

Satellite DNA from the Chinese Fat Newt, *Pachytriton brevipes*, and Related Species

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(With 26 Text-figures)

ABSTRACT

The highly repetitive sequences of genomic DNA from the Chinese fat newt, *Pachytriton brevipes*, were examined by the methods of restriction endonuclease digestion, electrophoretic separation and molecular hybridization. Two satellite DNAs cut with *Bgl*II and *Bam*HI were isolated from the genomic DNA of the fat newt. Their monomers were about 330 bp and 400 bp, respectively. These two monomers were named as PBr(*Bgl*II)-1 and PBr(*Bam*HI)-1, respectively. Southern blot-hybridization of partially digested DNA of this species with ³²P-labeled monomer fragments of PBr(*Bgl*II)-1 or PBr(*Bam*HI)-1 showed a typical ladder of bands corresponding to multiples of the 330 bp or 400 bp bands.

Hybridization experiments indicated that these two satellites belong to two different satellite families. Both monomers were cloned into the *Bam*HI site of pBR322, and the recombinants were named as pPbrS1–5 and pPbrS2–9.

Hybridization experiments indicated that the 330 bp *Bgl*II satellite is conserved in *Cynops orientalis* from China, *C. pyrrhogaster pyrrhogaster* from Japan, *Triturus alpestris apuanus*, *T. cristatus carnifex* and *T. vulgaris meridionalis* from Europe, and *Notophthalmus viridescens* and *Taricha granulosa* from North America in an approximately equal amount, while the 400 bp *Bam*HI satellite is conserved in varying contents in the foregoing newt species except *T. vulgaris meridionalis*. The conservation of five cloned satellites, Nv1, Nv2, TcS2, TcS1 and Tvm1, obtained from the DNAs of American and European newts was examined and their relationships with the DNAs of Japanese and Chinese newts were discussed.

INTRODUCTION

High nuclear DNA content is one of the principal molecular features of amphibian cells. The DNA content shows a very high degree of interspecific variability in amphibians. Studies on reassociation kinetics have confirmed the hypothesis that the difference in genome size is due to the difference in the repetitive DNA content in both urodeles and anurans (BIRSTEIN, 1982).

A considerable amount of information has been accumulated on satellite DNA in certain groups of urodeles since 1980 (VARLEY et al., 1980a; VARLEY et al., 1980b; DIAZ et al., 1981; BALDWIN and MACGREGOR, 1985; EPSTEIN et al., 1986;

BARSACCHI-PILONE et al., 1986). The clones of the satellite DNA fragments were obtained from an American newt, *Notophthalmus viridescens* (pNv15 and pNv13) and European newts, *Triturus cristatus carnifex* (pTcS275-1 and pTcS1), *T. vulgaris meridionalis* (pTvm1) and *T. cristatus karelinii* (pTkS1 and pTkS2). In contrast, no clone has been obtained from Asian newts. In order to fill this blank and gain more insight into the problem, it seems desirable to analyze the nucleotide sequences of satellite DNA isolated from related species in Asia.

In the beginning of this study, the author ascertained that the genome of the Chinese fat newt, *Pachytriton brevipes*, contained a significant amount of highly repeated DNA. Then, he proceeded to isolate and characterize the nucleotide sequences by cloning, and to search for similar sequences in some other newt species. In the present paper, the author will report two satellite DNA sequences, 330 bp *Bgl*II and 400 bp *Bam*HI, isolated from the genomic DNA of the fat newt, *Pachytriton brevipes*, and will describe their clones, pPbrS1-5 and pPbrS2-9. The conservation of these satellite sequences in allied species distributed in different areas of the world will also be discussed.

MATERIALS AND METHODS

Pachytriton brevipes and *Cynops orientalis* were collected from Anhwei province, east China. Their genomic DNAs were prepared at the Institute of Developmental Biology, Beijing, People's Republic of China. The genomic DNA from *Notophthalmus viridescens*, *Taricha granulosa*, *Triturus alpestris apuanus*, *T. cristatus carnifex* and *T. vulgaris meridionalis* were given by Professor Joseph G. GALL, Department of Biology, Yale University. The genomic DNA of *Cynops pyrrhogaster pyrrhogaster* was prepared at the Laboratory for Amphibian Biology (Professor M. NISHIOKA), Faculty of Science, Hiroshima University, Japan.

Plasmid pBR322 DNA (BOLIVAR et al., 1977) and *Escherichia coli* HB101 (BOYER and ROULLAND-DUSSOIX, 1969) were used for cloning. Restriction enzymes were obtained from Boehringer-Mannheim Biochemicals, Bethesda Research Laboratories, TAKARA Shuzo Co., and New England Biolabs. *Escherichia coli* DNA polymerase and calf intestinal alkaline phosphatase were obtained from Boehringer-Mannheim, T₄ DNA ligase was from New England Biolabs, RNase was from Worthington, and bacterial alkaline phosphatase was from Bethesda Research Laboratories. Proteinase K was obtained from Merck and Agarose HGT (V) and LGT (VII) from Sigma. Other enzymes were supplied by Boehringer-Mannheim Biochemicals. SSC and DENHARDT's solution were prepared (DENHARDT, 1966).

DNA extraction. Livers or whole carcasses of newts were used after removal of the gut. The genomic DNA was isolated according to KAVENOFF and ZIMM (1973) with slight modifications as described previously (WU et al., 1986). The main innovation in this paper on the preparation of genomic DNA samples is to repeat the spool of DNA after the RNase treatment. This step makes DNA samples purer and does not necessitate further purification by equilibrium centrifugation in CsCl in most cases prior to Southern blot-hybridization.

Restriction endonuclease digestion. Nine kinds of restriction endonucleases, *Bgl*II, *Bam*HI, *Taq*I, *Eco*RI, *Hind*III, *Cla*I, *Kpn*I, *Pst*I and *Hinf*I, were used according to the manufacturers' instructions. Digestions using these enzymes were carried out at 37°C (except for *Taq*I, which was used at 65°C) for 2~4 hours under the conditions indicated by the manufacturers. After digestion, DNA samples in 25 μ l of reaction mixture were mixed with 2.5 μ l of 10 \times stopping mixtures (SCHAFFNER et al., 1976).

Gel electrophoresis and blot-hybridization. Agarose gel electrophoresis was performed using the method of HELING et al. (1974). The vertical slab gel apparatus used in this work was the product of ATTO Electrophoresis (Tokyo, Japan) or Bio-Rad Laboratory (Richmond, California). Electrophoresis was carried out with constant voltage (65~80 V) at 18°C. Agarose gel was made 1.5~2% (w/v) in the standard buffer (TAE). After electrophoresis, the gel was removed, stained with ethidium bromide (0.5 μ g/ml) and photographed under ultraviolet light of long wave length. *Hind*III and *Bgl*II-digested λ phage DNA or *Hinf*I-digested pBR322 DNA was routinely included in the electrophoresis as molecular weight standards (HELLING et al., 1974; PRASSOLOV et al., 1986). The method described by SOUTHERN (1975) was used in order to transfer DNA directly from the gel onto a nitrocellulose filter. Prior to DNA/DNA hybridization, the filters were incubated for 4~6 hours at 42°C in 50 ml solution of 40% formamide, 4 \times SSC, 10 \times DENHARDT's solution (DENHARDT, 1966), and 0.1% SDS, plus heat-denatured calf thymus DNA and sodium pyrophosphate. Heat-denatured ³²P-DNA was added to the pre-incubation mixture. Hybridization was usually carried out overnight at 42°C. The filters were then washed several times with 2 \times SSC+0.1% SDS at room temperature by circular shaking motion. They were again washed three times with 1 \times SSC+0.1% SDS for at least 2 hours at 60~65°C. Filters were exposed at -80°C on Kodak XR-5 films, Shanghai X-ray films or Fuji RX films, using Dupont Lightning Plus, Fuji Hi-Screen B-2 or Beijing Chemical Factory's High Speed Intensifying Screens (LASKEY and MILLS, 1977).

Clones. Five clones, pNv15, pNv13, pTvm1, pTcS275-1 and pTcS1, provided by Professor J. G. GALL, Department of Embryology, Carnegie Institution, were used. Clone pNv15 consists of a dimer (222 \times 2 bp) of satellite 1 (Nv1) from *Notophthalmus viridescens*. It contains a *Bgl*II fragment from the genomic DNA of *N. viridescens* inserted in the *Bam*HI site of pBR322 (DIAZ et al., 1981; EPSTEIN et al., 1986). Clone pNv13 consists of a monomer (330 bp) of satellite 2 (Nv2) from *N. viridescens*. Clone pTvm1 consists of a monomer (310 bp) of satellite DNA (Sat G) from *Triturus vulgaris meridionalis*. It contains a *Hind*III fragment from satellite DNA (Sat G) of *T. vulgaris meridionalis* inserted in the *Hind*III site of pBR322 (BARSACCHI-PILONE, 1986). Clone pTcS275-1 (TcS2) consists of a monomer (330 bp) of satellite DNA from *Triturus cristatus carnifex*. It contains a *Bgl*II fragment from the genomic DNA of *T. cristatus carnifex* inserted in the *Bam*HI site of pBR322 (VARLEY et al., 1980b). Clone pTcS1 consists of a monomer (400 bp) of satellite DNA of *Triturus cristatus carnifex*. It contains a *Bam*HI fragment from the genomic DNA of *T. cristatus carnifex* inserted in the *Bam*HI site of pBR322 (VARLEY et al.,

1980b; BALDWIN and MACGREGOR, 1985).

Preparation of radioactive probes. Double-stranded DNA (0.5~1 μ g) was 32 P-labeled by nick-translation (MANIATIS et al., 1975), using three non-radioactive dNTPs and one radioactive dNTP (Amersham or ICN). The (α - 32 P)dNTP had specific activities ranging from 800 to 3200 Ci/mmol and 25, 50 or 180 pmol/ μ g of DNA were used in each reaction. *E. coli* DNA polymerase I was used at 2 units per reaction. The volume of the mixture was 100 μ l. The probes had specific radioactivities ranging from 1.0×10^7 to 1.0×10^8 cpm/ μ g.

OBSERVATION

I. Identification and characterization of satellite components in the genomic DNA of *Pachytriton brevipes*

The fat newt, *Pachytriton brevipes*, is mainly distributed in the southeastern part of China. The satellite components in the genomic DNA of *Pachytriton brevipes* were studied by the methods of restriction endonuclease digestion, electrophoretic separation and molecular hybridization.

According to SINGER (1982), the repeat units in the genomic DNAs of eukaryotic organisms and their tandem organization can be revealed by restriction endonuclease digestion. When a recognition site for a particular restriction endonuclease occurs within a typical repeat unit, digestion converts a tandem array to a set of DNA fragments of a repeat unit length. Partial digestions produce "ladders" of fragments which can be demonstrated by electrophoresis. The "ladder" fragments are integral multiples of the basic repeat unit length, providing evidence for the tandem organization of the satellite.

In order to detect bands, which might represent highly repetitive sequences, the genomic DNA of *Pachytriton brevipes* was digested with several restriction endonucleases, electrophoresed on agarose gel, and stained with ethidium bromide. Examples of agarose gel electrophoresis of *Bgl*II, *Taq*I, *Bam*HI, *Hind*III, *Hin*fI, *Kpn*I and *Eco*RI-digests of the DNA of *Pachytriton brevipes* are shown in Fig. 1. Single or several well-defined bands were seen against a background smear in the digests of *Bgl*II, *Taq*I and *Bam*HI, but no discrete bands were produced by digestion of the same DNA with *Hind*III, *Hin*fI, *Kpn*I, *Eco*RI and *Pst*I (data not shown). In some of these digests, a band of dimer-length was also seen, indicating that these DNA sequences may be arranged in tandem.

By digestion with endonuclease *Bgl*II, a prominent band corresponding to a sequence length of 330 bp (base pairs) was visualized by illumination with ultraviolet light (Fig. 2,b). A ladder of bands corresponding to multiples of the 330 bp band appeared by partial digestion, indicating that this sequence is present in substantial tandem repeats. The gel slice containing this band (330 bp) was taken out from the preparative gel and purified as follows: Agarose gels (low gelling temperature agarose, type VII, Sigma) were poured and allowed to harden at 4°C for 1 hour. Gels were electrophoresed, stained, and photographed.

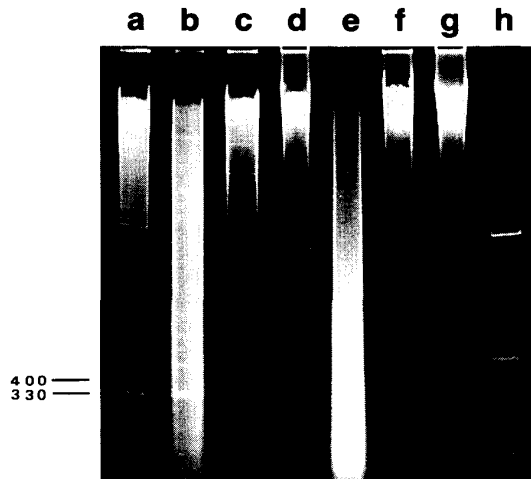


Fig. 1

Fig. 1. Ethidium bromide-stained gel of the genomic DNA of *Pachytriton brevipes* digested with several restriction endonucleases, *Bgl*II (a), *Taq*I (b), *Bam*HI (c), *Hind*III (d), *Hinf*I (e), *Kpn*I (f) and *Eco*RI (g). The molecular weight size markers were pBR322 digested with *Hinf*I (h). Size of the monomers in lanes (a) and (c) is shown in base pairs.

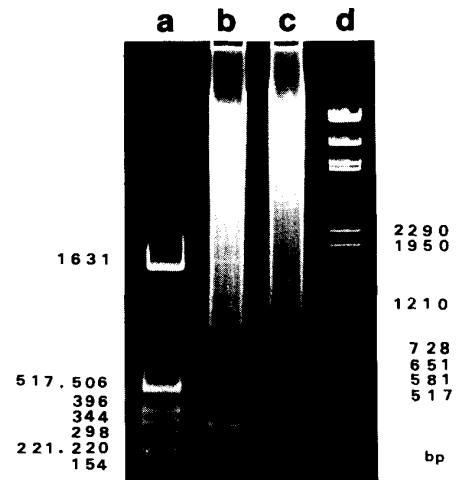


Fig. 2

Fig. 2. Digestion of the genomic DNA of *Pachytriton brevipes* with restriction endonucleases, *Bgl*II (b) and *Bam*HI (c). The DNA of the smallest bands was eluted. Molecular weight size markers were pBR322 digested with *Hinf*I (a) and λ phage DNA digested with *Hind*III and *Bgl*II (d). 1.5% agarose gel was stained with ethidium bromide. Marker bands are shown in base pairs.

Monomer bands were taken from the gel. The volume of the gel containing the monomer bands was estimated, 1/10 of its volume of 3M sodium acetate (pH 5.2) was added, and the tube was held at 65°C until the agarose melted. The DNA was extracted twice with phenol, once with a mixture (1:1) of phenol and chloroform, and three times with ether. It was precipitated twice with ethanol. The 330 bp fragment of the genomic DNA obtained by digestion with *Bgl*II was then separated. This *Bgl*II repeat unit was designated as PBr(*Bgl*II)-1 fragment.

By digestion with endonuclease *Bam*HI, a ladder of bands corresponding to multiples of 400 bp was visualized (Fig. 2,c). The smallest fragment (about 400 bp) was purified by the same method as described above. The *Bam*HI repeat unit was designated as PBr(*Bam*HI)-1 fragment.

When the DNA of the oriental newt, *Cynops orientalis*, was digested with *Bgl*II, *Taq*I, *Bam*HI, *Hind*III, *Hinf*I, *Kpn*I, *Eco*RI and *Pst*I (data not shown), electrophoretic patterns similar to those of *Pachytriton brevipes* appeared (Fig. 3). Prominent bands corresponding to sequence lengths of 330 bp and 400 bp were visualized by *Bgl*II and *Bam*HI digestions, respectively (Fig. 4,b and c).

There are three questions to be answered here: (1) Is there any sequence homology between *Bgl*II and *Bam*HI satellites in *Pachytriton brevipes*? (2) Is there any sequence homology between the *Bgl*II satellite of *Pachytriton brevipes* and that of *Cynops orientalis*? (3) Is there any sequence homology between the *Bam*HI satellite of *P. brevipes* and that of *C. orientalis*?

Autoradiograph of the gel shown in Fig. 2 after Southern blot-hybridization with 32 P-PBr(*Bgl*II)-1 fragment revealed a ladder of multimeric bands of radioactivity

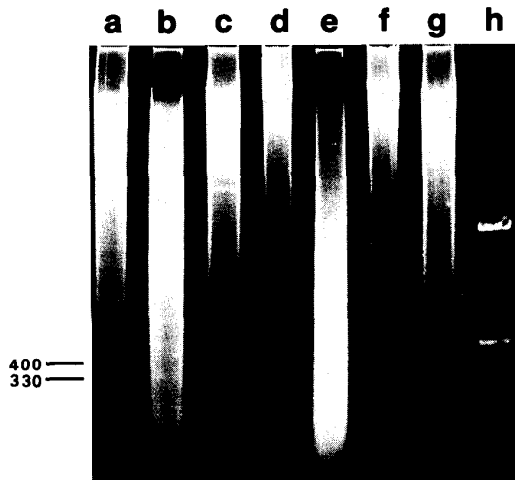


Fig. 3

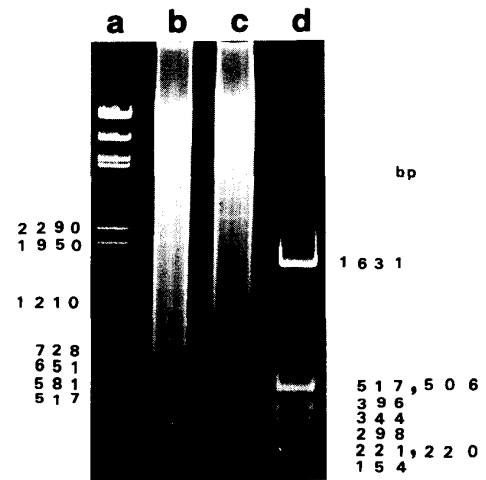


Fig. 4

Fig. 3. Ethidium bromide-stained gel of the genomic DNA of *Cynops orientalis*. The DNA was digested with several restriction endonucleases, *Bgl*II (a), *Taq*I (b), *Bam*HI (c), *Hind*III (d), *Hinf*I (e), *Kpn*I (f) and *Eco*RI (g). The molecular weight size markers were pBR322 digested with *Hinf*I (h). Size of the monomers in the lanes (a) and (c) is shown in base pairs.

Fig. 4. Digestion of the genomic DNA of *Cynops orientalis* with restriction endonucleases, *Bgl*II (b) and *Bam*HI (c). Molecular weight size markers were obtained by λ phage DNA digested with *Hind*III and *Bgl*II (a), and by pBR322 digested with *Hinf*I (d). 1.5% agarose gel was stained with ethidium bromide. Marker bands are shown in base pairs.

in the *Bgl*II-digest as predicted (Fig. 5,b). The lowest molecular weight band of the ladder corresponded to the 330 bp fragment found in the gel shown in Fig. 2. These results show that the *Bgl*II satellite is typical tandemly-repeated DNA sequences found in the genomic DNA of *P. brevipes*. It was interesting to note that the *Bam*HI-digests showed positive hybridization only with the fragments of higher molecular weight. This indicates that 330 bp *Bgl*II satellite and 400 bp *Bam*HI satellite may belong to different satellite families (Fig. 5,c).

Autoradiograph of the gel in Fig. 4 after Southern blot-hybridization with 32 P-PBr(*Bam*HI)-1 fragment revealed a ladder of multimeric bands of radioactivity in the *Bam*HI-digest of the genomic DNA of *Cynops orientalis* (Fig. 6). The band of the lowest molecular weight corresponded to the 400 bp fragments. The *Bgl*II-digests showed positive hybridization with the higher molecular weight fragment only. Therefore, it seemed that within the genomic DNA of *C. orientalis*, there exist highly repetitive sequences which are homologous to PBr(*Bam*HI)-1 fragments of *Pachytriton brevipes*.

When 32 P-labeled PBr(*Bgl*II)-1 fragment was used as a probe to hybridize with *Taq*I, *Bgl*II, *Eco*RI and *Bam*HI-digests of the genomic DNA of *P. brevipes*, and to hybridize with *Bgl*II, *Eco*RI and *Bam*HI-digests of the genomic DNA of *C. orientalis*, both the *Bgl*II-digests of the genomic DNAs of *P. brevipes* and *C. orientalis* showed ladder patterns representing repeats of 330 bp fragment (Fig. 7).

It was interesting that *Hinf*I-digest of the genomic DNA of *C. orientalis* showed a single strong monomer band (330 bp) (Fig. 8B, e). On the other hand, when the PBr(*Bam*HI)-1 probe was used to hybridize with the genomic DNAs of *P. brevipes*

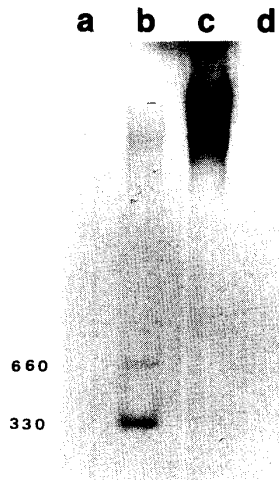


Fig. 5

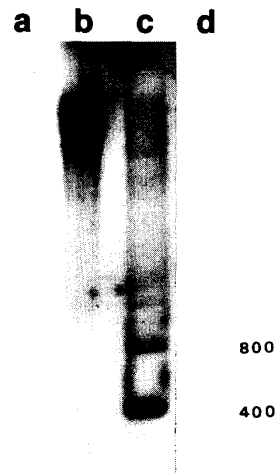


Fig. 6

Fig. 5. Autoradiograph of the gel in Fig. 2 after Southern blot-hybridization with ^{32}P -PBr(*Bgl*II)-1 fragment. Digestion of the genomic DNA of *Pachytriton brevipes* with restriction endonucleases, *Bgl*II (b) and *Bam*HI (c). Molecular weight size markers were pBR322 digested with *Hinf*I (a) and λ phage DNA digested with *Hind*III and *Bgl*II (d). Only the *Bgl*II-digest showed a ladder of hybridization. Size of the monomer and dimer is shown in base pairs.

Fig. 6. Autoradiograph of the gel in Fig. 4 after Southern blot-hybridization with ^{32}P -PBr(*Bam*HI)-1 fragment. Digestion of the genomic DNA of *Cynops orientalis* with restriction endonucleases, *Bgl*II (b) and *Bam*HI (c). Molecular weight size markers were λ phage DNA digested with *Hind*III and *Bgl*II (a) and pBR322 digested with *Hinf*I (d). Only the *Bam*HI-digest showed a ladder-like pattern of hybridization. Size of the monomer and dimer is shown in base pairs.

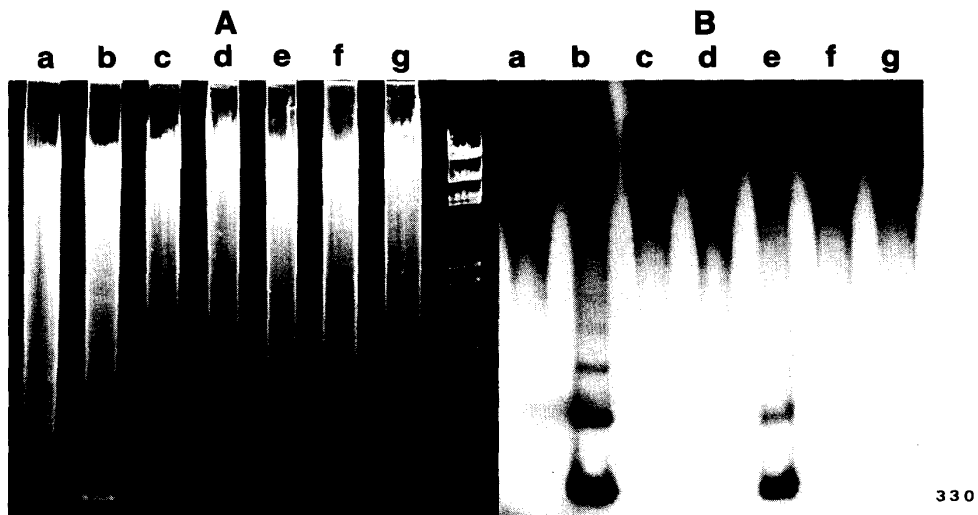


Fig. 7. A. Ethidium bromide-stained gel of the genomic DNA of *Pachytriton brevipes* digested with *Taq*I (a), *Bgl*II (b), *Eco*RI (c) and *Bam*HI (d); and that of the genomic DNA of *Cynops orientalis* digested with *Bgl*II (e), *Eco*RI (f) and *Bam*HI (g). The rightmost lane contains λ phage DNA digested with *Hind*III and *Bgl*II. B. The gel shown in <A> was blotted onto a nitrocellulose filter and hybridized with ^{32}P -labeled PBr(*Bgl*II)-1 fragment. *Bgl*II-digests (lanes b and e) showed a ladder-like pattern of hybridization. Size of the monomer is shown in base pairs.

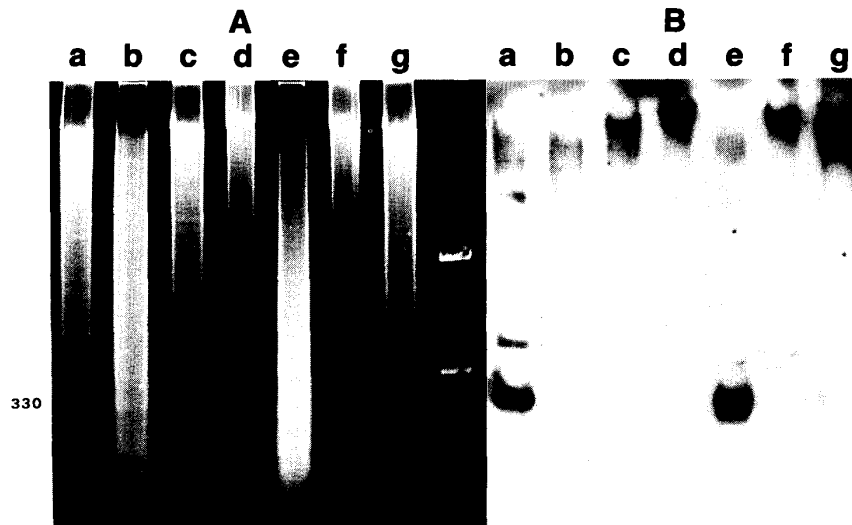


Fig. 8. A. Ethidium bromide-stained gel of the genomic DNA of *Cynops orientalis* digested with *Bgl*II (a), *Taq*I (b), *Bam*HI (c), *Hind*III (d), *Hin*fI (e), *Kpn*I (f) and *Eco*RI (g). The rightmost lane contains pBR322 DNA digested with *Hin*fI. B. Autoradiograph of the gel in <A> after transferring to a nitrocellulose filter and hybridized with 32 P-labeled PBr(*Bgl*II)-1 fragment. A strong 330 bp band is shown in *Hin*fI-digestion.

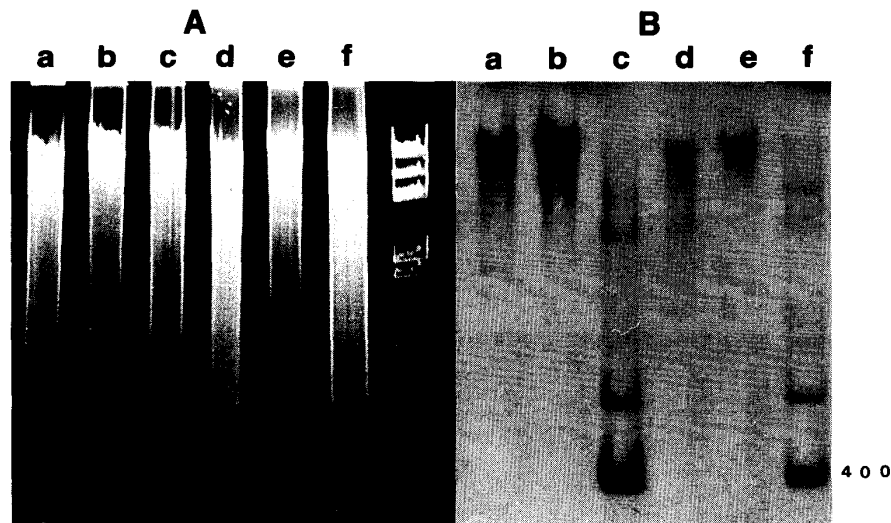


Fig. 9. A. Ethidium bromide-stained gel of the genomic DNA of *Pachytriton brevipes* digested with *Bgl*II (a), *Hind*III (b) and *Bam*HI (c); and that of the genomic DNA of *Cynops orientalis* digested with *Bgl*II (d), *Hind*III (e) and *Bam*HI (f). The rightmost lane contains λ phage DNA digested with *Hind*III and *Bgl*II. B. The gel shown in <A> was blotted onto a nitrocellulose filter and hybridized with 32 P-labeled PBr(*Bam*HI)-1 fragment. *Bam*HI-digests (lanes c and f) showed a ladder-like pattern of hybridization. Size of the monomer is shown in base pairs.

and *C. orientalis* digested with different restriction enzymes, *Bgl*II, *Hind*III and *Bam*HI, they all revealed positive hybridization with higher molecular weight fragments, except that the *Bam*HI-digests of these two species showed a ladder pattern representing repeats of 400 bp (Fig. 9B, c and f). The *Taq*I and *Eco*RI-digests of the DNA of *P. brevipes* showed weak but distinct ladder-like pattern (Fig. 10B, a and c)

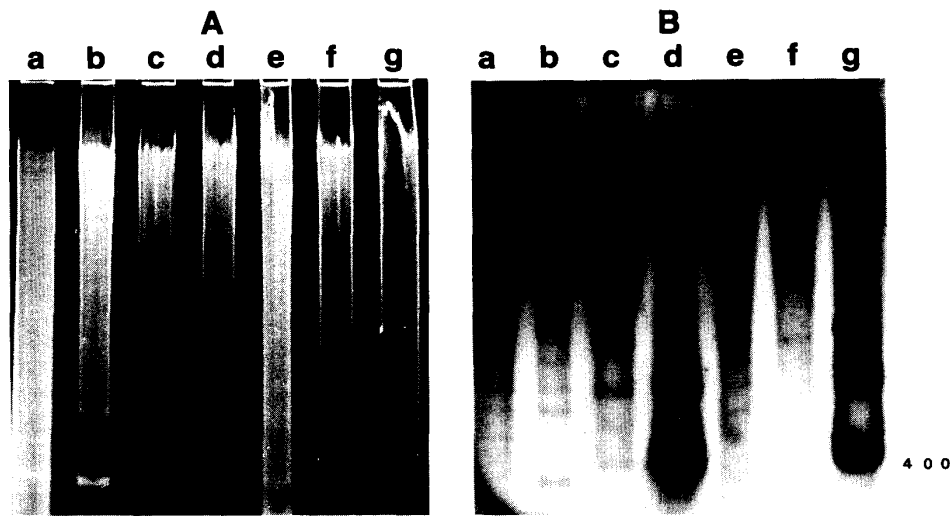


Fig. 10. A. Ethidium bromide-stained gel of the genomic DNA of *Pachytriton brevipes* digested with *TaqI* (a), *BglII* (b), *EcoRI* (c) and *BamHI* (d); and that of the genomic DNA of *Cynops orientalis* digested with *BglII* (e), *EcoRI* (f) and *BamHI* (g). B. The gel shown in <A> was blotted onto a nitrocellulose filter and hybridized with ^{32}P -labeled PBr(*BamHI*)-1 fragment. Size of the monomer is shown in base pairs.

In any case, the results of hybridization experiments indicated that there are two different satellite DNAs, *BglII* satellite and *BamHI* satellite, present in the genomic DNAs of *P. brevipes* and *C. orientalis*, respectively. The monomer of *BglII* satellite is about 330 bp, while the monomer of *BamHI* satellite is about 400 bp. Hybridization results indicated that the sequences of these two kinds of satellites had little homology, if any.

II. Conservation of *BglII* satellite and *BamHI* satellite of *Pachytriton brevipes* in various newt species

The presence of the sequences of PBr(*BglII*)-1 fragment in the genomes of several other newt species was examined. Hybridization using ^{32}P -labeled PBr(*BglII*)-1 as a probe with the *BglII*-digested DNAs (same quantity, 6 μg) from various species showed that there are almost the same amounts of PBr(*BglII*)-1 fragment conserved in American newt (*Taricha granulosa*), European newts (*Triturus alpestris apuanus*, *T. cristatus carnifex* and *T. vulgaris meridionalis*), and Asian newt (*Cynops orientalis*) (Fig. 11). Hybridization occurred almost as strongly as that with the homologous DNA of *P. brevipes*. Therefore, these results indicate that a satellite homologous to the *BglII* satellite of *P. brevipes* is highly conserved in each of these species.

The hybridization of the *BamHI*-digest of the same quantity (6 μg) of the DNAs from various species with ^{32}P -labeled PBr(*BamHI*)-1 probe showed that moderate hybridization occurred with *Cynops orientalis*, and only weak hybridization occurred with *Taricha granulosa*, *Triturus alpestris apuanus* and *T. cristatus carnifex*. Hybridization with *Triturus vulgaris meridionalis* did not show any ladder-like pattern (Fig. 12). Obviously, there were wide variations in the amount of *BamHI* satellite among

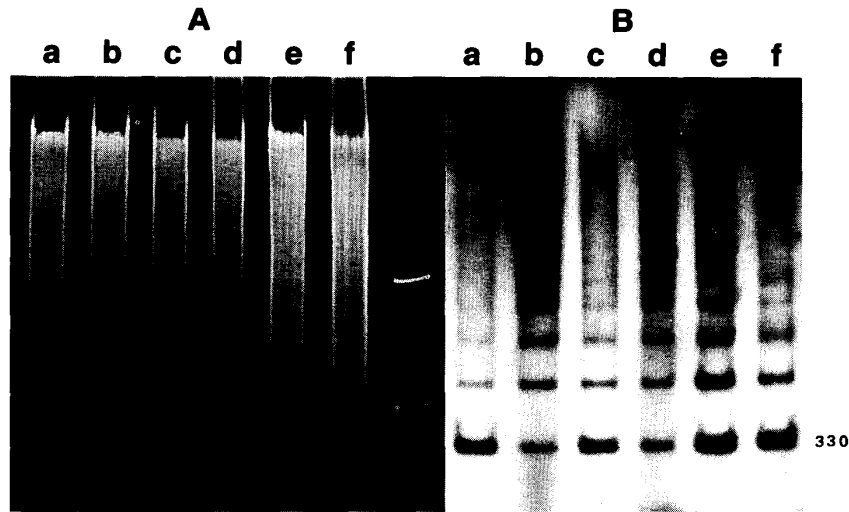


Fig. 11. A. Ethidium bromide-stained gel of the genomic DNAs of *Taricha granulosa* (a), *Triturus alpestris apuanus* (b), *T. cristatus carnifex* (c), *T. vulgaris meridionalis* (d), *Pachytriton brevipes* (e) and *Cynops orientalis* (f) digested with *Bgl*II. The rightmost lane contains pBR322 digested with *Hinf*I. B. Autoradiograph of the gel in <A> after transfer to a nitrocellulose filter and hybridization with ³²P-labeled PBr(*Bgl*II)-1 fragment. All the species showed ladder-like pattern of hybridization which were almost as strong as that with homologous DNA of *Pachytriton brevipes*. Size of the monomers is shown in base pairs.

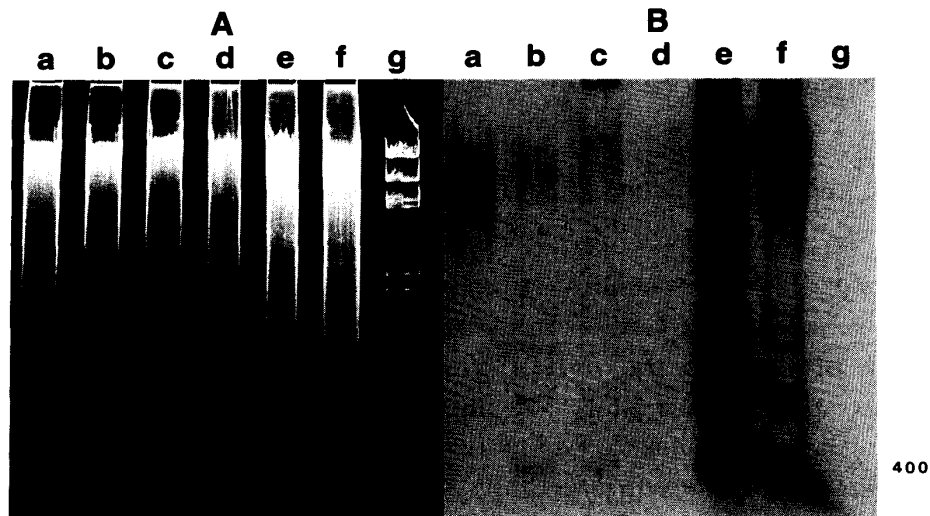


Fig. 12. A. Ethidium bromide-stained gel of the genomic DNAs of *Taricha granulosa* (a), *Triturus alpestris apuanus* (b), *T. cristatus carnifex* (c), *T. vulgaris meridionalis* (d), *Pachytriton brevipes* (e) and *Cynops orientalis* (f) digested with *Bam*HI. The lane (g) contains λ phage DNA digested with *Hind*III and *Bgl*II. B. Autoradiograph of the gel in <A> after Southern blot-transfer to a nitrocellulose filter and hybridization with ³²P-labeled PBr(*Bam*HI)-1 fragment. Moderate hybridization occurred with *Cynops orientalis* (f), and weak hybridization occurred with *Taricha granulosa* (a), *Triturus alpestris apuanus* (b) and *T. cristatus carnifex* (c). *T. vulgaris meridionalis* (d) showed no ladder-like hybridization. The quantities of the sequences of PBr(*Bam*HI)-1 fragment in different species were different. Size of monomers is shown in base pairs.

these species. The conservation of the satellite homologous to the *Bam*HI satellite of *P. brevipes* was not the same among different species.

III. Cloning of the *Bam*HI and *Bgl*II satellite DNA sequences of *Pachytriton brevipes*

It is necessary to obtain the clones of the satellite DNA sequences in order to advance the studies on their structure and function, their localization on the chromosomes and their transcription. Therefore, PBr(*Bam*HI)-1 fragments of the genomic DNA of *P. brevipes* were ligated to *Bam*HI-cleaved pBR322 as described by BOLIVAR et al. (1977). *Escherichia coli* HB101 cells were used to obtain transformants by the method of COHEN et al. (1972). Nine transformants that contained plasmids with inserts were selected on an ampicillin plate and a tetracycline plate (CLEWELL and HELINSKI, 1969) and were screened with nick-translated PBr(*Bam*HI)-1 fragments isolated from the *Bam*HI-digest of the DNA of *P. brevipes* (GRUNSTEIN and HOGNESS, 1975). The plasmid DNA was purified by the method of CLEWELL and HELINSKI (1969). The nine recombinant clones were analyzed by agarose gel electrophoresis and Southern blot-hybridization with the probe of ³²P-labeled PBr(*Bam*HI)-1 fragment. These experiments demonstrated that recombinant plasmids have inserts of about 400 bp, consisting of monomer units of *Bam*HI satellite. One of the clones containing a single repeat of *Bam*HI satellite of *P. brevipes* was called pPbrS2-9 (Fig. 13). Gel electrophoresis of *Bam*HI-restricted DNA from pPbrS2-9 showed that the plasmid has inserts of about 400 bp (Fig. 14).

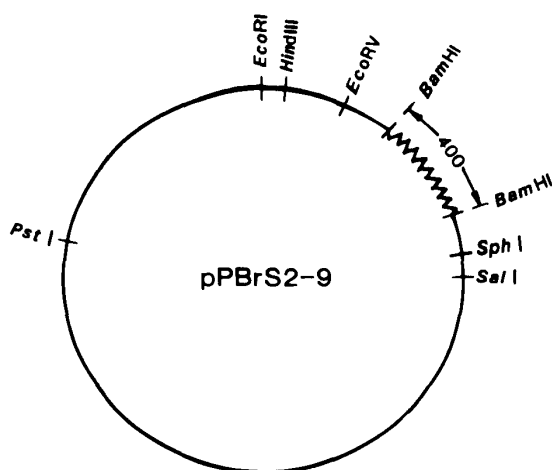


Fig. 13

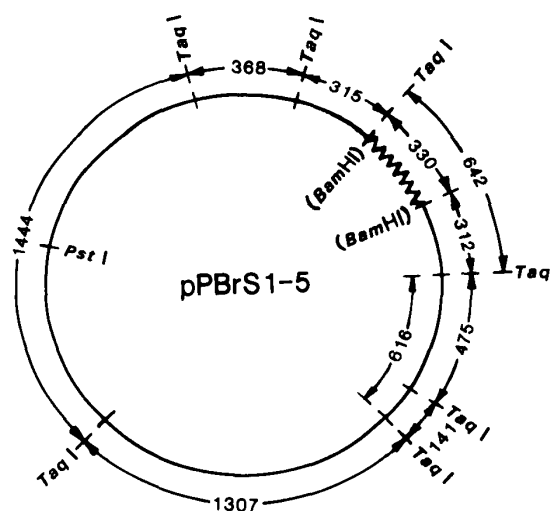


Fig. 15

Fig. 13. Restriction enzyme map of plasmid pPbrS2-9. This plasmid contains a monomer of *Pachytriton brevipes* *Bam*HI satellite DNA cloned in pBR322. The monomer is a 400 bp *Bam*HI fragment isolated from genomic DNA; it is cloned in the *Bam*HI site of the vector.

Fig. 15. Restriction enzyme map of plasmid pPbrS1-5. This plasmid contains a monomer of *Pachytriton brevipes* *Bgl*II satellite DNA cloned in pBR322. The monomer is a 330 bp *Bgl*II fragment isolated from genomic DNA; it is cloned in the *Bam*HI site of the vector.

PBr(*Bgl*II)-1 fragments of the genomic DNA of *P. brevipes* were also ligated to *Bam*HI-cleaved pBR322. Five transformants containing plasmids with inserts were selected on an ampicillin plate and a tetracycline plate and were screened with nick-translated PBr(*Bgl*II)-1 fragments isolated from *Bgl*II-digest of the DNA of *P. brevipes*. The clone containing a single repeat of *Bgl*II satellite of *P. brevipes* was designated as pPbrS1-5. It was not possible to excise the *Bgl*II-satellite DNA sequence out of the recombinant plasmid, as the two restriction endonuclease-recognizable sequences, AGATCT for *Bgl*II and GGATCC for *Bam*HI, were lost. However, gel electrophoresis of *Taq*I-restricted DNA from pPbrS1-5 showed that the plasmid has inserts of about 330 bp within the 642 bp fragment (i.e. 312 bp *Taq*I fragment of pBR322 plus 330 bp of insert) (Figs. 15 and 16).

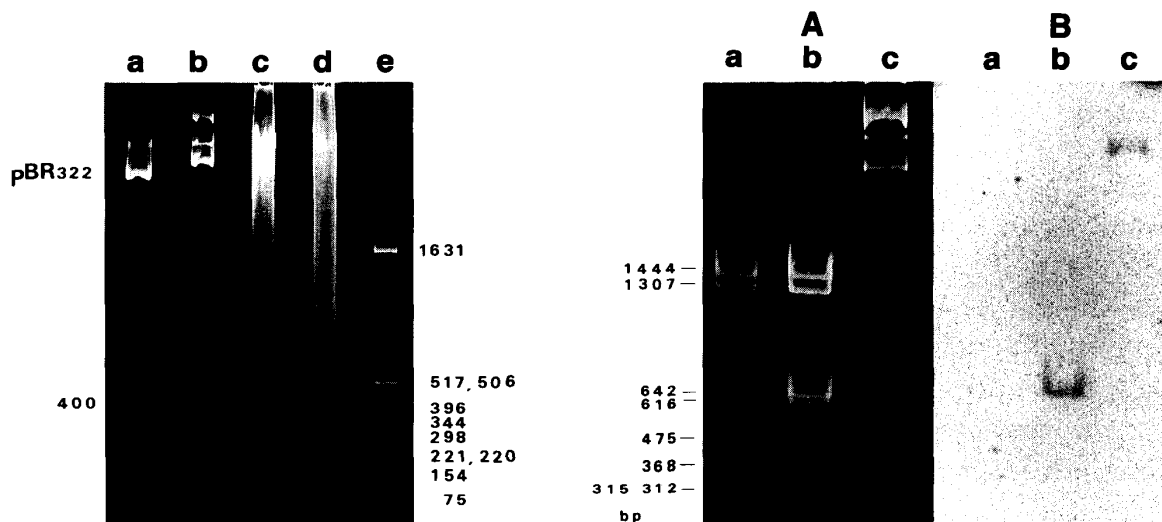


Fig. 14

Fig. 16

Fig. 14. Ethidium bromide-stained gel of clone pPbrS2-9 digested with *Bam*HI (a), uncut clone pPbrS2-9 (b), the genomic DNAs of *Pachytriton brevipes* (c) and *Cynops orientalis* (d) digested with *Bam*HI. Plasmid DNA (pBR322) digested with *Hin*I was used as a size marker (e). Size of molecular weights is shown in base pairs.

Fig. 16. A. Ethidium bromide-stained gel of plasmid DNA (pBR322) (a) and clone pPbrS1-5 digested with *Taq*I (b) and uncut clone pPbrS1-5 (c). The 642 bp (312+330) fragment contained the 330 bp PBr(*Bgl*II)-1 fragment of *Pachytriton brevipes*. B. Southern blot of <A> hybridized with some of the 330 bp PBr(*Bgl*II)-1 genomic fragment used for cloning (³²P-labeled). A single restriction fragment showed hybridization, indicating that the clones contained *Pachytriton brevipes* DNA. Size of molecular weights is shown in base pairs.

The present author examined the occurrence of clone pPbrS1-5 sequences in the genomes of *C. orientalis* and *C. pyrrhogaster*. Hybridization of *Bgl*II-digests of similar DNA amounts (6 μ g) from the two species with cloned ³²P-pPbrS1-5 showed that a satellite homologous to pPbrS1-5 sequences is conserved in both *C. orientalis* and *C. pyrrhogaster* (Fig. 17B). Hybridization occurred almost as strongly as that with the homologous DNA of *P. brevipes*.

The conservation of the satellites homologous to clone pPbrS2-9 in *C. orientalis*

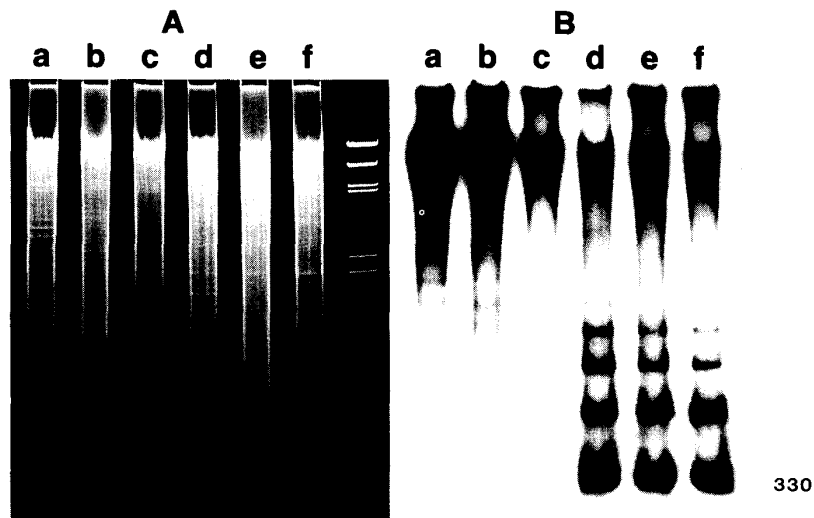


Fig. 17. A. Ethidium bromide-stained gel of the genomic DNAs of *Pachytriton brevipes* (a, d), *Cynops orientalis* (b, e) and *C. pyrrhogaster* (c, f) digested with *Bam*HI (a, b, c) and *Bgl*II (d, e, f). The rightmost lane contains λ phage DNA digested with *Hind*III and *Bgl*II. B. Autoradiograph of the gel in <A> after Southern blot-transfer to a nitrocellulose filter and hybridization with 32 P-labeled clone pPbrS1-5. Size of the monomers is shown in base pairs.

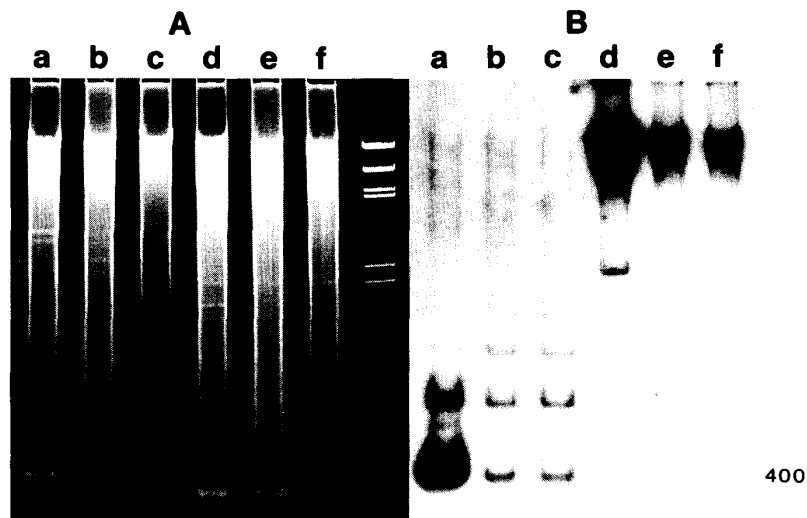


Fig. 18. A. Ethidium bromide-stained gel of the genomic DNAs of *Pachytriton brevipes* (a, d), *Cynops orientalis* (b, e) and *C. pyrrhogaster* (c, f) digested with *Bam*HI (a, b, c) and *Bgl*II (d, e, f). The rightmost lane contains λ phage DNA digested with *Hind*III and *Bgl*II. B. Autoradiograph of the gel in <A> after Southern blot-transfer to a nitrocellulose filter and hybridization with 32 P-labeled clone pPbrS2-9. Size of the monomers is shown in base pairs.

and *C. pyrrhogaster* was investigated by digesting the corresponding genomic DNAs with the same battery of enzymes as used for the homologous species. The resulting patterns were very similar to those obtained in the homologous species. However, the intensity of hybridization showed that there is a large difference in the amount of clone pPbrS2-9 sequences between *Pachytriton* and *Cynops* (Fig. 18). A consistent, although weak, cross-hybridization occurred with *Taricha granulosa*, *Triturus alpestris apuanus* and *T. cristatus carnifex*, while *T. vulgaris meridionalis* did not

show any ladder-like pattern of hybridization (data not shown).

IV. The conservation of the other satellite DNAs of some other newt species

Besides the clones pPbrS2-9 and pPbrS1-5, there are so far seven satellite DNA sequences obtained from some newts and purified by molecular cloning: pNv15, pNv13, pTcS275-1, pTcS1, pTvm1, pTkS1 and pTkS2 (MACGREGOR and SESSIONS, 1986). Of these clones, pNv15 and pNv13 have been derived from *Notophthalmus viridescens*. Clone pNv15 contains a dimer (222×2 bp) *Bgl*II fragment of satellite 1 (DIAZ et al., 1981; GALL et al., 1983), and clone pNv13 contains a monomer (330 bp) of satellite 2 (EPSTEIN et al., 1986). Clones pTcS275-1 and pTcS1 have been derived from *Triturus cristatus carnifex*. Clone pTcS275-1 contained *Bgl*II fragment (330 bp, previously reported as 275 bp), and clone pTcS1 contained *Bam*HI fragment (390 bp, originally reported as 330 bp, and then as 380 bp) (VARLEY et al., 1980a ; BALDWIN and MACGREGOR, 1985). Clone pTvm1 has an insert of *Hind*III-cleaved fragment, being 310 bp of Sat G derived from *Triturus vulgaris meridionalis* (BARSACCHI-PILONE et al., 1986).

The presence of pNv15 sequences in the genomes of various newts was examined. The autoradiographs of genomic DNAs of various newts after *Bgl*II-digestion, gel electrophoresis and Southern blot-hybridization with ³²P-pNv15 showed that a satellite homologous to the pNv15 sequences is conserved only in *Taricha granulosa* (Fig. 19), although the hybridization is weak. The European newts, *Triturus alpestris apuanus*, *T. cristatus carnifex* and *T. vulgaris meridionalis* do not possess a related satellite DNA (data not shown). Therefore, satellite 1 from *Notophthalmus viridescens* is not a widely conserved repetitive sequence among newt species.

The properties of satellite 2 from *N. viridescens* are not shared by satellite 1. Hybridization of *Bgl*II-digests of similar amounts of the DNAs of various newts with ³²P-labeled pNv13 showed that a satellite homologous to the pNv13 sequence is conserved both in the American newt, *Taricha granulosa*, and the European newts, *Triturus alpestris apuanus*, *T. cristatus carnifex* and *T. vulgaris meridionalis* (Fig. 20).

The presence of pTvm1 sequences in the genomes of other newt species was also examined. Hybridization of *Hind*III, *Taq*I and *Bgl*II-digests of similar amounts of DNA with ³²P-labeled pTvm1 showed that a satellite homologous to the pTvm1 sequence is conserved in *Notophthalmus viridescens*, *Triturus alpestris apuanus* and *T. cristatus carnifex*, while this satellite is not conserved in *Taricha granulosa* (Figs. 21 and 22). Additional bands in the *Taq*I-digests of *Triturus cristatus carnifex* indicated the occurrence of the second *Taq*I site within the repeat units (Fig. 21, f).

The results of hybridization experiments indicated that a satellite homologous to the pTcS275-1 is conserved in the American newts, *Notophthalmus viridescens* (lane a) and *Taricha granulosa* (lane d), and the European newts, *Triturus alpestris apuanus* (lane b), *T. cristatus carnifex* (lane e) and *T. vulgaris meridionalis* (lane f) (Fig. 23).

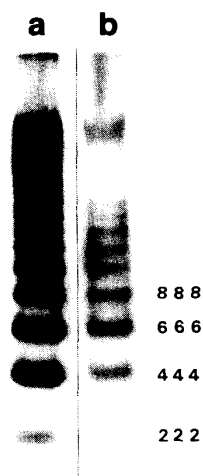


Fig. 19

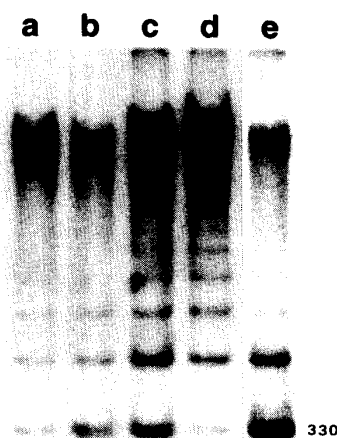


Fig. 20

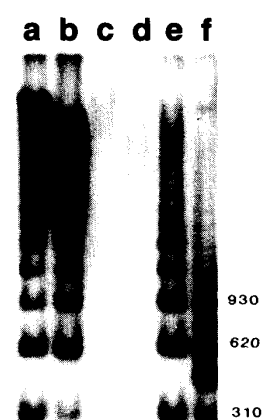


Fig. 21

Fig. 19. Autoradiograph of the genomic DNAs of *Notophthalmus viridescens* (a) and *Taricha granulosa* (b) after *Bgl*II-digestion, gel electrophoresis and Southern blot-hybridization with ^{32}P -pNv15. A ladder of hybridization was shown in both species. Size is shown in base pairs.

Fig. 20. Autoradiograph of the genomic DNAs of five newt species after *Bgl*II-digestion, gel electrophoresis and Southern blot-hybridization with ^{32}P -pNv13. *Triturus alpestris apuanus* (a), *Taricha granulosa* (b), *Triturus cristatus carnifex* (c), *T. vulgaris meridionalis* (d) and *Notophthalmus viridescens* (e). In each reaction (a~d), 6 μg genomic DNA was used except *Notophthalmus viridescens* (e), in which 0.3 μg genomic DNA was used. Size is shown in base pairs.

Fig. 21. Autoradiograph of the genomic DNAs of *Triturus alpestris apuanus* (a, b), *Taricha granulosa* (c, d) and *Triturus cristatus carnifex* (e, f) after *Hind*III-digestion (a, c and e) and *Taq*I-digestion (b, d and f), gel electrophoresis and Southern blot-hybridization with ^{32}P -pTvm1. A satellite homologous to the pTvm1 sequence was conserved in *Triturus alpestris apuanus* and *T. cristatus carnifex*, but was not conserved in *Taricha granulosa*. Additional bands in the *Taq*I-digests of *T. cristatus carnifex* (f) indicated the occurrence of the second *Taq*I site within the repeat units. Size is shown in base pairs.

Finally, from the results of hybridization experiments, it was found that a satellite homologous to the pTcS1 is conserved in the American newts, *Notophthalmus viridescens* and *Taricha granulosa*, and the European newts, *Triturus alpestris apuanus*, *T. cristatus carnifex* and *T. vulgaris meridionalis* (Figs. 24 and 25).

Extensive digestion of the genomic DNAs of *Pachytriton brevipes*, *Triturus cristatus carnifex* and *Triturus vulgaris meridionalis* with various restriction endonucleases yielded several discrete fragments which are well separated by electrophoresis on 1.5% agarose gel and are readily detected by ethidium bromide staining. When the genomic DNA of *T. cristatus carnifex* was digested with *Bgl*II, the band with the highest mobility contained a DNA fragment of about 330 bp in contrast to 275 bp which was described by VARLEY et al. (1980b). In the meantime, when the genomic DNA of *T. cristatus carnifex* was digested with *Bam*HI, the band with the highest mobility contained a DNA fragment of about 400 bp in contrast to 330 bp which was described by VARLEY et al. (1980a). A digest of *T. vulgaris meridionalis* DNA with *Hind*III gave discrete bands at intervals of about 360 bp on the agarose gel (Fig. 26,g). A digest of *T. vulgaris meridionalis* DNA with *Bam*HI gave discrete bands at intervals of about 460 bp on the agarose gel (Fig. 26,h).



Fig. 22

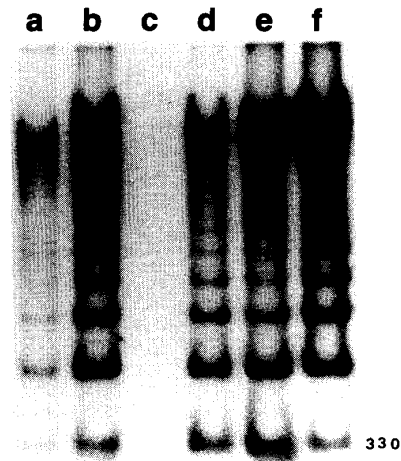


Fig. 23

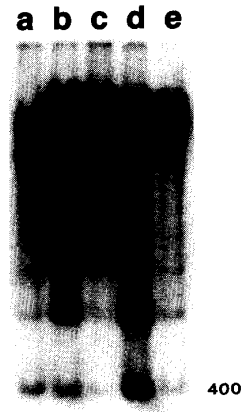


Fig. 24

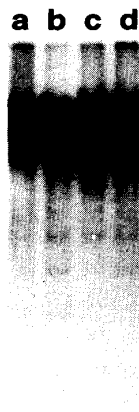


Fig. 25

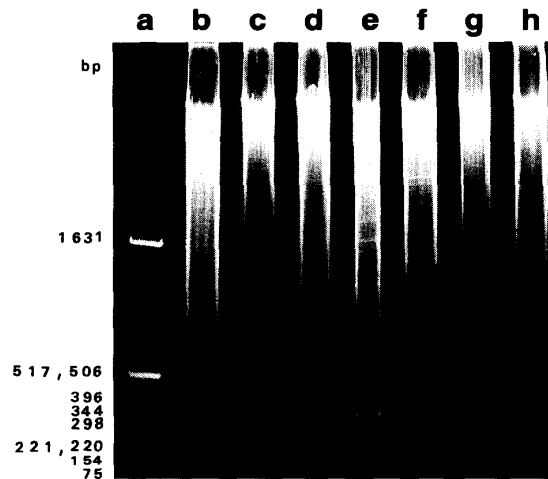


Fig. 26

Fig. 22. Autoradiograph of the genomic DNAs of *Notophthalmus viridescens* (a), *Triturus alpestris apuanus* (b), *Taricha granulosa* (c), *Triturus cristatus carnifex* (d) and *T. vulgaris meridionalis* (e) after *Bgl*II-digestion, gel electrophoresis and Southern blot-hybridization with ^{32}P -pTvml. A satellite homologous to the pTvml sequence was conserved in all the species except *Taricha granulosa* (c).

Fig. 23. Autoradiograph of the genomic DNAs of *Notophthalmus viridescens* (a), *Triturus alpestris apuanus* (b), *Taricha granulosa* (d), *Triturus cristatus carnifex* (e) and *T. vulgaris meridionalis* (f) after *Bgl*II-digestion, gel electrophoresis and Southern blot-hybridization with ^{32}P -pTcS275-1. A satellite homologous to pTcS275-1 was conserved in all the species tested. Lane c, λ phage DNA digested with *Bgl*II and *Hind*III was used as the molecular weight size marker. Size is shown in base pairs.

Fig. 24. Autoradiograph of the genomic DNAs of *Notophthalmus viridescens* (a), *Triturus alpestris apuanus* (b), *Taricha granulosa* (c), *Triturus cristatus carnifex* (d) and *T. vulgaris meridionalis* (e) after *Bam*HI-digestion, gel electrophoresis and Southern blot-hybridization with ^{32}P -pTcS1. A satellite homologous to the pTcS1 sequence was conserved in all the species tested.

Fig. 25. Autoradiograph of the genomic DNAs of *Notophthalmus viridescens* (a), *Triturus alpestris apuanus* (b), *Taricha granulosa* (c) and *Triturus cristatus carnifex* (d) after *Bgl*II-digestion, gel electrophoresis and Southern blot-hybridization with ^{32}P -pTcS1. A satellite homologous to the pTcS1 sequence was conserved in all the species tested.

Fig. 26. Ethidium bromide-stained gel of *Triturus cristatus carnifex* (b~d), *Pachytriton brevipes* (e, f) and *Triturus vulgaris meridionalis* (g, h) after digested with *Bgl*II (b, e), *Kpn*I (c), *Bam*HI (d, f, h), and *Hind*III (g). Plasmid DNA (pBR322) digested with *Hinf*I was used as the size marker (a).

DISCUSSION

Genomic DNA of eukaryotic organisms contains a large number of repetitive sequences. What is called satellite DNA is composed of short, tandemly repeated sequences, each usually less than 1000 nucleotide base pairs (bp) in length and present in more than 10^4 copies per haploid genome. At one time, two methods dominated the analysis of repeated DNA sequences: one is the method of equilibrium density gradient centrifugation in CsCl and Cs₂SO₄ (SZYBALSKI, 1968) and the other is that of measurement of DNA renaturation kinetics (BRITTEN et al., 1974). These conventional methods were available for general quantitative studies of repetitive DNA sequences in eukaryotic genomes. However, it was difficult to obtain precise information on individual repetitive DNA sequences by these methods.

Genetic engineering technique has provided a method for purifying specific sequences (single genes and their associated sequences) from complex genomes. The cloning technique has not only permitted purification of the specific sequence but also its amplification in bacteria. Therefore, large quantities of cloned sequences can be prepared and their physical properties can be characterized. The discovery of restriction endonucleases and the development of molecular cloning and DNA sequencing techniques have revolutionized the study of the structure of complex genomes. In principle, the sequences which are repeated sufficiently to represent at least 0.5% of genome often appear as distinct bands on the gel against the background smear after restriction endonuclease digestion (SINGER, 1982). After partial digestion of genomic DNAs by any restriction endonuclease, some repetitive DNA sequences may generate a characteristic ladder of fragments which are demonstrable upon gel electrophoresis. They are integral multiples of the basic repeating units. It is sometimes possible to select such a restriction endonuclease which digests genomic DNA into tandemly arranged repeats. This kind of satellites could be called restriction satellite, in contrast to gradient satellite DNA demonstrable by gradient technique (BERIDZE, 1986).

In this paper, two satellites cut with two restriction endonucleases, *Bgl*II and *Bam*HI, have been reported for the first time in Chinese fat newt, *Pachytriton brevipes*. Their monomers measure about 330 bp and 400 bp. These two satellite DNA sequences are very abundant and each of them represents about 1% of the whole genomic DNA. When the whole genomic DNA is digested with *Bgl*II or *Bam*HI and hybridized with ³²P-labeled PBr(*Bgl*II)-1 or PBr(*Bam*HI)-1 probes, a ladder of bands corresponding to multiples of the 330 bp band or 400 bp band can be seen, indicating that these sequences are present in substantial tandem repeats. A ladder-like hybridization pattern is regarded as typical for tandemly-repeated, simple sequence DNAs (EPSTEIN et al., 1986). The Southern blot-experiments seemed to indicate that the *Bgl*II satellite is little homologous, if any, to the *Bam*HI satellite, and these two repetitive sequences might belong to two different satellite families.

The existence of *Bgl*II and *Bam*HI satellite sequences obtained from *Pachytriton brevipes* in the genomes of other newt species was examined (Table 1). The results showed that the *Bgl*II satellite is not only well conserved in the Asian genus *Cynops* (*C. orientalis* from China and *C. p. pyrrhogaster* from Japan), but also is even shared by the European genus *Triturus* (*T. alpestris apuanus*, *T. cristatus carnifex* and *T. vulgaris meridionalis*) and the North American genera *Notophthalmus* (*N. viridescens*) and *Taricha* (*T. granulosa*). Up to date, three 330 bp *Bgl*II satellites have been cloned from different newt species. One of them is Nv2 (satellite 2) (pNv13) obtained from *Notophthalmus viridescens*, another is TcS2 which is originally named as pTcS275-1 and obtained from *Triturus cristatus carnifex*, and the remainder is pPbrS1-5 obtained from *Pachytriton brevipes* by the present author. In the nucleotide sequences of satellite DNAs, TcS2 is remarkably similar in both length and sequence to Nv2 (VARLEY et al., 1980b; EPSTEIN et al., 1986). These two sequences show 80% homology in the first 220 bp, and 50% homology in the remaining 110 bp (MACGREGOR and SESSIONS, 1986).

TABLE 1

Comparison of binding intensity in Southern blot-hybridization between genomic DNAs of several newt species and the satellite DNA sequences purified from several newt species by molecular cloning

Satellite DNA	China		Japan	Europe			America	
	<i>Pachy. brev.</i>	<i>Cynops orien.</i>	<i>Cynops pyrrh.</i>	<i>Trit. a. ap.</i>	<i>Trit. c. ca.</i>	<i>Trit. v. me.</i>	<i>Notoph. virid.</i>	<i>Taric. gran.</i>
pPbrS1-5	+++	+++	+++	+++	+++	+++	+++	+++
pPbrS2-9	+++	++	++	+	+	-	+	+
pNv15 (Sat. 1)	?	?	?	-	-	-	+++	+
pNv13 (Sat. 2)	?	?	?	++	++	++	+++	++
pTvm1	?	?	?	++	++	+++	+	-
pTcS275-1	?	?	?	++	+++	++	++	++
pTcS1	?	?	?	++	+++	++	++	++

+++ , indicates that the intensity of DNA binding is as strong as that with the original species from which the satellite DNA is derived. ++ , a lower binding intensity but none the less distinct level of binding. + , a level of binding that is about 10% or less of that obtained for the species of origin. - , indicates no detectable binding. A question mark indicates that no data are yet available.

It would be interesting to learn how widely the *Bgl*II satellite found in *Pachytriton brevipes* is distributed in the other newt species. When a similar amount of genomic DNA digested with *Bgl*II was hybridized with ³²P-pPbrS1-5, it was confirmed that a satellite homologous to the pPbrS1-5 sequence is conserved in all of the following seven species, *Cynops orientalis*, *C. p. pyrrhogaster*, *Triturus alpestris apuanus*, *T. cristatus carnifex*, *T. vulgaris meridionalis*, *Notophthalmus viridescens* and *Taricha granulosa*. These species were almost equal in intensity of hybridization to *Pachytriton brevipes*. This seems to indicate that the same or a very closely related sequence is present in an approximately equal amount in all these newt species. Both Nv2 and TcS2 are present in two European species, *Triturus*

alpestris apuanus and *T. vulgaris meridionalis*, and one American species, *Taricha granulosa*. According to the model suggested by MACGREGOR and SESSIONS (1986), the 330 bp *Bgl*II satellite may be a very old satellite.

In contrast to the *Bgl*II satellite, the *Bam*HI satellite found in *Pachytriton brevipes* shows considerable degrees of species specificity. Although this satellite is present in *Cynops orientalis*, *C. p. pyrrhogaster*, *Notophthalmus viridescens*, *Taricha granulosa*, *Triturus alpestris apuanus* and *T. cristatus carnifex*, except *T. vulgaris meridionalis*, there is a wide variation in their quantities among these species.

The present author examined the existence of the satellites homologous to pPbrS2-9 in various newt species by Southern blot-hybridization method. It was found that the inserts homologous to *Bam*HI satellite show a moderate degree of hybridization with *Cynops orientalis* and *C. p. pyrrhogaster*, while they show only a weak degree of hybridization with *Triturus alpestris apuanus*, *T. cristatus carnifex*, *Notophthalmus viridescens* and *Taricha granulosa*.

Recently, two satellite families, *Bgl*II and *Bam*HI satellites, have been described in *Triturus vulgaris meridionalis* by BARSACCHI-PILONE et al. (1986). However, the 310 bp pTvm1 insert is neither homologous to the *Bam*HI nor to the *Bgl*II satellite. In the present author's viewpoint, 330 bp *Bgl*II satellite from *P. brevipes* is a very common satellite family conserved widely in different species of Salamandridae. On the other hand, the 400 bp *Bam*HI satellite from *Pachytriton brevipes* seems to have been abundantly conserved in special species. This satellite is present in different amounts and shows various degrees of species specificity. The monomer length of the *Bam*HI satellite, pTcS1, obtained from the *Triturus cristatus carnifex* has been described at first as 330 bp by VARLEY et al. (1980a), later rectified as 380 bp by BALDWIN and MACGREGOR (1985), and recently recorrected by MACGREGOR and SESSIONS (1986) as 390 bp. However, according to the present author, the ethidium bromide-stained gel photograph of *Bam*HI-digested genomic DNA of *T. c. carnifex* evidently indicated that the monomer fragment is 400 bp which is slightly larger than the 396 bp side marker band (Fig. 26, d). Even though there may be large sequence diversion between pPbrS2-9 and pTcS1, the present author assumes that the clone pPbrS2-9 is possibly related to pTcS1, and the size of the insert of pTcS1 was given incorrectly in MACGREGOR and SESSIONS' paper (J. G. GALL, personal communication).

It is the present author's belief that further studies on the organization and chromosomal location of the *Bgl*II and *Bam*HI satellites of *Pachytriton brevipes* should provide new information on the evolution of these families of repetitive sequences. Examination of the presence of repeated sequences in the genomic DNAs of various amphibian species will lead to greater understanding of the amphibian evolution. Since amphibians have a large haploid DNA content and gigantic lampbrush chromosome, new experimental approaches to the question of the function of repeated sequences will be offered by amphibians in the near future by combining genetics or cytogenetics with molecular analysis.

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LITERATURE

- BALDWIN, L. and H. C. MACGREGOR 1985. Centromeric satellite DNA in the newt *Triturus cristatus karelinii* and related species: Its distribution and transcription on lampbrush chromosomes. *Chromosoma (Berl.)*, **92**: 100-107.
- BARSACCHI-PILONE, G., R. BATISTONI, F. ANDRONICO, L. VITELLI and I. NARDI 1986. Heterochromatic DNA in *Triturus* (Amphibia, Urodela). I. A satellite DNA component of the pericentric C-bands. *Chromosoma (Berl.)*, **93**: 435-446.
- BERIDZE, T. 1986. Satellite DNA. Springer-Verlag, Berlin Heiderberg, 149 pp.
- BIRSTEIN, V. J. 1982. Structural characteristics of genome organization in Amphibians: Differential staining of chromosomes and DNA structure. *J. Mol. Evol.*, **18**: 73-91.
- BOLIVAR, F., R. L. RODRIGUEZ, P. J. GREENE, M. C. BETLACH, H. L. HEYNEKER and H. W. BOYER 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. *Gene*, **2**: 95-113.
- BOYER, H. W. and D. ROULLAND-DUSSOIX 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. *J. Mol. Biol.*, **41**: 459-472.
- BRITTEN, R. J., D. E. GRAHAM and B. R. NEUFELD 1974. Analysis of repeating DNA sequences by reassociation. *Methods Enzymol.*, Vol. 29(E). Academic Press, New York, pp. 363-418.
- CLEWELL, D. B. and D. R. HELINSKI 1969. Supercoiled circular DNA-protein complex in *Escherichia coli*: Purification and induced conversion to an open circular DNA form. *Proc. Natl. Acad. Sci. USA*, **62**: 1159-1166.
- COHEN, S. N., A. C. Y. CHANG and L. HSU 1972. Nonchromosomal antibiotic resistance in bacteria: Genetic transformation of *Escherichia coli* by R-factor DNA. *Proc. Natl. Acad. Sci. USA*, **69**: 2110-2114.
- DENHARDT, D. T. 1966. A membrane-filter technique for the detection of complementary DNA. *Biochem. Biophys. Res. Commun.*, **23**: 641-646.
- DIAZ, M. O., G. BARSACCHI-PILONE, K. A. MAHON and J. G. GALL 1981. Transcripts from both strands of a satellite DNA occur on lampbrush chromosome loops of the newt *Notophthalmus*. *Cell*, **24**: 649-659.

- EPSTEIN, L. M., K. A. MAHON and J. G. GALL 1986. Transcription of a satellite DNA in the newt. *J. Cell Biol.*, **103**: 1137-1144.
- GALL, J. G., M. O. DIAZ, E. C. STEPHENSON and K. A. MAHON 1983. The transcription unit of lampbrush chromosomes. In *Gene Structure and Regulation in Development*. S. SUBTELNY and F. C. KAFATORS, editors. Aln R. Liss, Inc., New York, pp. 137-146.
- GRUNSTEIN, M. and D. S. HOGNESS 1975. Colony hybridization: A method for the isolation of cloned DNAs that contain a specific gene. *Proc. Natl. Acad. Sci. USA*, **72**: 3961-3965.
- HELLING, R. B., H. M. GOODMAN and H. W. BOYER 1974. Analysis of Endonuclease R·*EcoRI* fragments of DNA from lambdoid bacteriophages and other viruses by agarose-gel electrophoresis. *J. Virol.*, **14**: 1235-1244.
- KAVENOFF, R. and B. H. ZIMM 1973. Chromosome-sized DNA molecules from *Drosophila*. *Chromosoma (Berl.)*, **41**: 1-27.
- LASKEY, R. A. and A. D. MILLS 1977. Enhanced autoradiographic detection of ³²P and ¹²⁵I using intensifying screens and hypersensitized film. *FEBS Lett.*, **82**: 314-316.
- MACGREGOR, H. C. and S. K. SESSIONS 1986. The biological significance of variation in satellite DNA and heterochromatin in newts of the genus *Triturus*: an evolutionary perspective. *Phil. Trans. R. Soc. Lond. B*, **312**: 243-259.
- MANIATIS, T., A. JEFFREY and D. G. KLEID 1975. Nucleotide sequence of the rightward operator of phage λ. *Proc. Natl. Acad. Sci. USA*, **72**: 1184-1188.
- PRASSOLOV, V. S., Y. KUCHINO, K. NEMOTO and S. NISHIMURA 1986. Nucleotide sequence of the *Bam* HI repetitive sequence, including the *Hind* III fundamental unit, as a possible mobile element from the Japanese monkey *Macaca fuscata*. *J. Mol. Evol.*, **23**: 200-204.
- SCHAFFNER, W., K. GROSS, J. TELFORD and M. BIRNSTIEL 1976. Molecular analysis of the histone gene cluster of *Psammechinus miliaris*: II. The arrangement of the five histone-coding and spacer sequences. *Cell*, **8**: 471-478.
- SINGER, M. F. 1982. Highly repeated sequences in mammalian genomes. *Int. Rev. Cytol.*, **76**: 67-112.
- SOUTHERN, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.*, **98**: 503-517.
- SZYBALSKI, W. 1968. Equilibrium ultracentrifugation in the caesium sulphate density gradient. In: GROSSMAN, L., MOLDAVE, K. (eds) *Methods Enzymol.*, Vol. 12(B). Academic Press, New York, pp. 330-360.
- VARLEY, J. M., H. C. MACGREGOR and H. P. ERBA 1980a. Satellite DNA is transcribed on lampbrush chromosomes. *Nature (Lond.)*, **283**: 686-688.
- VARLEY, J. M., H. C. MACGREGOR, I. NARDI, C. ANDREWS and H. P. ERBA 1980b. Cytological evidence of transcription of highly repeated DNA sequences during the lampbrush stage in *Triturus cristatus carnifex*. *Chromosoma (Berl.)*, **80**: 289-307.
- WU, Z., C. MURPHY and J. G. GALL 1986. A transcribed satellite DNA from the bullfrog *Rana catesbeiana*. *Chromosoma (Berl.)*, **93**: 291-297.