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Genotypic Variation in Geographical Isolates of *Oryctes* Baculovirus

By ALLAN M. CRAWFORD,[1]* BERNHARD ZELAZNY[2] AND AMBROSIO R. ALFILER[3]

[1]*Entomology Division, Department of Scientific and Industrial Research, Private Bag, Auckland, New Zealand,* [2]*UNDP/FAO Coconut Pests and Diseases Project, P.O. Box 1864, Manila, Philippines and* [3]*Philippine Coconut Authority, Albay Research Center, Guinobatan, Albay, Philippines*

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SUMMARY

Twelve geographical isolates of *Oryctes* baculovirus were cloned in DSIR-HA-1179 cells. The DNA of these isolates was analysed by restriction endonuclease digestion with the enzymes *Bam*HI, *Eco*RI, *Hin*dIII and *Pst*I. Each isolate showed slightly different restriction fragment electrophoresis profiles. Most of the changes were due to small insertions or deletions of DNA, although two isolates lacked a single restriction site. The sites of genotypic change were not randomly distributed but were mainly associated with regions that have been shown to contain reiterated sequences.

The use of *Oryctes* baculovirus to control pest populations of the coconut palm rhinoceros beetle, *Oryctes rhinoceros*, is well documented (Bedford, 1981). Its main success has been in the South Pacific when the disease was introduced into several islands with very serious pest damage. The virus was first discovered in Malaysia (Huger, 1966) and is endemic in other neighbouring countries (Zelazny, 1977). This paper examines genotypic variation amongst *Oryctes* baculovirus isolates by the analysis of DNA restriction digests and mapping DNA insertions, deletions and restriction site changes. Twelve isolates of *Oryctes* baculovirus were examined. All except two of the isolates were collected from areas where the virus occurs naturally.

The history of the twelve isolates is given in Table 1. All virus isolates were twice cloned by endpoint dilution as previously described (Crawford & Sheehan, 1985). Isolates were replicated in DSIR-HA-1179 cells (Crawford, 1982) and the DNA was purified from pelleted, extracellular virus as previously described (Crawford *et al.*, 1985). The DNA of each isolate was digested with each of four restriction enzymes, *Bam*HI, *Eco*RI, *Hin*dIII and *Pst*I. The cleaved DNA was run on 0·7% horizontal, submerged, agarose gels using 0·089 M-Tris, 0·089 M-boric acid, 0·002 M-EDTA pH 8·0 as the electrophoresis running buffer. DNA fragments were stained with ethidium bromide (0·5 mg/l) and examined with a u.v. transilluminator.

The results of these digestions are shown in Fig. 1; a summary of the differences observed is given in Fig. 2. All the strains could be distinguished from each other on the basis of their restriction fragment profiles. All strains were compared with our type strain, PV505, with which most of the molecular biological investigations have been carried out (Payne *et al.*, 1977; Crawford & Sheehan, 1985) including a physical map of its DNA (Crawford *et al.*, 1985).

The major differences between strains were the presence or absence of small pieces of DNA varying between 30 and 150 bases in size. In general, the greater the geographical separation of isolates the greater the genotypic variation. Differences in the *Bam*HI, *Hin*dIII, *Eco*RI and *Pst*I patterns of the five isolates from Southern Luzon (22A, 46P, AP371, V23B and PV505) were due to the presence or absence of a 100-base insert in the *Eco*RI fragment S and a 30-base insert in the *Pst*I fragment G. Also, deletions of between 40 and 70 bases occurred within *Eco*RI fragment e. Of the three other Philippine isolates, DVO (Mindanao) showed similar changes but the two isolates from Bugsuk Island (X2B and B36) each had an additional insert of 70 and 90 bases

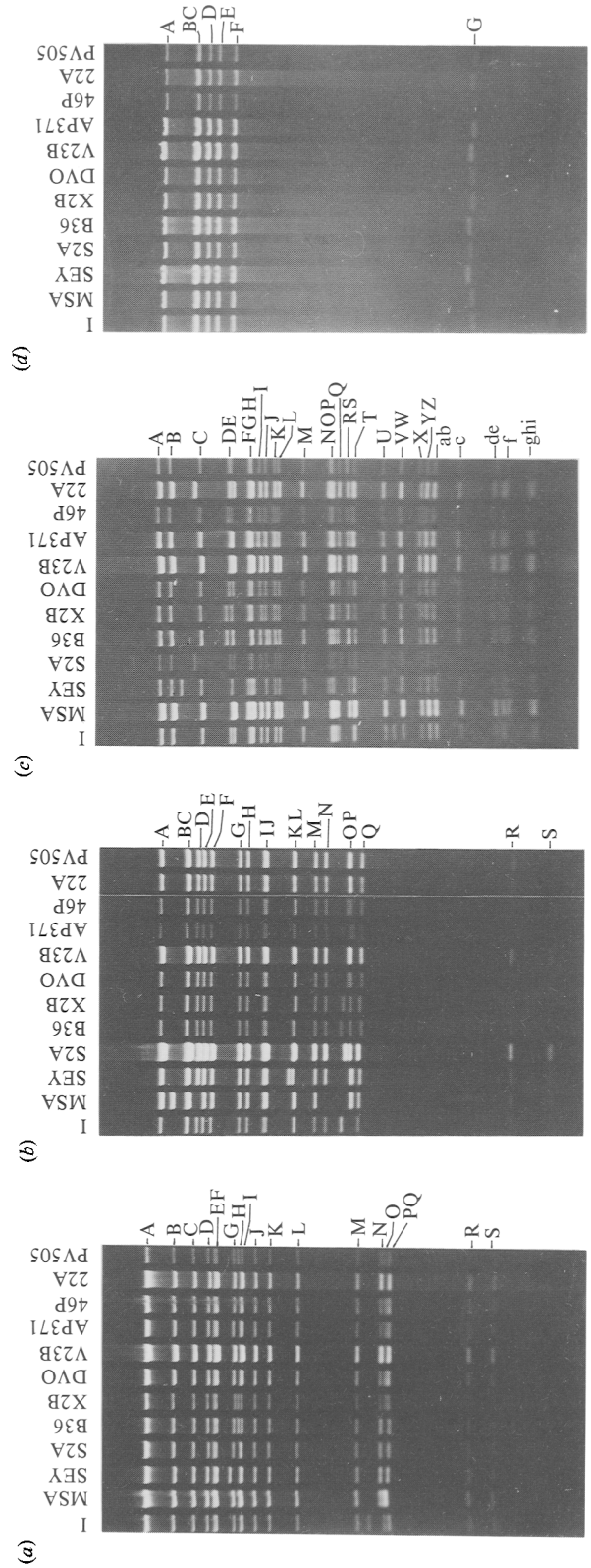

Fig. 1. Analysis of *Oryctes* baculovirus DNA digested with (*a*) *Bam*HI, (*b*) *Hin*dIII, (*c*) *Eco*RI and (*d*) *Pst*I restriction endonucleases. Gels prepared as described in the text were run overnight at a constant 1·5 V/cm. The letters on the right of each gel refer to the fragment designation of the PV505 strain.

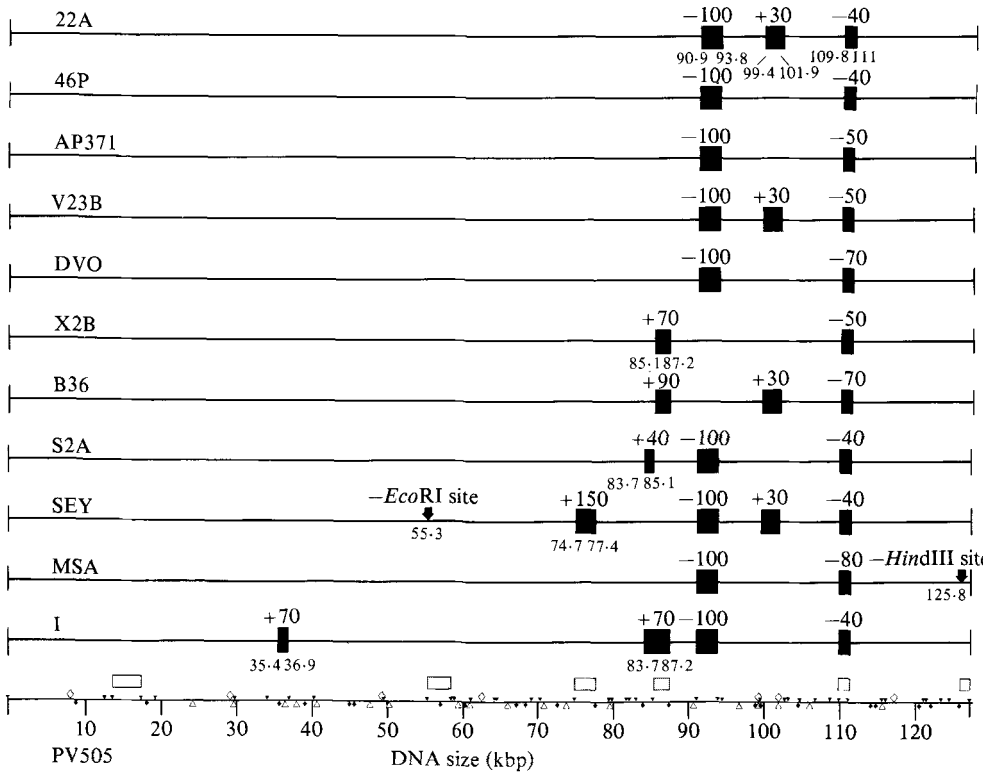

Fig. 2. Genotypic changes in the isolates compared with the PV505 strain. The positions of restriction sites are shown on the PV505 map. ▼, *Eco*RI; △, *Bam*HI; ◆, *Hin*dIII, ◇, *Pst*I. The location of regions containing reiterated sequences is shown by the shaded boxes above the PV505 genome. The approximate location of the DNA insertions and deletions is given by the black boxes on the other 11 genomes. Above the boxes, + represents an insertion and − a deletion, and the number following is an estimate of the number of bases involved. The figures below the boxes show the position of the box in kilobases from the left-hand end of *Eco*RI fragment A, the designated start of the *Oryctes* baculovirus physical map (Crawford *et al.*, 1985).

respectively in *Bam*HI fragment H. It is noteworthy that virus from other parts of the Philippines has been released on Bugsuk Island.

Both the Tanzanian and Seychelles isolates originated from Samoa and passed through a less susceptible species, *Oryctes monoceros*. The virus was released into the *O. monoceros* populations of the Seychelles in 1972 and in 1979 *O. monoceros* cultures were infected in Tanzania. In turn, the virus had been introduced into Samoa from Malaysia in 1964. This makes it hardly surprising that isolates S2A and SEY are different. The Malaysian (MSA) isolate had a unique *Hin*dIII restriction site loss in *Eco*RI fragment T and the Indian isolate had a unique 70-base insertion in *Hin*dIII fragment O.

Many of the genotypic changes occurred in the same regions of the genome in which reiterated sequences have been shown to occur (shaded boxes in Fig. 2; Crawford *et al.*, 1985). Until we understand the significance of these reiterated sequences it is difficult to interpret this observation. Fraser *et al.* (1985) showed that at least some spontaneous baculovirus mutants were due to the insertion of a foreign DNA sequence which had the properties of a transposon of the host cell. Perhaps these regions contain small inverted repeats which increase the rates of DNA excision and insertion. Another simpler possibility is that insertions and deletions occur throughout the genome but these are the only regions where such changes are not lethal. The sequencing of one or more of these areas of reiterated sequence will be necessary to understand these processes further.

Table 1. *Origin of the analysed isolates of* Oryctes *baculovirus*

Designation	Origin	Date isolated
PV505	Southern Luzon, Philippines	1977
22A	Southern Luzon, Philippines	May 1983
46P	Southern Luzon, Philippines	May 1983
AP371	Southern Luzon, Philippines	May 1983
V23B	Southern Luzon, Philippines	1980
DVO	Southern Mindanao, Philippines	1984
X2B	Bugsuk Island, Palawan, Philippines	Feb. 1983
B36	Bugsuk Island, Palawan, Philippines	1984
S2A*	Tanzania	1981
SEY*	Seychelles	Unknown
MSA	Malaysia	1984
I	Kerala, India	April 1983

* Virus taken from infected *O. monoceros*. The Tanzanian isolate was from a laboratory colony and Seychelles isolate from a field population. All other isolates were from field populations of *O. rhinoceros*.

At the moment, we have no information on the naturally occurring rate of genotypic change in *Oryctes* baculovirus or any other baculoviruses. As part of a virus release programme on the islands of the Maldives, Indian Ocean, several of the *Oryctes* baculovirus clones described here have been released into isolated, previously unexposed *O. rhinoceros* populations. It will be interesting to re-isolate the virus from these populations and study the genotypic changes that occur in time.

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