Ecological Characterization of the Aquatic Habitats of Mosquitoes (Diptera: Culicidae) in Enzootic Foci of Venezuelan Equine Encephalitis Virus in Western Venezuela

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ABSTRACT We studied the aquatic mosquito habitats in and around enzootic foci of Venezuelan Equine Encephalitis virus (VEE) in western Venezuela. Specimens were sampled for 5 mo in three types of vegetation: tall lowland tropical forests, short inundated/secondary growth forests, and pastures/herbaceous vegetation around forests. Ground pools, flooded pastures, swamps, ponds, and canals predominated. We used a multivariate statistical approach to quantitatively assess the relationships of mosquito species with broad categories of the landscape, and with environmental variables within each aquatic habitat. Twenty-four mosquito species in the genera *Aedes*, *Psorophora*, *Culex*, *Mansonia*, and *Uranotaenia* were collected. Species richness was higher in the tall forests than in other types of vegetation. Discriminant Function Analysis showed a strong association between landscape category and mosquito species assemblage and identified *Culex erraticus* Dyar & Knab and *Mansonia titillans* Walker as indicator species of open areas, and *Aedes serratus* Theobald as an indicator of tall forests. *M. titillans*, *Uranotaenia geometrica* Theobald, *Cx. erraticus*, and *Culex dunni* Dyar were associated with unshaded, warm, vegetated waters in flooded pastures and swamps, whereas *Ae. serratus*, *Aedes fulvus* (Wiedemann), *Psorophora albipes* Theobald, *Psorophora ferox* (Humboldt), *Culex caudelli* Dyar & Knab, and *Culex pedroi* Sirivanakarn & Belkin were associated with small, shaded ground pools within the tall forests. *Culex coronator* Dyar & Knab was associated with partially exposed sites within short forests. These results allowed us to interpret better our previous studies on mosquito adult spread in the study area and their possible role as VEEV disseminators.

KEY WORDS mosquito ecology, spatial distribution, aquatic habitats, Venezuelan equine encephalitis vectors, Venezuela

VENEZUELAN EQUINE ENCEPHALITIS (VEE) is a reemerging arboviral disease (Togaviridae: *Alphavirus*) that has caused repeated epizoodemics in the Americas (Walton and Grayson 1988, Weaver et al. 1996, Rivas et al. 1997, Weaver 1998). The VEE complex includes two main phenotypes: epizootic or equine-virulent varieties (IAB and IC), which have been isolated only during epizootics; and enzootic varieties (ID, IE, IF, II, IIIA-D, IV, V, and VI) that circulate in swamps and riparian and lowland tropical forests throughout the Americas (Weaver et al. 2000, 2004). Epizootic virus variants descend from enzootic strains occurring in northern South America (Weaver et al. 1992); however, the mechanisms for the evolution of these strains are not completely understood. Epizootic and enzootic cycles differ in a variety of components, such as their main hosts and vectors, and they generally do not overlap spatially. Epizootic foci are usually open areas devoted to cattle ranching and/or associated with extensive wetlands. Open areas around enzootic foci may act as natural vaccination belts for nearby equines (Chamberlain 1972, Groot 1972) because enzootic viruses immunize equines against epizootic VEEV (Wang et al. 2001). This raises the question of how epizootic strains that may be generated via mutation in enzootic foci are transported across this "halo" of equine immunity.

Comparative ecological studies of VEEV enzootic foci in Venezuela and Colombia revealed the presence of a greater genetic diversity of subtype ID strains in the Venezuelan foci (Moncayo et al. 2001), which was associated with greater forest fragmentation, smaller stands, larger interpatch distances, and augmentation of the forest perimeter (Barrera et al. 2001, 2002). Increased ecotone forest/open areas may facilitate virus exchange between open-area mosquitoes and infected enzootic hosts. Among the possible mechanisms of emergence of epizootic viruses is their transport by alternate mosquito vectors to distant locations with susceptible epizootic hosts and vectors. Epizootic transmission could follow the movement of epizootic variants generated in enzootic hosts or via the move-

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ment of epizootic precursors (before mutation) followed by mutation and selection in the epizootic vector species or in susceptible equine amplification hosts that reside in open areas (Mendez et al. 2001).

Likely mosquito vectors capable of virus export from the enzootic foci are those that are found in large numbers in both the open areas and in the forest, have a wide range of vertebrate hosts, can disperse over long distances, and have vectorial competence for VEEV. Investigations of adult mosquitoes along transects from open areas into nearby forests in enzootic foci in western and northern Venezuela, and in the middle Magdalena Valley of Colombia (Mendez et al. 2001, Salas et al. 2001, Barrera et al. 2002), indicated very little adult mosquito activity during the day outside the forests; this implied that any movement of viruses should occur at other times. Mosquito species seeming to fulfill the abovementioned conditions included *Culex nigripalpus* Theobald, *Aedes scapularis* (Rondani), *Aedes angustivittatus* Dyar & Knab, *Psorophora cingulata* (F.), and *Mansonia titillans* (Walker). Several species, including the principal enzootic vectors of enzootic subtype ID VEEV (Walder et al. 1984, Ferro et al. 2003), were sylvan mosquitoes with very little movement outside the forests [e.g., *Aedes serratus* (Theobald), *Aedes fulvus* (Wiedmann), *Psorophora ferox* (Humboldt), *Psorophora albipes* (Theobald), *Culex spissipes* (Theobald), *Culex pedroi* Sirivanakarn & Belkin, *Culex vomerifer* Komp, *Culex adamesi* Sirivanakarn & Galindo, *Culex crybda* Dyar, *Culex dunni* Dyar, and several Sabethini species]. Thus, strictly sylvan mosquitoes would be mainly involved in the maintenance of the VEEV enzootic cycle, whereas the open-area mosquitoes could potentially move the virus away from the enzootic foci.

To understand better the spatial spread of adult mosquitoes and their possible role as virus exporters from enzootic foci (lowland tropical forests), we studied what aquatic habitats were being used by mosquitoes and their location in the main types of vegetation in and around enzootic foci of ID VEEV in Zulia State, western Venezuela. The results of this study are discussed in conjunction with our previous observations on adult mosquito distribution in the study area (Barrera et al. 2001, 2002; Mendez et al. 2001). We used a multivariate statistical approach to quantitatively assess the relationships of mosquito species with broad categories of the landscape (open areas, tall and short lowland forests) and with environmental variables within each aquatic habitat.

Materials and Methods

Study Site. The aquatic habitats of immature mosquitoes were located and sampled in forests and open pastures next to forests on the tropical plains of the Catatumbo River, Zulia State, western Venezuela (40–70-m elevation). The study area has been described in detail previously (Barrera et al. 2001). Tall lowland tropical forests have been cleared for cattle ranching, African oil palm cultivation, and oil extraction. The main landscape elements, as detected by remote sensing (1996) and ground proofing (Barrera et al. 2001), were pasture (41%), tall primary forest (24%), tall logged forest (10%), palm/narrow-leaf tree stand (9%), short forest (8%), bare/urban/eroded soil (5%), and water (3%). Mean annual temperature and rainfall in the area were 27.2°C (1978–1984) and 2,991 mm (1978–1996), respectively. There is a short dry season from December to March and two main rainy seasons (April–May and October–December). This region suffers frequent floods due to high precipitation, low slopes ($<0.05\%$), and poor drainage. Reticular soil erosion produces small canals and pools of varying dimensions on the ground (locally called *s.* zuro, *pl.* zuros) that fill with water during the rainy season and become the main mosquito larval habitats. In the cleared or open areas, other mosquito habitats such as flooded pastures, lagoons, and swamps predominated. Wetlands supported a variety of aquatic plants, including emergent (e.g., *Thalia geniculata* L., *Heliconia marginata* (Griges) Pittier, *Cyperus* sp. L., and *Echinochloa* sp. P. Beauv.), submerged (*Eleocharis* sp. R. Br. and *Utricularia* sp. L.), and floating (*Pistia* sp. L., *Ludwigia* sp. L., and *Eichornia* sp. A. Rich.) plants.

Mosquito Sampling. To study the spatial distribution of mosquito aquatic habitats and species, we sampled 235 water bodies in 17 localities, including seven tall forests (TF), two short forests (SF), and eight surrounding open areas (OA). Names and locations of the tall forests were Doña Bertha I (9° 4′ 10″ N–72° 34′ 6″ W), El Bajo (9° 1′ 40″ N–72° 37′ 32″ W), Las Nubes (9° 3′ 51″ N–72° 36′ 59″ W), Los Angeles (8° 42′ 5″ N–72° 34′ 14″W), Madre Vieja I (9° 8′ 8″ N–72° 40′ 48″ W), Madre Vieja II (9° 8′ 16″ N–72° 40′ 30″ W), and Río Claro (9° 0′ 53″ N–72° 41′ 56″ W). Short forests were located at: Doña Bertha II (9° 3′ 58″ N–72° 33′ 2″ W), and Playa Bonita (9° 2′ 3″ N–72° 32′ 53″ W).

Sampling sites were selected to obtain replicates of each of the main breeding sites for each habitat category (Table 1). Larvae were sampled from each site with standard (300 or 500 ml) mosquito dippers during May, June, July, September, and October 1998, and the number of larvae per dip was calculated from 30 samples for each breeding site. Samples were always taken carefully by the same individuals, dragging the dipper over the water surface for 30–50 cm. Third and fourth instars and pupae were removed from the dipper, transported to the laboratory in individual vials, and reared to the adult stage; the associated larval and pupal exuviae were used for identification by using the characters described by Lane (1953), Bram (1967) and Sirivanakarn (1983). Results were expressed as mean number of individuals per liter of sample. Vouchers of all examined species were deposited in the Collection of Laboratorio de Biología de Vectores (LBV), Instituto de Zoología Tropical, Universidad Central de Venezuela, Caracas, Venezuela.

Environmental Variables. Seven environmental variables were characterized in each breeding site: 1) water surface; 2) exposure (fully exposed, partially exposed, and shaded); 3) type of aquatic vegetation (emergent, floating, and submerged); 4) total cover of aquatic vegetation (mean from six replicates, 0.5- by 0.5-m quadrats, Braun-Blanquet method; Mueller-Dumbois and Ellenberg 1974); 5) mean water depth

Table 1. Environmental characteristics (mean ± SE) of mosquito breeding sites sampled in and around enzootic VEEV foci in the Catatumbo River Basin, Zulia State, western Venezuela, from May to Oct. 1998.

Habitat type/breeding site	Open area					Short forest		Tall forest	
	Ground pool	Flooded pasture	Swamp	Lagoon	Canals	Ground pool	Swamp	Ground pool	Swamp
n (samples)	5	38	10	6	2	6	10	112	7
Water surface	L	L	L	L	L	L	L	S	L
Type of aquatic vegetation	F	F, E, S	E	F, E, S	F, E, S	A	A	A	A
Canopy	A	A	A	A	A	C	P	C	C
Aquatic vegetation cover (%)	68 ± 10	76 ± 3	67 ± 7	51 ± 6	87 ± 7				
Depth (cm)	16 ± 3	31 ± 4	20 ± 5	28 ± 4	39 ± 15	13 ± 2	10 ± 1	27 ± 2	18 ± 4
Temp (°C)	28 ± 1	31 ± 1	31 ± 1	31 ± 1	26 ± 6	27 ± 1	27 ± 1	27 ± 1	27 ± 1
Conductivity (mOhm/cm^2)	32 ± 15	89 ± 17	69 ± 12	31 ± 10	38 ± 27	62 ± 6	206 ± 11	207 ± 28	3063 ± 518

Water surface: L, large (>12 m^2); M, medium (6.1–12m^2); S, small (0.1–6.0 m^2); type of aquatic vegetation: A, absent; E, emergent; F, floating; S, submerged; and canopy: A, absent; P, partial; and C, complete.

(six readings); 6) mean water temperature (six readings); and 7) mean water conductivity (six readings). Portable field meters were used for conductivity and water temperature (model 33, Yellow Springs Instrument Co., Inc., Yellow Springs, OH).

Data Analysis. The $\log_{10}(x)$ transformation (species abundance, water depth, temperature, and conductivity) and angular transformation (vegetation cover) were applied before statistical analyses to normalize the data (Legendre and Legendre 1998). The type of aquatic vegetation and habitat, and the relative size of the breeding place were coded as dummy variables. Species with very low abundance (<5% of samples) were excluded from the analyses.

Discriminant function analysis (DFA) was used to examine the correspondence between habitat category (e.g., tall forest, short forest, and open area) and mosquito assemblages. Here, we addressed the question of whether each habitat category supported a distinct mosquito community, with this analysis being a measure of the similarity of the mosquito fauna within each habitat category, and the divergence of the mosquito community among habitats. Thus, the within-habitat variance–covariance matrices were assumed to be homogeneous among habitats. The standardized coefficients for discriminant functions were used to determine the contribution of each mosquito species to habitat separation (Legendre and Legendre 1998). By plotting the DFA scores for the first two discriminant functions, we examined the spread of samples among habitats and the result of the classification. We used the Statistical Package for the Social Sciences for Windows program, version 10 (1999) (SPSS Inc., Chicago, IL) to run the analyses.

Canonical correspondence analysis (CCA; ter Braak and Smilauer 1998) was used to determine the amount of variation in the mosquito data that could be explained by various sets of variables (e.g., breeding site conditions). CCA is an ordination technique, the results of which are based on species abundance and values of environmental variables simultaneously. In the CCA, axes are constrained to optimize their relationship with a set of environmental variables, the direction of which can be indicated in the ordination diagram by arrows with lengths proportional to their importance. Statistical validity of resulting environmental axes, the model, and the selected environmental variables explaining the variation of species abundance were evaluated by means of unrestricted Monte Carlo permutation tests. The computer program CANOCO 4 (ter Braak and Smilauer 1998) was used for this analysis.

Results

Habitat Characterization, Mosquito Species Richness, and Spatial Distribution. Breeding sites in open areas had more vegetation and lower conductivity and were deeper, warmer, and more exposed than those located in the forests (Table 1). In total, 3,424 individuals from 24 mosquito species were collected during the sampling period. They belonged to the genera *Aedes, Anopheles, Culex, Mansonia, Psorophora,* and *Uranotaenia* (Table 2). The most common species were *Psorophora ferox* (Humboldt), *Aedes serratus*

Table 2. Relative abundance (%) of mosquito species per type of vegetation and percentage of total numbers collected in the Catatumbo River Basin, Zulia State, western Venezuela, from May to Oct. 1998

Species	Habitat type			
	Open area	Short forest	Tall forest	% total no. collected
Ae. (*Ochlerotatus*) *fulvus*	0	0	100	7.5
Ae. (*Och.*) *scapularis*	0	0	100	<0.1
Ae. (*Och.*) *hortator*	0	0	100	0.1
Ae. (*Och.*) *serratus*	0	0	100	24.6
An. (*Nys.*) *marajoara*	100	0	0	<0.1
Cx. (*Culex*) *coronator*	23.8	76.2	0	0.7
Cx. (*Cux.*) *habilitator*	0	27.3	72.7	0.7
Cx. (*Cux.*) *mollis*	3.0	14.2	82.8	4.3
Cx. (*Melanoconion*) *albinensis*	100	0	0	0.1
Cx. (*Mel.*) *caudelli*	1.1	0	98.9	11.8
Cx. (*Mel.*) *conspirator*	0	0	100	2.2
Cx. (*Mel.*) *distinguendus*	0.8	7.3	91.9	3.9
Cx. (*Mel.*) *dunni*	58.3	0	41.7	0.8
Cx. (*Mel.*) *erraticus*	74.2	0	25.8	1.0
Cx. (*Mel.*) *intrincatus*	11.3	88.7	0	2.0
Cx. (*Mel.*) *pedroi*	0	11.3	88.7	2.3
Ma. (*Mansonia*) *titillans*	100	0	0	2.9
Ps. (*Grabhamia*) *cingulata*	38.5	0	61.5	0.4
Ps. (*Jantinosoma*) *albipes*	0	0	100	3.0
Ps. (*Jan.*) *ferox*	0.3	0	99.7	30.2
Ps. (*Jan.*) *cyanescens*	0	0	100	0.1
Ps. (*Psorophora*) *cilipes*	0	0	100	0.4
Ps. (*Pso.*) *lineata*	0	0	100	0.3
Ur. (*Uranotaenia*) *geometrica*	59.3	0	40.7	0.9

(Theobald), *Culex caudelli* Dyar & Knab, and *Aedes fulvus* (Wiedemann). Species richness in the forests (19 species; 11 species restricted to forests) was higher than in the open areas (13 species; three species restricted to open areas), and reached values in the tall forest habitat (19 species; nine species restricted to tall forests) greater than in the short forest category (six species; none endemic to short forests; Table 3). *Culex mollis* Dyar & Knab and *Culex distinguendus* Dyar occurred in every habitat and a total 10 species were collected in both forest and open areas.

The spatial patterns of mosquito species were further explored by the discriminant function analysis (Fig. 1). This analysis showed that most samples could be correctly assigned either to open areas or tall forest habitats on the basis of the 14 most common mosquito species. The Wilks lambda statistic was significant ($P < 0.05$) and the overall success of habitat separation was 86.6%, with the DFA plot showing relative homogeneity within groups (Fig. 1). Standardized coefficients for the first and second discriminant functions indicated three main, distinct groups of mosquito assemblages. Two of the groups were separated by the first discriminant function, and the third group was classified by the second discriminant function. Along the first function, a group of species was classified according to their association with the open area [*Mansonia titillans* (Walker), *Culex erraticus* Dyar & Knab, and *Uranotaenia geometrica* Theobald]. The second group of mosquito species discriminated along this first function showed a positive relationship with the tall forest habitat category [*Ps. ferox*, *Ae. serratus*, *Cx. caudeli*, *Psorophora albipes* (Theobald), *Culex pedroi* Sirivanakarn & Belkin, and *Ae. fulvus*]. The species contributing more to habitat separation on this first discriminant function were *Cx. erraticus* and *Ma. titillans*, as indicator species of open areas, and *Ae. serratus* as an indicator species of tall forests. The final group classified by the second discriminant function was *Culex coronator* Dyar & Knab, *Culex mollis* Dyar & Knab, and *Cx. distinguendus.* These species were distributed in open areas as well as in short and tall forests. The spatial distribution of *Culex dunni* Dyar was poorly described by this analysis.

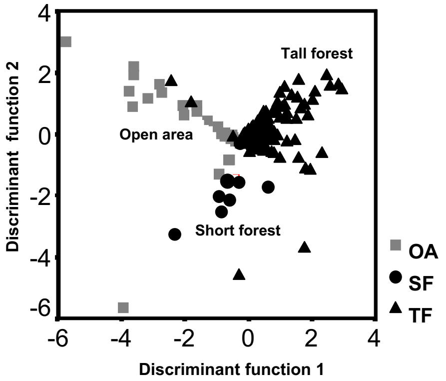

Fig. 1. Plot of the first two discriminant functions showing individual sample scores based on the mosquito species composition for each of the major vegetation types in the Catatumbo River Basin, Zulia State, Venezuela, from May to October 1998. Separation of samples in three groups reflects the success of the discriminant function analysis (86.6% correct classification).

Table 3. Mean density of mosquito species (× 100; larvae per liter) and SE in each type of aquatic habitat in the Catatumbo River Basin, Zulia State, western Venezuela (May –October 1998)

Breeding sites/species	Habitat type						
	Open area					Forest	
	Ground pool ($n = 3$)	Flooded pasture ($n = 25$)	Swamp ($n = 11$)	Lagoon ($n = 8$)	Canal ($n = 2$)	Ground pool ($n = 127$)	Swamp ($n = 17$)
Ae. fulvus	0	0	0	0	0	53.3 (6.3)	0
Ae. hortator	0	0	0	0	0	17.8 (−)[a]	0
Ae. scapularis	0	0	0	0	0	8.9 (−)	0
Ae. serratus	0	0	0	0	0	65.4 (11.1)	53.3 (−)
An. marajoara	0	2.2 (−)	0	0	0	0	0
Cx. albinensis	0	0	0	0	6.7 (−)	0	0
Cx. caudelli	0	7.8 (1.4)	0	0	0	46.0 (3.7)	0
Cx. conspirator	0	0	0	0	0	77.8 (2.5)	0
Cx. coronator	6.7 (−)	17.8 (−)	4.4 (5.3)	0	0	0	17.8 (4.4)
Cx. distinguendus	0	0	4.4 (−)	0	0	48 (5.3)	20 (5.3)
Cx. dunni	4.4 (−)	7.4 (0.4)	4.4 (−)	7.8 (2.5)	0	22.2 (−)	0
Cx. erraticus	0	10 (1.9)	11.8 (1.6)	8.8 (1.9)	0	17.8 (−)	0
Cx. habilitator	0	0	0	0	0	24.4 (0.6)	0
Cx. intrincatus	0	31.1 (−)	0	0	0	244.4 (−)	0
Cx. mollis	0	0	17.8 (−)	0	0	41.7 (4.4)	16.9 (2.4)
Cx. pedroi	0	0	0	0	0	26.4 (2.2)	17.8 (1.5)
Ma. titillans	11.7 (4.2)	20.6 (3.0)	8.9 (−)	4.2 (0.09)	6.7 (−)	0	0
Ps. albipes	0	0	0	0	0	19.7 (1.8)	0
Ps. cilipes	0	0	0	0	0	18.9 (0.4)	0
Ps. cingulata	0	22.2 (−)	0	0	0	11.1 (1.1)	0
Ps. ferox	13.3 (−)	0	0	0	0	60.2 (6.6)	0
Ps. lineata	0	0	0	0	0	5.5 (0.2)	0
Ur. geometrica	7.2 (1.5)	5.8 (1.2)	0	4.4 (−)	0	12.2 (−)	0

[a] −, species was collected only in one sample.

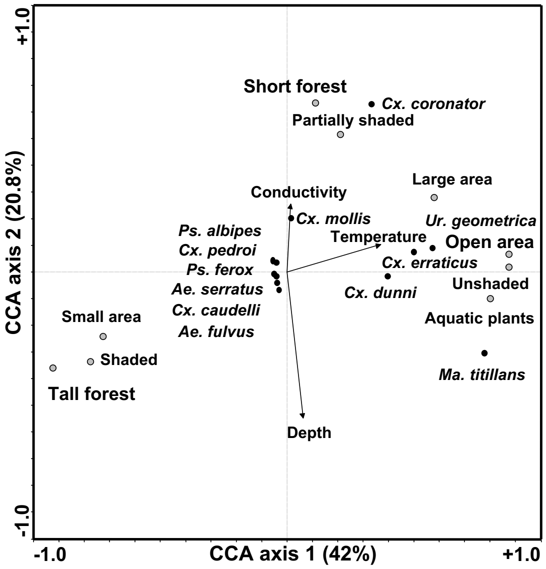

Fig. 2. Ordination by canonical correspondence analysis of mosquito abundance (dark dots) and environmental variables ($P < 0.05$) of aquatic habitats in the Catatumbo River basin, Zulia state, Venezuela (May–October 1998). The percentage of total variation in mosquito abundance accounted for by each canonical axis is reported in parentheses on each axis. Location of a species on the biplot represents its optimum. Continuous environmental variables are represented by arrows (water temperature, conductivity, and depth), and ordinal variables by gray dots (type of terrestrial vegetation, water shading, surface, and presence of aquatic vegetation). Perpendicular projection from a species' centroid to an environmental factor's arrow depicts its degree of association with such factor. Proximity of a species' centroid to an ordinal variable's gray dot denotes association.

Forest ground pools (zuros) showed a greater species richness than the other larval habitats. Most of the mosquito species collected within the forests came from this type of aquatic habitat. The most abundant species collected in ground pools, in their order of abundance, were *Culex intrincatus* Brèthes, *Culex conspirator* Dyar & Knab, *Ae. serratus*, *Ps. ferox*, *Ae. fulvus*, *Cx. distinguendus*, *Cx. caudelli*, and *Cx. mollis.* Mosquito species richness in open areas was greater in swamps and flooded pastures than in ground pools, lagoons, and blind rivers. *Ma. titillans*, *Ur. geometrica*, *Cx. erraticus*, and *Cx. dunni* were the most abundant species in these water bodies.

Spatial Abundance and Associated Habitat Factors. There was a significant relationship between mosquito abundance and habitat variables (CCA analysis: $F = 4.896$, $P < 0.05$; Fig. 2), with the first two axes accounting for 62.8% of the explained species–environment relationship, and a strong species–environment correlations ($r = 0.93$ and 0.70 for axes 1 and 2, respectively). Four mosquito species dominated the ordination: *Ma. titillans*, *Cx. erraticus*, *Ur. geometrica*, and *Cx. dunni* (Fig. 2). The first CCA axis reflected the difference in mosquito species composition along the two broad habitat categories (open area versus forest), as well as the following ecological gradients: microhabitat heterogeneity (e.g., reflected by the presence of aquatic vegetation) and physical features of the breeding site (e.g., degree of sun exposure and accordingly, water temperature). Consequently, two distinct mosquito assemblages were clearly identified along this axis. *Ma. titillans*, *Ur. geometrica*, *Cx. erraticus*, and *Cx. dunni* were positively associated with the open-area habitat, unshaded and warm waters, and the presence of macrophytes. In the opposite direction on the first CCA axis, the distribution of *Ae. serratus*, *Ae. fulvus*, *Ps. albipes*, *Ps. ferox*, *Cx. caudelli*, and *Cx. pedroi* was positively linked to the shaded and small water bodies (zuros) of the tall forest habitat. Finally, the second ordination axis, largely a measure of water depth and conductivity, separated *Cx. coronator*, which was associated to the partially shaded sites of the short forest habitat; whereas *Cx. mollis* was linked with shallow and high water conductivity sites (swamps).

Discussion

Mosquitoes and Enzootic Landscape. The distribution and abundance of mosquitoes in any given location are influenced by variables operating across spatial and temporal scales. In our study at the landscape level, the discriminant function analysis (Fig. 1) revealed the existence of groups of mosquito species that were useful in characterizing the tall forests and open areas around them. Separation of samples in discriminant space from open areas and tall forests (along the abscissa in Fig. 1) was due to the existence of species only found in each of those habitats. For example, *Ma. titillans* was found only in permanent, exposed water bodies with aquatic vegetation, where their immatures attach their siphons to the submerged roots of plants for air exchange (Clements 1999). The results also showed that a greater richness of mosquito species was undergoing immature development in the tall forests than in their surrounding open areas, despite the existence of a greater variety of aquatic habitats in open areas (flooded pastures, lagoons, swamps, ground pools, and canals; Table 1). Tall forests contained the largest number of mosquito species that were restricted to this type of habitat, both as immatures and adults (see below).

Because of habitat specificity, the current landscape configuration partly determines the composition and abundance of the mosquito species at this regional scale. Changes in the main components of the landscape, such as further conversion of forest stands into pasturelands, are expected to modify the fauna and the role of surviving mosquito species as vectors of pathogens. The greater genetic diversity of VEEV subtype ID strains that has been reported for this area (Moncayo et al. 2001), compared with that of a Colombian study site 200 km away, probably results from a greater forest fragmentation, smaller stands, larger interpatch distances, and augmentation of the forest perimeter (Barrera et al. 2001, 2002).

Local Gradient Forest–Open Areas. The results of the canonical correspondence analysis were useful at ordering immature mosquito species along environmental gradients (Fig. 2). This technique also showed discernible separation of aquatic habitats and their associated mosquitoes in relation to their terrestrial habitats (open areas and short and tall forests). The best separation of species along the environmental gradients was achieved for the open-area mosquitoes (greater spacing of species in Fig. 2), meaning that observed habitat variables could explain a larger portion of variation in the abundance of those species. Therefore, although forest and nonforest mosquitoes were clearly separated by the set of environmental variables used, they provided few clues as to what variables determined the abundance of immature mosquitoes within tall forests. Most of the aquatic habitats in the forests were ground pools and swamps. The forests in the Catatumbo River basin are characteristic because of their reticular erosion, which produces ground pools and canals of varying sizes that can cover over one-half the surface of the forest floor. The dynamics of these aquatic habitats has not been studied in any detail. However, we anticipate that in addition to the importance of the spatial dimension, the temporal patterns in occupancy or species succession (temporal, nonseasonal replacement of species) may explain to some degree the large number of mosquito species observed in that habitat. Several of the abundant mosquito species found in aquatic forest habitats (*Aedes* and *Psorophora* species) are known to produce eggs resistant to desiccation, and their seasonal numbers likely respond to the pluvial regime (Barrera et al. 2001). Conversely, many *Culex* species probably use more stable aquatic habitats. However, many of the aquatic habitats found in open areas were rather large and likely permanent, as indicated by the presence of aquatic vegetation. The temporal component of aquatic habitats in open areas is probably less important, and local variations in shading, aquatic plants, and predators could be the ecological factors driving this mosquito community.

Patterns of Mosquito Distribution. Knowledge of the immature aquatic habitats of mosquitoes in this area allows us to integrate those results with our previous studies on the spatial distribution of adult mosquitoes (Mendez et al. 2001, Barrera et al. 2002). 1) Few adult specimens were attracted to CO_2-baited CDC miniature light traps in open areas during the day. 2) Most adult specimens captured in open areas at night belonged to species that develop in open areas (*Ma. titillans*), or in both habitats (*Ps. cingulata* and *Cx. mollis*). 3) Adult mosquitoes that develop in open areas (*Ma. titillans*) or in both areas (*Ps. cingulata, Cx. mollis,* and *Cx. dunni*) were captured inside the forest. 4) Species that develop only inside the forest are captured in low numbers as adults at night in the open (*Ae. serratus, Ae. scapularis, Ps. albipes, Cx. pedroi,* and *Cx. spissipes*). 5) Some species that develop only in the forest do not leave it as adults (*Ae. fulvus* and *Ps. ferox*). 6) A group of species found in breeding places in the study area do not seem to be attracted to the CO_2-baited CDC miniature light traps [*Cx. coronator, Cx. habilitator, Cx. albinensis, Cx. conspirator, Cx. erraticus, Cx. intrincatus, Psorophora cyanescens* (Coquillett)], and therefore their spatial distribution was not completely ascertained. We did not encounter the aquatic habitats of several mosquito species that occurred in previous adult collections in the study area [*Anopheles mattogrossensis* Lutz & Neiva, *Anopheles rangeli* Gabaldon, Cova-Garcia & Lopez, *Anopheles nuneztovari* Gabaldon, *Coquillettidia juxtamansonia* (Chagas), *Coquillettidia nigricans* (Coquillett), *Cx. nigripalpus, Cx. ocossa, Cx. spissipes,* and *Mansonia pseudotitillans* (Theobald)]. It is noteworthy that *Cx. nigripalpus* was very abundant in adult traps in both open areas and forests at night (Mendez et al. 2001, Salas et al. 2001, Barrera et al. 2002); its breeding places are more likely to be found in the open areas (Forattini 1965). Another important species whose breeding places were not found was *Cx. spissipes.* This species (reported in error as *ferreri;* Walder et al. 1984) was found infected with enzootic ID VEE virus in the study area.

Our results suggest that some adult mosquitoes reside part of the time inside the forests, regardless of where they underwent immature development. Therefore, there is a possibility that some species could become infected and transport enzootic VEEV to other forests and open areas where they could transmit the viruses to a variety of hosts, including equines and humans. Assuming that some of these infected mosquitoes could fly up to 3 km from any forest patch (Edman and Bidlingmayer 1969, Morris et al. 1991), it has been shown that they could cover 97% of the total land area in the study area (Barrera et al. 2001). If the protective equine immunity halo hypothesis holds true, then most equines within the study area should be naturally vaccinated, or if some epizootic variants reach nonimmunes, herd immunity would prevent an outbreak. However, if other hosts, particularly birds or bats, which can cover large distances, are infected with enzootic VEEV or epizootic precursors and transport these viruses to areas with susceptible, unvaccinated equines, then it would be feasible for an outbreak to occur. An alternate or concurrent mechanism for virus export from enzootic foci would be through the bites of infected mosquitoes on highly mobile, susceptible vertebrates within the enzootic foci. However, there is little evidence for the involvement of alternate hosts in the emergence of VEEV epizootics, perhaps because very few studies have been conducted between epizootics.

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