

Table 1 Nest size, length and weight of queen

	Normal nest	Nest with polygamy
Nest volume (cm <sup>3</sup> )	6405.9 ± 825.3	6292
Nest weight (kg)	3.07 ± 0.28	3.315
Diameter of royal chamber (cm)	4.42 ± 0.05	4.5
Weight of queen (mg)	111.5 ± 62.35	24.6 ± 10.66
Length of queen (cm)	2.1 ± 0.04	0.9 ± 0.04

More than one pair of reproductives in certain species of termites have been reported as unusual cases<sup>1-5</sup>. This phenomenon was unknown in the present termite, *M. championi* and this seems to be the first record of polygamy in this species.

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## PRODUCTION OF MOSQUITO GENE BANK

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THE concept and the development of recombinant DNA technology has made it possible to introduce segments of prokaryotic or eukaryotic genes into bacteria<sup>1-5</sup>, yeast<sup>6,7</sup> and cultured mammalian cells<sup>8-10</sup>. Gene banks have been constructed using the shot-gun approach. Eukaryotic systems for which gene banks have been constructed include among others the yeast chromosome<sup>11</sup>, *Drosophila* embryo DNA<sup>12</sup>, goose europygial gland DNA<sup>13</sup>, random sequences from normal chicken DNA<sup>14</sup>, human X chromo-

some<sup>15,16</sup> and chromosomal DNA of *Drosophila melanogaster*<sup>17</sup>.

In a given organism, genes may be turned 'on' or 'off' in response to specific stimuli or may be modulated as a part of a developmental programme, several approaches are now available to study the mechanism of the gene activation during development<sup>8,9</sup>. Anopheline mosquitoes are vectors for malarial parasites and viruses<sup>18</sup>. In our laboratory it is shown<sup>19</sup> that the pesticide, Sumithion, induces a specific puff in the salivary gland polytene chromosome of *Anopheles stephensi* and we are interested in studying the structure and regulation of pesticide responding locus. With this in view, we set out to construct a gene bank of *A. stephensi* using the 'shot-gun' approach.

From the pathogen-free inbred strain of *A. stephensi* (National Institute of Virology, Pune) larvae were reared in enamel trays under controlled temperature and humidity till the fourth instar. Salivary glands were taken out and stored at -70°C.

Nuclei were isolated by homogenizing glands (in Tris-HCl, pH 7.4 10 mM, KCl 60 mM, NaCl 15 mM, sucrose 340 mM, EDTA 2 mM, Spermine 0.15 mM, Spermidine 0.05 mM, PMSF 0.2 mM, DTT 5 mM and Triton X-100, 0.01 %) lysed in SDS-proteinase K (1 % and 20 µg/ml respectively) (37°C, 6 hr) and DNA extracted by chloroform-isomyl alcohol (24:1)<sup>20</sup> and precipitated in ethanol. Purified DNA was treated with N-cetyl-N, N, N-trimethyl ammonium bromide to remove carbohydrates<sup>21</sup> and digested to limit with the restriction endonuclease Bam H1 (Tris-HCl, pH 7.4, 10 mM, MgCl<sub>2</sub> 10 mM, NaCl 60 mM, BSA 200 µg/ml and DTT 1 mM). Figure 1 shows the electrophoretic mobility of the uncleaved and Bam H1 cleaved DNA from the salivary glands of *A. Stephensi* in a 0.8 % neutral agarose gel<sup>22</sup>. The total mixture of the Bam H1 fragments was used for cloning into *E. coli* HB101 using plasmid pBR 322 as the vector.

The relaxed replicating plasmid<sup>23</sup> pBR 322 was grown in *E. coli* HB 101 and amplified in the presence of chloramphenicol<sup>24</sup>. The plasmid DNA was extracted

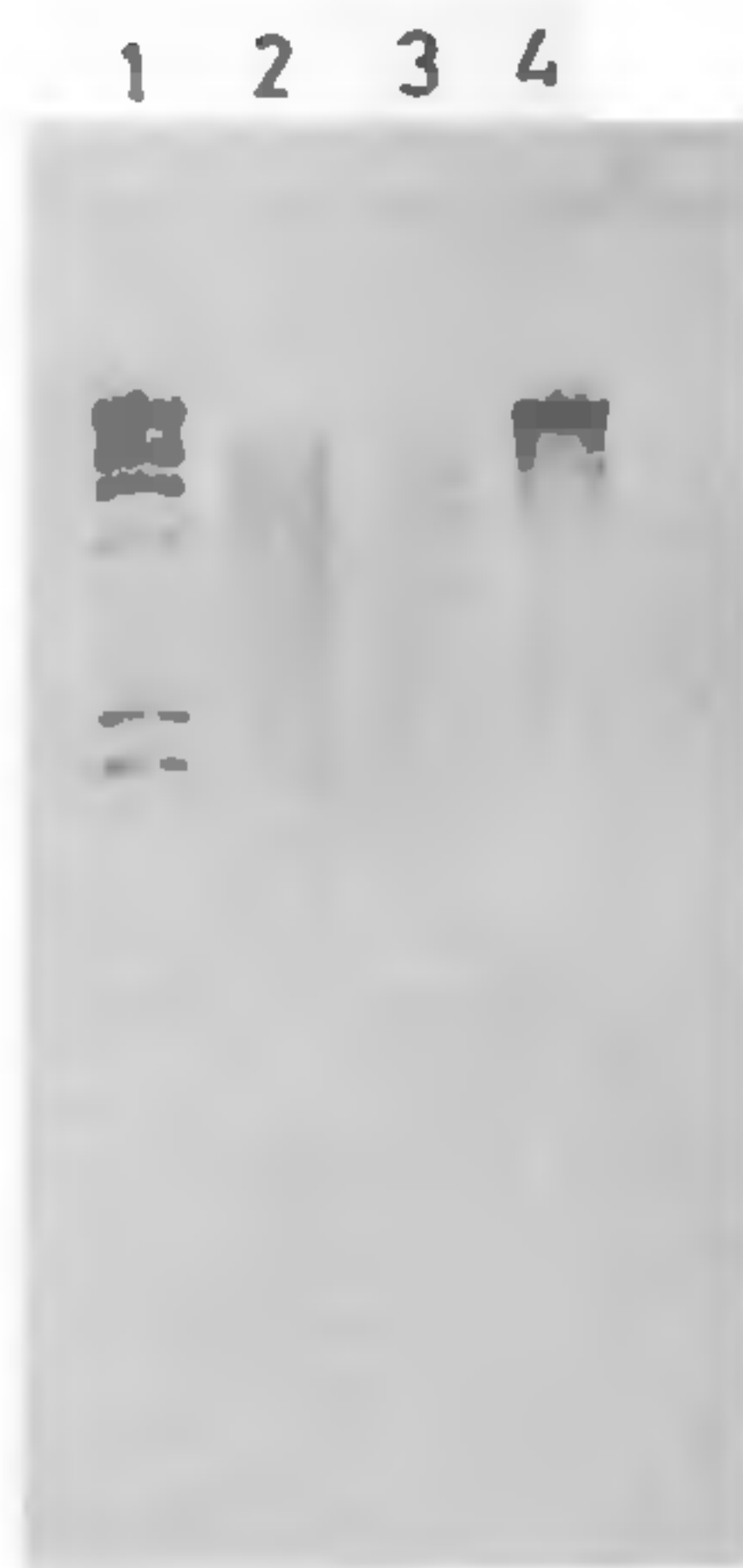


Figure 1. 0.8% neutral agarose gel showing the electrophoretic pattern of *Anopheles stephansi* salivary gland DNA. Track 1 is of  $\lambda$  DNA digested with Hind III, Track 2 is of *A. stephansi* DNA digested with Bam HI before treatment with CTAB, Track 3 is of *A. stephansi* DNA digested with Bam HI after CTAB treatment and Track 4 is of *A. stephansi* DNA before restriction digestion.

from the cleared lysate obtained by lysozyme (2 mg/ml), EDTA, (150 mM) and Triton X-100 (0.1 %) treatment extracted by phenol chloroform<sup>25</sup> and ethanol precipitated. Greater than 80 % of the DNA was in the supercoiled form and was directly digested with Bam HI. Cleaved plasmid DNA was treated with bacterial alkaline phosphatase (in Tris-HCl pH 8.0, 100 mM) to remove the 5' phosphate of the linearised DNA to prevent self ligation.

The Bam HI fragments of *A. stephensi* DNA were mixed with the linear pBR 322 molecules in a ratio of 1:100 and allowed to anneal (in Tris-HCl buffer pH 7.4, 10 mM, KCl 30 mM, MgCl<sub>2</sub> 30 mM and EDTA 0.5 mM). Circularised chimeric plasmids were ligated with 4 units of T<sub>4</sub> DNA ligase (in Tris-HCl pH 7.4, 40 mM, KCl 30 mM, MgCl<sub>2</sub> 30 mM, ATP 1 mM, BSA 1 mg/ml, spermidine 1 mM and DTT 10 mM).

*E. Coli* strain HB101 cells made competent by calcium chloride treatment<sup>26</sup> were transformed with the chimeric plasmid DNA (ratio of 10<sup>4</sup>:1) so that no more than one plasmid transformed a single bacterium. The transformants were selected on Luria agar plates containing ampicillin (100  $\mu$ g/ml). Replica-plated transformants were again selected on ampicillin (100  $\mu$ g/ml) and tetracycline (10  $\mu$ g/ml) as well as ampicillin (100  $\mu$ g/ml) alone. Colonies capable of growing only in the presence of ampicillin alone were selected as those having the chimeric plasmid with the

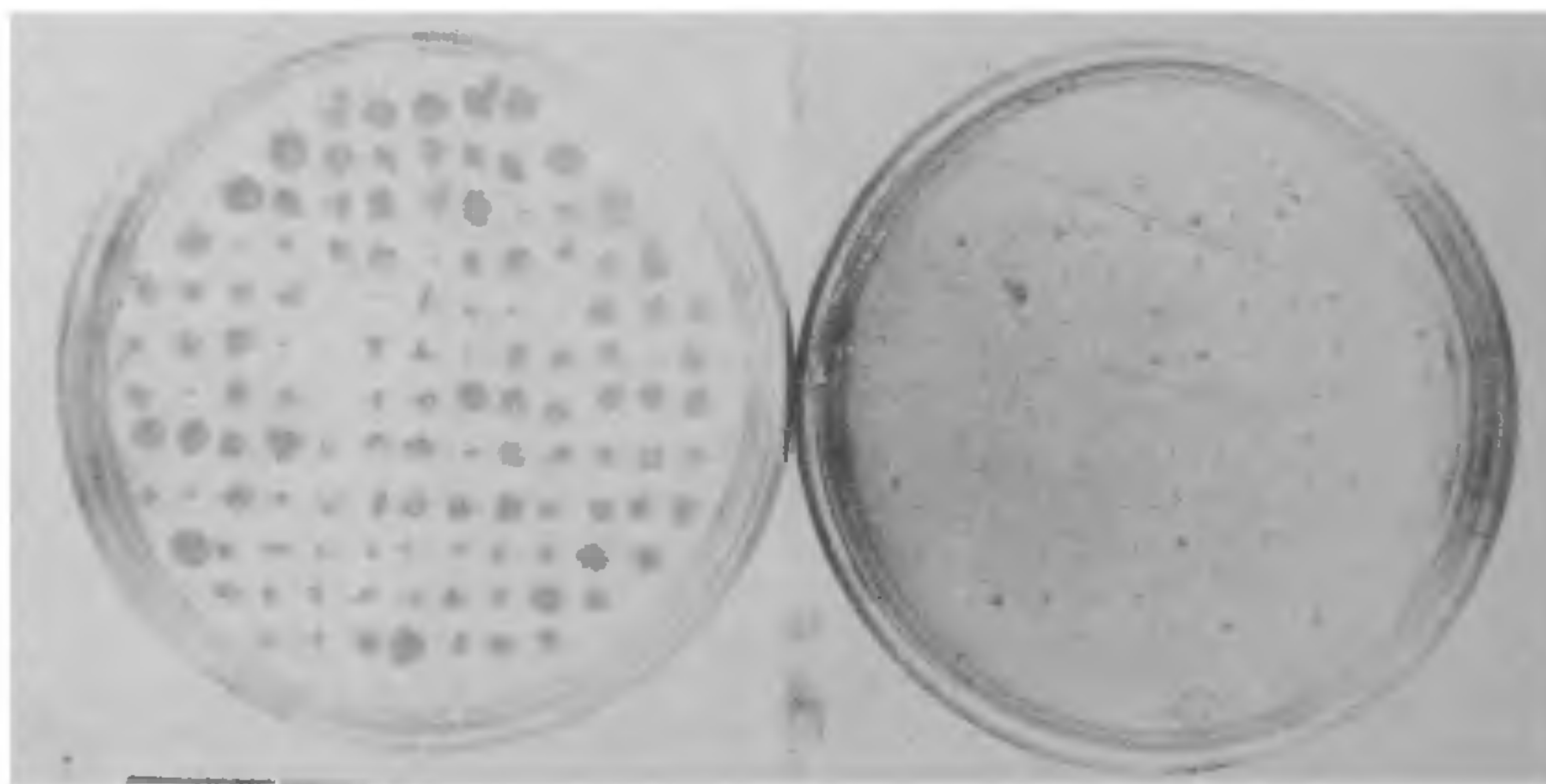


Figure 2. Screening of colonies based on antibiotic resistance. Left: Luria agar plate supplemented with ampicillin (100  $\mu$ g/ml). Right: Luria agar plate supplemented with ampicillin (100  $\mu$ g/ml) and tetracycline (10  $\mu$ g/ml).

Table 1 Transformation frequency

Organisation used for transformation	DNA	Number of colonies per ml of transformed culture medium		Transformation frequency
		Luria agar	Luria agar antibiotics*	
<i>E. coli</i> HB101	pBR322	$1.25 \times 10^8$	$8.5 \times 10^6$	6.08 %
<i>E. coli</i> HB101	pBR322 + Mosquito DNA	$7.50 \times 10^7$	$1.9 \times 10^6$	2.50 %

\* Tetracycline (10  $\mu\text{g/ml}$ ) and ampicillin (100  $\mu\text{g/ml}$ ) were used when pBR 322 DNA was used for transformation and only ampicillin (100  $\mu\text{g/ml}$ ) was used when pBR 322 + mosquito DNA was used for transformation.

mosquito DNA fragment (figure 2). The transformation frequency of *E. coli* HB101 cells with the chimeric plasmid was about 60 % lower than that with pBR 322 (table 1).

Selected colonies ( $\text{Amp}^+/\text{Tet}^-$ ) were further tested for the presence of the *A. stephensi* DNA by colony hybridization<sup>27</sup> with  $\alpha\text{-}^{32}\text{P}$  labelled nick translated<sup>28</sup> (in Tris-HCl pH 7.5, 50 mM,  $\text{MgCl}_2$  25 mM, DTT 10 mM, BSA 500  $\mu\text{g/ml}$  and the four cold dNTP's 2 mM each in addition to  $\alpha\text{-}^{32}\text{P}$  labelled dCTP and  $10^{-3}$  mg/ml of DNase I and 4 units of DNA polymerase I) DNA Colonies were first hybridized with denatured salmon sperm DNA and then with nick translated *A.*

*stephensi* salivary gland DNA and then autoradiographed. Figure 3 shows that the colonies are lighted up. Plasmid DNA was isolated from these colonies and electrophoresed on 0.4 % neutral agarose gel. As seen in figure 4, the chimeric plasmid DNA showed lower electrophoretic mobility than pBR 322 DNA. Some of these were found to contain as big as  $10^7$  dalton segments.

We wanted to recover mosquito DNA fragment inserted into pBR 322 by digesting the chimeric plasmid with Bam HI. Surprisingly, instead of the expected two fragments (one corresponding to the linear pBR 322 DNA and the other to the mosquito DNA

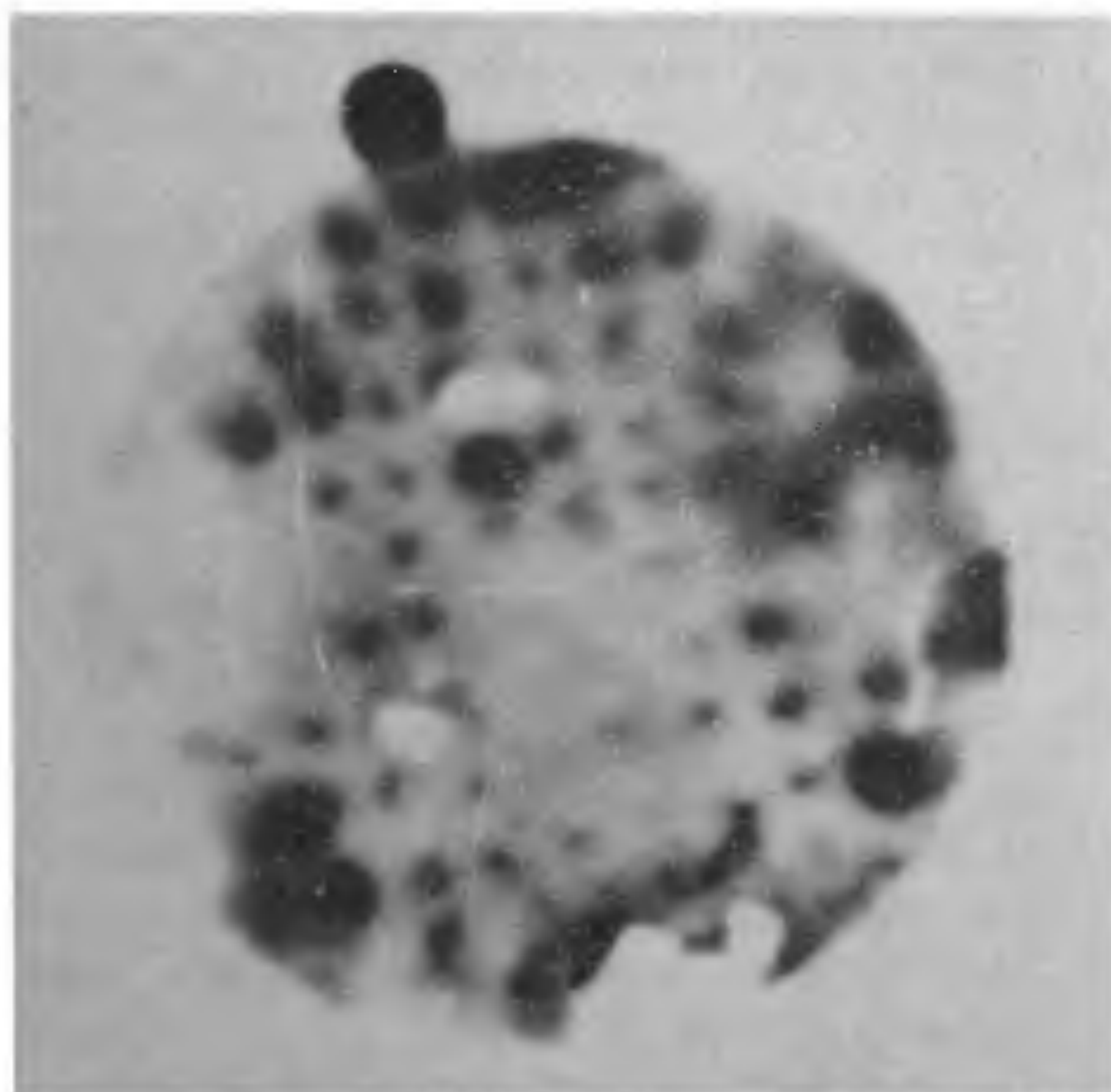


Figure 3. Colony hybridization using  $\alpha\text{-}^{32}\text{P}$ -dCTP nick translated *A. stephensi* salivary gland DNA as the probe.

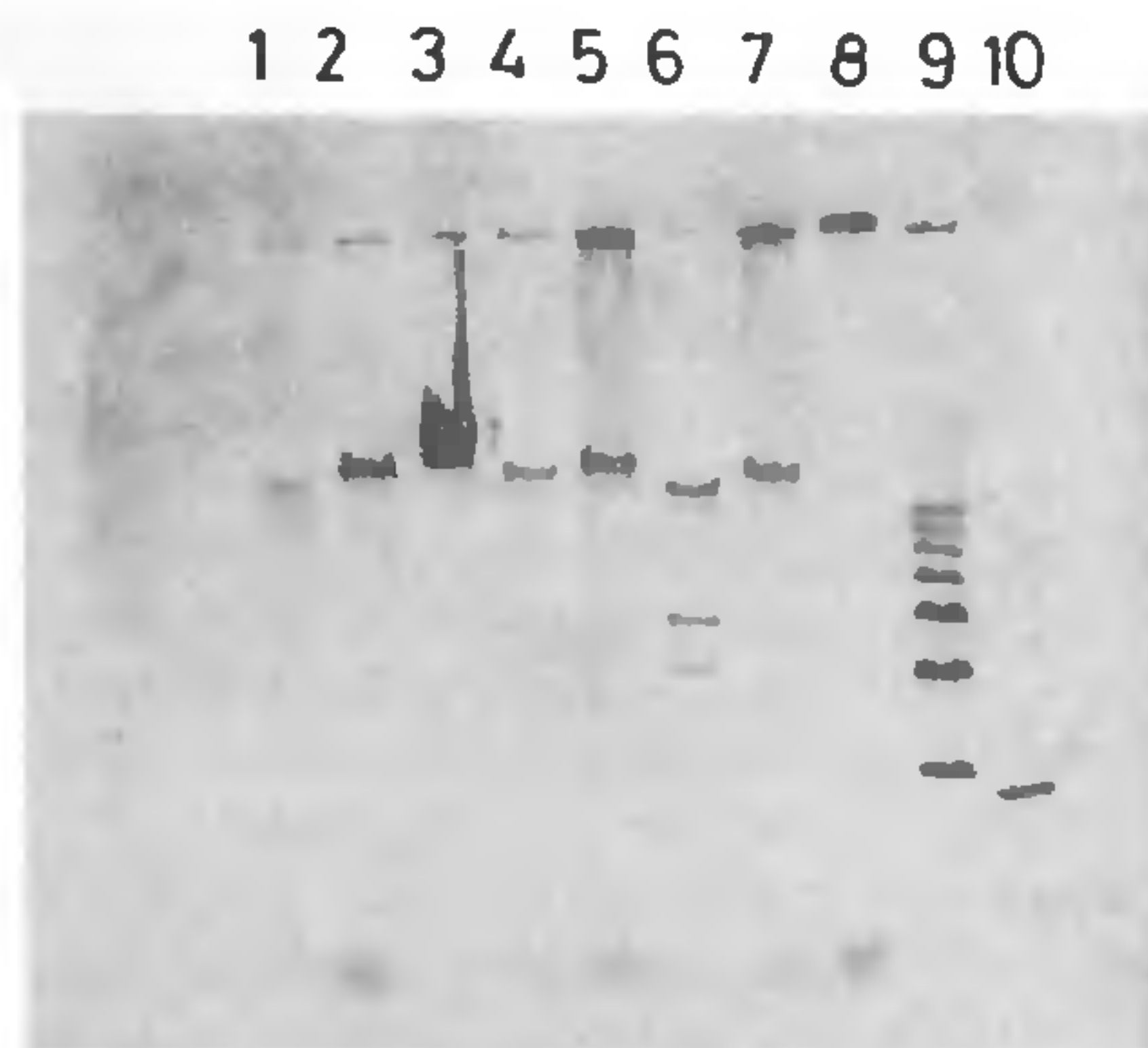
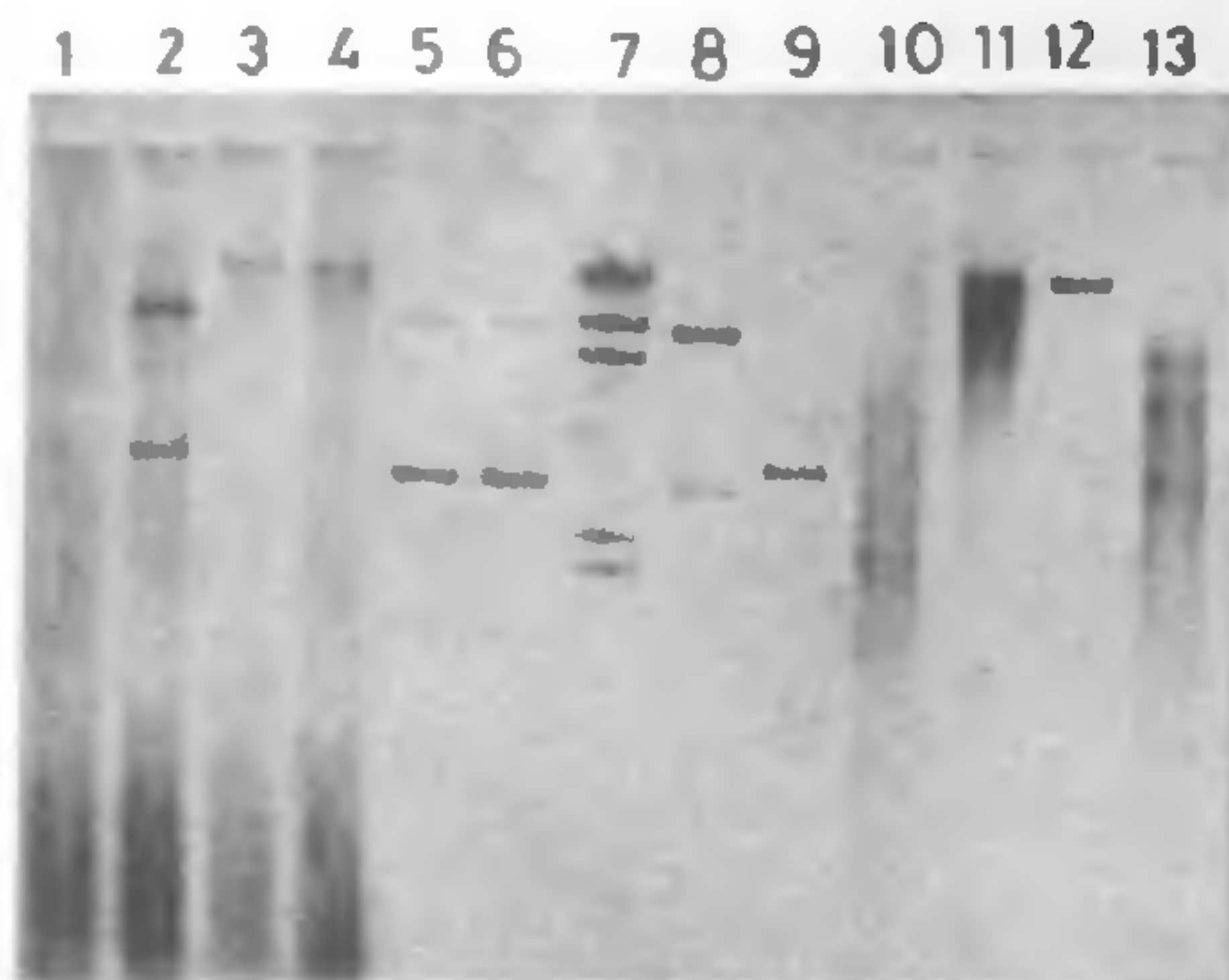


Figure 4. 0.4 % neutral agarose gel showing reduced electrophoretic mobility of the chimeric plasmids with the mosquito DNA inserts (tracks 1, 2, 4, 5, 7 and 8) as compared to that of the plasmid pBR322 (track 10). Tracks 3 and 6 are  $\lambda$  DNA and its digest with Hind III respectively, Track 9 contains linear oligomers of  $\lambda$  dv 21 DNA. (Boehringer Mannheim).



**Figure 5.** Electrophoretic pattern of restriction endonucleases digests of chimeric plasmids on a 1.2% neutral agarose gel. Tracks 1, 2 and 4 are of DNA from clone No. 2 digested with Hinc II, Bcl I and Bam HI respectively, track 3 is of uncleaved DNA from clone No. 2, tracks 10, 11 and 13 are of DNA from clone No. 4 digested with Bam HI, Hinc II and Bcl I respectively, track 12 is of uncleaved DNA from clone No. 4, tracks 5 and 6 are of uncleaved pBR322 DNA, tracks 8 and 9 are of pBR322 DNA digested with Bam HI and Hinc II respectively and track 7 is the Hind III digest of  $\lambda$  DNA.

fragment) many more DNA fragments including that corresponding to linear pBR 322 were obtained (tracks 4 and 10, figure 5). This probably suggests that a number of Bam HI recognition sequences on the genomic *A. stephensi* DNA are not accessible to Bam HI. Bam HI has been found to be inactive on DNA where the internal cytosine residue of the Bam HI recognition sequence is methylated<sup>29,30</sup>. However, it is not excluded that either some of the Bam HI fragments are derived from contaminating *E. coli* DNA or from tandemly ligated multiple genomic inserts.

Further work is in progress to study the extent and specific sites of methylation in the *A. stephensi* salivary gland DNA. We are also trying to pick from the gene bank, by *in situ* hybridization the clone(s) containing the fragment of DNA in the pesticide responding locus.

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## ANNOUNCEMENTS

### INDIAN SOCIETY OF DEVELOPMENTAL BIOLOGISTS—YOUNG SCIENTIST AWARD

The Indian Society of Developmental Biologists invites nominations from institutions/individuals for the Swami Pranvanand Award for Young Scientist in Developmental Biology. The award carries a sum of Rs. 3,000/- and a citation. The awardee must be below 32 years of age as on 1 January 1984, and should have made outstanding contribution in the field of Developmental Biology. The awardee will be re-

quested to deliver an oration during the next symposium by the Society.

Nominations should be sent along with five typed copies of a statement of the contributions made by the scientist and should reach Dr Suresh C. Goel, Secretary, Indian Society of Developmental Biology, Department of Zoology, University of Poona 411 007 on or before 15 October 1984.

### SPRING COLLEGE ON RADIATION IN PLASMAS, MIRAMARE, TRIESTE, ITALY

The International Centre for Theoretical Physics will hold a College on Plasma Physics from 27 May to 21 June 1985. It will be organized and directed by the ICTP Plasma College Committee and the ICTP Advisory Group on Plasma Physics.

While the main theme for the College is "Charged Particle Transport in Plasmas" there will be three separate sessions on the following topics: "Space Plasma Physics" (27 May–4 June) including a mini-symposium on 'Results from the 1985 International Comet missions'; "Laser/Beam-Plasma Interactions" (4–12 June) including a mini symposium on 'Recent advances in Particle/Plasma acceleration'; "Magnetically Confined Plasmas" (12–21 June) including two mini-symposia on 1. "Recent results from large Tokamaks, JET, TFTR, JT-60, T-10";

2. "Applications of new advances in non-linear dynamics to plasma physics".

In addition to the above programme, on 4 and 5 June there will be a 'Special session on Science and Development'. The College will also include workshops on several different topics.

The College is open to scientists from all countries that are members of the United Nations, IAEA or UNESCO. Participants should preferably have completed several years of study and research after a first degree. English will be the working language. The last date for requesting participation is 15 November 1984.

Further particulars may be had from: International Centre for Theoretical Physics, College on Radiation in Plasmas, P.O. Box 586, I-34100 Trieste, Italy.