The Biosystematics of *Culex (Melanoconion) taeniopus sensu lato* in relation to Venezuelan Equine Encephalomyelitis

by

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Species of the genus *Culex*, subgenus *Melanoconion*, are important components in the enzootic transmission cycle of Venezuelan equine encephalomyelitis (VEE) viruses. Three species in this subgenus have been shown to be natural vectors of these alphaviruses (see Cupp et al., 1979). Numerous isolations of VEE, eastern equine encephalomyelitis alphavirus (Galindo, 1978; Walder et al., 1984) and several group C bunyaviruses (Woodall, 1979) have also been made from other *Melanoconion* species. In epidemiological studies, it is crucial to make accurate identifications of field populations and relate distribution of potential vector species to relatively large geographic areas where virus transmission is ongoing. Therefore, a stable and functional nomenclature has been badly needed for this important subgenus.

Toward that end, Sirivanakarn (1982) reviewed and devised a preliminary classification of *Melanoconion* species. In it, he proposed taxonomic changes that incorporated previous suggestions made by Galindo (1969) concerning the "*Cx. spissipes*-group," a collection of species that contains several known or suspected VEE vectors. It also incorporated earlier changes that attempted to resolve problems of identity and synonymy of *Cx. taeniopus* Dyar and Knab and related species (Sirivanakarn and Belkin, 1980), a complex that occurs throughout northern South America, Middle America, the Caribbean and south Florida.

Of particular interest to the study of VEE ecology was the vector populations from southern Florida that were identified as *Cx. opisthopus* Komp by Pratt et al. (1945) and later renamed *Cx. cedecei* by Stone and Hair (1968), both of which were synonymized with *Cx. taeniopus* by Sirivanakarn and Belkin

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(1980). However, because a mixture of male characters had been incorrectly incorporated into the description of *Cx. taeniopus* by the earlier work by Dyar (1928), Sirivanakarn and Belkin (loc. cit.) discovered that only those mosquitoes fitting the description of the single holotype female should be designated as *Cx. taeniopus* and that those individuals formerly described under this designation but that also possess broad white femoral knee spots should be identified as *Cx. pedroi* Sirivanakarn and Belkin (1980).

To test the assumption of synonymy of the Florida population of *Cx. opisthopus* (= *Cx. cedecei*) with *Cx. taeniopus*, we have evaluated several biological characters of *Cx. taeniopus sensu lato*, using both colonized and wild-caught material from Florida and Guatemala, as well as compared published information by other investigators. The data include: (1) several morphological features: (2) genetic analysis consisting of hybridization and isoenzyme characterization; and (3) two important aspects of vector biology -- host selection and vectorial efficiency for several VEE virus subtypes.

Both populations were compared for several external characters that are used for the separation of several members of the *Cx. taeniopus* group. There were no significant differences between the male genitalia of either population nor were knee spots present. However, the hind tarsal markings of adults from Guatemala were well developed, whereas those on the Florida population are weakly developed or inconspicuous. Indeed, this character, along with slight variation in the male terminalia, led Stone and Hair (1968) to separate *Cx. cedecei* from *Cx. opisthopus* as a new species.

A cross-mating study suggested that the two colonized strains of *Cx. taeniopus* were different species (Dziem and Cupp, 1983). In homologous crosses, insemination rates were approximately 85 and 90%, but heterologous crosses revealed several interesting differences between the populations. There was a very low rate of insemination for the Florida female-Guatemala male cross, suggesting that precopulatory mechanisms were important. This was not the case for the Guatemala female-Florida male cross where a high rate of insemination coupled with a low rate of embryonation indicated that a postcopulatory, prezygotic isolating mechanism was involved. Of the 11,926 eggs obtained in this cross, 16 larvae hatched. Of these, only three survived beyond the second instar, ultimately producing one female and two male adults. Dissection and examination of the reproductive organs revealed normal development.

An isozyme study comparing 27 gene loci was conducted on females from both colonies. Six enzymes produced fixed, differently migrating allomorphs, i.e. it was possible to identify mosquitoes from each laboratory colony by any one of these six enzymes. For purposes of comparison, it is worth nothing that in another enzyme study of two closely related species of *Melanoconion* that had formerly been lumped under the designation of *Cx. aikenii*, species-specific allomorph differences for *Cx. ocossa* and *Cx. panocossa* were detected for seven enzymes (Kreutzer and Galindo, 1980).

These enzyme data were also used to calculate genetic identity and distance between the two populations. The proportion of genes that are identical in structure in these populations is between 0.777 and 0.704, and the number of allelic substitutions per 100 loci is between 25.4 and 35.1. In studies on *Drosophila* spp., values such as these have been reported for groups at the subspecies/incipient species level of evolutionary divergence (Ayala, 1975). When these data are considered with those from the hybridization and morphological studies, the conclusion that the two populations represent separate species seems warranted.

Several other biosystematic characters derived from the vector biology of the two populations also support this contention and relate the process of speciation to the evolution of vector competency. During the course of a four-year field study in an enzootic focus of VEE on the Pacific Coast of Guatemala, 915 bloodmeals from *Cx. taeniopus* were identified using an agar gel double-diffusion technique (Cupp et al., 1986). This information, when compared to that in a report on the blood-feeding habits of the Florida population in the Everglades (Edman, 1979), offers an interesting contrast. The latter population was found to be predominantly mammalophilic, with rodents being heavily utilized as hosts. Conversely, *Cx. taeniopus* in Guatemala is highly opportunistic, selecting not only mammals for blood-feeding, but also birds and occasionally reptiles. While this situation may reflect, in part, a more catholic feeding pattern due to a more diverse array of hosts in Guatemala than in Florida, a basic difference in host selection is strongly suggested.

A coevolutionary pattern of VEE virus susceptibility by the two populations can also be discerned by cross-infection studies. The vectorial efficiency of each mosquito population for a range of VEE viral subtypes has been evaluated and serves as a taxonomic character.

As a brief digression and for the purpose of general review, it should be noted that Venezuelan equine encephalomyelitis viruses exist as antigenic subtypes possessing distinctive serological characteristics based on haemagglutination inhibition assay. The subtypes also differ in their pathogenicity for humans and horses. Certain subtypes termed "enzootic" may be highly focalized in distinctive habitats that occur over a relatively wide geographic area. Others termed "epizootic" may occur sporadically, causing human epidemics and equine epizootics in northern South America and, rarely, in Middle America. Practically nothing is known of the origin and/or maintenance of these epidemic-epizootic forms. As an example, the source of the epizootic strains that appeared in Middle America and eventually intruded into Texas during the early 1970's is unknown. Indeed, no maintenance cycle for an epidemic-epizootic VEE subtype has ever been described. During outbreaks, these subtypes are transmitted by mosquito species belonging to several genera, particularly floodwater *Aedes*, *Psorophora* and occasionally *Mansonia*. Because of the bionomics of the vectors, epizootic virus sweeps through a population of

susceptible vertebrates in synchrony with rising mosquito populations. This contrasts sharply with the enzootic cycles of other VEE subtypes that are ecologically stable and use rodents, marsupials and possibly birds as amplifiers and *Culex (Melanoconion)* species as vectors.

As additional biological characters, the abilities of the two *Cx. taeniopus* populations to serve as vectors of VEE virus subtypes from Middle America and Florida were compared (Scherer et al., 1981; Weaver et al., 1986). The strain of virus subtype IE used is enzootic and was isolated in Guatemala from sentinel hamsters. The two strains of I-AB used are both epizootic and were isolated from humans during the 1971 outbreaks in Central America. Virus Subtype II, also called Everglades virus, is enzootic and was isolated in Florida from mosquitoes.

The two populations of *Cx. taeniopus* exhibited markedly differing abilities to become infected and transmit VEE viruses. The Guatemalan population was easily infected by and transmitted its sympatric subtype, IE. However, at lease 10^4 CEC pfu of the epizootic or Florida enzootic viruses were required to initiate infection of the midgut. In either case, no virus transmission occurred. As noted in an earlier study by Weaver et al. (1984), with mean bloodmeal titers of up to $10^{5.3}$ CEC pfu, only 20% or less of a *Cx. taeniopus* population from Guatemala became infected with epizootic VEE viruses, and virus replication was limited to the mesenteron. This mechanism of vector incompetency has been referred to as the midgut escape barrier (Kramer et al., 1981; Scherer et al., 1986).

The Florida population is susceptible to all three viral subtypes and does not possess any detectable barrier to infection (Weaver et al., 1986). This sharp difference in vectorial efficiency illustrates several interesting points. The Guatemalan population exhibits a specialized vector competency for subtype IE and an incompetence for the epizootic-epidemic forms of the virus that swept through that area in the early 1970's. This suggests that this species is not involved in the postepizootic survival of subtypes I-AB. In contrast, the Florida population is a generalist and accepts all three subtypes. Based on this composite of vector competence information, it is evident that the Florida population of *Cx. taeniopus* is biologically distinct from its Guatemalan counterpart and warrants a separate species status.

Based on taxonomic precedent, the name *Cx. cedecei* should be considered distinct from *Cx. taeniopus* and used for the Florida population. Further studies should be conducted for populations in the Caribbean Region. From an epidemiological perspective, it is also obvious that a systematic-biogeographical study of the *Cx. taeniopus* group can further our understanding of the evolution of virus vector competency, particularly in cases of incipient mosquito speciation. Such studies may also be useful in better defining the natural corridors of VEE virus movement and provide new ways to detect possible "silent cycles" of the epizootic-epidemic forms of this virus group.

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