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Drosophila S Virus Is a Member of the *Reoviridae* Family

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The S character of *Drosophila simulans*, the absence or malformation or both of bristles and other cuticular structures, was described by Comendador (Drosophila Inf. Serv. 55:26–28, 1980.) Its characteristics (maternal transmission, low pathogenicity, and sensitivity to temperature) suggested the existence of a virus as the causative agent. Indeed, reoviruslike particles were found in subcuticular cells of S individuals, and its association with S phenotypic expression was shown. This virus was called *Drosophila* S virus (DSV) (C. Louis, M. López-Ferber, N. Plus, G. Kuhl, and S. Baker, J. Virol. 62:1266–1270, 1988). We report here the purification and analysis of some properties of DSV particles, the morphology (spherical, 60 nm in diameter with an electron dense central core and less dense shell) and genome composition (double-stranded RNA divided into segments), which classify DSV as a new member of the family *Reoviridae*.

The S morphological character was first described by Comendador (4, 5) in *Drosophila simulans* imagos from a natural population of the Azores. It was characterized by loss or abnormalities of bristles, especially the dorsocentral and scutellar bristles (4). This phenotype shows a rapid response to directional selection in penetrance (mean number of flies showing the S character among the offspring of an S pair) and expressivity (mean number of deformed or missing bristles per imago). A small but statistically significant reduction of fitness parameters is observed in S individuals (6). It correlates with the expressivity of the S character. At high levels of expressivity, fecundity is strongly reduced and the offspring do not present the character. Thus, it is not possible to fix the S character.

It seems likely that the S character is present in nature at low expressivity levels, allowing it to be conserved. Under laboratory conditions without selection, the expressivity of the S character decreased to the level of natural bristle number variability in a few generations; therefore, selection is necessary at each generation to maintain an almost constant level of expressivity and penetrance. A genetic analysis of this character showed that its transmission was transgametal and non-Mendelian (4).

Reoviruslike particles have been detected in flies from the strain of *D. simulans* selected for high expressivity of the S character (strain SimES-st) and bred under axenic conditions (7). This virus was named the *Drosophila* S virus (DSV) (12).

We checked for the presence of the S character in flies from 10 natural populations of *D. simulans*; in one, named the Israel-st strain, we were able to select a phenotypically similar trait. Morphologically analogous virus particles were also observed in ultrathin sections of S flies from this second strain.

DSV seems to be the causative agent of the S character. It is absent from the tissues of normal flies and is present in the gonads and epidermal cells of S flies; moreover, the quantity of visible virus particles correlates with the intensity of the S phenotype (12). DSV, like most hereditary viruses, is produced in small quantities in infected flies. In fact, statistical analysis of the spatial distribution of affected bristles revealed that little or no transmission from cell to cell occurs (M. López-Ferber, Ph.D. thesis, Université des Sciences, Montpellier, France). It should be emphasized that it is difficult to obtain the quantities of DSV necessary for analysis because the amount of virus particles in a *Drosophila* fly, the average weight of which is 1 mg, is small.

In preliminary purification attempts, a discrepancy was evident between the relatively large number of entire virus particles observed in ultrathin sections of S flies (12) and the small number of particles (often damaged) recovered. This suggested that S virus particles are very fragile. Conditions for improving the virus yield after extraction and purification was explored by using batches of 35 S flies. The virus yield was estimated by electron microscopic count of intact particles negatively stained with ammonium molybdate under standard conditions.

Various media, supplemented or not supplemented with a protease inhibitor (phenylmethylsulfonyl fluoride or fetal bovine serum) and detergent (Triton X-100), and various pHs were tested with poor results. Only few particles were recovered, those recovered were mostly damaged, and recovery was obtained only at pH 7.0. This led us to use coupling agents (formaldehyde and glutaraldehyde tested at various concentrations and for various times) to prevent disruption of the virus particles during purification. This treatment increased the virus particle yields 10 to 100 times.

Formaldehyde-treated samples showed large aggregates of intact particles without a clear outer structure. Sometimes a fibrillar matrix was observed between particles. Glutaraldehyde did not produce agglutination, fixed particles showed a precise external structure, and the total virus yield was greater, since no loss occurred during sedimentation. Good results were obtained when S flies were crushed directly into the glutaraldehyde-containing buffer Britton Robinson II (19) (Fig. 1). Under these conditions, fixation was not sufficient to produce tissue rigidization.

For preparative purification, 25,000 individually selected S flies were crushed in a solution of Britton Robinson II buffer containing 4% glycerol and 1% glutaraldehyde (pH 7.0) at 0°C. The suspension was filtered through muslin cloth and clarified by centrifugation at 5,000 × *g* for 10 min. The supernatant was kept cold, and the pellet was suspended and clarified as described above. The two supernatants were

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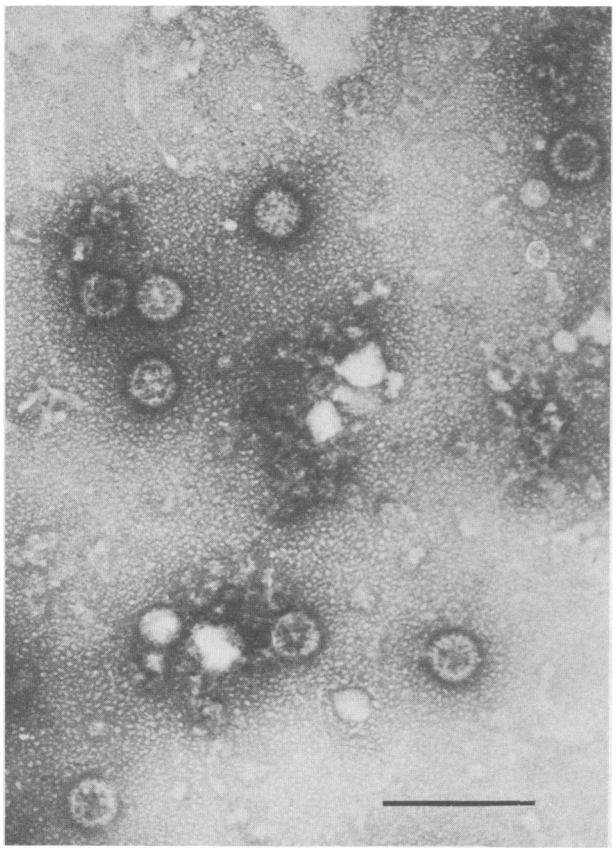

FIG. 1. Partially purified DSV particles fixed with 1% glutaraldehyde and negatively stained with 4% ammonium molybdate. The virus particles are dispersed. The virus particle in the upper right corner is oriented on a fivefold symmetry axis, and 10 peripheric capsomers are visible. Bar, 200 nm.

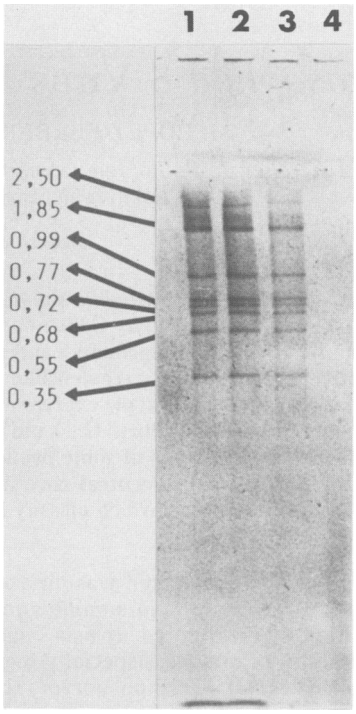

FIG. 2. Silver-stained gel electrophoresis of DSV nucleic acid submitted to different enzymatic treatments. As a control of enzymatic digestion, rRNA was used. It is visible at the bottom. Lanes: 1, no treatment; 2, DNase treatment; 3, RNase treatment at a high saline concentration (control single-stranded RNA was digested); 4, RNase treatment at a low saline concentration (single- and double-stranded RNAs were digested). DSV segments disappeared. The molecular weights shown (10^6) were estimated from parallel electrophoresis with reovirus 3 (Dearing strain) RNA segments.

pooled and centrifuged at 150,000 × *g* for 2 h. The pellet was dispersed in the same medium, and its contents of intact virus particles was checked by electron microscopy before further purification by density gradient centrifugations.

In sucrose density gradients (15 to 45% [wt/vol]), two peaks were detected, as usual in reovirus purifications. The lighter band contained some empty particles and disrupted shells; the heavier band was composed of intact virus particles. Further centrifugation in a CsCl density gradient revealed a peak of absorbance at 1.34 g/cm^3. Observation of this fraction after dialysis and staining revealed only disrupted particles (data not shown).

In Renografin (Radioselectane; Schering Laboratories, Lys lez Lannoy, France)-preformed density equilibrium gradients (20 to 76% [wt/vol], 15 h at 30,000 × *g*), no top component band but instead an unique band of mostly intact particles was found. It was collected, dialyzed, and centrifuged (2 h at 150,000 × *g*), and the pellet was used to analyze the structure of the virus particles by electron microscopy and also the nucleic acid content.

The diameter of these purified particles was 57.00 ± 0.58 nm, in good agreement with the diameter of particles in ultrathin sections or extracted without fixation.

Virus particles observed after glutaraldehyde fixation by the rotation technique of Markham et al. (13) appeared on a five- or sixfold symmetry axis (data not shown).

In previous experiments, the regressive Bernhard staining technique of ultrathin sections (2) was used to indicate the nucleic acid nature (DNA or RNA). The results were in favor of the RNA nature of the nucleic acid (12).

The nature of the nucleic acid of DSV was analyzed by enzymatic treatment as described by Hull (10). Samples of DSV nucleic acid were treated by DNase (15 min at 37°C in 10 mM Tris hydrochloride [pH 7.5]–2 mM $MgCl_2$) or RNase (15 min at 25°C) at a high (2× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate]) or low (0.1× SSC) saline concentration and compared with an untreated sample by polyacrylamide gel electrophoresis under Laemmli conditions (11). As a control of RNase activity, we added single-stranded rRNA to each sample. This single-stranded rRNA disappeared in RNase-treated samples. The nucleic acid of DSV was degraded only in the sample treated by RNase at a low saline concentration but not in the other solutions, confirming that its nature is double-stranded RNA. Figure 2 shows the results of electrophoresis of these samples.

The genome is segmented with at least eight different segments. The difference of intensity of band 2 might indicate that two segments comigrate.

The smudge between bands 1 and 2 was observed in all preparations and disappeared in samples treated with RNase at a low saline concentration. Thus, it corresponds to a heterogeneous class of double-stranded RNA which copurifies with the virus particles. It could be a partially degraded or heterogeneous genome segment. Further investigations are necessary to resolve this question.

The apparent molecular weights of those double stranded RNA segments were estimated from parallel electrophoresis

with double-stranded RNA genome segments of human reovirus 3, (strain Dearing) as the standard (Fig. 2). The molecular weights of these control segments have been published by Ramig et al. (18).

Nucleic acid extracted from virus particles of the two different *D. simulans* strains carrying the S character, SimES-st and Israel-st, revealed no difference between the migratory patterns (data not shown). These two viruses are also similar in morphology and morphogenesis. Although these data are not sufficient to certify that the two viruses are identical, the fact that the two strains that carried them were selected not for its presence but for phenotypic expression of the S character did suggest this.

Although the data presented here confirm that DSV is a member of the Reoviridae family, they do not permit us to ascribe it to any of the established genera. On the basis of its morphology and morphogenesis, DSV seems to be related to other reoviruses of *Diptera* (for example, the I virus of *Ceratitis capitata* [16, 17], the F virus of *D. melanogaster* [9, 17], the HFV virus of *Musca domestica* [14, 15], the reovirus of *Dacus oleae* [1; M. Agnanou-Veroniki, personal communication]) and *Hemiptera* (the reovirus of *Cimex lectularius* [8]). Still, the DSV genome segment distribution is different even from that of the diverse strains of the F virus of *D. melanogaster*, a species close to *D. simulans*.

The fragility of DSV particles enabled us to extract intact virions. We used coupling agents to recover undamaged particles for analyses of structure and nucleic acid composition, but this treatment does not permit protein analysis or preservation of infectivity to formally establish the role of DSV in the S character.

However, the facts that the two strains selected for expression of the S character are infected by apparently identical viruses and none of the wild-type *D. simulans* strains analyzed contained similar particles support the hypothesis of the causative role of DSV in the S character.

What can be the mechanism for the conservation of this low level of infection in the two populations at the origin of SimES-st and Israel-st strains?

Drosophila natural populations are frequently infected by viruses (3); some of them show vertical transmission and do not seem to have an effect on the fitness of flies. A beneficial action of the DCV picornavirus of *D. melanogaster* when it infects via the chorion has been reported by Thomas-Orillard (20), whereas upon injection into imagos, DCV is highly pathogenic.

Whether low levels of DSV infection confer any kind of selective advantage to *Drosophila* flies is under investigation.

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