose gel, 9°C. We found no evidence that the five species of YLS examined here possess chromosomal DNA larger than 10 Mbp.

Nilaparvata lugens

In *N. lugens* DNA chromosomes of YLS were separated into three bands (Fig. 1, lane 2; Fig. 2A). The largest chromosome band was larger than the largest one of *S. pombe* (5.7 Mbp) and DNA size standards of this size were not available. It was therefore roughly estimated at 7.5 Mbp. This band is faint in Figs. 1 and 2A (arrow). The sizes of the other two bands were estimated as 4.2 and 1.4 Mbp using DNA chromosome standards of *S. pombe*, *H. wingei*, and *S. cerevisiae*. The middle-sized band (4.2 Mbp) was brighter (Fig. 1, lane 2) and broader (Fig. 2A) than the two other bands when viewed under uv light, indicating probable doublets or two very closely migrating bands. With four chromosomes present, the size of the genome was estimated as 17.3 Mbp (Table 1).

Sogatella furcifera

Three chromosomal bands, about 6.3, 3.5, and 2.1 Mbp, were detected in *S. furcifera* under the conditions suitable for the separation of DNA chromosomes of *S. pombe* (Fig. 1, lane 3). The 6.3-Mbp band was further resolved into 6.3- and 5.7-Mbp bands (Fig. 2B, arrows) using raised switching intervals. Thus, four chromosomal DNA molecules were at least detected with a genome size of approximately 17.6 Mbp (Table 1).

Laodelphax striatellus

The smallest band in *L. striatellus* was almost the same size as the largest one of *S. cerevisiae*, 2.2 Mbp, and the largest band, 7.0 Mbp, was larger than the largest one of *S. pombe*. Four chromosome bands were usually detected (Fig. 1, lane 4) and the third largest one could be further separated into two, 3.1 and 2.8 Mbp (Fig. 2C, arrows). Although the largest 7.0 Mbp band was brighter than the second one (Fig. 1, lane 4), it is uncertain whether the largest 7.0-Mbp band is doublets. Therefore, five resolvable chromosome bands

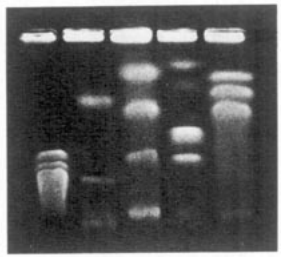

FIG. 1. Separation of chromosomal DNA of YLS of the three species of rice planthoppers on a CHEF gel. A 0.8% agarose gel electrophoresed for 140 hr at 50 V with 1500- to 5000-sec ramped switching interval. Lane 1, *S. cerevisiae* DNA size standard; lanes 2–4, YLS of *N. lugens*, *S. furcifera*, and *L. striatellus*, respectively; lane 5, *S. pombe* DNA size standard.

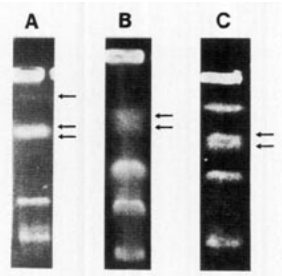

FIG. 2. Resolution of the similarly sized chromosomal DNA of YLS in the rice planthoppers. (A) YLS of *N. lugens;* a 0.8% agarose gel electrophoresed for 140 hr at 50 V with 1500- to 5000-sec ramped switching interval; single arrow, 7.0-Mbp band; double arrows, probable comigrating bands. (B) YLS of *S. furcifera;* a 0.8% agarose gel electrophoresed for 168 hr in total, (43 hr at 60 V, 53 hr at 50 V, and 72 hr at 40 V), with 2000- to 9000-sec ramped switching interval; arrows, 6.3- and 5.7-Mbp bands. (C) YLS of *L. striatellus;* a 0.8% agarose gel electrophoresed for 144 hr at 50 V with 1200- to 3600-sec ramped switching interval; arrows, 3.1- and 2.8-Mbp bands.

were at least recognized with a total genome size of approximately 20.1 Mbp (Table 1).

Lasioderma serricorne

The size range of chromosome bands of YLS of *L. serricorne* was more limited than that of YLS of the rice planthoppers and more bands were fractionated. The largest chromosome band was 3.0 Mbp and the smallest was 1.0 Mbp (Fig. 3), the range corresponding to that of *H. wingei* (1.05–3.13 Mbp). Eleven chromosomes were resolved and the genome size was estimated at 20.9 Mbp (Table 1).

Stegobium paniceum

The size range of chromosomal DNA in *S. paniceum* was yet smaller than that of YLS of *L. serricorne* and was similar to that of *S. cerevisiae.* DNA molecules were well resolved even at high field strength, and therefore electrophoresis was completed within 24 hr. The largest chromosome band was 1.83 Mbp and the smallest was 0.52 Mbp (Fig. 4A). The fourth (1.2 Mbp) and seventh (0.93 Mbp) largest bands were probably doublets (Figs. 4A and 4B, arrow, respectively), because these bands were more intensely stained. The second smallest band in Fig. 4A was composed of two closely migrating DNA molecules of 0.59 and 0.57 Mbp (Fig. 4C, arrows). Fifteen bands of DNA molecules were detected and the genome size was approximately 15.1 Mbp (Table 1).

Identification of the Chromosomes That Possess rDNA

Chromosomes on which rRNA genes are located were determined by transferring the separated chromosomal DNAs to a nylon membrane, followed by successive hybridization with ECL-labeled probes of PCR-amplified rDNA. The rDNA probes hybridized with DNA of the 4.2-Mbp doublet band in YLS of *N. lugens* (Fig. 5, lane 3). One of or both of the two comigrating 4.2-Mbp chromosomes contained 18S rRNA genes. In YLS of *S. furcifera* and *L. striatellus,* the 3.5- and 5.0-Mbp bands, respectively, were positive using the rDNA probes amplified from genome DNA of YLS of *N. lugens* (Fig. 5, lane 4 and 5, respectively). The ECL reaction was also detected on the 1.7-Mbp band in YLS of *L. serricorne* using rDNA probe of the same species (Fig. 6, lane 2). In YLS of *S. paniceum,* hybridization was observed in two (or three) chromosomal DNA, 1.0 Mbp band, and 0.93 Mbp doublet with rDNA probes of *L. serricorne* and *S. paniceum* (Fig. 7, lane 2). The probes used in the present study also hybridized to the smallest chromosomal DNA (3.7 Mbp) of *S. pombe* and the largest one (2.2 Mbp) of *S. cerevisiae* (Fig. 5, lane 6, and Fig. 6, lane 1, respectively). These two chromosomes are reported to have rDNA clusters (Vollrath and Davis, 1987).

DISCUSSION

In the present study, each band of YLS appears to represent a respective chromosome. The chromosomal size varies widely from 0.52 Mbp of YLS of *S. paniceum* to 7.5 Mbp of YLS of *N. lugens.* The largest chromosomal YLS bands of the three species of rice planthoppers were larger than the largest band in the chromosomal DNA of *S. pombe,* 5.7 Mbp, and no molecular weight marker exists in this size range; therefore, the size estimates of these large chromosome bands must be considered tentative. The chromosomal organization of YLS of the two anobiid beetles differed from each other, the chromosome size of *S. paniceum* being smaller than that of *L. serricorne* and the chromosome number being larger. However, their YLS were interchangeable and both were able to live in abnormal host beetles (Pant and Fraenkel, 1954). This suggests that the two YLS of the beetles have similar function and physiology and are closely related; they probably originate in the same ancestral species. The difference in the chromosomal organization is a reflection of their long separation from each other. The chromosomes may be comparatively changeable within their organization but their functional genes remain unchanged.

Application of PFGE to separate fungal chromosomes is an excellent technique by which to analyze fungal genomes (Mills and McCluskey, 1990). The PFGE technique seems to be a useful method for characterizing intracellular YLS of insects in particular, because no other analytical means are yet available for genetic study owing to the difficulties of culturing and to a lack of genetic background. It is also suggested that molecular karyotyping becomes one of the taxonomic tools for identifying and classifying microorganisms (Mills and McCluskey, 1990). To compare a microorganism with an already known authentic sample of that microorganism, this method is surely useful.

TABLE 1
Size Estimates (Mbp) of Chromosomal DNA in YLS of Rice Planthoppers and Anobiid Beetles

Band No.	Rice planthoppers			Anobiid beetles	
	N. lugens	*S. furcifera*	*L. striatellus*	*L. serricorne*	*S. paniceum*
1	7.5	6.3	7.0	3.0	1.83
2	4.2*	5.7	5.0*	2.8	1.48
3	4.2(*)	3.5*	3.1	2.5	1.39
4	1.4	2.1	2.8	2.4	1.20
5			2.2	2.1	1.20
6				1.7*	1.11
7				1.6	1.00*
8				1.5	0.93*
9				1.2	0.93(*)
10				1.1	0.85
11				1.0	0.79
12					0.72
13					0.59
14					0.57
15					0.52
Genome size[a]	17.3	17.6	20.1	20.9	15.11

Note. Asterisks show the chromosomal DNAs which contain the 18S rDNA sequence. Chromosomal DNA bands with asterisks in parentheses may also contain 18S rDNA sequences.

[a] Genome size estimates based on sum of the individual chromosome sizes.

The following evidence also supports the usefulness of identifying microorganisms: chromosomal organization of eukaryotic microorganisms varies among species and chromosomal length polymorphism within one species is also known (Carle and Olson, 1985; Ono and Ishino-Arao, 1988; Mills and McCluskey, 1990; Bastien *et al.*, 1992). A electrophoretic pattern of chromosomes, however, is not very useful for classifying microorganisms. It is hard to find a definite tendency in chromosomal organization in one group of microorganisms. Actually, the karyotypes of YLS of *L. serricorne* and *S. paniceum* were similar to those of *H. wingei* and *S. cerevisiae,* respectively, but the similarity does not indicate the their relatedness. Instead of the electrophoretic karyotype, molecular analysis of genes is usable to classify the YLS. Nucleotide sequences of PCR-amplified 18S rDNA of YLS of the planthoppers, one of which was used as a hybridization probe in the present study, were determined, and 18S rDNA was shown to be a good index for classification of the YLS (Noda *et al.*, 1995).

The present study was undertaken to clarify whether the chromosomal organization and genome size of YLS were different from pathogenic or free-living

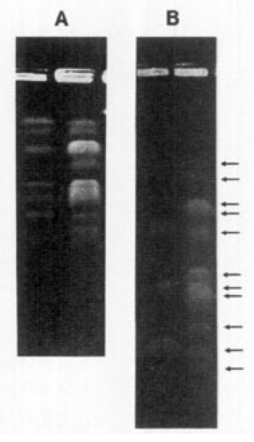

FIG. 3. Separation of chromosomal DNA of YLS of *Lasioderma serricorne.* (A) 0.8% Agarose gel electrophoresed for 242 hr at 40 V with 500- to 2800-sec ramped switching interval; lane 1, *H. wingei* DNA size standard; lane 2, YLS of *L. serricorne.* (B) 0.8% Agarose gel electrophoresed for 495 hr at 40 V with 400- to 2900-sec ramped switching interval; lane 1, *S. cerevisiae* DNA size standard; lane 2, YLS of *L. serricorne;* each arrow indicates chromosomal band.

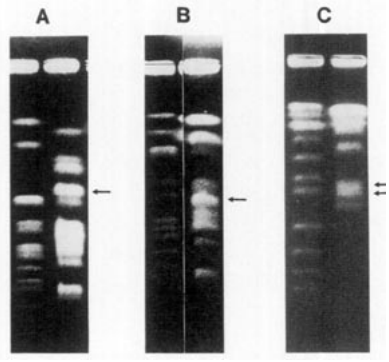

FIG. 4. Separation of chromosomal DNA of YLS of *Stegobium paniceum.* Lane 1, *S. cerevisiae* DNA size standard; lane 2, YLS of *S. paniceum.* (A) 1% Agarose gel electrophoresed for 24 hr at 200 V with 60- to 150-sec ramped switching interval; arrow, 1.20-Mbp doublet. (B) 1% Agarose gel electrophoresed for 24 hr in total at 200 V, for 14 hr with 70-sec switching interval, for 8 hr with 90-sec switching interval, and for 2 hr with 100-sec switching interval; arrow, 0.93-Mbp doublet. (C) 1% Agarose gel electrophoresed at 200 V for 8 hr with 40-sec switching interval and for 13 hr with 60-sec switching interval; arrows, 0.59- and 0.57-Mbp bands.

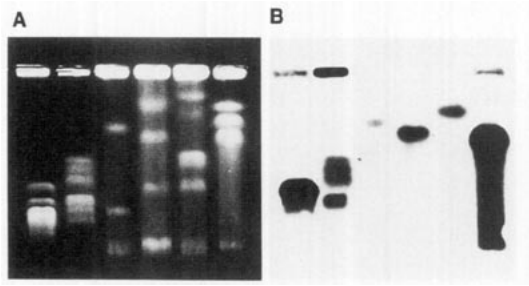

FIG. 5. Southern DNA hybridization analysis of YLS chromosomal DNA containing rDNA in the three species of rice planthoppers. (A) 0.8% Agarose gel electrophoresed for 212 hr at 40 V with 1500- to 6000-sec ramped switching interval. (B) DNA transferred filter probed with PCR amplified 18S rDNA of YLS of *N. lugens* using ECL detection systems. Lane 1, *S. cerevisiae* DNA size standard; lane 2, *H. wingei* DNA size standard; lanes 3–5, YLS of *N. lugens*, *S. furcifera*, and *L. striatellus*, respectively; lane 6, *S. pombe* DNA size standard.

yeasts and fungi, because genome size in some intracellular symbiotes is considered to be made smaller by transfer of genes to host cell nuclei and deletion of genes which are used for living outside the cell (Schwemmler, 1983). However, the YLS of the insects showed no significant difference in chromosomal organization nor in genome size from the nonsymbiotic yeasts and fungi. The number of YLS chromosomes of the rice planthoppers and the anobiid beetles, 4 to 15, is within the range of chromosome number of yeasts and fungi which have been studied by PFGE, from 3 in *S. pombe* (Smith *et al.*, 1987) to 20 in *Ustilago maydis* (Kinscherf and Leong, 1988). The size range of YLS chromosomes is also within that of yeasts and fungi, from about a few hundred kilobasepairs for example in the smaller-sized chromosomes of *S. cerevisiae* to more than 10 Mbp in the largest chromosome of *Pyricularia oryzae* (Hayashi, 1993). The genome size of all five species of YLS, 15.1–20.9, was also similar to that of non-

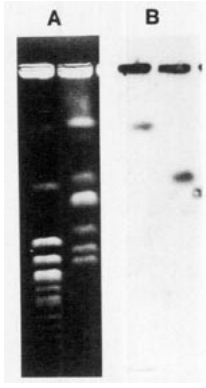

FIG. 6. Southern DNA hybridization analysis of YLS chromosomal DNA containing rDNA in *Lasioderma serricorne*. (A) 1% Agarose gel electrophoresed for 24 hr at 200 V with 100- to 180-sec ramped switching interval. (B) DNA transferred filter probed with PCR amplified 18S rDNA of YLS of *L. serricorne* using ECL detection systems. Lane 1, *S. cerevisiae* DNA size standard; lane 2, YLS of *L. serricorne*.

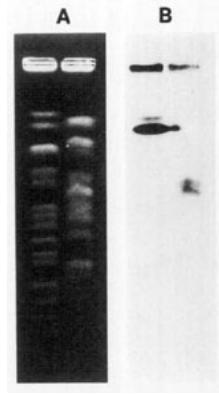

FIG. 7. Southern DNA hybridization analysis of YLS chromosomal DNA containing rDNA in *Stegobium paniceum*. (A) A 1% agarose gel electrophoresed at 200 V for 15 hr with 70-sec switching interval and for 9 hr with 100-sec switching interval. (B) DNA transferred filter probed with PCR amplified 18S rDNA of YLS of *S. paniceum* using ECL detection systems. Lane 1, *S. cerevisiae* DNA size standard; lane 2, YLS of *S. paniceum*.

symbiotic yeasts and fungi. The largest genome sizes reported are 47.7 Mbp in *Neurospora crassa* (Orbach *et al.*, 1988) (this may be an overestimation because the size estimate of standard DNA was larger than the present one) and 46.6 Mbp in *Phytophthora megasperma* (Toolley and Carras, 1992). Small genome size is observed in *Rhizoctonia solani*, 11.6 Mbp (Wako *et al.*, 1991), and in a highly virulent isolate of *Leptosphaeria maculans*, 8.6 Mbp (Taylor *et al.*, 1991). The fact that the YLS of the anobiid beetles can be cultured *in vitro* also indicates that the YLS still maintain most of the necessary genes for living outside a host cell. In this connection, the YLS of the anobiid beetles have a life stage outside the host beetles when they infect the next generation. They are smeared on the egg surface by female beetles and must live outside for at least several days until they are taken in by newly hatched larva (Jurzitza, 1979). This seems to be the reason that the YLS of the anobiid beetles are easily cultured *in vitro*. As for the YLS of the rice planthoppers, however, they are inherited transovarially and do not have any life stage outside the host planthopper: *in vitro* culturing of the YLS has failed. There is a question whether the inability to culture the YLS of the planthoppers *in vitro* is due to inadequate nutrition for them in the culture media or to a loss of some function (or gene) which is necessary for them to support themselves. If the latter is true, culturing the YLS of the planthoppers alone is impossible.

Using the hybridization technique, the rDNA was mapped on chromosomes of YLS in each insect species. The present technique is applicable for mapping various genes on the chromosomes of YLS and can be used to clone genes from individual chromosomes. If a gene which is involved in a process of infection with host insects is found in pathogenic fungi, for example, we would be able to map a homologous gene on the chro-

mosomes of the YLS. It is of interest how the gene in the YLS differ from that in the pathogen. If we could obtain the homologous genes, we might be able to start a new comparative study of mutualism and parasitism.

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