

MOSQUITO VECTOR CONTROL AND BIOLOGY IN LATIN AMERICA—A TWELFTH SYMPOSIUM

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ABSTRACT. The 12th annual Latin American symposium presented by the American Mosquito Control Association (AMCA) was held as part of the 68th Annual Meeting in Denver, CO, in February 2002. The principal objective, as for the previous 11 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 35 presentations that were given orally in Spanish or presented as posters by participants from 7 countries in Latin America. Topics addressed in the symposium included results from chemical and biological control programs and studies; studies of insecticide resistance; and population genetics, molecular, ecological, and behavioral studies of vectors of dengue (*Aedes aegypti* and *Aedes albopictus*) and other arboviruses, malaria (*Anopheles albimanus* and *Anopheles pseudopunctipennis*), leishmaniasis (*Lutzomyia*), and Chagas Disease (*Triatoma*). Related topics included biology and control of *Culiseta inornata*, *Ochlerotatus taeniorhynchus*, scorpions, *Chironomus plumosus*, and *Musca domestica*.

KEY WORDS Mosquitoes, mosquito control, bionomics, *Aedes*, *Anopheles*, *Culiseta*, *Ochlerotatus*, *Lutzomyia*, *Triatoma*, scorpions, *Loxosceles*, *Musca domestica*, *Chironomus*, insecticide resistance

INTRODUCTION

The American Mosquito Control Association (AMCA) is dedicated to the study and control of mosquitoes, other vectors, and other arthropods and promotes cooperation and interaction among professionals and students in this field both in the USA and internationally. To promote greater and more active participation among and with a portion of its international membership, a Spanish language symposium was held 1st at the AMCA Annual Meeting in 1991 and at all 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 35 presentations that were given in Spanish by participants from 7 countries in Latin America. Topics addressed in the symposium included results from chemical and biological control programs and studies; studies of insecticide resistance; and population genetics, molecular, ecological, and behavioral studies of scorpions, *Chironomus plumosus*, and *Musca domestica*, and vectors of dengue (*Aedes aegypti*) and other arboviruses, malaria (*Anopheles albimanus* and *Anopheles pseudopunctipennis*), leishmaniasis (*Lutzomyia*), and Chagas Disease (*Triatoma*). Other subjects included biology and control of *Culiseta inornata* and *Ochlerotatus taeniorhynchus*. Summaries of 10 previous symposia

have been published (Clark and Suarez 1991, 1992, 1993; Clark 1995, 1996; Clark and Rangel 1997, 1998, 1999; Clark et al. 2000; Clark and Quiroz-Martinez 2001).

Financial support for this session was provided by the following sponsors and individuals: Clarke Mosquito Control Products (Lyell Clarke III), H. D. Hudson (Manuel Lluberas), Vopak (Benjamin Gomez), Wellmark (Larry Smith), Rockefeller Foundation, and the Pan American Health Organization. Funds from these sponsors provided Annual Meeting registration and partially defrayed travel expenses for many symposium participants. Enthusiasm and interest for this symposium among Spanish- and non-Spanish-speaking participants was high and it will continue to be a part of future meetings.

SUMMARIES

Characterization of anopheline breeding habitats in the Upper Orinoco River, Amazonas State, Venezuela

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Between October 1999 and January 2000, anopheline breeding habitats were identified and characterized in Ocamo area, Municipality of Upper Orinoco, Amazonas State, in southern Venezuela. During the study, 46 samples were taken

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from 4 types of larval habitats: lagoon (37), ponds (2), pools (1), and streams (6). Anopheline larvae were present in 38 of the habitats sampled. A total of 1,303 anopheline larvae were collected, belonging to 7 species: *Anopheles oswaldoi*, *An. darlingi*, *An. triannulatus*, *An. mattogrossensis*, *An. punctimacula*, *An. mediopunctatus*, and *An. braziliensis*. Not all of the larvae collected were identified to species because many 1st and 2nd instars failed to reach the 4th larval instar or adults. The most abundant and frequent species collected was *An. oswaldoi*, followed by *An. triannulatus* and *An. darlingi*. The remaining species accounted for 10% of the collections. Lagoons were the most productive breeding sites in terms of the total number of larvae collected (1,034) and species diversity, although density was low (0.59 larvae/liter). The biotic and abiotic factors that influenced the species collected were determined by an analysis of canonic correspondence (CANOCO). The biotic factors analyzed were type of vegetation, percentage of cover, and anopheline larval instars collected, whereas the abiotic factors considered were type of larval habitat, water depth (cm), pH, temperature (°C), shade, and water permanence (permanent vs. temporal). The CANOCO showed that water depth significantly determined species abundance, whereas pH and water temperature had little effect. *Anopheles triannulatus* was found in deeper waters, whereas *An. darlingi*, *An. oswaldoi*, and *An. mattogrossensis* were found in shallower sites. Regarding species association with biotic factors, a positive association was found between *An. triannulatus*, *An. darlingi*, and *An. mattogrossensis* and 4 variables: pupal density, larval density, submerged vegetation, and floating seeds. On the other hand, these species showed a negative relation with 4 other variables: shrubs, floating vegetation, algae, and grasses.

Bionomics of *Anopheles* mosquitoes in malaria residual foci of Oaxaca, México

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We conducted studies on the bionomics of *Anopheles* mosquitoes in malaria foci of Oaxaca, México. Few studies, if any, have been carried out in the area, so more knowledge on the dynamics of mosquitoes was needed. Fluctuations in mosquito population densities, larval habitats, host-seeking behavior, and feeding patterns were assessed between July 2000 and June 2001 in 2 coastal villages and 2 mountainous villages.

Three species of anopheline malaria vectors were collected on humans or mammals. In coastal villages, collections yielded *Anopheles albimanus* (93–97%), *An. pseudopunctipennis* (1–7%), and *An. punctimacula* (2%), whereas mosquitoes found in villages of the foothills area were *An. pseudopunctipennis* (90–99%), *An. punctimacula* (8%), and *An. albimanus* (1–2%). *Anopheles albimanus* was active throughout the night, showing a significant increase in biting rate before midnight, whereas the *An. pseudopunctipennis* biting peak occurred after midnight during the wet season in villages located at a lower elevation. Blood-meal analysis indicated that goats (38.7%) and humans (35.5%) were most preferred by *An. albimanus* in coastal villages, whereas <47% of female *An. pseudopunctipennis* and 100% of *An. punctimacula* fed on a horse in the same area. Both horses (20.6%) and dogs (20.6%) were selected by *An. pseudopunctipennis* over human hosts (10.7%) in villages in the foothills.

Biological variation among *Anopheles vestitipennis* populations in southern México

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Studies that used genetic and egg ornamentation of populations of *Anopheles vestitipennis* revealed the possible existence of distinct anthropophilic and zoophilic sympatric populations. In this study, we compared the length of the gonotrophic cycle, survival, and the oogenic development of both populations. The study was carried out in Nueva Independencia, Chiapas, in southern México. Mosquitoes were collected with sets of 2 traps baited either with 2 men or a horse between 1800 and 2400 h. The gonotrophic cycle was estimated with a cross-correlation analysis, whereas the survival rate was assessed with a vertical method. Daily changes of parity rates gave significant correlation indices at 3 days ($r = 0.292$, $P < 0.001$) and 4 days ($r = 0.918$, $P < 0.5$) in the zoophilic and anthropophilic populations, respectively. The survival rate was 0.93 and 0.88 and the minimum time required to develop mature eggs after bloodfeeding was 54 h and 60 h in females from zoophilic and anthropophilic populations, respectively. Differences observed in these biological parameters provide additional support for the possible existence of subpopulations with distinctive feeding preferences within *An. vestitipennis*.

Field evaluation of Agnique MMF® to control *Anopheles albimanus*, a malaria vector, in Chetumal, Quintana Roo, México

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The Malaria Control Program in Quintana Roo State has relied in recent years on the following activities: wall spray with Deltamethrin WP, ultra-low-volume space-spraying with permethrin, liquid Temephos (50%) against larval populations, and infrequently, cleaning of green algae in vector breeding sites. Beginning in 2000, the state malaria control program started evaluating products that were less toxic to the environment and human health. The program tested Agnique MMF® as a potential choice against larval and pupal populations of *Anopheles albimanus* (Wiedemann). Agnique MMF produces a monomolecular film that acts on all aquatic instars of Culicidae. Agnique MMF reduces water surface tension at the air-water interface, thus suffocating larvae and pupae, which drown and sink to the bottom. Newly emerged male and female mosquitoes also drown. Ovipositing females stick to the water surface and die. To evaluate its effectiveness in reducing immature and adult stages of *An. albimanus*, Agnique MMF was evaluated in the field by using a Hudson X-pert backpack to dispense the material at a rate of 4.7 liters/ha. Larval densities were measured 3 days before treatment with 100 dips per day and for 15 days after treatment. We found that larvae started dying 24–48 h after applications, with 100% mortality at 72 h. Human landing collections recorded the impact of Agnique MMF on adult *An. albimanus* populations. Landing collections by 2 collectors were performed for 15 days after treatment and a 60% reduction of female mosquitoes was found compared to the untreated area. Changes in age structure of females confirmed reduction of older individuals with an increase of nullipars from 37% before treatment to 72% after treatment. The untreated area located 2 km away had results similar to the pretreatment period. Because of the prolonged 21-day residual effect and safety to nontarget aquatic organisms, Agnique MMF was incorporated as a new tool for control of larval *An. albimanus* in the Quintana Roo Vector Control Program.

Large-scale evaluation of Vectobac® AS and Vectolex® CG for the control of *Anopheles pseudopunctipennis* in Oaxaca, México

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A large-scale study was carried out during the dry seasons of 2000 and 2001 to evaluate the impact of 2 bioinsecticides, VectoBac® AS (*Bacillus thuringiensis israelensis*) and VectoLex® CG (*Bacillus sphaericus*) on larval and adult populations of *Anopheles pseudopunctipennis* in Oaxaca, México. Experimental areas included 3 groups of 3 villages, with each group corresponding to 1 treatment and 1 control. Larvicide was applied with hand compression pumps (2000) or mist blowers (2001) on all water sites located within a 1.5-km radius surrounding each group of villages, at rates of 4 liters/ha (VectoBac) or 11.2 kg/ha (VectoLex). Entomological evaluations included larval surveys and adult collections conducted inside or outside houses of the area with ultraviolet-updraft light traps. Because bioinsecticide applications were made mostly in slow-running waters where larvae of *An. pseudopunctipennis* prevail, larvicidal effect persisted less than 7 days for both products. However, this was sufficient to sharply reduce the number of mosquitoes for more than 14 days after application of larvicide, especially in the VectoBac area. We concluded that the application of VectoBac could be a good complement to suppress mosquito larvae in this area, where environmental manipulation of filamentous algae, the primary source for *An. pseudopunctipennis* breeding, has been very successful.

Preliminary evaluation of indoor house spraying of biphenthrine 10 WP on *Anopheles albimanus* in malarious areas of Chetumal, Quintana Roo, México

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Studies of the residual effects and behavioral changes of *Anopheles albimanus* to biphenthrine were conducted in 5 localities near Chetumal City,

Quintana Roo State, in southern México from July to September 2001. Wall bioassays to determine residuality showed 100% mortality after 15 and 30 days after treatment when a Hudson X-pert backpack was utilized, whereas 94.1% mortality resulted when fast spraying was done with low-volume-dispensing backpacks for the same periods. Human landing mosquito collections showed ranges of 1–5 bites/human/h indoors compared to 10–24 bites/human/h outdoors. Temporal changes of female age structure of *An. albimanus* showed a reduction in parity from 54 to 21%. Endophilic behavior of indoor-resting females also was assessed. We found mosquitoes in only 7 of 94 sprayed houses, with a total of 8 *An. albimanus*; however, after 24 h they were all dead. All 8 females had ovaries in Sella's stages 1 or 2, suggesting that they were freshly fed. A Colombian curtain was set to measure repellency and irritability to biphenthrin and both behaviors were recorded. Female *Ae. albimanus* rested on biphenthrin-sprayed walls for a 2-h prefeeding period inside the experimental house and tried to exit at least 2 h after feeding. A group of 560 field-collected mosquitoes were released and only 10% tried to escape. These individuals had 100% mortality after 24 h.

Integrated control of *Anopheles albimanus* larval populations in southern Chiapas, México

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We are investigating the integrated control of larval *Anopheles albimanus* and its impact on the number of biting adults in neighboring villages along the coastal plain of Chiapas, México. Tactics being evaluated separately or together, and being applied as needed, include environmental management (i.e., elimination of vegetation that favors breeding and elimination of puddles within villages due to tire tracks, open sewage canals, and so on), all with strong community participation; application of *Bacillus sphaericus* (VectoLex® CG) with a mist blower at rate of 1 g/m²/sec; and application of larvivorous fish (*Poecilia sphenops*) only in artificial water collections (e.g., irrigation canals, drainage canals, roadside ditches, and large puddles due to recurrent tire tracks on the streets of villages) at rates of 150 fish/ha. All these tactics were compared with an untreated control. Tactics are being applied in all breeding sites situated within a 2-km radius around the villages. Evaluations include weekly larval surveys and indoor-outdoor adult

collections (landing counts and ultraviolet-updraft light traps). Thus far, $\geq 80\%$ and $\geq 50\%$ reductions of larval and adult populations of *An. albimanus*, respectively, have been obtained by integrating all control tactics compared to untreated locations.

Biology of *Triatoma picturata* (Hemiptera: Reduviidae) under laboratory conditions

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Triatoma picturata is one of the most important vectors of Chagas Disease in México. In western México, *T. picturata* is commonly found in human habitations, chicken coops, and in some sylvatic habitats. The biology of this species is poorly understood, in spite of its active domestication, frequent contact with humans, and high *Trypanosoma cruzi* infection rates. Taking into account previous information on the host preference of *T. picturata*, the objective of this study was to understand its life cycle, number of blood meals required before molting, and parameters related to vectorial capacity of a triatomine species fed on leghorn hens under laboratory conditions. Time until feeding began, time required to get a complete meal, and defecation patterns were recorded. Specimens were separated in groups of 12 1st-stage nymphs, placed in plastic containers (10/container), fed weekly on leghorn hens, and checked daily for molting and mortality. The mean time for the life cycle was 165 ± 6.7 days, with fewer than 10 blood meals needed for molting 5 times from 1st instar to adult. All stages, including adults, began feeding within 5 min and were in contact with the host less than 5 min. First, 3rd, and 5th nymphal instars and adults defecated soon (less than 5 min) after feeding. With the short life cycle, aggressiveness, and rapid defecation behavior of *T. picturata*, it can be considered an important vector of *T. cruzi* to humans.

Control of the black salt-marsh mosquito *Ochlerotatus taeniorhynchus* at the Panama Canal

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Ship transits through the Panama Canal require more than 30 h. Annoyance by mosquito bites represents one of the main complaints of discomfort by the Canal's labor force. Unusually high

tides and rains in salt-marsh areas near the Canal in May 2001 increased the populations of the black salt-marsh mosquito (*Ochlerotatus taeniorhynchus*). The nuisance produced by large swarms of this mosquito and its role in the transmission of Venezuelan equine encephalitis virus compelled us to double our efforts and treat this mosquito problem as an emergency situation within our interagency pest management program. Efforts stressed physical control through ditching and draining of swampy areas, maintenance of tide gates, and chemical control with larvicides and adulticides. In addition, recommendations for the use of long-sleeve shirts and repellents on exposed areas of the body, and the installation of mosquito screens on doors and windows, as well as curtains in shop doors, were issued. In 1 month, we managed to reduce the number of black salt-marsh mosquitoes collected in New Jersey light traps from 2,500 to less than 100 mosquitoes per trap night.

Aquatic Diptera of the Pesqueria River: entomological risks of polluted waters

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Polluted waters are ideal breeding sites for several species of aquatic insects, especially those included as r-selected; among them several species can be an entomological risk to human health. Our objective was to identify the aquatic Diptera of Pesqueria River, one of the most polluted aquatic systems in Nuevo Leon State. Three square meters of the bottom of this aquatic system were taken from 6 study sites along the Pesqueria River. An entomological net was introduced after the substrate was removed and the organisms were transferred into the net. All insects were preserved in ethyl alcohol and identified. A total of 116,076 Diptera larvae were collected in 10 sample dates. These were from 9 families that included Chironomidae, Ceratopogonidae, Culicidae, Psychodidae, Stratiomyidae, Tabanidae, Muscidae, Syrphidae, and Ephydriidae. The dominant species was *Chironomus plumosus* (99.73%). We also found *Culex pipiens*, *Cx. interrogator*, *Cx. erraticus*, *Anopheles pseudopunctipennis*, and *Chrysops* sp.

Serologic survey of arboviruses in Lacandon Forest area from Chiapas, México

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A serologic survey in domestic animals (birds and mammals) was conducted in 5 communities in the Lacandon Forest, Chiapas, during May 28–June 2, 2001, with the objective of identifying arboviruses circulating in this area. We collected sera from healthy domestic chickens, domestic geese, domestic ducks, domestic turkeys, horses, and cattle. The samples were tested by plaque-reduction neutralization test for antibodies to West Nile, western equine encephalomyelitis, eastern equine encephalomyelitis, Venezuelan equine encephalitis (VEE), St. Louis encephalitis (SLE), and Ilheus and Bussuquara viruses. Neutralizing antibodies to SLE were detected in 2 (2%) of 91 chickens, 2 (7%) of 27 turkeys, 16 (24%) of 67 cattle, and 3 (60%) of 5 horses. Antibodies to VEE were detected in 27 (40%) of 67 cattle and 1 (20%) of 5 horses. No neutralizing antibodies against the other viruses were detected, although cross-reacting flavivirus antibody was detected in a single cattle specimen. Our results of arbovirus activity are the 1st reported from Chiapas.

Preventing dengue: the Nuevo Leon Vector Control Program model in northeastern México

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Dengue fever is considered a priority for public health authorities in México. Disease incidence is accentuated in urban areas because of the indoor-resting behavior of *Aedes aegypti*. Control experts believe that the most important dengue prevention measure will be attained by promoting community participation, and an efficient education program to change human behavior is under way. However, chemical control interventions need to be conducted when outbreaks appear. In México, when important dengue epidemics occur, vector control measures with short-term duration are applied. In Nuevo León State, short-term and simultaneous prevention interventions were applied in 2000 and 2001. In other words, we acted as though an emer-

gency had occurred, when none existed. The successful prevention model reduced cases from 11,000 in 1999 to 25 and 10 cases for 2000 and 2001, respectively. The latter were confirmed by enzyme-linked immunosorbent assays in the state laboratory. Our experience led us to build a prevention model based on epidemiological and entomological history. In Nuevo León State, monthly dengue frequency was detected in August and September (higher rainfall and fall-like temperatures favoring vector longevity) when the 1st outbreaks appeared. Considering virus extrinsic and intrinsic incubation periods, earlier cases started occurring in July and August. These months coincided with the summer vacations of university students and their increased travel activity. Similarly, we found that house indices of *Ae. aegypti* increased in June and peaked in July. These data came from 2 years of entomological monitoring. We decided to intervene in 3-month cycles throughout the year. In these activities, we visited 600,000 houses to check and eliminate breeding sites and covered 30,000 blocks with ultra-low-volume space-spraying. As a result, the house index was reduced to 5% in June, July, and August. Similarly, larval indices were lowered more than 6% below significant epidemiological values. We summarized our model in the following way: analysis of historical epidemiological and entomological data may help launch intensive, short-term, integrated adult and larval control measures in a timely manner to prevent risky vector-dengue-environment situations efficiently, and therefore avoid or reduce dramatically dengue outbreaks during the peak transmission season. We also agreed that community participation in the midterm would have the highest impact on control of *Ae. aegypti*.

Baseline space treatments of Aqua-reslin Super®, Anvil®, and Solfac® for the control of *Aedes aegypti*

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We studied the efficacy of space treatments of 3 insecticides for the control of the dengue virus vector *Aedes aegypti*. Insecticides tested were water-based Aqua-Reslin Super®, and mineral oil-based Anvil® or Solfac®, applied at rates of 11, 6, and 2 g/ha, respectively, with London®, HP®, or Microgen® pumps mounted on vehicles. Studies included standard linear tests situating sets of 2 cages with 20 F₁ mosquitoes held on T-poles at 20, 40, 60, 80, and 100 m; and house tests situating sets of 2 cages holding 20 F₁ mosquitoes on the front porch, living room, bedroom, and backyard. Exposure times

were 30 and 60 min, respectively, for the linear tests and house tests. Overall results indicated that mineral oil-based insecticides (Anvil and Solfac) were more effective than water-based insecticide (Aqua Reslin-Super). Effective average mortality ($\geq 75\%$) in linear tests was observed up to 80 m in Anvil and Solfac tests, whereas 69% mortality could only be observed at 40 m in Aqua Reslin-Super tests. In house tests, Anvil yielded mosquito mortalities $\geq 85\%$ in the front porch, living room, bedroom, and backyard. Therefore, Anvil performed better than the other insecticides evaluated to control *Ae. aegypti* in Chiapas, México.

Impact of insecticide space treatments on *Aedes aegypti* adult population of Tapachula, Chiapas, México

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To determine whether the dengue virus vector *Aedes aegypti* is controlled by space treatments of insecticide, we assessed the impact of 3 insecticide products, Aqua Reslin-Super®, Anvil®, and Solfac®, in 7 experimental sets of 9 blocks in Tapachula, Chiapas, México. Insecticides were applied on 1 or 2 consecutive days during each of 3 fortnight rounds at rates of 11, 6, and 2 g/ha for Aqua Reslin-Super, Anvil, and Solfac, respectively. Entomological impact was assessed by oviposition rates, number of eggs per ovitrap, house index for adults, and mean number of adults per house throughout the trial. Results indicated that oviposition rates were not reduced by any of the treatments compared with the control, perhaps because of input of mosquitoes from surrounding untreated houses. However, the mean number of eggs per ovitrap, house index for adults, and mean number of adults per house was significantly reduced in the Anvil area with respect to the control, followed by Aqua Reslin-Super and Solfac. Comparing 1- or 2-day consecutive treatments, no differences were observed in the Anvil area. Therefore, although populations of *Ae. aegypti* were not eliminated by ultra-low-volume treatments, significant reductions were observed after 1-day application of Anvil.

Susceptibility of *Aedes aegypti* larvae to organophosphates and synthetic pyrethroids insecticides in Nuevo Leon, México

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The Nuevo León State, in northeastern México, officially reported 4,769 cases of dengue fever in

1999; 32.5% of all cases reported in México. This is the 1st study to document the susceptibility of historically used insecticides against the dengue vector, *Aedes aegypti*, in Nuevo León State. Mean lethal concentrations (LC_{50} s) in natural mosquito larval populations from dengue-endemic cities were determined. The chemicals evaluated were malathion 50%, temephos 50%, permethrin 95.92%, and bifenthrin 93.25%. Bioassay methods followed the 1981 World Health Organization standard protocol and results were analyzed by probit analysis. The LC_{50} results for malathion were 0.326, 0.101, 0.247, 0.207, 0.176, and 0.209 ppm for the cities of Monterrey, San Nicolás, Villa de Santiago, Allende, Guadalupe, and Escobedo, respectively. The LC_{50} results for temephos were 0.00016, 0.00058, 0.00054, 0.00050, and 0.00072 ppm for the municipalities of Monterrey, Villa de Santiago, Allende, Guadalupe, and Escobedo, respectively. The LC_{50} results for permethrin were 1.3×10^{-5} , 1.8×10^{-5} , and 1.5×10^{-5} for the cities of Monterrey, Guadalupe, and Villa de Santiago, respectively. Concentration ranges of bifenthrin were between 0.0001 and 0.00055, 0.0001 and 0.0004, 0.00015 and 0.0005, and 0.0009 and 0.0025 ppm for the cities of Monterrey, Linares, Villa de Santiago, and Allende, respectively.

Adult and larval *Aedes aegypti* susceptibility to bifenthrin in Nuevo León, México

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Dengue control has been hampered in México and other Latin American countries by increasing resistance to commonly used chemical insecticides. Resistance has extended to most toxicological groups, including organochlorines, organophosphates, and synthetic pyrethroids. The absence of a dengue vaccine or preventive medications increases the importance of mosquito control activities. Therefore, the search for new and more efficient chemicals has become an important activity for vector control programs in developing countries. This study was conducted to assess the susceptibility and resistance of *Aedes aegypti* to bifenthrin (0.1%), a pyrethroid recently approved by the World Health Organization (WHO) and the México Vector Control Program. We evaluated this new chemical on mosquito populations in 4 dengue-endemic cities in northeastern México. The WHO bioassay methodology for adult mosquitoes was used. Susceptibility results showed 100% mortality after 24 h on *Ae. aegypti* from Guadalupe, Allende, Apodaca, and Villa de Santiago. Likewise, the me-

dian lethal time with 0.1% bifenthrin was 36.73 min for the entire mosquito female population. Analysis of our data indicated that bifenthrin could quickly replace traditionally used adulticides in campaigns against *Ae. aegypti*. However, more research is needed to evaluate potential cross-resistance and use of bifenthrin as a new tool for integrated vector control programs.

Preliminary field evaluation of Bistar 1.5% ULV (bifenthrin) against the dengue vector *Aedes aegypti* in Allende, Nuevo León, México

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The decision of México at the end of the 1990s was to ban malathion for control of adult populations of the dengue vector *Aedes aegypti*; this decision was supported by strong suspicions of resistance to this organophosphate, which had been used for 30 years. Consequently, this motivated control programs to search for alternate adulticides for use against vector populations because of the increasing public health problem caused by dengue viruses. Bifenthrin (Bistar® 1.5 ULV) in an oil-based formulation, a synthetic pyrethroid recently introduced in México, was evaluated in the field. Study sites were established in Allende, 50 mi east of Monterrey. A field test was conducted in the residential area Colonia Los Perales, which has 2-level concrete houses. Adulticide effectiveness was determined through ultra-low-volume (ULV) space-spraying against indoor and outdoor cages of female *Ae. aegypti*. A total of 20 nylon mesh cages measuring 30×15 cm contained laboratory-reared, 3-day-old female *Ae. aegypti*. Ten cages were placed in each of 2 inhabited houses in typical indoor and outdoor resting places for this species, such as kitchen, living room, dining room, under beds, closets, gardens, backyards, and laundry. Bistar 1.5 ULV was sprayed at a flow rate of 2.5 oz/min with a 2-nozzle Typhoon II vehicle-mounted ULV generator, driven at 10 mph. Teflon-coated slides measured droplet size averaging 20.5 μ m. More than 87% of droplets were between the ranges of 15 and 25 μ m. Wind speed was less than 2 mph, and ambient temperature and relative humidity were 25°C and 91%, respectively. Two cages with 20 mosquitoes each were set as controls about 10 mi away from the study site. Mortality was recorded at 1 h after treatment, and total mortality was recorded at 24 h. Mean indoor and outdoor mortality at 24 h was 75.2%; indoor mor-

tality was higher (78%) as compared to outdoors (72%). The highest mortality (88%) was found in living rooms, whereas the lowest mortality (60%) was found in kitchen areas. Outdoor sites produced only 68% mortality. Control cages had 2% mortality. We conclude that bifenthrin (Bistar 1.5% ULV) is a potential tool for control programs against *Ae. aegypti*, however, more research is still needed to improve effectiveness, including testing slightly higher flow rates.

Preliminary identification of acetylcholinesterase gene (*Ace^R*) in *Aedes aegypti* from 10 ecological settings of Veracruz State, México

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The target site of the organophosphate and carbamate insecticides is acetylcholinesterase (AChE), a serine esterase that hydrolyzes the neurotransmitter acetylcholine. Once these insecticides inhibit AChE, paralysis or death of the insect occurs. Two mechanisms confer high resistance to organophosphorus insecticides: increased detoxification by overproduced esterases and insensitive AChEs. These mechanisms have been described in many insect species. Normal and insensitive AChEs are transmitted as monofactorial characters (*Ace* gene). Formal genetics studies have shown 2 AChE forms: a normal form coded by allele *Ace^S* and a form insensitive to inhibition coded by *Ace^R*. The use of malathion to control mosquitoes in México during the last 22 years has selected for those mechanisms and it is obvious that after decades of prolonged use, selection pressure has been exerted on natural mosquito populations. The latter effect also has been augmented by concurrent use of the same chemicals for agriculture pests. To conduct research on population genetics of resistance, field strains of *Aedes aegypti* were selected to be used as reference strains in the laboratory. This study evaluated resistance mechanisms and therefore usefulness of organophosphorus and other insecticides for dengue fever vector control in México. In August 2001, larval sampling of *Ae. aegypti* was carried out from 10 localities in Veracruz State, southeastern México. Larvae were transported to the Laboratory of Medical Entomology at the University of Nuevo León for rearing. A polymerase chain reaction-based method was used to detect *Ace^R* in the genome of single mosquitoes. The distribution of the

Ace^R is reported in *Ae. aegypti* from northern to southern Veracruz, México.

Population genetics of *Anopheles albimanus* in Latin America using microsatellite markers

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Anopheles albimanus, one of the main vectors of malaria in Latin America, has a wide geographical distribution and a variable phenotype that makes it essential to evaluate the structure of its populations. Proper identification combined with epidemiological and transmission studies can improve malaria control. Four microsatellite markers (MS1-90, MS2-14, MS6-41, and MS2-25) were used to establish the population structure and phylogenetic relationships of *An. albimanus* in Latin America. Mosquitoes were collected in 9 countries, with a total of 30 collections and 1,412 individuals. After extraction of DNA from individual mosquitoes, microsatellite markers were amplified by polymerase chain reaction. The genotype for each locus was determined for individual mosquitoes in polyacrylamide sequencing gels. The markers MS1-90, MS2-14, MS2-25, and MS6-41 presented 23, 25, 39, and 13 alleles, respectively. In an analysis of variance of genotype frequencies in 5 regions (Cuba, México to Nicaragua, Costa Rica–Panama, Venezuela, and Colombia), 89.91% of total variation in allele frequency arose among individuals in collections, whereas only 1.55% of variation arose among collections in groups and 8.55% of variation arose among groups or regions. The fixation index for the regions was estimated at 0.085. The results suggest partial genetic isolation with respect to Central America, especially of Venezuelan and Colombian populations.

Presence of large genetic distances in *Aedes aegypti* populations on México's Pacific coast

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A population genetic analysis was conducted among 20 populations of *Aedes aegypti* from 19 cities along the southern Pacific coast in the states of Guerrero, Oaxaca, and Chiapas of México and in Coatepeque, Guatemala. Markers included 131

random amplified polymorphic DNA (RAPD) loci amplified by polymerase chain reaction. Regression analysis of geographic distances and pairwise F_{st} estimated from RAPD markers indicated that populations are isolated by distance. Furthermore, cluster analysis of $F_{st}/(1 - F_{st})$ failed to group collections by geographic proximity and as much genetic variation was found among collections within a 60-km radius as was found among all collections throughout the approximately 900-km range. This large genetic differentiation found in southern coastal México may be due to reduced gene flow arising through reduced human commerce and a greater diversity of habitats and altitudes among collection sites.

Protection of *Aedes aegypti* eggs in common breeding sites from Mérida, Yucatán, México, during dry periods

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Aedes aegypti is the vector of dengue viruses in the Yucatán Peninsula of México. Mosquito breeding sites frequently are small water containers distributed indoors and outdoors. Mosquito eggs are resistant to desiccation and can remain viable for long periods of time. When the rainy season begins, eggs hatch and adult populations increase rapidly. Adult mosquito abundance depends in part on the protection that the container's microenvironment provides for the eggs during the dry season. The objective of this study was to evaluate the capacity of selected container breeding sites in Mérida to enhance survivorship of eggs of *Ae. aegypti* during the dry season. This study was conducted from January to July 2000. Eggs were obtained from a colony of *Ae. aegypti* from Mérida and collected on paper strips containing about 100 eggs each. The strips were placed inside organly bags. Six bags with 3 strips each were placed inside 6 different containers: aluminum can, tire, glass bottle, plastic vessel, ceramic flower pot, and concrete water container. One bag was collected from each container and from the insectary (control) after periods of 15, 30, 60, 90, 120, and 150 days. Every strip was immersed in a plastic vessel containing 20 ml of water. The number of emerged larva was recorded at 12, 24, and 48 h. Larvae were transferred to containers with 100 ml of water. The proportion of adults that successfully emerged was determined for each container type. Most of the containers pro-

vided environmental conditions that protected the eggs during the dry period; the proportions of adults that successfully emerged from these containers were similar to the control. However, the proportions of adults that emerged from plastic bottles and concrete water containers were significantly reduced compared to the control. This suggested that these types of containers do not protect mosquito eggs against desiccation.

The lethal effects of *Eugenia melanadenia* and *Psidium rotundatum* on *Aedes aegypti*

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Dengue is a mosquito-borne disease for which there is no vaccine, and mosquito control constitutes the principal means of preventing this disease. The larvicidal effect of certain plants has been recognized as an environmentally safe vector control option since ancient times, although it is seldom exploited. Commercial larvicides are expensive, inaccessible, and have limitations on their use; therefore, plant extracts represent an attractive control alternative. In an effort to discover novel compounds with biological activity against larval *Aedes aegypti*, we conducted laboratory assays with essential oils obtained from leaves from 2 Cuban plants in the family Myrtaceae (*Eugenia melanadenia* and *Psidium rotundatum*) by a hydrodistillation method. Plant assays were replicated 3 times and each test had at least 4 concentrations. Percent mortality was registered at 24 h after each application. Results analyzed by probit-log software demonstrated that essential oils had a larvicidal effect, with median lethal concentration and 95% lethal concentration values of 0.0085% and 0.0104%, respectively, for *E. melanadenia* and 0.0063% and 0.0071%, respectively, for *P. rotundatum*. Diagnostic concentrations with values of 0.0022% and 0.0164% for *E. melanadenia* and *P. rotundatum*, respectively, also are provided. The possible implications of these findings on field populations of *Ae. aegypti* are discussed.

Larval control of mosquitoes with *Macrocyclus albidus* (Copepoda: Cyclopoidae) under laboratory conditions

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Predatory cyclopoid copepods have been used successfully in some countries for controlling mos-

quitoes inhabiting both artificial and natural containers. For the 1st time in Cuba, we evaluate the predatory activity of *Macrocyclus albidus* on larvae of *Aedes aegypti* and *Culex quinquefasciatus*, mosquitoes that frequently cohabitate domestic containers in urban areas. Experiments were performed under laboratory conditions at $25 \pm 2^\circ\text{C}$ and a 12:12 h light:dark cycle. Plastic 7-liter containers with 5 liters of dechlorinated water and 3 g of leaf litter were used. Two combinations of predatory densities (10 and 100 *M. albidus*) and 3 combinations of prey densities (300 *Ae. aegypti* and 0 *Cx. quinquefasciatus*, 150 of each species, and 0 *Ae. aegypti* and 300 *Cx. quinquefasciatus*) were replicated 5 times. Separate controls with no copepods and the same 3 combinations of mosquito densities were studied in similar containers. Surviving larvae and remaining copepods were counted 6 days after the experiment began. Analysis of variance showed a significant reduction of mosquito larvae in all the treatments with copepods. Predatory activity was greater on larval *Ae. aegypti*, and this effect was not affected by the association with *Cx. quinquefasciatus*.

Ecological diversity of the aquatic insects associated with *Culiseta inornata* (Diptera: Culicidae) breeding sites

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The knowledge of the aquatic insect communities that live in breeding sites of *Culiseta inornata* could help develop the appropriate strategies to control this species. We evaluated the diversity of the aquatic insects in breeding sites with *Cs. inornata* in San Antonio Peña Nevada (an ecotourism area). Each month from July 2000 to June 2001, we took 10 samples from permanent pools with an entomological net and the insects were identified, and their densities were compared on the basis of indices of Simpson, Shannon, Taylor, and Green. We found a total of 31 genera and species in 5 orders; Diptera was the most important, with 7 species. Maximum diversity with the Simpson index was $DV = 14.6763$ at 2,111 m elevation; the minimum value was $DV = 0.4311$ at 3,542 m. The highest value for the Shannon index was $H' = 1.0399$, this aquatic system was at 1,860 m; the lowest Shannon index was $H' = 0.4311$ at 3,560 m. The majority of distribution index was an aggregate distribution of 74.19% for Taylor and 58.33% for Green of the total taxa collected.

Larval habitats of *Aedes albopictus* and its associated fauna in Nuevo León, México

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Two localities (Allende and Villa de Santiago) have been reported positive for *Aedes albopictus* in Nuevo León State. Larvae are found most frequently in tires; however, the importance of other breeding sites is unknown, as well as the relation of this species to other arthropods in the same habitat. From April to October 2001, monthly collections were conducted in Allende and Villa de Santiago. A group of 644 containers was inspected and divided into the following categories: 49.6% discarded tires, 38.0% flower pots, 6.0% plastic containers, 4.0% metal containers, 0.3% Bromeliaceae plants, 0.62 small basins, and 1.2% ceramic pots. Results showed that 106 (96.3%) tires, 2 (1.8%) Bromeliaceae; 1 (0.9%) metal container, and 1 (0.9%) plastic container had larval *Ae. albopictus*. Several other mosquito species (*Aedes aegypti*, *Culex pipiens*, and *Toxorhynchites rutilus*) were also found in breeding sites of *Ae. albopictus*.

Influence of the blood-meal source on the development of *Triatoma longipennis* (Hemiptera: Reduviidae)

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Triatoma longipennis is among the most important vectors of *Trypanosoma cruzi* (Chagas Disease) in México because of its occurrence in houses and chicken coops, frequent contact with humans, and high *T. cruzi* infection rates in villages of western México. As with some other triatomine species, *T. longipennis* usually feeds on mammals or birds, depending on the availability of the hosts. Studies of some *Triatoma* species (e.g., *T. infestans*, *T. brasiliensis*, *T. sordida*, and *T. pseudomaculata*) have shown that specimens fed on mammals (mice) have shorter developmental times and lower mortality rates than those fed on birds (pigeons). With the information on the host preference of *T. longipennis*, the objective of this study was to determine the influence of the blood-meal source on the life cycle of *T. longipennis*, number of blood meals needed before the molting, and reproductive development. Specimens were separated in 2 groups of 150 1st-stage nymphs, placed in plastic containers (10 each), fed weekly on Winstar rats or leghorn

hens, and checked daily to record molting and mortality. The specimens fed on hens had a shorter life cycle (mean 209.7 ± 41.8 days) than those fed on rats (259.8 ± 28.7 days), needed fewer meals to molt, laid more eggs/female after 90 days (mean 161.3 ± 5.7 vs. 147.3 ± 7.4), but had higher mortality rates in each stage. Our results showed that, in contrast to previous studies, the nutritional characteristics of blood from hens were higher than those of blood from rats. These results support the idea of a strong association of *T. longipennis* with domestic birds, as cited in previous studies.

Some cases of myiasis in Nuevo León, México

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The term myiasis was used for the 1st time in 1840. It has been used to describe the presence of dipteran larvae in organs and tissue of mammals. Although myiasis frequently has been found in tropical zones, several cases have been reported to our laboratory in Nuevo León State. The 1st case was a 70-year-old woman with oestrid larvae in her eyes. The 2nd case was a 33-year-old woman with sarcophagid larvae in her reproductive system (ovaries). The 3rd case was a 2-year-old girl with a calliphorid larva that suddenly appeared in her reproductive system. These 3 cases are described in detail.

Spatial distribution of *Chironomus plumosus* (Diptera: Chironomidae) egg masses in a man-made lake

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Chironomus plumosus is the most common midge species in polluted waters in Nuevo León and has been collected throughout the metropolitan area of Monterrey. To increase our knowledge of the oviposition behavior and to improve control strategies for this midge, we evaluated spatial distribution of eggs masses in an artificial lake. We placed 25-cm² artificial substrates as oviposition sites. These were located apart and connected by a synthetic cord. After 24 h of exposure to natural oviposition, the materials were removed and taken

to the laboratory. Egg masses were counted and the distribution was analyzed statistically. An average of 381 egg masses was found per substrate. We found no statistical difference between the substrate locations and concluded that female *C. plumosus* only need a substrate upon which to lay their eggs.

Effectiveness of Pounce® 0.4G on *Musca domestica* larvae

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As a 1st step in the treatment of water entering sewage treatment plants, solid materials such as plastic, wood, and gravel are removed. All other solids are covered by organic matter and become ideal breeding sites for several species of Diptera, where the dominant species has been *Musca domestica*. We evaluated the efficacy of Pounce® 0.4G to control larvae of *M. domestica* under field conditions. Our test systems were small cages with 1 kg of dry gravel (stored for at least 1 day), wet gravel, a mixture of gravel and plastic, and recently removed gravel. A commercial concentration of Pounce was applied to each test system and then 10 3rd-stage larvae were added. Mortality was registered at days 7, 14, 21, and 28 after treatment with 5 replications and the data were analyzed by analysis of variance. The mortality caused by Pounce 0.4G on larval *M. domestica* was between 72 and 100% in all substrates evaluated and statistical analysis showed no difference between treatment and posttreatment time.

Field evaluation of polymer-based granular formulations of *Bacillus thuringiensis israelensis*

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Bacillus thuringiensis israelensis (Bti) extract was produced, evaluated, and formulated with 3

polymers (gelatin, acacia gum, and paraffin) and a solar protectant (malachite green). The extract was tested for efficacy with 200-liter plastic containers, previously colonized with mosquito larvae, in a random square design with 4 treatments, applied at a dose of 13.7 mg/liter. Larval population densities were assessed every 3–4 days after treatment, by taking 10 dipper samples from each treatment, and a test for residual activity was done at days 21 and 33 after treatment against laboratory 4th-stage larvae of *Aedes aegypti*. The data showed that the 3 *Bti* formulations, with arbitrary potency of 196.8 ITU/mg, were equally effective at 4 and 7 days post treatment against larvae of *Ae. aegypti* and *Culex* sp. The average number of pupae was significantly higher in control than in treatment containers until day 7 after application, but after day 11, no differences were detected. After 21 and 33 days after treatment, residual activity produced 59–85% and 54–69% mortality, respectively, in laboratory-reared 4th-stage larvae of *Ae. aegypti* in water samples obtained from treated containers.

Scorpions (Arachnida: Scorpiones) from San Antonio Pena Nevada, México, with notes on their spatial and elevational dispersal

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The region of San Antonio Pena Nevada is located in Zaragoza, Nuevo León, México. This region is a candidate to become a Protected Natural Area. It is characterized for having a diverse vegetation where pine forest predominates. As part of the characterization of the flora and fauna of this region, monthly collections of scorpions were made, where sampling (polygons) at different elevations of the zone were established. Inside every polygon, 5 points of samples were taken (circular plots) from June 2000 to June 2001. In this region, a total of 375 specimens with 3 species of scorpions was found. The most abundant species was *Vaejovis rossamani*, with 355 specimens; this species was distributed from an elevation of 2,169 m to 3,307 m. *Diplocentrus ferrugineus* was 2nd in abundance with 12 specimens collected from 2,199 to 2,282 m. Finally, 8 specimens of *Vaejovis bilineatus* were collected from 1,983 to 2,282 m. We concluded that species diversity was highest in pine forest vegetation, whereas the largest number of individuals was captured in oak forests.

New breeding places for *Anopheles* in Colombia

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The finding of immature *Anopheles* mosquitoes in artificial breeding containers is unusual, because these mosquitoes usually breed in natural habitats such as rain water ponds, lagoons, fish ponds, bromeliads, and streams. This study reports primary malaria vectors in Colombia breeding in artificial containers: *Anopheles nuñeztovari* was found in a concrete water tank in Cimitarra (Santander State) and in a concrete drain in Itsmina (Chocó); *An. albimanus* was found in an underground well in Moñitos (Córdoba State); *An. darlingi* was reported in a water jar in Hatillo de la Sabana (Magdalena State) and in an elevated water tank in Cazarito (Vichada State); and *An. punctimacula* was found in a concrete trough, a puddle of water on a concrete floor, and in a laundry tank in La Tebaida (Quindío State). All of these artificial containers had 4th-stage larvae, which suggests a good adaptation of these species to these breeding places. These results have been reported previously in Brasil by Carreira in 2001, but not from Colombia. In view of these findings, it is very important to consider artificial containers in the epidemiological and entomological surveillance of malaria because these vector species might colonize these and other artificial breeding sites.

Natural enzootic vectors of Venezuelan equine encephalitis virus in the Magdalena Valley, Colombia

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Phylogenetic studies indicate that epizootic and epidemic strains of Venezuelan equine encephalitis virus (VEE) have evolved at least 3 times from an enzootic, subtype ID lineage that occurs in Colombia and western Venezuela. To characterize the enzootic transmission cycle of these epizootic progenitors, we incriminated natural mosquito vectors in a sylvatic VEE focus in the middle Magdalena Valley of Colombia. Modified Trinidad no. 10 traps were baited with hamsters and placed in the forest,

and daily mosquito collections were frozen for laboratory analysis. When hamsters died from a VEE infection, mosquitoes collected from the corresponding trap were sorted by species, triturated, and assayed for virus. In 18 cases, a single, initial, high-titered mosquito pool representing the vector species was detected 2–3 days before hamster death, whereas subsequent collections yielded nearly complete infection, reflecting viremic blood meals from the hamster. Vectors incriminated in this manner included *Culex (Melanoconion) vomerifer* (11 cases of transmission), *Cx. (Mel.) pedrofi* (5 transmissions), and *Cx. (Mel.) adamesi* (2 transmissions). These results extend the number of incriminated enzootic VEE vectors to 7, all of which are members of the Spissipes section of the subgenus *Melanoconion*. Our findings challenge the dogma that a single mosquito species generally serves as the principal enzootic VEE vector at a given location.

Entomological study in an urban area with malaria transmission in the Colombian Pacific coast

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An epidemiological and entomological study was carried out from June 1993 to May 1995 in urban and neighboring communities of Buenaventura, the main Colombian port on the Pacific Ocean, which is highly vulnerable to malaria transmission. We have previously reported *Anopheles albimanus* as the most frequently collected species in urban areas, and *An. nuñeztovari*, as the most frequently collected species in neighboring communities. We estimated the sporozoite rate (SR) and entomological inoculation rate (EIR) and correlated them with rainfall, mosquito density, and malaria cases. Two of 2,499 *An. albimanus* were positive for *Plasmodium vivax* (SR = 0.08%), with 375 and 116 sporozoites, respectively. One of the positive mosquitoes was collected during the peak biting rate in 1994, and the 2nd was collected during a similar peak in 1995. Both mosquitoes were collected in the urban area between 1800 and 1900 h. An EIR of 10.5 infective bites per person per year was calculated. This study increased our knowledge of vector bionomics, biting habits, behavior, and preferred breeding places to better understand malaria incidence and establish more effective control strategies.

Population genomics of dengue susceptibility in *Aedes aegypti*

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We tested the hypothesis that the early trypsin gene conditions midgut susceptibility of *Aedes aegypti* to dengue virus. Specifically, could early trypsin cleave specific proteins from the surface of the dengue genome necessary for viral attachment to mosquito midgut epithelial cells? Previous research has shown that a minimum of 2 genomic regions control dengue susceptibility in *Ae. aegypti*. Bosio showed that alleles at the early trypsin gene site strongly cosegregate with dengue midgut susceptibility and that no other genes cosegregate with early trypsin. The early trypsin gene apparently is a part of a unique signal transduction system. Its function may be to taste the incoming meal to determine if protein is sufficient to support a gonadotrophic cycle. If so, the signal transduction pathway activates late trypsin transcription to digest the blood meal. We showed linkage disequilibrium among segregating sites in early trypsin in *Ae. aegypti*. Presently, we are examining genes, including the early trypsin gene, among >1,000 mosquitoes from 38 collections throughout México. We have sequenced the entire early trypsin gene in 52 individuals from Miguel Aleman, the site that presented the greatest early trypsin gene diversity. The primers that we used amplify a portion of the gene encoding amino acids 89–167 of early trypsin. This region contains the active site and all but 1 of the conserved trypsin residues. A Fortran program PGENOME was used to compare genotype frequencies among 52 mosquitoes from Miguel Aleman. Kristine Bennett fed these mosquitoes per os with blood containing dengue 2 strain JAM1409 to determine if each mosquito had a midgut infection barrier (MIB) and midgut escape barrier (MEB). She demonstrated 3 phenotypes: infected heads (MIB– and MEB–); uninfected heads and infected midguts (MIB– and MEB+), and uninfected heads and midguts (MIB+). We compared differences in early trypsin nucleotide frequencies between midgut infected vs. uninfected *Ae. aegypti* from México ($n = 1,470$), and between head infected and uninfected ($n = 1,070$). We failed to detect any quantitative trait nucleotides (QTN) for MEB. We concluded that single-strand conformation polymorphism (SSCP) analysis is a sensitive and specific means to assay for single nucleotide polymorphisms (SNPs) in individual (diploid) genotypes. We have applied population genomics toward identifying a putative QTN in the early trypsin gene associated with dengue midgut susceptibility in *Ae. aegypti*. Although the early trypsin gene does not have any QTN that segregates with MEB, 1–3 QTNs apparently are correlated to MIB.

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