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Drosophila S Virus, a Hereditary Reolike Virus, Probable Agent of the Morphological S Character in *Drosophila simulans*

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Isometric reolike virions were found in all the examined *Drosophila simulans* flies from two strains (SimES-st and Israel-st) presenting the S phenotype, a maternally inherited morphological trait (abnormalities of bristles). Normal flies of both strains appeared virus-free. Virions were found in the cytoplasm of male and female gonads and epidermal cells, including the bristle-forming cells, which appeared disorganized. Steps of virogenesis were described. A positive correlation was demonstrated between expressivity of the S phenotype and degree of viral infection. This hereditary reolike virus seems to be responsible for the S character of *D. simulans* and was named DSV (*Drosophila* S virus).

Examples of maternally inherited physiological traits caused by microorganisms are known in *Drosophila* species. The best studied are the sensitivity to CO_2 caused by the rhabdovirus sigma (3) and the lethality of male zygotes induced by spiroplasmas (19).

In *Drosophila simulans*, the hereditary S character, found by Comendador (4, 5), does not follow Mendelian laws. This morphological trait may affect 50 to 60% of the individuals and consists of bristle abnormalities or loss of bristles, mostly dorsocentral and scutellar.

The S trait presents the following other characteristics. (i) It is quantitative: zero to eight thoracic bristles may be missing; (ii) it is strongly asymmetric, and S bristles are localized more often on the same side of the thorax; (iii) it is very responsive to selection (5); (iv) it is transmissible not only by females, but also by males, though to a very low degree (M. Lopez-Ferber, Ph.D. thesis, Université des Sciences, Montpellier, France, 1987).

The original *D. simulans* strain from the Azores islands was infected with two pathogenic microorganisms. The strain cleared of these pathogens (SimES-st or S-st strain) retained the S phenotype. The S flies of the S-st strain had fitness parameters somewhat lower than those of wild-type flies of the same genotype (6). Comendador et al. (6) postulated that a third microorganism, a hereditary virus, was the causal agent of the S character. Three arguments were advanced in favor of this hypothesis: (i) the S phenotype is very sensitive to temperature (E. Garcia-Vazquez, M. Lopez-Ferber, and M. A. Comendador, manuscript in preparation); (ii) it is not affected by the addition of antibiotics to the culture medium; (iii) the factor responsible for the S trait presents a very low but significant contagious power when an extract of S flies is deposited on SimES eggs (6).

Nevertheless, all prior attempts to multiply the hypothetical virus by injections of acellular suspensions of S flies into normal flies have had negative results (Lopez-Ferber, Ph.D. thesis).

The hypothetical virus should be, a priori, hereditary; slightly pathogenic or nonpathogenic; weakly invasive, owing to the asymmetry of the S trait; and more or less defective, owing to the failure of its transmission through injections. Finally, it should affect the epidermal cells responsible for chaetal formation.

We present here the discovery of a reolike virus in the epidermal tissue of the S flies that, according to its properties, could be the agent of the S phenotype. We describe the main steps of virogenesis of this virus, its role in the target cells which are responsible for chaetal production, and the existence of a correlation between the intensity of the phenotype and the density of the virions in epidermal tissue. We demonstrate its presence in other tissues of the S flies, in particular the male and female gonads, and its absence in the corresponding tissues of normal flies of the same strains.

MATERIALS AND METHODS

Electron microscopy. (i) Preparation and examination of ultrathin sections. Flies (2 to 3 days old) were anesthetized with ether and dissected in a cold 2.5% solution of glutaraldehyde in 2.1% cacodylate buffer. Tissues were postfixed in osmium tetroxide, dehydrated in acetone, embedded in Epon resin, sectioned, contrasted, and examined by transmission electron microscopy (TEM).

(ii) Regressive staining. Sections of infected epidermal tissue fixed only in glutaraldehyde were stained by floating for 1 min on a 3% uranyl acetate (aqueous solution) and for 30 min on a 10% EDTA solution at pH 7. They were then rinsed and contrasted for 1 min with lead citrate. Control sections were floated on distilled water instead of EDTA solution (1).

(iii) Rapid staining for TEM examination of squashes from infected tissues. Fragments of cuticle and epidermis of S flies were placed on grids in a drop of Schneider culture medium (13) containing 10% calf serum and then rinsed and negatively stained with a 4% solution of ammonium molybdate at pH 7.4. The entire process was done at 0°C.

***D. simulans* strains.** The S-st strain was derived from flies captured in the Azores islands (4, 5). These flies had been submitted to egg disinfection by dechorionation to eliminate the nonhereditary microorganisms (6).

The Israel-st strain, produced by egg dechorionation of flies collected in nature in 1983, had the S trait with about the same characteristics as the S-st strain (Lopez-Ferber, Ph.D.

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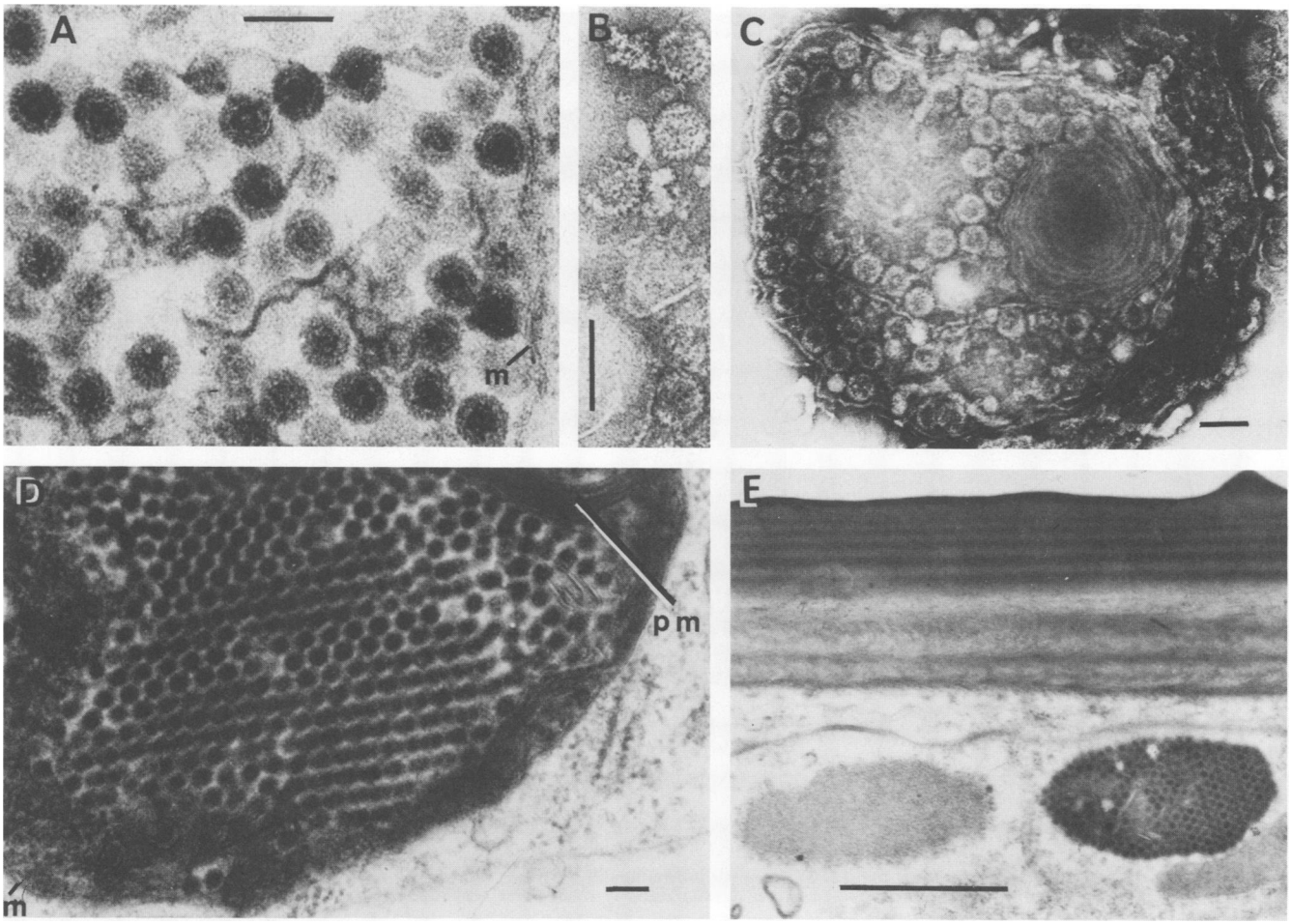

FIG. 1. Virions of *D. simulans* virus (DSV) from S flies of the SimES-st strain (A to D; bars = 100 nm) and from S flies of the Israel-st strain (E; bar = 1 μm). (A) Ultrathin section of fixed tissue; virions seem to be well preserved. m, cytoplasmic membrane. (B and C) Squashes showing disrupted cells; (B) most of the virions are damaged; (C) virions are included in a membranous system; some of them seem intact. (D) Ultrathin section showing pseudocrystalline arrangements of DSV virions. m, Cytoplasmic membrane; pm, plurimembranous formations. (E) Ultrathin section showing viroplasm and pseudocrystalline arrangements of virions in an epidermal cell of an Israel fly.

thesis, 1987). Both strains were continuously selected for the maintenance of the S phenotype.

Culture conditions. All *Drosophila* strains were reared and kept on axenic medium (7) at 24 ± 0.1°C with a 12-h/12-h photoperiod.

RESULTS

Observation of ultrathin sections. In searching for virions, epidermal tissues were systematically examined by TEM on the thorax where the S macrochaetae had been first observed and also on the abdomen, which had abnormal or missing microchaetae (5). Observations of abdominal epidermal cells were easier than those of thoracic cells, which were in part replaced by muscular insertions.

The ultrathin sections of S flies all revealed the presence of isometric spherical particles in the cytoplasm. These were about 60 nm in diameter, with a dense central core and a less dense double shell. They were found in the S flies of the S-st strain (Fig. 1A to D and 2A to D) and of the Israel strain (Fig. 1E).

In these infected cells, the virions were observed in different formations: (i) lysosomal-like bodies with pseudomyelin formations where partially damaged virions were found in electron-dense structures; (ii) viroplasms composed of a granulofibrillar zone surrounded by virus particles and by viral shells (Fig. 1E and 2B and C); (iii) virions dispersed in the cytoplasm, appearing alone or in small clusters between the cellular organelles; (iv) formations apparently surrounded by a membrane and containing virions dispersed in a matrix more or less electron dense (Fig. 2B and D); (v) pseudocrystalline arrangements of numerous virions, protected by one or two membranes (Fig. 1D and E).

In S flies, the virions were observed in subcuticular (epidermal) cells of thoracic and abdominal sections, in follicular cells of the ovarioles, in the male gonads, in tracheal cells, and occasionally, in the periplasm of muscular cells (Fig. 2C). They were also observed in wing imaginal disks of L3 larvae from S × S crossbreedings (Lopez-Ferber, Ph.D. thesis, 1987). Various stages of virogenesis took place in cells associated with the S bristles (trichogen or tormogen cells, or both) (Fig. 2A and B). In normal flies, these types of cells regress and are difficult to distinguish from the other cells of the epidermis at the adult stage (2). The other tissues examined, particularly the adipose tissue and the midgut, were apparently virus-free.

Attempts to correlate the expressivity of the phenotype to the degree of viral infection were made by TEM examination

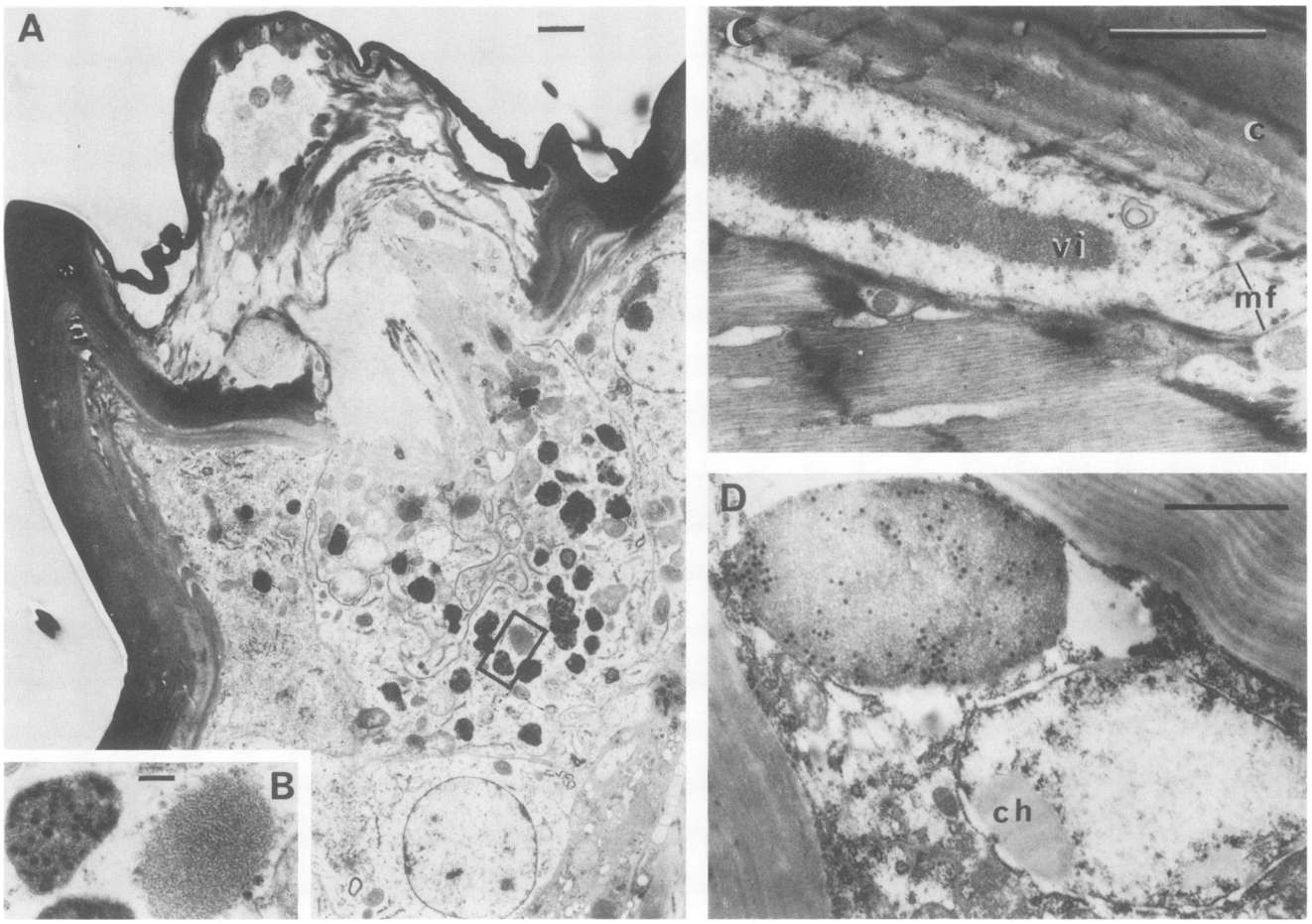

FIG. 2. Ultrathin sections of epidermal cells of S-st flies. (A) Base of an abnormal thoracic macrochaeta. Residual cells responsible for bristle production are filled with virions at different stages of virogenesis. Bar = 1 μm. (B) Detail of panel A (box): viroplasm and virions surrounded by a membrane. Bar = 200 μm. (C) Viroplasm at the level of a muscular insertion. The viral replication obviously disturbs the links between muscular fibers and cuticle. vi, Viroplasm; mf, microfilaments; c, cuticle. Bar = 1 μm. (D) Section treated by the EDTA regressive technique of Bernhard (1). Virions are intensely contrasted, chromatin (ch) is unstained. On control sections (not shown), both virions and chromatin are intensely stained. Bar = 1 μm.

of at least two whole cross-sections of each of 43 sister flies (28 S and 15 wild type). Figure 3 shows the relationships between the phenotype expressivity (number of missing bristles per fly) and the viral density calculated in millimeters of epidermal tissue. To estimate this last value, we classified the viral clusters as small, intermediate, large, and very large and gave them values of 1, 2, 5, and 20, respectively. The Spearman's r_s ordinal coefficient of correlation corrected for the presence of twin values (14) was utilized to calculate the relationship between the intensity of the phenotype and the viral density. The coefficient $r_s = 0.8176$ was significant at $\alpha = 0.01$. Thus, a positive correlation exists between these two variables.

Neither virion nor viroplasm was seen in the 15 wild-type flies which were sisters of the 28 S flies. The difference between 28 positive and 15 negative flies is highly significant ($\chi^2 = 43.35$ for 1 df). In the Israel strain, five S flies examined were infected and three wild-type flies were virus-free.

Preferential RNA staining. Preferential RNA staining (1) resulted in a nonstaining of the host nuclear DNA. The center of virions appeared electron dense (Fig. 2D), as for *Drosophila* picornavirus P (16).

Electron microscopic observations of S fly squashes. Virions were observed either isolated (Fig. 1B) or grouped in inclusion bodies surrounded by a membrane (Fig. 1C) or at the periphery of areas presenting a loose structure, probably indicating granulofibrillar viroplasms. A large proportion of these virions appeared disrupted. The intact virions had a morphology and size very similar to those seen in the ultrathin sections.

DISCUSSION

The results of the present study are in agreement with the hypothesis that the nonchromosomal causative agent of the S character in *D. simulans* is a hereditary cytoplasmic virus. The virus is clearly hereditary, as both *Drosophila* strains were derived from dechorionated eggs and were constantly maintained on axenic medium. Indeed, viral particles have been seen by TEM in the female and male gonads of the S flies.

Four categories of facts support the causative role of this virus in the S phenomenon. One is the statistically significant association shown between the presence of viral particles and the S phenotype and, more importantly, the positive correlation existing between the intensity of the phenotype and the viral density. The second is the tissue affinity of the virus; in S flies, virions were found preferentially in the

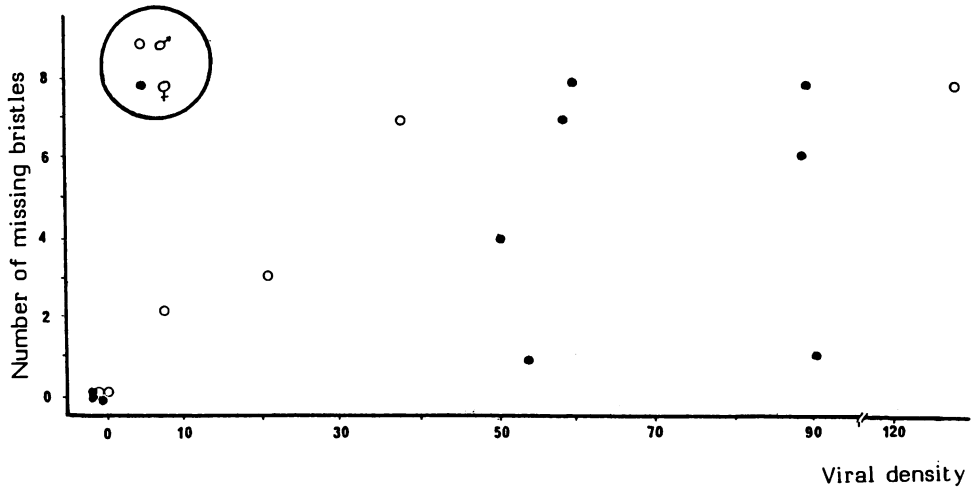

FIG. 3. Correlation between the expressivity of the S phenotype and the viral density. The Spearman's ordinal coefficient of correlation $r_s = 0.8176$ was significant at $\alpha = 0.01$.

subcuticular cells, including those lying under the abnormal bristles. Viral replication may be stimulated by the intense cellular activity of rapid chaetal development which occurs during the pupal stage. In turn, this viral replication may impair the cellular metabolism, resulting in production of abnormal bristles. The third fact is the presence of an apparently identical virus with the same tissue affinity in the geographically distant Israel strain which has the same non-Mendelian S character. The fourth is the low invasive power of the virus: in ultrathin sections we never observed phenomena known for vertebrate reoviruses (15, 17) such as virions in adsorption or penetration phases, extracellular virions, or lysed cells. The infected cells could have received the virions from the mother cells during cellular division. Such a reduced invasive power of the virus inside the host is consistent with the pattern of missing chaetae in S flies, which is preferentially asymmetric. An invasive hereditary virus would have systematically infected both sides of the thorax. The known bristle malformations in *Drosophila* species caused by chromosomal genes such as the bobbed gene present a symmetric pattern. An abnormal degree of lability of the virions could explain their low invasive power in vivo and the high percentage of damaged virions in vitro in squashes, even when the action of proteases was reduced by the addition of 10% calf serum.

Finally, the ultrastructural characteristics of *D. simulans* virions and of their virogenesis led us to consider them as putative members of the reovirus group of the *Reoviridae* family (9, 10, 17, 18). In sections and in squashes, *D. simulans* virions are spherical, about 60 nm in diameter, and are composed of a dense central core, a double layer of proteins, and no envelope. The virogenesis also resembles that of the *Reoviridae*, particularly those described in *Drosophila* species (F virus [8]) or in other Diptera Brachycera such as *Ceratitis capitata* (I virus [12]) and *Musca domestica* (HF virus [11]). The contrasted virions obtained by the EDTA technique of Bernhard (1) are an indication of their RNA nature.

To our knowledge, a virus with a morphological effect has not yet been described in insects. The reolike virus found in S strains has been named *Drosophila* S virus (DSV). It is very likely the agent responsible for the S morphological trait in *D. simulans* as it fulfills the conditions required of such a candidate: it is hereditary, is present in female and male gonads, replicates preferentially in epidermal tissues, disturbs the cellular functions of the cells responsible for chaetal formation, does not cause cellular lysis, and seems to have a reduced capacity of invasion. The intensity of the S phenotype is correlated with the density of virions present in epidermal cells. The apparent lability of DSV virions hinders their biochemical characterization and the direct demonstration of their causative role in the S phenotype.

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