Togavirus Interference in *Culex annulirostris* Mosquitoes

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**SUMMARY**

*Culex annulirostris* mosquitoes infected with SFV-S, a temperature-sensitive, small-plaque mutant of Semliki Forest virus isolated from cultured *Aedes albopictus* cells, could not be infected later with wild-type virus (SFV-W). Interference occurred as soon as 24 h after infection with SFV-S. In mosquitoes infected with a mixture of the two viruses, both replicated normally.

Mosquitoes infected with alphaviruses show no obvious signs of infection, nor do virus-infected mosquito cell cultures, which, if suitably maintained, continue to yield virus particles indefinitely (reviewed in Dalgarno & Davey, 1973). However, virus produced in cultured mosquito cells often exhibits a gradual alteration in virion size, plaque morphology, temperature sensitivity and antigenicity (Brown & Gliedman, 1973; Salazar & Thomson, 1973; Davey & Dalgarno, 1974; Shenk et al. 1974; Stollar et al. 1974).

It has been well documented that cells in persistently infected cultures resist superinfection by homologous but not heterologous viruses (Stollar & Shenk, 1973; Peleg & Stollar, 1974). However, there have been few reports of studies on viral interference in intact mosquitoes. Early studies on *Aedes aegypti* infected with Semliki Forest, Sindbis or West Nile viruses, failed to demonstrate interference in virus growth (Lam & Marshall, 1968a). However, Semliki Forest virus damaged the salivary glands of the mosquito and thus prevented transmission of the superinfecting virus (Lam & Marshall, 1968b). Peleg (1975) infected *A. aegypti* mosquitoes with a small-plaque mutant of Sindbis virus and subsequently challenged with the wild-type strain of Sindbis. He found a slight decrease in the amount of the wild type virus in the mosquitoes; however, it was not transmitted to suckling mice.

In this paper we describe the complete suppression of the growth of a superinfecting homologous virus in *Culex annulirostris* mosquitoes.

Two strains of Semliki Forest virus were used in this study; the wild strain, SFV-W (strain 25639, Rockefeller Foundation Research Laboratory) and a virus isolated from persistently infected *Aedes albopictus* cells, SFV-S. This latter isolate was described by Davey & Dalgarno (1974). It is temperature sensitive, forming plaques in Vero cell monolayers at 34 but not at 37 °C; SFV-W forms plaques at both temperatures. SFV-S interferes with the growth of SFV-W in cultured *A. albopictus* cells (Regnery, 1976).

The mosquitoes used were the F₁ progeny of *C. annulirostris* captured at Griffith, N.S.W. Adults, 7 to 14 days old were infected intrathoracically with 0·3 ml borate-buffered saline, pH 9·0, containing 0·2 % bovine serum albumin and 10⁵ p.f.u./ml of virus. Mosquitoes were maintained at 28 °C until killed by freezing. Samples were assayed on Vero cell monolayers as previously described (Davey et al. 1973).

Fig. 1 shows the growth of SFV-W and SFV-S in separate groups of *C. annulirostris*. Both viruses attained the same titre in the insects and there was no significant decrease in virus concentration during the experiment. In the next experiment, groups of mosquitoes were infected either with SFV-S or with virus-free medium. After seven days, half of the individuals from each group were superinfected with SFV-W. The SFV-W grew normally in the
Fig. 1. Concentration of SFV-W and SFV-S in *Culex annulirostris* mosquitoes at different times after inoculation. Mosquitoes were inoculated intrathoracically with SFV-W (●) or SFV-S (○) in borate buffered saline, pH 9.0, containing 0.2% bovine serum albumin and maintained at 28 °C. At the times indicated, two mosquitoes were killed by freezing, sonicated in 1 ml phosphate buffered saline, pH 7.4, containing 30% calf serum and assayed at 37 °C, for SFV-W, or 34 °C, for SFV-S, on Vero cell monolayers (Davey & Dalgarno, 1974).

Fig. 2. Concentration of SFV-W in *Culex annulirostris* at different times after some had been infected with SFV-S. Mosquitoes were inoculated intrathoracically either with SFV-S or with buffered saline alone. After 7 days at 28 °C, mosquitoes were inoculated with SFV-W. Samples were taken and assayed at 37 °C as described in Fig. 1. ●, SFV-W growth in mock-infected *C. annulirostris*; ○, SFV-W growth in 7 day SFV-S infected *C. annulirostris*. ■, SFV-W growth after co-infection of *C. annulirostris* with a mixture of SFV-W and SFV-S.

Mosquitoes which had been inoculated with it alone, but no growth of SFV-W could be detected in mosquitoes injected first with SFV-S (Fig. 2). Assay of the superinfected mosquitoes at the permissive temperature for SFV-S, showed that the first virus was still present and at the same titre (2 × 10^5 p.f.u./mosquito) as in the non-superinfected mosquitoes.
Subsequent experiments showed that the superinfected SFV-W was totally excluded as early as 24 h after inoculation with SFV-S. However, in *C. annulirostris* inoculated with a mixture of SFV-W and SFV-S, both viruses grew as in the single infection (Fig. 2).

Thus, as has been found in cultured mosquito cells, infection of mosquitoes with SFV-S completely inhibits the growth of SFV-W inoculated 1 day or more later, whereas when injected at the same time, both viruses grow independently as if only one were present. This result is in contrast to the results of Peleg (1975) where the superinfecting virus replicated but attained a lower titre than normal. Possibly the mechanism of interference differs for different viruses or hosts.

The significance of these results to studies on the ecology of alphaviruses is uncertain because these viruses are usually isolated from wild mosquitoes using mammalian cells maintained at 37 °C or animals. Hence we do not know whether isolates like SFV-S occur naturally.

**REFERENCES**


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