ılly overwintering, and to determine me of spring emergence.

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COLONIZATION OF SIX SPECIES OF MOSQUITOES IN JAPAN [1]

A. BURNS WEATHERSBY, CDR, MSC, USN

Division of Parasitology, Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland

Laboratory colonies of mosquitoes are value to military Preventive Medicine nits for training personnel within the nit, ships or stations, and for determin- g insecticide resistance and relations to sease. To meet these needs colonization several species of mosquitoes was at- mpted at U. S. Navy Preventive Medi- ne Unit No. 8 at Yokosuka, Japan, in ugust 1955. Two species, *Armigeres (Armigeres) subalbatus* (Coq.) and *nopheles (Anopheles) sinensis* Wied, ıve not been reported in laboratory lonization; *Culex (Culex) tritaenio- ynchus* Giles and *Aedes (Finlaya) togoi* Theo.) recently have been reported in ccessful colonization (Newsom, *et al.*,)56; Lien, 1959); and *Aedes (Stegomyia) albopictus* (Skuse) and *Culex (Culex) pipiens* Linn. have been in colonization for many years but *Culex (Culex) pipiens* var. *pallens* Coq. has not been reported in colonization. Species previously colonized are mentioned here because of the ease with which they adapted to laboratory conditions.

MATERIALS AND METHODS. Larvae of these mosquitoes were collected from various habitats on the Miura Peninsula. They were reared in the laboratory by well-established techniques (Trembley, 1955). The *Armigeres* and *Aedes* were reared in mouse jars and fed Purina guinea pig and dog chow and aeration of the cultures kept them free of surface scum. The *Anopheles* and *Culex* were reared in white enamel photographic pans. The *Anopheles* fed on finely ground Purina guinea pig chow and the *Culex* fed on the pellets. The adults were maintained initially in 14" x 18" screened cylin-

[1] The opinions and assertions contained herein e the private ones of the writer and are not be construed as official or reflecting the views the Navy Department or the Naval Service at ge.

drical cages. These were replaced by 18" x 18" x 18" cages for *Armigeres* and *Aedes* and 3' x 3' x 3' cages for *Anopheles* and *Culex*. *C. tritaeniorhynchus,* being eurygamous, required a 4' x 4', floor to ceiling cage.

Temperature and humidity were not controlled except by providing steam heat during cold weather and draping wet burlap over the cages or from the ceiling, kept wet by a small spray pipe to maintain relatively acceptable humidity.

The *Armigeres* and *Aedes* oviposited on strips of paper towelling lining crystallizing dishes ¼ full of water. The *Anopheles* and *Culex* oviposited in any large container of water in the cages.

RESULTS AND DISCUSSION. *A. subalbatus* adults, reared from larvae collected from night soil storage tanks, were reluctant to feed on animals but readily bit man. The adults of subsequent generations fed on chicks, guinea pigs and rabbits. The egg papers could be dried and stored for 2 to 3 months, and would hatch in a matter of minutes after being placed in culture water. Duration in days of various stages was: egg, 4–5; larvae, 7–12; pupae, 2–3; eclosion to blood meal, 4; and oviposition, 5. Optimum larval development occurred when culture had been set up and allowed to age or ferment. The colony was carried through an estimated 26th generation in 23 months. Some authors believe that *A. obturbans* (Walker) on the mainland of Southeast Asia most likely is *A. subalbatus* (Coq.). Russell and Mohan, 1942, colonized *A. obturbans* in India.

The first attempts to colonize *C. tritaeniorhynchus* in 1955 ended in failure when wild caught specimens were not available for replenishing the colony. In June, 1956, tremendous numbers of larvae were collected from rice paddies and rain barrels and were introduced into a 4' x 4', floor-to-ceiling, screened cage in a corner of a permanent insectary. The cage remained in subdued light most of the day.

The adults from these larvae did not feed well on animals and only sparingly on man. A volunteer (the same each time) entered the case at 2030 and remaine for 1 to 2 hours. The first evidence swarming was noted at 2100, 10 Jul in the subdued light from a flashligh covered with a paper towel. The swarn ing was within 2 feet of the floor and th mosquitoes bit readily during this perio Tape recordings of mosquito hums an high pitched sounds from electronic osci lators were employed without success stimulate swarming. Dawn and dus lighting conditions were employed, fo lowing the observation of swarming in th light from the flashlight. A manuall controlled dawn-dusk period was replace by an automatic apparatus fabricated fro timer and motor from a New Jersey ligh trap. The motor controlled 2 large shu terlike blades which opened and closed light box at prescribed times. This co trolled daylight-dawn-dusk lighting 14–16 hours was essential in the earl phases of colonization (Brennan and Ha wood, 1953).

About 1,500 first instar larvae we noted in an 18" x 10" wooden tub of wat in the cage on 16 July although egg raf had not been found. These remained i the tub until pupation on the 10th da Five egg rafts were collected from the tu during this period and were set up an reared like *C. pipiens*. From this time o there were no additions of adults fro wild caught larvae. The population i the cage was reduced to about 200 adul in mid-August but the larval cultures we developing and the colony increase rapidly. It was estimated to be in th 6th generation on 1 November when ten peratures exceeded 120° F. for about 1 hours. Heat was required in the hospit area that night and valves on radiato installed in the insectary had not bee closed.

The 1st generation required 24 da but this period had been reduced to 1 days for the 5th and 6th generations. Th minimum periods of development were eclosion to oviposition, 8; egg, 2; larva 6; and pupae, 2. A few larvae survive in the larval insectary but the adults fro these did not maintain the colony.

An attempt was made to establish a olony during the winter from egg rafts llected on Okinawa. Through the urtesy of Lt. A. A. Hubert, MSC, USA, the Army Preventive Medicine Unit on kinawa, where colonization was proessing, many egg rafts were collected om nature and shipped by air to Japan test tubes lined with wet filter paper. large adult population was established om these eggs but without successful olonization. A colony was re-established 1957 from locally collected specimens ith results comparable to 1956.

Pupae of *A. togoi,* collected in great mbers from rock pools near the tide-ne, were reared in the laboratory. The ck pools contained pure sea water and l individuals were in the same developental stage. Transfer from sea water tap water was without apparent ill fect. Adults were set up in 18" x 18" 18" breeding cages in May 1956. These osquitoes feed vigorously on man and imals. Heavy egg deposits were made the papers in the oviposition dishes d the aquatic stage development parleled that of *A. albopictus.* Colonizan was effected without difficulty with erage generation development of 25 ys. The time in days for the various ges was: blood meal to oviposition, 6; egg, 4–5; larvae, 8–10; pupae, 2–3; osion to blood meal, 4. The colony s in the 24th generation by July 1957 d was not adversely affected by the gh temperature in the adult insectary e to the large larval population at that ne.

Inoki, 1951, mentioned laboratory rear-g of *A. togoi* and Lien, 1959, reported lonization of this species from northern iwan.

Attempts to colonize *A. sinensis* in 1956 re unsuccessful. Breeding cages were tocked in early May 1957 and 47 eggs re collected from oviposition pans on May and other eggs were harvested about weekly intervals. By mid-June 3' x 3' x 3' rearing cage was stocked with out 250 first generation adults from these eggs. The colony was well stocked and vigorous by 30 July, although the adults did not feed vigorously on either man or laboratory animals. Duration in days of the various stages was: egg, 3–4; larvae, 17–18; pupae, 2–3; eclosion, 5; and blood meal, 4. The colony was discontinued in the 4th generation when additional space for *C. tritaeniorhynchus* over-wintering studies was required.

Colonization of *A. albopictus* and *C. pipiens* var. *pallens* was effected without difficulty in 1955. *A. albopictus* adults, reared from wild caught larvae and pupae, fed vigorously on man or laboratory animals. The adults of *C. pipiens* var. *pallens* like *C. pipiens* were reluctant to feed unless given special conditioning. They fed sparingly on chicks in the dark after a period of 24 hours under illumination and denial of sugar water. The colonies were continued for 28–29 generations.

SUMMARY. Colonization of six species of mosquitoes in Japan is reported. Two species, *Anopheles sinensis* and *Armigeres subalbatus,* have not been reported in colonization. *Aedes togoi* and *Culex tritaeniorhynchus* recently have been reported in colonization. The colonization of *Culex pipiens* var. *pallens* is reported although *Culex pipiens* has been colonized previously. *Aedes albopictus* has been colonized in other laboratories. Duration of the various stages is reported and pertinent information regarding special techniques is included.

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BIOLOGICAL EVALUATION OF THE C-47 AERIAL SPRAY SYSTEM FOR LARVAL MOSQUITO CONTROL

CYRIL J. HODAPP,[1] DALE W. PARRISH [2] AND FRANK H. DOWELL [3]

During August and September 1961, studies were conducted at Eglin Air Force Base and Destin, Florida, to evaluate the effectiveness of aerially dispersed insecticides (DDT) against DDT-susceptible anopheline and culicine mosquito larvae. A C-47 aircraft equipped with underwing discharge booms, as described by Husman (1949), was used.

Two areas of twenty acres each were selected as the test plots. Test plot number 1 was in a coastal freshwater swamp with high, dense tree cover accompanied by heavy growths of aquatic vegetation. Test plot number 2 was a coastal piney wood habitat, i.e., low tree cover accompanied by dense growths of brush and aquatic grasses. One area, approximate ¼ acre, in this plot was void of tree cov but was partially covered with growths aquatic grasses.

Pre-spray mosquito density surveys we conducted in both test plots four hou preceding the insecticide application (T bles 1 and 2). Larvae in the 1st, 2nd, 3r and 4th instar were present. Twenty-s oil-sensitive cards were placed througho both test plots immediately preceding t insecticide application in order that t atomization and actual quantity of t insecticide solution reaching the grou

TABLE 1.—Pre- and post-spray larval coun test area No. 1

Station No.	Larval rates (pre-spray) (Total/10 dips)	Larval rates (post-spray) (Total/10 dips	
		14 hrs.	20 h
1	51	62	67
2	37	35	36
3	15	14	15
4	12	11	12
5	7	7	6
6	10	12	14
7	6	6	6

[1] Capt. USAF, MSC, Director, Entomology and Parasitology Dept. 6570th Epidemiological Laboratory, USAF Aerospace Medical Division (AFSC), Lackland AFB, Texas. The opinions stated herein are the private ones of the authors and are not to be considered the views of the United States Air Force.

[2] Capt. USAF, MSC, Medical Entomologist, 6570th Epidemiological Laboratory, USAF Aerospace Medical Division (AFSC), Lackland AFB, Texas.

[3] USAF Special Aerial Spray Flight Langley AFB, Virginia.