THE INFLUENCE OF SOIL FERMENTATION ON OVIPOSITION SITE SELECTION BY MOSQUITOES

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Studies of the natural history of mosquitoes show that each species has rather characteristic breeding habits. The habits of the family as a whole are sufficiently diverse that the larvae of one or more species of mosquitoes are able to inhabit almost every type of terrestrial water accumulation.

Field observations indicate clearly that the ecological distribution of larvae is strongly influenced by the oviposition habits of the adults of different species. While the degree of habitat specificity of mosquito species is variable there is no evidence that adults deposit their eggs in all types of water, with observed distribution resulting from a selective mortality governed by the relative compatibility with the particular environment thus chosen at random.

Laboratory experiments on oviposition behavior have, in general, given unsatisfactory results. The reactions of mosquitoes in the restricted environment of a cage often seen quite unrelated to the reactions that we know or suspect exist in nature. In the laboratory, mosquitoes often will oviposit in anything that contains water, even solutions lethal to eggs or larvae. When mosquitoes have evidenced discrimination toward a series of oviposition media, the basis of selection has been difficult to evaluate.

Many authors have dealt with the influence of inorganic compounds dissolved in breeding waters on oviposition site selection. This work has been adequately reviewed by other authors, particularly Wallis (1954) and Hudson (1956) and will not be repeated here.

Few investigators have considered the possibility that organic compounds attract adult mosquitoes to oviposition sites. Rudolfs (1922), in a study of the chemotropisms of mosquitoes, demonstrated that adult female mosquitoes are attracted through olfactory perception toward several compounds.

Rudolfs and Lackey (1929) studied two pools that differed greatly in production of mosquitoes. Mosquito breeding could not be correlated with pH, free carbon dioxide, chlorides, sulfates, acid carbonates, free ammonia, or albuminoid ammonia. However, a marked difference was noted in the decomposition products of the mixtures of muck and organic material obtained from the bottoms of the pools. It appeared that selection of breeding site might have been influenced by the presence of specific organic substances in the water produced from the decomposition of organic matter.

OBSERVATIONS. In California, the most important species in vector control work is *Culex tarsalis* Coquillett, the principal vector of encephalitis. This ubiquitous species commonly breeds in rice fields, a well-defined environment.

The amounts of organic material in the soil of rice fields are moderate to large, depending on the agricultural practice followed between rice crops, during the winter. Some growers disk the rice straw into the soil in the fall, some burn (incompletely) and disk in the spring, others grow vetch over the rice stubble and disk all the plant material into the soil in the spring. Field observations indicate that increased mosquito breeding is associated with the latter practice, which places the greatest amount of plant material in the soil at the time the fields are flooded.

In well-aerated soils plant material is normally decomposed through oxidation by aerobic bacteria and fungi. In contrast, submergence of the soil permits only anaerobic bacterial decomposition of the plant material, producing butyric acid, propionic acid, lactic acid, acetic acid, methane, hydrogen, and carbon dioxide. This fact is well known, and was shown by Acharya (1935 a and b) to be specifically true of rice straw.

These reduced organic compounds diffuse into the overlying water where their fate is determined by a number of factors. All are subject to oxidation, ultimately to carbon dioxide, by aerobic bacteria in the surface water. Hydrogen and methane escape readily into the atmosphere. Acetic acid and butyric acid, although soluble in water, exert sufficient vapor pressures that considerable proportions are liberated into the air. Carbon dioxide remains in the water, partly as carbonates, and is available for assimilation by algae.

In 1956, the author treated experimental rice field plots with specific chemical algicides. Certain treatments markedly depressed algal growth with a resulting reduction in the biochemical oxygen demand (BOD) of the water. The appreciably reduced pH of the water probably resulted from the accumulation of organic acids. The lower BOD values most probably indicated a lag in oxidation of the organic acids produced from the underlying soil. Oviposition by *C. tarsalis* was greatly favored in the algicide-treated plots. The observations suggest that, because of inefficient oxidation in the water, some product of anaerobic decomposition was accumulating and serving as an attractant for the adult female mosquitoes. The basis for choice was evidently a preference for algicide-treated plots (Gerhardt, 1957).

The unanswered questions posed by these observations show the need for investigation of the influence of decomposition of organic material in rice field soil on preferential selection of oviposition sites.

FIRST EXPERIMENT

METHODS. In July, 1957, at a location near Davis, California, 13 small plots were constructed by digging shallow depressions two-feet square and lining them with polyethylene sheeting. The plots were in 2 rows about 3 feet apart, with those in each row separated by one foot of soil

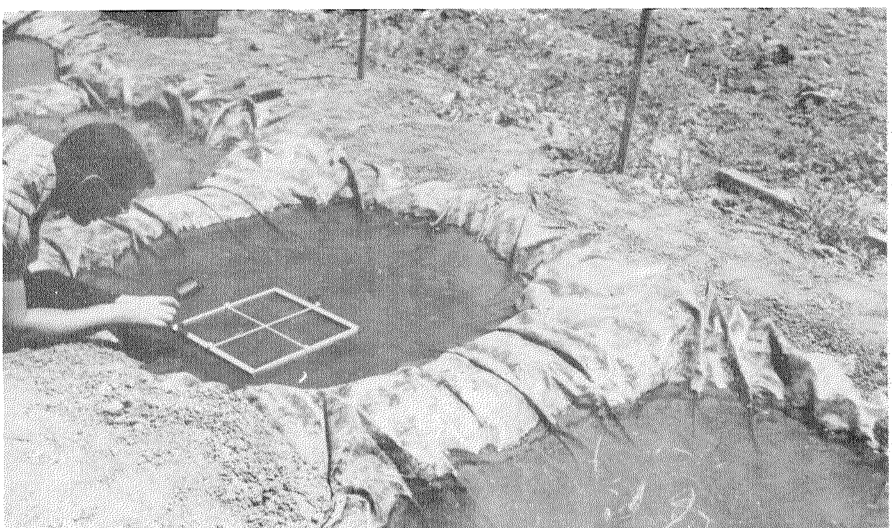

FIG. 1.—Counting larvae—oviposition study plots

covered with the continuous polyethylene sheet. A 6-inch layer of Stockton clay adobe soil (a common California rice soil) was placed on the sheeting in the plots.

For extra organic matter, 5 of the 13 plots each received 2 pounds of a prepared dog meal (gross composition listed as 25 percent protein, 6 percent fat, 5 percent fiber, 10 percent ash). The meal was supplied to the soil below the depth to which reducing conditions would extend.

The plots were flooded with tap water to a depth of six inches, and the depth was restored daily. No circulation was provided, and the only water loss was through evaporation.

The progress of decomposition of the organic material supplied to the plot soils was closely followed. The accumulation of organic acids in the surface water was determined by periodic pH measurements and by chemical analysis of the water for volatile acids. The development of reducing conditions in the soil was determined with a platinum electrode.

Oviposition preference was measured by counting egg rafts deposited on the plot surfaces and by counting first instar larvae only. Larvae were calculated from counts of those enclosed in a rack one foot square, floating on the water (Figure 1).

RESULTS OF FIRST EXPERIMENT. Egg rafts, first observed in the organically fortified plots six days after flooding, were left in place. The resulting larvae were later identified as *Culex peus* (= *stigmatosoma* Dyar). Determination of pH of the water and reduction-oxidation potentials of the soil showed that reducing conditions were well established in the treated plots, with acids accumulating in the surface water.

Oviposition continued throughout several weeks. Larvae of *C. peus* predominated throughout the test period, but, during the last week, specimens of *C. tarsalis* were abundant and several specimens of *Anopheles freeborni* Aitken were recovered. The control plots remained completely free of egg rafts and larvae throughout the test period. A summary of the results is presented in Table 1.

SECOND EXPERIMENT

METHODS. Following the first experiment, all of the plots were drained, cleaned, and flushed out with fresh water. The major portion of the added organic material had been fermented; the rapid evolution of gases that characterized the early stages of the experiment had stopped.

Three plots previously used as controls were chosen for a supplementary experiment, as follows: (a) one pound of sucrose in strong solution was injected into the soil of one plot; (b) the same amount of casein solution was injected into the soil of the second plot; (c) the third plot received one pound of hydrogenated vegetable oil.

RESULTS OF SECOND EXPERIMENT. Five days after these organic materials were introduced into the three test plots egg rafts were found on the water surface of all test plots. None were observed on the control plots. The resulting larvae proved to be *Culex peus*.

Rafts continued to appear in the sucrose plot for 15 days after they were first found. The casein and fat plots continued to attract ovipositing female mosquitoes for 24 and 31 days, respectively, after the first rafts were found.

TABLE 1.—Main oviposition study. Data collected from June 6, 1957, through July 12, 1957

	Total no. egg rafts	Average no 1st instar larvae/sq. ft.	Average pH water	Volatile acids mg/liter
Test plots (5) organic material added to soil	20	123	8.06	54.2
Control plots (8)	0	0	8.70	30.7

A summary of the results is presented in Table 2.

TABLE 2.—Supplementary oviposition study

	No. rafts recovered	Av. pH during period of oviposition	Av. pH after oviposition ceased	Period of active oviposition (days)
Sucrose added	10	8.1	9.0	15
Casein added	14	8.4	9.0	24
Fat added	16	8.5	8.8	31
Control	0	8.9	8.9	(observed for 35 days)

DISCUSSION. The foregoing experiments suggest that adult female mosquitoes were attracted to the test plots by compounds resulting from the anaerobic decay of organic materials. A relatively simple, easily fermentable substance (sugar) produced a brief response by the mosquitoes; more complex materials (casein and fat) produced longer responses.

Eggs were first deposited on the water on the day when the pH values started to drop. In the plots studied, this increase in acidity can be attributed to the accumulation of organic acids from fermentation of the organic materials supplied to the plots.

A shallow-water environment includes two distinct components of vastly different characteristics. The water-logged soil becomes anaerobic shortly after being flooded, while the soil-water interface and the overlying water remain aerobic, increasingly so as photosynthetic organisms become established. The metabolism of the main body of soil is fermentative, and that of the water is oxidative. Under ordinary circumstances, the two functions are approximately balanced.

The environment can be artificially altered so that, for a time, the fermentative metabolism is faster than the oxidative metabolism. If extra organic material is supplied to the soil, fermentation products accumulate in the water. Similarly, when the oxidative biota of the water is reduced—by such artificial means as treatment with copper sulfate or other algicidal materials—the fermentation products accumulate.

With adequate knowledge of the particular environment, it is possible to judge the nature of its predominant metabolism as excessively fermentative, approximately balanced, or predominantly oxidative. These three characteristics have long been recognized by limnologists in regard to larger bodies of water. The oxidative environment is designated as oligotrophic, the balanced environment as eutrophic, and the fermentative environment as dystrophic.

The writer prefers not to borrow the exact terms of the limnologist to describe the trophic relationships of shallow bodies of water. The condition of dystrophy in lakes is intense, hardly comparing with the condition found in shallow-water environments.

If the limited data of the experiments are to be interpreted precisely, the appearance of *C. peus* in the study plots during the period of rapid fermentation confirms the preference of this species for foul water. The succession of *C. tarsalis* and *A. freeborni* in apparent response to reduced fermentative activity, conforms to the oviposition preferences of these species. However, these observations are in need of exhaustive investigation.

Apparently *Culex peus* prefers environments that display the characteristics of the fermentative, anaerobic soil. The exclusive manner in which the test plots were selected suggests that these characteristics were detected from a considerable distance. It would seem that the ovipositing females were attracted by olfactory stimuli directly to the plots that had extra organic matter in the soil.

The exact nature of the attractants remains unknown, but the short-chain volatile acids known to arise from water-logged soils seem, logically, to be the compounds most directly associated with the attraction of the mosquitoes.

References Cited

ACHARYA, C. N. 1935a. Studies on the anaerobic decomposition of plant materials. I. Anaerobic decomposition of rice straw. Biochem. Jour. 29:528–41.

———. 1935b. Studies on the anaerobic decomposition of plant materials. II. Some factors influencing anaerobic decomposition of rice straw. Biochem. Jour. 29:1116–20.

GERHARDT, R. W. 1957. The occurrence of *Culex tarsalis* Coq. in California rice fields as related to dissolved nitrogenous compounds and biological productivity. Ph.D. thesis, University of California (microfilm, U. C. library).

HUDSON, B. N. A. 1956. The behaviour of the female mosquito in selecting water for oviposition. Jour. Exptl. Biol., 33:478–92.

RUDOLFS, W. 1922. Chemotropism of mosquitoes. New Jersey Agric. Exp. Sta. Bull. 367.

RUDOLFS, W., and LACKEY, J. B. 1929. The composition of water and mosquito breeding. Amer. Jour. Hyg. 9:160–80.

WALLIS, R. C. 1954. A study of oviposition activity of mosquitoes. Amer. Jour. Hyg. 60:135–68.

A LIST OF NEVADA MOSQUITOES, WITH FIVE NEW RECORDS

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Some of the first records of mosquitoes collected in Nevada were by Dyar (1917, 1922), who reported 8 species from the State. Carpenter and LaCasse (1955) listed 12 species from Nevada. The most recent list by Richards *et al.* (1956), contains 23 species and is based on their published records plus their personal collections.

While surveying areas in the counties surrounding Reno, the writer collected larvae of four species not heretofore reported from the State. An additional species was identified from larval material collected by R. C. Bechtel, of the Nevada State Department of Agriculture. These records are as follows:

Aedes communis (DeGeer): Douglas County: Spooner Summit, III-19–59; Glenbrook, IV-24–59. Washoe County: Lake Tahoe, IV-9–59; Mt. Rose, IV-21–59.

A. hexodontus Dyar: Douglas County: Spooner Summit, III-19–59; Glenbrook, IV-24–59. Ormsby County: Lake Tahoe, IV-9–59. Washoe County: Mt. Rose, IV-21–59. Elko County: Lamoille Canyon, VI-2–59.

A. pullatus (Coq.): Elko County: Lamoille Canyon, VI-18–58 (collected by R. C. Bechtel), VI-2–59.

A. schizopinax Dyar: Washoe County: Mt. Rose, IV-21–59; Hunter Lake, VI-11–59.

Culex territans Walker: Douglas County: Lake Tahoe, IX-1–58. Ormsby County: Lake Tahoe, X-10–58. Washoe County: Lake Tahoe, VII-4–59.

A record apparently overlooked by both Carpenter & LaCasse and Richards *et al.* is that of an *Anopheles* reported by Dyar (1922) as *maculipennis* Meigen from Steamboat Springs, VIII-19–15. The wing tips of the species known by Dyar as *maculipennis* have a brassy reflection and must be either *occidentalis* Dyar and Knab or *earlei* Vargas. The former species is supposedly restricted to a rather narrow strip along the Pacific coast from California to Canada; hence the Nevada record probably is *earlei*.

[1] In cooperation with the Nevada Agricultural Experiment Station.