Mosquitoes as carriers of viral diseases

James L. Hardy □ Edward J. Houk □ Laura D. Kramer □ Richard P. Meyer

Encephalitis (inflammation of the brain) can be caused by several arthropod-borne viruses, or arboviruses. Genetic alteration of field populations of mosquitoes, besides reducing vector (or carrier) populations, might serve to control encephalitis by making the mosquitoes less able to carry the arboviruses that cause this clinical disease. During the past six years, the Arbovirus Research Program at the University of California, Berkeley, has evaluated the feasibility of this approach.

We had observed that field populations of *Culex tarsalis*, the primary vector of western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) viruses during the summer in California, varied significantly in their ability to become infected with WEE virus. In the laboratory we were able to select strains of *Cx. tarsalis* that genetically were either highly resistant or highly susceptible to infection with WEE virus. These results supported the concept that the susceptibility of mosquitoes to infection with at least one arbovirus is a genetically inherited trait and suggested that insertion of genes for WEE viral resistance into field populations of *Cx. tarsalis* might make them less competent carriers of encephalitis viruses.

Before beginning work on this method of encephalitis control, we believed that it was necessary to understand the underlying biochemical or biophysical mechanisms that control vector competence (the ability of mosquitoes to become infected after ingesting an arbovirus and to subsequently transmit the virus by bite to various hosts, including human beings and horses). Further, we wanted to establish if variations in vector competence of different geographical populations of mosquitoes were correlated with differences in viral activity in these areas.

Barriers to infection/transmission

In a competent female mosquito, WEE virus is ingested with the blood meal from an infected bird or jackrabbit and then completes a series of multiplication cycles before it is transmitted in the saliva when the mosquito subsequently feeds on another host. Multiplication first occurs in the cells of the mosquito midgut. The newly produced virus then escapes from the midgut into the blood-containing cavity (hemocoelom), where it undergoes secondary multiplication in various cells and tissues. Finally, the virus invades the salivary glands and multiplies there.

In studies using both competent and incompetent mosquito species or strains, we have identified several barriers that prevent either the infection of the mosquito or the sequential spread of a virus in infected females. The first barrier to infection that confronts a virus is called the "midgut barrier" and determines if the virus can initiate infection in the mosquito. Incompetent females that have this barrier apparently lack sites for viral attachment to their midgut epithelial cells; these sites are required to initiate a viral infection. This type of barrier has been reported previously for other arboviruses and mosquito species, but its existence in genetic strains of the same mosquito species is a new observation.

Two other barriers may exist in infected female *Cx. tarsalis*: one prevents escape of an infective virus from the midgut; the other apparently prevents infection of the salivary glands. Finding these barrier systems in incompetent mosquitoes was a surprise, because it was formerly thought that any mosquito, once infected with an arbovirus, eventually would be able to transmit the virus by bite. However, these findings do explain why we found that up to 75 percent of experimentally infected females from field populations of *Cx. tarsalis* in California were unable to transmit virus after the normal 14-day incubation period. The purpose of current research is to elucidate the biochemical/biophysical mechanisms involved in these barrier systems.

Enzyme markers

The presence and absence of various enzymes have been used as phenotypic expressions or markers for genetic traits in insect populations. We have found enzymatic patterns of nonspecific esterases in the midguts of WEE viral-resistant mosquitoes that will differentiate them from susceptible mosquito species and strains. We plan to use this marker to determine if we can quickly evaluate the vector competence of field populations of mosquitoes for WEE virus. At this time we do not know if WEE viral susceptibility or resistance is directly related to these nonspecific esterases, since both markers could be controlled by separate genes that are closely linked.

Geographical differences

We have reported that different geographical populations of *Cx. tarsalis* vary in their susceptibility to infection with WEE and perhaps SLE viruses. Until recently, however, we had not attempted to correlate our indices for vector competence with levels of viral transmission or disease in areas where mosquitoes were collected for evaluation. This is because arboviral activity in our study areas in the California Central Valley has been low or nonexistent since we initiated our studies on vector competence seven years ago.

Since SLE viral activity was found every year of the decade in the Coachella and Imperial valleys of southern California, we began a study in 1978 to compare the vector competence for SLE virus of *Cx. tarsalis* and *Culex pipiens quinquefasciatus* populations in the Central, Coachella, and Imperial valleys. Results from the first year of study suggested that these populations of mosquito species differed in susceptibility to infection after ingestion of SLE virus.

In a follow-up study we plan to evaluate the ability of mosquitoes both to become infected and to transmit virus, because transmission might be a better measure of vector competence than is susceptibility to infection. We also are expanding these studies to include collections of *Cx. tarsalis* and *Cx. pipiens quinquefasciatus* or *Cx. pipiens* from Oregon, Utah, Minnesota, and Texas. The *Cx. pipiens* complex of mosquitoes serves as the primary vector of SLE virus in midwestern United States, whereas *Cx. tarsalis* is the primary vector in the western United States. We hope this study will allow us to conclude that the vector competence of mosquitoes for arboviruses is a critical determinant in the geographical distribution of diseases caused by these viruses.

James L. Hardy is Professor of Medical Virology, Department of Biomedical and Environmental Health Sciences; Edward J. Houk is Assistant Research Entomologist, Naval Biosciences Laboratory; and Laura D. Kramer is Post-doctoral Fellow, Naval Biosciences Laboratory. All are with the School of Public Health, University of California, Berkeley, CA 94720. Richard P. Meyer is Post-doctoral Fellow at the Arbovirus Field Station, School of Public Health, Bakersfield, CA.