

## MOSQUITO VECTOR CONTROL AND BIOLOGY IN LATIN AMERICA—A TENTH SYMPOSIUM

GARY G. CLARK,<sup>1</sup> HUMBERTO QUIROZ MARTÍNEZ<sup>2</sup> AND YADIRA N. RANGEL<sup>3</sup>  
(ORGANIZERS)

**ABSTRACT.** The 10th annual Latin American symposium presented by the American Mosquito Control Association (AMCA) was held as part of the 66th Annual Meeting in Atlantic City, NJ, in March 2000. The principal objective, as for the previous 9 symposia, was to promote participation in the AMCA by vector control specialists, public health workers, and academicians from Latin America. This publication includes summaries of 57 presentations that were given orally in Spanish or presented as posters by participants from 9 countries in Latin America. Topics addressed in the symposium included results from chemical and biological control programs and studies; studies of insecticide resistance; and molecular, ecological, and behavioral studies of vectors of dengue (*Aedes aegypti*), malaria (*Anopheles albimanus* and *Anopheles aquasalis*), leishmaniasis (*Lutzomyia*), and Chagas' disease (*Triatoma*). Related topics included biology and control of scorpions and *Chironomus plumosus*.

**KEY WORDS** Mosquitoes, mosquito control, *Aedes*, *Anopheles*, *Culex*, *Lutzomyia*, *Triatoma*, scorpions, *Chironomus* resistance

### INTRODUCTION

The American Mosquito Control Association (AMCA) is dedicated to the study and control of mosquitoes and other vectors and promotes cooperation and interaction among professionals and students in this field both in the USA and internationally. To promote greater involvement of one segment of the international membership, a Spanish language symposium was held at the AMCA Annual Meeting in 1991 and symposia have been held at subsequent meetings. In addition to providing a forum for scientists whose primary language is Spanish, the session promoted interaction and esprit de corps within this group, interaction with control industry representatives, and interaction with professional colleagues in the USA who are involved in mosquito vector control, training, and research at the university level, and with state and federal government officials.

This publication includes summaries of 57 presentations that were given in Spanish by participants from 9 countries in Latin America. Topics addressed in the symposium included results from chemical and biological control programs and studies, studies of insecticide resistance, and molecular, ecological, and behavioral studies of scorpions and *Chironomus*, vectors of dengue (*Aedes aegypti* (L.)), malaria (*Anopheles albimanus* Wiedemann and *Anopheles aquasalis* Curry), leishmaniasis (*Lutzomyia*), and Chagas' disease (*Triatoma*). Summaries of 8 previous symposia have been published

(Clark and Suarez 1991, 1992, 1993; Clark 1995, 1996; Clark and Rangel 1997, 1998, 1999).

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### SUMMARIES

#### Integrating vector control with the World Health Organization's healthy cities and primary environmental care strategies

Steven K. Ault

*Pan American Health Organization (PAHO),  
World Health Organization (WHO), Brasilia,  
Brazil*

In developing countries, vector control programs need to leverage their scarce resources with environmental and public health programs at the national, state, and municipal levels in order to optimize surveillance, prevention, and control of vectors. Dozens of cities worldwide are participating in the World Health Organization's (WHO's) Healthy Cities program, which addresses local environmental and public health needs in a community-based manner. As well, WHO's Primary Environmental Care strategy places emphasis on managing local environmental health problems in both rural and urban areas. These programs are described with examples, and examined as to how they have been and can be linked to vector control

<sup>1</sup> Dengue Branch, Centers for Disease Control and Prevention, 1324 Calle Cañada, San Juan, PR 00920-3860.

<sup>2</sup> Laboratorio de Entomología, Facultad Ciencias Biológicas, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, México.

<sup>3</sup> Laboratorio de Biología de Vectores, Instituto de Zoología Tropical, Universidad Central de Venezuela, Caracas, Venezuela.

programs. Special emphasis is given to integrating control of dengue and malaria vectors in the local Healthy Cities program (e.g., potable water, sanitation, drainage, and solid waste management interventions) and a Primary Environmental Care strategy (e.g., use of local environmental health indicators and community participation in prevention, surveillance, control, and evaluation).

### **The dengue situation in the Americas with special reference to the Brazilian Control Program**

Christian Frederickson

*Communicable Disease Control Program, Pan American Health Organization (PAHO), World Health Organization (WHO), Brasilia, Brazil*

Dengue and its severe form, dengue hemorrhagic fever, is the most important and rapidly spreading arbovirus infection in the Americas. The number of cases reported in countries from Mexico to Brazil have increased dramatically, as have the number of reported deaths. The response of affected or threatened countries has ranged from limited action to the reinitiation of an *Aedes aegypti* eradication program. The dengue epidemiologic situation in Latin America for the past 5 years is reviewed. More than 70% of the cases in 1998 have been reported from Brazil. To combat this, Brazil launched an aggressive and massive eradication campaign. Details of the control efforts are examined and the results are critically evaluated.

### **Spatial and temporal analysis of dengue cases in the metropolitan area of Monterrey, Nuevo León, México, during 1998**

Saúl Lozano-Fuentes, Roberto Mercado-Hernández and Ildelfonso Fernández-Salas

*Universidad Autónoma de Nuevo León (UANL), Facultad de Ciencias Biológicas, Laboratorio de Entomología Médica, San Nicolás de los Garza, Nuevo León, México*

The number of dengue cases and their severity have increased in recent years in Nuevo León State. Development of systems to evaluate and support vector control actions are needed. Epidemiologic information on these cases was provided by the Nuevo León State Health Department and layered polygons of the geostatistic basic area were taken from the maps digitized by the National Institute of Statistics, Geography and Informatics. The cases were divided by 15-day time intervals from July to December 1998, and a map was created for each period. The distribution map of cases for 1998 showed that dengue vectors were distributed in a large part of the metropolitan area. Of the 968 geostatistic basic areas, 314 contained at least 1 case.

A continuous area was identified where 60% of the dengue cases occurred. Ten principal components were obtained; component 1 presents a variability of 34.68%. Ninety-one percent of variability was obtained in 6 components.

### **Spatial analysis of dengue cases in Guadalupe, Nuevo León, México, 1995–96**

Roberto Mercado, Ildelfonso Fernández and Saúl Lozano

*Universidad Autónoma de Nuevo León, Facultad de Ciencias Biológicas, San Nicolás de los Garza, Nuevo León, México*

The spatial and temporal distribution of dengue fever cases in Guadalupe, Nuevo León, México, was analyzed using a geographic information system. Data were obtained from the state health department and mapped for the municipality of Guadalupe, using CARTALINX® and IDRISI® programs. Confirmed cases (689) of dengue occurred most frequently in October (42.3%) and November (43.9%) and more cases were reported in women (59.1%) than men. The age group from 21 to 30 years had the most (25.3%) cases. The dengue cases were ranked at 5 levels: 1–2 cases, 3–5, 6–7, 8–9, and >9 cases, and then mapped. In 1995, the spatial distribution of 545 dengue fever cases was concentrated in the south, but in October they were concentrated in the south-central sector and in November they were concentrated in the west-central area. In 1996, most (145) were distributed in the east-central area. Factors such as human population density and availability of basic sanitary services influenced dengue incidence.

### **Effect of an educational campaign for *Aedes aegypti* control in Colima, Mexico**

F. Espinosa Gómez, C. M. Hernández Suarez, X. Montoy Guzmán and I. Fernández Salas

*Faculty of Medicine, University of Colima, Mexico; and Department of Medical Entomology, Faculty of Biological Sciences, Universidad Autónoma de Nuevo León (UANL), San Nicolás de los Garza, Nuevo León, México*

As a part of a community participation program for *Aedes aegypti* control in Colima, México, 187 houses were sampled for presence of larval positive containers following the recommendations of the Pan American Health Organization. The Breteau index and positive container/house (C+/H) were determined for each dwelling at the beginning of the study and after 6 months. A total of 374 observational units were computed. Simultaneously, a pre-mise condition index (PCI) was assessed according to Tun-Lin. Spearman's correlation between C+/H and PCI showed a weak, but significantly positive

correlation ( $r = 0.2$ ,  $t = 4.02$ ,  $P = 0.0005$ ); meanwhile a logistic regression between PCI and positive-negative houses revealed a significant correlation ( $\chi^2 = 3.4$ ,  $P < 0.04$ , OR = 1.61, CI 1.0–2.61). These results support the original hypothesis stated by Tun-Lin: that it is possible to estimate accurately the risk for house infestation with *Ae. aegypti* from a simple inspection. This strategy could reduce costs and time when *Ae. aegypti* surveillance campaigns are planned.

#### **Evaluation of a natural fertilizer infusion as an attractant for *Aedes aegypti* oviposition**

María de Lourdes da Graça Macoris, María Teresa Macoris Andrighetti, Luiz Takaku, Vanessa Camargo Garbeloto and Vanusa Cristina Busato Cirino

*Superintendencia de Control de Endemias, Servicio Regional Marília, Superintendencia de Control de Endemias, Suceen, Serv. Reg. de Marília, Brazil*

Flower vases are one of the most important breeding sites of *Aedes aegypti* in Brazil. A natural fertilizer for plants (diluted cow excrement) was evaluated as an attractant for *Ae. aegypti* oviposition in the laboratory and under field conditions. In the laboratory, a cage with 200 female and 200 male *Ae. aegypti* was used. Three glasses filled with water and with filter paper around the edge were used as oviposition sites. The glasses were filled with tap water, the pure fertilizer (100%), or the diluted fertilizer (10%). A strong preference for oviposition in the pure fertilizer (57% of the eggs) was observed, followed by oviposition in the glass with diluted fertilizer (33.8%), and 9.2% in tap water. In the field, the product was evaluated with paired ovitraps (with tap water and the 10% fertilizer). The paired ovitraps were placed in 65 houses of an urban area of Marília, Sao Paulo State, Brazil. They were serviced weekly for 1 year (March 1998–March 1999). No significant differences were found between positivity in ovitraps with the fertilizer and with plain water but a significantly higher number of eggs were laid in the ovitraps with fertilizer ( $t = 3.1617$ ,  $P = 0.95$ ).

#### **Gonotrophic cycle, survival, and pregravid rate of *Aedes aegypti* with mark–release–recapture method in Monterrey, northeastern Mexico**

M. L. Rodríguez-Tovar, M. H. Badii, A. Flores-Suárez and I. Fernandez-Salas

*Department of Invertebrate Zoology and Laboratory of Medical Entomology, School of Biological Sciences, UANL, San Nicolás de los Garza, Nuevo León, México*

Feeding frequency and survivorship along with multiple partial blood meals represent important

vectorial capacity variables for the dengue vector, *Aedes aegypti*. As a part of a project to study the bionomics of *Ae. aegypti*, this field study aimed to determine the length of the gonotrophic cycle and the pregravid rate, or the need of females to have a 2nd blood meal in order to stimulate oogenesis. The study site was located in Guadalupe City, part of the metropolitan area of Monterrey, in northeastern México. This region has reported serious dengue outbreaks since 1995. A group of 1,100 field-caught females reared in the laboratory were released in the field. They were marked with fluorescent powder 24–48 h after emergence. Three releases were conducted in order to obtain consistency of results. A recapture period of 9 days postrelease using human bait collections was conducted. Recaptured females that had been marked were taken to the laboratory and classified according to trophic stage and ovaries were dissected for oogenesis progression. Results showed that most *Ae. aegypti* females were recaptured at 4.5 days. Trophic stages of recaptured *Ae. aegypti* females showed 66% (75/113) were unfed, 24% (27/113) had fed, and 8% (11/113) were pregravid. Daily survivorship calculated by Guilles' regression method was 0.86, 0.83, and 0.78, for each of the 3 repetitions. Discussion on the importance of field studies to redefine known variables of the vectorial capacity for *Ae. aegypti* is also included.

#### **Evaluation of the perifocal application of Cypermethrin 40 WP for the control of *Aedes aegypti* in Brazil**

I. Braga, R. Pimenta Faria, C. Frederickson, A. K. Ribeiro Galardo and S. da Silva Soares

*Fund. Nac. de Saude, Brasilia, Brazil*

A comparative study to determine the effectiveness of Cypermethrin 40 WP as a perifocal treatment for the control of *Aedes aegypti* was conducted in Novo Iguaçu, Rio de Janeiro, and Manaus, Amazonas. Three application rates (125 mg active ingredient [AI]/m<sup>2</sup>; 250 mg AI/m<sup>2</sup>, and 500 mg AI/m<sup>2</sup>) were used to treat the external surfaces of large water storage containers such as 50-gal plastic or painted steel drums and asbestos water tanks. Bioassay results indicated that mortality decreased from 95% on day 1 after the treatment to less than 20% in the asbestos water tanks (treated with 125 mg AI/m<sup>2</sup> and 250 mg AI/m<sup>2</sup>). At the highest application rate, mortality decreased from 98 to 47%. With the plastic tanks the mortality decreased by approximately 50%. The results indicate that Cypermethrin is ineffective for perifocal treatment and does not give more than 1 wk of control for *Ae. aegypti*. The results were compared with previous studies using fenitrothion 40 WP applied at 1 g AI/m<sup>2</sup>.

### Evaluation of the residual effectiveness of temephos 1g against *Aedes aegypti* larvae in Rio de Janeiro State, Brazil

I. A. Braga, A. K. Ribeiro Galardo and  
S. da Silva Soares

*Fund. Nac. de Saude, Brasilia and Rio de Janeiro, Brazil*

Temephos 1g has been used in Brazil for the control of *Aedes aegypti* for more than 15 years. Reportedly this formulation has a residual effect of approximately 3 months. The duration of effectiveness was evaluated in 55 houses in 2 municipalities in Rio de Janeiro State using paired tire section larvitrap. One larvitrap was treated with temephos 1g at a rate of 1 ppm and the adjacent larvitrap was used as an untreated control. Traps were inspected weekly and larvae were removed and counted from positive traps before the original water level was restored. Tests were also conducted in a series of standard water tanks containing 200 liters of water and treated once with temephos at 1 or 2 ppm. Trials were conducted with and without partial water replacement to simulate normal household usage. Once a week for 5 wk, larvae were introduced to the tanks and mortality was recorded after 24 h. The results indicated that larvitrap treated with temephos became positive for larvae in 4 of the 6 groups beginning in the 2nd week. The remaining groups became positive in the 4th week of the test. In the water tank tests that had partial water replacement, larval survival was noted after the 3rd week at both concentrations. Water tanks without water replacement did not have any larval survival until the 5th week of the test.

### Impact of residual spraying of Cypermethrin on an *Aedes aegypti* population

María Teresa Macoris Andrighetti, María de Lourdes da Graça Macoris, Luiz Takaku, Vanessa Camargo Garbeloto and Vanusa Cristina Busato Cirino

*Superintendencia de Control de Endemias, Servicio Regional de Marília, Brazil*

Residual spraying for *Aedes aegypti* control in Brazil has been restricted to perifocal spraying in places with large numbers of breeding sites. The objective of this study was to evaluate the impact of this strategy in urban areas. Two districts of Marília (Sao Paulo State) with high *Ae. aegypti* infestations were chosen. Rosalia and Nobrega are urban districts with about 500 houses each. Vector infestation was monitored by the Breteau index and female index (number of females per inspected houses multiplied by 100). In the summer of 1998, the Breteau indices were 22.6 and 14.5 and female indices were 50.9 and 52.6 in Rosalia and Nobrega,

respectively. After the winter, the infestations rose again and 90% of Rosalia houses were treated with Cypermethrin (250 CE) in November 1998. In the following months, we observed an increased infestation in Nobrega, where the Breteau index reached the levels of the previous summer (14.7), whereas in Rosalia it was only 2. The female index per house reached 20 in Nobrega, whereas the maximum observed in Rosalia was 4.2. This method of control should be considered for special situations where traditional methods cannot be used.

### Evaluation of resistance of *Aedes aegypti* to temephos in Rio de Janeiro State, Brazil

I. A. Braga, A. K. Ribeiro Galardo, A. Costa Garcia and J. Bento Lima

*Fund. Nac. de Saude and Inst. Biol. do Exercicio, Brasilia and Rio de Janeiro, Brazil*

Temephos susceptibility was evaluated in 7 municipalities in Rio de Janeiro State. This insecticide has been used intensively to control dengue epidemics since 1986. *Aedes aegypti* were collected and pooled from several locations in each community using enhanced Centers for Disease Control ovitraps, and the F<sub>1</sub> generation was compared with a standard Rockefeller strain using the diagnostic dosage of 0.012 mg active ingredient/liter. The results indicated the presence of resistance in all of the municipalities evaluated. The resulting mortalities were 23% for São Gonçalo, 32% for Rio de Janeiro, 34% for Niterói, 34% for Caxias, 44% for São João de Meriti, 58% for Novo Iguaçu, and 74% for Campos. The Rockefeller strain had a mortality rate of 100%.

### Different levels of insecticide resistance in larvae and adults of *Aedes aegypti* from Venezuela

D. M. Molina de Fernández, Magdalena Rodríguez and Juan Bisset

*Servicio Autónomo de Altos Estudios en Salud Pública "Doctor Arnoldo Gabaldón," Ministerio de Salud y Desarrollo Social, Maracay, Venezuela; and Instituto de Medicina Tropical "Dr. Pedro Kourí," Havana, Cuba*

Since 1989, malathion has been the principal adulticide and temephos has been the principal larvicide used for controlling *Aedes aegypti* in Venezuela. As a result, adult *Ae. aegypti* have become resistant to malathion but no temephos resistance in larvae has yet been detected. Nine field strains of *Ae. aegypti* from 9 states from Venezuela were bioassayed to malathion and other organophosphate and pyrethroid insecticides. No malathion resistance was detected in larvae in any of the strains evaluated, whereas adults from the same strains

showed high levels of resistance to malathion, using the diagnostic dose recommended by the World Health Organization. In addition, some adult resistance was found to fenitrothion and some cross-resistance to 1 carbamate (propoxur) in the 9 strains tested. Incipient resistance to pyrethroids has been found in 9 field strains of *Ae. aegypti* from Venezuela in both larvae and adults. In vivo analysis of resistance mechanisms suggest that esterases and mixed-function oxidase cause resistance in larvae. We are investigating the resistance mechanism that is involved in generating different levels of resistance between larvae and adults.

#### **Estimation of insecticide resistance of *Aedes aegypti* from Trujillo, Venezuela**

L. Alvarez González, E. Rojas and J. V. Scorza  
*Laboratorio de Control del Vectores, Centro de Investigaciones "J. W. Torrealba," Trujillo, Venezuela*

Dengue and its severe form, dengue hemorrhagic fever, are transmitted by *Aedes aegypti*, and have become an important worldwide public health problem. Unfortunately, this vector has demonstrated resistance for DDT, dieldrin/benzene hexachloride, organophosphates, and carbamate insecticides in different states of Venezuela. In Trujillo, *Ae. aegypti* has exhibited resistance against propoxur and lambda-cyhalothrin. Resistance to the carbamate propoxur was found to be low (5.2-fold) and resistance to lambda-cyhalothrin was moderate (9.6-fold). Susceptibility to malathion was found (2.9-fold). These results must be considered in the future for *Ae. aegypti* control programs because organophosphates and pyrethroids are currently used in vector control in most countries of Central and South America.

#### **In vitro assay of lambda-cyhalothrin in two populations of *Aedes aegypti* from Trujillo, Venezuela**

L. Alvarez González, E. Rojas and J. V. Scorza  
*Laboratorio del Control de Vectores, Centro de Investigaciones "J. W. Torrealba," Trujillo, Venezuela*

*Aedes aegypti* populations collected from 2 localities in the state of Trujillo, Venezuela, were studied for their susceptibility to the pyrethroid lambda-cyhalothrin. Fourth-stage larvae from both strains (IVSS and San Martin) were used for a bioassay test and their median lethal concentrations were estimated. When these strains were compared with the reference strain (Rockefeller), we found a low resistance with the strain IVSS (4.24-fold) and a moderate resistance with the San Martin strain (9.6-fold). These data should be considered in the

selection of insecticides to be used in Venezuela against *Ae. aegypti*.

#### **Cross-resistance to pyrethroid and organophosphate insecticides, selection with temephos in *Aedes aegypti* from Cuba**

M. M. Rodríguez, J. Bisset, M. Ruiz and A. Soca  
*Instituto de Medicina Tropical "Pedro Kourí," Havana, Cuba*

A strain of *Aedes aegypti* from Cuba was subjected to temephos selection to evaluate the usefulness of this organophosphate insecticide for mosquito control. High resistance developed after 6 generations of selection. Little or no cross-resistance was observed to several organophosphates (malathion, fenitrothion, and fenthion), but high cross-resistance was found to the pyrethroid deltamethrin. Esterases and glutathion-S-transferase could be the resistance mechanisms responsible for this phenomenon.

#### **Negative factors affecting dengue control programs in Guadalupe, Nuevo León, México**

K. M. Valdez, H. Quiroz, V. A. Rodríguez,  
K. L. Saavedra, F. Gonzalez and M. A. Rodríguez  
*Laboratorio de Entomología, Facultad de Ciencias Biológicas, Universidad Autónoma Nuevo León, San Nicolás de los Garza, Nuevo León, México*

Socioeconomic level and knowledge about dengue are 2 factors that adversely affect public health control programs. Pareto graphics can be used to detect these problems. Surveys were conducted to detect negative factors that affect dengue control programs. Two hundred people were surveyed in Guadalupe, Nuevo León. The survey included 25 questions about dengue, mosquito breeding, mosquito control, and other factors. The most notable result was that people with low salary (about US \$200 per month) did not worry about dengue but were concerned about how to get more money to cover their basic needs such as housing and food.

#### **Use of copepods (*Mesocyclops longisetus*) in the *Aedes aegypti* control program of Cali, Colombia**

Marcela Suárez, Marco F. Suárez and María Eugenia Cuadros

*Departamento de Biología, Universidad del Valle and Secretaría de Salud Pública Municipal, Cali, Colombia*

Dengue is considered the most important arbovirus disease in the world. Because of the absence of a vaccine, lack of specific medical treatment, and

limited long-term vector control effects, creative approaches should be tested. In Cali, with its 2 million inhabitants, dengue cases were drastically reduced with integrated and selective vector control strategies, including biological control. The primary *Aedes aegypti* breeding places in Cali are catch basins. They almost always contain water, provide a good environment, and have larval food throughout the year. They produce 27 times more pupae than all other breeding places combined. *Aedes aegypti* populations in Cali are resistant to temephos, one of the most commonly used larvicides. *Mesocyclops longisetus*, a copepod, was mass produced and approximately 500 specimens were placed in each catch basin. In 1999, 412 basins were treated. None of the treated basins had *Ae. aegypti* larvae after 3 months of copepod use. This effect extended as long as 6 months after treatment. Copepods are able to maintain the basins free of *Ae. aegypti* for extended periods of time if external contaminants such as car oil are not present.

#### **The use of palm-sized computers in entomological surveys of *Aedes aegypti* in Barbados: pilot project**

Marco F. Suárez, Dale Ifill and Emerson Yearwood

*Pan American Health Organization, World Health Organization and Vector Control Unit, Ministry of Health and the Environment, Barbados, West Indies*

This pilot study was initiated to examine the utility of palm-sized computers as an appropriate technology to improve data and information management for the reduction of mosquito infestation levels in Barbados. Traditionally, health inspectors visit hundreds of houses and record data on the status of mosquito breeding sites on paper. The volume of the data collected is often quite large, making it difficult to associate and summarize rapidly and produce meaningful information that can be used to make appropriate decisions about vector control interventions. An electronic version of the traditional paper data collection form was created and enhanced on a Casiopeia palm-sized computer. For this study, 10 health inspectors were trained to use the palm-sized computers in the field. During a survey and evaluation period of over 6 wk, 2 palm-sized Windows CE Casiopeia computers with 16 MB of memory were used. Based on a questionnaire designed to evaluate the ease of use and acceptability of these machines by health inspectors in their field work, a comparison was made between palm-sized computers and traditional data collection, classification, and summarization techniques. A database report was designed for a desktop computer to summarize data and produce the standard indices used for measuring mosquito infestations.

As in other industries, such as electrical utilities, data will be captured and recorded electronically at the point of inspection, thus minimizing errors due to recording and subsequent transfer.

#### ***Aedes albopictus* and its association with *Aedes aegypti* in Allende, Nuevo Leon, Mexico**

Juan de Dios Aguilar-Gueta, Roberto Mercado-Hernández and Ildefonso Fernández-Salas

*Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, México*

*Aedes albopictus* has been reported in northern México since 1988. This paper reviews the *Ae. albopictus*—*Ae. aegypti* association in Nuevo León. During the colonization phase, we assumed that the presence of *Ae. albopictus* was associated with *Ae. aegypti*. For this study, 175 ovitraps were placed from December 1998 through August 1999 in several zones of Allende, Nuevo León, including locations with high, medium, and low mosquito density. All mosquito larvae were collected and identified. *Aedes albopictus* was recorded in April 1999 and subsequently in other zones. Through July, no association between the 2 species was demonstrated ( $c^2 = 3.30$ ,  $P > 0.05$ ), and *Ae. albopictus* was more abundant.

#### **Preliminary incrimination of *Culex (Melanoconion) vomerifer*, *Cx. (Mel.) adamesi*, and *Cx. (Mel.) pedroi* as enzootic vectors of subtype ID Venezuelan equine encephalomyelitis viruses in a sylvatic focus in the Magdalena River Valley, Santander, Colombia**

C. Ferro, J. Boshell, M. L. Ahumada, M. Gonzalez, S. Pérez and S. C. Weaver

*Instituto Nacional de Salud, Santa Fe de Bogotá, Colombia; and University of Texas, Medical Branch, Galveston, TX*

As part of studies to delineate the generation of epizootic Venezuelan equine encephalomyelitis virus (VEE) from enzootic progenitors, field studies were carried out in an active and continuous focus of enzootic subtype ID virus transmission in a forest in the Magdalena Medio River Valley, Colombia. Hamster-baited traps were used to monitor virus circulation and to identify natural enzootic vectors. Mosquitoes were collected daily for 1st 6 days after hamster exposure or until hamster death. When VEE was isolated from a sentinel hamster, mosquitoes from positive traps were sorted by species and pools were tested for VEE. Generally, the 1st or 2nd day's collection contained a pool with a single infected mosquito species, representing the incriminated vector, whereas the last day's collec-

tion before hamster death contained pools that were all infected because of viremic blood meals. Initial vector incrimination results were *Culex (Melanocnion) vomerifer*, 3 natural transmissions; *Cx. (Mel.) adamesi*, 1 transmission; and *Cx. (Mel.) pedroí*, 1 transmission. These results challenge the dogma that a single mosquito species serves as the principal enzootic VEE vector in a given focus.

**New record of *Pseudouroctonus redelli* (Scorpiones: Vaejovidae) in México**

C. Solís-Rojas, J. J. Flores-Maldonado, C. B. Ramos-Silva, H. Quiroz-Martínez, R. M. González-Iglesias and R. Garza-Cantú  
*Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, México; and Departamento de Recursos Naturales, Instituto de Estudios Superiores, Monterrey, Nuevo León, México*

México's vaejovid scorpion fauna is one of the most diverse in the world. A small percentage of the members of this family is known because of the limited investigations that have been conducted in northeastern México. *Vejovis*, *Serradigitus*, *Frankus*, and *Paruroctonus* are the genera reported for this family in this area. We examined 1,000 specimens of the family Vaejovidae and preserved them in the arachnid collection of the Biological Science Faculty-UANL. *Pseudouroctonus redelli* is reported as a new record in several localities of the states of Nuevo León and Coahuila, México. The specimens of *P. redelli* were collected at elevations between 800 and 1,550 m and identified with keys of Gertsch and Soleglad (1972) and Stockwell (1992).

**Presence of *Centruroides elegans* (Scorpiones: Buthidae) in Nuevo León, México: a new distribution record?**

C. Solís-Rojas, J. J. Flores-Maldonado, K. Arevalo-Niño, S. Morales-Arroyo, W. A. Albeldaño-Vázquez and I. Iracheta-Tamez  
*Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, México*

Members of the family Buthidae are among the most medically important scorpions in the Americas because of their sting. The genus *Centruroides* belongs to this family and is found exclusively in the Americas. Some of the central states of Mexico have some of the most toxic scorpion species known. In these areas, scorpion envenomization is considered one of the most important public health problems and high human mortality is reported in this region. *Centruroides vittatus* and *C. gracilis* are native scorpions in Nuevo León. However, since 1980, *Centruroides elegans*, one of the most toxic

scorpions in Mexico, has also been reported in this state. Approximately 5,000 specimens of Buthidae were reviewed from the arachnid collection at the Biological Science Faculty at the State University of Nuevo León. The specimens were identified using keys written by Hoffman. *Centruroides elegans* may have arrived in Nuevo León State by transportation of fruit products, and not as a general expansion of its previous range.

**Morphometric comparison among *Triatoma longipennis* (Hemiptera: Reduviidae) from the west coast of México**

José A. Martínez-Ibarra, Jesús Romero-Nápoles, Ricardo Alejandro-Aguilar, Gabriel Rincón-Enríquez and Juan C. Zaragoza-Ramírez  
*Instituto Fitosanidad, Colegio Postgraduados and Laboratorio Parasitología, Escuela Nacional Ciencias Biológicas and Laboratorio Marcadores Genéticos, Texcoco de Mora, Mexico*

*Triatoma longipennis* is one of the most important vectors of *Trypanosoma cruzi* (the cause of Chagas' disease) to humans in western México. Some ethological differences were detected in vector populations sampled from 7 localities across its range along the west coast of México. Five measurements were performed on 10 adults from each of these populations. The populations were not statistically different ( $P > 0.05$ ) morphologically, and they were concluded to represent a single population in spite of the different ethological habits recorded.

**Epidemiologic and entomological characterization of a leishmania focus in Venezuela**

M. Dora Feliciangeli, Jazzmin Arrivillaga, Irma Agrela, Noris Rodríguez, Olga Zerpa, Angela Hamilton, José Plata and Leyda Gudiño  
*Universidad de Carabobo, Aragua, Maracay; Instituto de Biomedicina, Caracas; Servicio de Dermatología Sanitaria, La Grita; and Malariología, San Felipe, Venezuela*

Cutaneous leishmaniasis (CL) in Venezuela is scattered all over the country but mainly concentrated in the foothills of the Andes and the Serranía de la Costa, with an elevational distribution from sea level to 2,800 m. From 1955 to December 1998, a total of 41,250 cases of CL were registered by the National Department of Dermatology, now the Venezuelan Institute of Biomedicine. American visceral leishmaniasis (AVL) in Venezuela is found mainly in 3 areas: in the east (the island of Margarita and states of Sucre and Anzoátegui), in the center (Aragua, Carabobo, Guárico, and Cojedes states), and in the west from Lara State to Zulia

State. A total of 925 cases were reported from 1955 to 1998. However, underreporting is common and, as for CL, true figures may be at least 3 times the number that has been reported. We have studied several foci of CL and AVL in Venezuela and gathered evidence on domestic and peridomestic transmission that is supported by epidemiologic and entomological data. The epidemiologic criteria for characterization of foci includes the distribution of the disease among the population in relation to demographic and social characteristics, which helps in the definition and recognition of risk factors. Long-term temporal (seasonal) and spatial (indoors and outdoors) catches of phlebotomine sand fly species tested by the dot-enzyme-linked immunosorbent assay have shown that humans are the main source of blood meals. Further, naturally infected sandflies with *Leishmania* spp. were found to be indistinguishable from those collected from people living in endemic foci. *Lutzomyia ovallesi*, *L. panamensis*, *L. gomezi*, *L. spinicrassa*, and *L. youngi* are recognized as the vectors of LC and *L. longipalpis* and *L. evansi* as vectors of AVL. Techniques such as geographic information systems will allow the stratification of the leishmaniasis in Venezuela and will be helpful in the decision-making process for prevention and control of the disease and produce a more adequate allocation of resources. This work was supported by a Venezuelan Government-World Bank project (PCEE-VEN.96-002).

#### Chagas' disease and leishmaniasis: new tools for control of vector-borne diseases

E. Villegas-Avila

Laboratorio de Control del Vectores, Centro de Investigaciones "J. W. Torrealba," Trujillo, Venezuela

Vector-borne diseases represent a significant public health problem for many countries. Among the diseases that have shown a recrudescence in recent years are malaria, Chagas' disease, schistosomiasis, onchocercosis, dengue, filariasis, leishmaniasis, and encephalitis. The use of control measures such as spraying of the dwellings with insecticides is a common measure for controlling vector-borne diseases. This control measure involves different problems: high cost of insecticides, support of highly specialized personnel, economics, and politics. The use of insecticide-impregnated curtains and bednets to interrupt the human-vector contact have been implemented as new advances for control of vector-borne diseases. The efficacy of this measure has been demonstrated against different species of bloodfeeding mosquitoes. In Latin America, mosquito nets and curtains were used in Colombia and Venezuela for control of Chagas' disease and leishmaniasis. This method was very effective in reducing the number of *Rhodnius prolixus* and *Lutzomyia*

*youngi*. The use of mosquito nets and curtains is a promising technique for the control of vector-borne diseases.

#### Characterization of *Anopheles* breeding habitats in Sifontes, Bolivar State, Venezuela

Jorge E. Moreno

División de Investigaciones, Escuela de Malariología y Saneamiento Ambiental "Dr. Arnoldo Gabaldón," Maracay, Venezuela; and Centro de Investigación de Campo "Dr. Francesco Vitanza," Bolivar, Venezuela

Sifontes is a city in northeastern Bolivar State and is the main focus of malaria in Venezuela. From 1992 to 1998, an average of 4,180 cases was reported annually from this area, where mining is the principal activity. To locate and identify *Anopheles* breeding sites and to determine the species present in this area, a longitudinal study was initiated in the localities of Las Claritas, El Dorado, San Martín de Turumbán, Tumeremo, and Bochinche. *Anopheles* larvae were collected with a dipper and later identified to species. Some physicochemical and botanical observations were made of the breeding site. Twelve species of *Anopheles* plus *Chagasia bathana* were collected. Of these, 6 belong to the subgenus *Nyssorhynchus*, 5 to the subgenus *Anopheles*, and 1 to the subgenus *Lophopodomomyia*. The most abundant species in the area is *An. triannulatus*, followed by *An. marajoara*. The main vector of the area, *An. darlingi*, is not very abundant but is widely distributed. Canonical correspondence analysis allowed us to determine the decisive biotic and abiotic factors influencing the presence, abundance, and distribution of these species.

#### *Anopheles albimanus* mortality in an indigenous area of Panama

Argentina Y. de Turner, Gloria de Obaldía, A. García and C. Campos

University of Panama, Panama Canal Commission, and the Ministry of Health, Panama City, Panama

Problems of insecticide resistance in *Anopheles albimanus*, the main malaria vector in an indigenous area dedicated to agriculture and cattle, were studied. The incidence of malaria in this area has progressively increased since 1993, and the number of cases of malaria doubled in 1998 compared with the same dates for 1997. The study area was in the province of Bocas del Toro, located on the Atlantic coast, 500 km west of Panama City. It is a rural area with houses of mud and palm trees or of wood and zinc and has an average temperature of 28°C and a relative humidity of 85%. Over a 1-year period, we made 2,640 biological assays on walls to



evaluate resistance of k-othrine (deltamethrin, 5%) on the different surfaces. In the area, we evaluated the percentage of mortality in 39,600 female *An. albimanus* collected with human bait. We found an average 78% mortality with a residual effect of 34 wk during the 1st 6 months and 93% mortality with 28 wk of residual effect.

### Comparison of four methods for collecting adult anopheline mosquitoes from a malaria endemic area in Rondonia, Brazil

F. Santos, J. B. P. Lima and I. A. Braga

Fundação Nacional de Saúde and USAMRU/B, Brasília, Brazil

For many years, the most common method for collecting anopheline mosquitoes has been the human biting collection, but this method increases the chances of contracting malaria. Four methods were compared in the endemic malaria area in Porto Velho, Rondonia, Brazil. These included the Shannon modified trap, the Shannon trap with a light source, a person using a black cotton sock, and collections on human legs. These methods were used outside at 4 locations at 4 times during 1998. Human biting collections proved to be more effective than other methods. The most abundant species collected was *Anopheles darlingi*. The nulliparous rate was significantly higher than that of the parous rate in all methods. These results showed that for monitoring anopheline populations, the best method was the human biting collection; however, other efforts should be made to find an alternative method that does not compromise the health of the human volunteers.

### Morphologic effects in *Anopheles pseudopunctipennis* (Diptera: Culicidae) caused by control strategies in an artificial pool

F. González, H. Quiroz, V. A. Rodríguez, L. M. Villegas, L. O. Tejada and M. H. Badii

Laboratorio de Entomología, Facultad de Ciencias Biológicas, UANL, and Programa Graduado de Agricultura, San Nicolás de los Garza, Nuevo León, México

We used Bactimos (*Bacillus thuringiensis* var. *israelensis* [Bti]) and *Buena scimitra* as control strategies alone and together against anopheline mosquitoes and studied their effect on surviving mosquito larvae in an artificial pool. The treatments applied were Bti, *B. scimitra*, Bti plus *B. scimitra*, and control. Our test systems were 20 3rd- and 4th-stage *Anopheles pseudopunctipennis* larvae. After 24 h of exposure, mortality was recorded and surviving larvae were taken to the laboratory, where adults were reared. Body measurements were taken and the data were analyzed statistically with anal-

ysis of variance. A statistically significant difference was found among treatments ( $P < 0.05$ ) and mortality was similar among the treatments. Ninety adults were obtained, including 35 females and 55 males. Female body measurements were 8.92-mm hind legs, 4.33-mm thorax-abdomen, and 3.80-mm wings.

### Effect of algal extraction on *Anopheles pseudopunctipennis* larval populations in southern Chiapas, México

Juan G. Bond-Compeán, Juan I. Arredondo-Jiménez and Mario H. Rodríguez

Centro de Investigaciones Paludismo, Instituto Nacional de Salud Pública, Tapachula, Chiapas, México; and Centro de Investigaciones Sobre Enfermedades Infecciosas, Instituto Nacional de Salud Pública, Cuernavaca, Morelos, México

We investigated the effects of mechanical extraction of filamentous algae on the dynamics of *Anopheles pseudopunctipennis* larvae from river pools of southern Chiapas, México. The study took place throughout a dry season, over a 6-km river-wide belt transect divided in 500-m sections. Six of these 500-m sections were selected randomly and assigned to be treatments (where all algal mats were mechanically extracted) or controls (where the normal dynamics of algal mats were followed). *Anopheles pseudopunctipennis* larvae from all belt sections were monitored fortnightly. A total of 138 larval habitats distributed along the 6 transects were counted. They were composed of 2 green algae genera, *Spirogyra* (at early succession of the algal mat) and *Cladophora* (at later stages of succession). The extraction of algal mats had a significant effect on the reduction of *An. pseudopunctipennis* larvae ( $F = 31.98$ ,  $df = 5$ ,  $P < 0.001$ ), with an overall mean of 2.5 larvae/dip in the treatment belt sections and 11.4 larvae/dip in the control. Total mosquito larval control lasted for 45 days postextraction. This complementary, naturalistic vector control tactic is now being implemented with excellent results by the National Malaria Control Program through community participation.

### Innate host preferences in *Anopheles vestitipennis*

Armando Ulloa, Juan I. Arredondo-Jiménez, Mario H. Rodríguez and Ildefonso Fernández-Salas

Centro de Investigaciones de Paludismo, Instituto Nacional de Salud Pública, Tapachula, Chiapas, México; Centro de Investigaciones Enfermedades Infecciosas, Instituto Nacional Salud Pública, Cuernavaca, Morelos, México; and Laboratorio de Entomología Médica, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, México

We assessed the existence of innate host preferences in *Anopheles vestitipennis* using  $F_1$  mosqui-

toes from parental females with known feeding histories. The study was conducted in southern Chiapas, México, in an experimental hut constructed with local materials and divided into 3 compartments, each lined inside with netting. The side compartments were occupied by 2 humans and 1 horse, 1 cow, or 2 pigs. Two types of experiments were conducted: 1 with females collected unfed and released in the experimental hut, and 1 with  $F_1$  females collected from animal or human hosts. Mosquitoes of each population were marked with different fluorescent dust colors. Mosquitoes were released from the central room at 1930 h, with hosts already positioned, and recaptured from each compartment at 0500 h. We recorded the number of marked bloodfed and unfed mosquitoes selecting each host. Using wild-collected mosquitoes, the recapture rates on the human or animal compartment were 24 and 76%, respectively ( $\chi^2 = 427.84$ ,  $P = 0.0001$ ). With  $F_1$  mosquitoes, we released 773 and 522 anthropophilic and zoophilic mosquitoes, respectively, whereas recapture rates were 59 and 80% ( $\chi^2 = 61.66$ ,  $P = 0.0001$ ). These results confirmed the existence of innate host preferences in *An. vestitipennis*.

**Comparative study of the genetic identity of sympatric and allopatric populations of *Anopheles vestitipennis* using random amplified polymorphic DNA–polymerase chain reaction techniques**

Juan I. Arredondo-Jiménez, Hortensia Murillo-Sánchez, Fidel C. Hernández, Cuauhtémoc Villarreal and Mario H. Rodríguez

*Centro de Investigaciones de Paludismo, Instituto Nacional de Salud Pública, Tapachula, Chiapas, México; Laboratorio Entomología Molecular, Centro Investigaciones y Estudio Avanzados, México Distrito Federal, México; and Centro de Investigaciones Sobre Enfermedades Infecciosas, Instituto Nacional de Salud Pública, Cuernavaca, Morelos, México*

Our aim was to gather evidence to support the hypothesis of the existence of anthropophilic and zoophilic populations within *Anopheles vestitipennis*. To do this, we investigated the presence of random amplified polymorphic DNA markers that could differentiate mosquitoes collected on human and animal hosts. After an initial screening of 62 random primers (kits A, B, C, and D, Operon Technologies, Alameda, CA), 10 primers (A08, A09, A14, A17, B04, B05, C01, C10, C13, and D01) produced 21 consistent polymorphic markers that were chosen for further analysis of indoor human landing and animal bait populations from 4 villages of Chiapas (3) and Tabasco (1) states. Nei's genetic distance (Nm) found among *An. vestitipennis* human landing and animal bait collections averaged

0.2318, with smaller genetic distance between anthropophilic mosquitoes (0.2050) than among zoophilic mosquitoes (0.2487). The variance in allele frequency, Wright's F statistic, averaged  $0.32 \pm 0.16$ , and effective migration rate Nm was  $<1$  in 14 of 21 studied markers, averaging 0.5, or 1 migrant individual every 2 generations, which indicates a restricted gene flow. The dendrogram of genetic distances yielded 2 branches, grouping 3 of 4 anthropophilic populations in 1 branch and 3 of 4 zoophilic populations in the other branch. These findings provide additional evidence to the existence of a species complex within *An. vestitipennis*.

**Gene flow among *Anopheles albimanus* populations as determined by random amplified polymorphic DNA–polymerase chain reaction in México**

C. Villarreal Treviño, J. I. Arredondo-Jiménez, M. H. Rodríguez L. and I. Malo-García

*Centro de Investigaciones de Paludismo, Instituto Nacional de Salud Pública, Tapachula, Chiapas, México; and Centro de Investigaciones Sobre Enfermedades Infecciosas, Instituto Nacional de Salud Pública, Cuernavaca, Morelos, México*

The gene flow among populations of the malaria vector *Anopheles albimanus* was investigated by amplification of random regions in genomic DNA using random amplified polymorphic DNA (RAPD) markers (RAPD-polymerase chain reaction). Variation patterns in gene flow among neighboring and distantly located populations were investigated by studying 20 populations collected from various agroecological areas of southern Chiapas, México, and 10 populations collected from various states of Mexico (9) and Guatemala (1). After an initial screening of 60 random primers (kits A, B, and C, Operon Technologies, Alameda, CA), 5 primers (A05, A20, B04, B11, and B18) produced 47 consistent polymorphic markers for microgeographic analysis, whereas for the macrogeographic analysis, 7 primers (A05, A08, A12, A19, A20, B04, and B11) that produced 54 markers were selected. In Chiapas, the average F statistic value was  $0.117 \pm 0.073$  and  $Nm = 1.9$  migrants/generation, whereas in Mexico, the average F statistic was  $0.1967 \pm 0.141$  and  $Nm = 1$  migrants/generation. The dendrogram of Chiapas indicated incipient clusters of localities by agroecological areas, whereas the dendrogram of macrogeographic analysis yielded 2 principal clusters, 1 from the northern states of Mexico and the other from the south. The small number of migrants could explain the focalization of malaria transmission in México.

### Raw plant extracts influencing oviposition in *Anopheles albimanus* under laboratory conditions

José L. Torres-Estrada, Rosa A. Meza-Alvarez, Juan Cibrián-Tovar, Juan I. Arredondo-Jiménez, Leopoldo Cruz-López and Mario H. Rodríguez

*Centro de Investigaciones de Paludismo, Instituto Nacional de Salud Pública, Tapachula, Chiapas, México; Instituto de Fitosanidad, Montecillos, México; El Colegio de la Frontera Sur, Tapachula, Chiapas, México; and Centro de Investigaciones Sobre Enfermedades Infecciosas, Instituto Nacional de Salud Pública, Cuernavaca, Morelos, México*

Some plant species are very attractive as oviposition sites for some anopheline species, indicating that such places are specifically selected by gravid females that are guided using chemical cues released from the larval habitat, perhaps from plants. In this investigation, we assessed the effect of raw aqueous extracts of aquatic plants on the oviposition behavior of *Anopheles albimanus* in southern Chiapas, México. Aqueous extracts of 5 plant species were tested at 6 concentrations (50, 10, 1, 0.1, 0.01, and 0.001% dilutions), compared to plain water. Five replicates per plant species of 2 dishes containing 200 ml of each treatment were set side-by-side in a cage containing 30 gravid *An. albimanus* females. Dishes were inspected for eggs after 24 h. Results indicated that 50 and 10% plant extracts elicited repellence (*Brachiaria mutica*, *Fimbristylis spadicosa*, *Cynodon dactylon*, *Ceratophyllum demersum*, and *Jouvea straminea*), whereas plant extracts at 0.01 and 0.001% concentrations showed an attraction response. These results indicate that some plants need to be studied in detail for their attraction or repulsion properties towards gravid *An. albimanus* females. Gas chromatography-mass spectrometry analysis showed mixed terpenoid and alcohol compounds of aqueous extracts, including guaiacol, phenol, isoeugenol, longifolene, caryophyllene, phenyl ethyl alcohol, and *p*-cresol.

### Residual effect of *Bacillus sphaericus* (VectoLex® CG) for the control of *Anopheles darlingi* in Iquitos, Perú

Ernesto Berrocal, Cristiam Carey, Luis Rodríguez, Carlos Calampa and Luis Valdivia

*Dirección Regional de Salud Loreto, Ministerio de Salud del Perú, Iquitos, Perú*

The proliferation of fish farms at the community level has provided suitable breeding sites for *Anopheles darlingi*, the main malaria vector in the region of Loreto, in northeastern Perú. Eight fish farms and 1 natural pond with active *An. darlingi* breeding, which had no history of larval control

measures, were selected at 3 localities near Iquitos. Two of the breeding sites were used as untreated controls and 7 of the sites were treated with a granular formulation of *Bacillus sphaericus* (VectoLex® CG, supplied by Abbott Laboratories, North Chicago, IL) at rates of 5, 10, and 20 kg/ha by manual application. Larval populations were monitored weekly for 1 month before application, and at 1, 2, 3, 7, 14, 21, 30, and 45 days postapplication. One hundred percent reduction of all larval instars was obtained by 2 days postapplication in all treated sites. Residual control was maintained for up to 45 days, as measured by the presence of late instars. No evidence was found of adverse effects on the fish. Because of the ease of use, this granular formulation of *B. sphaericus* offers a promising tool for community-based control of *An. darlingi*.

### Larvicidal effect of *Bacillus thuringiensis* var. *israelensis* H-14 (*Bti*) in fish-farm ponds near Iquitos, Perú

P. Ventosilla, J. Chauca, H. Guerra, J. Merello, B. Infante, C. Calampa, J. Aramburú, H. Jabá, J. Gonzales, W. Tello, E. Ruiz, D. Arévalo, C. Ramal and R. Naupay

*Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Perú; and Dirección Regional de Salud de Loreto, Iquitos, Perú*

*Bacillus thuringiensis* var. *israelensis* (*Bti*) is one of the most successful bacterial entomopathogens used for controlling insect vectors of human disease pathogens. We report on the toxic effect of a commercial *Bti* formulation on *Anopheles* larvae in the field. Assays were performed in Quistococha, Loreto, Perú, using Vectobac G Abbott (200 ITU/mg), donated by Rivers of the World. The *Bti* was hand-applied at about 2 g/m<sup>2</sup> in 5 test fish-farm ponds, whereas 5 other ponds served as controls. All ponds were comparable, as determined by studying their flora, fauna, soil type, and selected chemical parameters. We evaluated mortality and duration of toxic effect per larval instar in *Bti*-inoculated ponds, and mean fluctuations of larval population in control ponds. The toxic effect of *Bti* against *Anopheles triannulatus*, *An. nuneztovari*, and *An. albitarsis* larvae lasted ( $P < 0.005$ ) for 23 days compared with initial populations, with 100% reductions in all ponds for all larval instars during the 1st 4 days and 99–100% at 7 days. The fluctuation of mean larval populations was significant ( $p < 0.005$ ) for different larval instars and different control ponds. If used weekly or even fortnightly, *Bti* may effectively control *Anopheles* larvae in fish-farm ponds.

### Molecular characterization of B esterase-amplified haplotypes related to insecticide resistance in *Culex quinquefasciatus* from Cuba

J. A. Bisset, M. M. Rodríguez, E. Calvo, C. Díaz and A. Soca

*Instituto de Medicina Tropical "Pedro Kourí,"  
Havana, Cuba*

Overproduction of esterases is a common insecticide resistance mechanism in *Culex quinquefasciatus*. The same amplified haplotypes (e.g., B1 and B2) have been found in different regions and a large polymorphism in the esterase B locus has been reported in susceptible populations, which has arisen as a migration hypothesis for amplified genes. In this paper, we report the results of restriction fragment length polymorphism and restriction mapping assays on 3 strains of *Cx. quinquefasciatus* from Cuba. These strains were obtained from natural populations from Santiago de Cuba and Havana City and another was generated by selection in our laboratory. The same pattern of amplified B1 haplotypes was detected in mosquitoes from both strains, which also was very similar to the amplified B1 haplotypes of the temephos reference strain, although a difference accounted for a HindIII site in the flanking sequences that coamplify with the concerned esterase gene. These results suggest a probable migration of B1 genes in our country after an initial event of different amplification that is independent from the event that generated the amplified B1 haplotypes in the reference strain. This information supports the theory of gene migration after considering probable independent amplification events of the same gene in different regions under well-determined conditions.

### Oviposition in artificial substrates for *Chironomus plumosus*

B. Medellín, V. A. Rodríguez, H. Quiroz, V. Robledo and X. Martínez

*Laboratorio de Entomología, Facultad de Ciencias Biológicas, UANL, San Nicolás de los Garza, Nuevo León, México*

Presence of adult midges is 1 cause of economical loss in real estate business and tourism because chironomids occur in large swarms and can cause panic among residents and visitors. The objective of this study was to determine oviposition sites preferred by *Chironomus plumosus*. White, yellow, red, and green panels were used as artificial substrates for *C. plumosus* oviposition. The panels were placed in a large pool in a wastewater treatment plant and egg masses were taken from each panel each week. The egg masses were counted and differences were analyzed statistically. No difference was found among the colors ( $P > 0.05$ ).

### Mosquito control strategies in artificial containers and their morphologic effects

V. A. Rodríguez, H. Quiroz, L. Torres, F. González, L. O. Tejada and M. H. Badii

*Laboratorio de Entomología, Facultad de Ciencias Biológicas, UANL, San Nicolás de los Garza, Nuevo León, México*

The most acceptable mosquito control strategies are those with minimum effect on the environment. Many integrated mosquito management programs have been established, but a question that has arisen is what happens to the survivors. The objectives of this study were to evaluate the effectiveness of Agnique and *Buenoa scimitra* alone and together as mosquito control strategies in artificial containers and their effects on survivors. The study was conducted in 200-liter plastic containers, and 4 treatments were applied (Agnique, *B. scimitra*, Agnique plus *B. scimitra*, and an untreated control). Every week, 15 samples were taken and the *Culex quinquefasciatus* larval densities were recorded. Treatment differences were tested by analysis of variance and backswimmers after 3 months. Differences were not statistically different except in the case of the joint application of Agnique. A total of 115 *Cx. quinquefasciatus* adults were obtained; 63 males and 52 females. Wing size was similar in all treatments.

### The mosquitoes of Puerto Rico: distribution and habitats (1993-99)

E. A. Kenny-Elliot

*Department of Biology, University of Puerto Rico, San Juan, Puerto Rico*

The habitat, diversity, and distribution of mosquitoes in Puerto Rico has not been studied extensively. Since 1993, I have collected mosquitoes on the main island. Preliminary results of these collections include *Anopheles albimanus*, collected from light traps in Cabo Rojo (west coast), and Arecibo (north coast); *Anopheles grabhamii*, from light traps in Cabo Rojo, and larvae from brackish ground water in Añasco (west coast); *Aedes taeniorhynchus* larvae, collected from a rainfall muddy ground pool at Piñones State Mangrove Forest (north coast) and from light traps at Cabo Rojo, Arecibo (north coast), Fajardo (east coast), and Salinas (south coast); *Aedes sollicitans*, from light traps next to mangroves at Piñones Forest, Arecibo, and Cabo Rojo; *Aedes mediovitatus*, from tire traps at Añasco and Vega Alta State Forest (north coast), and also from foul water in artificial containers at Gurabo (east-central); *Psorophora jamaicensis*, found in temporary rainpools in Gurabo; *Toxorhynchites portoricensis*, collected in tire traps in Gurabo mountains, Camuy (north coast), and Guajataca

State Forest in Quebradilla (north coast); *Wyeomyia* sp., found in bromeliads at Yunque mountains in Rio Grande (northeast coast); *Culex quinquefasciatus*, found in foul water in an artificial container at Cabo Rojo, Gurabo, San Juan, Fajardo, Maricao (west-central), and Susua State Forest in Yauco/Sabana Grande (south-central); *Culex secutor*, found in tire traps, fallen palm bracts, and terrestrial bromeliads at Yunque forest; *Culex janitor*, collected from tire traps at Susua forest; and *Culex habitator*, found in brackish ground pools and tire traps in Añasco.

### Posters

#### Correlation between premise condition index and Breteau index

F. Espinosa Gómez, C. M. Hernández Suárez, X. Montoy Guzmán and I. Fernández Salas

Faculty of Medicine, University of Colima, México; and Department of Medical Entomology, Faculty of Biological Sciences, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, México

To compare the effect of an educational campaign (EC) for *Aedes aegypti* elimination versus vehicle-mounted ultra-low volume (ULV) applications of malathion, we examined 187 houses in Colima, México, during a 6-month period. Larval densities were expressed as positive container/house index (C+/H) and were determined at the beginning and at the end of the study. Houses were divided into 4 treatment blocks: A) treated with ULV alone, B) control (untreated), C) ULV application plus EC, and D) EC only. Baseline C+/H values were not significantly different in any block ( $F = 2.56$ ,  $P > 0.05$ ). Changes in the C+/H index at the end of the study were  $-0.108$  for A,  $+0.31$  for B,  $-0.183$  for C, and  $-0.870$  for D. A 2-way analysis of variance showed a significant reduction in EC vs. control ( $F = 8.4$ ,  $P < 0.0042$ ), a more discrete reduction with ULV ( $F = 0.38$ ,  $P > 0.5$ ), and a negative interaction between EC and ULV ( $F = 6.51$ ,  $P < 0.01$ ). Our results suggest that an EC is more economical, effective, and sustainable for *Ae. aegypti* breeding site reduction than traditional ULV applications, and that ULV applications could reduce the efficacy of an EC, possibly by creating a false sense of security in the community.

#### The vector control program of the Panama Canal Commission (1914–2000)

G. Dávila de De Obaldia and O. E. Pinzón

Panama Canal Commission, Environmental Division, Panama City, Panama

The construction and continued operation of the Panama Canal are made possible through the san-

itation and vector control program developed during the 86 years of operation that the Panama Canal will have had in the year 2000. Diseases such as yellow fever and malaria constantly threatened the labor force of the Panama Canal and the human population of surrounding areas. We have developed an integrated vector control program that includes the following components: channeling of standing water; ditch cleaning; collection and disposal of solid waste; monitoring of light traps; collection and identification of samples from light traps; continued search for new vectors; the elimination of breeding sites using mechanical, physical, chemical, and biological controls; and an educational and research program in the area. With this successful integrated control program, we have an interoceanic route free from tropical vector-borne diseases.

#### Contribution to knowledge of the culicids of Coahuila, Mexico

S. Vergara Pineda, O. García Martínez and H. Quiroz Martínez

Laboratorio Entomología y Acarología, Centro Nacional Ref. Fitosanitaria Gmo. Pérez Valenzuela, Coyoacán, México; and Departamento Parasitología, Universidad Autónoma Agraria Antonio Narro, Laboratorio Entomología, Facultad Ciencias Biológicas, Monterrey, Nuevo León, México

We examined 592 microscope slides of mosquito larvae and 1,447 pinned adults. From these materials, the following 22 species were identified: *Aedes albopictus*, *Ae. aegypti*, *Ae. epactius*, *Ae. scapularis*, *Ae. trivittatus*, *Ae. vexans*, *Ae. zoosophus*, *Anopheles franciscanus*, *An. quadrimaculatus*, *An. pseudopunctipennis*, *An. punctipennis*, *Culex arizonensis*, *Cx. coronator*, *Cx. erythrothorax*, *Cx. quinquefasciatus*, *Cx. stigmatosoma*, *Cx. tarsalis*, *Cx. thriambus*, *Culiseta particeps*, *Psorophora confinis*, *Ps. signipennis*, and *Uranotaenia* sp. When diversity indexes were applied, *Cx. quinquefasciatus* was the most representative in larval and adult stages, with  $P_i = 0.4375$  and  $0.466$ , respectively, whereas the least diverse was both larval and adult *An. franciscanus*, with  $P_i = 0.0016$  and  $0.0007$ , respectively. We divided the state into 3 zones (north, center, and south) and an overlapped niche was observed for larval stages between north and south zones with  $I_M = 0.9544$  and for the adult stage between north and central with  $I_M = 0.9516$ . Higher diversity was found for the south zone both for larval and adult stages with  $E = 0.8100$  and  $0.6689$ , respectively.

### Dengue stratification in a hyperendemic city using a geographical information system

R. Barrera, N. Delgado, M. Jiménez, Iris Villalobos and I. Romero

*Laboratorio Biología Vectores, Instituto de Zoología Tropical, Facultad de Ciencias, Universidad Central de Venezuela, Caracas, Venezuela; Instituto de Zoología Agrícola, Universidad Central de Venezuela, Maracay, Venezuela; and Corposalud Aragua, Maracay, Venezuela*

We analyzed the dynamics of dengue transmission in a hyperendemic city. Results were used to stratify the city for control purposes. A geographical information system incorporated the spatial nature of the disease and related environmental variables. Analyses were performed at the levels of counties (7) and neighborhoods (349) in Maracay, Venezuela, with its population of 1 million people. Variables investigated were dengue incidence, prevalence, and persistence (number of consecutive months a locality had cases) from 1993 to 1998; *Aedes aegypti* house, Breteau, and container indices; pupal production; adult mosquito density; inhabitants; area; quality of water supply and refuse services; and human mobility. Limits of counties and neighborhoods, roads, hydrology, and elevation were digitized from maps (1:5,000). Dengue prevalence was high (171 dengue and 42 dengue hemorrhagic fever [DHF] cases per 100,000 a year; 245 DHF cases per 1,000 dengue cases) with relatively low mortality (8 deaths). A temporal analysis revealed that the same neighborhoods consistently produced most of the cases every year. The relationship between dengue incidence and persistence allowed us to make a preliminary stratification of the city, so that immediate control would need to be applied to 34% of the area or 15% of neighborhoods. Further analyses are under way to determine the effect of environmental variables on dengue transmission.

### Effectiveness of Permanet® (permethrin 55%)-impregnated traps against *Chironomus plumosus*

A. Escalera, V. A. Rodríguez, H. Quiroz, K. L. Saavedra, K. Ochoa and E. Melendez

*Laboratorio de Entomología, Facultad de Ciencias Biológicas, UANL, San Nicolás de los Garza, Nuevo León, México*

The chironomid midge *Chironomus plumosus* is a special problem in public health because its annoying swarms continuously cause phobias in people who live near wastewater treatment plants. The effectiveness of Permanet® (permethrin 55%) impregnated on metal light traps against *C. plumosus*

adults was evaluated around a wastewater facility in San Nicolás de los Garza, Nuevo León, México. Four concentrations of permethrin were used as treatments and they were impregnated in the white surface of the trap. The adult mortality was registered weekly for 1 month. The results were analyzed with an analysis of variance and significant differences among concentrations and time postimpregnation were found ( $P > 0.05$ ).

### Entomological risks associated with wastewater treatment plants

M. M. Ríos, V. A. Rodríguez, K. M. Valdez, C. A. Hernández and L. Rosales

*Laboratorio de Entomología, Facultad de Ciencias Biológicas, UANL, San Nicolás de los Garza, Nuevo León, México*

Risk factors are all circumstances that affect human health. In this study, our objective was to identify the insects inside and outside of a wastewater treatment plant that could cause a public health problem. Every week, samples were taken inside and outside of the Agua Industrial Monterrey S. de U. facility. Samples were taken with an entomological net in the general vegetation, and samples were taken directly from the water. Insects were preserved in ethyl alcohol and later identified. Five insect orders were collected, which included 16 families, 15 genera, and 16 species. Of these insects, only *Chironomus plumosus* is likely to become a public health problem, because its swarms can cause panic among the public.

### Coconut and yucca agars to produce *Bacillus thuringiensis* var. *israelensis*

J. Merello, P. Ventosilla, J. Chauca, H. Guerra, B. Infante and E. Pérez

*Instituto de Medicina Tropical Alexander von Humboldt, and Universidad Peruana Cayetano Heredia, Lima, Perú*

The entomopathogen *Bacillus thuringiensis* var. *israelensis* (*Bti*) is produced commercially using expensive, complex chemical fermentation media. We produced *Bti* using coconuts (*Coco nucifera*) and yucca (*Manihot esculenta*). To 100 ml of raw coconut water and yucca infusion (15 g yucca, peeled, chopped, boiled 2 min in 150 ml of water, then filtered), we added 0.18 g of agar and then autoclaved and poured the mixture into Petri dishes. *Bacillus thuringiensis* var. *israelensis* (strain IPS-82) was plated and incubated for 96 h at 30°C. The bacteria were harvested, washed by centrifugation, and inactivated (75°C, 15 min). The coconut water agar yielded 80–85% sporulation,  $3.49 \times 10^6$  spores/ml, and a median lethal concentration ( $LC_{50}$ ) =  $2.41 \times 10^4$  spores/ml; yucca infusion agar yield-

ed 87–90% sporulation,  $1.18 \times 10^6$  spores/ml, and  $LC_{50} = 2.66 \times 10^4$  spores/ml. Dr. de Barjac's standard medium yielded 85–90% sporulation,  $10^6$  spores/ml, and  $LC_{50} = 10^4$  spores/ml. For bioassays, we used field-collected, 3rd-stage *Anopheles pseudopunctipennis* larvae. The  $LC_{50}$ s showed no significant difference between the 3 media,  $P = 0.6342$ . Triptose phosphate broth and nutrient yeast supplemented mineral agar media take longer (120 h) to produce spores. Coconut and yucca agars are appropriate alternatives for local production of *Bti*. This work was funded by Rivers of the World.

### Population fluctuations of the malaria vector *Anopheles darlingi* in southern Venezuela

Yasmín Rubio-Palís, Magda Magris, Ramón Paz and Jo Lines

*Escuela de Malariología y Saneamiento Ambiental "Dr. Arnoldo Gabaldon," Maracay, Venezuela; CAICET, Puerto Ayacucho, Venezuela; and London School of Hygiene and Tropical Medicine, London, United Kingdom*

Malaria is one of the major causes of morbidity among the Yanomami Amerindian population in southern Venezuela. Traditional vector control with residual indoor spraying of insecticides and outdoor space spraying have failed due to several factors. These include the geographic situation, with difficult access; sociocultural characteristics of the Yanomami population, with frequent migrations and housing with incomplete or no walls; circulation of *Plasmodium falciparum* strains with multiple drug resistance; and biting behavior of the incriminated vector *Anopheles darlingi*. To evaluate the impact on the vector population of an intervention involving insecticide-treated bed-nets, monthly mosquito collections were made beginning in June 1998 in 18 villages located along the Orinoco, Mavaca, and Ocamo rivers in the Upper Orinoco, Amazonas State, Venezuela. Mosquitoes were collected with Centers for Disease Control light traps set from 1800 to 0600 h within selected dwellings in each of the 18 villages for 4 nights per month every month. In the morning, mosquitoes were taken to the field base in Ocamo, identified, and counted. Mosquitoes were kept dry on silica gel for future sporozoite detection using enzyme-linked immunosorbent assay. In December 1998, bed-nets treated with  $10 \text{ g/m}^2$  of lambda-cyhalothrin were distributed to the entire population in 9 villages, whereas the population of the remaining 9 villages were provided with placebo-treated bed-nets. More than 250,000 anophelines belonging to 4 species (*An. darlingi*, *An. oswaldoi*, *An. strodei*, and *An. triannulatus*) were captured. *Anopheles darlingi* accounted for 99.8% of the mosquitoes identified. The number of mosquitoes caught per trap varied from 12 to 5,970 among villages. The village with

consistently higher mosquito densities was Ishinotheri, located on the Ocamo River. Preliminary results showed seasonal fluctuations in the abundance of *An. darlingi* in all villages, with a major peak during May 1999. Anopheline fluctuations were strongly correlated with rainfall and river level ( $P < 0.05$ ), whereas the effect of insecticide-treated nets on mosquito densities was not clear and more studies are required.

### Toxicity and sublethal effects of *Bacillus thuringiensis* var. *israelensis* (Vectobac G®) on *Aedes aegypti*

G. Ponce, A. E. Flores, M. H. Badii, M. L. Rodríguez and I. Fernández

*Department of Invertebrate Zoology and Laboratory of Medical Entomology, and Faculty of Biological Sciences, University of Nuevo León, San Nicolás de los Garza, Nuevo León, México*

*Bacillus thuringiensis* var. *israelensis* is highly toxic to mosquito larvae. The objective of this research was to determine toxicity of Vectobac® to 2nd and 3rd instars of *Ae. aegypti* and the sublethal effect of Vectobac on biological parameters of adults under laboratory conditions. On the basis of 24-h bioassays, the results indicated 0.0094, 0.0168, and 0.0302 ppm for the 30% lethal concentration (LC), median LC, and 70% LC, respectively. The biological parameters such as growth rate, survival capacity, and reproductive potential of this species under toxic effect of this pathogenic bacteria were determined and compared with the untreated control. The implications of these findings and their bearing on field-population dynamics of *Ae. aegypti* are discussed.

### Ecological aspects of *Anopheles* spp. production in excavations of abandoned mines in Matupá and Peixoto de Azevedo/Mato Grosso, Brazil

A. L. M. Ribeiro, R. H. Zimmerman and R. Dijunko Miyazaki

*Mato Grosso/Brazil's Federal University, Mato Grosso, Brazil*

We studied ponds created by abandoned mines, where anophelines are produced. Seventy-four excavations were classified according to year since abandonment (2–4.0, 4.1–6.0, 6.1–8.0, 8.1–10.0, and more than 10.1 years). We studied the distance to the city, vegetation, and limnological features of the ponds. After rainy periods, 87.8% of the excavations were positive for anopheline larvae and during droughts, 86.7% were positive. Seven species of *Anopheles* were identified. They were *Anopheles albitarsis* (s.l.), *An. darlingi* group, *An. galvaoi*, *An. nuneztovari*, *An. strodei*, *An. triannulatus* (s.l.), and *An. evansae*. *Anopheles albitarsis*

occurred both near and distant from cities; *An. darlingi* was found only 8 km from the city. Of ponds with mosquitoes, 84% contained emergent plants. The length of time of abandonment of the mines did not influence the distribution of anopheline species.

### Laboratory studies of plant extracts for controlling *Aedes aegypti*

Julian García-Rejón, Juan Chable-Santos, José Farfán-Alé, Luis Flores-Flores and María Alba Loroño

Laboratorio de Arbovirología, Centro de Investigaciones Regionales "Dr. Hideyo Noguchi," Universidad Autónoma Yucatán, Mérida, Yucatán, México

In this study, we evaluated the extracts of plants from Yucatán State, México, that could be used for the control of the mosquito *Aedes aegypti*, the main vector of dengue in Yucatán. The extracts were obtained from the mature leaves of 16 plant species, mainly ornamental varieties. The leaves were dried at 90°C in an oven and were allowed to cool to ambient temperature. Dry leaves (25 g) were added to 500 ml of water. This mixture was heated and then boiled for 3 min, and then allowed to cool for 24 h and filtered with cloth. The resulting infusion was used in the toxicity tests. For the tests, 1 ml of each infusion was added to 100 ml of water, to which were added 25 4th-stage *Ae. aegypti* larvae produced at the Centro de Investigaciones of the University of Yucatan. At 24 and 48 h postapplication, percent mortality was determined by counting larvae with abnormal movements versus normal larvae. Three repetitions were made for each treatment and respective control. Two of the 16 species tested demonstrated a larvicidal effect; lady of the night (*Cestrum nocturnum*) and the bougainvillea (*Bougainvillea butiana*), with 100 and 20% mortality at 24 h, respectively. Untreated larvae had no mortality. These preliminary results indicate that plants exist that currently only are used as ornamentals that potentially could be used as biological insecticides in México. More plant species are being tested and the close response of the 2 plants mentioned is being studied.

### Population genetics of *Anopheles albimanus* in Latin America using genetic markers: random amplified polymorphic DNA-polymerase chain reaction and mitochondrial DNA

Ana María P. de Mérida, Alvaro Molina C., Eduviges Molina de Archila, Yadira Rangel and W. C. Black IV

Universidad del Valle de Guatemala, Guatemala City, Guatemala; Instituto de Zoología Tropical, Universidad Central de Venezuela, Caracas, Venezuela; and Department of Microbiology, Colorado State University, Fort Collins, CO

*Anopheles albimanus* is a species with a wide geographic range and an extremely variable phenotype. Differing behavior, biologies, and vectorial status of many isomorphic *Anopheles* spp. make it essential to evaluate not only the seasonal population structure but also the spatial one, within the same geographic region. Little is known about molecular genetics, population structure and dynamics, barriers to gene flow, and phylogeny. Previous studies in Guatemala and a few other Central and South American countries, showed populations within <200 km of one another were panmictic. However, at distances >200 km F statistic and geographic distances were correlated, suggesting that populations are isolated by distance. In this sense, a study of this species in 10 Latin American countries is proposed, the main objective being to yield information covering these topics. The DNA was extracted by the cetyltrimethylammonium bromide method and the mitochondrial DNA (mtDNA) (ND5) region was amplified by polymerase chain reaction (PCR) by using primers M2 and P1. Single-strand conformation polymorphisms analysis was done on the PCR products. After revealing the different haplotypes found in each country, most common ones will be selected and sequenced. Sequences will be aligned in both directions (SEQUAID program) to correct the sequences. Parsimony, distance, and maximum likelihood analysis (Phylip and PAUP programs) will be performed. The 14GS06 random amplified polymorphic DNA-PCR and mtDNA markers indicate that *Aedes aegypti* populations along the northeastern coast of Mexico are isolated by distance.

### Random amplified polymorphic DNA-polymerase chain reaction and mitochondrial DNA markers indicate *Aedes aegypti* populations along the northeastern coast of Mexico are isolated by distance

Norma Gorrochotegui-Escalante, M. L. Muñoz, Ildefonso Fernandez-Salas, B. J. Beaty and W. C. Black IV

Centro de Investigaciones y Estudios Avanz, Instituto Politécnico Nacional, Mexico City, México; Department of Entomology, University of California, Riverside, CA; MEL, UANL, San Nicolás de los Garza, Nuevo León, México; and Department of Microbiology, Colorado State University, Fort Collins, CO

A population genetic analysis of gene flow was conducted among 10 *Aedes aegypti* populations along the northeastern coast of Mexico. Approxi-



mately 58 mosquitoes were sampled in each collection site from 7 cities. Four collections were made from Monterrey to examine local patterns of gene flow. Markers included 60 random amplified polymorphic DNA (RAPD) loci amplified by the polymerase chain reaction (PCR) single-strand conformation polymorphism (SSCP) analysis of variation in a 387-base pair region of the reduced nicotinamide adenine dinucleotide dehydrogenase subunit 4 (ND4) from the mitochondrial DNA (mtDNA). Seven mitochondrial haplotypes were detected and phylogenetic analysis of the sequences identified 2 well-supported clades. Regression analysis of geographic distances and pairwise F statistics estimated from RAPD markers indicated that populations are isolated by distance and suggested free gene flow among collections within 90–250 km. However, isolation by distance was not detected in a similar analysis of mtDNA haplotypes. A collection from Nuevo Laredo had a unique pattern of RAPD and mitochondrial haplotype frequencies and reduced heterozygosity, suggesting that the Nuevo Laredo population was probably founded by very few mosquitoes.

#### **Enzyme polymorphism among *Triatoma longipennis* from the west coast of Mexico: baseline data**

José Alejandro Martínez-Ibarra, Nina Malena Barcenás-López, Benjamín Noguera-Torres, Mario Henry Rodríguez-López and Porfirio Ramírez-Vallejo

*Laboratory of Genetic Markers, Colegio de Postgraduados, Texcoco, México; Parasitology Laboratory, National School of Biological Science, Ayala, México; Centro de Investigaciones de Enfermedades Infecciosas, INSP, Cuernavaca, México*

Allozyme variability in populations of the Chagas' disease vector *Triatoma longipennis* was investigated by means of starch gel electrophoresis. Samples were taken from 7 localities across the range of this insect on the west coast of Mexico where the insect is the prime vector. Zymograms for proteins coded by a total of 7 loci on 5 enzymes (esterases [ES EC 3.1.1.1.], catalase [CAT EC 1.11.1.6], malate dehydrogenase [MDH EC 1.1.1.37], malic enzyme [ME EC 1.1.1.40], and phosphoglucosomerase [PGI EC 5.1.2.9]), were obtained. All populations were highly polymorphic for these enzymes, and also had some unique alleles. Allele frequencies, proportion of polymorphic loci (P), mean heterozygosity per locus (H), and gene flow among populations will be estimated.

#### **Phylogenetic tree based on genetic distance: testing congruences in *Albimanus* Section**

Hermes Piñango and Yadira Rangel

*Instituto de Zoología Tropical, Laboratorio de Genética de Poblaciones y Vectores, Universidad Central de Venezuela, Caracas, Venezuela*

Congruence of trees from morphologic and molecular data is a theme in many recent systematic papers. However, the congruence between isozyme and morphologic or molecular trees has been underemphasized. The underlying notion is that any 2 data sets from a single population sample must be consistent, regardless of the apparently discordant topologies of the trees. To perform a preliminary evaluation of this issue, we used data of allele frequency obtained by protein electrophoresis of 4 Venezuelan species belonging to the *Albimanus* Section of *Anopheles* (*Nyssorhynchus*) and from a species belonging to the sister section (*Albitarsis*). Such data were analyzed from the standpoint of population genetics and phylogenetic framework, and contrasted with the prevailing classification for the section. We found partial correspondence both from population genetic and phylogenetic analyses. However, we must use the complete array of biochemical and molecular characteristics of species to establish phylogeny.

#### **Infestation rates for the triatomines in the west coast of Mexico**

J. A. Martínez-Ibarra, R. Alejandro-Aguilar, N. M. Barcenás-Ortega, B. Noguera-Torres, E. Magallón-Gastélum and L. M. Rodríguez-Sánchez

*Colegio de Postgraduados, IFIT, México-Texcoco, Texcoco, México; Laboratorio de Parasitología, E NCB/IPN Mar Carpio y Plan de Ayala, México, Distrito Federal; and Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Jalisco, México*

Chagas' disease is a serious public health problem in most American countries. In Mexico, little is known about this triatomineborne disease and the risk of *Trypanosoma cruzi* infection for human populations. Few studies have been conducted on entomological indicators recommended by the Pan American Health Organization and World Health Organization for evaluating human risk. Our team found 3 *Triatoma* species in Jalisco state: *T. longipennis* (3 localities), *T. picturata* (1 locality), and *T. pallidipennis* (1 locality). Only *T. longipennis* had 2 indicators suggesting risk for humans: colonization indicator (41.7%, 5 of 12 houses), and natural infestation (19.6%, 28 of 143 triatomines). In Nayarit, indicators for only 2 species were found: *T. longipennis* colonization was 50–100% and nat-

ural infestation was 19.6–100%, whereas for *T. picturata*, colonization was 100% in all the localities and natural infestation was 19.6–100%. According to analysis of these data, human populations in both study areas are at risk of being infected with *T. cruzi* by the triatomines.

### Resting site selection in *Triatoma gerstaeckeri* determined by its physiologic stages

J. L. Torres-Estrada, J. A. Martínez-Ibarra,  
J. A. García-Pérez and F. Santillán-Valenzuela

*Centro de Investigación de Paludismo, INSP/ISSA, Tapachula, Chiapas, México; Colegio de Postgraduados, IFIT, México-Textcoco, Textcoco, México; and Facultad de Ciencias Biológicas, UANL San Nicolás de los Garza, Nuevo León, México*

Few studies have been conducted in Mexico on triatomine behavior. *Triatoma gerstaeckeri* is one of the most dangerous vectors of *Trypanosoma cruzi* in northeast Mexico. The influence of combinations of 2 different physiologic stages in the selection of resting sites on adults of *T. gerstaeckeri* was studied. An 8-m<sup>3</sup> light-proof cage was built using glass and steel. Three simulated indoor walls were built of concrete block, cardboard, or ditched reed grass (1 of each) and 2 simulated mouse shelters were added, 1 with a mouse inside and the other empty. Four groups of 10 adult triatomines, each with a combination of fed or fasted and fecund or unfecund stage were released in the center of the cage, 1 by 1. A typical rural house was used for a similar field experiment. Under laboratory conditions, most fed triatomines preferred climbing the concrete block wall as a resting site, whereas most fasted bugs preferred climbing the cardboard wall, all of them independently of their stage of fecun-

dity. Under field conditions, most triatomines preferred to stay on the ground without climbing any surrounding wall, independently of their stage of fecundity. Results from these field experiments were consistent with previous experiences in finding triatomines under natural conditions.

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