AEDINE MOSQUITOES OF MANITOBA : EGG IDENTIFICATION

A

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ABSTRACT

The mosquito fauna in Manitoba is composed of six genera, of which the genus Aedes is the most predominant. As Aedes species spend the majority of their life cycle in the egg stage, a key to the identification of the aedine eggs of Manitoba would undoubtedly be useful. Sanitarians could use the information obtained from the eggs of Aedine species in planning their abatement operations, and ecologists could use a knowledge of egg identification in population survey studies.

Eggs for the present studies were obtained generally from wild caught females from ten locations, extending as far north as Baker Lake in the North West Territories. The adult females were given blood, then placed in individual cages for oviposition. Eggs were conditioned by incubating them first at 20°C for three months and then placing the embryonated eggs in a constant cold temperature (4°C) incubator for four months. Hatching of the conditioned eggs was most satisfactory when eggs were placed directly from the cold into the hatching medium at 65°F (15°C). Larvae were reared in alternating temperature incubators to the fourth instar stage, and then preserved for identification. After the species was known,

similar eggs were grouped.

In order to study the egg characteristics the whole eggs were examined above a black background in intense reflected white light. Detailed examination of the chorionic reticulation was made with phase contrast illumination. Eggs were measured using an optical micrometer. The dorsoventral view was sketched using a Zeiss drawing apparatus fitted to the stereomicroscope. Photographs of silhouettes and chorionic detail are given.

Aedine eggs show a variation in shape, size, colour and chorionic sculpturing according to species. The present study also shows that eggs possess characteristics indicating certain phylogenetic relationships. Four species had two or more significantly different sizes and/or shapes within the species. This may be an indication of sub-speciation. Twenty-seven species are reported as having been found in Manitoba, four of which are reported for the first time as being present in the Province.

CHAPTER I

INTRODUCTION

Man has suffered from the activities of mosquitoes from time immemorial, and to all of us these insects are familiar as pests that attack persons and livestock. Mosquitoes often render life almost unendurable during certain seasons of the year. In addition to the annoyance caused by mosquitoes to humans, it is a well known fact that certain species are capable of transmitting pathogens of a few dreadful diseases such as yellow fever, dengue, filariasis, malaria and equine encephalitis. None of the mosquitoes in Manitoba is known to be involved in the transmission of pathogens, although there is a possibility that western equine encephalitis may be transmitted by mosquitoes. At the present time, however, the primary importance lies in their nuisance value.

In Manitoba, several species of Aedes Meigen have become disgustingly annoying in parks, camp sites, some urban areas, and around areas of commercial importance. Mosquitoes are known to have been the causative factor in the reduction of real estate values in certain localities. With the constantly increasing human activity in the northern regions and the proposals for further development of the north, it is of

great importance to learn everything possible about the distribution and abundance of these mosquitoes to permit effective control. Hence, mosquitoes have become extremely important to the economy of the province of Manitoba.

The mosquito fauna of Manitoba is comprised of six genera: Aedes, Culex, Anopheles, Culiseta, Wyeomyia and Mansonia. The present studies are confined solely to the genus Aedes which comprises the majority of the mosquito population in the province and is also the most predominant genus in Canada.

The world over, mosquito studies have centered on the all important genus Anopheles with work of a supplementary nature on a few Culex and Aedes. It is only quite recently that several workers have begun to show an interest in the Nearctic aedine mosquitoes which in Canada areman's most troublesome summer time problem.

Edwards (1932) listed about 400 species as belonging to the genus Aedes. Since then several hundred more have been recognized. McLintock (1944) was the first to list the mosquito species in the Greater Winnipeg Area. Of the twenty-two species reported by him, fourteen belong to the genus Aedes. In the present study the author reports twenty-seven species of Aedes found in Manitoba.

To aid in the identification of the North American adult mosquitoes, and fourth instar mosquito larvae, there are several taxonomic keys. Two of the most satisfying are found in the works by Carpenter

and La Casse (1955) and Barr (1958). Recently even a key to the identification of the first instar larvae has been published by Dodge (1966).

Aedine mosquitoes spend most of their lives in the egg stage and most unfortunately workers have been considerably handicapped because of a lack of knowledge about these eggs and their identification. The eggs of many aedine mosquitoes are sufficiently distinct so that they can be identified with a stereomicroscope at a magnification of about 80 times. Yet, only a little is known about the eggs of the aedine mosquitoes in Manitoba. Work in this field had been hindered because until recently there were neither suitable methods devised for obtaining eggs from wild caught female mosquitoes, nor appropriate survey methods developed to obtain aedine eggs from their natural oviposition sites. Further, delayed hatching and difficulties in rearing larvae made laboratory investigations very difficult and almost impossible (Craig 1956). With most of these factors now overcome and with the use of phase microscopy it has become possible to make minute examinations of the surface sculpturing of the chorion of aedine eggs, with a possibility of separating these into different species.

The present work therefore, deals with identification of the aedine mosquitoes of Manitoba based solely on the characteristics of the eggs, the stage in which these creatures spend most of their lives.

Malcolm MacGregor (1927) quite explicitly stated:

> "..... there is no doubt that the specific differences in the structure of the eggs would prove a valuable and interesting subject for research, and I commend it to the attention of future workers."

THE PROBLEM

The studies were concerned with the detailed description of the eggs of aedine mosquitoes, in Manitoba, to help in the identification of, and the production of a taxonomic key to, the aedine eggs in this province. Characteristics of the whole egg, namely, size, shape and colour had to be described. To study the details of the surface sculpturing, chorionic mounts were observed with the aid of a phase contrast microscope. Photomicrographic techniques were utilized to obtain plates of the chorionic pattern.

An attempt was made, very briefly though, to study the distribution of the species and also the types of pools in which larvae of aedine species were found.

LOCATION OF THE STUDY

The adult female mosquitoes were collected from the following areas:

(a) Greater Winnipeg Area

(b) Sandilands Forest Reserve

(c) Whiteshell Forest Reserve

(d) Turtle Mountain Forest Reserve

(e) Riding Mountain Forest Reserve

(f) Duck Mountain Forest Reserve

(g) Porcupine Mountain Forest Reserve

(h) Flin Flon

(i) Churchill

(j) Baker Lake

Soil samples for obtaining eggs as outlined by Horsfall (1956) were taken from certain locations within the Greater Winnipeg area and from the banks of pools located in the Sandilands Forest Reserve, from the same locations where adults were collected.

All laboratory studies were undertaken in the Department of Entomology, University of Manitoba.

IMPORTANCE OF THE PROBLEM

So far the work done in relation to the mosquitoes of Manitoba seems to be chiefly related to the control of these insects, with a few studies on the biology of one or two important species (Tully 1928; Brust 1960).

As mosquitoes will probably continue to be one of the major pests causing annoyance in spring and summer in this province for years to come, a recognition of the species involved and a knowledge of their

habitat and distribution are important in the planning of effective abatement operations. In order to make such studies feasible it is better to be able to identify the respective aedine eggs, the stage in which they spend most of their lives.

The chorion of aedine eggs bears characteristics sufficient to permit recognition of species. Egg identification would also be very helpful for reasons mentioned hereunder:

(a) Very often, in field collections, the females are rubbed or damaged making identification impossible. Eggs from such females are adequate for identification. Even eggs obtained from dried gravid females could help in the identification of such adults.

(b) It is a well known fact that the difficulty in the identification of the adult females of the black legged group (Appendix VI) has made biological studies on the species extremely difficult (Vockeroth 1950; Hocking et al 1950; Jenkins & Hassett 1951; Beckel 1954). Egg characteristics of these species should help to overcome the identification difficulty encountered with the adults.

(c) As adults disperse widely over feeding areas, it is the egg stage that is most available for reliable survey studies. Aedine eggs are immobile and are present for the greater part of the year in the soil on the banks of pools which are subject to transient inundation. Surveys based on distribution and abundance of eggs provide information which allows prediction of future populations of adults in ecological

studies similar to the work of Shotwell (1935) who observed that data on distribution of grasshopper eggs are the most reliable for forecasting abundance.

(d) Oviposition in the field is a subject which needs more intensive study. Aedine egg recognition would give more information on the oviposition habits of females.

(e) Craig and Horsfall (1958) report that the activities of investigators and sanitarians have been hindered by their inability to recognize eggs. Sanitarians could use information obtained from the eggs of aedine species in planning their abatement operations.

(f) To the research worker in the laboratory, egg identification would undoubtedly be of tremendous value in experiments pertaining to thermal studies on development, or the role of diapause in the egg stage. Experiments could be performed well in advance, even before eggs are conditioned, hatched, and reared to fourth instars for identification of species.

It is for these reasons that investigations were carried out to obtain a rapid and dependable means of recognizing the egg characteristics, and the construction of a taxonomic key to the eggs of aedine mosquitoes in Manitoba. In my opinion it will serve as a convenience to the ecologist, physiologist, sanitarians and economic entomologist, and to all others interested in mosquito work.

CHAPTER II

REVIEW OF LITERATURE

Since the turn of the century many workers have reported that eggs of mosquitoes have characteristic features which would help in their identification. Theobald (1901) observed that the eggs of Culicidae differed in each genus. Giles (1902) writing on the eggs of Culex appeared to have noted that the surface of the egg shell had spiny outgrowths which differed in the different species and which he thought might assist in the differentiation of species belonging to this genus.

Most of the earlier work was mainly in relation to the genus Anopheles because of its importance in transmitting diseases. The eggs of Anophelines have very often been figured and described in several faunal papers. Nuttall and Shipley (1901) described in detail the shape and surface sculpturing of the egg of Anopheles maculipennis Meigen. Nicholson (1921) also described the external morphology of the egg of Anopheles maculipennis. In spite of the recognition of the diversity of the anopheline eggs for a long time, little importance was attached to the differences until Falleroni (1926) showed that the eggs of mosquitoes provided certain important characters

for segregating them into smaller categories. This work enabled him to break up the Anopheles maculipennis complex, and set up six sub-species within the group. Martini, Missiroli, and Hackett (1931 cited in Bates 1949) discovered that, the eggs provided the easiest and only reliable method of separating the various populations related to Anopheles maculipennis.

With most of the work having been focussed on the anophelines, detailed observations of the eggs of the other genera of Culicidae had been neglected (Giles 1902). With regards to aedine eggs, Goeldi (1905) was the first to attribute specific characters to them. Mitchell (1907) writing concerning investigations by herself and J. W. Dupree in Louisiana wrote the first key to the eggs of mosquitoes using for the first time chorionic sculpturing for taxonomic purposes, and hence showing its importance in systematic studies.

Howard, Dyar and Knab (1912-1917) in their monographs, inter alia, briefly described eggs of ten species of Aedes. Abdel - Malek (1949), and Breland (1951) have given descriptions to eggs of a single species of Aedes. The former described the egg of Aedes trivittatus (Coq) and the latter that of Aedes infirmatus Dyar and Knab. These are isolated descriptions and are not of any comparative nature. Eight species of Aedes from Ohio have been very briefly described by Newkirk (1955).

Horsfall, Miles and Sokatch (1952) gave detailed descriptions

of the size, shape and chorionic structure of seven species of Psorophora. For the first time phase microscopy was used to make detailed studies of the chorionic sculpturing, which enabled these workers to obtain specific differences for eggs of Psorophora. This work was also the first presentation of a workable key to the eggs of a homogenous group of culicine mosquitoes.

The most significant recent contribution to the systematics of aedine mosquitoes was by Craig (1956) and Craig and Horsfall (1958). The most important contribution made by Craig was that the external stuctures of eggs appeared to indicate some phylogenetic relationship among aedine mosquitoes. He also provided a key to the identification of eggs of the Nearctic aedine mosquitoes. Ross and Horsfall (1965) published an "egg - key" to the species recorded from Illinois.

CHAPTER III

BIONOMICS OF AEDINE MOSQUITOES

Aedine mosquitoes belong to that category referred to as flood - water mosquitoes, because they are found in areas that are subjected to seasonal temporary flooding (Horsfall 1963). They exhibit two general bionomic patterns: (a) species which have a single generation a year, called univoltine species and whose eggs must be cold conditioned for several months before they hatch and (b) the multi-voltine species which have a number of generations per year, a brood following each inundation provided the water temperature is suitable for hatching and development.

The former group generally hatches from eggs in very cold water, often near freezing, and matures before the onset of hot weather. Observations made in some pools at Sandilands, Manitoba, in the early spring of 1966 (7th May) revealed that certain pools contained 1st and 2nd instar larvae of A. punctor (Kirby) and A. communis (De Geer) at water temperatures of 1 degree Centigrade. There are, however, other univoltine species which require a higher temperature (above 10°C) for hatching and develop-

ment. A. pionips (Dyar) from Sandilands, Manitoba belongs to this group.

Some aedine species, reported as being univoltine, have now been shown to be partly multivoltine when reared in the laboratory. A. cinereus Meigen, A. canadensis (Theobald), and A. spencerii (Theobald), which were thought to be univoltine, may have repeated generations in the laboratory.

OVIPOSITION AND HATCHING

The aedine mosquitoes in Manitoba, other than the tree hole species Aedes triseriatus (Say), deposit their eggs in the moist soil on the margins of pools, between the current water line and the level of maximum flood. Gentle slopes present a greater surface area to the females and are therefore better oviposition sites than the more precipitous slopes. The shaded sides of the pools are preferred to the sunnier sides. Eggs are concentrated in the moist areas rather than in the drier areas. The eggs are rather susceptible to drying and collapse under such conditions. Collapsed eggs will not hatch.

The Aedes species in Canada overwinter in the egg stage. The embryos within the eggs develop normally to the point of hatching and then remain in a state of suspended development, or diapause, until some stimulus reactivates the embryonic larva to hatch. This phenomenon is now the subject of a great deal of investigation.

The eggs of the multi-voltine species have the ability of terminating this dormant period during the summer in which they are deposited and hatch whenever their sites are inundated. On the other hand, the eggs of the univoltine aedine mosquitoes lack this faculty of terminating the dormant period and therefore must remain in diapause during the summer in which they are deposited, and throughout the first winter until spring. That is, the eggs of univoltine species hatch only after a period of conditioning which takes place during the 8 to 9 months they are in diapause.

Several workers, including Gjullin et al 1941; Borg and Horsfall 1953; Horsfall 1956; Horsfall et al 1958, have investigated the nature of the hatching stimulus and have come to the conclusion that hatching of aedine eggs is due directly to a reduction in the concentration of the dissolved oxygen of the medium in which the eggs are immersed. In nature, microbial activity in the medium reduces the oxygen content of the water. In the laboratory various artificial media are used to lower the oxygen concentration in the medium.

The egg spine on the dorsum of the head helps to rupture the chorion along a line of dehiscence and a circular rent separates the anterior cap from the rest of the eggs enabling the larva to emerge into the external aquatic medium.

Mosquitoes, like other holometabolous insects, pass through two distinct immature stages, the larva and pupa, before becoming adults. Both immature forms are aquatic. The larvae require about