MOSQUITO FAUNAL SURVEY OF SAIPAN, MARIANA ISLANDS (DIPTERA: CULICIDAE): TAXONOMY AND LARVAL ECOLOGY

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ABSTRACT. Mosquito collections made on Saipan during September–October 1991, as part of a retrospective investigation of a Japanese encephalitis (JE) outbreak, are summarized. Forty-two larval habitats were sampled at 21 different sites. Most larvae were individually link-reared to the adult stage. Nine species, representing three genera and five subgenera, were collected. Numerous specimens of *Culex tritaeniorhynchus*, an established JE vector, were collected. Data on larval habitats including physical-chemical parameters and species associations are presented.

INTRODUCTION

During October 1990, a discrete outbreak of Japanese encephalitis (JE) occurred on Saipan, Commonwealth of Northern Mariana Islands (CNMI). Ten human cases were identified, all with onset during a four-week period (Paul et al. 1993). This was the first documented outbreak of JE on Saipan and the first incursion of JE into Oceania since an outbreak on Guam during 1947–48 (Hammon et al. 1958).

As part of a follow-up investigation, entomological studies were conducted in September–October 1991. This period corresponds to the same time of year that JE virus amplification would have occurred during the preceding year. Collections included adult mosquitoes to be processed for virus isolation (Mitchell et al. 1993), and larval collections made for taxonomic and ecological studies. Herein, we report the results of larval collections and taxonomic studies.

Saipan, the capital of the CNMI, lies at 15°N latitude and about 120 km north of Guam. The island is volcanic in origin, approximately 42 km long and 16 km wide at the widest point, and reaches an elevation of 474 m at Mt. Tapotchau. Much of the native vegetation was destroyed during World War II. Denuded portions were reseeded with tangen-tangen, *Leucaena glauca* Benth. Hook, which now forms dense thickets, especially over much of the northern half of the island. The human population of about 40,000 is concentrated on the southern half of the island and along the west coast.

The climate is tropical and thus warm and humid throughout the year with little variation in temperature (Commonwealth of Northern Mariana Islands 1990). The average annual temperature is 25.6°C, and humidity averages between 79 and 86 percent. Annual rainfall averages about 212 cm. There are two main seasons: a wet period usually occurring between July and October, and a dry period usually occurring between January and April, with periods of transition in between. Tropical storms and typhoons occur, especially during late summer and early autumn. During this survey, typhoon Muriel visited Saipan with high winds and dropped more than 25 cm of rainfall in 24 h.

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MATERIALS AND METHODS

Collections included adult mosquitoes to be processed for virus isolation, and larval collections made for taxonomic and ecological studies. Adult mosquitoes were aspirated from human bait, pig bait, and collected with CDC light traps baited with CO_2. A total of 7,250 specimens representing seven species were pooled by taxa and tested for virus isolation with negative results (Mitchell et al. 1993).

Larval and pupal collections were made from 42 different habitats at 21 sites on Saipan between September 18 and October 3, 1991 (Fig. 1). At sites 1–2, 4–7, both adult mosquito collections for virus isolation and larval collections were made. At site 3, only adult collections were made (Mitchell et al. 1993), and at sites 8–22, only larval collections were made.

A collection record describing each habitat was completed, and for selected habitats, parameters such as water temperature, conductivity, and pH were measured. Aquatic stages were transported to a field laboratory and the majority were individually link-reared to the adult stage. Adults were pinned on paper points and appropriately numbered. Larval and pupal exuviae and some larvae and pupae were preserved in alcohol, numbered, and subsequently slide-mounted. Specimens were identified based upon examination of adults and associated exuviae when possible.

Specimens and collection records are deposited in the U.S. National Museum of Natural History, Washington, DC, and in the C.P. Gillette Entomology Museum, Colorado State University, Fort Collins.

Relationships among the 36 mosquito-positive habitats based on species composition (Fig. 2) were investigated by use of McQuitty or WPGMA cluster analysis (SAS Institute 1987). For each habitat-pair, the Dice Coefficient (C_D), a coefficient based on the presence or absence of taxa which emphasizes similarity or common taxa (Dice 1945, Hall 1969), was calculated. Cluster analysis was conducted on a distance (D) matrix with values calculated by subtracting each C_D value from 1.

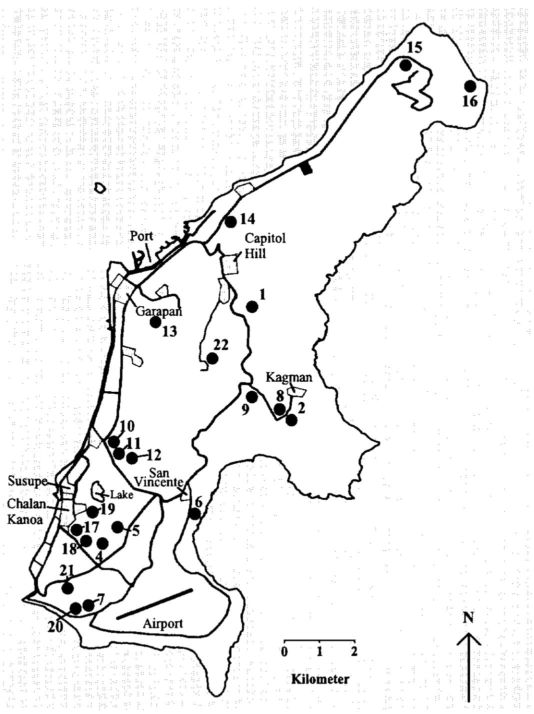

Fig. 1. Larval collection sites, 1–2, 4–22, on Saipan, September–October 1991.

RESULTS

The 986 collected specimens, the majority link-reared, represent nine species (Tables 1,2). Numerous specimens of *Culex* (*Culex*) *tritaeniorhynchus* Giles, an established JE vector, were collected, which constitutes the first published record of this species on Saipan (Mitchell et al. 1993). Four of the 13 species previously or presently reported to occur on Saipan, *Aedes* (*Stegomyia*) *aegypti* Linn., *Ae.* (*Stg.*) *guamensis* Farner and Bohart, *Cx.* (*Cux.*) *annulirostris marianae* Bohart and Ingram, and *Cx.* (*Cux.*) *litoralis* Bohart, were not represented in our larval collections (Table 1).

Forty-two larval habitats, consisting of 10 (23.8%) surface water and 32 (76.2%) container habitats, were sampled at 21 different sites (Fig. 1). The 10 surface water habitats included the following six habitat-types: three ground pools (GP), one ditch (D), one flooded pasture (FP), two phragmites marsh (M) habitats, two temporarily flooded terrestrial grass (TFG) habitats, and one sewage pond (SP). Container habitats included nine (28.1%) natural containers (NC) and 23 artificial con-

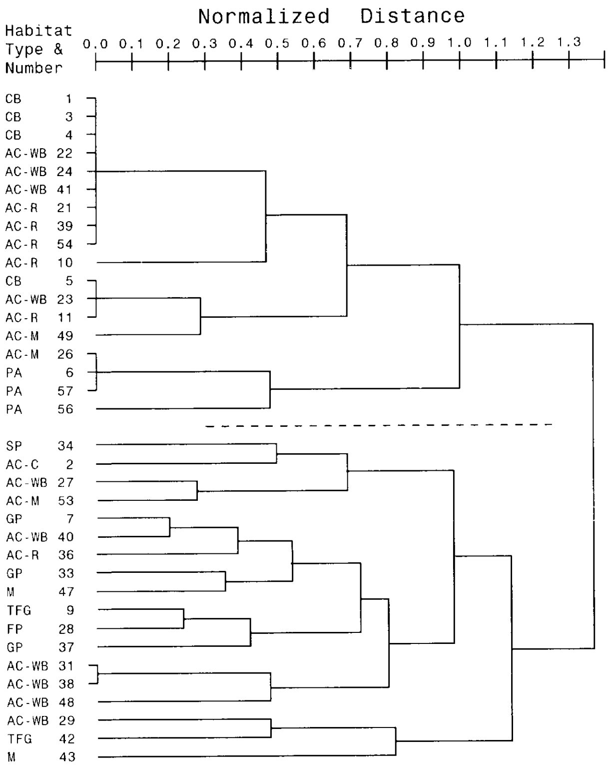

Fig. 2. Relationships, resulting from cluster analysis, among 36 mosquito-positive habitats based on species composition. See materials and methods section for details and abbreviations. Dashed line separates upper cluster, which includes all container breeding *Aedes* habitats, from the lower cluster. Lower cluster includes all surface water habitats and some artificial container habitats in which all species of *Culex* and *Anopheles indefinitus* were collected.

tainer (AC) habitats (71.9%). The nine natural containers represented two habitat-types: four cut bamboos (CB), and five *Pandanus* spp. axils (PA). The 23 artificial container habitats were divided into four habitat-types for ease of discussion as follows: one concrete cistern (AC-C); 10 water barrels (AC-WB), which were generally in or near animal barns or pens; seven water-holding refuse containers (AC-R) of various sorts such as broken Styrofoam ice chests, gallon-sized cans, and discarded pesticide containers; and five miscellaneous containers (AC-M) including war memorials and buckets.

Thirty-six (85.7%) of the 42 sampled habitats contained mosquito larvae. The six (14.3%) mosquito-negative habitats included: one of one D habitat (habitat #8); two (40.0%) of five PA habitats (#55,58); one (14.3%) of seven AC-R habitats (#52); and two (40.0%) of five AC-M habitats (#25,35). All GP, FP, TFG, M, SP, CB, AC-C and AC-WB habitats sampled contained mosquito larvae. Fifteen (41.7%) of the 36 mosquito-positive habitats contained only a single mosquito species. *Aedes* (*Stg.*) *albopictus* (Skuse) was the most frequently encountered solitary species, occurring in nine (60.0%) of the 15 single-species habitats, followed by *Ae.* (*Stg.*) *saipanensis* Stone in three (20.0%), *Cx.* (*Cux.*) *quinquefasciatus* Say in two (13.3%) and *Cx. tritaeniorhynchus* in one (6.7%). All single-species habitats were containers, except for one TFG habitat (#42) that contained *Cx. tritaeniorhynchus*. The remaining 21 (58.3%) of the 36 mosquito-positive habitats contained two or more species. The most species-rich habitats were a FP (#28) and a TFG (#9) habitat, each with six species. The number of specimens collected by taxa, and the number of times each taxon was collected in each habitat-type are summarized in Table 2.

Table 1. A checklist of the mosquitoes of Saipan.

Taxon	Previous records	This study
An. (*Cel.*) *indefinitus*	Pratt and Siren 1971	+
Ae. (*Adm.*) *oakleyi*	Bohart 1957	+
Ae. (*Adm.*) *nocturnus*	Bohart and Ingram 1946 Bohart 1957	+
Ae. (*Stg.*) *aegypti*	Esaki 1939 Kuwabara 1941 Bohart and Ingram 1946	–
Ae. (*Stg.*) *albopictus*	Kuwabara 1941 Bohart and Ingram 1946 Bohart 1957 Pashley and Pashley 1983	+
Ae. (*Stg.*) *guamensis*	Stone and Farner 1945 Bohart 1957	–
Ae. (*Stg.*) *neopandani*	Bohart 1957	+
Ae. (*Stg.*) *saipanensis*	Stone 1945 Bohart and Ingram 1946 Bohart 1957 Pashley and Pashley 1983	+
Cx. (*Cux.*) *annulirostris marianae*	Bohart and Ingram 1946 Bohart 1957	–[1]
Cx. (*Cux.*) *quinquefasciatus*	Esaki 1939 Kuwabara 1941 Bohart 1957	+
Cx. (*Cux.*) *litoralis*	Bohart 1957	–
Cx. (*Cux.*) *tritaeniorhynchus*		+, new record[2]
Cx. (*Lut.*) *fuscanus*	Pratt and Siren 1971	+

[1] Adults of *Cx. a. marianae* were collected in separate collections made for virus isolation during the same time period (Mitchell et al. 1993).
[2] First published record based on same specimens and reported in companion paper by Mitchell et al. 1993.

Environmental parameters measured at 12 mosquito positive habitats varied as follows: conductivity ranged from 34.5–670 microSiemens (μS) ($\bar{x}$ = 240.7); pH from 7.0–9.4 ($\bar{x}$ = 7.6); and water temperature from 25–34°C ($\bar{x}$ = 27.8). Water temperature and pH values were homogenous among the three habitat categories: natural container habitats, artificial container habitats and surface water habitats. Conductivity values for surface water habitats ($\bar{x}$ = 343.5 μS, n = 7, range = 34.5–670) were generally higher than values from the two container habitat categories ($\bar{x}$ = 96.7 μS, n = 5, range = 52.5–190.0) with differences approaching statistical significance (t = 2.14, P = 0.058).

Cluster analysis on the 36 mosquito-positive habitats based solely on species composition was conducted (Fig. 2). The analysis resulted in two large, basal clusters which reflect the dichotomy between container breeding *Aedes* habitats, the upper cluster, and surface water and artificial container habitats, the lower cluster (Fig. 2). The upper cluster includes all the natural container habitats, CB and PA, as well as some artificial container habitats. Only *Aedes* mosquitoes were collected from habitats included in the upper cluster with *Ae. albopictus*, *Ae. saipanensis* and *Ae.* (*Stg.*) *neopandani* Bohart being representative. The lower cluster includes all the surface water habitats and some artificial container habitats. All species of *Culex* occurred in habitats within this cluster, along with the single anopheline, *Anopheles* (*Cellia*) *indefinitus* (Ludlow). In addition, the surface water *Aedes* species, *Ae.* (*Aedimorphus*) *nocturnus* (Theobald) and *Ae.* (*Adm.*) *oakleyi*

Table 2. Larval mosquitoes collected on Saipan, September-October, 1991. The following abbreviations are used for the larval habitat-types: AC = artificial containers, AC-C = cistern; AC-R = refuse; AC-WB = water barrel; AC-M = miscellaneous (bucket, memorial); CB = cut bamboo; FP = flooded pasture; GP = ground pool; M = marsh; PA = *Pandanus* axils; SP = sewage pond; and TFG = temporarily flooded grasses.

Taxon	No. of times collected[1]	No. of specimens collected	Larval habitat-types[2]
An. (*Cel.*) *indefinitus*	3	43	FP, M, TFG
Ae. (*Adm.*) *oakleyi*	6	112	GP(3), FP, TFG, AC-M
Ae. (*Adm.*) *nocturnus*	6	18	GP, M, FP, TFG, AC-C, AC-R
Ae. (*Stg.*) *albopictus*	20	300	AC-R(6), AC-WB(5), AC-M, CB(4), GP(2), TFG, M
Ae. (*Stg.*) *neopandani*	1	2	PA
Ae. (*Stg.*) *saipanensis*	9	120	PA(3), AC-M(2), AC-WB(2), AC-R, CB
Cx. (*Cux.*) *quinquefasciatus*	12	199	AC-WB(5), AC-R, AC-M, GP(3), FP, M
Cx. (*Cux.*) *tritaeniorhynchus*	8	149	M(2), TFG(2), FP, GP, AC-WB(2)
Cx. (*Cux.*) *sitiens* group	2	3	M
Cx. (*Lut.*) *fuscanus*	10	40	GP(2), TFG, FP, SP, AC-WB(3), AC-C, AC-M
Total		986	

[1] Total of 42 habitats sampled.
[2] The number of taxa-positive habitats for each habitat-type is given in parentheses following the habitat-type if greater than one.

Stone, were common in habitats of this cluster, along with the ubiquitous *Ae. albopictus.*

DISCUSSION

The date of introduction of *Cx. tritaeniorhynchus* onto Saipan is unknown, but appears to have occurred during the interval between 1970 and 1981. The 1970 date corresponds to survey work conducted by Siren on Saipan (Pratt and Siren 1971) in which no mention of *Cx. tritaeniorhynchus* was made. In 1981, Dr. K. Ando, in an unpublished trip report submitted to the World Health Organization, reported that *Cx. tritaeniorhynchus* was collected from a small marsh near the airport (Mitchell at al. 1993). By the time of our survey in September 1991, *Cx. tritaeniorhynchus* was well established on Saipan in M, FP and TFG habitats.

Of the four species previously reported from Saipan that were not represented in our collections (Table 1), *Cx. annulirostris marianae* was represented by a few specimens in separate adult collections made for virus isolation (Mitchell et al. 1993). Bohart (1957) reported that larvae of *Cx. annulirostris marianae* were collected in a wide variety of fresh and brackish, surface water habitats as well as assorted container habitats in the Marianas, and that this species was particularly common from August to March, which includes the time period of our visit. Whether the apparent rarity of *Cx. annulirostris marianae* during our survey truly reflects the population levels of this species on Saipan or is an artifact related to the short duration and time of our visit is unknown.

The absence of *Ae. aegypti*, *Ae. guamensis* and *Cx. litoralis* in our collections reflects the likely absence, or restricted distributions of these species on Saipan. Bohart and Ingram (1946) reported *Ae. aegypti* to have been present on Saipan, but with distribution limited to selected urban areas. In 1957, Bohart suggested that extensive control efforts conducted on Saipan, Tinian, Rota and Guam during 1945–49 may have eliminated *Ae. aegypti* from Saipan, Tinian and Rota. No collections or records of *Ae. aegypti* on Saipan are available for the period after 1946. The distribution of *Ae. guamensis* is limited to Micronesia, and this species has been reported to be common only on Guam and Rota (Bohart and Ingram 1946, Bohart 1957). *Aedes guamensis* is a container breeding species that is more commonly collected in areas away from human habitation (Bohart and Ingram 1946, Rozeboom and Bridges 1972). Our collection activity focused on residential areas, with the exception of four CB and five PA collections, and this may explain our failure to collect this relatively rare species. To date, only 14 specimens of *Ae. guamensis* have been reported from Saipan based on two separate collections (Stone and Farner 1945, Bohart 1957). On Guam, *Ae. guamensis* decreased in abundance as measured by the percentage of larva-positive AC habitats between 1948 and 1970 (Reeves and Rudnick 1951, Rozeboom and Bridges 1972), and Rozeboom and Bridges (1972) speculated that this reduction was associated with the introduction of *Ae. albopictus* onto Guam in 1944. The replacement of *Ae. aegypti* by *Ae. albopictus* in the southern U.S. (Hobbs et al. 1991) strengthens this argument and suggests that the presence of *Ae. albopictus* on Saipan accounts in part for the absence of *Ae. aegypti* and apparent rarity of *Ae. guamensis* on Saipan. The absence of *Cx. litoralis* in our collections likely results from our failure to sample the preferred habitat of this species, which is slightly brackish water in coral rock holes and occasionally artificial containers along the beach. Time was not allocated to sample these habitats because *Cx. litoralis* is not believed to be of medical importance.

The mosquito negative-habitat rate of 14% found in this survey is probably lower than the actual negative-habitat rate. Due to the very limited length of our stay on Saipan, the larval sampling program focused on habitats that seemed very likely or those known to contain mosquitoes, and those habitats likely to contain possible JE vectors.

The low conductivity values reported herein for mosquito habitats on Saipan reflect the lack of extensive brackish water coastal marshes and mangrove areas on this oceanic island, and our failure to sample coral rock holes and beach habitats. The nearly significant dichotomy ($P = 0.058$) in conductivity values between container habitats and surface water habitats reflects the greater input from aerosols and the solution of coral substrates in larger surface water habitats. Conductivity values measured on Saipan from container habitats, from 52.5–190.0 μS, are very low and correspond to low values reported for rainwater on Upolu and Manono islands, Western Samoa, and Nukulaelae atoll, Tuvalu (Laird 1988).

All species, except for *Ae. neopandani*, which was collected from a single PA, were collected from three or more different habitat-types (Table 2). Strong associations between particular species, except for *Ae. neopandani*, and habitat-types as defined herein appear weak or nonexistent. However, at the higher level of habitat categories, i.e., surface water, artificial container and natural container habitats, species-specific patterns become apparent (Fig. 2). Most species, including *Ae. oakleyi*, *Ae. nocturnus*, *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus* and *Cx. (Lutzia) fuscanus* Wiedemann, occurred in both surface water and artificial container habitats, but never in natural container habitats. *Anopheles indefinitus* occurred only in surface water habitats. *Aedes saipanensis* occurred in artificial and natural container habitats, but never in surface water habitats. Only one species, *Ae. albopictus*, occurred in all three habitat categories.

Cluster analysis on the 36 mosquito-positive habitats based solely on species composition resulted in two large, basal clusters; however, smaller clusters assignable to habitat-types are not apparent (Fig. 2). The mosquito fauna is composed of two primary units:

those taxa, e.g. *Ae. saipanensis*, *Ae. neopandani* and *Ae. albopictus*, which evolved in natural containers and presently occur in both natural and artificial containers; and those taxa including the majority of species which evolved in surface water habitats. A few species of this latter group, particularly *Cx. quinquefasciatus* and *Cx. fuscanus*, have successfully colonized artificial container habitats and along with *Ae. albopictus* account for nearly all of the habitat overlap between members of these two groups.

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