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## WORLD EVENTS CONCERNING MOSQUITOES IN 1967

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### INTRODUCTION

Tissue culture of insect cells is a rapidly expanding field which you may expect to hear much about in the next few years. At present, the chief reason for culturing these cells is to study arboviruses in insect tissues *in vitro*.

Attempts to accomplish growth of such cultures were made over 25 years ago. W. Trager (*Amer. J. Trop. Med.* 18(4) :387-393, 1938) cultured western equine encephalitis virus in surviving mosquito tissues. Although other workers reported efforts to propagate cells, T. D. C. Grace (*Nature (London)* 211(5047) :366-367, 1966) of Australia is the first to establish a line of mosquito cells *in vitro*. This important event was reported in 1966. Alimentary canals and bodies of *Aedes aegypti* (L.) larvae were the sources of his cells. Larvae were grown in a sterile environment. The most common type of cells were spindle shaped and grew in clumps. Cultures of Grace's cells have been distributed in numerous laboratories in the world.

Following this accomplishment, E. C. Sutor, L. L. Chang and H. H. Liu (*Expt. Cell Res.* 44(2/3) :572-578, 1966) have cloned a spindle-shaped cell from Grace's *A. aegypti* line. They believe that this is the first reported cloned line of continuously cultured insect cells.

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K. R. P. Singh (Current Sci. (Bangalore) 36(19) :506-508)<sup>2</sup> working in India, reported in 1967 the establishment of two cell lines from *Aedes aegypti* and three from *A. albopictus* (Skuse). The tissues were obtained from newly hatched larvae of both species. The two cultures from *A. aegypti* have reached the 30th and 15th passage while those of *A. albopictus* have reached the 30th, 25th and 21st passage.

Rarely have I discussed control of mosquitoes by biological agents because our most dramatic successes since DDT have been with chemicals. Owing to the great amount of research now being conducted on the potential of biological agents for control, I will mention some of the activities in this field. The range of organisms reported to attack mosquitoes include what might be considered an odd assortment: Spiders, bats, bacteria, protozoans, mites, fish, fungi, beetles, flies, nematodes and viruses.

In Poland, studies on the use of spiders to reduce mosquito abundance have been made by J. Luczak and E. Dabrowska-Prot (Acad. Pol. Sci. Ser. Sci. Biol. 14(5) :315-320, 1966) and Dabrowska-Prot and associates (ibid. 14 (11/12) :771-775; 777-782).

At least 16 species of mosquitoes in the United States appear attractive to nematodes. According to H. C. Chapman, D. B. Woodard, J. J. Petersen (Mosquito News 27(4) :490-492), *Aedes sollicitans* (Wlk.) is attacked by *Agamomermis culicis*. Young mosquito larvae acquire the infection which is then passed through the pupae to the adults as parasitic juveniles. These finally emerge as postparasitic nematodes. Large numbers (10 or more) may kill first instars. Adults may die when the nematode emerges, but the female mosquitoes which survive apparently have a lowered egg production. Although infections of this nematode in southwestern Louisiana are widespread, the only other area from which the species is recorded is New Jersey, according to Petersen, Chapman and Woodard (ibid. 27(4) :493-498).

Perhaps, the best known fungi attacking mosquito larvae are the *Coelomomyces* of which there are now over 38 species. Members of this genus are aquatic and seem to prefer mosquitoes. J. Muspratt (WHO<sup>3</sup> Bul. 29(1) :81-86, 1963) reported that *Coelomomyces* caused a 95 percent mortality of *Anopheles gambiae* Giles larvae in some localities. Marshall Laird (WHO Chron. 21(1) :18-26) used large samples of *Coelomomyces* to infect instars of *Aedes polynesiensis* Marks in an island in the South Pacific, in 1958. Although infections were produced, the larvae died before reproductive stages of the fungus could be reached. However, many larval habitats were seeded with *Coelomomyces*. As a result, 13 of the 35 larvae of *A. polynesiensis* found in 1963 were infected.

<sup>2</sup>All dates refer to 1967 unless otherwise stated.

<sup>3</sup>WHO is the abbreviation for the World Health Organization.

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Although *Thelohania*, a protozoan, is apparently cosmopolitan in distribution, there are a few published records in the United States. Only five States had reported it until D. L. Bailey, W. W. Barnes and R. W. Dewey (J. Invertebrate Path. 9(3) :354-356) found *T. inimica* in larvae of *Aedes canadensis* (Theo.) in Worcester Co., Maryland. H. C. Chapman, T. B. Clark, J. J. Petersen and W. D. Woodard (Pest Control 36(5) :64-73, 1968) found numerous species of *Thelohania* in larvae of *Culex*, *Anopheles*, *Culiseta inornata* (Will.), *Aedes* and *Psorophora confinnis* (L. A.).

When I first saw the name *Culicoides anophelis* Edw., I passed it by as a reference. Then I discovered that this sand fly is an ectoparasite on several species of mosquitoes in the genera *Anopheles*, *Mansonia*, *Culex* and *Aedes*. C. Chastel, J. Rageau and E. Abonnenc (Soc. de Path. Exot. Bul. 59(1) :151-155, 1966) have recorded the species in Cambodia where it was found attached to the abdomen of *Aedes* sp. This is apparently the first time that adults have been reported attached to mosquito abdomens. Of nearly 10,000 specimens examined, only 28 mosquitoes were found to be attacked. *Culicoides cavaticus* Wirth and Jones larvae were observed by T. B. Clark and T. Fukuda (Mosquito News 27(3) :424-425) to prey on fourth instars and pupae of *Aedes sierrensis* (Ludl.) in California. They may even kill this species.

An interesting virus, designated in 1965 as mosquito iridescent virus (MIV), has been found in several species of *Aedes* and *Psorophora ferox* (Humbt.). The disease causes an external iridescence in the thorax of third and fourth instars. According to H. C. Chapman, T. B. Clark, D. B. Woodard and W. R. Kellen (J. Invertebrate Path. 8(4) :545-546, 1966), heavily infected larvae died before pupation. The few which lived to pupate failed to produce adults. Although more than 3,000 mosquito larvae were found infected with MIV in areas of southwest Louisiana, the infection rate in all hosts has been low.

While biological agents have their value, they certainly are in no position to replace chemicals. Numerous pathogenic agents may be found in the exact area where control is being conducted. If these agents are transported to another area, will they be effective in keeping the same mosquito species under even partial control? M. Laird showed how difficult it was to make any headway with *Coelomomyces* in the South Pacific.

When a predator population suddenly fails, it might be useful to know why. If the reasons were known, it might be possible to predict an outbreak of mosquitoes in a given area.

Although much investigation needs to be conducted, it is indicated that some species of mosquitoes will be partly held in check by biological agents.

Ultra-low-volume (ULV) sprays are becoming popular for mosquito control. Such chemicals as malathion and naled have been approved for ULV use. ULV for naled has been approved for mosquito control only on noncropland. During 1966, an outbreak of St. Louis encephalitis in Texas was halted in 8 days through the use of 3 fluid ounces of undiluted malathion per acre. Mortality of *Culex pipiens quinquefasciatus* Say in the spraying operation reached 90-95 percent.

Three of the world's best known malariologists expired in 1967.

Dr. Louis L. Williams, Jr. responsible for designing and spearheading the eradication of malaria from the United States, died at the age of 78 on May 6, 1967. Following the complete success of his work, the concept of world eradication of malaria evolved in the early 1950's. Dr. Williams headed the Malaria Commission to the China-Burma Highway in 1939-1940. In the early part of World War II, he conceived, developed and directed Malaria Control in War Areas. This was a huge program for controlling malaria and other tropical diseases in and around war-related cantonments. He was perhaps the person most responsible for convincing the Department of State that public health programs are an important instrument of foreign policy. From 1948-1953, Dr. Williams was chief of the International Health Division of the U. S. Public Health Service and from 1954-1963 was consultant to the Pan American Health Organization. Among the honors he received were the J. A. LePrince Medal and the Chevalier de la Santé Publique award. He worked tirelessly for the cause of global malaria eradication. Dr. Williams, born in Hampton, Virginia, on February 21, 1889, had boundless enthusiasm, great human understanding and a host of friends and disciples all over the globe. Further details on his life are found in *Tropical Medicine and Hygiene News* 6(2) :4-5, 1957; 16(3) :17-19, 1967. I am indebted to Dr. Williams for his wise counseling over many years in connection with this annual world review of mosquito activities.

Another world famous figure was Professor George Macdonald who died on December 10, 1967, at the age of 64. Following research posts in Sierra Leone, India and Assam, he became Assistant Director of the Ross Institute at the London School of Hygiene and Tropical Medicine and Director in 1945. He was also professor of tropical hygiene at the University of London for many years. During World War II, he was in charge of malaria field laboratories and then served as consultant malariologist in the Middle East and Central Mediterranean Forces. He was particularly noted for his application of modern mathematical techniques to the epidemiology of malaria. His mathematical models presented in papers from 1952-1957 and in his book "Epidemiology and Control of Malaria" have been widely accepted and adapted to computer calculation

to help design and assess malaria control programs. He spent his life in attempting to ameliorate disease, poverty and famine in several tropical countries. Those of us who knew him were impressed with his dynamic personality and his kindness. Prof. Macdonald was President of the Royal Society of Tropical Medicine and Hygiene in 1965-1967. Additional details on his life are given by P. C. C. Garnham (Roy. Soc. Trop. Med. and Hyg. Trans. 62(1) :160, 1968).

The third outstanding malariologist, Dr. Justin M. Andrews, died on June 29, 1967, at the age of 64. Dr. Andrews also was a distinguished teacher, author and public health administrator. During World War II, he was Theater Malariologist in the North African Theater of Operations from 1943-1944 and Theater Malariologist for the Armed Forces in the Pacific in 1945. In the Communicable Disease Center, he rose to Officer in Charge in 1952. He became Assistant Surgeon General of the U. S. Public Health Service in 1953. From 1957 until 1964 when he retired, he was Director of the National Institute of Allergy and Infectious Diseases. Dr. Andrews received the Legion of Merit for his contribution to malaria control in North Africa and Italy, the LePrince Award of the American Society of Tropical Medicine and Hygiene and the Meritorious Service Medal of the Public Health Service. He has been President of the following societies: National Malaria Society, American Society of Tropical Medicine and Hygiene and the American Society of Parasitologists. Dr. Andrews, who was one of the principal executors of the plan to eradicate malaria from the United States, was born on August 8, 1902, in Providence, Rhode Island.

Charles Ronald Ribbands, a malariologist during World War II, died in a road accident, at the age of 53. In West Africa, he studied the attractiveness of humans to mosquitoes and fluctuations in the size of mosquito populations. According to Trevor Lewis (Nature (London) 5093 :1171), he "was a pioneer in the uses of DDT and gammexane as mosquito larvicides. In 1943 he was posted to South East Asia and India as officer commanding No. 2 Entomological Field Unit, to plan and conduct research on new insecticides and methods of controlling malaria and scrub typhus."

William C. McDuffie, who had a long and distinguished career with the U. S. Department of Agriculture, expired at the age of 57, on October 8, 1967. He began work on mosquitoes at New Smyrna Beach, Florida and conducted special studies of these insects in Churchill, Canada. In 1962, he became Chief of Man and Animals Research Branch, where he devoted part of his time to mosquito research and control. Mr. McDuffie was born on January 25, 1910, in Nettleton, Mississippi.

Among the new periodicals which appeared in 1967, the Michigan Entomologist, Journal of Virology, Journal of General Virology and Pesticides Monitoring Journal are of interest to workers in our field.

Since 1947, the solution of the urban yellow fever problem, through eradication of *Aedes aegypti*, has been entrusted to the Pan American Sanitary Bureau. Although eradication has been achieved in most of the originally infested areas of the Western Hemisphere, some of these are being reinfested from non-eradicated areas. In addition to El Salvador, which I mentioned last year, Guatemala has become infested. It is believed that the yellow fever mosquito was introduced from El Salvador.

It is not surprising that the Pan American Sanitary Bureau should be disturbed over present events. At the Conference on *Aedes aegypti* Eradication in the Americas, held April 3-5, 1967, it was pointed out that the problem of reinfestation is not only extremely costly but is a threat to the entire eradication campaign in the Americas. The presence of *aegypti* in the United States and other areas where eradication has not been achieved is a potential danger. Not only does *aegypti* carry yellow fever but dengue and Asiatic hemorrhagic fever.

Dengue, though seldom fatal, proves costly in two ways: First, in man hours lost due to illness; and second, in the loss of tourist trade. Recent epidemics, such as those of the Caribbean area, could affect the overall economy of the countries involved. Although mosquito-borne hemorrhagic fever appears to be confined to Asia, there is no surety that it will not invade this hemisphere. Fatalities of this disease range 10 - 15 percent.

It was urged by the Conference that countries and territories still infested with *aegypti* should . . . "give the highest priority to the provision of funds, personnel, and supplies needed to complete those campaigns as soon as possible."

Yellow fever has been isolated for the first time from an additional species of mosquito, *Aedes fulvus* (Wied.). According to the Pan American Sanitary Bureau (Wkly. Epidemiol. Rpt. 39(48) :269) a pool of 33 specimens was collected on June 28, 1967, in the forest of Amapá Territory of Brazil. This could be significant because *A. fulvus* is known to enter the forest canopy where it comes in contact with the monkey population. In October of the same year, a dead howler monkey was found in a state of putrefaction near the area where *A. fulvus* was collected. The finding of the monkey may have been a coincidence but further studies are underway.

Did you know that there are now more than 800 current periodicals in the life sciences? Current Contents, a highly expensive, weekly journal is an excellent way to review all of these periodicals without the manual labor of obtaining journals and looking through the contents of each.

Names of senior authors and their addresses are found at the back of each issue. For those of us who must read selectively and keep pace with the latest developments in biological sciences, chemistry and medicine, Current Contents is an immense time saver.

TAXONOMY

New mosquito names which have appeared in the 1967 literature are as follows:

- |   |   |
|---|---|
| <i>Aedes chamboni</i> Cornet                          | <i>Culex isabelae</i> Duret                                   |
| <i>Aedes cretatus</i> Delfinado                       | <i>Culex mijanae</i> Brunhes, Adam and Baily-Choumara         |
| <i>Aedes crossi</i> Lien                              | <i>Culex neolitoralis</i> Bram                                |
| <i>Aedes gibbosus</i> Delfinado                       | <i>Culex nicaroensis</i> Duret                                |
| <i>Aedes hispidus</i> Delfinado                       | <i>Culex orstom</i> Brunhes, Adam and Baily-Choumara          |
| <i>Aedes hurlbuti</i> Lien                            | <i>Culex pavlovskiyi</i> Casal and García                     |
| <i>Aedes latipennis</i> Delfinado                     | <i>Culex pereyrai</i> Duret                                   |
| <i>Aedes notabilis</i> Delfinado                      | <i>Culex peytoni</i> Bram and Rattanarithikul                 |
| <i>Aedes protuberans</i> Delfinado                    | <i>Culex pholeter</i> Bram and Rattanarithikul                |
| <i>Aedes ramachandrai</i> Reuben                      | <i>Culex quintetti</i> Brunhes, Adam and Baily-Choumara       |
| <i>Aedes torosus</i> Delfinado                        | <i>Culex ronderosi</i> Quiroga de Linero                      |
| <i>Anopheles tigerti</i> Scanlon and Peyton           | <i>Culex scanloni</i> Bram                                    |
| <i>Culex ameliae</i> Casal                            | <i>Culex shoaie ugandae</i> van Someren                       |
| <i>Culex barrinus</i> Bram                            | <i>Culex silvai</i> Duret                                     |
| <i>Culex brami</i> Forattini, Rabello and Lopes       | <i>Culex spiculosus</i> Bram and Rattanarithikul              |
| <i>Culex castroi</i> Casal and García                 | <i>Culex spiculothorax</i> Bram                               |
| <i>Culex chitae</i> Duret                             | <i>Culex thurmanorum</i> Bram                                 |
| <i>Culex ernanii</i> Duret                            | <i>Eretmapodites caillardi</i> Rickenbach, Ferrara and Eouzan |
| <i>Culex eukrines</i> Bram and Rattanarithikul        | <i>Eretmapodites salauni</i> Rickenbach, Ferrara and Eouzan   |
| <i>Culex faurani</i> Duret                            | <i>Haemagogus aeritinctus</i> Galindo and Trapido             |
| <i>Culex ferreri</i> Duret                            | <i>Toxorhynchites cavalierii</i> García and Casal             |
| <i>Culex fuscisiphonis</i> Bram and Rattanarithikul   |   |
| <i>Culex hamoni</i> Brunhes, Adam and Baily-Choumara  |   |
| <i>Culex helenae</i> Brunhes, Adam and Baily-Choumara |   |
| <i>Culex hepperi</i> Casal and García                 |   |
| <i>Culex incomptus</i> Bram and Rattanarithikul       |   |

H. H. Wang and C. Y. Feng (*Acta Zootaxonomica Sinica* 1 (1) : 37-41, 1964) described the following new species of *Culex*: *pilifemoralis*, *szemaonensis* and *harpagophallus*.

In order to keep up with revisions in mosquito names, Dr. Alan Stone's Supplement III to "A Synoptic Catalog of the Mosquitoes of the World" is a must. This was published in *Ent. Soc. Wash. Proc.* 69(3) : 197-224.

Ralph A. Bram has produced several valuable publications during the year. One of the most extensive of these is entitled "Contributions to the Mosquito Fauna of Southeast Asia. II. The Genus *Culex* in Thailand (Diptera: Culicidae)" (*Amer. Ent. Inst. Contrib.* 2(1) :1-296). Although a taxonomic paper, it included data on distribution and biology of 60 species and subspecies found in Thailand. Of this number, 19 were new to the country and 5 were described as new (see beginning of this section). Seven names were placed in synonymy, another seven dismissed as misidentifications and one subspecies was elevated to specific rank. A description of the male, female and fourth instars has been included. Numerous keys and excellent illustrations are found throughout the work.

Another paper by Dr. Bram is "A Revision of the *Culex* Section of the Preliminary Keys to the Mosquitoes of Vietnam." This has 14 pages and was published by the Smithsonian Institution.

A third publication by Dr. Bram (*U. S. Nat. Mus. Proc.* 120 (3557) :1-122) concerned the classification of *Culex* subgenus *Culex* of the New World. Descriptions of adults and larvae and data on distribution were included. Keys to the species of this subgenus, based on male terminalia and larvae, are most useful. Although *Culex alani*, *bickleyi*, *covagarciae* and *oswaldoi* were described as new species, they should be credited to Forattini who unfortunately published these names in 1965.

Bram and M. Rattanarithikul (*Ent. Soc. Wash. Proc.* 69(1) :1-17) described six new species of *Culex* from Thailand (see beginning of section).

This year, Mercedes D. Delfinado (*Amer. Ent. Inst. Contrib.* 1(8) : 1-56) has written another valuable paper on the taxonomy of mosquitoes of the Oriental region. The present one concerned a revision of *Aedes*, subgenus *Neomacleaya*, in Thailand. Data on 20 species were included. Of these, 7 were described as new (see beginning of this section). Keys to males, females, pupae and fourth stage larvae were included. Taxonomic characters for these stages, distribution and biological notes for all species were furnished.

Worthwhile illustrated keys to third and fourth stage larvae as well as to females of the anophelines of the Philippines were published by F. E. Baisas and F. H. Dowell (*J. Med. Ent.* 4(1) :11-23).

*Anopheles nivipes* Theo., long considered a synonym of *A. philippinensis* Ludl., has been observed in Malaya by J. A. Reid (J. Med. Ent. 4(2) :175-179) to have characters which differed from those of the latter species. These differences, particularly in pupae and males, were pointed out in detail and well illustrated. The problem of distinguishing these two forms from *A. pallidus* was discussed. Although Dr. Reid believes that there is no reason to doubt that *A. nivipes* and *A. philippinensis* are species, he stated that their validity should be confirmed by "examining series of progeny reared from single females."

See also Cova Garcia et al. in Distribution Section.

"An Atlas of Common Queensland Mosquitoes," by Elizabeth N. Marks, concerns 23 common species known from Queensland. This most useful booklet of 87 pages contains characters for identification which may be discerned with a 10-power lens and with occasional access to a microscope. Under each species, Dr. Marks has included distinctive characters, distribution, biological notes, relation to disease if any and species with which each may be confused. The keys and large illustrations are important features of the atlas.

"Bloodsucking Mosquitoes of the Subtribe Culisetina (Diptera, Culicidae) of the World Fauna," by A. V. Maslov (Akad. Nauk SSSR. Zool. Inst. Opredeliteli Faune SSSR 93, 181 pp.) was written in Russian. A systematic section (with keys), biological data, general morphology and world-wide distribution of the group were included.

C. M. Myers (Canad. Ent. 99(8) :795-806) presented a key and measurements for eggs of 23 species of *Aedes* from California and Nevada. Eggs of 11 species are described for the first time. The illustrations are exceptionally clear.

See also Eddleman in Anatomy, Morphology and Physiology Section.

#### DISTRIBUTION

Ellinor C. C. van Someren (Bul. Ent. Res. 57(2) :207-220) has published a valuable check list of 159 culicines of Tanganyika. Of these, three probably have not been described. Notes on distribution of each species in Tanganyika and systematics when of interest were included.

C. T. O'Connor, Jr. (Mosquito News 27(1) :42-54) recorded 34 species and 2 subspecies of *Anopheles* from Ethiopia. The distribution by species, was shown on numerous maps. An additional feature was a table which showed the distribution of the species by provinces in that country. Although the collection records were made chiefly by personnel of the Ethiopian Malaria Eradication Service, other sources were included.

The distribution of the *Anopheles* of the Republic of Rwanda and the Republic of Burundi has been presented by M. Vermynen (Riv. di Malariol. 46(1/3) :13-22).

A study of the culicine larvae of North Iraq was made by J. Abul-Hab (Bul. Ent. Res. 57(2) :279-284). Brief biological notes were furnished on each of the nine species discussed.

As a result of a survey on the Andaman Islands, K. S. Krishnan and P. G. Helernkar (Indian Soc. Malaria and other Commun. Dis. Bul. 4(1) :35-42) reported nine species of *Anopheles* to be present there.

T. Ramachandra Rao (WHO Bul. 36(4) :547-551) furnished data on the localities in which *Aedes aegypti* (L.) has been found in India, Pakistan, Burma and Ceylon.

The discovery of *Culex infantulus* Edw. in Malaya has been reported by Dr. D. H. Colless (J. Med. Ent. 4(4) :519-520). Specimens were collected in Segambut, Selangor.

P. Palicka (Acta Ent. Bohemoslovaca (Praha) 64(1) :69-78 has published in English, "Contribution to the Study of Mosquitoes (Diptera, Culicinae) Occurring in some Areas of Central and South Moravia." A total of 26 species of mosquitoes now has been recorded from four areas in Central and South Moravia.

"Mosquitoes (Culicinos) de Venezuela," by P. Cova Garcia, E. Sutil and J. A. Rausseo, included keys and illustrations to the mosquitoes of that country. Volume 1 containing 410 pages and volume 2, 406 pages were both published in 1966 at Caracas.

R. A. Brust and K. S. Kalpage (Mosquito News 27(1) :117-118) have reported four species of *Aedes* new to Manitoba, Canada. These were *abserratus* (F. and Y.), *triseriatus* (Say), *decticus* (H. D. and K.) and *barri* (Rueger). In Canada, *Aedes decticus* has been reported from Labrador and Ontario and *A. barri* only from Ontario.

J. E. Porter (Mosquito News 27 (1) :35-41) presented a check list of mosquitoes recorded from the Greater Antilles, as well as the Bahama and Virgin Islands.

The "Mosquitoes of Colorado," was written by F. C. Harmston and F. A. Lawson who have spent many years collecting mosquitoes in the field and identifying them. The book of 140 pages, contains keys to males, females and larvae and full-page plates with details of 42 species in 6 genera.

Additional distribution records of mosquitoes in Michigan from 1948-1963 were presented by C. B. Obrecht (Mich. Ent. 1(5) :153-158).

In a study of the distribution of *Aedes sollicitans* (Wlk.) and *A. taeniorhynchus* (Wied.) in the United States, K. L. Knight (Georgia Ent. Soc. J. 2(1) :9-12) found the range to be correlated with the average annual minimal temperatures. *Aedes sollicitans* apparently could overwinter at an average minimum temperature of -30°F. and *A. taeniorhynchus* at 0°F. Records for the two species, obtained from 65 sources, were clearly plotted on maps. Distribution was shown by counties.

New State records\* of mosquitoes in United States follow:

<i>Species</i>	<i>State</i>	<i>Reported By</i>
<i>Aedes abserratus</i> (F. and Y.)	Indiana	R. E. Siverly
<i>Aedes fitchii</i> (F. and Y)	Delaware	R. W. Lake
<i>Anopheles earlei</i> Vargas	New Jersey	W. J. Crans
<i>Culiseta morsitans</i> (Theo.)	Indiana	R. E. Siverly
<i>Orthopodomyia alba</i> Baker	Delaware	R. W. Lake

### TECHNIQUES

One of the most interesting studies of colonization of mosquitoes during the year was that of a crab-hole breeding mosquito, *Deinocerites pseudus* D. and K. by P. Galindo (Mosquito News 27(2) :187-190) in Panama. This is apparently the first successful colonization of any species in this genus. Other colonies of mosquitoes have been established as follows: In Connecticut, R. C. Wallis and J. Frempong-Boadu (ibid. 27 (1) :9-11) described a method for rearing *Wyeomyia smithii* (Coq.). In Canada, R. A. Brust and K. S. Kalpage (ibid. p. 117) discovered a way to rear *Aedes abserratus* (F. and Y.) in the laboratory. Although *Culex univittatus* Theo. had been colonized in Egypt, P. G. Jupp and R. G. Brown (Ent. Soc. South. Afr. J. 30(1) :34-39) had difficulty in establishing a colony in South Africa. After determining that a large space and special lighting conditions were necessary before the mosquitoes could mate, they succeeded in establishing a colony in Johannesburg. By 1967, they estimated that it was in the seventh generation.

I. A. Urgenson and V. S. Teplykh (Ent. Obozr. 46 (2) :295-298, in Rus., Engl. Sum.) have suggested a technique to detect yellow bodies in the ovaries of insects, including *Aedes aegypti* (L.), by luminescent microscopy.

A modification of the Greenberg artificial feeding device has been described by R. Behin (Mosquito News 27(1) :87-90). A membrane prepared from the crop of a chicken and a stirring rod to prevent sedimentation of suspended materials were the improvements suggested.

E. I. Hazard (Mosquito News 27(1) :115-116) has modified A. B. Weathersby's ice water technique for harvesting mosquito pupae. A mechanical instrument was devised so that two men could harvest 20,000 pupae of *Anopheles* or *Culex* in less than an hour. Ice water was used in the device.

A number of timely methods for dissecting mosquitoes have been described by Jack C. Jones (Mosquito News 27(1) :76-82). He not only included his own techniques but those of many other workers who

\*Noted in the 1967 literature, but collections may have been made earlier.

had written him about their special methods. The paper needs to be read in full to obtain the numerous helpful suggestions.

Mechanical aspirators are continually being devised and improved. The "CDC Sweeper," somewhat similar to the three-volt suction apparatus described by E. N. De Freitas, R. E. Shope and O. R. Causey (Mosquito News 26(3) :368-372, 1966) has been developed by R. O. Hayes, G. E. Kitaguchi and R. M. Mann (ibid. 27(3) :359-363) into a six-volt aspirator. Although mosquito collections could be made from foliage by moving the unit slowly back and forth, the aspirator was more useful in collecting resting mosquitoes and recently disturbed flying ones. Details of the mechanism were included.

R. C. Husbands and J. R. Holten (Calif. Vector Views 14(12) :78-80) have developed an improved battery operated mechanical aspirator which is more efficient, convenient and rugged than that of Husband's in 1957. The aspirator was constructed for both laboratory and field use. Details of construction and illustrations were included.

According to R. O. Hayes and H. K. Maxfield (Mosquito News 27 (4) :458-461), diapause was interrupted in *Culiseta melanura* (Coq.) by using a 20-hour photoperiod and feeding larvae on ground chicken feed. In 11 tests, they obtained 95 percent pupation. By this technique, adults were produced routinely for 2 years during the winter months in Massachusetts.

W. R. Horsfall and M. L. Taylor (Ent. Soc. Amer. Ann. 60(1) : 118-120) have found that temperature and age are important factors in inducing insemination of mosquitoes under laboratory conditions. A method for manually inducing insemination was given in detail. By mating 14-day old males with females of any age between 3 and 14 days, a high level of insemination of a wild strain of *Aedes vexans* (Meig.) was obtained. Females, fully engorged with a honey-water mixture and those held in a relative humidity of 80 percent or higher, were most receptive. For successful copulation of any mosquito, the apex of abdominal segment nine needed to be extended well beyond segment eight.

## GENETICS

One of the most significant contributions in a long time is a paper by Mario Coluzzi and A. Sabatini (Parassitologia 9(2) :73-88) on identification of species A and B of the *Anopheles gambiae* complex. Fourth instars, reared under laboratory conditions, were distinguished by the polytene chromosomes in the salivary glands. Chromosome maps were included in this article.

G. Frizzi (Inst. Marine Med. Gdansk Bul. 18(3/4) :121-129) has shown the importance of cytogenetics in the investigation of mosquito

species complexes. By using *Anopheles maculipennis* complex as an example, he pointed out that the salivary gland chromosomes have general similarities in the six sibling species in Europe. Of these, *A. sacharovi* Favr. not only may be distinguished by a rearrangement of the chromosomes "but also morphologically, in that it reveals a very large number of branches of antepalmate hairs the lowest number of which exceeds even the highest number found in the other species. It also has a thorax of a constantly pale colour, besides laying its practically floatless eggs in a uniform grey pattern. But the most interesting fact is that these chromosome rearrangements found in every species are peculiar to the whole species regardless of its geographical position."

One of the most fascinating studies being conducted in genetics is the projected biological control of *Culex pipiens quinquefasciatus* Say by genetic incompatibility. H. Laven (Nature (London) 216(5113) :383-384) achieved eradication of this species in a limited area of Burma during a pilot project sponsored by the World Health Organization there in 1966. An incompatible strain of *C. p. quinquefasciatus* with the cytoplasm from a strain from Paris and the genome from a strain from Fresno, California, was used in this study. At Okpo, a village near Rangoon, adult mosquitoes ranged from 4,000 to 20,000 per day. Incompatible males were released from February until early May 1967, usually at the rate of 5,000 per day. By the fifth week, the percentage of eggs not hatching was almost 20 percent. This rose to 39 percent in the eighth week and 70 percent in the tenth. By May 9 and 10, 100 percent non-viable egg rafts were obtained. Eradication was accomplished in about 3 months.

In another paper, Dr. Laven (WHO 37(2) :263-266) suggested a possible model for speciation by the cytoplasmic isolation in the *Culex pipiens* complex.

The number of mutants of *Aedes aegypti* (L.) had risen to 87 before a new one called bronze (bz) was added by S. C. Bhalla and G. B. Craig, Jr. (J. Med. Ent. 4(4) :467-476). This female sterile mutant was found to turn the darker part of all stages of *A. aegypti* pale tan or bronze. Although bronze females laid sterile eggs, males remained fertile.

G. A. H. McClelland (WHO Bul. 36(1) :176-178) has discovered a spontaneous dominant mutant called *Gold*, in *Culex pipiens* L. The abdominal tergites in the *Gold* phenotype were completely covered with pale golden scales. This is apparently the first mosquito mutant found to show recessive lethality.

Another mutant, designated *Black larva* (*Bl*), has been reported from *C. pipiens* by R. C. Vandehey (Mosquito News 27(1) :69-73). The larva of *C. pipiens* normally has a translucent head capsule or has only a small pigmented bar on each gena. In the *Bl* mutants, a greatly

enlarged bar was found as well as heavy pigmentation on the frons and labial areas.

A number of valuable articles on mosquitoes are to be found in the book "Genetics of Insect Vectors of Disease," edited by J. W. Wright and R. Pal. The work was published in Amsterdam and contains 794 pages. J. B. Kitzmiller and G. F. Mason (pp. 3-15) discussed the formal genetics of anophelines while H. Laven (pp. 17-65) presented the formal genetics of *Culex pipiens* L. G. B. Craig, Jr. and W. A. Hickey (pp. 67-131) described the genetics of *Aedes aegypti* (L.). Kitzmiller, G. Frizzi and R. H. Baker (pp. 151-210) discussed the evolution and speciation within the *Anopheles maculipennis* complex. The *Anopheles gambiae* complex was explored by G. Davidson and Coauthors (pp. 211-250). Dr. Laven (pp. 251-275) presented studies on speciation and evolution in *Culex pipiens*, while G. A. H. McClelland (pp. 277-311) discussed speciation and evolution in *Aedes*. A. Spielman and Dr. Kitzmiller (pp. 459-485) included mosquitoes in their discussion of the genetics of populations of medically-important arthropods. The genetics of insecticide resistance in insects, including mosquitoes, was treated by A. W. A. Brown (pp. 505-552). P. F. Mattingly presented the genetics of behavior on pages 553-566. W. W. Macdonald (pp. 567-584) showed the influence of genetic and other factors on vector susceptibility to parasites.

#### BEHAVIOR, BIOLOGY AND ECOLOGY

The discovery of *Culiseta melanura* (Coq.) larvae breeding in artificial containers in Connecticut in 1965 and 1966 has been reported by R. H. Wallis and L. Whitman (J. Med. Ent. 4(3) :273-274). This natural occurrence in tractor tires is a departure from its usual habits. In both areas where the larvae were found there was no contact with ground water, plant roots or soil.

G. R. DeFoliart (J. Med. Ent. 4(1) :31) observed *Aedes canadensis* (Theo.) feeding in nature on Blanding's turtle (*Emydoidea blandingi*) in Wood County, Wisconsin. Many mosquitoes were hovering over the turtle and engorging.

A number of timely papers by Botha De Meillon and associates, concerning habits of *Culex pipiens quinquefasciatus* Say, appeared in volume 36, number 1 of the WHO Bulletin. One of the most significant concerned the habit of adults feeding on cane sugar by De Meillon, A. Sebastian and Z. H. Khan (pp. 53-65). Cane-sugar feeding delayed oviposition in gravid females. Not only is this find important for laboratory workers who keep adults alive on cane sugar but for field personnel who find this habit occurring in nature. If conditions are not favorable for oviposition, cane-sugar feeding could delay deposition of eggs until suit-

able conditions occur. In nature, feeding on cane sugar also prolonged the life of the mosquito. During the monsoons, many gravids came to the cane fields to feed as the rains washed out the breeding places. These adults, deprived of the stimulus of the breeding areas, flew about in a disoriented state.

The time of arrival of gravid *C. p. quinquefasciatus* at an oviposition site, the oviposition cycle and the relation between the time of feeding and time of oviposition were reported by De Meillon, Sebastian and Khan (pp. 39-46).

The duration of the egg, larval and pupal stages of *C. p. quinquefasciatus* in Rangoon, Burma, was discussed by De Meillon, Sebastian and Khan (pp. 7-14). Studies on flight activities by De Meillon and Khan (pp. 15-20) revealed that the species moves about more in the daylight and dark than was usually suspected.

Sebastian and De Meillon (pp. 47-52) reported that successful matings of *C. p. quinquefasciatus* occurred in relatively small cages. Swarming of males, normally under field conditions, was not necessary for copulation.

Another valuable study by De Meillon, Myo Paing, Sebastian and Khan (pp. 67-73) revealed that *C. p. quinquefasciatus* rested outdoors in Rangoon, Burma. Usually, it was assumed that this species fed and rested indoors. In Rangoon, females which rested indoors, fed indoors whereas those which rested outdoors appeared to feed outdoors. The authors concluded that the risk of infection from filariasis was as great indoors as outdoors.

J. E. Wright and C. E. Venard (Ent. Soc. Amer. Ann. 60(4) :861-862) demonstrated that females of *Aedes triseriatus* (Say) were just as attractive to males whether they had blood meals or not. They found that copulation time had little effect upon the percent of the females inseminated.

A Spielman (WHO Bul. 37(2) :271-276) discussed the population structure in the *Culex pipiens* complex. In the tropical areas, *C. p. quinquefasciatus* Say fed on blood of many different hosts whereas *C. p. pipiens* L. appeared to be more adapted to birds. The autogenous *C. p. molestus* Forsk. was essentially a non-blood feeder. In areas where these forms overlapped, *pipiens* and *quinquefasciatus* appeared to interbreed as well as *molestus* and *quinquefasciatus*. On the other hand, *pipiens* and *molestus* apparently were isolated by mating barriers.

According to N. V. Volozina (Ent. Obozr. 46(1) :49-59, in Rus., Engl. Sum.), the minimal amount of blood which could cause development of follicles in *Aedes intrudens* Dyar and *A. diantaeus* H. D. and K. was equal to 0.5-0.7 mg. "Maturation of an incomplete portion of eggs on account of one incomplete bloodsucking may take place when the ratio of

consumed blood weight to the body weight of unfed female makes no less than 0.4-4.6." As the physiological age increased, the quantity of blood sucked by a female at a complete bloodsucking decreased. Carbohydrate deficiency resulted in abortion of follicles and mortality of females in a number of cases.

A valuable piece of research concerning reproduction in *Aedes impiger* (Wlk.) and *A. nigripes* (Zett.) was conducted by P. S. Corbet (Nature (London) 215(5101) :662-663) on Ellesmere Island, Canada. It has been assumed previously that, in the first gonotrophic cycle, an individual mosquito has the potentiality to mature eggs autogenously or anautogenously but not in both ways. Experiments showed that a few females of each species show oligate autogeny but that the majority are anautogenous or autogenous, depending on whether or not they obtain a blood meal. The numbers of eggs maturing after facultative autogeny, obligate autogeny and anautogeny were about 5, 25 and 50 respectively.

J. D. Gillett (Roy. Soc. (London) Proc. Ser. B. 167(1008) :316-329) has postulated that mosquitoes which complete blood meals quickly and are slow to produce irritation in the host are likely to deposit more eggs. Also, these traits "will be maintained by natural selection, with the onset of irritation in the host acting as the main selection force." Experiments conducted with laboratory-reared *Aedes aegypti* (L.) and wild-caught *A. africanus* (Theo.) showed that ". . . feeding was unusually fast in the wild species, which had presumably been subjected to rigorous selection against slow feeding, but variable and often slow in the captive species, which had been reared for generations in the absence of this pressure. The results also indicated a slight compensatory delay in the onset of irritation following slow feeding in the wild mosquitoes . . ."

P. H. Thompson (Amer. Midland Natur. 77(2) :533-535) observed swarms of *Coquillettidia perturbans* (Wlk.) in an oak-maple forest near Madison, Wisconsin, in June 1961. About 200 mosquitoes were seen in a ball about 1.5 m. above the ground on one evening and a smaller swarm was noted the next evening. Secondary swarms on both evenings probably used the author's head as a marker rather than a host.

Swarms of *Culex tritaeniorhynchus* Giles were investigated by S. Kawai, Y. Wada and N. Omori (Trop. Med. (Nagasaki) 9(1) :58-64, Engl. Sum.) in the field near Nagasaki and Isahaya Cities, Japan, from early spring until late fall in 1965 and 1966. They were ellipsoid in shape and formed about 1.5 m. high in the air. Mating often was observed in the swarm.

A rather comprehensive investigation of the flight habits of *Anopheles freeborni* Aitken, over a 4-year period in California, was made by S. F. Bailey and D. C. Baerg (Calif. Mosquito Cont. Assoc. Proc. 35:55-69). A total of 54,800 marked specimens was used in the release-recapture

tests. In the major rice breeding areas, mosquitoes tended to disperse in all directions but more generally towards the south. The major fall flight varied from year to year but usually occurred in late September. Some individuals flew as far as 17.5 miles but the majority sought hibernation sites within a 5-mile radius from the breeding site in the rural areas. In the Sacramento Valley, populations hibernated mainly in the valley but some remained in the foothills. Semi-dormant females moved about from niche to niche except when the temperature fell below 40-50°F. Females generally lived about 5 months in winter and their resting places often were miles from the point of origin.

A study of the circadian flight activity of *Anopheles gambiae* Giles was conducted by M. D. R. Jones, M. Hill and A. M. Hope (J. Expt. Biol. 47(3) :503-511). Under conditions of alternating 12 hours' light and 12 hours' dark, mosquitoes were inactive in light but active during the first-half-hour following darkness. Although activity continued, it became less frequent during the rest of the dark period. In constant dark, there was cyclical activity and peaks appeared about every 23 hours. It was thought that an inhibitory neurohormone was the cause for inactivity associated with light and an excitory neurohormone with flight activity during darkness.

By using a cartop-mounted funnel trap, K. L. Knight and C. Henderson (Georgia Ent. Soc. J. 2(3) :63-68) found that *Aedes vexans* (Meig.) had a crepuscular peak at the third 15-minute period after sunset at Ames, Iowa. Activity steadily declined until about 11 P.M., then became low but constant until the fourth 15-minute period before sunrise. A lower crepuscular peak occurred in the third 15-minute period before sunrise.

A. W. Lindquist and Coauthors (WHO Bul. 36(1) :21-37) found that radioactive *Culex pipiens quinquefasciatus* Say dispersed rather evenly over a concentric test area. The study was conducted in the Kemmendine area of Rangoon, Burma. Following release, the mosquitoes flew upwards for 20-25 feet, then away over housetops and trees. In this densely populated area, concentric circles, 150 feet apart, were laid over a map. Some mosquitoes could fly more than a third of a mile over water and more than one-half mile from the release point without lures or traps. As the mosquitoes were within several feet of ample sources of nectar, blood meals and water suitable for oviposition, there was speculation as to the reason for dispersal up to 600 yards. They may fly in response to an urge to fly, and in this sense the first flight may be a migration.

Perhaps the most unusual mating behavior among mosquitoes is that of *Opifex fuscus* Hutton and *Deinocerites cancer* Theo. M. W. Provost and J. S. Haeger (Ent. Soc. Amer. Ann. 60(3) :565-574) have presented an interesting paper on the mating habits of *D. cancer*. Males of

*D. cancer*, the crab-hole mosquito, often skate or walk over the water to seek pupae which they seize. They make circles about the pupal horns with the tips of their extremely long antennae. As soon as a female emerges, copulation begins but in some cases this act begins just before she leaves the pupal skin. Coitus usually lasts 40-50 minutes but sometimes continues up to 2 hours. The male often drags the attached female to the shore and may even fly with a female dangling in coitus. Males mate many times but females usually only once. When females emerge unattended, males start copulating with them immediately. Males are aggressive and will fight over pupae, virgin females or recently cast pupal skins. The authors made a detailed comparison with *O. fuscus*, the only other mosquito known to have similar mating habits. [See also my annual review in N. J. Mosquito Extermin. Assoc. Proc. 53:23-24, 1966, for additional data on *O. fuscus*.]

In connection with research on selection of oviposition sites of *Aedes taeniorhynchus* (Wied.), W. H. McGaughey and K. L. Knight (Ent. Soc. Amer. Ann. 60(1) :107-115) conducted a study on the oviposition activity of this species under laboratory conditions. Females became relatively inactive following a blood meal. When ovarian development was completed, the first onset of darkness initiated a period of flight activity. This activity occurred even when suitable oviposition substrates were available. Flight apparently was necessary for oviposition and was the result of an appetite drive. The observations suggested "that unnatural conditions imposed in the laboratory may result in acceptance of substrates not normally utilized in nature."

Another phase of the above mentioned subject concerned the oviposition movements of *Aedes* females. Dr. Knight and D. Sulzbach (Calif. Mosquito Contr. Assoc. Proc. 35:100-103) described the oviposition movements of *Aedes aegypti* (L.) *A. trivittatus* (Coq.) and *A. taeniorhynchus* under microscopic conditions. According to the authors, this is the first time that these movements have been recorded microscopically.

The series of studies on the eggs of floodwater mosquitoes has been continued by W. R. Horsfall and Milam Trpis. Part 10 (Ent. Soc. Amer. Ann. 60(5) :1021-1025) concerned the conditioning and hatching of winterized eggs of *Aedes sticticus* (Meig.) in Illinois. Embryos withstood a limited exposure to post-cold temperatures between 8° and 32° C. The optimum temperature for conditioning and hatching was 15° C. Part 11 by Trpis and Horsfall (ibid. 60(6) :1150-1152) dealt with hatching media for *A. sticticus*. "Microbial activity in different dilutions of nutrient broth hatched eggs at all temperatures between 8° and 25° C . . . ."

The pupation rhythm in *Aedes taeniorhynchus* (Wied.) was reported by several workers during the year. In part one of this study, M. W. Provest and P. T. M. Lum. (Ent. Soc. Amer. Ann. 60(1) :138-149) showed

that this species followed a diurnal rhythm during alternating light and dark with a period of about 22.2 hours, which may be truly circadian. Temperature was not found to affect the period of the pupation rhythm but food deficiency during larval development may lengthen the pupal period.

J. K. Nayar (ibid. 60(5) :946-971) concluded that nutrition, density of larval population and salinity of the water are the major environmental factors during larval life that influence the pattern and periodicity of the pupation rhythm of *A. taeniorhynchus*. The hypothesis, that the emergence rhythm was found to be dependent on the pupation rhythm and separated by a period affected by temperature but not light regime, was confirmed.

An interesting paper on larval habitats of culicines in the South African highveld region was published by P. G. Jupp (Ent. Soc. Southern Afr. J. 30(2) : 242-250). The study area, Olifantsvlei, is a cattle farm and sewage effluent disposal area. Of the seven species observed, *Culex pipiens* L. and *C. theileri* Theo. were the most frequently collected in the effluent habitat. *Culex univittatus* Theo., *Aedes lineatopennis* (Ludl.) and *A. caballus* (Theo.) also were found in the effluent but to a much lesser extent. *Aedes lineatopennis*, *A. dentatus* (Theo.), *A. caballus* and *A. mixtus* Edw. all preferred rainwater for breeding. *Culex theileri* had a slight preference for polluted ground pools supplied by rainwater over the effluent, but was found in all the types of habitat. Although *C. pipiens* was found in all habitats, rainwater was selected only to a small degree.

W. Hanec and R. A. Brust (Canad. Ent. 99(1) :59-64) studied the effect of temperature on the immature stages of *Culiseta inornata* (Will.) under laboratory conditions. The optimum temperature for rearing was near 21°C. When reared at 29°C., the highest mortality occurred during molting. Although most larvae attempted to molt, molting was incomplete or death occurred after molting. Fourth instars, reared at 29°C., seemed to be oversized and either died during molting or if they lived to pupate, died shortly after.

Brust (ibid. 99(9) :986-993) demonstrated that weight and development time stages of *Aedes vexans* (Meig.), *A. nigromaculis* (Ludl.) and *Culiseta inornata* differed under various rearing temperatures. With each increase of 5.5°C. in rearing temperature, weight decreased in larvae, pupae and adults. In all three species, the fourth instar period was the longest and the weight gain the greatest. The optimum survival temperature for *A. nigromaculis* and *Culiseta inornata* was 21°C. while that for *A. vexans* was 26.5°C.

In order to determine the effect of photoperiod and temperature on diapause in *Aedes triseriatus* (Say), K. D. Kappus and C. E. Venard (J. Insect Physiol. 13(7) :1007-1019) experimented with eggs from

two strains maintained for a number of years under laboratory conditions. One strain originally came from Hocking County, Ohio, and the other from Montgomery, Alabama. The strain from Alabama diapaused as eggs or fourth instars but the Ohio strain diapaused only as eggs. Direct photoperiodic treatment had a marked effect on eggs. When adults received the same treatment, there was no apparent effect on induction of diapause in their subsequent eggs. "Both temperature and photoperiod caused differences in diapause induction and there was a significant interaction between these factors."

An investigation of possible competitive displacement between populations of *Aedes aegypti* (L.) and *A. albopictus* (Skuse) in Calcutta was carried out by S. K. Gilotra, L. E. Rozeboom and N. C. Bhattacharya (WHO Bul. 37(3) :437-446). Oviposition traps showed that *A. aegypti* was more prevalent than *A. albopictus* in houses and tenements in the cities whereas *A. albopictus* was predominant in rural areas. In small city gardens, the population of both species was about equal. If *A. aegypti* should be eradicated in Calcutta, the authors recognize the possibility of *A. albopictus* replacing *A. aegypti*.

Research on the histological development of organs of genotypic males of *Aedes stimulans* (Wlk.) and *A. aegypti* (L.) was conducted by J. F. Anderson (J. Expt. Zool. 165(3) :475-484). "Intersexuality of homologous and nonhomologous organs was shown to arise in a manner that has not been previously reported in insects. Homologous organs develop similarly . . ." in males and females up to a critical stage when morphogenesis commences. "This critical period occurs at an earlier stage of ontogeny in males than it does in females. At elevated temperatures and during their period of plasticity, organs of genotypic males developed in a feminine manner. Determination began approximately at the time of divergent development." When the temperature was consistently high, the organs continued their feminine development but lowered temperatures prior to feminine specialization resulted in masculine development. The influence of temperature on development of the sexes occurs during the larval stage.

Biological notes on a few species of *Anopheles*, *Culex*, and *Aedes* found in the arid zone of Australia have been given by Elizabeth N. Marks (Austral. Nat. Hist. 15(10) :331-336).

J. Lukasiak (Wiad. Parazytol. 13(6) :751-758, in Pol., Engl. Sum.) made a study of 17 species of mosquitoes in water reservoirs of the Warsaw area. Both seasonal pattern and appearance of pupae were discussed. Characteristics of pupae were described. The mass occurrence of mosquitoes in Swinoujscie, Poland, in August 1965 also was reported by Dr. Lukasiak (ibid. 13(1) :113-115).

"Mosquito Ecology," a report of a WHO Scientific Group (WHO Tech. Rpt. Ser. No. 368, 22 pp.) stressed the need for techniques to determine actual numbers of all stages of mosquitoes in a population. Better measurement of mosquito numbers are necessary to assess the effectiveness of control. Due to the rapid growth of cities and irrigation projects in rural areas, mosquito populations have changed. Ecological studies were recommended to help forecast changes in such populations.

Y. Wada and Coauthors (Trop. Med. (Nagasaki) 9(1) :45-57) reported the results of their studies in 1965 on the life history and seasonal prevalence of *Culex tritaeniorhynchus* Giles in the Nagasaki area of Japan. From collections in animal shelters, human dwellings and baited traps, and dry ice traps, nearly 150,000 mosquitoes were captured from early spring to early winter. The dominant species collected was *C. tritaeniorhynchus* which had a strong preference for animals. Day length, rather than temperature, apparently was a controlling factor in determining the gonioactivity of female *C. tritaeniorhynchus*.

M. Shimogama and Y. Takatsuki (Endemic Dis. Bul. (Nagasaki Univ.) 8(4) :159-165) observed the seasonal distribution and abundance of mosquitoes in a cave dug in a hill in Nagasaki City, Japan. Throughout the year the chief mosquito found was *Culex pipiens pallens* Coq. Few males were found from February to April but began to appear in May. Both males and females reached high population levels in November. Females reached their maximum numbers in December, but decreased sharply in February.

#### ANATOMY, MORPHOLOGY AND PHYSIOLOGY

R. H. White has continued his valuable research on the mosquito eye. An earlier study was mentioned in my review (N. J. Mosquito Extermin. Assoc. Proc. 51:31, 1964). He has begun a series of papers on the effect of light and light deprivation on the ultrastructure of the larval mosquito eye. Part I by White and C. D. Sundeen (J. Expt. Zool. 164(3) : 461-478) concerned polyribosomes and endoplasmic reticulum. They showed that the larval eye was affected by light. Part II by White (ibid. 166(3) :405-425) dealt with the rhabdom. The surface area of rhabdom membrane was found to be 2-3 times greater in mosquito larvae reared in darkness than those reared in light. In both experiments, *Aedes aegypti* (L.) was used.

C. W. Schaefer, J. P. Vanderberg and J. Rhodin (J. Cell Biol. 34(3) : 905-911) have observed the midgut muscle of *Anopheles quadrimaculatus* (Say) to be very similar to other described insect visceral muscles. "Tubules of the T-system and cisternae of the sarcoplasmic reticulum are sparse, mitochondria are few and their cristae are poorly developed, and

the muscle is poorly tracheolated . . . Myosin myofilaments appear to be surrounded by 12 actin filaments, and there is little clear evidence of banding."

W. S. Romoser and C. E. Venard (Ent. Soc. Amer. Ann. 60(3) : 617-623) have continued their valuable study of the oesophageal diverticula in *Aedes triseriatus* (Say). They have discovered that "The dorsal diverticula develop mitotically from the oesophageal tissues of the proventriculus and in this way are different from the ventral sac which develops as a result of mitotic divisions in the anterior imaginal ring . . . The dorsal sacs are inflated by the aspiration of air soon after the emergence of the adult."

W. B. Owen has continued his competent studies of the chemoreceptor organs on the tarsi and labella of *Culiseta inornata* (Will.). In this paper (J. Expt. Zool. 166(3) :301-305), he reported his investigations on the "interaction in the central nervous system of sensory input arising from simultaneous application of opposing stimuli to receptors on the tarsi and labella . . . Stimulating chemosensory hairs on the tarsi with 5 M NaCl failed to inhibit the labellar response to sucrose at concentrations above 0.125 M. At lower sucrose concentrations there was a decrease in the number of mosquitoes responding. The application of 5 M NaCl to the tarsal receptors elevated the labellar threshold for sucrose in all subjects tested . . . Stimulating the tarsal hairs with 2 M sucrose resulted in a labellar response to NaCl at concentrations of 0.50 M and lower. Ordinarily NaCl is rejected at all concentrations by this mosquito. Evidence was obtained which indicated that the labellar response to NaCl was mediated by the water receptor rather than by the salt receptor."

Research conducted by V. Lacher (J. Insect Physiol. 13(10) : 1461-1470, Engl. Sum.) revealed that there are three olfactory receptor cells located on the antenna of female *Aedes aegypti*. These hairlike, thin-walled setae were designated as A 1, A 2 and A 3. The A 1 sense cells were excited by fatty acids but their resting activity was depressed by essential oils. On the other hand, A 2 cells showed a decreased activity under stimulation with lower fatty acids but increased activity when stimulated with higher fatty acids.

M. Bar-Zeev (Nature (London) 213 (5077) :737-738) observed that gravid *Aedes aegypti* would deposit only 6 or 7 eggs each when enclosed in a dry screen over a Petri dish holding wet filter paper. No direct contact was permitted with the wet paper. When antennae were removed, the female laid an average of 4 eggs each and when the proboscis was removed, the average was reduced to less than one. Normally, 100 eggs per female were deposited on wet paper whether antennae were removed or not. It was suggested that moisture receptors might be involved in oviposition under normal circumstances.

Experiments on the orientation of *Aedes aegypti* in vertical air currents were conducted by P. N. Daykin (Canad. Ent. 99(3) :303-308). Tests indicated that the species has a "vertical-air-speed sensor, of a mechanical type, to direct movement up or down in a vertical air stream. The cybernetic system must be complex and requires cooperative use of mechanical and optical sensory information channels. The initial action of repellent vapor appears to be peripheral rather than central to the nervous system, and to affect only the mechano-, chemo-, and thermo-receptors."

A. N. Clements and S. A. Potter (J. Insect Physiol. 13(12) :1825-1836) have described in detail the minute structure of spermathecae of *Aedes aegypti*. The two chief types of cells of both the spermathecae and spermathecal ducts were found to be epithelial and glandular. The epithelial cells secreted cuticular components. Glandular cells of the spermatheca were grouped near the entrance of the spermathecal duct and discharged their "secretion through short cuticle-lined ductules into the lumen of the spermatheca. Isolated glandular cells are also present on the surface of the spermathecal duct, near the spermatheca, and they discharge their secretion through long cuticle-lined ductules into the lumen of the spermathecal duct." The spermatozoon was observed to have a "narrow head region followed directly by a very elongate tail region."

A significant paper on spermatocysts in *Aedes aegypti* is one by Jack C. Jones (Biol. Bul. 132(1) :23-33). Testes in larvae of the Bangkok strain of this species increased vastly in size and number of germinal cells. The number of compartments (spermatocysts) reached a maximum of 24 in larvae just prior to pupation, 29 in pupae and 34 in newly emerged adults. In unmated males, the number of testicular compartments declined with age. Following multiple matings, two to three days were necessary to replenish the supply of sperms. It was calculated that 12,000 to 13,000 spermatozoa were formed by both testes but spermatozoa were never observed in the spermathecal ducts of pupae. In the fourth stage larva, one testis was usually smaller than the other and this formation tended to persist throughout the adult life.

Research conducted by A. Spielman, M. G. Leahy and V. Skaff (Biol. Bul. 132(3) :404-412) demonstrated that female *Aedes aegypti* in nature, apparently under normal conditions, make use of sperm from only one male. Although multiple inseminations did occur, sperm was effective only on occasion when less than 5 hours separated first and second matings. Semen from a second mating apparently was discharged by the female from the copulatory bursa after the withdrawal of the aedeagus.

V. P. Sharma and K. S. Rai (Canad. Ent. 99(10) :1116-1118)

found that apholate-sterilized males of *Aedes aegypti* depleted their sperm earlier than normal ones. This seemed to be due to reduced fecundity.

A. Spielman and V. Skaff (J. Insect Physiol. 13(7) :1087-1095) revealed in a valuable paper that a derivative of farnesoic acid (DFA) inhibited development of *Aedes aegypti* and *Culex pipiens quinquefasciatus* Say larvae. Although DFA has been known to have juvenile-hormone like activity in some Lepidoptera, this is the first detailed account of its effect on dipterous larvae. Preliminary observations were published by Spielman and C. M. Williams in 1966 (Science 154(3752) :1043-1044, 1966). Following exposure to DFA, *A. aegypti* larvae in some cases kept larval characteristics in the pupal stage, others failed to undergo adult ecdysis by hardening within the pupae, and some which developed into adult males had terminalia which failed to rotate. DFA prevented ecdysis of adult *A. aegypti* only when applied at about the time when normal larval feeding stopped. *C. p. quinquefasciatus* was relatively sensitive to DFA in the fourth instar. Although a number died in larval through adult stages, many adult males which emerged were abnormal.

A. O. Lea (J. Insect Physiol. 13(3) :419-429) has demonstrated that the median neurosecretory cells (mnc) are as essential as the corpora allata in egg development of mosquitoes. Each, however, controlled different processes in egg maturation. Removal of the mnc in *Aedes taeniorhynchus* (Wied.) suppressed egg development, but the process could be reversed by transplantation. When this species was blood-fed, only one pair of mnc was needed from an anaesthetized donor but when sugar-fed, four pairs were needed. If the donor mosquito was killed, four pairs were necessary to restore maturation. Removal of mnc had little effect in older female mosquitoes.

A. L. Hylton (J. Insect Physiol. 13(1) :153-157) studied the water retention ability of *Eretmapodites chrysogaster* Graham and *Aedes albopictus* (Skuse) under conditions of low humidity. The former was observed to conserve body fluids and death did not appear to be due to desiccation but may result from flooding of the vital organs with metabolic wastes. Despite the fact that *A. albopictus* lost body fluids, this mosquito outlived *E. chrysogaster* under all conditions of the experiment.

Four gonotrophic cycles for *Anopheles sinensis* Wied. were reported by M. Taketomi (Endemic Dis. Bul. (Nagasaki Univ.) 8(4) :170-190). Each one lasted 3 days except the first which took 4 days. Physiological changes of the follicular tubes under various temperatures and seasonal changes were discussed in detail.

Preliminary studies by C. L. Judson (Biol. Bul. 133(2) :369-377) revealed that apholate, when added to larval rearing pans of *Aedes aegypti*, resulted in a rudimentary, non-functional ovary in emerging females. Implantation of a normal ovary in addition to an accessory gland into such

females, produced the mated biting pattern. Although virgin females matured the same number of eggs as mated females, these were retained. But implantation of the male accessory gland into virgin females caused them to deposit their eggs upon maturation. Material from the male accessory gland seemed to influence oviposition behavior in female *A. aegypti*.

A paper entitled "The Effect of some Anions and Cations upon the Fluxes and Net Uptake of Chloride in the Larva of *Aedes aegypti*, and the Nature of the Uptake Mechanisms for Sodium and Chloride" was published by R. H. Stobbart (J. Expt. Biol. 47(1) :35-37). He pointed out that the anal papillae of larvae of this mosquito were responsible for 90 percent of the steady-state exchange of chloride and that the "relationships between chloride flux and external chloride concentration are approximately described by the Michaelis equation."

As little morphological study has been conducted on early larval stages of mosquitoes, C. D. Eddleman (Ent. Soc. Amer. Ann. 60(1) : 33-41) began a series of observations and measurements to determine if basic generic characters for instar identification exist among mosquitoes. The first of these studies concerned *Culex territans* Wlk. Instars from two through four apparently may be recognized mainly by the length of the antennae and respiratory siphon. Growth of the larvae was noted to be almost linear instead of geometric as defined by H. G. Dyar (Psyche 5:420-422, 1890).

See also Kappus and Venard in Behavior, Biology and Ecology Section.

## ARBOVIRUSES

One of the problems concerning virus nomenclature is that new viruses are often named in the literature before descriptions are published. Virus nomenclature is now under consideration by an International Commission on Nomenclature of Viruses.

New mosquito-borne viruses which have come to my attention in the 1967 literature are as follows:

1. Bushbush, Ieri and Lukuni viruses in Trinidad from mosquitoes by L. Spence, C. R. Anderson, T. H. G. Aitken and W. G. Downs (Soc. Expt. Biol. and Med. Proc. 125(1) :45-50). Bushbush was isolated from *Culex amazonensis* (Lutz), Ieri from *Psorophora* spp. and Lucuni from *Aedes scapularis* (Rond.).

2. Nodamura virus from *Culex tritaeniorhynchus* Giles in Japan by W. F. Scherer and H. S. Hurlbut (Amer. J. Epidemiol. 86(2) :271-285).

3. Restan virus from *Culex* spp. in Trinidad and Surinam by A. H. Jonkers, D. Metselaar, A. H. Pães de Andrade and E. S. Tikasingh (Amer. J. Trop. Med. and Hyg. 16(1) :74-78)

4. Tanga virus from *Anopheles* spp. in Tanzania by J. P. Woodall and M. C. Williams (East Afr. Med. J. 44(2) :83-36).

5. Tlacotalpan virus from *Mansonia titillans* (Wlk.), *Anopheles albimanus* Wied., *Anopheles* spp. and *Aedes taeniorhynchus* (Wied.) in Mexico by W. F. Scherer, C. Campillo-Sainz, R. W. Dickerman, A. Diaz-Najera and J. Madalengoitia (Amer. J. Trop. Med. and Hyg. 16(1) : 79-91).

One of the most valuable books I have ever seen on arboviruses is the "Catalogue of Arthropod-Borne Viruses of the World," by R. M. Taylor. The first edition, printed as Public Health Service Pub. No. 1760, contains 898 pages. Dr Taylor has listed the arboviruses by number but has an alphabetical index to them. The country of origin, mosquito or other vectors, hosts and much relevant data are included.

R. W. Emmons and E. H. Lennette (Soc. Expt. Biol. and Med. Proc. 125(2) :443-447) reported the isolation of St. Louis encephalitis from a naturally-infected gray fox, *Urocyon cinereoargenteus*.

M. A. Grayson, S. Srihongse and P. Galindo (Mosquito News 27(2) : 204) have isolated St. Louis encephalitis from a pool of *Deinocerites pseudus* D. and K. in Panama.

A study was conducted by S. Srihongse, W. F. Scherer and P. Galindo (Amer. J. Trop. Med. and Hyg. 16(4) :519-524) to determine if adult golden hamsters could be used as sentinels to detect arboviruses during periods of very low transmission. These animals proved much more sensitive than sentinel mice, captured wild rodents used as sentinels or baby mice inoculated with ground-up wild-caught mosquitoes. Sentinel hamsters proved highly valuable for detecting Venezuelan equine encephalitis and group C arboviruses during periods of low transmission.

Outbreaks of EEE<sup>5</sup> have been known among horses in Panama since 1946 but the first strain of EEE to be isolated from mosquitoes was reported by Srihongse and Galindo (Mosquito News 27(1) : 74-76). This strain, isolated from *Culex taeniopus* D. and K., was collected in the Almirante area in September 1964.

More than 72,000 mosquitoes were collected in Sarawak during arbovirus studies by W. W. Macdonald and Coauthors (J. Med. Ent. 4(2) : 146-157). From among the nearly 80 species identified, five strains of Japanese encephalitis virus were isolated from *Culex gelidus* Theo., one strain from *C. tritaeniorhynchus* and one from *Aedes curtipes* Edw. In addition, one strain came from a pool of mixed *Culex* spp.

As a result of vaccinating 367 horses and 1 donkey with a live WE<sup>6</sup> virus, J. P. Hughes and H. N. Johnson (Amer. Vet. Med. Assoc. J. 150 (2) :167-171) found 41 horses had developed antibodies to the virus.

<sup>5</sup>Eastern equine encephalitis.

<sup>6</sup>Western encephalitis

In recent years, two outbreaks of WE have occurred in Saskatchewan, Canada: One in 1963 involving 39 confirmed human cases and the other in 1965 involving 72 confirmed human cases. In addition, nearly 400 cases in horses were reported in all. J. McLintock, A. N. Burton and J. G. Rempel (N. J. Mosquito Extermin. Assoc. Proc. 54 :97-104) revealed that the virus was delayed in wildlife but became numerous in August during the epidemic years. Also, WE involved chiefly nestling house sparrows and high infection rates were observed in *Culex tarsalis* (Coq.) as well as in domestic poultry. "In the interepidemic years, wildlife infections appeared early and, after the latent period, they persisted at a high level throughout the summer but involved mainly nestling birds other than house sparrows, mosquitoes other than *C. tarsalis* and were accompanied by low infection rates in domestic poultry." There was evidence that garter snakes and leopard frogs were overwintering hosts for WE and that ground squirrels as well as birds might be involved, but no evidence that mosquitoes served as overwintering hosts in Saskatchewan.

The first recorded isolation of California encephalitis virus from mosquitoes in Canada was made by O. Morgante and J. A. Shemanchuk (Science 157(3789) :692-693). The virus was isolated from two pools of *Culiseta inornata* (Will.) from burrows of mammals in Alberta in 1965.

H. Aspöck and C. Kunz (Zentralbl. Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene 203(1) :1-24) discussed research on the ecology of Tahyna virus.

Guaroa virus was isolated from *Anopheles neivai* H. D. and K. in the Pacific lowlands of Colombia by V. H. Lee and C. Sanmartin (Amer. J. Trop. Med. and Hygiene 16(6) :778-781). Previously, the only record of the virus from mosquitoes was made in Panama where it was found in a mixed pool of anophelines including *A. neivai*.

Under experimental conditions, T. Ramachandra Rao, K. R. P. Singh and P. N. Bhatt (Indian J. Med. Res. 55(12) :1306-1310) successfully transmitted Chandipura virus from mouse to mouse by *Aedes aegypti* (L.), *A. albopictus* (Skuse), *Anopheles stephensi* (List.) and *Culex tritaeniorhynchus*.

## FILARIASIS

As a result of laboratory experiments in Burma, B. De Meillon, S. Hayashi and A. Sebastian (WHO Bul. 36 (1) :81-90) have indicated that a *Culex pipiens quinquefasciatus* Say which would pick up an infection of *Wuchereria bancrofti* early in its life, could be reinfected by subsequent feedings and probably remain infected for the rest of its life. An average of 6.1 larvae of *W. bancrofti* per infected mosquito was found following the first blood meal. After the second blood meal, however, the figure

was only 3.6, showing that nearly half of the *W. bancrofti* larvae had been lost. It was believed that a combination of passive pressure of engorged blood in the abdomen and active movement of the *W. bancrofti* larvae themselves, accounted for the loss. It was shown that only about two larvae of *W. bancrofti* were deposited on the skin of the host by the infective mosquito when it fed. This fact was supported by field data.

De Meillon, B. Grab and Sebastian (ibid. pp. 91-100) proposed an index, based on entomological data, to evaluate the risk of infection to man with *W. bancrofti* in Rangoon.

Almost an entire issue of WHO Bulletin 36(1) was devoted to *Culex p. quinquefasciatus* in relation to filariasis. Abstracts of some of these will be found as follows: De Meillon, Sebastian, Khan, Paing, and Lindquist et al. in Behavior, Biology and Ecology Section; Tadano and Brown in Resistance and Susceptibility Section.

A. Ewert and B. C. Ho (Roy. Soc. Trop. Med. and Hyg. Trans. 61(5) :659-662) described a method to determine how infective filarial larvae, following feeding by mosquitoes, could be estimated. In this experiment, *Aedes togoi* (Theo.) with a 77 percent rate of infection and an average of 7.5 *Brugia pahangi* larvae per mosquito were fed on mice. The results were as follows: 22.3 percent of the expected number of *B. pahangi* larvae were found in tissues of the mice; 32.3 percent remained in the mosquito; and 45.4 percent apparently were lost on the surface of the skin. About one half of the mice, exposed to *A. togoi* harboring *B. pahangi* larvae, had no larvae in their tissues.

Ho and Ewert (ibid. pp. 663-666) also observed the experimental transmission of *B. pahangi* larvae in relation to feeding behavior of *A. togoi*.

"WHO Expert Committee on Filariasis (*Wuchereria* and *Brugia* infections)" is the title of the Committee's second report, published as WHO Tech. Rpt. Ser. No. 359, 47 pp.). Recent advances in the knowledge of parasites, methodology to assess the standardization of techniques, chemotherapy, mosquito vectors, and control of the disease by drugs and measures against vectors were included.

V. P. Sinha (Patna J. Med. 41(5) :193-217) has published a literature review entitled "Global Studies on *Culex fatigans* (Wied. 1828) - - A Vector of Filariasis." Life history, habits and various types of control were included. The over 200 references cover the period from 1878 to 1966 inclusive.

The accomplishments in the control of filariasis in Brazil have been outlined by O. Franco and D. M. Da Silva Lima (Rev. Brasil. de Malar. 19(1) :73-89). Studies revealed that the disease was endemic in that country, the largest foci being in Belém and Recife. *Culex pipiens quinquefasciatus* was determined to be the chief vector there. Treatment of individuals was with diethylcarbamazine.

Dr. Frank Hawking (Roy. Soc. (London) Proc. Ser. B. Biol. Sci. 169(1014) :59-76) has presented a review of his work and that of his colleagues on the 24-hour periodicity of microfilariae and the biological mechanisms for its production and control. *Brugia* and *Wuchereria* as well as *Dirofilaria* of dogs and monkeys were considered.

B. R. Laurence and F. R. N. Pester (J. Helminthol. 41(4) :365-392) have described the successful adaptation of *Brugia patei* to a new mosquito host, *A. togoi*, over a 6-year period under laboratory conditions.

S. Uemura (Trop. Med. (Nagasaki) 9(1) :24-38) found a local strain of *Anopheles sinensis* Wied. to be fairly susceptible to *Wuchereria bancrofti* in the Kin area of Okinawa. He concluded however, that this may not have any effect upon the spread of filariasis there in recent years, as *A. sinensis* was found in stables and pigstys rather than dwellings in the area.

The use of repellents as one means to control filariasis was explored by Brian Hocking (WHO Bul. 37(2) :323-327). Under laboratory conditions, it was observed that microfilariae of *Wuchereria bancrofti* died almost immediately when they came in contact with dimethylphthalate. When this repellent is used, it seems unlikely that infection of *W. bancrofti* would occur under normal circumstances. However, much more needs to be known concerning morphology, such as the receptor organs, of the vector, *Culex pipiens quinquefasciatus*.

## MALARIA

Imported cases of malaria since the mass eradication of the disease in the USSR have concerned the Soviets for a number of years. I. M. Groshkova, M. S. Pavlova and E. F. Petrova (Med. Parazit. i Parazit. Bolezni 36(3) :348-352, in Rus., Engl. Sum.) have suggested that towns and villages in the Kazakh SSR be grouped according to the risk of imported malaria as follows: Those which have a high risk of spread, those with low risk and the rest with no practical possibility of spread if the infection is imported. This proposal allows more flexibility in planning control and in checking the results.

Although malaria was practically eliminated in the RSFSR in 1960, S. N. Pokrovsky and V. K. Spudis (ibid. pp. 345-348, in Rus., Engl. Sum.) reported cases in 4 subsequent years as follows: 109 in 1962, 108 in 1963, 140 in 1964 and 201 in 1965. The source of these was mainly from foreigners and Soviet citizens from other republics of the USSR. The rest were from blood transfusions and local cases. In 1964, 89 percent of all malaria cases in the USSR were attributed to foreign importation but in 1965 this figure rose to 96 percent. DDT and BHC were used to control mosquitoes in areas where malaria cases were found.

A. Ya. Lysenko, I. N. Semashko and K. S. Fonareva (ibid. pp. 737-744, in Rus., Engl. Sum.) presented a paper dealing with the importation of malaria from Africa. A map, indicating initial endemicity and present status of malaria "eradication" programs in Africa, was included.

In another paper, Spudis and K. K. Vostokova (ibid. 36(6) :733-736, in Rus., Engl. Sum.) stated that 43 indigenous cases of quartan malaria were reported in 16 regions, territories and autonomous republics of the RSFSR from 1959 to 1965. Of these, 15 cases occurred in the North Caucasus.

An extensive paper on malaria in the middle Nile basin and surrounding areas has been presented by G. Wernsdorfer and W. Wernsdorfer (Z. f. Tropenmed. u Parasitol. 18(1) :17-44, in Ger., Engl. Sum.). Vectors, plasmodia and control were discussed.

"Malaria in Tanzania" has been written by D. F. Clyde who has worked on the disease in that part of Africa since 1952. The book, containing 167 pp., was published in London, New York and Toronto. The present status of malaria, vectors and results of control measures were discussed. Chemotherapy, drug resistance and timely data on immunology are important features of the book. P. E. C. Manson-Bahr (Amer. J. Trop. Med. and Hyg. 16(6) :803) has said: "The book is a good example of the knowledge that must be acquired before malaria-eradication schemes are attempted in Africa, and should be required reading for all malariologists interested in the eradication of malaria."

A very readable account of the history of malaria eradication efforts in Asia has been written by D. R. Johnson (*In Infectious Diseases, Their Evolution and Eradication*. Edited by A. Cockburn. pp. 292-308, Springfield, Illinois). Most of the chapter was devoted to the Indian program as it is the largest. Jordan was pointed out as an example of a small country which stopped eradication measures too soon because of lack of funds. However, malaria cases appeared so rapidly that the program there had to return to the attack phase. The present status of a number of other Asian countries was discussed.

The current status of malaria eradication campaign in Cuba was discussed by J. M. Argudin Romero (Rev. Cubana Med. Trop. 19(1) : 75-87, in Sp., Engl. Sum.). Malaria was still present in areas of the Oriente Province, where 36 cases were reported in 1966. However, in November and December of that year no case was reported from anywhere on the island. According to the author, this was the first time that no cases of malaria have been reported.

The WHO Expert Committee on Malaria has published its thirteenth annual report (WHO Tech. Rpt. Ser. No. 357, 59 pp.). An assessment was made of the status of malaria eradication programs as of June 1966 and results shown on a world map. They also analyzed the factors affect-

ing the progress of the programs since the global malaria eradication concept of 1955.

Another World Health Organization publication (WHO Tech. Rpt. Ser. No. 374, 32 pp.) concerned the prevention and re-introduction of malaria in areas freed from it. Methods for screening tourists, immigrants, migratory workers and other travellers were discussed. It also was pointed out that malaria training must continue as long as the disease exists in the world.

Malaria transmission has been studied for a number of years in Kenya and Tanzania. G. Pringle (Roy. Soc. Trop. Med. and Hyg. Trans. 61(1) :69-79) presented part three of the investigation carried out in the Pare area of Tanzania. As a result of a 3-year residual spraying with dieldrin (ending in 1959), *Anopheles funestus* Giles apparently was eliminated from the sprayed zone and *A. gambiae* Giles reduced to about one-fifth its former density. Although malaria transmission was almost completely stopped, a small residue continued. Much of the area was formerly malarious but increased malaria transmission following the suspension of residual spraying was unexpectedly delayed. "This delay has been attributed largely to changes brought about in the original vector mosquito populations, changes originating during the residual spraying campaign but persisting well beyond the interval during which the mosquitoes were exposed to the direct activity of the residual insecticide. For instance, the once important local vector, *A. funestus*, had failed to re-establish itself completely in the area as recently as mid-1966; furthermore, it now seems probable that the South Pare *A. gambiae* underwent selection during the residual spraying campaign, resulting in the ascendancy of a relatively exophilic population of the species which lingered for several years after selection pressure was lifted.

"A further factor that appears to be restraining any overall increase in malaria parasite rates, despite a recent rise in malaria risk, is the increased popularity of modern antimalarial drugs for the treatment of fever. In addition, there is a public awareness of the value of these remedies in coping with the current seasonal epidemic waves of malaria transmission."

A. Smith and associates have investigated the disease in the Taveta area of Tanzania and Kenya since 1954. [See my review — N. J. Mosquito Extermin. Assoc. Proc. 54 :207.] Smith and Pringle (East Afr. Med. J. 44(11) :469-474) pointed out that malaria transmission by 1959 had almost ceased for a period of 3 years in the area following 6 cycles of residual dieldrin sprayings. The parasite rate was down to about 5 percent but began to rise during the latter half of 1960. Despite the high risk of infection by the return of *A. funestus*, parasite rates began a decline after 1963. The same explanation offered by Dr. Pringle in the pre-

ceding paper apparently was the reason for the decline of the parasite rates in the Taveta area.

The susceptibility of a new world monkey, the owl monkey (*Aotus trivirgatus*) to a human malaria parasite (*Plasmodium falciparum*) was reported by Q. M. Geiman and M. J. Meagher (Nature (London) 215 (5099) :437-439). Following splenectomy, monkeys were inoculated with a Vietnam strain of *P. vivax*. Parasitemia developed. Similar tests were made with *P. falciparum* (Uganda strain) with resultant parasitemia. With additional passages, *P. falciparum* became adapted to monkeys with spleens.

P. I. Trigg (Nature (London) 213(5080) :1019-1020) has described a simplified method for the *in vitro* growth of *Plasmodium knowlesi* and *P. falciparum*, from ring to schizont stage. This was designed for use in large scale investigations of acquired immunity to *P. falciparum*.

A. B. Weathersby (Georgia Ent. Soc. J. 2(2) :31-35) made a study of the survival time for sporozoites of *Plasmodium gallinaceum* in the haemocoels of refractory *Culex pipiens pipiens* L. He concluded that a "short term exposure to the adverse environment in the refractory host stimulated the parasites to greater infectivity."

In another paper Weathersby and J. W. McCall (J. Parasitol. 53(3) : 638-640) reported the survival of sporozoites of *P. gallinaceum* for 767 days in liquid nitrogen (-197C).

R. L. Kisiuk, M. Friedkin, L. H. Schmidt and R. N. Rossan (Science 156(3782) :1616-1617) showed tetrahydrohomopteroic acid to be a new type of antifolate effective against malaria parasites resistant to pyrimethamine. Doses of 80 mg per kilogram given daily for 5 days eliminated "parasites from the blood of monkeys infected with pyrimethamine-sensitive or pyrimethamine-resistant strains of *Plasmodium cynomolgi*."

See also Najera et al. in Adulticides and Larvicides Section.

## YELLOW FEVER

Jungle yellow fever outbreaks in Panama in 1948 and 1956 involved both man and monkeys. According to P. Galindo and S. Srihongse (WHO Bul. 36(1) :151-161), surveillance has continued there but no evidence of the disease was found in the 1963 survey. However, surveys in 1966 revealed that within the 2 previous years an epizootic apparently had occurred in eastern Panama. Howler monkeys, which readily succumb to the disease, were greatly reduced in numbers in the Darien Province. In one area of the Province, 40 percent of the spider monkeys, which seldom die of the infection, had antibodies for yellow fever. A number of these were less than 2 years old. There was no indication that the virus

had spread to other areas of Panama. It was suspected that virus transmission could have been permanently interrupted because of a drought in 1965 as no other reports of jungle fever had been received by the end of 1966. [According to available sources, there apparently was no active jungle fever virus in Panama during 1967. hsr.]

Odair Franco (Rev. Brasil. de Malariol. 19(2) :219-225) described the various epizootics of jungle yellow fever in Brazil from 1932 to 1966. In January 1966, seven fatal cases of yellow fever occurred in Paraná State, nine in Santa Catarina and five in Rio Grande do Sul. A total of 37,566,936 persons was vaccinated against yellow fever in Brazil from 1937 to 1966. As *Aedes aegypti* (L.) had been eradicated from Brazil, there was no possibility of an epidemic of urban yellow fever there. Furthermore, the Ministry of Health maintains a vigilance service to prevent re-introduction of that mosquito.

#### ADULTICIDES AND LARVICIDES

An imposing list of 42 new compounds which showed promise as residuals against *Anopheles quadrimaculatus* Say was presented by J.B. Gahan, H. G. Wilson and G. C. LaBrecque (N. J. Mosquito Extermin. Assoc. Proc. 54 :145-152). Nineteen of these gave 100 percent kill over a 24-week test period. The most promising of the 19 insecticides because of their reported low toxicity to warm-blooded animals were as follows: Union Carbide UC-8454, Bay 38799, Hooker HSR-1634, Mobil MC-A-600 and Neopynamin. On two occasions Neopynamin gave only 98 percent kill.

In order to find a substitute for DDT, J. B. Moore and S. G. Breeland (Mosquito News 27(1) :105-111) tested Abate and Dursban along the shorelines of a lake near Lexington, Tennessee. As larvicides, both compounds appeared to be good substitutes for DDT. Dursban in some cases had a residual effect of 7-10 days but Abate had none that could be observed.

Tests conducted by C. S. Lofgren, J. E. Scanlon and V. Israngura (Mosquito News 27(1) :16-21) showed fenthion to be the most toxic of 6 compounds dispersed as thermal fogs against *Aedes aegypti* (L.) and *Culex pipiens quinquefasciatus* Say in Bangkok, Thailand. Over 98 percent kill was obtained. Sumithion [Bay 41831] and malathion were slightly less effective. Under laboratory conditions, Dursban and Abate were both effective against larvae of the two species. In concrete water storage jugs, Dursban, Abate, and Shell SD-8211 killed third and fourth instars of *A. aegypti* placed in the water each week for a period of 6 weeks. "In field tests, Abate, Dursban (0.005 and 0.025 pound/acre), fenthion (0.05 and 0.25 pound/acre), and malathion (0.25 pound/acre) gave

99+ percent control of *C. p. quinquefasciatus* when applied as water emulsion sprays."

Malathion was tested under large scale field conditions in southern Uganda for malaria control by J. A. Najera, G. R. Shidrawi, F. D. Gibson and J. S. Stafford (WHO Bul. 36(6) :913-935). House and animal shelters were sprayed with an emulsion prepared from 50 percent malathion. For 2 years, three rounds of spraying were conducted. Densities of *Anopheles funestus* Giles and *A. gambiae* Giles, the vectors, fell from 66 per shelter per day in 1960-1961 to 0.0011 by the end of 1964. Malaria transmission in humans apparently was interrupted. It was learned that imported cases in the area were the cause of all remaining cases of malaria.

Of three carbamates and two organophosphorus compounds tested against *Anopheles* and *Mansonia* in Upper Volta, J. Coz, P. Venard and M. Eyraud (Med. Trop. (Marseille) 27(3) :303-312) found Dursban and OMS 227 (a carbamate) to give the best results. Effective mortalities of adults were obtained for 3 months. The formulations were wettable powders, and were used mainly against *A. gambiae*.

T. A. Oktyabrskaya and L. N. Sherina (Med. Parazit. i Parazit. Bolezni 36(5) :604-608, in Rus. Engl. Sum.) found fenthion to be effective against larvae of *Anopheles maculipennis* Meig., *Culex pipiens* L. and *Aedes dorsalis* (Meig.) in water reservoirs in the Moscow countryside. "Dosage was changed according to the age of larvae and extent of overgrowth of the water reservoir." Against first to second instars, the following dosage rates were recommended: In shallow reservoirs with little growth 14 g/hectare, in reservoirs with average growth 28 g/hectare, and in extensively overgrown water reservoirs 56 g/hectare. For third and fourth instars the dosage should be increased to 28, 56, 112 g/hectare, respectively. Fenthion was used as a spray prepared from a 50 percent concentrated emulsion and as 2 percent granules. Granules were particularly useful in extensively overgrown water reservoirs.

Of several phosphates tested against larvae and pupae of *Aedes* spp., O. N. Vinogradskaya (Med. Parazit. i Parazit. Bolezni 36(1) :54-60, in Rus., Engl. Sum.) found methylnitrophose [Bay 41831] and trichlophose [trichlorfon?] to give the best results. Dichlophose [dichlorvos?] and 10 percent lindane dust were the most effective against adults.

Ultra-low-volume (ULV) application of insecticides against mosquitoes has been tested by a number of workers during the year. F. W. Knapp and C. H. Gale (Mosquito News 27(4) :478-482), in Kentucky, used this method with Baygon, Baygon-fenthion mixture, malathion, malathion-naled mixture and naled. The most effective adulticide was naled which had a rapid knock-down of *Aedes sollicitans* (Wlk.). Although Baygon and the Baygon-fenthion mixture seemed equally effective, the latter required less time for knock-down.

Malathion, naled and fenthion were compared as ULV and conventional aerial sprays in Florida against *Aedes sollicitans* and *A. taeniorhynchus* (Wied.) adults by G. A. Mount and C. S. Lofgren (Mosquito News 27(4) :473-477). Control with naled was essentially the same regardless of the method but fenthion was far better as a conventional spray. Malathion gave poor control, possibly because of resistance, but even the poor control obtained with ULV was better with the conventional spray.

L. F. Stevens and R. F. Stroud (Mosquito News 27(4) :482-485) reported that the combination of Baygon and Baytex [fenthion] applied as a ULV aerial spray appeared to be more effective than Baygon alone in controlling larvae and adults of *Culex restuans* Theo. The authors felt that Baytex might have been effective alone if adults only had been present. Temperature may have affected the results obtained with Baygon. "This relationship of temperature to the toxicity of Baygon and Baygon-Baytex to adult mosquitoes deserves further study."

A rather interesting situation exists concerning the status of control of *Culex pipiens quinquefasciatus* Say. This is best described by A. W. A. Brown (WHO Bul. 37(2) :297-299). "The present situation in the control of *C. p. fatigans* represents a victory for the adaptive powers of the mosquito over the chlorinated hydrocarbon insecticides. Most control operations in the tropics have reverted to the pre-1939 method of heavy applications of larvicidal oil, which give consistent if inadequate results. The organophosphorus insecticides have been used in larvicidal operations in California, later in Japan and occasionally in certain cities in Africa, where their effectiveness was eventually negated by the development of organophosphorus resistance in the field."

#### STERILIZATION METHODS

A noteworthy paper concerning sterilization of *Aedes aegypti* (L.) in a different manner was published by G. B. Craig, Jr. (Science 156 (3781) :1499-1501). When male accessory glands were implanted in virgin females, they remained sterile for life. Females were not inseminated even though they copulated freely with males. "Extract from one male could sterilize more than 64 females. The active principle may be a protein or peptide. Intraspecific transplant prevented insemination in 12 species including *Aedes*, *Anopheles*, and *Culex*; interspecific transplant gave partial protection." Dr. Craig pointed out that the substance secreted by the males might be used to control mosquitoes if a technique could be devised to sterilize young females by spraying them or by incorporating the substance in baits for feeding.

J. A. George (Mosquito News 27(1) :82-86) compared the relative competitiveness of spermatozoa from normal *Aedes aegypti* males and

those sterilized by gamma-irradiation as pupae. When females mated first with normal males, their egg hatch could not be reduced by subsequent matings with irradiated males. On the other hand, when females copulated with irradiated males, the egg hatch was not increased when they subsequently mated with normal males.

A. A. Abdel-Malek, A. O. Tantawy and A. M. Wakid have continued their worthwhile studies on the eradication of *Anopheles pharoensis* Theo. by the sterile male technique using Cobalt-60 [See my review in N. J. Mosquito Extermin. Assoc. Proc. 54 :211-212.] In part III, these authors (J. Econ. Ent. 60(1) :20-23) reported that complete sterility in both sexes was obtained when pupae were irradiated with a dose of 12,000 R. Part IV by Tantawy, Abdel-Malek and Wakid (ibid. pp. 23-26) concerned the mating behavior of sterilized *A. pharoensis*. They found that . . . "irradiated males were as effective as normal males in inseminating normal virgin females. Laboratory males could inseminate either wild females or laboratory females as efficiently as wild males, if each was confined to the same number of females." In part V, the same authors (ibid. 60(3) :696-699) showed that sterilized males were not as competitive as normal males when the ratio was 1 to 1 normal male and 1 normal female or when it was 5 : 1 : 1. When the ratio of sterilized males was 10 : 1 : 1 and 15 : 1 : 1, irradiated males could compete well. In part VI, Abdel-Malek, Tantawy and Wakid (ibid. 60(5) :1300-1302) demonstrated that irradiation with 12,000 R. did not damage the sperm, . . . "as the sperm of irradiated males competed successfully with that from normal males whether it was present in the spermatheca before or after copulation with normal males. Delaying the mating for 5 days after irradiation did not restore viability of the sperm."

According to C. N. Smith (WHO Bul. 36(4) :633-635), the use of chemosterilants to control natural populations of *Aedes aegypti* are not bright. However, new chemosterilants are being discovered and the picture may change.

I. Sandesco (Arch. Roum. Path. Expt. Microbiol. 26(1) :181-188) showed that hempa at concentrations of 100 to 150 ppm., applied to the rearing solution of third instar *Anopheles labranchiae atroparvus* Van Thiel, resulted in 80 percent sterility of adults. Thiotepa, in alcohol, was applied at the rate of 2.5 ppm. to larval rearing solution. Over 80 percent of the eggs produced failed to hatch.

#### RESISTANCE AND SUSCEPTIBILITY

according to E. E. Inwang, M. A. Q. Khan and A. W. A. Brown (WHO DDT-resistance in *Aedes aegypti* (L.), although known in the Caribbean area and Southeast Asia, had not developed in West Africa

Bul. 36(3) :409-421). Under laboratory conditions, West African strains developed a high resistance to DDT but at a much slower rate than Asian and American strains of similar initial susceptibility. Once the selected Upper Volta, West Pakistan and Thailand strains developed a full measure of resistance, they were crossed and backcrossed with susceptible strains, then crossed and backcrossed with visible mutant marker strains. It was found that resistance was due to a genetic factor linked with the marker *y* on chromosome 2. In the Upper Volta strain, dieldrin resistance was readily induced and was due to a gene also linked with *y*.

In another article, Dr. Brown (*ibid.* 36(4) :578-580) discussed the mechanisms of DDT-, dieldrin- and organophosphorus-resistance in *A. aegypti*, as well as their modes of inheritance and the resistance potential on this species under selection.

J. Mouchet and J. Laigret (*Med. Trop. (Marseille)* 27(6) :685-692) investigated five strains of *A. aegypti* from Tahiti (French Polynesia) and found them to be resistant to BHC and dieldrin but susceptible to DDT. Although susceptibility to malathion and diazinon was low, no resistance was observed. Under experimental conditions, fenthion appeared to be an effective larvicide.

Resistance in a strain of *C. p. quinquefasciatus* Say from Rangoon, Burma, to DDT was determined by T. Tadano and A. W. A. Brown (*WHO Bul.* 36(1) :101-111) to be due to a single principal gene allele which was almost entirely dominant. Dieldrin resistance, however, was due to a single gene allele which was neither dominant nor recessive. In the latter case, the hybrids were intermediate. The back-crosses with marker strains indicated that the DDT-resistance gene was on chromosome 2 and the dieldrin-resistance gene on chromosome 3.

J. Hamon, S. Sales and R. Subra (*Entomologie Médicale Cah.* 5(4) : 229-261, in Fr., Engl. Sum.) studied the resistance of *C. p. quinquefasciatus* to dieldrin in Bobo-Dioulasso, Upper Volta. They found that the selected character for dieldrin resistance to be "monofactorial, with incomplete dominance in the heterozygotes. The dominance is more conspicuous in the larval stage than in the adult one and for all genotypes males are more susceptible than females." In wild populations of this species, susceptible homozygotes were rare. "However the frequency attributed to heterozygotes varies enormously with the investigation methods used; besides this frequency is invalidated by the investigations carried out on  $F_1$  and  $F_2$  subsequent wild and laboratory-bred populations and is not compatible with the hypothesis of a natural population in equilibrium for the character of dieldrin resistance . . . The most promising hypothesis implies the occurrence of several non-cumulative genes for dieldrin-resistance within the wild population, only one major of these genes having

been selected in the resistant laboratory strain; this hypothesis accounts as well for discrepancies existing between the present laboratory and field observations, and previous ones, both published and unpublished."

In another paper, Hamon and J. Mouchet (WHO Bul. 37(2) :277-286, in Fr., Engl. Sum.) discussed the resistance of *C. p. quinquefasciatus* to numerous insecticides.

P. Rosen (WHO Bul. 37(2) :301-310) found *Culex pipiens quinquefasciatus* larvae to be naturally resistant to the chlorinated hydrocarbons in Rangoon, Burma. This confirms the findings made in other parts of the world. The resistance to these compounds was sufficient to prevent control of the species. Larvae were found to be susceptible to the organophosphorus compounds, particularly parathion and fenthion.

In the Philippines, R. E. Johnsen (Mosquito News 27(1) :22-26) found no evidence of resistance to DDT, BHC, dieldrin or malathion in larvae of *Aedes aegypti*, *A. albopictus* (Skuse) or *Culex pipiens* L. Most *C. pipiens* adults were susceptible to DDT and dieldrin but strains from the Dau area and Balibago showed partial resistance to dieldrin but not DDT. No resistance to DDT and dieldrin was evident in adult *A. aegypti* or *A. vexans* (Meig.). Resistance was determined by the use of the WHO test kit on laboratory strains of mosquitoes.

Irritability of *Anopheles pharoensis* Theo. to DDT, dieldrin, lindane and malathion was observed under laboratory conditions by M. Qutu-buddin (Sudan Med. J. 5(1) :18-28). Although the WHO test kit was used, the preconditioning period was omitted because this species was unable to withstand the constant exposure to artificial light for the 30 minutes recommended. A "wide range of variation occurred both in the excitation time as well as the number of take offs amongst the individuals of populations both bred in the laboratory and caught in nature." Of the insecticides tested, *A. pharoensis* was irritated most quickly with lindane.

J. Brengues and S. Sales (Entomologie Médicale Cah. 5(1) :21-42) determined (by WHO adult test kits) that impregnated papers of Bayer 39007 and Bayer 41831 should be used before they are 3 months old. Of several species tested, fed females of *Aedes aegypti* were the most susceptible to the two insecticides whereas unfed ones were less susceptible. *Culex pipiens quinquefasciatus* unfed females of a laboratory strain were most susceptible to Bayer 39007 but fed and gravid wild females of the latter species were the most tolerant of it. The authors suggested that a different scale of concentrations be adopted in the WHO adult test kit so that susceptibility and resistance might be tested with greater accuracy.

By treating larvae of *A. aegypti* with 1280 ppm of hempa, J. A. George and A. W. A. Brown (J. Econ. Ent. 60(4) :974-978) found that small intrachromosomal deletions after 36 hours and broken chromosomes after 48 hours had been induced. Larval selection with hempa

resulted in a decrease in the percent sterility, induced by the chemosterilant. This became evident at the F<sub>5</sub> generation; however, sterility was higher than ever in the F<sub>6</sub> generation. The results indicated the increase in tolerance to a chemosterilant that might be expected from an increase in defense mechanisms such as detoxication may sooner or later be negated by the appearance and accumulation of inheritable recessive genetic defects.

A laboratory colony of *Aedes aegypti* "which had been selected by exposing the larvae to 5-25 ppm of apholate for 43 generations . . ." was demonstrated by R. S. Patterson, C. S. Lofgren and M. D. Boston (J. Econ. Ent. 60(6) :1673-1675) to be 20 times as resistant as the regular colony owing to the sterilizing effect of apholate-treated larvae. Although little cross resistance to tepa was observed, there was resistance to metepa in the selected strain. All three compounds, at high concentrations, caused extensive mortality among pupae and adults. The colony resistant to apholate "could withstand higher dosages of apholate (but not tepa or metepa) than the regular colony without any detrimental effect on development."

See also Brown in Adulticides and Larvicides Section.

#### REPELLENTS

H. Gouck, T. P. McGovern and M. Beroza tested space repellents for use against *Aedes aegypti* (L.). Of 242 esters tested, these authors (J. Econ. Ent. 60(6) :1587-1590) found dipentyl malate, diisopentyl malate, bis(1-methylbutyl) malate, hexyl *p*-isopropylmandelate and bis(2-ethylhexyl)fumarate to be effective for over 100 days. In the second paper, McGovern, Beroza and Gouck (ibid. pp. 1591-1594) reported that the most effective repellent was N, N-Dipropyl 2-[(*p*-methoxybenzyl)oxy]acetamide which gave 266 days protection.

According to G. S. Pervomaisky and Coauthors (Med. Parazit. i Parazit. Bolezni 36(6) :730-733, in Rus., Engl. Sum.), a new insect repellent called hexamethylbenbutansulfamid (HMBS) was formulated. Under conditions in Leningrad, external applications of 30 percent ointment of HMBS protected against bites of *Aedes*, especially *A. communis* (De G.) for 16-20 hours. The activity of this repellent was close to diethyltoluamide and had a low toxicity for warm-blooded animals. HMBS could be made cheaply from readily available raw materials.

See also Daykin et al. in Anatomy, Morphology and Physiology Section.

#### ACKNOWLEDGMENTS

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PRESIDENT THORN: Thank you, Mrs. Sollers-Riedel. Are there any questions? Our next paper is on "Eastern Encephalitis in New Jersey in 1968," and will be given by Dr. Martin Goldfield of the State Department of Health, Trenton.