THE CONTINUOUS LABORATORY REARING OF Culiseta inornata (Will.) AND A STUDY OF THE STRUCTURE AND FUNCTION OF THE EGG-SHELL AND EGG-RAFT (DIPTERA : CULICIDAE)

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A thesis

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I. INTRODUCTION

In Canada mosquitoes are of importance primarily as pests of man and livestock. Their role as vectors of disease is confined solely to the virus of western equine encephalitis. Lacking a public health impetus, study of mosquito biology in Canada has lagged behind that of other countries where diseases such as malaria, yellow fever, filariasis and dengue exist. But in recent years, due to military activities in the arctic and subarctic of Canada, there has been an increasing interest in the biology of our pest mosquitoes. In these northern regions mosquitoes are sufficiently abundant to stop all effective human activity for a period of about two months during the summer (Twinn, 1949, 1950). On the other hand the study of mosquitoes in Canada has two serious handicaps. First is the comparative shortness of the season, and second is the lack of laboratory colonies of the indigenous species which could permit the study of these insects during the winter months.

In the family Culicidae the habit of depositing the eggs in boat-like masses or "rafts" which float on the surface of water is a characteristic of the genera Culex and Mansonia. The majority of
mosquito species (e.g. all Anopheles and Aedes) lay their eggs singly hence, as Christophers (1945) and Bates (1949) have suggested, the raft-building habit may be of some phylogenetic importance. It is therefore of considerable interest that in at least two genera some members in each lay their eggs singly while others deposit their eggs in rafts. Bates (1949) has drawn attention to the two habits in the primitive South American genus Trichoprosopon. In the northern genus Culiseta the eggs are laid in rafts only by members of the subgenus Culiseta.

Besides its obvious function as a protective covering for the egg, the egg-shell, or chorion, of a raft-forming species might be expected to possess certain structural adaptations associated with its ability to float and with its role as a component of the raft. A study of such adaptations is necessary before their affinities can be determined.

Nath (1925) and Roy and Majumdar (1939) traced the development of the egg of Culex fatigans within its follicle and gave structural details of the egg immediately before and after it is laid. Christophers (1945) described in detail the egg of Culex pipiens and compared the structure of its raft with those of several other species. Due to lack of material he was able to give only a preliminary description of the egg and egg-raft of one species of Culiseta (Theobaldia).
In the following pages a method is given for the continuous laboratory rearing of *Culiseta inornata*. This is the first species, indigenous to Western Canada, which it has been possible to adapt to continuous laboratory culture. Using material provided by such a colony, a study is presented of the structure of the egg-shell and egg-raft and their functions.

II. THE CONTINUOUS LABORATORY REARING OF *Culiseta inornata* (Will.)

During recent years the need for a laboratory-adapted representative of the hordes of "pest" mosquitoes which infest the Canadian plains and subarctic has become more urgent. Tests of insecticides and repellents made on a species such as *Aedes aegypti* have been found to give misleading results when applied in the field against northern mosquitoes. Studies in outdoor insectaries in Western Canada have had to be made with single generations of specimens reared from larvae taken in the field or from eggs laid by captive wild females; procedures which are usually hindered by high mortalities in the resulting mosquito populations. Studies of the biology of these important insects are invariably interrupted by lack of material which begins with the first frosts of the autumn. Of the six North American mosquito species which have been adapted to laboratory culture (*Anopheles quadrimaculatus* Say, *Aedes aegypti* (Linn.), *Culex pipiens* Linn., *Culex quinquefasciatus* Say, *Aedes atropalpus* (Coq.) (Trembley, 1944, 1945), *Anopheles albimanus* Wied. (Rozeboom, 1936; Burgess,
1950) none occurs in Western Canada. Of the 20-odd species which
were available in Southern Manitoba, where the present work was
started, only one, *Culiseta inornata* (Will.) - due to its known steno-
gamous habit - showed any promise of becoming a laboratory animal.

Dupree was probably the first to observe that *C. inornata*
(s. *C. consobrinus* Desv.) would mate in very confined quarters
(Mitchell, 1907). This fact was apparently overlooked until Owen
(1937) made the same observation and suggested that this species
(*Theobaldia inornata* Will.) would make a useful experimental animal.
In a footnote in the same publication he recorded his success in
maintaining a colony of this species through five successive genera-
tions. Later (1942) he reported success in the continuous rearing
of this species in the state of Wyoming using a "semi-balanced"
aquarium for the larvae. The principal ingredients of this aquarium
were obtained every three months from a "vernal-autumnal" pool.

Encouraged by Owen's success, attempts to rear *C.
inornata* in Canada were begun in the autumn of 1942 and these at-
ttempts have continued intermittently up until the present. It very
soon became apparent that establishing a colony in Western Canada
would be no simple problem. A balanced aquarium maintained by
additions of material from a natural pool was out of the question in
Manitoba. It was necessary to find an artificial medium in which the
larvae would grow. During the winter of 1942-43 a small colony was
maintained with difficulty for five generations using for the larval diet a hay infusion inoculated with paramecia. This larval rearing medium was difficult to duplicate. Numerous larval rearing media were tested during the summer of 1943 when an abundant supply of wild females was available. Again, during the winter of 1944-45 a small colony was kept alive through three to four generations using yeast alone in water for one generation and the proprietary product "Cerogras" in water until the colony died out. From August, 1946 to February, 1948 another colony was maintained through sixteen generations on a larval diet consisting of a mixture of flour and yeast in water. Here the larval mortality was variable and on more than one occasion the colony was reduced to a few individuals. The colonies mentioned were reared in Manitoba and Quebec (from Manitoba stock) and during the past three years another colony was established in Alberta. A routine method of rearing has now been developed which, it is believed, will be applicable anywhere that the simple necessary materials and equipment are available.

_C. inornata_ has much to recommend it as a laboratory insect. Besides its stenogamous habit, it is a large species, larger than the other North American mosquitoes which have been adapted to laboratory rearing. The male pupae are distinctly smaller than those of the females and the two sexes can readily be separated in this stage. The eggs are normally laid in the evening but if gravid females are
deprived of water on which to lay in the evening, they will deposit their eggs when placed over water at any time during the day, particularly against a dark background. The eggs are laid in "rafts" on the surface of the water which facilitates harvesting. The female requires about one-half hour to deposit her raft. The eggs are pearly-white when laid and turn dark brown in about two hours at 25°C, which simplifies the gathering of eggs of known age. The larvae can be reared in complete darkness or in light. The adults, both males and females, feed readily on almost any fluid diet presented to them. No preliminary warming of the diet is necessary to induce them to feed. The females will imbibe from a free fluid surface or through a membrane. The species is more available for colonization, having a wide distribution in North America which includes the entire United States and Mexican table land (Owen, 1942) and as far north as the 51st parallel in Manitoba and the 55th parallel in Alberta. Finally, *C. inornata* is a known laboratory vector of western equine encephalitis (Hammon and Reeves, 1943), St. Louis encephalitis (Hammon and Reeves, 1943a) and Japanese B encephalitis (Reeves and Hammon, 1946).

**A. REARING THE LARVAE**

The greatest obstacle to the continuous rearing of *C. inornata* in the laboratory has been the lack of a suitable technique for rearing the larvae. The successful rearing of mosquito larvae in general depends on obtaining a balance between the concentration of
food substances, volume and depth of medium, concentration of the larvae, and temperature. In rearing C. inornata it is also essential to keep the surface of the medium free of a pellicle which if allowed to form, will quickly smother the larvae.

The dietary requirements of the larvae of Aedes aegypti have been studied by Atkin and Bacot (1917), MacGregor (1929), Trager (1935, 1935a, 1937, 1948), Trager and Subbarow (1938), Goldberg and DeMeillon (1947, 1948, 1948a), Goldberg, DeMeillon and Lavoipierre (1945) and of Culiseta (Theobaldia) incidens by Frost, Herms and Hoskins (1936). The work on these two species indicates that mosquito larvae require in the diet proteins, lipoids, vitamins of the B group and various salts. During the present study no attempt was made to determine the specific dietary requirements of C. inornata but the above information was kept in mind when seeking possible culture media for the larvae. Nor was any attempt made to keep the cultures sterile although all apparatus used was kept as clean as it was convenient to do.

I do not intend to recount the many techniques and media which were tested during the course of this investigation. The object was to find a routine method for C. inornata, which would be as simple as possible and which could be duplicated anywhere. All the necessary dietary requirements for mosquito larvae are found in yeast, and the majority of the media recommended in the literature for mosquito
larvae include yeast or yeast extracts in one form or another. Early in this investigation the methods recommended by Trager (1935) (without sterilization), Woke (1937), Granett and Powers (1937), Granett and Haynes (1944) and Phillips and Swingle (1940) for *Aedes aegypti* and of Frost, Herms and Hoskins (1936) for *Culiseta incidens* were tried with *C. inornata*. Various modifications of their techniques were also tried as well as media which included hay infusions alone or containing wheat grains or cultures of paramecia, and media containing various proprietary preparations of powdered milk and baby foods. It is possible that these methods would have given better results had the necessity for preventing formation of a surface scum been recognized sooner. The most helpful publications encountered were those of Trembley (1944) and Bates (1941) and the medium eventually found to be most satisfactory was a modification of Bates' "standard" for anopheline larvae.

*C. inornata* should be reared at a temperature of 20-21°C. The species can be reared at 25°C and development is more rapid at this temperature, but larval mortality is slightly higher and the adults are not as large and robust as those reared at the lower temperatures.

The egg-rafts on being harvested were placed in enamel pudding pans measuring eight inches in diameter and 2-1/2 inches deep. Five to six rafts were placed in each pan which contained a medium consisting of 100 mg Difco Brain Heart Infusion, 50 mg Baker’s yeast
("Fleischmann's Royal") and 0.5 gm of ground whole wheat bread crumbs in 1250 ml of Bates' (1941) "medium S". The bread crumbs were prepared by slicing whole wheat bread, drying the slices in the sun or in an oven at 45°C then grinding as fine as possible in a mortar. Bates' "medium S" consists of 0.5 gm calcium sulphate (CaSO₄·2H₂O), 0.5 gm sodium chloride (NaCl) and 1 gm magnesium sulphate (MgSO₄·7H₂O) in 1 litre of distilled water. A hatching pan prepared in this way could accommodate about 1000 first instar larvae. The pan was covered with a piece of window glass and a slow stream of air bubbled continuously through the medium.

At 20° - 21°C the eggs hatch in about 57 hours and the resulting larvae were left in the hatching pans and allowed to complete their first moult there. The first moult occurs in 2 - 2-1/2 days after the eggs hatch and the larvae were not transferred to the larval trays until this moult was completed and the integument darkened. Each larval rearing tray received 150 second instar larvae, picked up, counted and transferred by means of a medicine dropper pipette.

The larval rearing trays were enamel instrument or photographic trays measuring 15 x 10 x 2 inches. Each tray contained 2-1/2 litres of Bates' "medium S" to which had been added 100 mg Difco Brain Heart Infusion, 50 mg Baker's yeast and 240 mg
of ground whole wheat bread crumbs. The trays, containing the preceding ingredients, were made up on the same day as the hatching pans i.e. on the day the eggs were laid. The trays also were covered with window glass and air was bubbled continuously through the medium. Each second day thereafter (from the day the trays were made up) until pupation began, 50 mg of the Baker's yeast and 240 mg of the bread crumbs were added to each tray. Since pupation began on about the tenth day after hatching, each tray received seven increments of the diet making a total of 350 mg of yeast and 1.7 gm of bread crumbs.

Average pupation in the trays was 90 per cent and was complete in from five to 12 days with the majority completing pupation in seven days. The males began to pupate first, followed about two days later by the first female. The pupae were removed from the trays each day by means of a dipping tube and transferred to an enamel pudding pan containing distilled water through which air was bubbled continuously. The pupal pan was kept in the emergence cage.

An air stream of 1 - 1-1/2 litres per hour delivered through a medicine dropper pipette to each hatching pan and larval rearing tray was sufficient to prevent the formation of a pellicle. Too fast an air flow caused the medium to splash on the glass cover and trap some of the younger larvae. As a source of air pressure
a standard laboratory compressed air system can be used with an air pressure regulator to reduce the pressure to a steady five pounds per square inch or lower, depending upon the number of trays to be aerated. A satisfactory source of air pressure was also obtained using a laboratory blower, or a "Cornelius" air compressor*, the compressed air being stored in a three to five gallon storage tank and brought up to 15 - 18 pounds pressure twice a day. The outward flow of air from the storage tank was controlled by a needle valve. The beneficial effect of the air stream was probably purely mechanical, for on many occasions I have taken the larvae of C. inornata from pools in which there was no detectable dissolved oxygen by the Winkler method.

Regarding the ingredients of the larval rearing medium, Bates' "medium S" was found to be superior to distilled water, tap water, well water or pond water. In Winnipeg, tap water that had been allowed to stand exposed to the air for two days before using was found to be satisfactory. But at Lethbridge mortality of the larvae was almost 100 per cent in media prepared with tap water. Well water and pond water gave erratic results in different localities. Difco Yeast Extract can be substituted for Baker's yeast but larval mortality with the former was slightly higher. After several days
the larval rearing media contained populations of bacteria and protozoa which probably originated in the bread. For some time domestic flour ("Canada Approved" vitamin B) was used instead of bread, but the larval mortality varied considerably from generation to generation and this was believed to have been due to greater variation in the numbers of micro-organisms present in the flour.

B. FEEDING AND HANDLING THE ADULTS

Blood is necessary for the production of eggs in C. inornata, although Owen (1942) obtained a few eggs on three occasions from females fed only on sugar solutions or raisins. The females when mated and hungry will feed readily on the human arm either when the arm is inserted in a cage or when the females have to feed through gauze when a cage or chimney is applied to the arm. I have never been able to induce unmated females to pierce the skin for a blood meal but an occasional female will do so while in the act of mating. However, such a method of providing a blood meal is too slow and tedious even for a colony of moderate size, so a quicker and more certain method was sought.

In studies of virus transmission by mosquitoes it is a common practice to infect the mosquitoes by allowing them to feed on a suspension of the virus soaked up in a cotton pledget. This method was adapted to feeding blood to C. inornata. For some unknown reason, the adults are unable to imbibe whole defibrinated,
heparinized, citrated or oxalated blood but both males and females readily take up such blood when it is diluted with a ten per cent solution of sucrose. Dilutions containing one volume of the sugar solution to six volumes or less of blood can be imbibed. The most satisfactory mixture was found to be one volume of the sugar solution to three volumes of defibrinated blood, mortality being lowest and egg production highest on this mixture. No comparative tests were made on the blood of different species, but for practical purposes beef, hog, or sheep's blood was satisfactory. A solution of the sugar in distilled water was kept on hand and added to the blood as soon as possible after it was collected and defibrinated (one to two hours). The mixture will keep in the refrigerator at 1°C to 5°C for two to three weeks. For cage feeding, the blood and sugar mixture, well shaken, was soaked up by absorbent cotton held in gauze bags. Much of the blood and sugar mixture passes through the insects undigested but this also occurs when the females feed on the arm.

The emergence cage - which was also used as a feeding and egg-laying cage - had a wooden frame 12 inches square and 22 inches high. The top and back were of three ply wood and the sides plates of window glass which slid in grooves in the wooden frame. The front was of unbleached cotton with a sleeve of the same material. The bottom three inches of the frame were made of 1 x 3 inch boards,
these boards being grooved on their upper edges to receive the window glass. The bottom of the cage was the bottom of an inverted galvanized iron tray with sides one inch high and perforated with nail holes one inch apart. On the inside, the bottom of the cage was covered with fine sand about one inch deep and a layer of blotting paper lay on the sand. The cage stood in a galvanized iron ice water pan 15 inches square and 4-1/2 inches deep. The ice water pan was kept filled with water to just above the level of the bottom of the cage; this served to keep the sand and blotting paper moist and maintained a satisfactory humidity in the cage. Food for the adults was provided by two gauze bags about one inch in diameter filled with absorbent cotton and soaked in the blood and sugar mixture described above. Two small gauze bags each containing four or five boiled raisins were also supplied. The four gauze bags were hung by strings about six inches long from the ceiling of the cage. The blotting paper became soiled with the faeces of the insects and had to be replaced about once a month. The growth of moulds on the blotting paper was retarded by sprinkling it with table salt. A cage of this type could accommodate about 1000 adult mosquitoes.

At 20°-21°C the adults emerge in about two days after pupation. The males begin to emerge first and when the females emerge mating takes place immediately, sometimes before the females are dry. Consequently it is almost certain that single females
observed resting on the walls of the cage have mated. Males and females will drink the blood and sugar mixture from the cotton pledgets. Unmated females which have fed on the blood will produce apparently normal rafts, but the eggs in these rafts never hatch.

The adults can be handled in different ways depending on the use to be made of the colony. For maintaining the colony, the adults were left in the emergence cage described above and a water surface for egg-laying was provided in the form of two pans measuring 10 x 4-1/2 x 1-1/2 inches half filled with water and placed in the bottom of the cage. The surface area of water provided for oviposition should be at least one half the area of the bottom of the cage. Under the artificial conditions in the cage the females apparently find the water surface by chance. If the free water area is too small, too many rafts are deposited on the moist blotting paper on the bottom of the cage. No air was bubbled through this water since movement of the water surface disturbed the females resting there, instead the water was renewed every second day. The egg-rafts were removed from the emergence cage each day. At temperatures fluctuating between 60°F and 70°F Owen (1942) obtained his first egg-rafts in from five to seven days following the first blood meal. At the comparatively constant temperatures of 20°C - 21°C I never obtained the first egg-raft earlier than seven days following the first meal of the blood and sugar mixture. If a number of egg-
rafts of approximately the same age were required, the females were deprived of a water surface until the 10th or 12th day.

If confined in lantern chimney cages for experimental purposes, the adults were supplied the blood and sugar mixture soaked up in one inch square thin gauze pledgets. The pledgets were placed on top of the gauze or marquisette covering the chimney and covered with a watch glass. These were renewed each day or alternated with boiled raisins.

The eggs of *C. inornata* cannot withstand freezing temperatures but they can be stored for a limited time at 4° - 5°C if first allowed to darken. At these temperatures development proceeds very slowly so that when placed in the refrigerator, after aging for two to three hours at 20° - 21°C, they will not hatch for about 21 days. Any time prior to this they can be removed from the refrigerator and will develop and hatch normally. Temperatures of 4° - 5°C are fatal for eggs which have not yet darkened.

By the method described the size of the colony which can be reared and maintained depends solely on the space and equipment available. With two constant temperature cabinets large enough to hold three hatching pans, 14 larval rearing trays and one emergence cage, a colony of 1500 - 2000 adults can be maintained throughout the year.
Culiseta inornata (Will.) is one of the most abundant mosquitoes on the plains and park lands of Western Canada. The adult females overwinter and it is one of the first species to appear in the spring. The population builds up during the summer, reaching its peak at the end of July or early in August. With falling mean temperatures its numbers gradually decline and finally disappear as the females go into hibernation.

The egg-rafts are inconspicuous objects. The trained eye can find them floating on the surface of the permanent or semi-permanent waters of sloughs, stagnant pools, ditches, pot holes in creek beds, or on water in artificial containers such as decorative ponds, rain water barrels, or the motley containers found around refuse heaps. The eggs, having been laid during the night, have usually darkened by daylight and the rafts then resemble pieces of floating wood, bark, dried leaf fragments or other debris on the surface of the water.

A. METHODS

The gross structure of the egg and egg-raft can be seen under the compound microscope, but the finer details of the egg-shell can only be seen in sections. To obtain serial sections of the eggs it was necessary to use a double-embedding technique. For this purpose the method of Crabb (1949), with a few modifications,
was found to be satisfactory.

Egg-rafts were killed in hot Bouin's fluid and could also be stored in this fluid after killing. When cool, or after storage, the eggs were punctured in the Bouin's and left in it for at least 18 hours after puncturing. The needles used for puncturing were made from minuten nadeln sharpened in acid. At least three punctures were made in each egg. After removal of the excess picric acid by 50 per cent tertiary butyl alcohol, dehydration was continued in increasing strengths of the same alcohol to 95 per cent (two changes). This was followed by carbol-xylol for 10 minutes, ether (three changes) for 10 minutes and while in the ether the eggs were transferred to a #00 gelatine capsule. The ether was then drained off and the capsule filled with the "Pi" solution of Crabb (l. c.) in which the eggs sank. On a weight basis the Pi solution, as given by Crabb, contains about 12 per cent rosin. To obtain satisfactory ribbons the rosin content had to be increased to about 13 per cent. The gelatine capsule, containing the eggs and Pi solution, was left uncovered under a 75-watt desk lamp for from eight hours to overnight then placed in chloroform vapour for eight to 24 hours. The capsule was next immersed up to its lip in water for ten minutes. The softened capsule could then be peeled off the firm block which was trimmed and placed in a vial of chloroform until it sank to the bottom. After the chloroform the block was passed through carbol-
xylol for fifteen minutes, xylol (three changes) for fifteen minutes then to 52°C wax, 60°C wax and embedded in the latter. Satisfactory ribbons down to five microns could be obtained. No attempt was made to orientate the eggs in the blocks, enough eggs being done together to ensure that satisfactory sections would be present in each block. Delafield's haematoxylin with eosin as a counterstain and Mallory's triple were the principal stains used.

To obtain a better understanding of the structure of the micropilar cup and exochorion, it was necessary to study the gravid ovaries in fresh dissections, preserved whole preparations and in serial sections. The usual procedures for fixing, dehydrating and clearing, remove the exochorions from the laid eggs and destroy the micropilar cups. But in the ovary treated thus the follicular epithelium holds the exochorions in place and preserves part of the cups. Females in the act of depositing rafts were etherized and the abdomens opened in Ringer's solution. Eggs could be moved out of the follicles into the oviducts by gentle pressure with a blunt needle. The ovaries and oviducts were also dissected out and preserved in Bouin's fluid or ten per cent formalin. The latter was the better preservative of the micropilar cup in its unruptured form. Whole preparations of the ovary and ovarian eggs were stained in borax-carmine. Ovaries for sectioning, after fixation in Bouin's fluid, were double embedded as above, but it was not necessary to puncture the ovarian eggs.
Whole mounts of the micropilar cup were obtained by placing a raft on a slide and covering it with a few drops of carbon bisulphide. After the carbon bisulphide had evaporated the eggs were removed leaving the micropilar cups adhering to the slide. These slides were stained and mounted in the usual manner.

In order to see the posterior polar specialised area the water droplet which covers that area had to be removed. A raft, in which the eggs had hatched, was placed on a coverslip. A few drops of acetone were then placed on the raft and allowed to evaporate. Transmitted light passing up through the empty egg-shells enabled the posterior polar specialised area to be seen under the microscope.

Other procedures used will be mentioned in the appropriate places in the text. Most of the drawings were made with the aid of the camera lucida.

**B. THE EGG-SHELL**

The egg of *Culiseta inornata* (Fig. 1) is cone-shaped, rounded, and broader at the anterior (lower) end. The posterior (upper) quarter of the egg is curved slightly. Using the position of the larva in the egg at hatching as a criterion, the posterior curve of the egg points either ventrally or ventro-laterally. When laid, the eggs have an average length of 0.71 mm and an average maximum diameter of 0.19 mm. During development the egg
increases slightly in size until, just before hatching, the average measurements are 0.83 mm and 0.21 mm.

The egg-shell, or chorion, is composed of two principal layers (Fig. 2) an outer, colourless, almost transparent exochorion and an inner pigmented endochorion. At the broad anterior end the egg carries a membranous structure (Fig. 1) which in the egg of Culex, Christophers (1945) has called the "micropilar cup" and Nath (1925) the "striated collar". At its anterior extremity the endochorion is modified to form the "micropilar area" (Fig. 3). The exochorion at the posterior extremity of the egg is modified to form the "posterior polar specialised area". The egg of C. inornata has no exochorionic structure resembling the "anterior polar specialised area" of the egg of Culex.

1. THE ENDOCHORION

When the egg is laid the endochorion is comparatively soft and pearly-white in colour. Within a short time after laying it becomes tough and amber-coloured but still remains flexible and semi-transparent. When the exochorion is stripped from an egg the endochorion appears uniformly smooth, shining and unbroken over the whole egg. Over most of its area the endochorion is about two microns thick but at the anterior tip of the egg there is a dark ring about three microns wide and about 35 microns in diameter.
This ring, which corresponds with the "supporting ring of the micropile" of *Anopheles* described by Nicholson (1921) and of *Culex fatigans* described by Nath (1925) indicates a region where the endochorion is thickened. It surrounds the micropilar area of the egg.

Within the micropilar area (Figs. 3, 4) the endochorion becomes thinner toward the center forming what Nicholson (1921) and Nath (1925) call the "micropilar disc". At the center of the disc, in a roughly triangular area measuring about six microns to a side, there is a thin membrane of irregular thickness. This thin triangular membrane covers the micropile and appears to be a remnant of the micropilar tube to be mentioned later. The hollowed-out portion of the endochorion which is the micropilar area is filled on the inside by what appears to be a gelatinous plug. This plug is seen most clearly in sections of gravid ovary (Fig. 4) where, under haematoxylin - eosin, it stains dark red and the endochorion blue. Under Mallory's triple stain the plug appears pale blue and the endochorion red.

The outer surface of the endochorion is strongly hydrophilic.

2. THE EXOCHORION

The exochorion (Fig. 2) is slightly over one micron thick. Over most of its area it consists of a double membrane in
which are set numerous evenly spaced papilliform structures or bosses (Fig. 6). Christophers (1945) has referred to similar structures in the exochorion of Culex and Culiseta annulata as being shaped like a "rifle bullet" and the same description could apply to those of C. inornata. In surface view they resemble the stippling of a newspaper picture.

The exochorion does not completely cover the egg. It terminates at the base of the micropilar cup, about 40 microns from the anterior end and in front of the widest diameter of the egg. At the posterior end of the egg, where it is reduced to a diameter of about 24 microns, the papilliform exochorion ends in a bevelled edge. The exochorion continues beyond this edge as six radially arranged tubes. Each of the tubes is about 1.5 microns in diameter and eight microns long. They leave a circular area about eight microns in diameter between their posterior ends. Within this circular area and between the tubes the hydrophilic endochorion is exposed. The posterior tip of the egg bearing the six exochorionic tubes (Fig. 5) is the posterior polar specialised area.

Almost as soon as an egg is laid a droplet of water forms between the ends of the exochorionic tubes and increases in size until it covers the posterior polar specialised area. The droplet remains there throughout the life of the egg, it persists after the egg has hatched and in fact until the disintegrating egg-raft
is finally submerged. In the laboratory this may be three to four weeks after the eggs have hatched.

The outer surface of the papilliform exochorion is strongly hydrophobic.

3. THE MICROPILAR CUP

At the anterior end of the egg, where its diameter is about 0.15 mm, the papilliform exochorion ends abruptly at a frill which girdles the egg. In a greatly magnified section (Fig. 2) this frill has the appearance of a flange arising from a stout base. The base is about five microns wide and the flange is a stout, flexible, transparent membrane about one micron thick. The membrane is faintly ridged which gives it the frilly appearance. Running through the membrane, from its base forward, are 16 evenly spaced and parallel supporting rays each about 16 microns long. The anterior edge of the frilled membrane is curved between the rays (Figs. 8, 9) giving the membrane, when extended, the appearance of an abbreviated parasol.

Attached to the anterior edge of the frilled membrane is another thinner, transparent membrane. The anterior edge of this second membrane is irregular in outline. Scattered at random over its surface are a number of spike-like processes (Figs. 2, 8, 9) which project inwards (or down, when the membranes are extended over the surface of the water). These processes have a diameter
of about two microns at their bases and are about 3.5 microns long. Their basal and distal portions give different staining reactions.

From the anterior edge of the base of the frilled membrane a third, delicate, transparent membrane extends forward. This membrane, which I shall call the basal membrane of the micropilar cup, covers the endochorion at the anterior end of the egg. It is possible that a small opening is present at the center of this membrane opposite the micropile but I was unable to confirm its presence.

The outer surface of the basal membrane is strongly hydrophilic. The inner surfaces of the frilled membrane and of the spike-bearing membrane are also strongly hydrophilic and their outer surfaces strongly hydrophobic. Since the frilled membrane is flexible at its base, it and the spike-bearing membrane stretch out over the surface of the water when the egg is floating. Consequently they, along with the basal membrane, present a strongly hydrophilic area measuring about 0.2 mm in diameter to the water surface beneath each egg in the raft. The three membranes together form the "micropilar cup" of the egg of Culiseta inornata.

When an egg is withdrawn from the water, the frilled and spike-bearing membranes close over and completely cover the anterior end of the egg. In doing so they enfold a layer of water
between their inner surfaces and the hydrophilic outer surface of the basal membrane (Fig. 8).

The origin of the micropilar cup of *C. inornata* is similar to that described for *Culex* by Nath (1925). It is formed of the remains of the follicular epithelium which surrounded the nurse cells. But when the egg of *C. inornata* is ready to be laid, all traces of the nurse cells have disappeared. At this time the follicular remnant forms a closed membranous sac at the anterior end of the egg (Fig. 7). In the mid line the anterior surface of the sac is drawn into an extremely delicate tube, the "micropilar tube", which extends back to the micropile in the endochorion. The anterior end of the sac is ruptured just before the egg is placed in the raft. Practically all trace of the micropilar tube disappears at this time.

4. THE INTERMEDIATE LAYER

It has been suggested by Christophers (1945) for *Culex* and by Gómez and Mañana (1948) for *Anopheles* that a third layer, with an oily or lipid nature, is situated between the exochorion and endochorion. This suggestion is based on observations made when egg-rafts or eggs are placed in fat solvents such as ether, xylol, gasoline, carbon bisulphide and liquid petroleum. When this is done, the eggs slip out of their exochorionic sheaths.

Similar observations have been made on the rafts of *C. inornata* using the above solvents as well as chloroform, benzene,
ethylene dichloride, and carbon tetrachloride. When the eggs are placed in any of these solvents a break occurs in the exochorion in the region of cleavage of the operculum of the egg about 0.09 mm behind (or above) the base of the frilled membrane. Each egg then slips out of the exochorionic sheath which covers the remainder of the egg. The eggs, with the membranes of the micropilar cups enclosing the layers of water, and the pieces of exochorion still attached, sink to the bottom of the solvent. Here, the micropilar cups with the pieces of attached exochorion become fixed to the bottom of the container. The eggs separate when they fall over.

The exochorion is not completely dissolved by any of the above solvents. In xylol and carbon bisulphide it is reduced to a gelatinous mass. In ether it shrinks but the raft of exochorions remains intact with a honeycomb appearance. In gasoline the exochorion is reduced to a very thin membrane. The solvents apparently affect only certain components of the exochorion.

None of the preceding solvents is miscible with water. When egg-rafts are placed in fat solvents such as absolute ethyl alcohol, dioxane, and acetone, which are miscible with water, a different reaction occurs. In these solvents the eggs merely collapse, the rafts remain intact, and there is no evidence of separation of exochorion from endochorion. This reaction does not suggest the presence of an oily or fatty layer. It does suggest
that if an intermediate layer be present it is composed of either water or of some hydrophilic substance. This view received support from observations made when egg-rafts were floated on aqueous solutions of stains.

Egg-rafts were floated on weak solutions (just enough dye in the water to detect it on filter paper) of acid fuchsin, Bismarck brown, methylene blue, methyl blue, orange G and fast green FCF contained in Syracuse watch glasses. In each case the dye was seen to move up the eggs between exochorion and endochorion and finally could be detected in the droplets at the posterior ends of the eggs. When the experiment was performed in covered watch glasses the dye moved very slowly, taking over six hours to traverse the length of the eggs. When performed in exposed watch glasses the dye reached the posterior tips of the eggs in about two hours. The dye did not enter the eggs and they hatched in the dye solutions. When the exochorion was stripped from an egg which had been floating on a dye solution, the dye was found to be adhering to the inner surface of the exochorion.

It was noted above that the outer surface of the endochorion is hydrophilic. That the inner surface of the exochorion is also hydrophilic can be shown by stripping a piece of the exochorion from an egg in water. When this is done the piece of exochorion - with its inside surface in contact with the water surface - spreads evenly
over the water. The floating piece of exochorion can be lifted off the surface of the water on a forked needle and replaced on the water surface in the same position. If now the piece of exochorion is lifted from the water surface on the forked needle and turned over to present the hydrophobic outer surface to the water, the exochorion will not spread but instead wraps itself around the tines of the fork.

C. THE EGG-RAFT

The well known description by Reaumur (1738) of raft formation by a species of Culex is the only original account of this process which I have encountered. Due to the conditions under which his observations were made, and to the limitations of his equipment, Reaumur was unable to see the part which the cerci and insula plate undoubtedly played in the formation of the raft. His observations were also influenced by his inability to conceive the possibility of a single egg, or even a small group of eggs, being able to float upright alone on the water. But considering that he had to study the insects with nothing but a hand lens, as they rested on the surface of the water in a tub in his garden, it is remarkable that he was able to see as much as he did.

Reaumur's observations though limited, had the virtue of having been made under natural conditions. The description of raft formation given here was gathered from observations of the females of a laboratory colony of C. inornata. They were observed
in the act of egg-laying in cages and under a stereoscopic microscope. In spite of these artificial conditions there was no noticeable difference, other than in size, between rafts laid in the laboratory and those found in natural pools.

When a female is ready to deposit her eggs (Fig. 12) she rests on the surface of the water with the hind legs extended backwards. Only the tarsal segments come in contact with the water surface. The distal ends of the two first, and longest, tarsal segments of the hind legs either cross or, more often, come together to form the apex of an open triangle. In the latter case the remaining tarsal segments of each hind leg lie side by side on the surface of the water. The open side of the triangle is thus directed toward the body of the female. Sometimes the female rests at the edge of the container and grasps its walls with the first, or the first and second, pairs of legs. In any case the hind tarsal segments are always in one of the characteristic positions. The open triangle formed by the first tarsal segments of the hind legs acts as a mould in which the floating raft is deposited. The first egg is pushed into the apex of the triangle, the succeeding two eggs are laid against it, and so on.

The location for each egg in the raft is found by the cerci. After an egg is deposited the abdomen moves laterally and as it does so the cerci move back and forth until the fine hairs at
the tip and side of one of them come in contact with two previously laid eggs, or with one egg and one of the tarsi. When this occurs the abdomen stops its lateral motion and another egg is deposited. The lateral movement of the abdomen is apparently random, for often it will move all the distance across the triangle before stopping to deposit the next egg. Or the eggs may be deposited side by side in a regular manner. At other times, after depositing a few eggs in a row beginning at one of the tarsi, the next egg may be laid against the same tarsus. These movements of the abdomen help to determine the shape of the raft. As the eggs fill the triangle, extension of the femora-tibial joints of the hind legs moves the raft back.

When the hind tarsi are crossed for egg-laying, they usually remain crossed until the raft is completed. But when the tarsi are parallel, they may remain so, or the tarsi of the two legs may gradually separate as the raft grows. The spreading apart of the legs is due to the pressure of eggs being laid against the tarsii. In this case the raft tends to be wider. It will be readily seen that a number of factors determine the shape of the raft. The rafts of *C. inornata* (Fig. 11) are most often oval or pear-shaped, but frequently they are triangular or long and narrow. These variations occur both in the laboratory rafts and in those found in natural pools.

The number of eggs laid in a raft varies considerably
depending on whether the female has previously laid (Owen, 1942) and probably also on the diet. For 17 rafts laid by wild females the average number of eggs per raft was 220 with the smallest containing 55 and the largest 376 eggs. For 100 rafts laid by laboratory bred females the average number of eggs per raft was 124 with the smallest containing 60 and the largest 283 eggs.

The eggs receive their posterior curvature while in the ovary, the posterior end of each egg being directed toward the oviduct. In passing down the oviducts the eggs turn about their longitudinal axes until, on reaching the gonopore, each egg lies on its dorsal surface with its posterior end directed upward. As the posterior end of the egg emerges from the gonopore, the sigma flexes upward, pressing the insula plate against the dorsal surface of the egg; this, along with the curvature of the egg, directs its posterior end upward. When the posterior end of the egg comes in contact with the ventral and median surfaces of the cerci, they flex upward and their median surfaces together form a shallow groove within which the egg slides as it emerges. As the wide anterior end of the egg clears the gonopore the egg is in a vertical position. The cerci - which by this time are against the middle region of the egg - return to their normal position, directed posteriorly, and in doing so push the egg, held vertically, against one of its predecessors in the raft, in the position previously
chosen by the cerci. The force by which the egg is pushed into position in the raft is sufficient to move the insect and her raft on the water surface. The median and ventral surfaces of the cerci are covered with short, fine hairs which probably assist in holding the egg while it is being deposited. And as the anterior end of the egg passes over the hairs on the upper surface of the insula plate these hairs rupture the delicate membranous sac which then forms the micropilar cup.

When it emerges from the gonopore, the outer surface of the egg is wet and for a short time after it has been placed in the raft a meniscus can be seen between the egg and its neighbours around their areas of contact. The source of this wet covering is unknown; it is believed to be the product of the accessory gland. A wet egg can be separated very easily from the raft without damage. If an egg is allowed to dry after being separated from the raft it cannot be rejoined to the raft. This moist layer is therefore an adhesive substance which plays some part in joining the eggs together in the raft.

In common with the rafts of some other species, those of C. inornata are concave on their upper surfaces and convex on their lower surfaces. The curvature of the raft of C. inornata is sometimes so pronounced, particularly in long narrow rafts, that some of the eggs at the narrowest end of the raft may be completely
out of the water when the raft is floating free on the water surface. The curvature of these concavo-convex rafts is usually attributed to the fact that the eggs are placed in the rafts with the broad anterior ends of the eggs down. But the shape of the egg and the length of its line of attachment to its neighbours in the raft also play a part in determining the curvature of the raft. In rafts of C. inornata the line of attachment between the eggs begins at the widest diameter of the egg and extends upwards for about 0.5 mm. That is, the eggs in the raft are attached to each other for well over half of their length. The rafts of some species such as those of Culex erraticus and Culex apicalis (Mitchell, 1907) are flat on both upper and lower surfaces.

The eggs are held together very firmly in the raft. Separating an egg from a raft more than an hour old requires two needles, one to hold the raft and the other to pry off the egg. An egg cannot be separated from the raft without rupturing the exochorion along the areas of contact between adjacent eggs. Even after the eggs have hatched the raft of empty shells holds together and, in the laboratory, does not begin to break up until about ten days later. At this time the raft may be completely submerged. An egg separated from the raft will float upright but if knocked over it will remain horizontal, being trapped on the surface by the strip of hydrophilic endochorion exposed when the egg was separated.
Another prominent characteristic of the raft is its great buoyancy. The slightest air movement or disturbance of the water is sufficient to move the raft rapidly across the water surface. It is almost impossible to submerge the raft by mechanical means. Attempts to push the raft beneath the water surface usually result in damage to the raft and it always returns to the surface floating upright.

D. STRUCTURE RELATED TO FUNCTION IN THE EGG-SHELL AND EGG-RAFT

Mosquitoes in general can be divided into those which lay floating eggs and those which lay non-floating eggs. Those which lay non-floating eggs include members of the large genus Aedes. The species of Aedes usually lay their eggs in mud, in damp depressions on the ground or among damp trash - locations which are likely to be flooded at a later date. Occasionally the eggs of Aedes are laid on water, but when this happens the eggs sink to the bottom.

Those mosquitoes which lay floating eggs include the species of Anopheles, Culex, Mansonia, Uranotaenia and the subgenus Culiseta of the genus Culiseta. The eggs of this group are always laid on the surface of water.* But whether a mosquito lays

* Under abnormal conditions mosquitoes will sometimes deposit their eggs in unusual situations. I have seen female Aedes vexans
tuck their eggs into the underside of a moist cellucotton pad that covered the lantern chimney in which they were confined. Bates (1949, p. 96) records a similar observation for Psorophora ferox. Gravid female C. inornata will deposit their rafts on moist filter paper if no free water surface is available.

A floating or non-floating egg, the egg is always deposited in a humid environment.

Associated with floating and non-floating eggs are two different hatching responses. The embryo of a non-floating egg, when laid in water, may proceed to develop and hatch soon afterwards if the aquatic environment is favourable (Gjullin, Hegarty, Bollen, 1941; Gjullin, Yates, Stage, 1939, 1950). But if the environment is unfavourable, or if the eggs are not deposited in water, hatching may be delayed for weeks, months, or years. These eggs can usually withstand temperatures below the freezing point of water and also considerable desiccation. On the other hand, when a floating egg is deposited, its embryonic development begins immediately and proceeds uninterrupted until the egg hatches. The development of the embryo may be greatly retarded by low temperatures, but as a rule the eggs cannot withstand freezing* or desiccation.

*Anopheles walkeri is apparently an exception. This species lays
both winter and summer eggs (Matheson and Hurlbut, 1937; Hurlbut, 1938) and the winter eggs are capable of withstanding an exposure to \(-21^\circ C\) for 72 hours (Peters, 1943).

The eggs of raft-forming mosquitoes are well known for their inability to withstand desiccation in the laboratory. This is said to be due to a very permeable chorion (Wigglesworth, 1949). But the report of Wilkins and Breland (1949) who recovered the eggs of *C. inornata* from "dry material", indicates that these eggs can withstand some desiccation under more natural conditions.

The phenomenon of an egg touching the surface of water with about one-fifteenth of its area, or completely out of water, and still surrounded by an aqueous layer is due apparently to capillarity between the exochorion and endochorion. The droplet at the posterior end of the egg will consequently have a hydrostatic function in assuring that a layer of water completely surrounds the egg. The egg can survive desiccation only as long as the droplet is present.

When a raft of *C. inornata* is placed on filter paper the eggs lose water and gradually collapse. If this is allowed to proceed until the droplets disappear, the eggs will not hatch after the raft is returned to the water surface. But if the raft is returned to the water at any time while the droplets are still present, the eggs fill again and subsequently hatch. Also, a raft can be made to float
upside down on the surface of water. When this is done the layer of water held by the membranes of the micropilar cup takes over the function of the posterior droplet. The embryos develop normally and can push off the hatching caps of the eggs in a raft made to float upside down, but being out of water the larvae are unable to free themselves from the egg-shells. Floating upright (anterior end down) is essential for hatching.

The ability to float is obviously a means of insuring that the egg remains in an environment suitable for the development of the larva. But a floating egg is at a disadvantage in its loss of anchorage or stability. It is more difficult for a larva to lever itself free from an egg floating lightly on the surface of water than from an egg anchored in mud or trash at the bottom of a pool. This disadvantage is partly overcome by the tendency of floating eggs to come to rest against emergent vegetation, floating trash, or the walls of a container. In eggs that float singly it is also partly overcome by the tendency of the eggs to gather in clusters on the surface of the water. In this way a heavier float is obtained. In Anopheles some of these egg clusters are so characteristic of the species that Saliternik (1942) has suggested their use for differentiating species in the field. The tendency to cluster is carried still further in the raft-laying species and has probably reached greatest development in the raft of Culiseta.
Christophers (1945) has noted that the eggs of *Culex* are held together rather loosely in the raft, that individual eggs can be separated from the raft by a light touch. As observed above, the eggs of *C. inornata* are held together firmly. The firm attachment between the eggs in the raft is due to interlocking of the exochorionic papillae and the adhesive action of the secretion which covers the eggs when they are laid. Sections of joined exochorions (Fig. 10) show the papillae of one exochorion between those of the other. Their distal ends do not quite reach the outer exochorionic membrane. The joined exochorions have a combined thickness of about 2.4 microns. Also, the interlocked papillae are no longer bullet shaped but conical, suggesting that pressure has been exerted on their walls. It appears then that the wet secretion covering the eggs when laid enables the papillae of each exochorion to slip into the interpapillary spaces of the other, aided by the thrust from the cerci.

Christophers (l. c.) observed that the combination of strongly hydrophobic exochorion and hydrophilic anterior surfaces contribute to the great buoyancy and stability of the *Culex* raft. The same combination is found in the raft of *C. inornata*. The freedom of translatory movement over the water surface is aided by the convex lower surface of the raft. The curvature of the raft however also has another important role. It was mentioned above
that some of the eggs at the narrow end of the raft are sometimes completely out of the water when the raft is floating free on the water surface. But in nature the rafts are rarely found floating free. Their great freedom of movement invariably brings them to rest against some object. Since these objects are usually wetted by water, the meniscus climbs their surfaces. Consequently when a raft comes to rest against these objects its curvature fits the meniscus and all the eggs are then in contact with the water.

E. DISCUSSION

From the descriptions given by Christophers (1945), Roy and Majumdar (1939) and Nath (1925) it is apparent that the eggs and egg-rafts of Culex and Culiseta have several features in common. The eggs of both are similar in shape, those of Culiseta being slightly larger. The rafts of both are similar in size, those of Culex tending to have a larger number of eggs in the raft. The rafts of both depend for their buoyancy and stability on a combination of hydrophobic and hydrophilic surfaces on the eggs. The papilliform exochorion with its strongly hydrophobic outer surface, the strongly hydrophilic outer surface of the endochorion and the posterior water droplet are common to the eggs of both groups.

There are also some notable differences. The micropilar cup of the egg of Culex is a conspicuous structure, while the egg of Culiseta must be examined carefully to detect the cup. When
found it resembles more closely a shallow, flat-bottomed bowl rather than a cup. The base of the cup on the *Culiseta* egg is much wider than in *Culex* due to a much wider circle of attachment to the exochorion. The attachment to the exochorion though flexible is strong while in *Culex* the attachment is tenuous and the cup easily dislodged. The wall of the cup of the egg of *Culiseta* is apparently a stouter structure and the spikes of the spike-bearing membrane have no counterpart in the micropilar cup of *Culex*. On the other hand the egg of *Culiseta* lacks the "anterior polar specialised area", this region of the egg being covered instead by the delicate hydrophilic basal membrane of the micropilar cup. Since the basal membrane of the cup is surrounded by the frilled and spike-bearing membranes, the hydrophilic area of the egg of *Culiseta* in contact with the water surface is almost double that of *Culex*. In the rafts of *Culiseta* the membranes of the micropilar cups of adjacent eggs form an almost continuous sheet under the raft when it is floating. The development of the preceding differences could be associated with the greater size of the *Culiseta* egg.

The posterior polar specialised area of the egg of *Culex pipiens* is an exclusively exochorionic structure composed of larger exochorionic projections, "surrounding a small central circular area of delicate exochorion devoid of any thickenings"
(Christophers, l.c.). In the egg of *Culiseta* the six radially arranged exochorionic tubes extend over an exposed area of the endochorion. It is this hydrophilic area of the endochorion which holds the droplet in position. In order to hold the droplet, the small circular area of exochorion of the *Culex* egg must be hydrophilic. Since the outer surface of the remainder of the exochorion is hydrophobic this small area might be composed of only the inner layer of the exochorion.

The only notable differences in the endochorions are found in the micropilar areas. In the egg of *Culex pipiens* the endochorion becomes thicker at the anterior pole and continues into the egg-spike. In the eggs of *Culiseta inornata*, *Culex fatigans* and in *Anopheles maculipennis* (Nicholson, 1921) there is no egg-spike, the thickening being confined instead to a ring at the anterior pole. Within this ring the endochorion becomes thinner towards the center to form the micropilar disc. The micropile is situated in the center of the disc. Christophers (l.c.) notes that in the newly laid egg of *C. pipiens*, the egg-spike "contains a spongy interior". Nicholson (l.c.) describes a thickening of "the inner wall" just below the micropilar funnel which he calls the "stopper". This, and the gelatinous plug beneath the micropile of the egg of *C. inornata* might possibly be homologous with the egg-spike of *C. pipiens*. No spike or stopper are present in the egg of
C. fatigans as described by Roy and Majumdar (l.c.) and Nath (l.c.). And I could find no evidence of a micropilar funnel in the ovarian egg of C. inornata.

In eggs which have been studied, the micropilar tubes and funnels disappear as soon as, or shortly after, the eggs are laid and the supporting ring of the micropile and egg-spike are variable in occurrence. It is therefore clear that these structures can play no part in the floating mechanism of the egg-raft. They appear to be associated solely with the process of fertilisation.

Nicholson (1921) believes that the egg of Anopheles maculipennis in passing down the common oviduct is under pressure by the muscular walls of the oviduct. This pressure causes the membranous disc to protrude forward. As the egg passes out the gonopore, posterior end first, the pressure on the egg is released, the disc returns to its normal position, and in doing so sucks the sperm down the micropilar tube and funnel. Nath (l.c.) and Roy and Majumdar (l.c.) believe that the same process occurs in Culex fatigans. Fertilisation of the egg of C. inornata is probably also similar to that claimed for Anopheles maculipennis. If, as Nicholson suggests, the "stopper" acts as a seal over the micropile after the sperm has entered the egg, then the egg-spike of C. pipiens would function both as a membranous disc and as a stopper. Compression of the wide anterior end of the egg would force the
spike into the mouth of the micropilar funnel and when released
the spike would retract and draw the sperm in; it would act like
a piston. But why a micropilar stopper is present in the eggs of
A. maculipennis, C. pipiens and C. inornata and absent in C.
fatigans still remains to be explained.

The size and shape of the rafts laid by different species
are sometimes claimed to be characteristic of those species.
Having observed the rafts of C. inornata in the course of con-
struction I doubt the diagnostic value of such characters. The
sometimes random movements of the abdomen when the eggs are
being deposited and the alternative positions of the tarsi (crossed
or parallel) give variety to the shape of the rafts. The rafts are
usually deposited at night, and under the comparatively quiet
conditions of a laboratory the rafts of different species might as-
sume a characteristic shape and size. But on a warm summer
evening there can be many agents both in the air and in the water
to disturb a mosquito in the act of depositing its raft in nature.

Comparative studies of the structure of the female
genitalia of mosquitoes have yielded little of value for taxonomic
purposes. If related to the structure of the egg-shells and to egg-
laying habits these studies might acquire new significance and
contribute to a knowledge of the evolution of the raft-forming
habit. The rafts and the shells of the eggs which compose the rafts
are highly specialised structures whose production involves modifications within the ovary and in the function of the cerci and post-genital plate. Such specialisation must have arisen gradually. Some specific differences in the structure of the eggs and rafts have been noted in the present study and others can probably be found. One would expect to find significant differences in the egg-shells of a genus in which some species lay their eggs singly and others in rafts.

IV. SUMMARY

A method is described for the continuous rearing of Culiseta inornata (Will.) in the laboratory. The larval rearing medium is a modification of one already recommended by Bates for Anopheles. Its successful application to C. inornata depends, (1) on the prevention of scum or pellicle formation on the surface of the medium, (2) on rearing the first instar larvae in a medium of high concentration, (3) on transferring the larvae only after the first moult is complete to a medium in which the concentration of food is gradually increased as the larvae grow and (4) on reducing the concentration of second and later instar larvae to 150 in 2-1/2 litres of the medium.

The adults are stenogamous. They feed readily and produce fertile eggs on a diet consisting of one volume of a ten per cent solution of sucrose to three volumes of defibrinated hog, beef,
or sheep's blood. This diet is presented to the adults soaked up in absorbent cotton or in thin gauze pledgets and requires no other treatment to induce the females to feed. The blood and sugar diet is supplemented by boiled raisins.

A study is presented of the structure of the egg-shell and egg-raft including a detailed description of the method by which the female builds the raft. The relationship between these structures is explained and also the role which they play in the formation of the raft and in maintaining the raft in an environment suitable for the development of the embryo and the larva. The structure of the egg-shell and egg-raft of *C. inornata* are compared with those of *Culex* as described by other workers and further studies are suggested.

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Figure 1. Egg of Culiseta inornata, lateral view.

Figure 2. Section of micropilar cup at junction with exochorion.
Figure 3. Micropilar area.

Figure 4. Section through micropile.
Figure 3

- base of frilled membrane
- supporting ring of the micropile
- micropile
- micropilar disc

Figure 4

- micropilar plug
- basal membrane of micropilar cup
- endochorion
- yolk

Scale: 0.02 mm and 0.05 mm
Figure 5. Posterior polar specialised area.

Figure 6. Exochorionic papillae.
exochorionic papillae
bevelled edge of exochorion
exochorionic tube
endochorion

0.02 mm

Figure 5

5 μ

Figure 6
Figure 7. Schematic section through anterior end of ovarian egg.

Figure 8. Micropilar cup detached from the egg. The dark stippled area at the top covering part of the frilled and basal membranes is the piece of exochorion attached to the cup. The bubble marks on the basal membrane are due to water trapped on its hydrophil outer surface. The dark structures around the outer edge of the mount are the spikes in the spike-bearing membrane. 230x
**Figure 7**

- spike-bearing membrane
- supporting ring of micropile
- micropilar tube
- spike
- micropile
- endochorion
- micropilar plug
- basal membrane
- micropilar tube
- frilled membrane
- exochorion

**Figure 8**
Figure 9. Micropilar cup extended. Partly diagrammatic.

Figure 10. Interlocked exochorionic papillae.
Figure 11. Egg-rafts of *C. inornata*; three not yet darkened. 8x

Figure 12. Female *C. inornata* in the act of depositing raft. 8x