OPERATIONAL AND SCIENTIFIC NOTES

AN "R" TYPE *IRIDOVIRUS* FROM *AEDES VEXANS* (MEIGEN)[1]

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The iridoviruses characteristically produce iridescent colors in infected insects. This iridescence results from constructive interference of visible wavelengths of light, and the color of the iridescence is a function of the interparticle spacing of the paracrystalline arrays formed by the virions in the tissues of the infected host (Fig. 1).

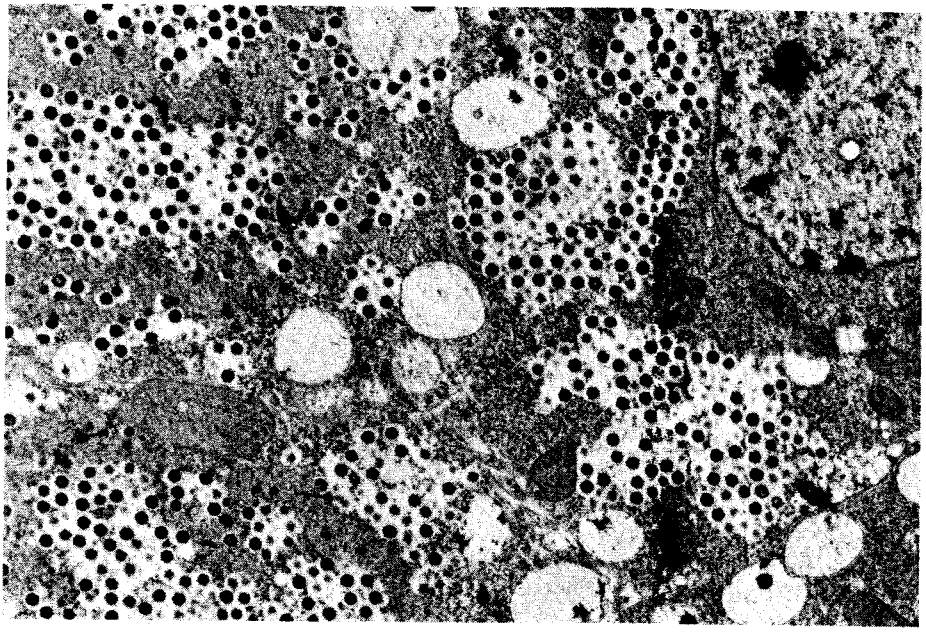

Fig. 1. *Iridovirus* in fat body cell of *Aedes vexans* x8000.

Iridoviruses have been reported from more than a dozen species of floodwater mosquitoes of the genera *Aedes* and *Psorophora* (Federici 1974). Most of these viruses produce green, blue, or purple iridescence.

Two iridoviruses have been reported from *Ae. taeniorhynchus;* one produces an orange iridescence and the other a turquoise iridescence. Matta and Lowe (1970) designated these the "R" (regular) and "T" (turquoise) strains respectively. The R and T strains from *Ae. taeniorhynchus* have been shown to be serologically identical but to differ in size and density (Hall and Lowe 1972; Wagner et al. 1973) with the R strain being approximately 35 nm greater in diameter than the T strain.

We have found an *Iridovirus* in *Ae. vexans* larvae collected in Hadley, Massachusetts which produces an orange iridescence nearly identical in color to that of the R strain virus from *Ae. taeniorhynchus*. Approximately 0.3% (11 of 3,515) of the larvae collected exhibited patent infections. Histological studies of infected larvae with the fluorochrome Coriphosphine O (Keeble and Jay 1962) have demonstrated large concentrations of viral DNA in the cytoplasm of cells of the fat body, epidermis, tracheal epithelium, imaginal discs, and salivary glands. This virus appears to have the same tissue specificity as the iridoviruses from *Ae. taeniorhynchus* (Anthony and Hall, 1970; Hall and Anthony, 1971) and *Ae. stimulans* (Anderson 1970).

Side-to-side measurements from electron micrograph negatives of virions oriented in the threefold axis of symmetry indicated a diameter of

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190±4nm, which is similar in size to the R virus from *Ae. taeniorhynchus*. An *Iridovirus* has previously been reported from *Ae. vexans* larvae collected in Louisiana, but this virus invariably produced green iridescence (Chapman et al. 1966). This is only the second known *Iridovirus* which produces orange iridescence. In both cases these viruses have been found in species of mosquitoes from which viruses producing green iridescence are also known. This fact suggests that R type viruses may also occur in some or all of the other species of *Aedes* and *Psorophora* in which iridoviruses with green or blue iridescence have been found. Efforts should be made to further characterize and to determine the distribution of the "R" and "T" types of virus so that their evolutionary relationships can be better understood.

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RAPID COUNTING METHODS FOR MOSQUITO LARVAE

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A careful balance of larval numbers with water surface, water volume and diet is required for survival and uniform, optimal growth and development of mosquito larvae reared for experimental purposes (Gerberg 1970). Bar-Zeev (1962) used 2 sizes of suction tubes stopped with sintered glass filters to obtain either 2100 or 15,000 newly hatched *Aedes aegypti L.*, on the basis of packed volume. Morlan et al. (1963) dispersed newly hatched *A. aegypti* in 2 liters of water with a food mixer and counted 20 2-ml. samples to determine the number of larvae per ml. of the suspension. For large-scale production the larvae were dispersed in a modified agitator-type washing machine and dispensed automatically with a tipping-bucket dispenser. Similarly, Gerberg et al. (1968) dispersed newly hatched *Anopheles stephensi* Liston in 3 liters of water with an electric stirrer and counted 25 1-ml. samples to determine the larval concentration.

The method of Bar-Zeev (1962) is not readily adaptable to counting varying numbers of larvae, and those of Morlan et al. (1963) and Gerberg et al. (1968) require one-by-one counting of larvae in a number of samples to achieve an acceptable degree of accuracy. The present paper describes 2 supplementary methods developed in our laboratory to simplify larval counting.

PHOTOGRAPHIC COMPARISON METHOD. Photographs were made of Petri dishes containing 100, 200,....900, 1000 dispersed 1st instar larvae of *A. aegypti* and reprinted as a single, full-sized strip photograph of the larval concentrations in serial order (Figure 1). The strip-photograph (comparator) is placed on a dark bench or table, and the number of larvae in a Petri dish is estimated by moving the dish alongside the comparator until a matching photograph is found. Larvae are then added or withdrawn from the dish with a dropper, and a new match is made, until the desired number of larvae is obtained. If equal numbers of larvae are to be