PNABM568

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A. Training of Mr. Fred Oduol from Kenya

Mr. Oduol completed a course in basic techniques of Electron Microscopy at the Feinberg Graduate School of the Weizmann Institute of Science. Thereafter, he recieved advanced training in fixation, staining and embedding of insect cells for electron microscopy by Prof. N. Kislev. Following the completion of this phase of his training, Mr. Oduol moved over to Prof. I. Edelman's laboratory to learn techniques of molecular biology as follows: isolation of high molecular weight and plasmid DNA, digestion of DNA by restriction enzymes and electrophoresis in agarose gels, sequencing of single strand DNA and computer analysis of nucleotides sequences, DNA ligation and cloning. Mr. Oduol is currently applying these techniques in the laboratory and continuing his training.

B. Scientific Results

We continued our studies on the replication of *S. exempta NPV in S. frugiperda* and *S. littoralis* cells in culture and in *S. littoralis* larvae. *S. littoralis* larvae were inoculated per *Os* by *S. exempta* NPV purified polyhedra (PIB), and diseased *S. exempta* larval homogenates collected from Sindo, Makende, and Gera Mbita in Kenya. Inoculum was in the range of 4×10^2 to 5×10^7 PIB per larva, which was sufficient to cause 90-100% mortality of *S. exempta* larvae in Kenya. The inoculated larvae were checked daily until the adult stage. No viral mortality or signs of viral disease were noticed. Percent mortality of inoculated larvae, excluding one experiment (No. 4; See table), was below or equal to mortality noted in uninoculated control larvae.

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Experiment Number	No. of *S.littoralis* Larvae	Inoculum Source *S.exempta* NPV	Range of *S.exempta* NPV PIB per *S.littoralis* larva	Percent mortality*a of S. littoralis larvae Control	inoculated
1	240	Purified PIB, Sindo,Kenya.	$8x10^3$ to $4x10^7$	4.2%	0.6%
2	228	Purified PIB,Makende, Kenya.	$4x10^2$ to $4x10^7$	4.3%	0.6%
3	158	Purified PIB, Gera, Kenya.	$5x10^2$ to $5x10^7$	2.2%	0.9%
4*b	192	4 larval homogenates from Sindo, Kenya.	$3.5x10^6$to $7x10^6$	9.9%	15.3%
5	280	Same as in No. 4	$1.5x10^2$ to $3.5x10^6$	6.7%	3.2%

*a Larval mortality excluding first day P.i.

*b High mortality in this experiment stemmed from contamination within the nursery colony and uncleaned Ricinus leaves which contained parasitoides. The larvae were inoculated singularly, but were grown 4 larvae per petri dish. Cadavrae (12) were checked for viral disease but polyhedra and viral replication were not observed. In some cadavrae bacterial contamination was noticed. Likewise 6 pupae were checked, one was found positive (see text below).

From each experiment, inoculated (and control) cadavrae, larvae, and pupae of *S. littoralis* were taken, (on 5 to 18 day p.i), surface sterilized, homogenized, filter sterilized and/or treated with alkali to liberate viruses from PIB. The homogenates were used to challenge *S. frugiperda* and *S. littoralis* cell lines in culture. In previous experiments when NPV diseased *S. exempta* larvae were treated as mentioned above and used as inoculum to challenge these cell lines, 10 out of 15 *S. exempta* larval homogenates were infectious to *S. frugiperda* cell lines and many PIB were observed; while only 2 out of 15 were found to produce PIB in a few *S. littoralis* cells in culture. In continuation, we homogenized 23 *S. littoralis* larvae, and 22 larval cadavrae that were infected with *S. exempta* NPV (see Table 1). No polyhedra were observed in either *S. frugiperda* or *S. littoralis* cell lines with either of the larval sources. When we checked 22 inoculated *S. littoralis* pupae homogenates in the same way, in only one case did we succeed to obtain virus replication. This isolate produced PIB readily in *S. frugiperda* cells and at a level of about 4% in *S. littoralis* cells in culture, similar to viral replication after inoculation with PIB of SeNPV or diseased *S. exempta* cadavrae homogenates. We have passed this virus isolate 3 times in cell cultures; samples from the first and second passes have been taken for ultrastructural studies.

Prof. N. Kislev carried out an examination of inoculated and control *S. frugiperda* and *S. littoralis* cells under the electron microscope. Inoculum source was homogenate of *S. littoralis* pupa, 18 day p.i. of its larva with 3.5×10^6 PIB of *S. exempta* NPV (experiment number 4, see above). Observations were carried out on first and second passes of inoculated cells. Viral replication and production of many PIB was noticed in many *S. frugiperda* cells, as described for *Autographa californica* NPV. Only a few *S. littoralis* cells with nuclei containing PIB were noticed; however the number of *S. littoralis* infected cells (containing viruses or viregenic stroma) was higher, but much

less then in *S. frugiperded* cells. Our assumption is that this virus isolate is *S. exempta* NPV and not *S. littoralis* NPV (*S. littoralis* NPV does not replicate well in *S. frugiperda* cells in culture; N. Kislev, 1986, J. Invertebr. Pathol. 47: 369-373.) DNA analysis of the viral genome by restriction enzymes will verify this point. Detail description and electron-micrographs will be given in the final report.