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Replication of the Densovirus of *Casphalia extranea* (Lepidoptera Limacodidae) on an Established Cell Line

Densoviruses belong to the Parvoviridae family (R. E. F. Matthews, *Intervirology* 17, 1–199, 1982). These small icosahedral nonoccluded viruses (diameter 20–22 nm) contain linear single-stranded DNA. The common name of densovirus, densonucleosis virus, was derived from the characteristic appearance of the nuclei in infected larvae of insects (C. Vago, G. Meynadier and J. L. Duthoit, *Ann. Epiphyt.* 15, 475–479, 1964).

Outbreaks of the moth *Casphalia extranea* (Lepidoptera, Limacodidae) occur periodically during the months of August and September in oil-palm and coconut plantations in Côte d'Ivoire, causing great damage. One of the main natural control agents of this pest was a densovirus. These viruses are potentially useful microbial control agent of insect pests (P. Genty, *Oléagineux* 30, 349–354, 1975). Some assays performed on rearings of this insect and the observation of the virus propagation in plantation showed the great pathogenicity of this densovirus. Field experiments have shown that when this virus is spread in plantation, it is able to control the pest satisfactorily. Two weeks after the treatment, the mortality reached 92% of the larvae (G. Fediere, P. Monsarrat, D. Mariau, and M. Bergoin, *Fundam. Appl. Aspects Invertebr. Pathol.*, 705, 1986).

The fact that the virus has been cultured only in living hosts limited its production. Insect tissue and cell culture is important for studying many aspects of virus–host relationships, for viral production, and for molecular biology. Since no *C. extranea* cell line has yet been developed, the different lepidopteran cell lines cultured in our laboratory were tested to replicate the virus. *Spodoptera frugiperda, S. littoralis, Mamestra brassicae, Trichoplusia ni,* and *Choristoneura fumiferana* did not permit the multiplication of the virus. Only the continuous cell line SPC BM40 (J. M. Quiot, *Serologia* 22, 25–31, 1982) was maintained in Grace medium containing 10% fetal bovine serum, in which calcium chloride, maltose, and β-alanine were omitted (X. Lery and G. Fediere, *J. Invertebr. Pathol.*, in press).

The densovirus was purified from diseased larvae collected in oil palm at the Palmindustrie plantations at Eloka, east of Abidjan (G. Fediere, Ph.D. thesis, Montpellier 1983). Log phase *Bombyx mori* cells (10×10^6) in 75-cm^2 tissue culture flasks (NUNC) were infected with a 2-ml virus suspension at 0.05 OD_{260}/ml or with viral DNA at 0.025 OD_{260}/ml, in Grace medium containing 3% fetal bovine serum, filtered at 0.22 μm. After a contact of 6 hr, with gentle agitation at 28°C, the virus or DNA was removed with medium and 10 ml of fresh medium containing 3% fetal bovine serum was added.

As no cytopathic effect was detected after 15 days, viral infection was revealed by different colorations. The perinuclear zone appeared intensely stained after acrydine orange or immunofluorescence assays under a compound microscope. Under the electron microscope, virions appeared in nuclei (Fig. 1) and caused modifications in the nuclear structure. The fact that this densovirus is found outside of the nucleus is of interest, because the replication site has generally been thought to be nuclear.

Supernatants from infected cultures, observed after negative contrast with phosphotungstic acid (PTA), showed high concentrations of virions in cellular fragments.

After 2 weeks, infected cells were scraped from flask walls, homogenized, and ultrasonicated in 0.05 M Tris buffer.

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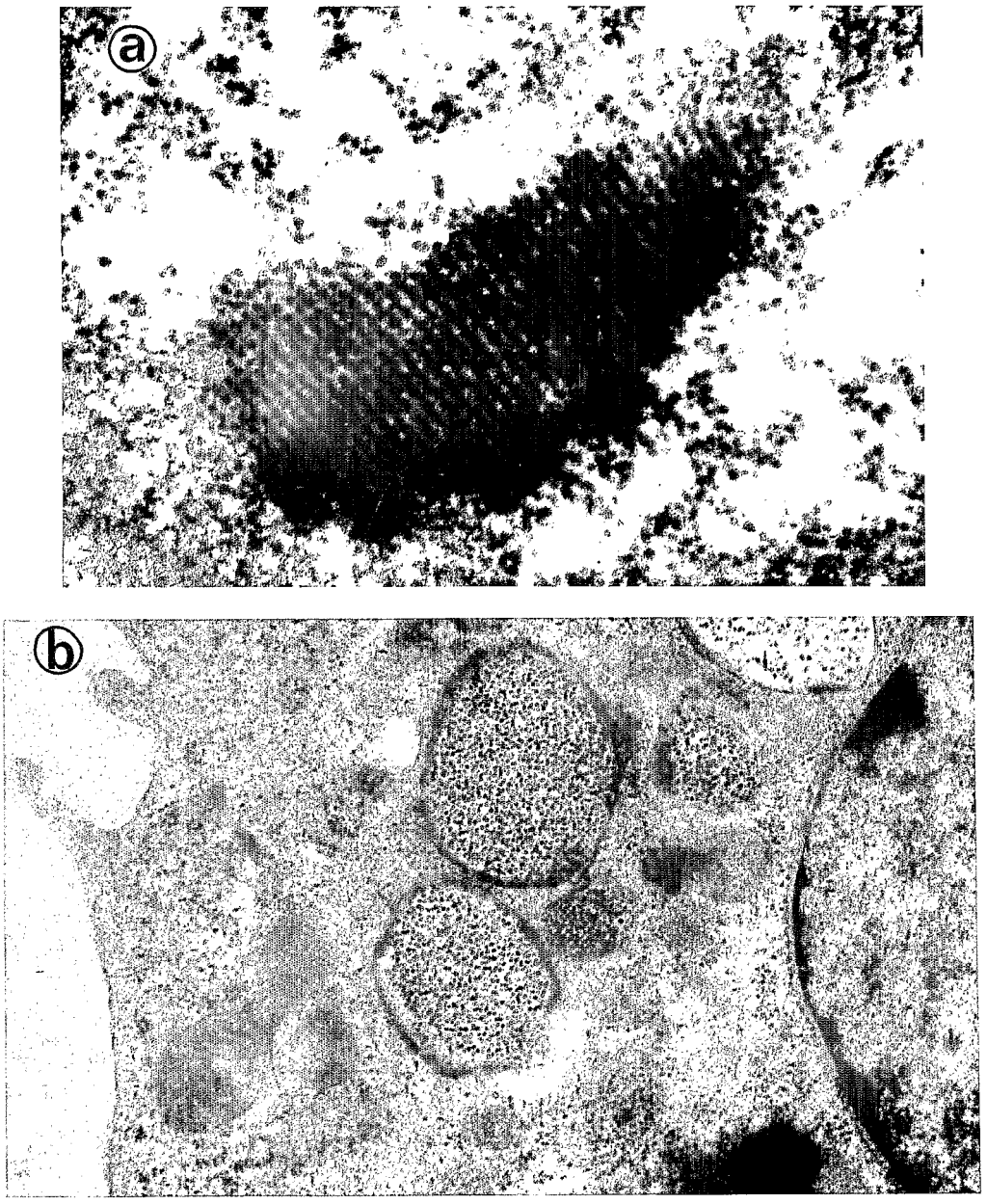

FIG. 1. Cell of the *Bombyx mori* SPC BM 40 line infected by the *Casphalia extranea* densovirus. (a) Portion of nucleus showing tightly packed virus particles arranged in crystalline arrays. ×66,000. (b) Virions in cytoplasmic vacuoles near the nucleus. ×35,000.

The suspension was clarified and the supernatant ultracentrifuged at 150,000*g* for 2 hr. The pellet was purified on sucrose gradient density (10/45) for 2-hr at 200,000*g*. The purified virus suspension obtained had a UV absorbance spectrum with an average 260/280 extinction ratio of 1.40 in comparison to 1.50 for the purified virus used for the infection. The rate of multiplication was ca. 10, corresponding to 1 OD_{260} per flask.

Serological studies indicated that the virus produced in cell cultures reacted identically to those used for infection.

Our observations confirmed the replication of the *Casphalia extranea* densovirus in the only continuous cell line SPC BM40 of *Bombyx mori*. It is the first time that a densovirus has been shown to replicate in an established cell line. The oil-palm insect pests studied in our laboratory, *Turnaca rufisquamata* (Lep., Notodontidae), *Pteroteinon laufella* (Lep., Hesperidae), *Latoia viridissima,* and *L. pallida* (Lep., Limacodidae), were not found to be infected by this virus, except *C. extranea*. These results confirmed the great specificity of this virus.

The virus produced could infect the larvae of *C. extranea* in laboratory rearings. The important rate of multiplication obtained in our cultures indicates a potential for the production of this virus for biological control applications.

KEY WORDS: Densovirus; *Casphalia extranea; Bombyx mori* cell line; virus production; transfection; Parvoviridae; DNA.

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