

NOTE

Virus-like Particles Found in the Ectoparasitic Bee Mite *Varroa jacobsoni* Oudemans

The ectoparasitic honey bee mite *Varroa jacobsoni* Oudemans (Acari: Varroidae) is a serious pest of the honey bee, *Apis mellifera* Linné (Hymenoptera: Apidae). This hemolymph-sucking mite not only weakens adult and larval bees but is also known as a vector of viral diseases of the honey bee, e.g., the acute bee paralysis virus (Ball, 1988). Recently, a putative iridovirus has been found in *Varroa* mites from one moribund honey bee colony in Pennsylvania (Camazine and Liu, 1998). A viral transmission within the colonies to kill both mites and honey bees is expected.

Due to the problems of pesticide applications in bee hives, there is increasing interest in the possible use of natural antagonists for control or suppression of the *Varroa* mite. During the search for diseases of this mite, in several parasitized bee colonies many mites were found with characteristic internal black-colored changes of the gut and of the fat body (Fig. 1). These signs were observed in various symmetrical and asymmetrical forms. Altogether 20.5% of living, moribund, and undecayed dead adult female mites carried such changes. On living bees, 3.6% of mites displayed this anomaly, in brood cells even 8%; also juvenile mites were affected. When dead mites were observed for 14 days, there was neither an increase of existing black areas nor did previously normal mites develop such signs. Decaying and postmortal processes obviously are not responsible for this phenomenon. Frequency and intensity of the symptoms can be enhanced by modification of the environmental conditions for the mites (deficiency of bee brood, deficiency of pollen, abnormal brood temperature, or death of the host). These modifications may reduce the natural resistance against pathogenic agents. Longevity of black-colored mites was significantly ($P = 0.012$) reduced by 43.8% compared to normal-looking individuals. However, there were no significant differences in longevity and vitality of bees parasitized by black-colored or normal mites, respectively. First observations also indicated a lower fertility of black-colored mites.

For cytopathological studies, series of tissue samples were dissected from symptomatic *Varroa* mites, fixed overnight in 2.5% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.2) and postfixed in 2.0% osmium tetroxide in the same buffer for 5 h. Then the tissue samples were

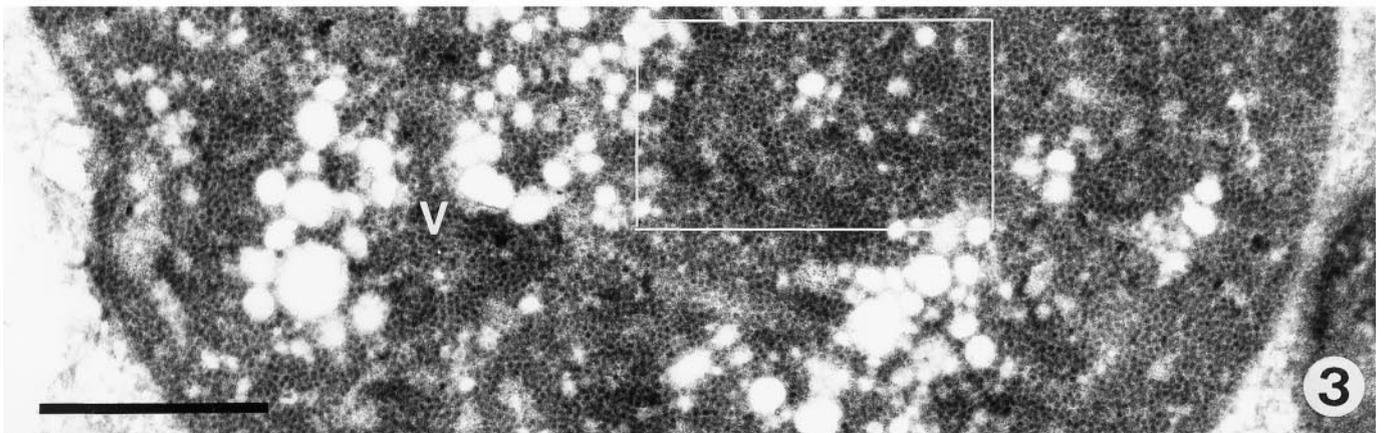
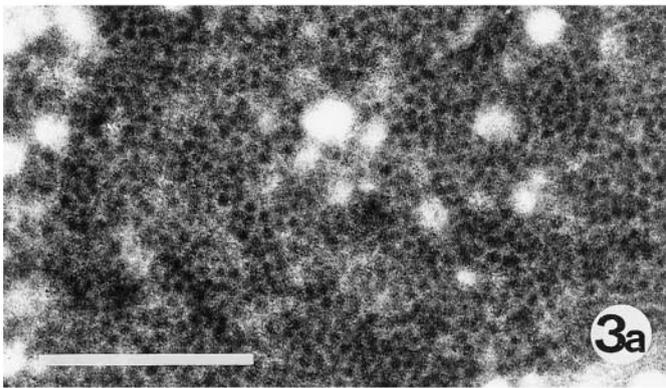
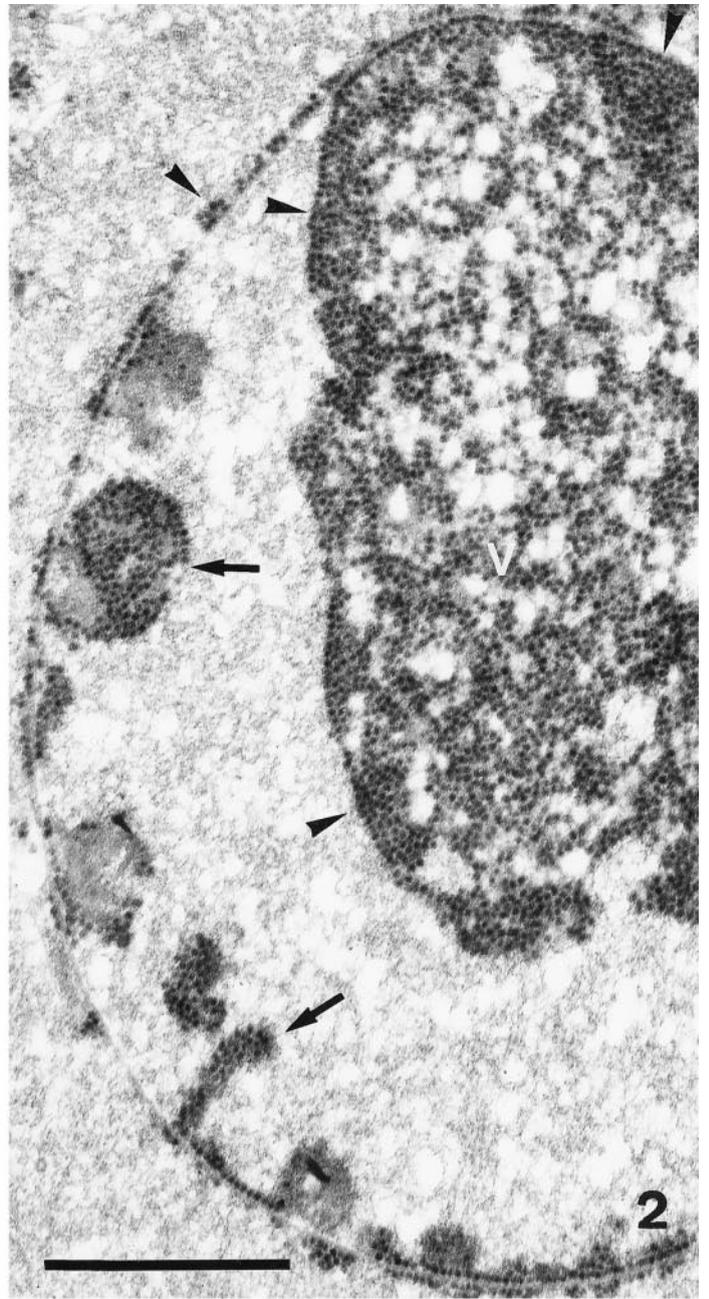
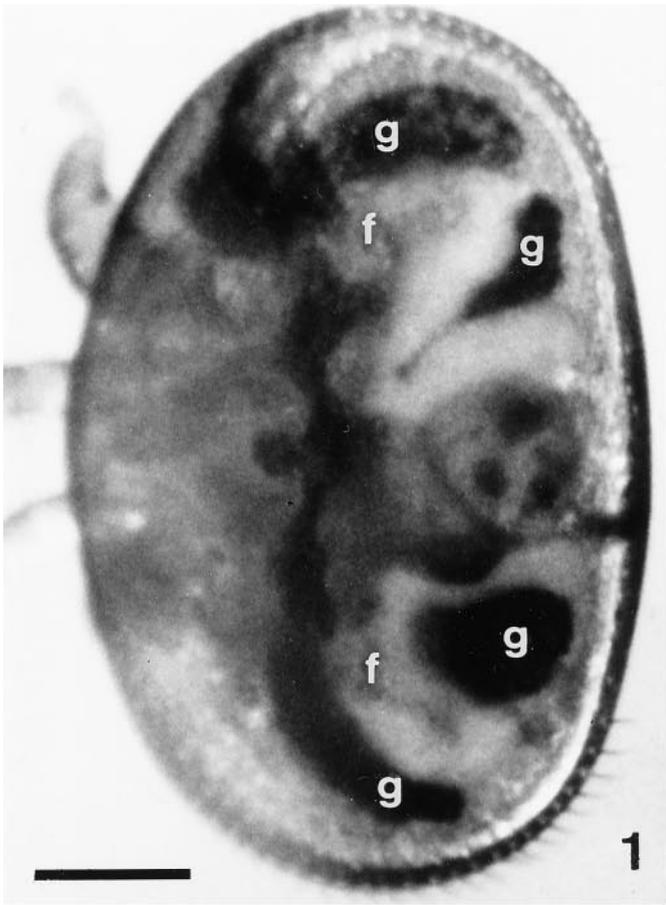
dehydrated through increasing concentrations of ethanol, followed by propyleneoxide, and embedded in epon. Thin sections were obtained with a Leica Ultracut S microtome and stained with 6% lead citrate, followed by 2% aqueous uranyl acetate. Sections were investigated using a transmission electron microscope (Zeiss 902). In symptomatic *Varroa* mites mostly myriads of spherical virus-like particles are observed primarily in the nuclei of the fat body (Figs. 2, 3, and 3a) and muscle tissues. The inner and the outer surface of the double-layered nuclear membrane seem to be preferred sites of initial occurrence of these particles (Figs. 2 and 3). Invaginations of the inner layer of the nuclear membrane with densely adhering virus-like particles are often observed (Fig. 2). In an advanced state of particle propagation the membrane of the nucleus is destroyed (Fig. 3). Masses of virus-like particles are often aggregated in irregular-shaped paracrystalline clusters of variable size (Figs. 4 and 5).

As determined from electron micrographs of thin sections of fat body tissues, the diameters of 50 of these particles occurring free in the cytoplasm resulted in a mean of 27.04 ± 1.88 nm when measured from side to side and 30.68 ± 2.44 nm from apex to apex. When aggregated in paracrystalline clusters, the particles have a periodicity of 22.41 ± 2.83 nm (side to side) and 28.9 ± 3.91 nm (apex to apex) ($N = 50$). As their size is similar to that of the acute bee paralysis virus (APV), parasitized bees were investigated for APV (Institute for Animal Health, Freiburg, Germany). The results were APV-negative.

Similar virus-like particles were found by Liu (1991) in the body cavity of the tracheal mite *Acarapis woodi* Rennie. It was not clarified if these particles reduced the longevity of the mites.

Attempts to purify the virus-like particles found in the present studies were conducted according to the methods of Williamson and von Wechmar (1992) with some modifications. However, the yield of particles was not sufficient for a biochemical characterization. Probably most of the particles were lost due to aggregation.

First *per os* transmission experiments were carried out with bee larvae and young *Varroa* mites. Bee larvae were fed with fat body extracts of symptomatic *Varroa* mites, and young, healthy *Varroa* mites were dipped



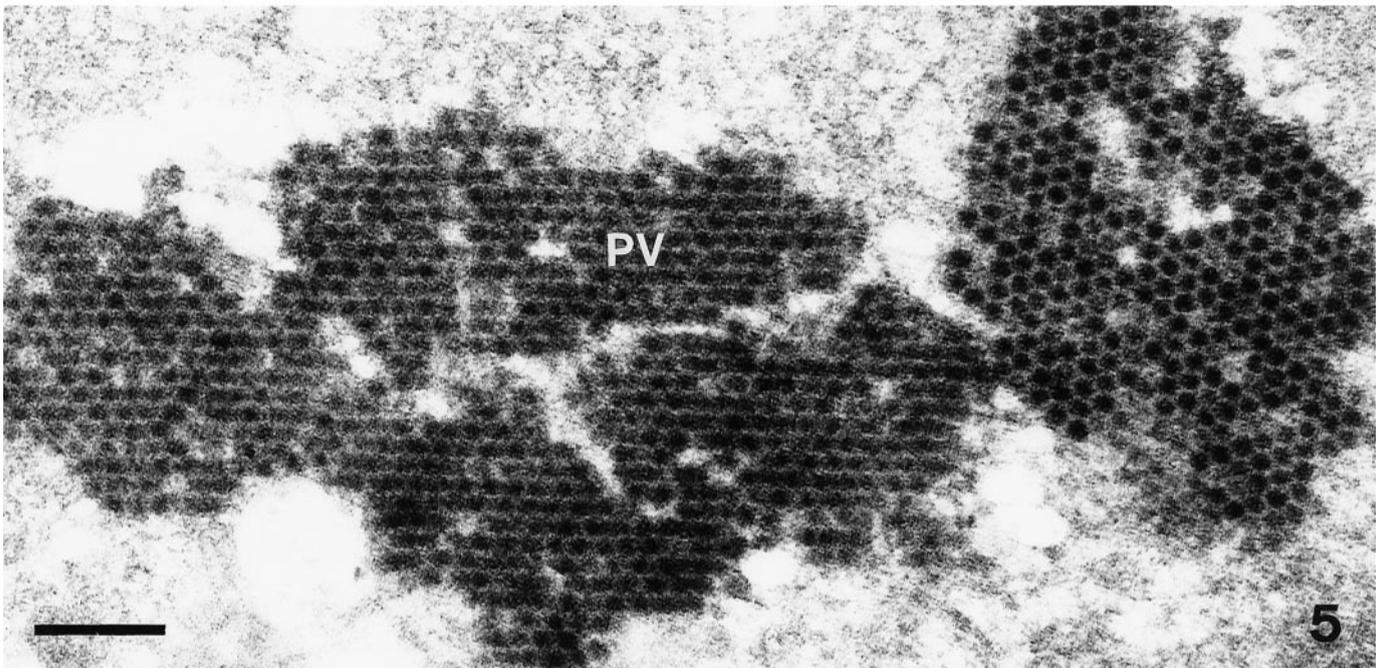
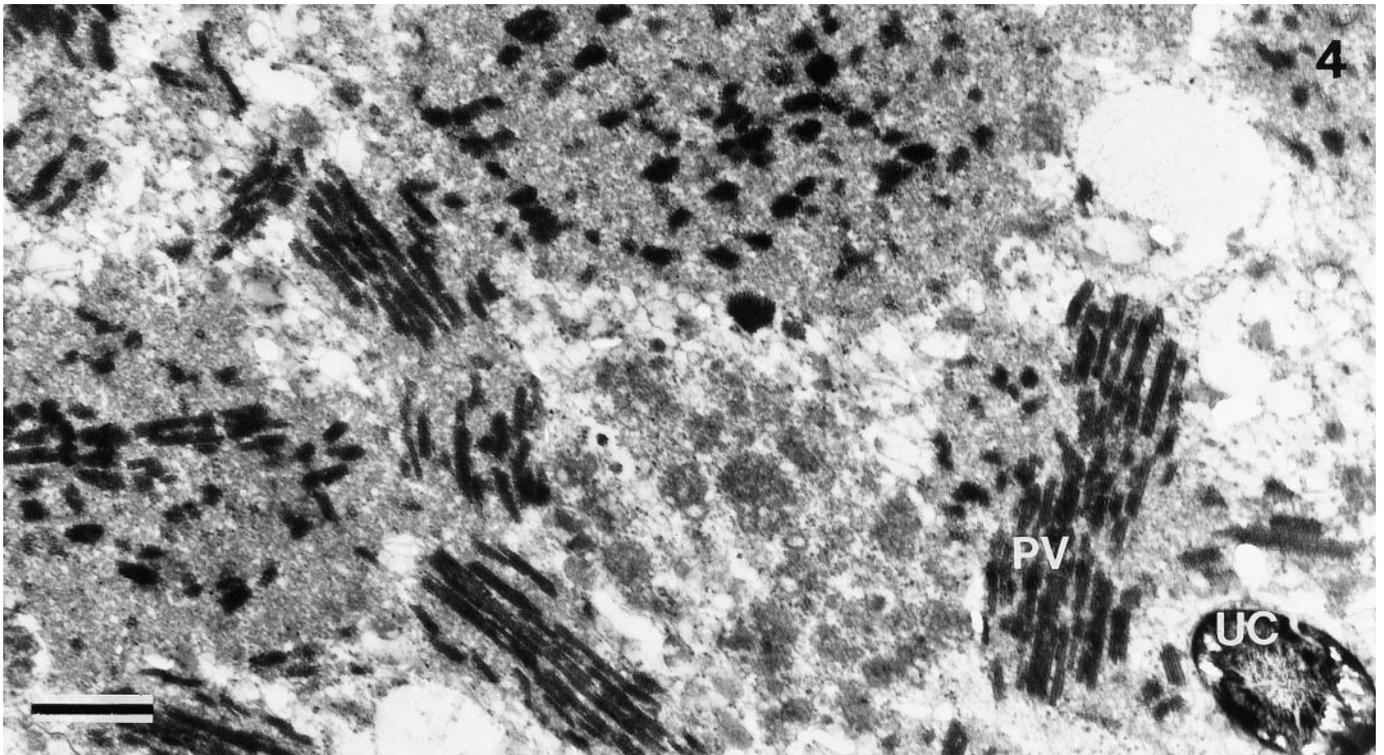


FIG. 4. Paracrystalline arrays of virus-like particles (PV) in the fat body of a *Varroa* mite; the nuclei are highly desintegrated. UC, uric acid crystal; bar = 1.0 μ m. **FIG. 5.** Paracrystalline arrays of virus-like particles (PV). Note the hexagonal contour of the particles. Bar = 0.2 μ m.

FIG. 1. Symptomatic *Varroa* mite with black-colored changes of different tissues, especially gut (g) and fat body (f); bar = 0.3 mm. **FIG. 2.** Nucleus of the fat body of a *Varroa* mite with increasing occurrence of virus-like particles. The inner and outer surfaces of the double-layered nuclear membrane may be preferred sites of virus adherence (arrowheads). Note detachment and invagination of the inner layer of the nuclear membrane densely occupied by virus-like particles (arrowheads); pockets of particles are drifting from the nuclear membrane into the nuclear area (arrows). V, virus-like particles; bar = 1.0 μ m. **FIG. 3.** Part of fat body nucleus stuffed with virus-like particles (V); bar = 1.0 μ m. **FIG. 3a.** Higher magnification of virus-like particles from Fig. 3; bar = 0.5 μ m.

into this extract. So far, the transmission tests failed, perhaps due to the method used or insufficient concentrations of virus-like particles.

Further studies are envisaged to elucidate the nature of these virus-like particles and their possible control capacity against the *Varroa* mite.

Key Words: virus-like particles; parasitic mite; *Varroa jacobsoni*; honey bee; *Apis mellifera*; electron microscopy; transmission tests.

The authors thank Dr. A. M. Huger (Federal Biological Research Center for Agriculture and Forestry, Institute for Biological Control) for his comments and helpful discussion and Dr. W. Ritter (Institute of Animal Health, Freiburg, Germany) for his investigations on bees with respect to APV.

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R. G. Kleespies
J. Radtke
K. Bienefeld

*Federal Biological Research Center for Agriculture and Forestry
Institute for Biological Control
Heinrichstrasse 243
D-64287 Darmstadt, Germany
E-mail: r.kleespies.biocontrol.bba@t-online.de*

*Institute of Bee Research
Friedrich Engels Strasse 32
D-16540 Hohen Neuendorf, Germany*

Received January 11, 1999; accepted July 21, 1999