LOW TITERS IN DROSOPHILA THAT REGULARLY TRANSMIT THE SIGMA VIRUS INFECTION¹

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MUCH information concerning the life cycle of the sigma virus in *Drosophila* was accumulated by means of an assay developed by PLUS (1954). The assay is used to determine the infectious virus content of extracts from infected flies and is based on an end-point dilution procedure. Aliquots from a series of dilutions are injected into virus-free imagos and the titers calculated from the distribution of infectious units among the dilutions.

Measurements thus obtained show that the regular maternal transmission of the virus, found in some *Drosophila* strains, does not depend simply on the flies' content of infectious material. PLUS (1954, 1955) and BRUN and SIGOT (1955) showed that flies transmitting the infection to relatively few offspring can contain higher virus titers than flies transmitting the infection to nearly all of their progeny. BRUN also showed that some fly strains which transmit the infection regularly to their offspring may remain free of infectious material for generations. Thus, generations of oocyte infection are not assured merely by invasion of infectious material from the soma but are determined by a continuing germ-line infection (BRUN 1963).

This conclusion depends, of course, on the efficacy of PLUS's assay. If there were a type of virus which was infectious in the animal of origin, but not in the inoculated test fly, the assay would not detect it and the interpretations would be wrong. The experiments described below were performed in order to obtain an independent estimate of virus content to serve as a check upon the assay results and, thereby, upon these interpretations.

No methods were available to measure virus content except as infectious units (IU) in PLUS's assay, but the injurious effect of high infectious-virus content upon developing flies was used to estimate the virus content indirectly. That is, high virus titers are known to be associated with deaths among developing strain-Lw flies (SEECOF 1964). Therefore, progeny survival was measured in a strain from which relatively little infectious material can be recovered (strain L) but which could be expected to contain a large virus content because its eggs are always infected. Progeny deaths, if found, would indicate a virus component invisible in the assay.

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MATERIALS AND METHODS

In the present investigation, strains L and Lws were investigated as to progeny survival, efficiency of transmitting virus to progeny, and imaginal virus titers. The results for the two strains were then compared to those already published for strain Lw (SEECOF 1964). The relationships among the genotypes of these three fly strains are given in this section and a summary of pertinent data for strain Lw is given in the DISCUSSION in order to facilitate the comparisons.

Culture conditions: The flies were raised under the same conditions as those described earlier (SEECOF 1964). Single young virgin females were placed with several males in individual shell vials. After the female had laid fertile eggs the flies were transferred daily to fresh vials and the eggs counted. A total of 72 to 170 eggs was scored for each female, and the progeny were later scored as imagoes that succeeded in emerging from their pupa cases.

Fly strains: Strain L is virus-infected. Strain LR originated from CO₂-resistant flies which appeared in strain L and is, therefore, composed of virus-free flies of similar genotype. Strain w is virus-free. The genotypes of strain-Lw and Lws flies were originally the same because both strains were derived from strain-w flies inoculated with an extract of strain-L flies. Their virus contents probably differ because strains Lw and Lws have been subjected separately to laboratory selection for many generations in order to maintain the infection. Strain Lw has also been selected for flies transmitting high virus titers (SEECOF 1964); strain Lws has never been selected to transmit high titers.

Assay for sigma titers: Sigma titers for strain Lw were measured at the time progeny deaths were detected (SEECOF 1964) by procedures published previously (SEECOF 1962). The titers for strains L and Lws were measured in the same way except for the following modifications: Titer values were calculated directly from the end-point dilution data instead of referring incubation times to a standard curve, incubation times for the injected test-strain flies were measured over 70-day periods, and the test-strain flies were injected 2.5 to 5.5 hours after eclosion. Too few data are available to statistically determine the precision of the assay under these conditions. However, the few end-point dilutions that have been done in duplicate have always yielded titer values that differed by less than a factor of two.

The modified assay is still basically the same as that presented and discussed by PLUS (1954) to determine titer by end-point dilution. The changes made, though, insured that the assay would detect slow-growing virus types such as described by BRUN (1963) if any were present. If such slow-growers had been present in strain Lw at the time it was investigated, the published titer values would have been underestimates. These values are used in the comparisons made below, regardless, because errors in this direction would only enhance the conclusion that strain-Lw flies contain higher average titers than strain-L or Lws flies.

RESULTS

Progeny survival for strains Lws and w: Females from strain Lws were crossed individually to males from the w strain. Each female was scored for number of eggs laid and number of resulting adult progeny. As controls, individual females from virus-free strain w were crossed to strain-w males and similarly scored. Data were compiled on progeny survival for the virus-infected strain-LWs females and for the strain-w females (Figure 1, Table 1). No significant differences between the two populations were detected in the variances or means for progeny survival.

Progeny survival for strains L and LR: Strain-L females were crossed to strain-LR males and, as controls, strain-LR females were crossed to strain-LR males. Each female was scored for number of eggs laid and number of resulting adult progeny (Figure 2, Table 1). No significant differences between the two populations were detected in the variances or means for progeny survival.

FIGURE 1.—The distribution of females in virus-free population (w) and virus-infected population (Lws) according to progeny survival.

FIGURE 2.—The distribution of females in virus-free population (LR) and virus-infected population (L) according to progeny survival.

TABLE 1

*Progeny survival data for virus-infected and virus-free Drosophila strains**

Fly strain	Number of females	Number of eggs	Average progeny survival	Standard error	Variance
Lws	27	2605	0.84	0.022	0.0138
w	27	2613	0.85	0.013	0.00435
L	26	3215	0.74	0.032	0.0261
LR	28	3257	0.72	0.034	0.0336

* Virus-infected flies of strains Lws and L have genotypes which are similar to virus-free flies of strains w and LR, respectively.

Virus transmission to progeny: Of the 30 females sampled in the experiment for strain Lws, 27, or 90%, were virus-infected. Surviving progeny from the virus-infected females (43 to 109 per female) were subjected to CO₂, and 0.82 were found to have received virus (Table 2). All 26 strain-L females sampled in this experiment were virus-infected. Their surviving progeny (38 to 149 per female) were tested and all were found virus-infected (Table 2). These estimates of the efficiencies with which flies transmit virus to their progeny were made

TABLE 2

Progeny infection data for strains Lws and L

Fly strain	Number of females	Number of progeny	Average progeny infection	IU per infected progeny
Lws	27	2125	0.82	3427
L	26	2414	1.0	1684

at the time their progeny survivals were measured and are, therefore, proper for the comparisons here. The observations that flies from strain L pass virus to nearly all their progeny and those from Lws and Lw pass virus to fewer of their progeny have, however, been made many times and are characteristic for the strains.

Titers associated with each fly strain: An extract of the CO₂-sensitive progeny from these crosses was made for each strain, about 1,500 progeny from strain-Lws and about 2,000 progeny from strain L. The extract of Lws flies gave a value of 3,427 IU per fly and the extract of L flies gave 1,684 IU per fly (Table 2).

DISCUSSION

The chief intent of this discussion is to compare strain-L flies, which include virus in nearly all of their eggs, to strain-Lw flies, which do not. The data for strain Lws are supplementary and are given to describe flies with a genotype like strain-Lw but an infection that does not cause progeny deaths. Data for strain Lw were presented earlier (SEECOF 1964) but are briefly reviewed below to facilitate the comparisons among strains.

Strain-Lw flies were found to average 9,000 IU per fly, and 25 of the 27 females tested, or 93%, contained virus. Deaths detected among their developing progeny were shown to be proportional to the titer of virus included in the eggs. These results showed that high average infectious virus content in adults is not sufficient to insure the infection of all eggs, but that it may be associated with the inclusion of so much virus in eggs that deaths are detectable among the progeny.

The data show that both strain-Lw and Lws flies produced many virus-free progeny, but they differed in that strain-Lws flies contained about 62% less infectious material and failed to display virus-dependent progeny deaths. Since the fly genotypes were the same (assuming no differences were fortuitously introduced by the selection procedures), the low number of deaths among Lws progeny supports the contention that strain-Lw deaths were attributable to extensive virus multiplication and not to any unusual incompetence of this fly genotype to survive an infection. Obviously, too, the results show that the kind of infection leading to many virus-free gametes need not also lead to fly deaths.

The total absence of virus-dependent progeny deaths from strain-Lws flies was surprising because their average titer was nearly half that of strain-Lw flies and was expected to lead to a detectable number of deaths. Strain-Lws flies are known to contain two or three types of virus, each distinguished by the rapidity with which single infectious units render inoculated flies CO₂-sensitive, while strain-Lw flies are pure or nearly so for only one of these virus types (SEECOF, unpublished results). The failure to find deaths among strain-Lws progeny may prove to be due to the mixture of virus types in the strain, but as such relationships are still obscure, virus types will not be discussed here.

Strain-L females included virus in a higher proportion of their eggs than did strain-Lw females although their content of infectious material (presumably virions) was about fivefold less. If the inclusion of virions into nearly all eggs

required that the female contain a high titer, these figures would indicate that strain-L flies supported a hidden virion component and might display progeny deaths. However, virus-dependent progeny deaths could not be detected. This does not exclude the possibility that the L-strain flies contain a hidden virus component but does mean that any hypothetical large component of virions must have two special properties: that of causing few deaths during multiplication, and that of being noninfectious in the assay procedure. To postulate such virions is unnecessary. The failure to detect deaths among strain-L progeny is most simply interpreted as confirming the low measured titers for the strain. The efficacy of PLUS's end-point assay and the conclusions that rest upon it are thereby supported.

None of the data presented here provides any new explanation for the behavior of strains like L, which include virus in their eggs so efficiently. The fact that strains Lws, L, and Lw contain more than one virus type suggests that the fly and virus genotypes have important effects; further work may clarify what these are.

SUMMARY

Drosophila females of strain L contained an average titer of 1,684 infectious units per fly and included virus in all of their eggs. No deaths attributable to the infection could be detected among their developing progeny. Strain-Lw females were known to omit virus from 7% of their eggs at a time when the females contained titers about fivefold higher than those in strain L. So much virus was included in the strain-Lw eggs that deaths attributable to the infection (about 20% of all deaths) could be detected. If the high efficiency with which strain-L females included virus in their eggs were due to a content of virus not detectable in the assay for infectious units, progeny deaths might have been detected. The failure to find deaths supports the results from the assay for infectious units. Namely, certain flies may regularly include virus in all of their eggs despite their containing relatively little infectious virus.

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