AEDINE MOSQUITOES OF MANITOBA : EGG IDENTIFICATION

A

Thesis presented to

The Department of Entomology

Faculty of Agriculture and Home Economics

The University of Manitoba

Winnipeg

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Kingsley Kalpage

October 1966

TABLE OF CONTENTS

APPENDIX

LIST OF TABLES

LIST OF FIGURES

FIGURE PAGE

ACKNOWLEDGEMENTS

Grateful acknowledgement is extended to my advisor, Dr. R. Brust, Assistant Professor, Department of Entomology, University of Manitoba for suggesting this study and for his continual interest, and most valued guidance throughout the study and in the preparation of this thesis. The writer is also grateful to Dr. A. G. Robinson, Professor and Acting Head, Department of Entomology for his helpful criticisms.

The writer expresses his appreciation to Mr. John Giardino and Mr. David Fox for the photographic work entailed in this study.

Thanks are due to Mr. Blaine Funk for his assistance in the laboratory during the winter of 1965/66. The writer also thanks his colleagues for the assistance given during the summer of 1965 in collecting the adult mosquitoes in the field.

Sincere appreciation is extended to Mrs. Wilson Barr Jones for the typing of this thesis.

The writer wishes to express his deep gratitude to the Canadian Commonwealth Scholarship and Fellowship Committee for the award of a Commonwealth Scholarship to study at the Department of Entomology, University of Manitoba.

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

7-15 days under normal conditions to become full grown fourth instars, at which stage they transform into pupae. In the immature stages, feeding takes place only during the larval period. The pupal stage lasts only about 2 days at normal temperatures but may be prolonged at lower temperatures. The larvae and pupae come to the surface of the water for breathing. Although the larvae possess gills they are not adequate for utilizing the oxygen dissolved in the water. During the pupal period the adult organs are fully formed, the pupal case cracks and the winged adult emerges.

The male mosquitoes usually begin to emerge a day or two before the females. Mating takes place within a couple of days after emergence during the "mating swarm flights" which are seen to occur at low light intensities in the evening or early morning.

The adults of both sexes will feed on various plant juices. This may be the only food which male mosquitoes take in the field. With very few exceptions, females must have one or two blood meals for the development of eggs. The females obtain the blood meal in the field from various mammals and to a lesser extent from birds, reptiles and amphibians.

DISTRIBUTION

It is not within the scope of this study to give a detailed account of the ecological distribution of the aedine mosquitoes in Manitoba, however I have endeavoured to give a very broad outline of

their distribution in relation to the natural vegetation of the province.

In Manitoba the vegetation may be very broadly divided into two distinct regions:

(a) The northern land mass above 60°N latitude (Fig. 188) is a vast cold treeless area known as the tundra. Baker Lake, in the North-West Territories (one of the locations from where adults were collected) is entirely tundra. This area is inhabited by a limited number of species. The tundra mosquitoes include Aedes nigripes (Zett), A. impiger (Walker), and A. hexodontus Dyar. Jenkins (1958) reports that A. nigripes is the most northern occurring mosquito in the world, and does not appear to breed south of the tundra. These three species constitute the true arctic species, and are most abundant beyond the tree line.

(b) Immediately south of the tundra is an enormous sub-arctic coniferous forest. This area is commonly referred to as the taiga or muskeg. Species typical of the taiga, or muskeg area are A. communis (De Geer), A. diantaeus H, D and K; A. excrucians (Walker); A. fitchii (Felt and Young); A. hexodontus Dyar; A. implicatus Vock; A. intrudens Dyar; A. pionips Dyar; A. punctor (Kirby); A. trichurus (Dyar), A. punctor and A. communis are the most abundant in this area and are considered to be the true forest species (Hocking et al 1950). A. campestris D and K; A. dorsalis (Meigen); A. flavescens (Muller); A. riparius D and

K, are wide ranging and have a scattered distribution in the north. In general, except for the tundra species, mosquitoes prefer a wooded habitat. The principal trees in these forests are white and black spruce, fir, jackpine, tamarack, aspen and willow.

Aedes, such as vexans, sticticus, spencerii and nigromaculis, are most abundantly found in extremely temporary pools. These species are more numerous in the summer especially after the July rains. A. vexans (Meigen) and A. sticticus (Meigen) are numerous during the summer and are found abundantly in and around Winnipeg. A. nigromaculis (Ludlow) is abundant further south in the St. Norbert area. In general multivoltine species are more numerous in southern latitudes, but some may range far northwards, for example: A. nigromaculis has been identified by Matheson among the mosquitoes collected at Churchill by McClure in 1936-37. A. spencerii and A. vexans are known to be migratory species, and it would be interesting to note how far north they could migrate.

In the present work four Aedes species, namely A. abserratus (Felt and Young); A. barri Rueger; A. triseriatus (Say) and A. decticus Howard, Dyar and Knab, not previously reported in Manitoba have been recorded.

CHAPTER IV

SYSTEMATIC LIST OF *AEDES* IN MANITOBA

The species and group names of the 27 species of *Aedes* recorded in Manitoba are given below. Species in the sub-genera *Aedes*, *Aedimorphus*, *Finlaya* and *Ochlerotatus* are recorded. The group arrangements in the sub-genus *Ochlerotatus* are taken from Edwards (1932). Group B in this sub-genus is the same as the *Aedes stimulans* group of Barr (1958), except that the latter has included *Aedes barri* Rueger as is done here. Within each group the species are alphabetically arranged. The nomenclature used is taken from Stone, Knight and Starcke (1959).

Genus:	*Aedes* Meigen 1818
Sub-genus:	*Finlaya* Theobald 1930
Species:	*triseriatus* (Say, 1823)
Sub-genus:	*Aedimorphus* Theobald 1903
Species:	*vexans* (Meigen 1830)
Sub-genus:	*Aedes* Meigen 1818
Species:	*cinereus* Meigen 1818
Sub-genus:	*Ochlerotatus* Lynch Arribalzaga

Group A: Laeniorhynchus group: Culicelsa

Species: nigromaculis (Ludlow 1906)

Group B: annulipes - group: Lepedoplatys

Species: barri Rueger 1958

excrucians (Walker 1856)

fitchii (Felt and Young 1904)

flavescens (Muller 1764)

riparius, Dyar and Knab, 1907

stimulans (Walker 1848)

Group E: dorsalis group: Acartomyia

Species: campestris Dyar and Knab 1907

canadensis (Theobald 1901)

dorsalis (Meigen 1830)

Group G: communis - group: Pseudoculex and Hyparcticus

Species: abserratus (Felt and Young 1904)

communis (De Geer 1776)

decticus Howard, Dyar and Knab 1917

diantaeus Howard, Dyar and Knab 1917

hexodontus Dyar 1916

impiger (Walker 1848)

implicatus Vockeroth 1954

intrudens Dyar 1919

nigripes (Zetterstedt 1838)

Group G:

Species: (continued)

pionips Dyar 1919

punctor (Kirby 1837)

spencerii (Theobald 1901)

sticticus (Meigen 1838)

Group H: *rusticus* group: *Feltianus*

Species: *trichurus* (Dyar 1904)

CHAPTER V

METHODS AND MATERIALS

This chapter is divided into two main sections. Section A describes the methods employed for obtaining eggs, conditioning of eggs, hatching, and rearing of larvae for identification.

Section B describes the techniques adopted for studying the diagnostic characters of the aedine mosquito eggs to facilitate a separation of the species.

Section A

METHODS FOR OBTAINING, CONDITIONING AND HATCHING EGGS; AND REARING LARVAE FOR IDENTIFICATION

Eggs of the aedine species vary in size, shape, colour and chorionic sculpturing. In order to study these characteristics, the eggs to be described were obtained by two distinct methods: (a) The majority of eggs were obtained from wild caught females which oviposited on moist cheesecloth in the laboratory. (b) Some eggs were obtained by separation of soil samples collected in the field. Details of these procedures are described in the succeeding paragraphs.

In the spring and summer of 1965 wild females were caught in

the field, with the aid of an aspirator, as they alighted on the collector's clothing. They were then placed in small plastic laboratory cages (6" x 1" x 1") which had plastic mesh on one of the long sides and nylon net on the opposite side. About 10 to 20 adults were placed in each cage. These cages were transported to the laboratory in coolers, which were kept cool with a few ice packs. Adult species from Flin Flon, Churchill and Baker Lake, were collected by Dr. R. Brust, Assistant Professor and Research Advisor. These were transported by air to Winnipeg in a manner similar to the above.

In addition to the above methods, adults collected at Sandilands were placed directly in large cages (20" x 10" x 7") each of which contained a guinea pig (Fig. 180). These cages are of plastic construction except for the top surface which is covered with 14 mesh per inch of plastic material. About 200 adults were placed in each cage. In this way the adults were able to have a blood meal immediately they were captured thus improving their survival rate under caged conditions.

In the laboratory the 6" x 1" x 1" cages were placed on moist cheesecloth (Fig. 181). All adult mosquitoes were kept in the constant temperature ($20^{\circ} \pm 1^{\circ}C$) room. The relative humidity in the room varied between 55% and 70%. The adults in the small cages were given at least three blood meals by feeding them on humans. The fe-

males in the larger cages were allowed to engorge on a guinea pig for four days. Feeding was confined to about four hours per day. In both instances, in addition to the blood meal, mosquitoes were also fed on honey. Cotton swabs soaked in clover honey were placed on and in the cages respectively.

After a period of seven to ten days the adults were anaesthetized with ether and each adult was transferred into a cylindrical cage (Fig. 182). The females regained consciousness rapidly and the anaesthetic had no apparent effect on the oviposition. Beckel (1955) states that most species which develop in ground pools will not lay their eggs readily on a surface as smooth as a filter paper. Therefore to enable the females to oviposit, each cage was placed on an individual pad of cheesecloth. The cages were arranged on a plastic tray placed in a pan half-filled with water. A wick from the water leading onto the plastic tray helped to keep the cheesecloth moist. This method enabled eggs laid by each individual female to be separate (Fig. 183). Each separate batch of eggs was then transferred to individual pads of moist filter paper in petri dishes. Several of these petri dishes were placed in a single large plastic container as illustrated in Fig. 187.

There was one species, however, that did not oviposit on moist cheesecloth. Aedes triseriatus the only tree hole species in Manitoba was collected at Charleswood, Manitoba. The adults were placed in 6" x 1" x 1" cages. These cages were then placed on moist brown coarse paper

towels in plastic pans. The females were fed on blood and honey, and oviposition resulted within ten days. Repass (1952) describes a method for the laboratory colonisation of this species. He used blocks of oak to facilitate oviposition. The author, however, observed that the females laid the majority of the eggs on the moist paper towel and only a very few on the moist piece of oak placed for oviposition.

Eggs of some species were also obtained by separation from soil samples which were taken from oviposition sites in the Fall of 1964 and 1965. Each sample was approximately 6" square and 1" in depth. The soil was cut with a pointed trowel. Each sample with the overburden of detritus was removed and placed in a cellophane bag.

In the laboratory, samples were stored at 40°F until they were to be separated for eggs. A modification of the techniques described by Horsfall (1956) for obtaining eggs from soil samples was adopted. The eggs were first removed from the leaves and sticks, then from the plant remains and silt and finally from the mineral components, by washing through three progressively smaller screens (Fig. 185). The eggs together with the mineral fraction were washed down to the bottom screen of 100 mesh. This mass was transferred to a percolation funnel containing a saturated solution of salt. The heavier material sank and the eggs together with similar dense detritus floated. A second treatment with saturated salt solution removed most of the extraneous material. The eggs together with the particles that floated on the surface were

collected on a 100 mesh sieve. The eggs were then rinsed from the sieve into a dish along with a small amount of water. The eggs were sorted out under a binocular microscope and transferred on to moist filter paper in petri dishes.

Wherever eggs are stored, the surface bearing them must remain moist for a few days after oviposition, in order to permit complete morphogenesis of the embryo. Eggs subjected to drying before the embryonic development is complete will collapse, and though they become turgid again they are not viable. After embryonic development is completed they are resistant to the normal desiccations which occur in nature and could remain viable 1 - 2 years. Thus in the laboratory the petri dishes containing the eggs were always kept moist.

As stated in the previous chapter the univoltine Aedes species enter an obligatory diapause in the egg stage. It appears that these forms must be exposed to low temperatures before they will hatch (Beckel 1954). Horsfall (1956) and Horsfall and Fowler (1961) report that the eggs would hatch only after a prior period of conditioning. The latter term refers to the sequence of events that predisposes them to hatch.

Eggs were first incubated at 20°C for three months. The resultant embryonated eggs were then placed in a constant cold temperature incubator at 5°C for 4 months. Unless the eggs of univoltine species are properly conditioned they will not yield to a hatching stimulus (Borg and Horsfall 1953).

The environmental feature that stimulates the hatching of conditioned eggs is the lowering of oxygen in the medium to a degree and at a rate that may vary according to the species (Borg and Horsfall 1953; Horsfall 1956; Horsfall et al 1958). It is remarkable that an aerobic organism should be stimulated to abandon its resting stage under anaerobic conditions. In the laboratory an artificial medium (Nutrient Broth, a Difco Laboratories product) was used to reduce the oxygen tension of the aqueous medium in which the eggs were immersed. In the present studies the laboratory observations indicated that a good hatch was obtained when conditioned eggs were placed in the hatching medium directly from the cold at a temperature of 65°F. Within 12 hours a great majority of the eggs had hatched into healthy larvae.

About ten eggs from each batch were hatched and the larvae reared to fourth instar for identification using the larval keys described in Carpenter and La Casse (1955) and Barr (1958). The larvae were reared in distilled water containing peat moss and yeast. The larvae were reared in alternating temperature incubators (CreLab Model 1222). Egg specimens from Flin Flon, Churchill and Baker Lake were reared in incubators where the temperature alternated daily from 5°C for a 12 hour period to 20°C for the remaining 12 hours. Specimens from Sandilands were reared at 10°C and 25°C for 12 hours at each temperature daily. Rearing under these conditions ensured that a majority of the

larvae grew to the 4th instar stage. Generally the 4th instar larvae were killed in hot water and preserved in 70% alcohol for identification. If, however, there was any uncertainty in the larval identification, a few larvae of that particular group were reared through to the adult stage. The 4th instar larval exuviae together with the adults were helpful in the identification. Identified eggs were grouped for a detailed study of the diagnostic characters of the egg. All eggs were stored at 5°C.

Section B

TECHNIQUES FOR STUDYING THE DIAGNOSTIC CHARACTERS OF AEDINE EGGS

The characters that help in the identification of eggs to species are the gross shape, size and the chorionic pattern. Very often the separation could be made by observing whole eggs, in reflected light at low magnifications, through a stereoscopic dissecting microscope.

Although the eggs within a given species or even those laid by a single female show a variation in shape, it is possible to ascribe a typical shape to each species. However, in the present studies instead of giving one single shape for a species, often three silhouettes depicting the shapes of eggs in a given species are shown, as in Figs. 1 to 93. Whole eggs for illustration and measurement were positioned so that the lateral aspect faced the observer. The dorsoventral aspect gives a distinct and characteristic silhouette, while silhouettes of a dorsolateral nature present very few distinctive charac-

terestics. Drawing of the silhouettes of whole eggs in the dorsoventral aspect, as seen by transmitted light, were made with a Zeiss drawing apparatus attached to a stereomicroscope. The magnification on the drawing surface was 160 X.

The measurements of the eggs were made with an ocular micrometer using a stereomicroscope at a magnification of 40 X. The length was measured from the anterior to posterior pole, and the width was measured across the dorsoventral diameter. All measurements are in microns. All mean measurements are for 100 eggs, unless otherwise stated (vide Table I). All measurements were made on eggs obtained from several females, except for the nine A. decticus eggs which were obtained from a single female. The dimensions of each species are listed under the specific descriptions and also in aggregate in Table I.

Whole eggs were best identified by placing them in water over a non reflective black background such as in the depression of a blackened "spot-plate". The eggs in the water were illuminated with an intense, sharply focussed, source of unfiltered, incandescent light. Magnifications of 10 X to 25 X were sufficient for manipulating, arranging and sorting of eggs, but magnifications of 40 X to 80 X were necessary for resolving the surface details sufficient for specific segregation. The characters that were revealed by this optical system

included shape, size and colour of the whole egg, the nature of the exochorion and the grosser aspects of the chorionic sculpturing.

For positive identification it was necessary to examine the finer details of the chorionic pattern which is more constant within each species. The surface sculpturing of whole eggs was examined by reflected light at a magnification of 80 X, however it was not always possible to see clearly the pattern on the surface of the chorion. This is because: (a) the surface is highly reflective, (b) certain species have a very faint sculpture, or (c) the thin exochorion, on which the pattern is quite distinct, is often rubbed off. In spite of these difficulties, it was possible to develop a technique to photograph the pattern on the whole egg of species possessing a distinct sculpturing while still maintaining the characteristic shape of the egg to a reasonable degree. The egg to be photographed was placed in the centre of a drop of water on a clean slide covered with a piece of Parafilm. Several sources of light (from microscope lamps) were focussed from above onto the egg lying within the drop of water. By manipulating the light into appropriate positions and intensities it was possible to minimize the highlights on the surface of the egg. Using a compound microscope with a camera attachment and a 16 mm luminar objective, photographs of the chorionic pattern on the whole egg were taken on a KB 14 film. Figures 94 to 99 show the chorionic pattern on the whole egg as photographed by the aforementioned technique. These were taken by

Mr. J. Giardino under the direction of Dr. R. Brust.

Due to the shiny surface of the chorion it was not always possible to study the minute surface sculpturing by the above-mentioned methods. After proper preparation, however, details of the chorionic pattern appeared quite distinct. In order to remove the colour and make the rigid shell pliable, the chorion has to be bleached. De Coursey and Webster (1952) used dilute aqua regia to bleach the chorion. Several investigators have used chlorine solutions (Mortenson 1950; Beckel 1953; Craig 1955; Christophers 1960). Hokama and Judson (1963) have used a readily available commercial preparation, developed for use as a hair bleach. This method avoids the discomfort caused by chlorine fumes, but is very laborious. The author adopted the method described by Craig (1955) for the preparation of the chorion for microscopy. The procedure is briefly described in the following paragraphs.

The gelatinous exochorion and any other adherent particles of dirt were removed by rolling the whole egg between two pieces of filter paper. After cleaning, the egg was placed under water in the depression of a blackened spot plate and the cap was removed from the anterior end. The larva and the vitelline membrane were carefully teased out using a pair of bent <u>minuten nadeln</u>. This operation should not be carried out under alcohol because the vitelline membrane adheres to the inner surface of the endochorion. In some instances the eggs used

for recognition were not injured as they were intended for rearing work. In such cases the shells were used after eclosion of the larvae. The empty shells were washed in absolute alcohol.

To remove the blackish pigmentation of the shell and also to make it pliable, the chorion had to be bleached. The shell was transferred from the alcohol into a spot plate depression which contained a few crystals of potassium chlorate. Concentrated hydrochloric acid was poured on the crystals. The free chlorine bleached the opaque dark colour from the shell, making it appear translucent. The author found that it was best to perform this chlorination in a small square plastic container covered with a lid. This method enclosed the chlorine within the container and bleached the shell in less time. Further, there was no possibility of losing the shells by being pushed out of the solution by the bubbles of free chlorine. The bleached shell was washed free of minute crystals by immersing in concentrated hydrochloric acid. Thereafter, washing in several changes of absolute alcohol removed all acid. During the bleaching process the shell lost its brittleness and inflexibility and became soft and pliable.

The shell was removed from the absolute alcohol with the aid of a small wooden spatula and placed in a drop of euparal on a slide. With the aid of a pair of <u>minuten nadeln</u> it was possible to tear the shell down the centre and spread it into 2 sheets. A No. 2 cover

slip was brought up to the drop of euparal so that the drop ran along the glass. Then the cover slip was gradually lowered until it came to cover the chorion. By exerting a certain amount of pressure on the cover slip it was possible to flatten the preparation.

Chorionic mounts were used to study the surface features by examining the slides in transmitted light through a phase contrast microscope. The phase contrast microscope is the most suitable as it gives the impression of added depth and dimension to the facets. The optical system used for the examination, and photographing the preparations shown in Figs. 100 to 179, consisted of a Cooke's phase contrast microscope and suitable light without filters of any sort. Most of the photographs were in phase. In certain instances photographs were made by racking the condenser downwards until it was out of focus. When the condenser is lowered in this manner the point of focus is below the centre cells of the specimen and only the margins are illuminated directly.

The chorionic pattern was also observed with a dark ground illumination effect which was obtained by using the 10 X phase objective with the condenser annulus that is normally matched with the 40 X phase objective. Chorionic mounts which showed a distinct pattern in this dark ground illumination were photographed.

Images were recorded by means of a 35 mm Zeiss camera mounted on the microscope in the usual manner. The film was Kodak High Contrast Copy Film, developed in Dekktol 18. The prints were enlargements on high contrast paper.

CHAPTER VI

DESCRIPTION OF EGGS AND LARVAE

Eggs of the different species of Aedes vary according to colour, shape, size and sculpturing on the egg shell.

COLOUR

The egg when laid is an off white colour, but within a few hours the endochorion hardens and darkens. When viewed in intense, reflected, white light, the colour could appear a bluish black or purplish black as in Aedes nigromaculis, a bronze as in A vexans; a dull brownish black as seen in A canadensis or a satiny black as seen in most of the other species. Kalmus (1941) has noted that as a general rule the dark colour of eggs is an adaptation to resist desiccation.

SHAPE

The shape varies considerably in the different species. Usually they vary from being fusiform to broadly obovate. The eggs are orientated by the micropyle which is at the anterior pole. This end of the egg is generally larger. The posterior portion tapers more than the

anterior end. The curvature on the dorsal and ventral surface varies. In Aedes, the eggs have a greater curvature on the ventral surface (Horsfall 1963). Figures 1 to 93 show the different shapes of the eggs of the species encountered and also the variation that is possible within each species. A description of the shape of each species is given under the species description of eggs.

SIZE

Only fertile eggs were measured, as sterile eggs were not only much larger but also mishapen. The length of eggs used in the present study ranged from 553 to 1285 microns; and the dorsoventral diameter from 154 to 450 microns. The dimensions of the eggs examined are given in Table I.

EGG SHELL

The egg shell consists of three distinct layers. An outer, thin translucent exochorion which deteriorates with age; an intermediate hard thick and opaque endochorion; and an inner thin vitelline membrane which surrounds the yolk and growing embryo. The latter is not of any systematic value.

The exochorion has a surface sculpturing which is identical with that occurring on the endochorion. This is not surprising as both layers are secreted by the same follicular cells of the ovary and probably the exochorion is moulded over earlier formed endochorion. The

thin translucent gelatinous exochorion is readily seen in newly laid eggs. Since the exochorion is quite easily rubbed off the newly laid eggs, it is of relatively limited value in the identification of eggs.

The endochorion or egg shell is the intermediate layer. At the time of oviposition it is soft and transparent but within a few hours it is a dark opaque hard covering. The chorion bears surface sculpturing which is a more constant characteristic within each species. The chorionic pattern usually consists of a reticulation over the entire surface though it may not be quite marked on eggs of all species. The network usually consists of cells which have four to six sides. Within each facet are roundish cells. The pattern could show a variation on the shell of a single egg, depending on which portion is being examined. In Aedes cinereus the pattern at the anterior pole is quite marked and distinct and differs from that on the median or posterior section of the egg. It has also been observed that in general the cells at the anterior pole have more pronounced walls and are generally longer or smaller than the middle area of the egg. The chorionic pattern is more reliable for recognition purposes than characters based on size or shape alone.

The characters of the eggs of the twenty-seven species of Aedes found in Manitoba are described for each individual species in the following pages. In addition to the egg characteristics, the author has also recorded any deviation from the larval description as given by Carpenter and La Casse 1955 and Barr 1958. Locations from where adults were obtained for eggs, by Craig (1956) and the author in the present studies are listed in Table II.

TABLE I

MEASUREMENT OF LENGTH AND DORSO-VENTRAL DIAMETER OF EGGS OF TWENTY-SEVEN SPECIES OF AEDES IN MANITOBA

Species	No. measured	SIZE IN MICRONS LENGTH MIN	LENGTH MAX	LENGTH MEAN & S.E.	DIAMETER MIN	DIAMETER MAX	DIAMETER MEAN & S.E.	RATIO: Length to diameter
abserratus	100	784	964	883±4	257	321	280±1	3.15
barri	22	810	934	892±8	244	283	257±3	3.47
campestris	100	681	822	758±3	180	218	197±1	3.85
canadensis	30	630	758	720±4	180	206	193±4	3.73
cinereus 'A'	50	771	925	835±4	167	193	188±1	4.44
'B'	18	694	797	766±5	167	193	179±2	4.28
communis 'A'	30	732	835	804±5	257	321	285±3	2.82
'B'	100	882	989	940±4	244	321	276±1	3.40
decticus	9	694	771	732±5	193	206	194±2	3.77
	*30	684	795	718±5	213	264	234±7	3.07
diantaeus	65	835	1002	905±5	257	321	270±2	3.35
dorsalis 'A'	46	553	643	606±3	167	206	181±15	3.35
'B'	100	655	732	684±2	167	206	189±1	3.61
excrucians	100	707	977	864±5	218	283	251±2	3.44
fitchii	100	681	861	763±3	193	244	210±1	3.63
flavescens	100	655	771	704±3	244	450	279±3	2.52

Species	No. measured	SIZE IN MICRONS LENGTH MIN	MAX	MEAN & S.E.	DIAMETER MIN	MAX	MEAN & S.E.	RATIO: Length to diameter
Hexodontus 'A'	30	668	822	737±7	231	321	288±5	2.56
'B'	100	822	1015	955±4	231	308	280±2	3.41
'C'	100	951	1272	1086±7	257	321	300±2	3.62
impiger	100	720	925	812±4	244	308	263±1	3.09
implicatus	100	643	835	739±5	193	296	236±2	3.13
intrudens	100	720	861	785±3	206	283	254±1	3.09
nigripes	75	964	1285	1090±10	283	386	326±2	3.34
nigromaculis	100	630	900	778±5	193	244	217±1	3.59
pionips	5	848	912	879±13	270	283	278±4	3.16
	*30	855	940	896±6	264	316	287±3	3.12
punctor	100	797	989	879±4	231	296	260±2	3.38
riparius	100	720	925	806±7	180	257	219±2	3.68
spencerii	100	591	771	676±4	167	218	196±1	3.44
sticticus	100	617	784	678±3	206	282	245±2	2.77
stimulans	*30	840	1040	936±7	220	320	282±3	3.32
trichurus	100	732	874	800±2	360	424	388±1	2.06
triseratus	100	591	784	684±4	154	218	186±1	3.68
vexans	100	630	745	705±4	167	205	194±2	3.63

* Measurements taken from Craig (1956).

TABLE II

COLLECTING SITES FOR AEDINE EGGS DESCRIBED TO DATE

Species	Craig (1956)	Present Study
A. abserratus		Sandilands Forest Reserve
A. barri		Turtle Mt.; Duck Mt., Porcupine Mt.
A. campestris		Churchill
A. canadensis	Circle and Fairbanks in Alaska	Duck Mt., Whiteshell, Porcupine Mt., Seton Prk.
A. cinereus	Fairbanks, Chitina, Circle in Alaska, Genesee Co. Mich.	Riding Mt., Porcupine Mt., Seton Park
A. communis	Knik, Fairbanks, Chitina in Alaska, Churchill, Man.Ft. St.John, BC	Sandilands, Churchill in Man.
A. decticus	Chatanika, Circle in Alaska	Churchill
A. diantaeus	"	Flin Flon
A. dorsalis	Yolo Co, Calif., Cook Co, Ill.	Sandilands Forest Reserve
A. excrucians	Chatanika Alaska, Clearwater Co, Minn.	Sandilands, Turtle Mt., Riding Mt., Duck Mt., Churchill, Flin Flon
A. fitchii	"	Sandilands, Turtle Mt., Riding Mt., Duck Mt., Porcupine Mt., Seton Prk., Churchill, Flin Flon
A. flavescens	Knik, Alaska	Sandilands Ft. Res.

Species	Craig (1956)	Present Study
A. hexodontus	*Thompson, Pass, Umiat Alaska	Churchill, Flin Flon in Man., Baker Lake, N.W.T.
A. impiger	"	Churchill
A. implicatus	Knik, Anchorage, Chitina in Alaska, Edmonton, Alberta	Sandilands Ft. Res.
A. intrudens		Sandilands Ft. Res.
A. nigripes		Churchill, Manitoba
A. nigromaculis	Fresno Co., Calif.; Orange Co., Calif.; Davis Co., Calif.	St. Norbert
A. pionips	Chatarika, Fairbanks in Alaska	Sandilands Ft. Res.
A. punctor	* "	Sandilands, Churchill, Flin Flon
A. riparius	Fairbanks, Alaska	Sandilands, Turtle Mt., Riding Mt., Duck Mt., Churchill
A. spencerii	not recorded	Sandilands Ft. Res.
A. sticticus	Champaign Co., and Platt Co., Ill., Benton Co., Ore.	Sandilands Ft. Res.
A. stimulans	Champaign Co., Ill., Harford Co., Md.	Sandilands Ft. Res.
A. trichurus	Glenesse Co., Mich.	Sandilands Ft. Res.
A. triseratus	Orange Co., Fla. Frederick Co., Md., Montgomery Co., Alaska	Charleswood, Manitoba
A. vexans	Benton Co., Ore., Calhoun Co., Iowa, Champaign Co., Ill., Harford Co., Md.	Winnipeg, Oak Bluff, Sandilands, Riding Mt., Porcupine Mt., Seton Prk.

* identification doubtful.

AEDES (OCHLEROTATUS) ABSERRATUS (FELT AND YOUNG 1904)

(FIGS.: 1-3; 100-103)

SHAPE: Broadly fusiform to obovate, anterior pole roundedly knobbed, posterior end gradually tapering, roundedly pointed. (Figs. 1-3)

SIZE: Length: 784-964 microns, mean 883±4 microns.
Dorso-ventral diameter: 257-321 microns, mean 280±1 micron.

COLOUR: Dull black.

EXOCHORION: Thin, transparent and somewhat adherent.

CHORION: Reticulation consists of irregularly hexagonal, less commonly pentagonal cells of variable size with a subdivided surface. Cells similar to subdivision in A. excrucians or A. riparius. Walls of contiguous cells bounded by slightly raised ridges not as distinct and tortuous as some of the other species, with similar reticulations. (Figs. 100-103)

REMARKS: Distinctive features of eggs are: (1) shape (2) chorionic reticulation of irregular hexagonal cells without prominent ridges separating cells.
This species has not been reported from Manitoba earlier. It was the most abundant species in the adult collections from the Sandilands Forest Reserve.

LARVAE: Same as in Carpenter and La Casse 1955.

AEDES (OCHLEROTATUS) BARRI RUEGER 1958

(Figs. 4-6; 104-106)

SHAPE: Broadly fusiform, with ends bluntly rounded, posterior end more tapering, dorsal side only slightly less crescentic than ventral. (Figs. 4-6)

SIZE: Length: 810 - 934 microns, mean 892±8 microns.
Dorso-ventral diameter: 244-283 microns, mean 257±3 microns.

COLOUR: Dull black.

EXOCHORION: Thin, transparent, adherent.

CHORION: Reticulation indiscernible or composed of faintly visible irregularly roundish to polygonal cells with indistinct boundaries similar to the sub-division of cells found in other species. (Figs. 104-106)

REMARKS: Distinctive features are: (1) shape, (2) reticulation of faintly visible roundish cells.
This species has not been reported from Manitoba earlier. A few larvae were collected from Sandilands. Adults were collected from practically all the collecting sites, given earlier in the text, except Baker Lake. Larvae may be confused with Aedes excrucians but are readily identified by using Barr's key (1958) which includes this species.

LARVAE: Same as in Barr (1958).

AEDES (OCHLEROTATUS) CAMPESTRIS DYAR AND KNAB 1907

(Figs. 7-9; 107 and 108)

SHAPE: Elliptical, ends rounded, posterior end tapering more strongly, dorsal side, only slightly less crescentic than ventral side. (Figs. 7-9)

SIZE: Length: 681-822 microns, mean 758±3 microns.
Dorsoventral diameter: 180-218 microns, mean 197 ± 1 micron.

COLOUR: Black with a faint greyish cast.

EXOCHORION: Reticulation composed of distinct pentagonal to hexagonal cells with narrow continuous slightly undulating ridges separating cells. Cell surface studded with characteristic roundish, varibly sized, saucer shaped craters. (Fig. 108) The internal cells are similar to the cells of A. sticticus. (Figs. 107 and 108)

REMARKS: Distinctive features are: (1) shape (2) characteristic reticulation with distinct broad cells within which are saucer shaped craters.

LARVAE: Same as in Carpenter and La Casse 1955.

AEDES (OCHLEROTATUS) CANADENSIS (THEOBALD 1901)

(Figs. 10-12; 109-112)

SHAPE: Sub-fusiform, cigar shaped, with anterior pole roundedly flattened, very slightly more crescentic ventrally. (Figs. 10-12)

SIZE: Length: 630-758 microns, mean 720±4 microns. Dorso-ventral diameter: 180 - 206 microns, mean 193 ± 4 microns.

COLOUR: Brownish black.

EXOCHORION: Thin and transparent, easily rubbed off.

CHORION: Quite distinct, reticulation uniform, composed of polygonal cells. In reflected light the surface appears distinctly sub-divided with the cells separated by raised, unbroken ridges. In transmitted light cells are very prominent, pentagonal to hexagonal in shape (Fig. 110). Walls of contiguous cells tortuous, raised, unbroken ridges (Fig. 112). Each cell is sub-divided into 6-14 smaller cells, walls of which are lower than main cell walls. The walls of the sub-divisions are less distinct than similar sub-divisions in A. excrucians. (Figs. 109-112)

REMARKS: The distinctive features are (1) cigar shaped egg (2) pentagonal to hexagonal cells with raised ridges.

LARVAE: Same as in Carpenter and La Casse 1955.

AEDES (AEDES) CINEREUS MEIGEN 1818

(Figs. 13-18; 94; 113-117)

SHAPE: Two distinct shapes were observed. Both types are fusiform having a cone shaped posterior end. There is a difference in the anterior end of the two kinds. Eggs of one type have a prominently tapered anterior end. (Fig. 13-15). Eggs of the other lack such a taper, and are bluntly rounded anteriorly. (Figs. 16-18)

SIZE: (A) Eggs with tapered anterior end: (Figs. 13-15)

Length: 771 - 925 microns, mean 835 $\pm$ 4 microns.

Dorso ventral diameter: 167 - 193 microns, mean 188 $\pm$ 1 micron.

(B) Eggs with rounded anterior end: (Figs. 16-18)

Length: 694 - 797 microns, mean 766 $\pm$ 5 microns.

Dorsoventral diameter: 167 - 193 microns, mean 179 $\pm$ 2 microns.

COLOUR: Dark brownish black.

EXOCHORION: Thin and adherent.

CHORION: In both types of eggs the chorionic sculpturing is similar. In refected light a few axially linear cells at the anterior pole only are visible (Fig. 94) remainder of the surface lacks a visible reticulation. The anterior cells are separated by slightly raised unbroken ridges.

In transmitted light a distinct difference in the reticulation at the anterior, median and posterior ends, is seen.

Anterior cells (Figs. 113, 114) are quite marked, narrow, axially linear hexagons with sub-divisions within each cell of circular mounds. Walls separating these cells are conspicuous, raised and unbroken at anterior end. The median cells (Fig. 116) are slightly irregular polygonal cells with slightly raised circular mounds. The walls separating these cells are less distinct just below the anterior hexagonal cells (Fig. 115), and become indistinguishable towards the median area. Posterior cells are hexagonal, smaller than those at anterior end and with walls slightly more distinct than median cells. (Fig. 117)

REMARKS: The distinguishing features of the egg of A. cinereus are (1) characteristic axially linear hexagonal pattern clearly distinct at anterior end only. (2) shape.

LARVAE: Same as in Carpenter and La Casse 1955.

AEDES (OCHLEROTATUS) COMMUNIS (DE GEER 1776)

(Figs. 19-23; 118-119)

SHAPE: Broadly fusiform, ventral surface distinctly crescentic, a very slight curvature on dorsum in the anterior third, both ends rounded with the posterior end tapering more, and the anterior end slightly knobbed, greatest diameter slightly before centre. (Figs. 19-23)

SIZE: Two distinct sizes were recorded.

(A) Smaller sized eggs from Sandilands Forest Reserve only (Figs. 19-21)

Length: 732 - 835 microns, mean 804 $\pm$ 5 microns.

Dorsoventral diameter: 257 - 321 microns, mean 285 $\pm$ 3 microns.

(B) Larger, broadly fusiform egg from Sandilands Forest Reserve, Churchill and Flin Flon. (Figs. 22-23)

Length: 822 - 989 microns, mean 940 $\pm$ 4 microns.

Dorsoventral diameter: 244 - 321 microns, mean 276 $\pm$ 1 micron.

COLOUR: Dull black.

EXOCHORION: Thin, transparent, easily removed.

CHORION: The sculpturing on both kinds of eggs similar. Reticulation consists of small, irregular, angular polygons, major cellular network wanting, absence of distinct separating

walls. No subdivision of large cells into a network of cells comparable to other species.

REMARKS: Distinctive features are: (1) shape with the anterior knobbed region, (2) absence of major cellular network.

LARVAE: Same as in Carpenter and La Casse 1955.

AEDES (OCHLEROTATUS) DECTICUS HOWARD, DYAR AND KNAB 1917

(Figs.: 24-27; 120-122)

SHAPE: Fusiform to columnar, both ends rounded, posterior and with a greater taper, ventral surface very gently crescentic, dorsal surface almost straight, greatest diameter at anterior third. (Fig. 24-27)

SIZE: Length: 694 - 771 microns, mean 732 ± 5 microns.
Dorso-ventral diameter: 193 - 206 microns, 194 ± 2 microns.

COLOUR: Dull black.

EXOCHORION: Thin, transparent.

CHORION: Reticulation consists of polygonal cells varying in size, few cells diamond shaped. Surface of cells subdivided into irregularly roundish cells, the boundaries of cells not well defined and indistinguishable from subdivisions, latter small and angular. (Figs. 120-122)

REMARKS: This is a rare northern species, reported for the first time as present in the Province. Eggs were obtained from adults collected at Churchill, Manitoba. Craig (1955) reports that eggs of this species are "similar to A. diantaeus, A. punctor and other related species". He obtained his collection from Alaska. The author observes that of the northern species, the eggs of A. decticus could be separated on the basis of the relatively narrow

and smaller size and the network of subdivided polygonal cells. (Figs. 120-122)

LARVAE: Same as in Carpenter and La Casse 1955.

AEDES (OCHLEROTATUS) DIANTAEUS HOWARD, DYAR AND KNAB 1917

(Figs. 28-29; 123-125)

SHAPE: Fusiform to obovate, both ends broadly rounded with posterior end with a greater taper, the dorsal surface almost straight, venter very slightly crescentic, greatest diameter at anterior third. (Figs. 28-29)

SIZE: Length: 835 - 1002 microns, mean 905 ± 5 microns. Dorsoventral diameter: 257 - 321 microns, mean 270 ± 2 microns.

COLOUR: Dull black.

EXOCHORION: Thin, transparent, adherent.

CHORION: Reticulation consists of irregularly roundish to polygonal cells of variable size, with faint walls. The spaces between the rounded cells distinct. These circular cells quite unlike the saucer shaped craters of A. dorsalis. (Figs. 123-125)

REMARKS: Chorionic sculpturing very much similar to A. punctor, however, whole egg of latter is smaller in size.

LARVAE: Same as in Carpenter and La Casse 1955.

AEDES (OCHLEROTATUS) DORSALIS (MEIGEN 1830)

(Figs. 30-33; 126-127)

SHAPE: Elliptical, often bilaterally symmetrical, some may be slightly crescentic on ventral side, anterior and posterior ends bluntly rounded, but posterior end tapering slightly more than anterior end. (Figs. 30-33)

SIZE: Two sizes were recorded:

(A) Eggs obtained from a soil sample taken from near the Winnipeg International Airport. (Figs. 32 and 33)

Length: 553 - 643 microns, mean 606 $\pm$ 3 microns.

Dorsoventral diameter: 167 - 206 microns, mean 181 $\pm$ 15 microns.

(B) Eggs obtained from wild caught females from Winnipeg and Sandilands Forest Reserve. (Figs. 30-31)

Length: 655 - 732 microns, mean 684 $\pm$ 2 microns.

Dorsoventral diameter: 167 - 206 microns, mean 189 $\pm$ 1 micron.

COLOUR: Shiny black.

EXOCHORION: Very thin and easily removed.

CHORION: Cells irregularly circular, clearly defined walls wanting. The surface of all cells have conspicuous ringed, roundish craters of variable size. Roundish cells not tightly packed, intercellular spaces visible. (Figs. 126-127)

REMARKS: Distinctive features are (1) elliptical shape, (2) small size, (3) chorionic pattern has conspicuous roundish large and small craters.

LARVAE: Same as in Carpenter and La Casse 1955.

AEDES (OCHLEROTATUS) EXCRUCIANS (WALKER 1856)

(Figs. 34-36; 95; 128-131)

SHAPE: Fusiform, both ends bluntly rounded, but with posterior end tapering more and bluntly pointed, greatest diameter towards anterior third. (Figs. 34-36)

SIZE: Length: 707 - 977 microns, mean 864 ± 5 microns. Dorsoventral diameter: 218 - 283 microns, mean 251 ± 2 microns.

COLOUR: Black with a very slight greyish tinge.

EXOCHORION: Thin, distinct, closely adherent.

CHORION: In reflected light, reticulation consists of rhomboidal to polygonal cells, size variable, surface of cells subdivided into smaller polygonal cells. (Fig. 95) Surface sculpturing in transmitted light shows distinct large rhomboidal to polygonal cells, surface of which is subdivided into smaller polygonal to irregularly roundish cells. (Fig. 129) Walls of large cells, distinct and thick. Cells towards micropyle smaller than the equatorial cells. (Figs. 128-131)

REMARKS: Distinctive features are (1) slender fusiform shape, (2) distinct chorionic sculpturing visible in reflected light against a black background, (3) cells subdivided into smaller polygonal cells.

LARVAE: Same as in Barr 1958.

AEDES (OCHLEROTATUS) FITCHII (FELT AND YOUNG 1904)

(Figs. 37-39; 96; 132-135)

SHAPE: Usually sausage shaped, similar to A. stimulans, but smaller, the anterior and posterior ends are broadly rounded, the greatest diameter is slightly anterior to the middle. (Figs. 37-39)

SIZE: Length: 681 - 861 microns, mean 763 ± 3 microns. Dorsoventral diameter: 193 - 244 microns, mean 210 ± 1 micron.

COLOUR: Greyish black.

EXOCHORION: Distinct, thin, adherent, rarely broken.

CHORION: Surface sculpturing in reflected light is similar to Aedes stimulans, Characteristic uniform reticulation composed of axially linear, hexagonal cells, walls of which are distinct, raised. (Fig. 96). In transmitted light the sculpturing appears as hexagonal, less commonly pentagonal cells. Cells longer than wide, surface of cells faintly pebbled with darker cellular cells. Boundaries of walls generally straight or slightly undulating and raised. (Figs. 132-135)

REMARKS: The distinctive features of these eggs are (1) sausage shaped with bluntly rounded ends, (2) greyish black colour, (3) hexagonal reticulation with no subdivisions. Similar to A. stimulans in most respects except the latter is larger in size.

LARVAE: Same as in Barr 1958. The best character for separating A. fitchii is the meso-thoracic dorsal principal which is longer than the head. This character is stated only in Barr's key and not in Carpenter and La Casse 1955.

AEDES (OCHLEROTATUS) FLAVESCENS (MÜLLER 1764)

(Figs. 40-42; 92; 136 and 137)

SHAPE: Venter very strongly arched, more so than A. sticticus and less than A. trichurus. (Fig. 41 and 92) Dorsal surface straight or concave; the anterior end is broadly rounded while the posterior end tapers, greatest diameter slightly past middle. (Figs. 40-42; 92)

SIZE: Length: 655 - 771 microns, mean 704 $\pm$ 3 microns. Dorsoventral diameter: 244 - 450 microns, mean 279 $\pm$ 3 microns.

COLOUR: Glossy black.

EXOCHORION: Thin and transparent.

CHORION: Reticulation composed of an irregular network of small, angular, irregularly roundish cells similar to the subdivisions within cells of A. excrucians. Cells lacking a definite orientation, walls faintly visible or apparently lacking.

REMARKS: Distinctive features are (1) shape with characteristic ventral hump and tapering posterior end, (2) glossy black colour, (3) lack of distinct cell margins around group of cells.

LARVAE: As in Carpenter and La Casse 1955.

AEDES (OCHLEROTATUS) HEXODONTUS DYAR 1916

(Figs. 43-51; 138 and 139)

SHAPE: Elliptical to broadly fusiform. The small eggs from Churchill had the dorsum slightly more curved than the larger eggs from Churchill and from Baker Lake. The eggs from Baker Lake had the dorsum more or less straight, venter gently crescentic; greatest diameter at anterior third. (Figs. 43-51)

SIZE: Three distinct sizes were recorded.

'A' (from Churchill): (Figs: 43-45)

Length: 668 - 822 microns, mean 737 $\pm$ 7 microns.

Dorsoventral diameter: 231 - 321 microns, mean 288 $\pm$ 5 microns.

'B' (from Churchill): (Figs. 46-48)

Length: 822 - 1015 microns, mean 955 $\pm$ 4 microns.

Dorsoventral diameter: 231 - 308 microns, mean 208 $\pm$ 2 microns.

'C' (from Baker Lake): (Figs. 49-51)

Length: 951 - 1272 microns, mean 1086 $\pm$ 7 microns.

Dorsoventral diameter: 257 - 321 microns, mean 300 $\pm$ 2 microns.

COLOUR: Black

EXOCHORION: Thin, easily rubbed off.

CHORION: Reticulation composed of irregularly roundish to polygonal cells of variable size; cells bounded by faint angular lines. Intercellular spaces distinct. (Figs. 138 and 139)

REMARKS: Chorionic pattern similar to A. punctor and A. pionips. The large size of the egg, and the large size of chorionic cells appear to be the best characters for separating the eggs.

LARVAE: Same as in Carpenter and La Casse 1955, except that certain larvae showed a variation in the number of comb scales from that described by the above author. Comb of eighth segment had 5-11 scales in a single or an irregular double row, instead of 5-9 scales. Comb scales 5-9 for A. hexodontus and 10-19 for A. punctor were used by Carpenter and La Casse (1955) to separate larvae of the two species. Instead of A. punctor having 10-19 comb scales, larvae from Manitoba have 5-25 comb scales. Previous keys to larval identification therefore, do not separate these two species in Manitoba. However egg characters described here do separate the two species and show no species overlap.

AEDES (OCHLEROTATUS) IMPIGER (WALKER 1848)

(Figs. 52-54; 140 and 141)

SHAPE: Broadly fusiform, venter crescentic, both ends roundedly pointed, the posterior end tapers only slightly more than the anterior end. (Figs. 52-54)

SIZE: Length: 720 - 925 microns, mean 812 $\pm$ 4 microns. Dorsoventral diameter: 244 - 308 microns, mean 263 $\pm$ 1 micron.

COLOUR: Shiny black.

EXOCHORION: Thin, easily rubbed off.

CHORION: In reflected light the surface appears smooth. In transmitted light the surface consists of a mixture of variably sized, roundish craters, there is a considerable and distinct variation between the size of the small and large craters. (Figs. 140-141)

REMARKS: Distinctive features are: (1) shiny black smooth surface lacking a surface reticulation, (2) surface packed with varying sized annular craters.

LARVAE: Same as in Carpenter and La Casse 1955.

AEDES (OCHLEROTATUS) IMPLICATUS VOCKEROTH 1954

(Figs. 55-57; 142 and 143)

SHAPE: Elliptical, both ends bluntly rounded. Venter gently crescentic, greatest diameter closer to centre than most of the other species. (Figs. 55-57)

SIZE: Length: 643 - 835 microns, mean 739 ± 5 microns. Dorsoventral diameter: 193 - 296 microns, mean 236 ± 2 microns.

COLOUR: Black.

EXOCHORION: Thin and transparent.

CHORION: Reticulation composed of polygonal to hexagonal cells often appearing as irregularly rounded cells, size variable, cells bounded by faint to distinct ridges, median cells often small, hexagonal, intercellular spaces markedly distinct. (Figs. 142 and 143)

REMARKS: Distinctive features are (1) the elliptical shape, (2) shape of cells with faintly distinct ridges.

LARVAE: Same as in Carpenter and La Casse 1955.

AEDES (OCHLEROTATUS) INTRUDENS DYAR 1919

(Figs. 58-60; 144 and 145)

SHAPE: Broadly fusiform to obovate; anterior end often roundedly knobbed, posterior end gradually tapering and roundedly pointed; greatest diameter at anterior third. (Figs. 58-60)

SIZE: Length: 720 - 861 microns, mean 785 ± 3 microns. Dorsoventral diameter: 206 - 283 microns, mean 254 ± 1 micron.

COLOUR: Dull black.

EXOCHORION: Thin, transparent, easily rubbed off.

CHORION: Reticulation composed of polygonal to hexagonal cells often appearing irregularly rounded. Cells bounded by faint ridges, intercellular spaces markedly distinct. (Figs. 144-145)

REMARKS: Similar to A. implicatus. Possibly separable on the basis of larger size of whole egg and slightly knobbed anterior end.

LARVAE: Same as in Carpenter and La Casse 1955.

AEDES (OCHLEROTATUS) NIGRIPES (ZETTERSTEDT 1838)

(Figs. 61-63; 146-149)

SHAPE: Columnar to broadly fusiform, dorsum almost straight, venter crescentic, ends roundedly blunt, occasionally anterior end knobbed, posterior end tapering more strongly. (Figs. 61-63)

SIZE: Length: 964 - 1285 microns, mean 1090 ± 10 microns. Dorsoventral diameter: 283 - 386 microns, mean 326 ± 2 microns.

COLOUR: Shiny black.

EXOCHORION: Thin, transparent, rubbed off easily.

CHORION: In reflected light surface sculpturing on whole egg indistinct. In transmitted light reticulation appears as an irregular network of angular polygons. These cells are comparable to the subdivisions of the cell surface in species like A. excrucians. The cells are bounded by slightly raised unbroken ridges. (Figs. 146-149)

REMARKS: Distinct features are (1) considerably large size, (2) chorionic sculpturing of angular polygons with slightly raised ridges.

LARVAE: Same as in Carpenter and La Casse 1955.

AEDES (OCHLEROTATUS) NIGROMACULIS (LUDLOW 1906)

(Figs. 64-67; 150-152)

SHAPE: Obovoid to broadly fusiform with both ends broadly pointed, often with the anterior end having a more prominant taper, ventral surface crescentic, greatest diameter slightly anterior to middle. (Figs. 64-67)

SIZE: Length: 630-900 microns, mean 778 $\pm$ 5 microns. Dorsoventral diameter: 193 - 244 microns, mean 217 $\pm$ 1 micron.

COLOUR: Shiny purplish black to bluish black.

EXOCHORION: Distinct and easily removed.

CHORION: Reticulation is formed of irregular polygonal cells, approximately as long as wide. Within the cells the surface has numerous circular punctate regions. The walls of the cells appear raised, distinctly irregular in shape, appearing as irregular sigmoids. (Figs. 150-152)

REMARKS: The distinctive features of these eggs are (1) colour, (2) anterior end often with a nipple like taper, (3) characteristic irregular polygonal reticulation with irregular sigmoid cell walls.

LARVAE: Same as in Carpenter and La Casse 1955.

AEDES (OCHLEROTATUS) PIONIPS DYAR 1919

(Figs. 68 and 69; 153 - 156)

SHAPE: Broadly fusiform, both ends rounded, posterior end more tapering, dorsum at times slightly crescentic, generally straight, venter often slightly more crescentic, greatest diameter at anterior third. (Figs. 68 and 69)

SIZE: Length: 848 - 912 microns, mean 879 ± 13 microns. Dorsoventral diameter: 270-283 microns, mean 278 ± 4 microns.

COLOUR: Dull black.

EXOCHORION: Thin transparent.

CHORION: Reticulation composed of irregularly roundish to angular polygons comparable to the subdivisions of the cell surface of A. excrucians or A. riparius. Walls between cells more prominent than A. punctor resulting in smaller intercellular spaces. There is no evidence of arrangement of cells into a network like some of the other species. (Figs. 153-156)

REMARKS: Distinctive features are (1) shape, (2) absence of cellular network.

LARVAE: Same as in Carpenter and La Casse 1955.

AEDES (OCHLEROTATUS) PUNCTOR (KIRBY 1837)

(Figs. 70-72; 157-159)

SHAPE: Broadly fusiform with bluntly rounded ends, posterior end tapering more; most eggs with a distinct knobbed anterior; venter gently crescentic; greatest diameter at anterior third. (Figs. 70-72)

SIZE: Length: 797 - 989 microns, mean 879 ± 4 microns. Dorsoventral diameter: 231 - 296 microns, mean 260 ± 2 microns.

COLOUR: Black.

EXOCHORION: Thin, transparent, adherent.

CHORION: Reticulation composed of irregularly roundish, to polygonal cells, less commonly pentagonal. Distinct intercellular spaces; no evidence of division into a network of cells. (Figs. 157 - 159)

REMARKS: Chorionic pattern similar to A. hexodontus, and A. pionips. Generally separated from the former on the basis of smaller size and the chorionic sculpturing in A. hexodontus composed of more uniform cells, and the eggs are much less rounded. A. pionips eggs are more rounded on the ends with a much less taper than A. punctor.

LARVAE: Same as in Carpenter and La Casse 1955, except that certain larvae showed a variation in the number of comb scales from that reported by Carpenter and La Casse. The comb of the

eighth segment was reported to have 10-19 scales, but the author found the range was as low as 5 scales and as high as 25 scales. Comb scales were arranged in a single or irregular double row. The only larval character known to separate A. punctor from A. hexodontus was the number of comb scales. However, since this character is not always applicable for the two species in Manitoba, the most reliable character is the shape and the size of the egg. There was no overlap between the two species when egg characters were used.

AEDES (OCHLEROTATUS) RIPARIUS DYAR AND KNAB 1907

(Figs. 73-75; 160-163)

SHAPE: Fusiform, very much similar to A. excrucians except that the ends are less broadly rounded, greatest diameter towards anterior third. (Figs. 73-75)

SIZE: Length: 720 - 925 microns, mean 806 ± 7 microns. Dorsoventral diameter: 180 - 257 microns, mean 219 ± 2 microns.

COLOUR: Black with greyish tinge.

EXOCHORION: Thin, transparent.

CHORION: Reticulation composed of large, distinct, irregular pentagonal to hexagonal cells of variable size, surface of these cells subdivided into 7-18 smaller irregularly roundish cells, and bounded by tortuous raised ridges, the subdivisions are slightly depressed, the boundaries of the contiguous cells are irregular. (Figs. 160-163)

REMARKS: Distinctive features are (1) shape, (2) reticulation of distinct cells with tortuous raised ridges and subdivision of irregularly roundish cells.

LARVAE: Same as in Carpenter and La Casse 1955, except that certain larvae were obtained where the number of comb scales varied from 5 - 12 scales. There was also a difference in the head hairs from that described by Carpenter and La Casse. Upper frontal 5 and lower frontal 6 usually double, occasionally single or triple.

AEDES (OCHLEROTATUS) SPENCERII (THEOBALD 1901)

(Figs. 76 - 78; 164 and 165)

SHAPE: Spindle shaped, both ends broadly pointed, anterior end appearing slightly nipple like, i.e. a distinct anterior taper, venter crescentic, greatest diameter near middle. (Figs. 76-78)

SIZE: Length: 591 - 771 microns, mean 676 $\pm$ 4 microns. Dorsoventral diameter: 167 - 218 microns, mean 196 $\pm$ 1 micron.

COLOUR: Shiny black.

EXOCHORION: Thin, transparent.

CHORION: In reflected light, the surface sculpturing is indistinct. In transmitted light, the reticulation consists of irregularly roundish cells appearing as large and small shallow craters, no distinct cellular network is visible. (Figs. 164 and 165)

REMARKS: The distinctive features of these eggs are (1) distinct spindle shape, with anterior end nipple like, (2) absence of cellular reticulation.

LARVAE: Same as in Carpenter and La Casse 1955.

AEDES (OCHLEROTATUS) STICTICUS (MEIGEN 1838)

(Figs. 79-81; 166 and 167)

SHAPE: Venter of eggs strongly arched (less than A. flavescens or A. trichurus), a very slight dorsal hump, greatest diameter near centre, ends bluntly pointed. (Figs. 79 - 81; 91)

SIZE: Length: 617 - 784 microns, mean 678 ± 3 microns. Dorsoventral diameter: 206 - 282 microns, mean 245 ± 2 microns.

COLOUR: Shiny black.

EXOCHORION: Thin, transparent, easily rubbed off.

CHORION: In reflected light, surface sculpturing on whole egg is distinct. In transmitted light the reticulation consists of a patchwork of large and small shallow craters, no distinct cellular network is visible, in contrast to that seen in A. campestris. (Figs. 166 and 167)

REMARKS: The distinctive features are (1) shape with slightly humped venter, (2) size, (3) absence of cellular reticulation.

LARVAE: Same as in Carpenter and La Casse 1955.

AEDES (OCHLEROTATUS) STIMULANS (WALKER 1848)

(Figs. 82; 97; 168-171)

SHAPE: Sausage shaped, similar to A. fitchii but considerably larger, slightly more crescentic ventrally than dorsally, greatest diameter in anterior third. (Fig. 82)

SIZE: Length: 840 - 1040, mean 936 ± 7 microns.
Dorsoventral diameter: 220 - 320, mean 282 ± 3 microns.
(These measurements are taken from Craig (1956) as sufficient eggs were not obtained for measurement.)

COLOUR: Black with a greyish tinge.

EXOCHORION: Thin, quite distinct and adherent, not so easily rubbed off.

CHORION: Surface sculpturing in reflected light (Fig. 97) seen as a characteristic uniform reticulation composed of linear hexagonal cells, bounded by raised unbroken ridges. Surface sculpturing in transmitted light appears as hexagonal cells, less commonly pentagonal. (Figs. 168-171) Cells 2-3 times as long as wide, surface irregularly punctate, cell boundaries generally straight or faintly undulating, raised.

REMARKS: The striking features are (1) large size, (2) greyish black colour, (3) characteristic reticulation seen clearly also in reflected light. The only other egg it could be confused with is A. fitchii, but the latter is considerably smaller and narrower.

LARVAE: Same as in Carpenter and La Casse 1955.

AEDES (OCHLEROTATUS) TRICHURUS (DYAR 1904)

(Figs. 83-84; 93; 172-174)

SHAPE: Very broadly obovate, triangular in outline. Dorsum slightly crescentic to straight; venter very strongly arched approximately near centre; anterior end bluntly rounded, posterior end roundedly pointed; greatest diameter approximately across the middle of egg. (Figs. 83-84)

SIZE: Length: 732 - 874 microns, mean 800 ± 2 microns. Dorsoventral diameter: 360 - 424 microns, mean 388 ± 1 micron.

COLOUR: Dull black.

EXOCHORION: Thin, transparent, easily rubbed off.

CHORION: In reflected light reticulation indistinguishable, surface often very finely granular and shagreened. In transmitted light surface consists of small, very closely set angular to roundish polygons, cells resemble the subdivisions of roundish cells found in other species. (Figs. 172-174)

REMARKS: The characteristic feature of these eggs when mixed with others is the strongly arched venter or "hump". Barr (1958) reports that females could easily be confused with A. implicatus. Eggs obtained from such doubtful females could very readily be distinguished.

LARVAE: Same as in Carpenter and La Casse 1955.

AEDES (FINLAYA) TRISERIATUS (SAY 1823)

(Figs. 85-87; 98; 175-176)

SHAPE: Elongate obovate, anterior end more broadly rounded than posterior end; ventral surface slightly more crescentic than dorsal surface; greatest diameter at anterior third. (Figs. 85-87)

SIZE: Length: 591 - 784 microns, mean 684 ± 4 microns. Dorsoventral diameter: 154 - 218 microns, mean 186 ± 1 micron.

COLOUR: Dull black with brownish tinge.

EXOCHORION: Quite distinct, thick, very strongly adherent.

CHORION: In reflected light the reticulation appears as pentagonal or hexagonal cells bounded by very pronounced walls. (Fig. 98) Cells deep set. In transmitted light, cells seen as being deeply concave, nearly uniform. (Fig. 175) Cells lobulate, with 3-4 lobes; cell walls broad, strongly elevated. (Figs. 175-176)

REMARKS: Distinctive features are (1) adhesive exochorion, (2) characteristic cellular reticulation with thick set walls and lobulated cells. This is a tree hole species, reported for the first time in the Province. Adults were collected from Charleswood, Manitoba where the principal trees were oak and elm.

LARVAE: Same as in Carpenter and La Casse 1955.

AEDES (AEDIMORPHUS) VEXANS (MEIGEN 1830)

(Figs. 88-90; 177-179)

SHAPE: Variable, being ovoid to spindle shaped with a distinct taper at anterior end, (Fig. 88) great curvature on ventral side, greatest diameter between anterior third and middle, sometimes eggs appear symmetrical. (Figs. 88-90)

SIZE: Length: 630 - 745 microns, mean 705 $\pm$ 4 microns.
Dorsoventral diameter: 167 - 205 microns, mean 194 $\pm$ 2.

COLOUR: Shiny bronze.

EXOCHORION: Thin transparent.

CHORION: In reflected light, surface sculpturing distinct. (Fig. 99) Reticulation seen as longitudinal striations, composed of axially linear polygonal to hexagonal cells, walls of which are irregularly angular. In transmitted light cells polygonal to hexagonal in shape, often longer than wide; cell walls chain like, appear to be formed of 2-3 bead like rows. (Figs. 178; 179) Cell walls more prominant towards anterior pole. There are distinct circular bossed areas within the large polygonal cells. (Figs. 177-179)

REMARKS: The distinctive features are (1) shiny bronze colour, (2) distinct with axially linear cells bounded by chain like walls, (3) fusiform shape.

LARVAE: Same as in Carpenter and La Casse 1955.

CHAPTER VII

TAXONOMIC KEY TO AEDINE EGGS OF MANITOBA

This key is only for the identification of eggs of Aedine mosquitoes of Manitoba discussed in the preceding chapter.

1. Shape of egg fusiform, elliptical or columnar; venter only slightly crescentic, the ventral surface is definitely not strongly arched and lacks a ventral "hump" ..... (2)

   Shape of egg generally obovate; triangular in outline, always with venter distinctly arched or with characteristic ventral "hump" ..... (27)

2. Eggs generally spindle shaped, often with a distinct anterior taper which is often nipple like ..... (3)

   Eggs lack characteristic anterior taper; generally the anterior end is bluntly or pointedly rounded or knobbed, but not nipple like ..... (6)

3. Colour shiny bronze, never black, reticulation composed of axially linear polygonal to hexagonal cells covering entire shell surface; boundaries of cell walls often bead like. (Figs. 88-90; 99; 177-179) ..... vexans

Colour not shiny bronze; lacking reticulation described above, but if surface composed of axially linear cells, confined only to anterior pole and not entire surface ..... (4)

4. Colour purplish black to bluish black; reticulation composed of irregular polygons; cell walls highly irregular, resembling irregular sigmoids; cell surface punctate. (Figs. 64-67; 150-152) ..... nigromaculis

Colour dull black, chorionic reticulation not as described above. ..... (5)

5. Surface reticulation varying from anterior to median area; reticulation at anterior end composed of axially linear hexagonal cells with prominent cell walls; median cells of irregular polygons with less distinct walls separating them. Eggs narrow and large, length greater than 771 microns, mean 835 microns. (Figs. 13-15; 94; 113-117) ..... (in part) cinereus

Surface reticulation constant over entire surface; lacking axially linear cells at anterior pole; reticulation composed of irregularly roundish polygons appearing as large and small craters. Eggs smaller than above. Length less than 771 microns, mean 676 microns. (Figs. 76-78; 164 and 165) ..... spencerii

6. Eggs sausage-shaped or subfusiform; anterior end roundedly blunt, often lacking a knobbed anterior taper. ..... (7)

Eggs broadly fusiform, obovate or columnar; eggs occasionally with knobbed anterior taper. ..... (9)

7. Only the cells at the anterior pole distinct in reflected light as axially linear hexagonal cells with distinct walls; median area shiny in reflected light, irregular polygons with less distinct walls. (Figs. 16-18; 94; 113-117) ... (in part) cinereus

Axially linear cells through entire surface; exochorion distinct; sculpturing distinct in reflected light. (Fig. 96 and 97) .. (8)

8. Eggs narrow and smaller in size with mean length 763 microns, range from 681 - 861 microns. (Figs. 37-39; 96; 132-135) ..... fitchii

Eggs broader and considerably larger than above; mean length 936 microns; range 840 - 1040 microns. (Figs. 82; 97; 168-171) ..... stimulans

9. Chorionic sculpturing distinct to faintly distinct as pentagonal to hexagonal cells when viewed at 80 X over a black surface in reflected light ..... (10)

Reticulation not distinctly visible, when viewed as above . (13)

10. Subfusiform in shape, often cigar shaped; blackish brown in colour. ..... (11)

Fusiform in shape. Dull black with greyish tinge. ..... (12)

11. Distinct large hexagonal to pentagonal cells, longer than wide; thick tortuous raised walls; each cell subdivided into about 6-14 smaller cells with walls lower than main cell walls. Exochorion thin, non adhesive; Length: mean 720 microns, range 630-758 microns. (Figs. 10-12 ; 109-112) .. canadensis

Cells considerably smaller than above, as long as wide; each cell lobulated consisting of 3-4 lobes. (Fig. 176) Exochorion thick and adherent; Length: mean 684, range 591-784 microns. Tree hole species. (Figs. 85-87; 98; 175-176) ..... triseriatus

12. Reticulation of characteristic rhomboidal to polygonal cells separated by thick, distinct walls. Surface of large cells, subdivided into smaller irregularly roundish to polygonal cells. (Figs. 34-36; 95; 128-131) ..... excrucians

Reticulation of irregular pentagonal to hexagonal cells, often the walls separating the contiguous subdivisions thinner. (Figs. 160-163) ..... riparius

13. Dull black colour, chorion composed of group of cells similar to subdivisions in A. excrucians. No prominent walls separating groups of cells. ..... (14)

Shiny black to a dull black colour, cells not arranged as above into a definite pattern. ..... (15)

14. Narrower egg with posterior end having a greater taper. Northern species never soth of Churchill, Manitoba. (Figs. 24-27; 120-122). ..... decticus

Broadly fusiform to obovate, posterior end gradually tapers. Shape distinctly unlike above; species does occur south of Churchill, Manitoba. (Figs. 1-3; 100-103). ..... abserratus

15. Eggs with a mean length less than 800 microns ..... (16)

Eggs with a mean length greater than 800 microns ..... (20)

16. Eggs broadly fusiform, a very slight bulge on the dorsal surface of the anterior third of egg often seen; reticulation consists of polygonal cells bounded by faint angular lines; cell surface lacks roundish craters of any sort. (Figs. 43-45; 132 and 139) ..... (in part) hexodontus

Eggs elliptical with both ends bluntly rounded; if fusiform lacks a bulge on dorsum of the anterior third. ..... (17)

17. A distinct pentagonal to hexagonal reticulation, especially when viewed in a dark ground illumination (Fig. 107), cells as long as wide. Cells studded with saucer shaped craters. (Fig. 108) ..... campestris

Absence of the above described reticulation in dark ground illumination, generally pattern shows small illuminated areas around dark patches. (Figs. 123; 126) ..... (18)

18. Eggs smaller, less than 700 microns; elliptical, often may appear bilaterally symmetrical. Reticulation consists of roundish craters of variable size, clearly defined walls wanting. (Figs. 30-33; 126-127). ..... dorsalis

Eggs larger, greater than 700 microns, elliptical to broadly fusiform. Reticulation not composed of roundish craters. .. (19)

19. Elliptical in shape, both ends bluntly rounded, eggs generally lack a knobbed anterior, eggs generally smaller, mean length 739 microns range 643-835 microns. (Figs. 55-57; 142 and 143) ..... implicatus

Broadly fusiform; posterior end gradually tapers: anterior end roundedly knobbed; eggs larger, mean length 785 microns, range 720-861 microns. (Figs. 58-60; 144 and 145) ..... intrudens

20. Surface of chorion consists of a mixture of variably sized roundish craters, distinct difference between the small and large craters. (Figs. 52-54; 140 and 141) ..... impiger

Chorionic sculpturing of irregularly roundish polygons of approximately equal size, definitely no difference in cells and no small and large craters as above. ..... (21)

21. Chorionic pattern indistinct, faintly visible as roundish polygons in transmitted light. (Figs. 4-6; 104-106) ..... barii

Chorionic pattern distinct of irregularly roundish to polygonal cells in transmitted light. ..... (22)

22. Very large shiny black egg, mean length greater than 950 microns. ..... (23)

Generally not as large as above, mean length less than 950 microns. ..... (24)

23. Reticulation of polygonal cells (Fig. 146) similar to subdivisions of A. excrucians. Cells bounded by raised unbroken ridges. (Figs. 61-63; 146-149) ..... nigripes

Reticulation of polygonal cells, no distinct walls separating cells. Distinct narrow ridges seen connecting adjacent cells. (Figs. 46-51; 138-139) ..... (in part) hexodontus

24. Eggs broadly fusiform often with anterior end roundedly knobbed. ..... (25)

Eggs broadly fusiform with both ends bluntly rounded generally lack a roundedly knobbed anterior. ..... (26)

25. Reticulation consists of small, irregular, angular polygons. (Figs. 19-23; 118 and 119) ..... communis

Reticulation consists of small irregularly roundish polygons with faint walls. (Figs. 28 and 29; 123 - 125) ..... diantaeus

26. Narrower egg, mean dorsoventral diameter 260 microns, range 231 - 296 microns. Posterior end with a greater taper. (Figs. 70-72) ..... punctor

Broader egg, mean dorsoventral diameter 278 microns, range 270-283 microns. Posterior end more rounded than above. (Figs. 68 and 69) ..... pionips

27. Very broadly obovate, triangular in outline (Fig. 84) dorsum generally straight, strongly humped venter, mean diameter 388 microns. (Figs. 83-84; 93; 172-174) ..... trichurus

Eggs not so broadly obovate or triangular in shape venter not as humped as above ..... (28)

28. Venter arched, generally slightly past middle (Fig. 40; 92) mean diameter 279 microns, dorsal surface straight, more often concave, posterior end tapers more. Glossy black. (Figs. 40-42; 92; 136 and 137) ..... flavescens

Venter not so distinctly arched, slight dorsal hump, greatest diameter near centre; mean diameter 245 microns; shiny black colour. (Figs. 79-81; 91; 166 and 167) ..... sticticus

PICTORIAL KEY

TO AEDINE EGGS OF MANITOBA USING PREPARED SLIDES OF CHORION

1a — Shape fusiform or elliptical or columnar. No ventral hump.

1 —

1b — Shape obovate, triangular in outline. Venter "humped". → (27)

2a — Spindle shaped with anterior tapered, often nipple like.

2 —

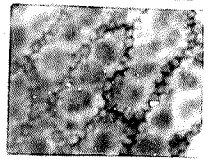
2b — Anterior end bluntly or pointedly rounded or knobbed, not nipple like.

3a — Colour shiny bronze. Reticulation axially linear over entire surface; walls bead like.

3 —

3b — Colour not shiny bronze.

VEXANS



6a — Sausage shaped. Anterior roundedly blunt, lacking knobbed anterior.

6 —

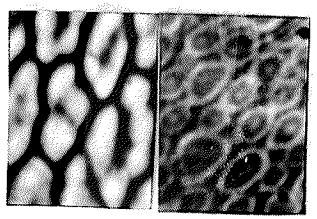
6b — Broadly fusiform, obovate, columnar, occasionally knobbed anterior. → (9)

7a — Only cells at anterior end axially linear.

7 —

7b — Axially linear cells over entire surface. Exochorion distinct. → (8)

CINEREUS (in part)



4a — Colour purplish black to bluish black. Reticulation of irregular polygons; walls irregular sigmoids.

4 —

4b — Colour dull black; Reticulation unlike other.

(4b)

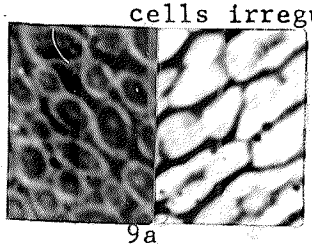
(6b)

(7b)

5a — 5 — 5b

8a — 8 — 8b

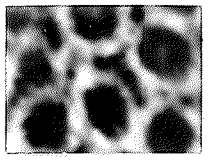
Anterior cells only axially linear. Median cells irregular polygons.



CINEREUS (in part)

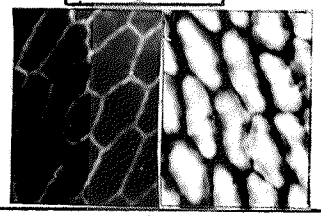
Smaller egg. Reticulation uniform over entire surface, composed of irregularly roundish polygons.

SPENCERII



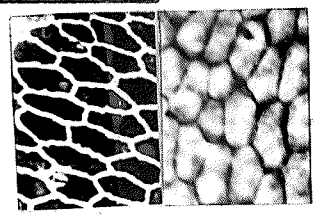
Eggs smaller and narrower. mean length 763 μ. Range: 681-861 μ.

FITCHII



Eggs broader and larger. mean length 936 μ. Range: 840-1040 μ.

STIMULANS



9a — 9 — 9b

Chorionic sculpturing distinct when viewed in reflected light against a black background.

Chorionic sculpturing not distinctly visible when viewed in reflected light against a black background.

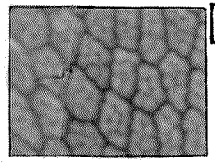
10a — 10 — 10b

Eggs sub-fusiform, "cigar shaped". Blackish brown tinge.

Eggs fusiform. Dull black with greyish tinge.

11a — 11 — 11b

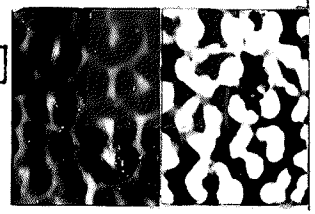
Distinct hexagonal to polygonal cells, longer than wide; each cell sub-divided into 6-4 cells; cells separated by thick tortuous walls. Larger egg, mean 720 μ.



CANADENSIS

Lacks distinct hexagonal to polygonal cells; as long as wide; cells lobulated consisting of 3-4 lobes, smaller egg, mean 684 μ. Tree hole species.

TRISERIATUS



(12)

(13)

(10b)

(9b)

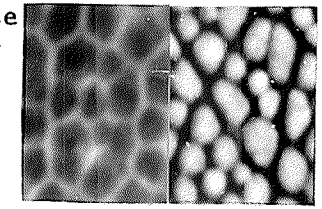
12a

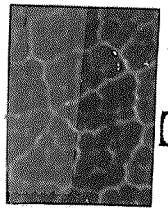
12

12b

Rhomboidal to polygonal colls. Separated by thick distinct walls. Surface subdivided into smaller roundish polygons.

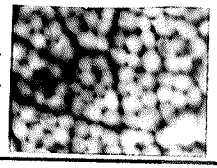
EXCRUCIANS





Irregular pentagonal to hexagonal cells. Walls separating continuous subdivisions thinner.

RIPARIUS



13a

13

13b

Dull black. Reticulation composed of group of cells equivalent to subdivisions of A. excrucians; no prominent wall separating group.

Shiny black to dull black; cells not arranged as the other.

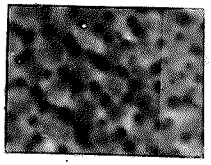
14a

14

14b

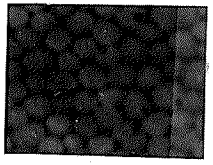
Width of egg narrower mean 194 μ; Posterior end tapers more; Northern species, only North of Churchill.

DECTICUS



Width broader, mean 280 μ; Posterior end with a gradual taper. Species occurs south of Churchill.

ABSERRATUS



15a

15

15b

Eggs small; mean length less than 800 μ.

Eggs large; mean length greater than 800 μ.

(16)

(20)

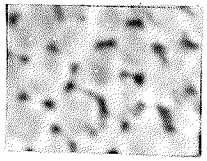
(15a)

16a — 16 — 16b

Broadly fusiform; slight bulge on dorsal surface in anterior third of egg; reticulation polygonal, cells bounded by angular lines. Lacks roundish craters.

Elliptical. Both ends bluntly rounded. If fusiform lacks dorsal bulge.

HEXODONTUS (in part)

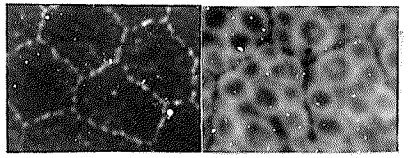


17a — 17 — 17b

Distinct reticulation of hexagonal to pentagonal cells when viewed in dark ground illumination; cells long as wide; cells studded with saucer shaped craters.

Absence of reticulation in dark ground illumination. Pattern shows as small illuminated areas around dark patches.

CAMPESTRIS

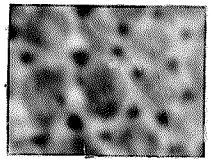


18a — 18 — 18b

Small egg, less than 700 μ; elliptical, Reticulation of roundish craters.

Eggs larger, greater than 700 μ. Reticulations do not show roundish craters.

DORSALIS



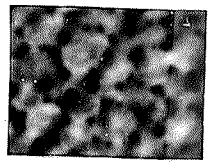
(18b)

19a — 19 — 19b

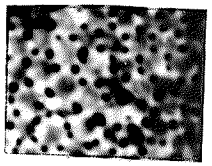
Elliptical; both ends bluntly rounded, absence of knobbed anterior; eggs smaller than other; mean 739 μ, range 643-835 μ.

Broadly fusiform; posterior with a greater taper, usually with knobbed anterior; eggs larger, mean 785 μ, range 720-861 μ.

IMPLICATUS



INTRUDENS

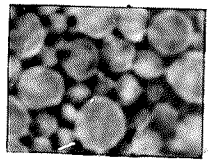


(15b)

20a — 20 — 20b

Reticulation composed of a mixture of variably sized roundish craters. There is a distinct difference in size of cells.

IMPIGER

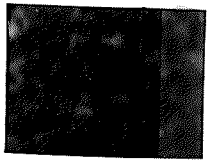


Reticulation composed of irregularly roundish polygons of approximately equal size. Absence of small and large craters.

21a — 21 — 21b

Pattern indistinct; faintly visible roundish cells.

BARRI



Distinct pattern of irregular roundish to polygonal cells.

22a — 22 — 22b

Very large, shiny black eggs. Mean length greater than 950 μ.

Not as large, mean length less than 950 μ.

(23)

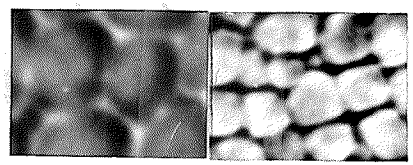
(24)

(22a)

23a — 23 — 23b

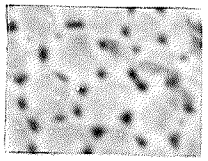
Polygonal cells, bounded by raised unbroken ridges.

NIGRIPES



Polygonal cells, no distinct walls separating cells, narrow ridges seen connecting adjacent cells.

HEXODONTUS (in part)



(22b)

24a — 24 — 24b

Broadly fusiform; generally anterior end roundedly knobbed.

Broadly fusiform; anterior end lacks rounded knob.

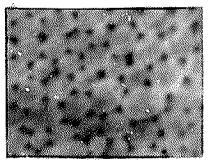
25a — 25 — 25b

Small irregular angular polygons.

COMMUNIS

Small irregular roundish polygons.

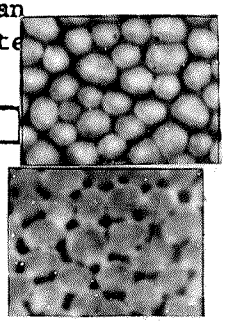
DIANTAEUS



26a — 26 — 26b

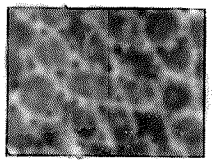
Width of egg narrower; mean mean diameter 260 μ. Poste end with a greater taper.

PUNCTOR



Width of egg greater than other, mean diameter 278 μ. Posterior end more rounded.

PIONIPS

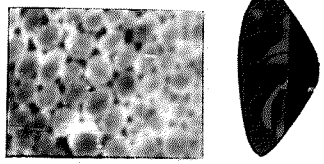


(1b)

27a — 27 — 27b

Very broadly obovate; triangular in outline, dorsum generally straight, venter strongly humped. mean diameter 388 µ.

TRICHURUS

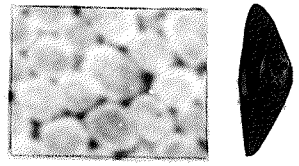


Eggs not so broadly obovate or triangular; Venter not as humped.

28a — 28 — 28b

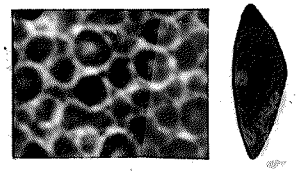
Venter arched, slightly past middle, dorsal surface straight more often concave, posterior end with distinct taper; mean diameter 279 µ; colour glossy black.

FLAVESCENS



Venter not so distinctly arched, slight dorsal hump, greatest diameter near centre, mean diameter 245 µ; colour shiny black.

STICTICUS



CHAPTER VIII

DISCUSSION

The increasingly widespread use of egg characters as an index of specific differences in the genus Anopheles gave a special interest to the study of egg variation. It does seem incredible that the egg stage should show such remarkable specific distinctions, since we generally expect the specific characters to develop late in the ontogenetic history of an individual. The egg shell, however, is a character of the mother. Its structure and pattern depend on the follicular cells in the ovary, which are responsible for the secretion of the distinctive layers of the shell. Differences in the chorionic pattern would therefore reflect differences in that part of the ovary, and hence differences in the species, in the same way that patterns of hairs or scales on the body of insects would vary according to species. The present observations indicate that there is a wide variation in the size and shape of the egg and pattern on the chorion, and hence we could infer that the ovarian follicles of aedine mosquitoes vary widely in these respects.

The descriptions and illustrations given in the present study clearly indicate that the eggs of aedine mosquitoes bear characteris-

tics for differentiation into species.

From measurements of the whole egg it is seen that each species has a typical mean, and range in length and width (dorsoventral diameter). However, as indicated in Table I the minimum and maximum vary greatly from the mean for any one species. In most of the species discussed, eggs were obtained from adults collected from more than one location except for A. decticus and A. pionips where eggs were obtained from a single female. Sufficient eggs of A. stimulans were not obtained, and the measurements given therein are from Craig (1956). The two A. stimulans eggs that were measured conformed to these dimensions. Care should be taken in the interpretation of size and shape for diagnostic purposes. Variation in the size is dependent upon locality, nutrition of the parent, oviposition, and numerous other factors. Laboratory studies on the effect of nutrition on egg formation would be helpful.

Shape of the egg aids in the specific differentiation to a very marked extent. Shape of eggs is distorted if eggs are infertile. The ratio of the length to the dorsoventral diameter is beneficial in certain instances. Narrower eggs have higher ratios and broader eggs lower ratios. A. cinereus has a ratio of 4.2 to 4.4, and A. trichurus a ratio of 2.06. These could be easily separated from the other species when studied solely on this basis. This ratio has been calculated from mean measurements and it could vary considerably when compared with single eggs. However, this ratio is less subject to variation than mean dimensions

alone because the variation in length and dorsoventral diameter are usually correlated.

For positive identification the finer detail of the chorionic sculpturing provides the best characteristics for separation into species. Figs. 100-179 indicate the wide variation in the chorionic reticulation depending upon the species. These characters are best observed in transmitted light using a phase contrast microscope.

In the present study in four species, namely A. cinereus, A. communis, A. dorsalis, and A. hexodontus, there was a significant difference within each species, in relation to the size and, or, shape of eggs. Two distinct types of A. cinereus eggs were observed, one type with a tapered anterior pole and the other with a roundedly blunt anterior pole. The former was also the larger egg. A. dorsalis and A. communis also showed two distinct sizes with a slight variation in shape as well. A. hexodontus showed three distinctly different sizes. Within each of these species the different sizes of eggs were statistically significant at the 5% level. However, there was no difference in the chorionic sculpturing among these different sizes of eggs. A distinct geographical variation in the size of egg was seen in A. hexodontus. In this species the eggs obtained from adults collected at Baker Lake in the North West Territories, were considerably larger (mean 1086 microns; range 951-1272 microns) than those collected at Churchill where 2 sizes were observed with means 737 microns and 955

microns. The samples of A. hexodontus obtained from Churchill and Baker Lake are conspecific, but as size of eggs are significantly different it may be an indication of subspeciation and these populations could be considered to be allopatric species. The smaller A. communis eggs were obtained only from certain adults collected in the Sandilands Forest Reserve. None of the adults obtained from Flin Flon or Churchill laid such eggs. Biometric studies on the relationship between geographic variation and size are needed before mean dimensions can have great taxonomic significance.

An analysis of the specific characters indicates that eggs of species examined in the present studies generally fall into categories which correspond to the system of classification proposed by Edwards (1932), which was based largely on characters of the female genitalia. Of the four sub-genera of Aedes covered in this study, three of them have only a single species recorded. The remaining twenty-four species belong to the sub-genus Ochlerotatus. The three species A. triseriatus, A. vexans and A. cinereus which belong to the sub-genera Finlaya, Aedimorphus and Aedes respectively, show a chorionic sculpturing which is quite distinct from each other and from those species in the sub-genus Ochlerotatus. In the sub-genus Ochlerotatus there is a variation among the groups. Of the species reported herein and belonging to the sub-genus Ochlerotatus, only one species belongs to group A. Eggs in this species, A. nigromaculis, are more distinctive and are more readily characterized from the eggs of species belonging to the groups B, E, G, and H. Group H

of Ochlerotatus may be separated on the basis of the sharply "humped" venter of the egg. Group B of Ochlerotatus consists of females which are very much similar and are difficult to separate as adults. The characters of eggs of species in this group indicate natural affinities. For example, the similarity of adults of A. stimulans and A. fitchii is reflected in similar eggs. It is of interest that A. barri where the larvae are separable from A. excrucians primarily by the spine on each ventral valve of the siphon, has a distinct and readily separable egg. Similarly in the case of A. excrucians A. fitchii and A. stimulans, where adult females are only poorly separable on the shape of the tarsal claw, these species could be separated readily by their distinct eggs. In general A. barri and flavescens have chorionic detail unlike the others of this group and this would indicate that neither species belongs in group B. A. canadensis, which is placed in group E of Edward's classification, has a chorionic reticulation quite unlike that of A. campestris or A. dorsalis which also belong to this group. The latter two, however, are very closely related in the chorionic sculpturing. The A. canadensis reticulation strongly resembles that of A. excrucians and related species and would best fit into group B. Hence by using egg characters in addition to larval and adult characters, species within the sub-genus Ochlerotatus could be more suitably grouped than was previously done using adult characters only.

There is great similarity between larvae of A. vexans and A. intrudens, as well as between A. sticticus and A. flavescens. These could,

however, be separated by an examination of eggs which are quite distinct. Similarly, larvae of A. hexodontus and A. punctor cannot be separated using the key by Carpenter and La Casse (1955). However, the egg characters of the two species are quite distinct.

The egg characters and the taxonomic key discussed herein are adequate for the use of investigators and sanitarians in the identification of eggs obtained in field collections or from the laboratory. Also of taxonomic significance is the fact that phylogenetic relationships within the genus can be more easily mapped using the egg characters illustrated and described herein.

CHAPTER IX

SUMMARY

1. Several workers have reported that eggs of mosquitoes possess certain important characters for segregating them into smaller categories. Most of the work done so far has been on the genus Anopheles. A few isolated descriptions of Aedes eggs have been reported, but they are not of a comparative nature. The work done by Craig (1956) has enabled the arrangement of aedine eggs into groups of less than generic rank. His work deals chiefly with the species collected from the U.S. and Alaska.

2. Ecologists, physiologists, sanitarians, and other investigators have been greatly handicapped by their inability to recognise the Aedes species in the egg stage, the stage in which these species spend most of their lives. In order to obtain a rapid and dependable means of recognizing aedine eggs and also to elucidate systematic relationships, eggs of the Aedes species in Manitoba have been examined to discover characters for specific differentiation.

3. With the development of newer methods for procurement and detailed examination of aedine eggs it has been possible to ascertain

characters sufficient for specific separation. Eggs were obtained from (a) wild caught females, each of which oviposited on separate pads of cheesecloth, (b) soil samples. Eggs were positively identified to species by hatching the conditioned eggs, and rearing to fourth instar larvae.

4. In the present studies, whole eggs have been observed, measured and illustrated by reflected light. A new technique, for photographing the chorionic pattern on the whole egg of a few species, was used. Fragments of cleared chorion have been observed and photographed by using a phase contrast microscope and transmitted light.

5. Illustrations and descriptions of twenty-seven species of Aedes recorded in Manitoba are presented in this work. Eggs differ among the species according to colour, size, shape and chorionic pattern. The latter is of particular assistance in segregating the species. A taxonomic key to the identification of Aedes species in Manitoba is presented.

6. Analysis of the specific characteristics indicates that eggs of Aedes fall into categories similar to the arrangement of groups in the phylogenetic system of classification of Edwards (1932). His system is based largely on adult female characters. The egg

characteristics also appear to indicate certain phylogenetic relationships of aedine mosquitoes, however, classification by eggs indicates certain exceptions to the system of Edwards. Species may be more suitably regrouped taking into consideration adult, larval and egg characteristics.

7. In four species, namely A. cinereus, A. communis, A. dorsalis and A. hexodontus, there was a significant difference in relation to different sizes and or shapes within each respective species. This may be an indication of sub-speciation.

8. Four species, A. abserratus, A. barri, A. decticus and A. triseratus, have not previously been reported from Manitoba.

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APPENDIX I

(Figs. 1 - 187)

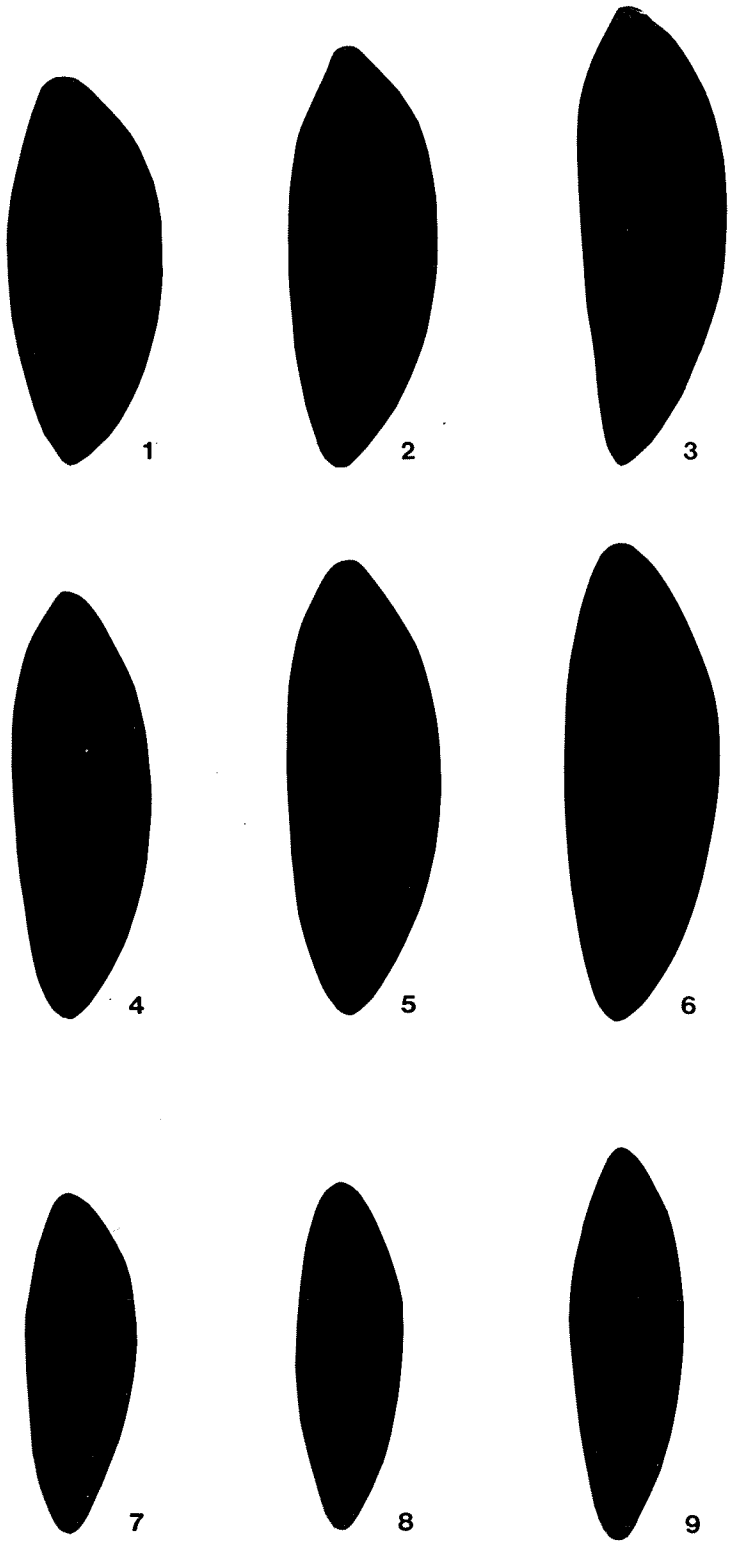
Figures 1 - 93.

Outline drawings of dorsoventral aspect of eggs of species of Aedes in Manitoba as seen with a stereo-microscope and a Zeiss drawing apparatus. Anterior pole towards top of the page. Dorsal aspect towards the left. All species drawn to scale.

Figures 1 - 3 *A. abserratus*

Figures 4 - 6 *A. barri*

Figures 7 - 9 *A. campestris*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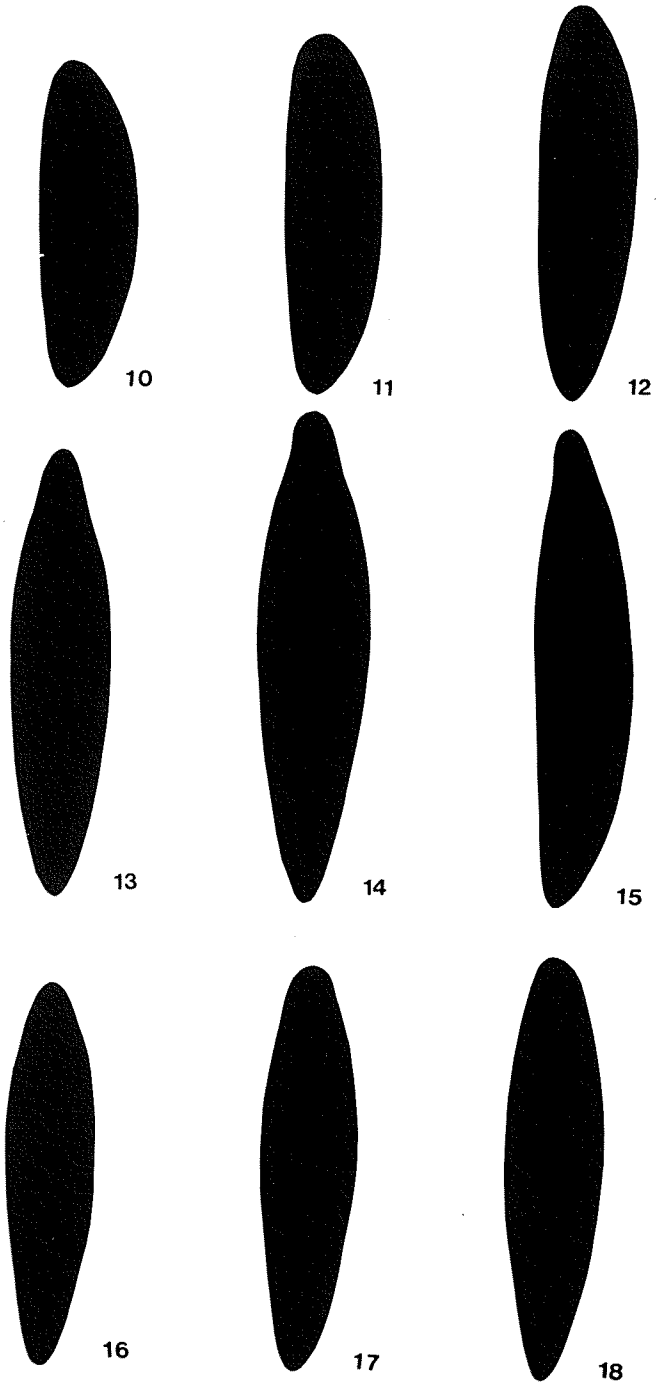
Figures 10 - 12 *A. canadensis*

Figures 13 - 15 *A. cinereus*
(Tapered anterior pole)

Figures 16 - 18 *A. cinereus*
(Rounded anterior pole)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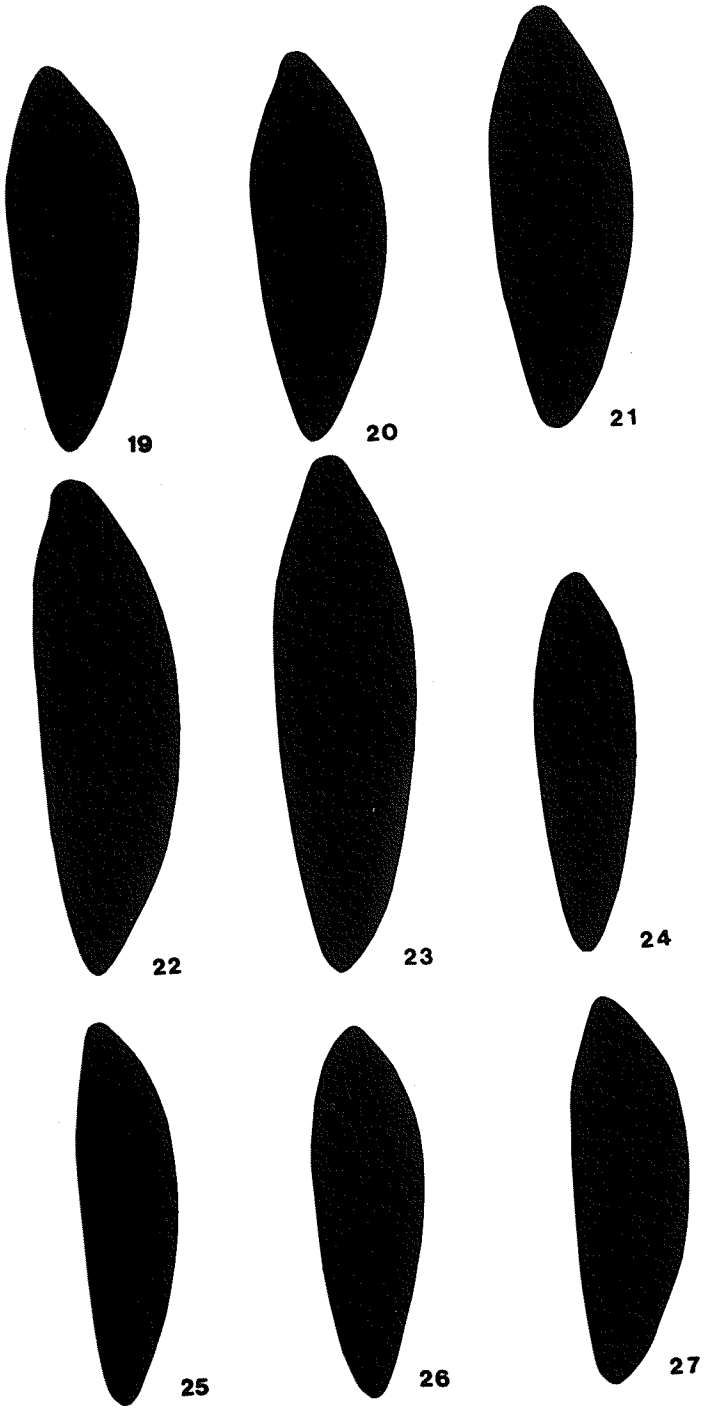
Figures 19 - 23 *A. communis*

Figures 24 - 27 *A. decticus*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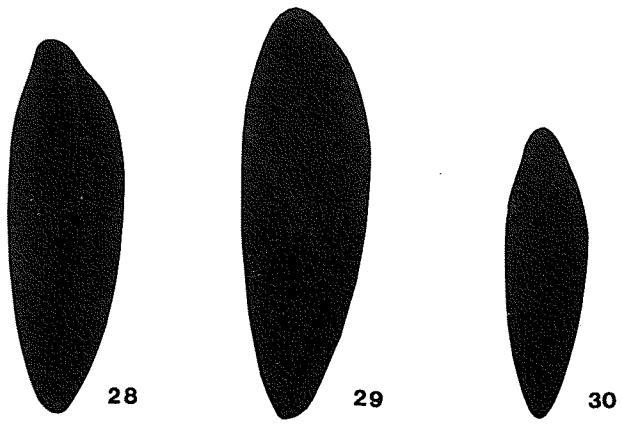
Figures 28 - 29 *A. diantaeus*

Figures 30 - 31 *A. dorsalis*

Figures 32 - 33 *A. dorsalis*

Figures 34 - 36 *A. excrucians*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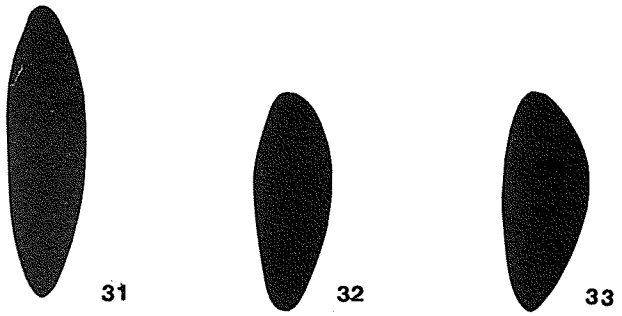
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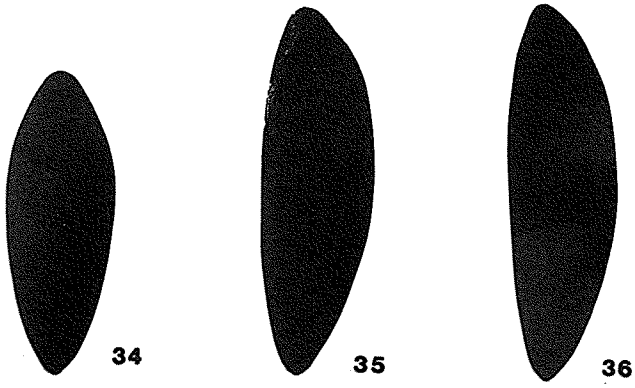
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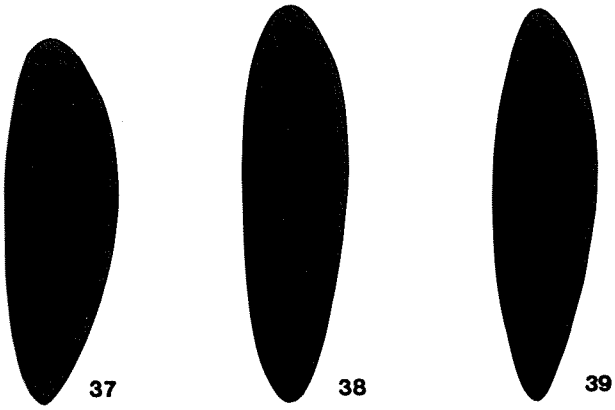
Figures 37 - 39 A. fitchii

Figures 40 - 42 A. flavescens

Figures 43 - 45 A. hexodontus

(From Churchill)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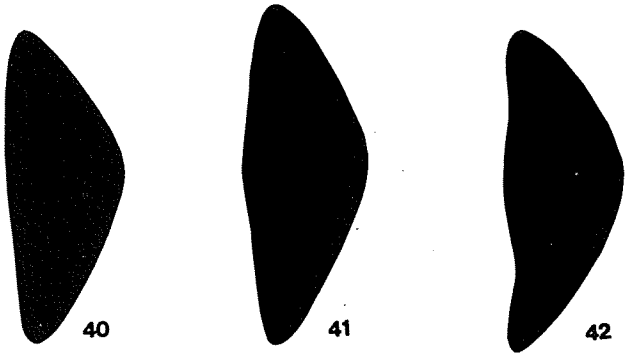
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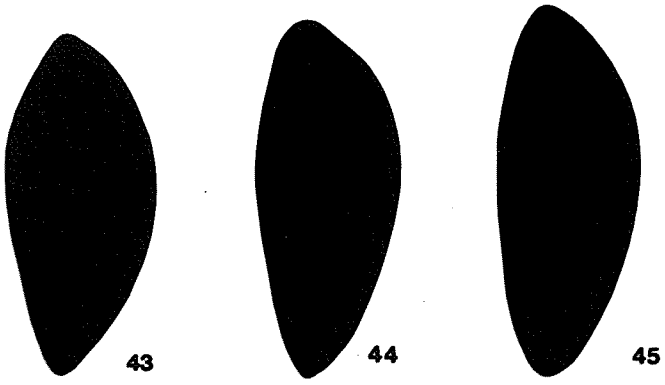
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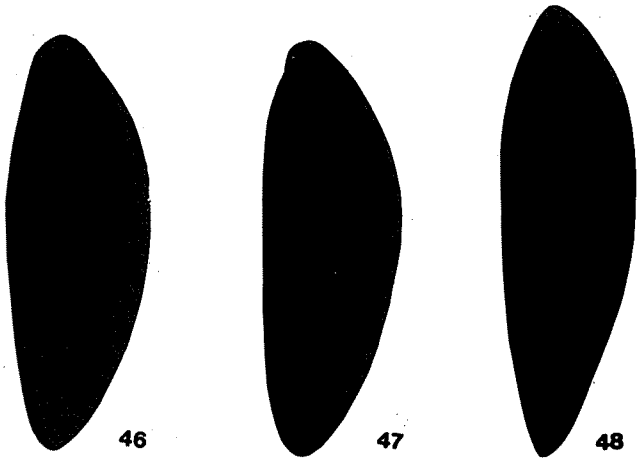
Figures 46 - 48 *A. hexodontus*

Figures 49 - 51 *A. hexodontus*



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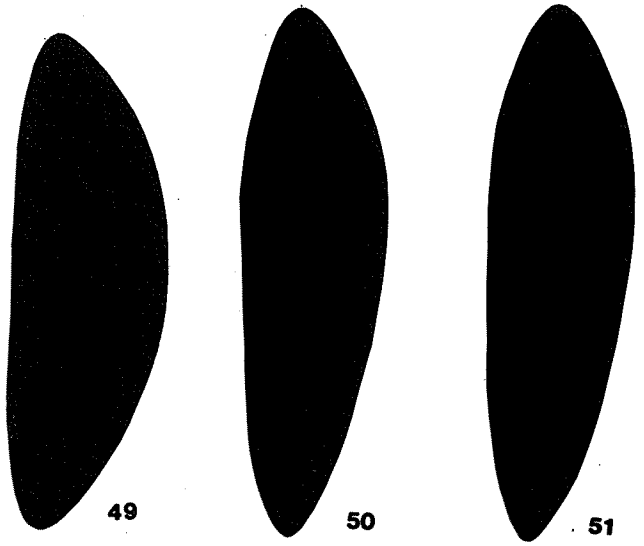

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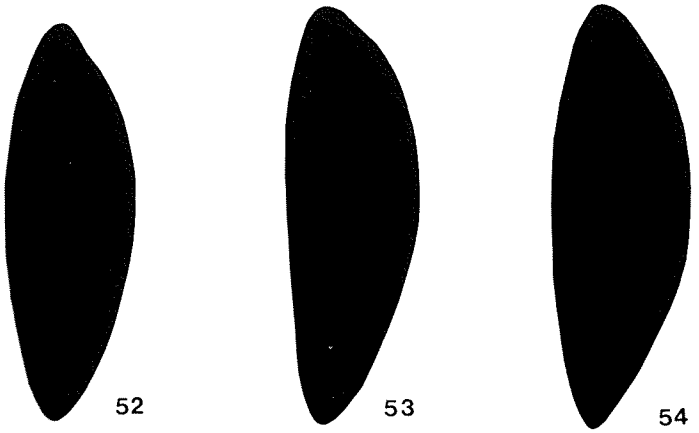
Figures 52 - 54 *A. impiger*

Figures 55 - 57 *A. implicatus*

Figures 58 - 60 *A. intrudens*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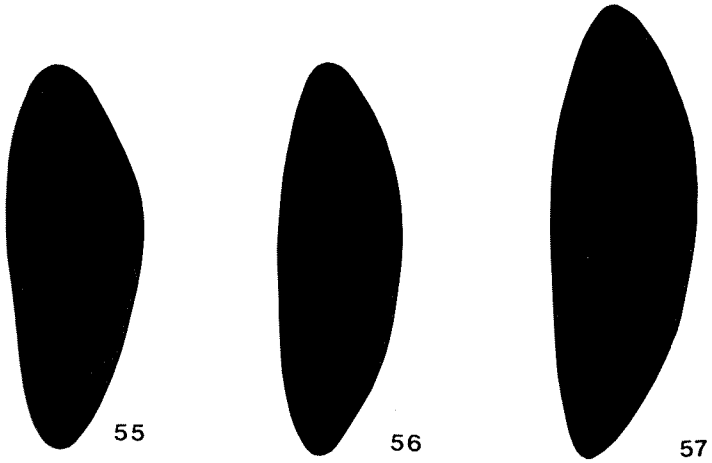
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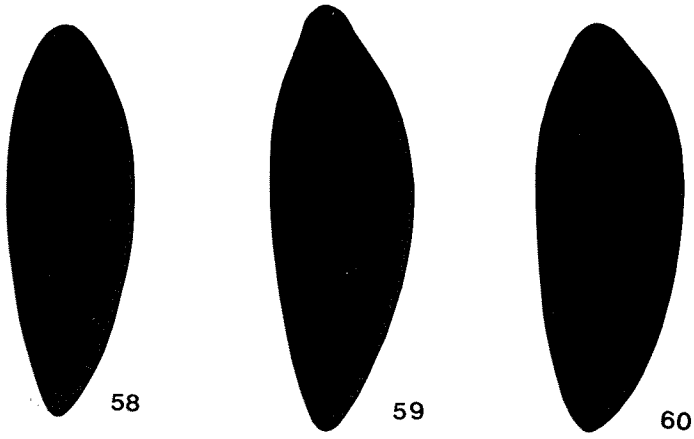
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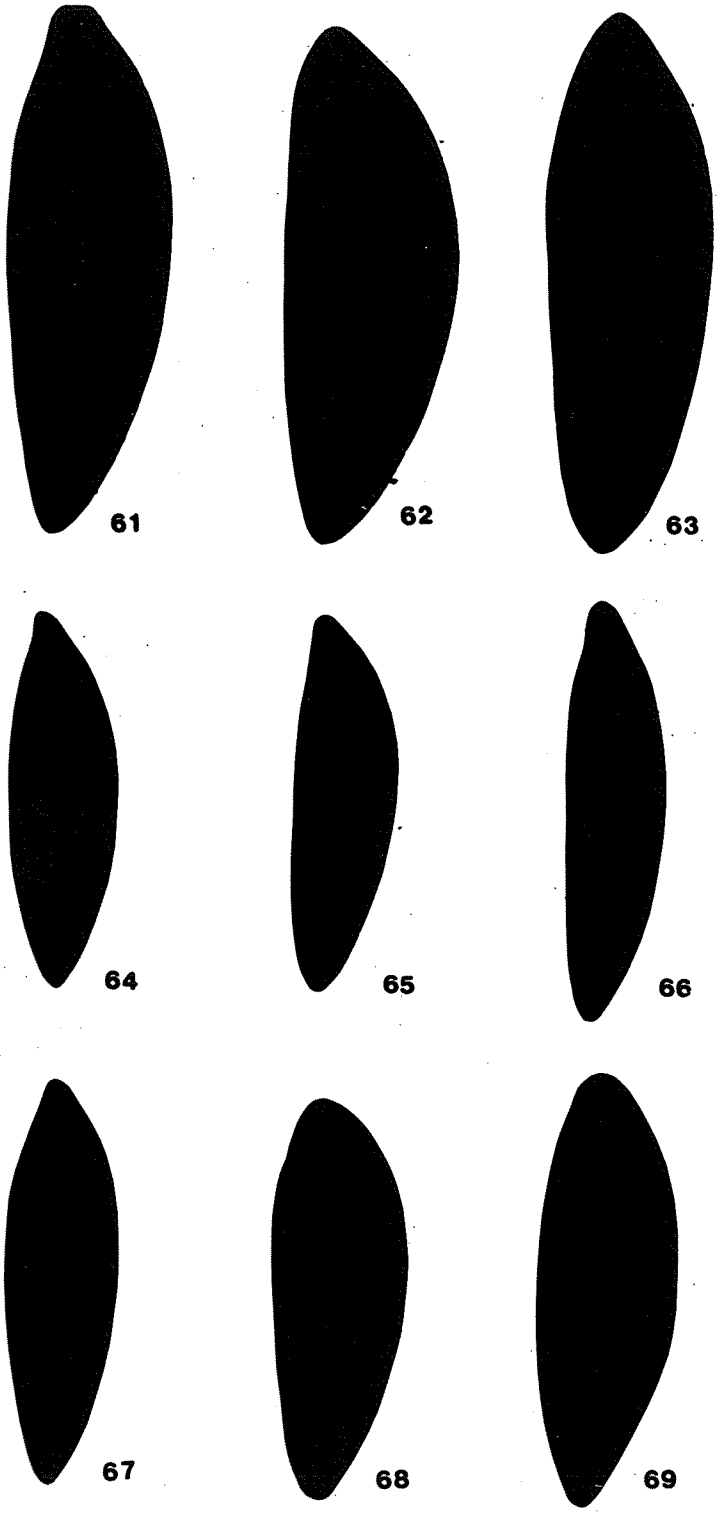
Figures 61 - 63 *A. nigripes*

Figures 64 - 67 *A. nigromaculis*

Figures 68 - 69 *A. pionips*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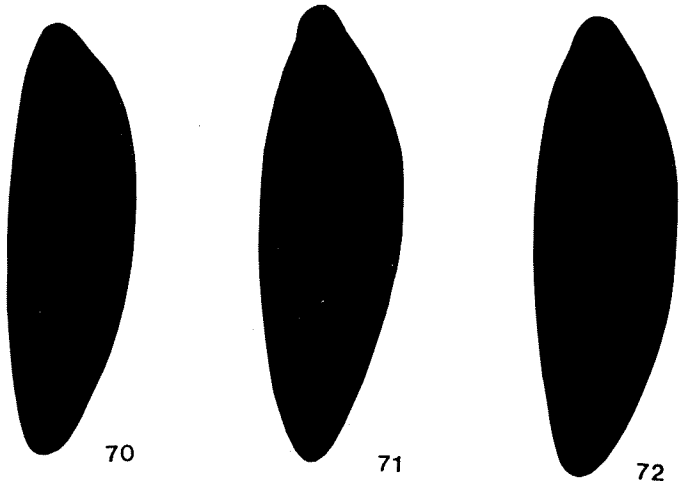
Figures 70 - 72 *A. punctor*

Figures 73 - 75 *A. riparius*

Figures 76 - 78 *A. spencerii*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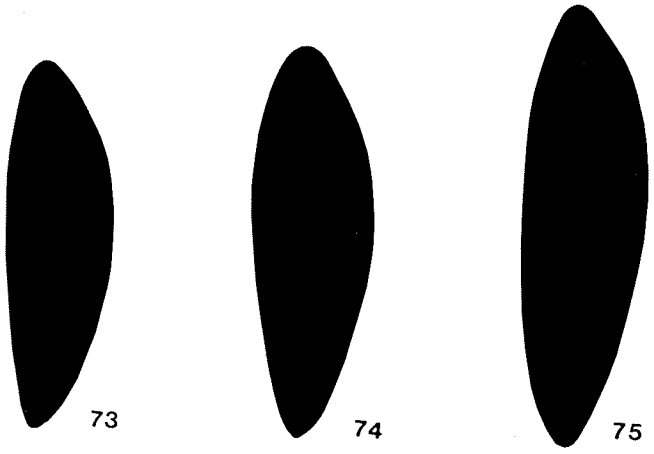
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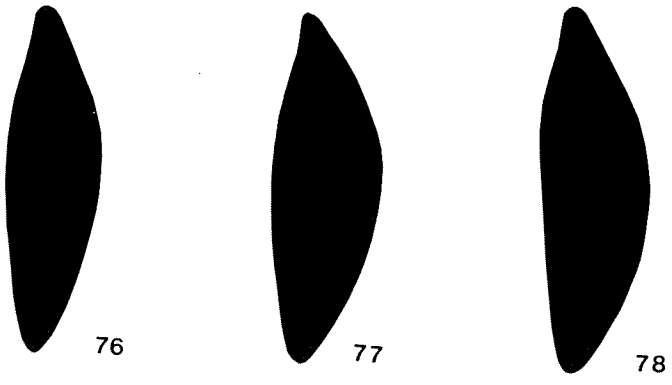
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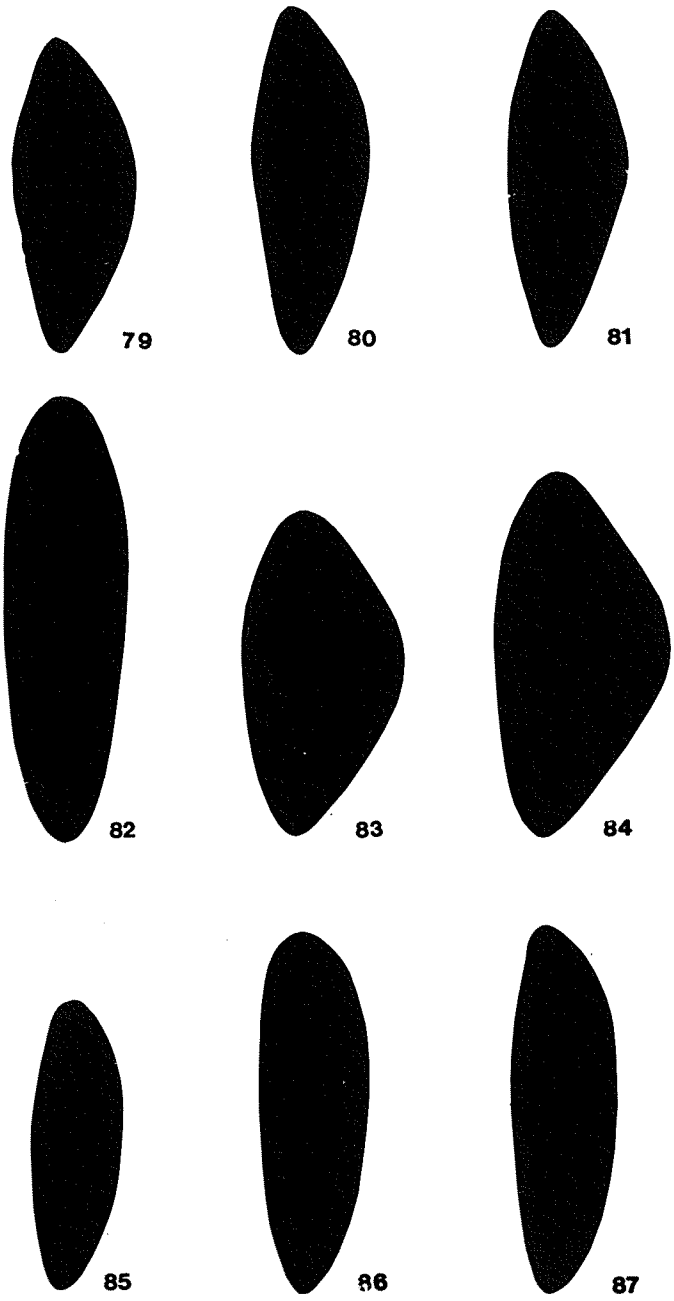
Figures 79 - 81 *A. sticticus*

Figures 82 - *A. stimulans*

Figures 83 - 84 *A. trichurus*

Figures 85 - 87 *A. triseratus*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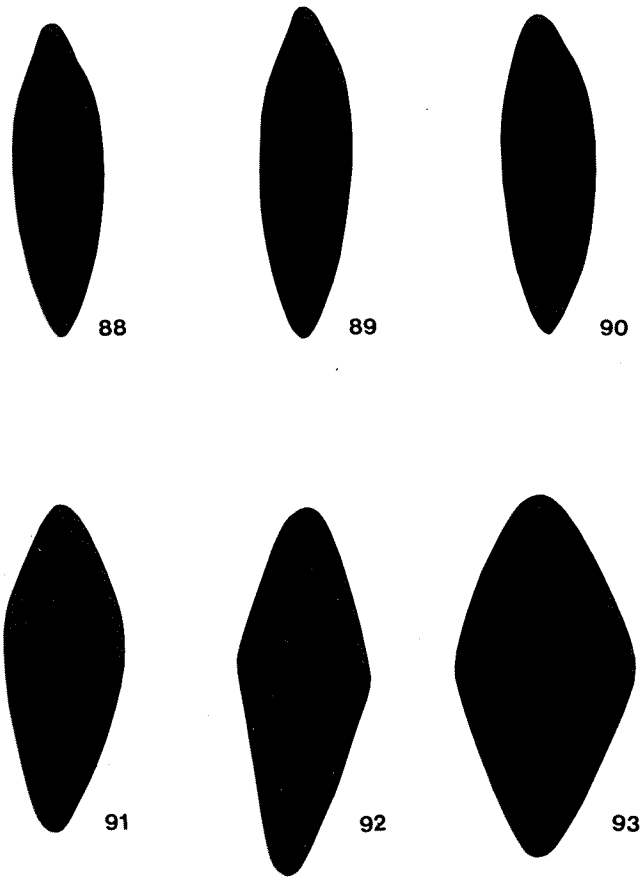
Figures 88 - 90 *A. vexans*

Figures 91 - 93 Lateral aspect of eggs of three species of *Aedes* in Manitoba.

Figures 91 *A. sticticus*

Figures 92 *A. flavescens*

Figures 93 *A. trichurus*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Figures 94 - 99 Photographs of whole egg showing chorionic sculpturing on surface.

Figure 94 *A. cinereus*

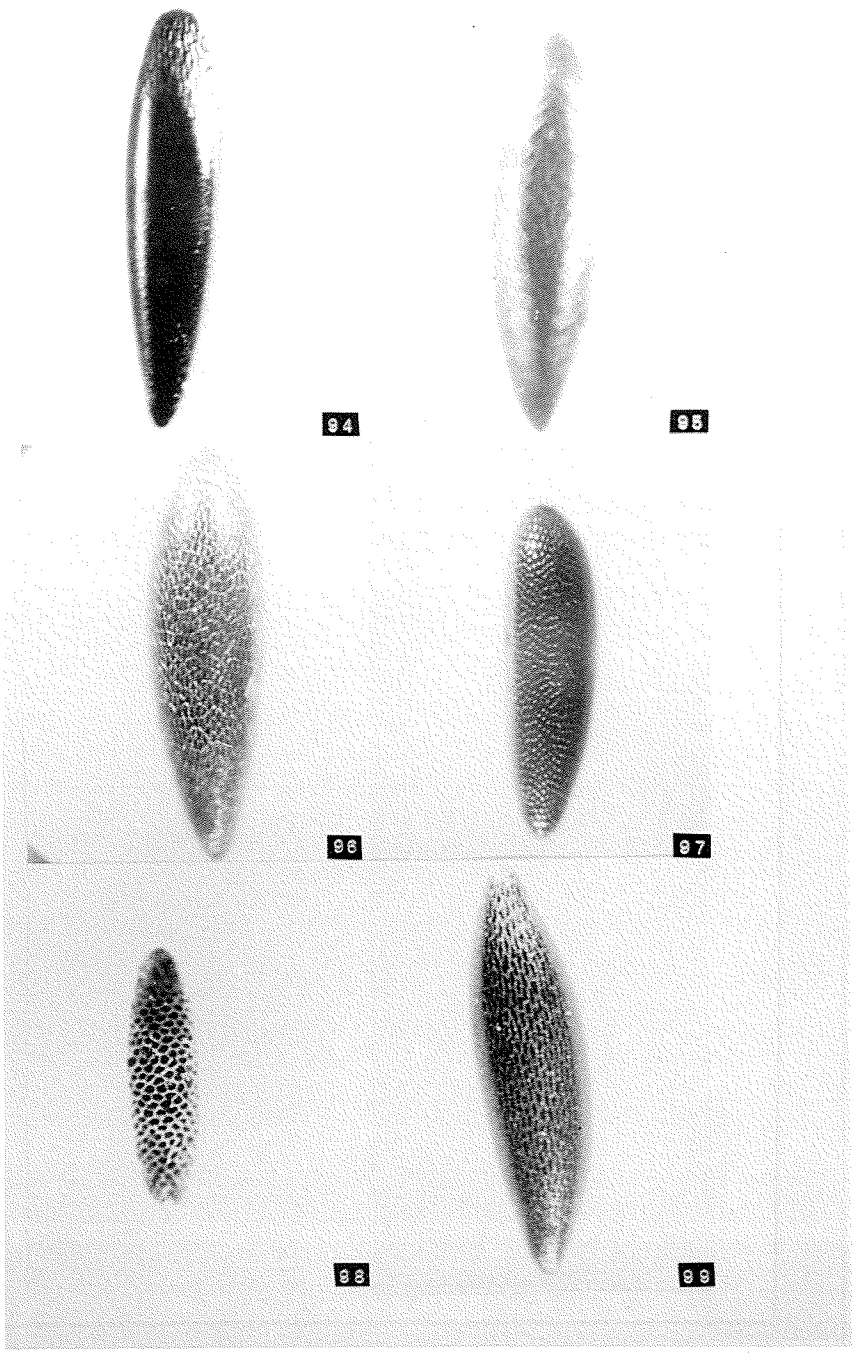
Figure 95 *A. excrucians*

Figure 96 *A. fitchii*

Figure 97 *A. stimulans*

Figure 98 *A. triseriatus*

Figure 99 *A. vexans*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Figs. 100 - 179 External chorionic detail of *Aedes* eggs of Manitoba as photographed through a phase contrast microscope.

Fig.	Species	Area of egg	Illumination	Magnification (-X)
100	A. abserratus	Median dorsal.	BM	170
101	"	"	BM	300
102	"	"	DL	800
103	"	"	DL	800
104	A. barri	"	BM	600
105	"	"	BM	800
106	"	"	BM	1500
107	A. campestris	"	*	300
108	"	"	BM	600
109	A. canadensis	Anterior dorsal.	BM	300
110	"	"	BM	680
111	"	Median dorsal.	BM	1600
112	"	"	BM	1600
113	A. cinereus	Towards anterior pole.	BM	600
114	"	"	BM	600
115	"	Transition from anterior to median.	BM	500
116	"	Median dorsal.	BM	450
117	"	Posterior dorsal.	BM	450
118	A. communis	Median dorsal.	BM	550
119	"	"	BM	1000

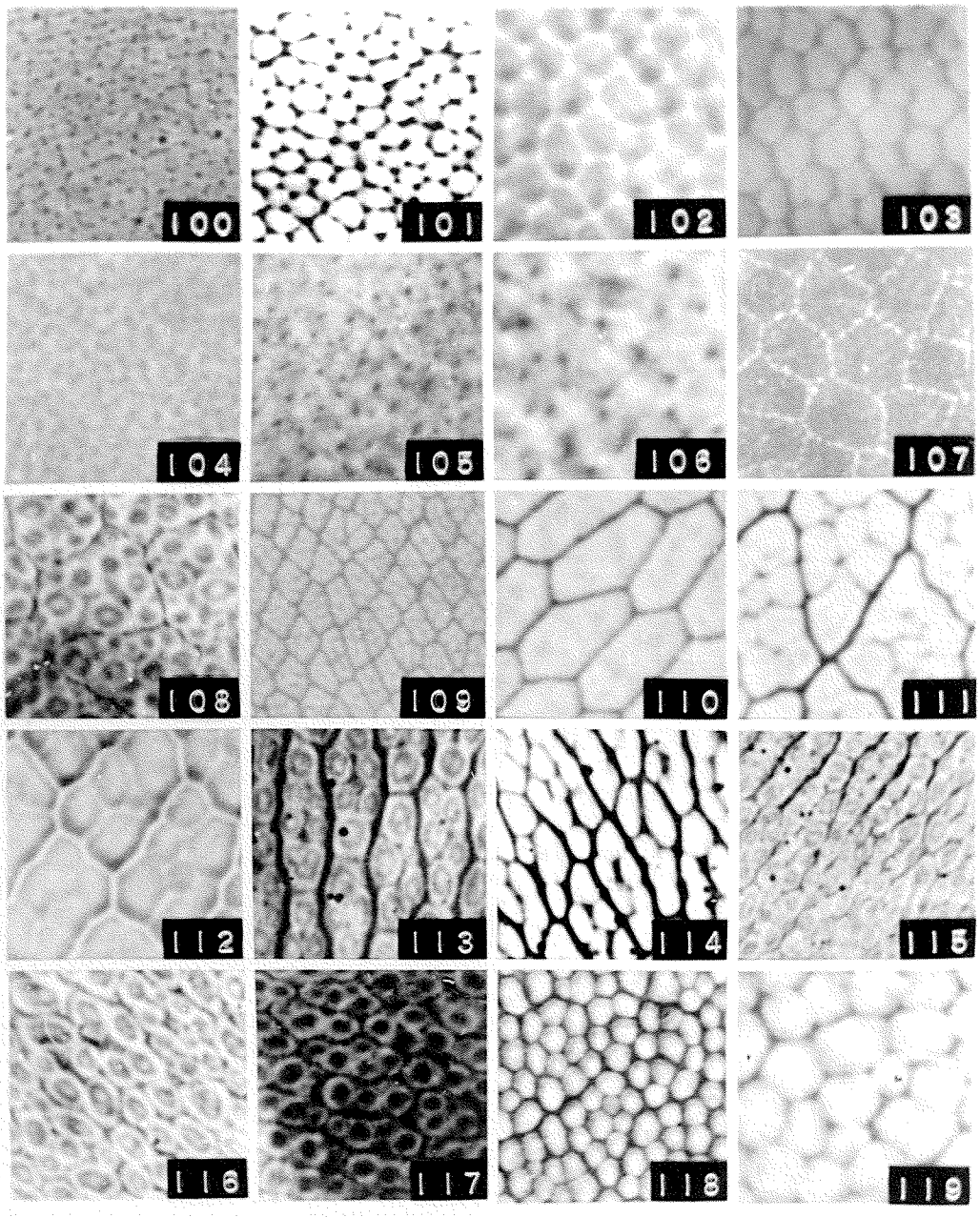
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Fig.	Species	Area of egg	Illumination	Magnification (-X)
120	A. decticus	Median dorsal	*	400
121	"	"	BM	700
122	"	"	DL	1000
123	A. diantaeus	"	*	500
124	"	"	BM	800
125	"	"	DL	1200
126	A. dorsalis	"	*	500
127	"	"	BM	1500
128	A. excrucains	"	*	650
129	"	"	DL	800
130	"	"	BM	800
131	"	"	DL	1000
132	A. fitchii	Median ventral	*	500
133	"	"	DL	300
134	"	"	BM	600
135	"	"	DL	800
136	A. flavescens	Median dorsal	*	600
137	"	"	DL	900
138	A. hexodontus	"	BM	800
139	"	"	BM	900

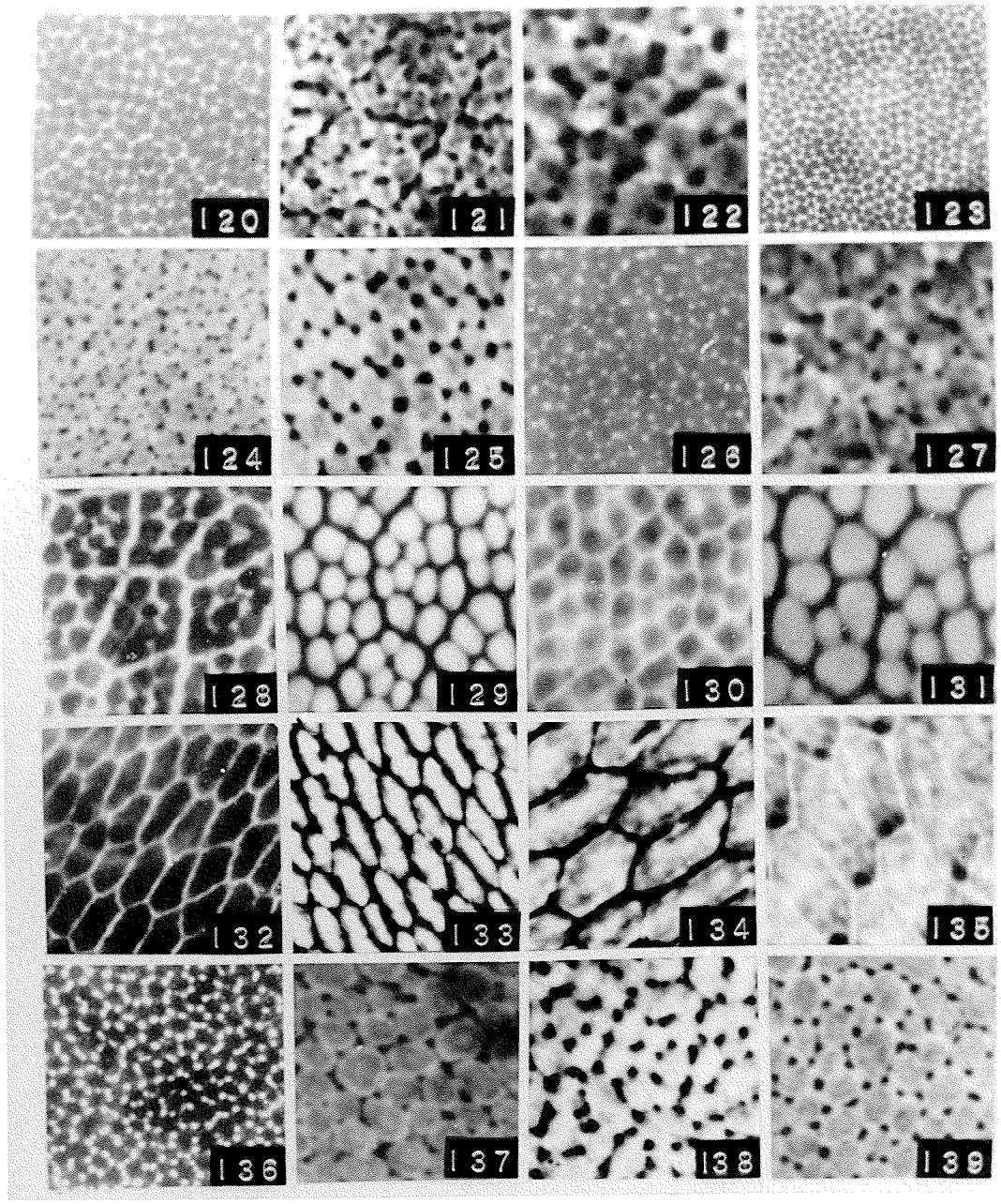
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Fig	Species	Area of egg	Illu-mination	Magni-fication
140	A. impiger	median dorsal	BM	1000
141	"	"	BM	2000
142	A. implicatus	"	*	400
143	"	"	BM	900
144	A. intrudens	"	*	500
145	"	"	BM	700
146	A. nigripes	"	DL	500
147	"	"	*	400
148	"	"	BM	850
149	"	"	BM	1000
150	A. nigromaculis	"	DL	400
151	"	"	BM	400
152	"	"	*	400
153	A. pionips	"	*	800
154	"	"	BM	700
155	"	"	BM	1400
156	"	"	DL	750
157	A. punctor	"	*	500
158	"	"	BM	700
159	"	"	BM	1300

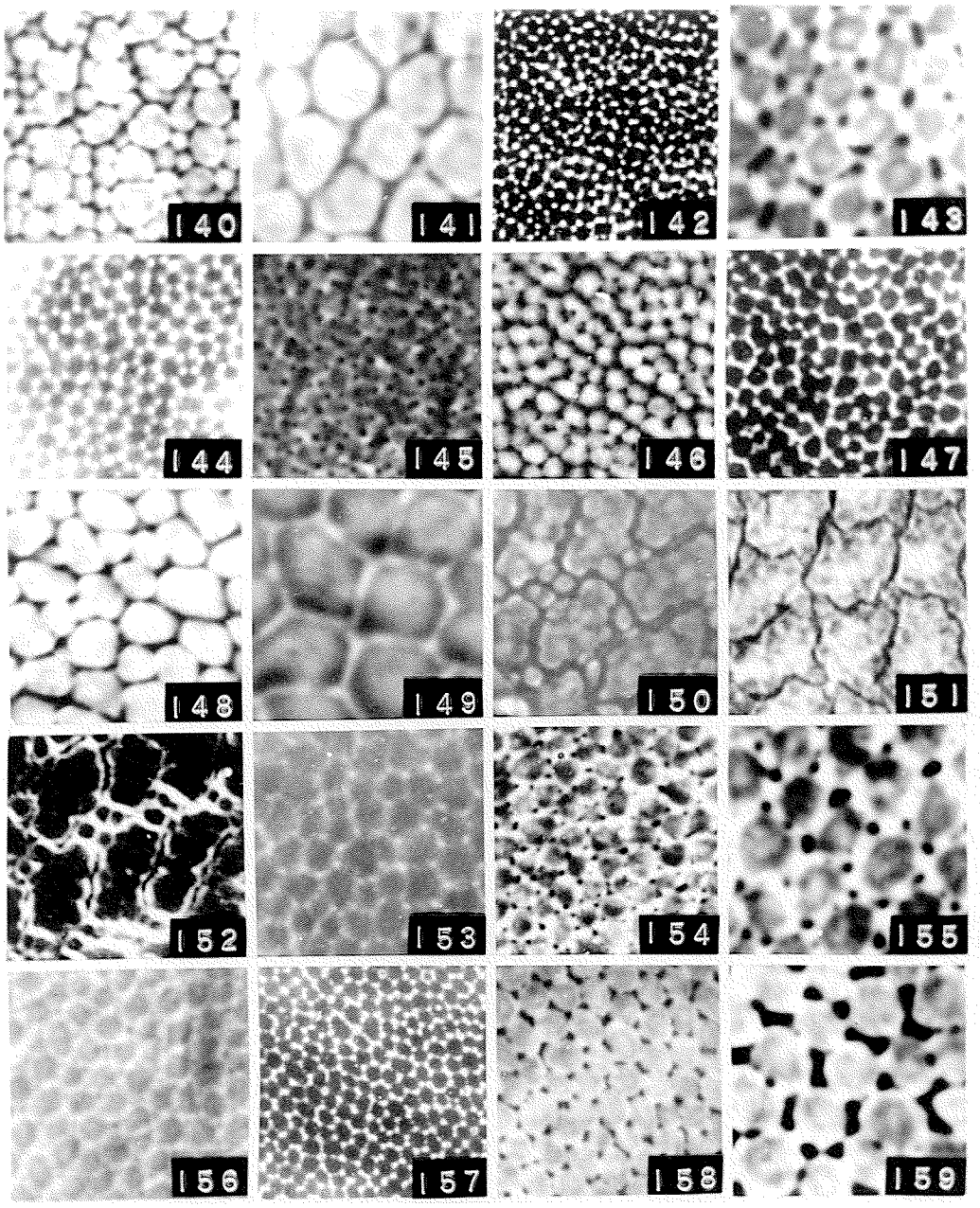
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Fig.	Species	Area of egg	Illu-mination	Magni-fication
160	A. riparius	Median dorsal	*	300
161	"	"	BM	450
162	"	"	BM	1200
163	"	"	DL	600
164	A. spencerii	"	BM	700
165	"	"	BM	1300
166	A. sticticus	"	BM	630
167	"	"	BM	1200
168	A. stimulans	"	*	400
169	"	"	BM	400
170	"	"	BM	800
171	"	"	DL	800
172	A. trichurus	"	*	400
173	"	"	BM	600
174	"	"	BM	1200
175	A. triseriatus	Median ventral	DL	600
176	"	"	BM	600
177	A. vexans	Anterior dorsal	*	400
178	"	"	BM	400
179	"	"	BM	700

BM Bright Medium

DL Dark Low

* Dark ground illumination effect. (see text p. 30)

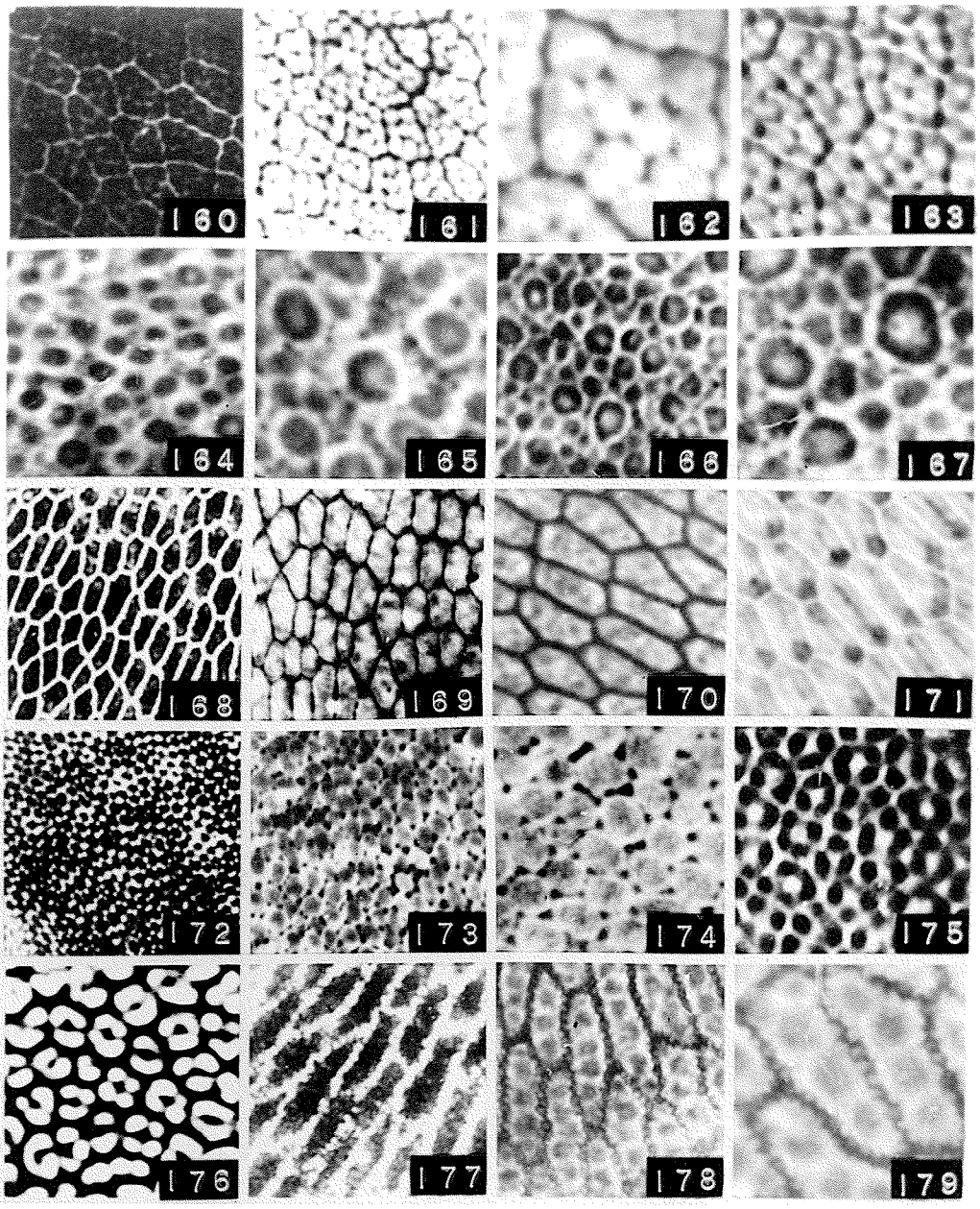
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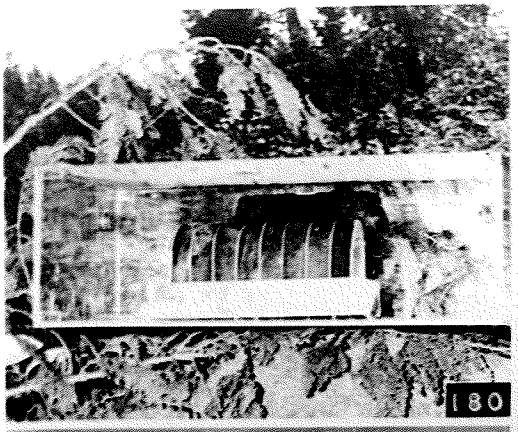
Figure 180. Large field collecting cage 20" x 10" x 7" with guinea pig inside.

Figure 181. Small mosquito cages 6" x 1" x 1" placed on moist cheesecloth in the laboratory. Cotton swabs soaked in clover honey are placed on the cage.

Figure 182. Cylindrical cages each containing a single female.

Figure 183. Six single female cages removed to show separate egg pads.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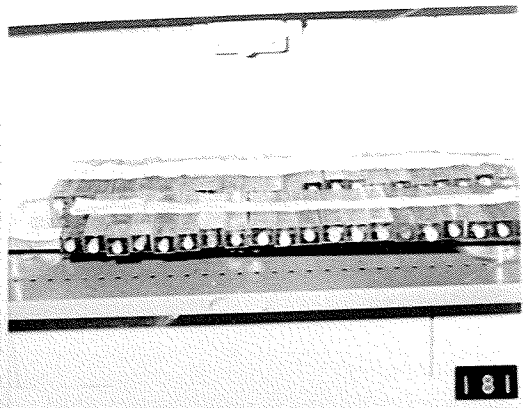
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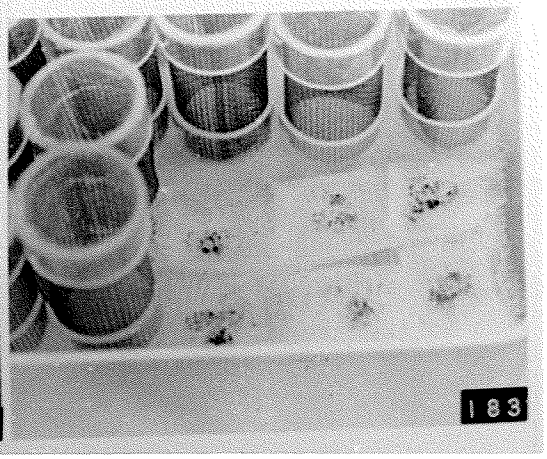
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Figure 184. Removing soil from edge of pool for egg recovery.

Figure 185. Egg separating screens.

Figure 186. Apparatus required for separating and sorting eggs from soil samples.

(A) Separating screens.

(B) Transfer screens.

(C) Percolation funnel containing saturated salt solution. Funnel shows dense material at bottom and less dense material with eggs floating on surface.

(D) Binocular microscope used for sorting eggs.

Figure 187. Eggs stores on moist filter paper in petri dishes.


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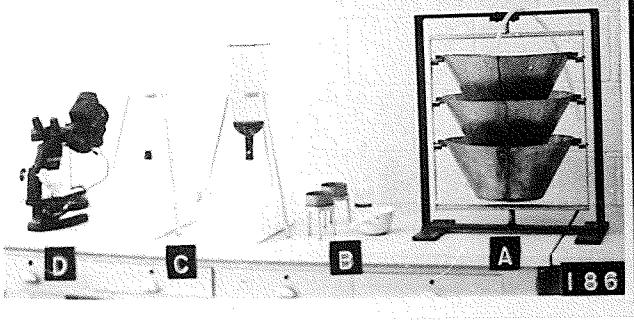

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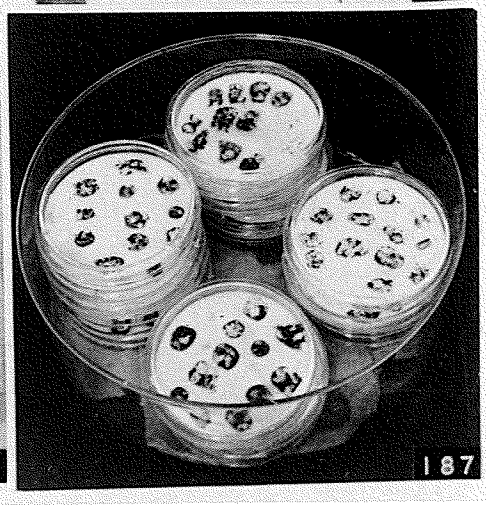
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Figure 188

Map of Manitoba indicating collecting sites.

(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.

Baker Lake in the North West Territories, 600 miles North of Churchill is not shown.

(Map reproduced from the Economic Atlas of Manitoba.)

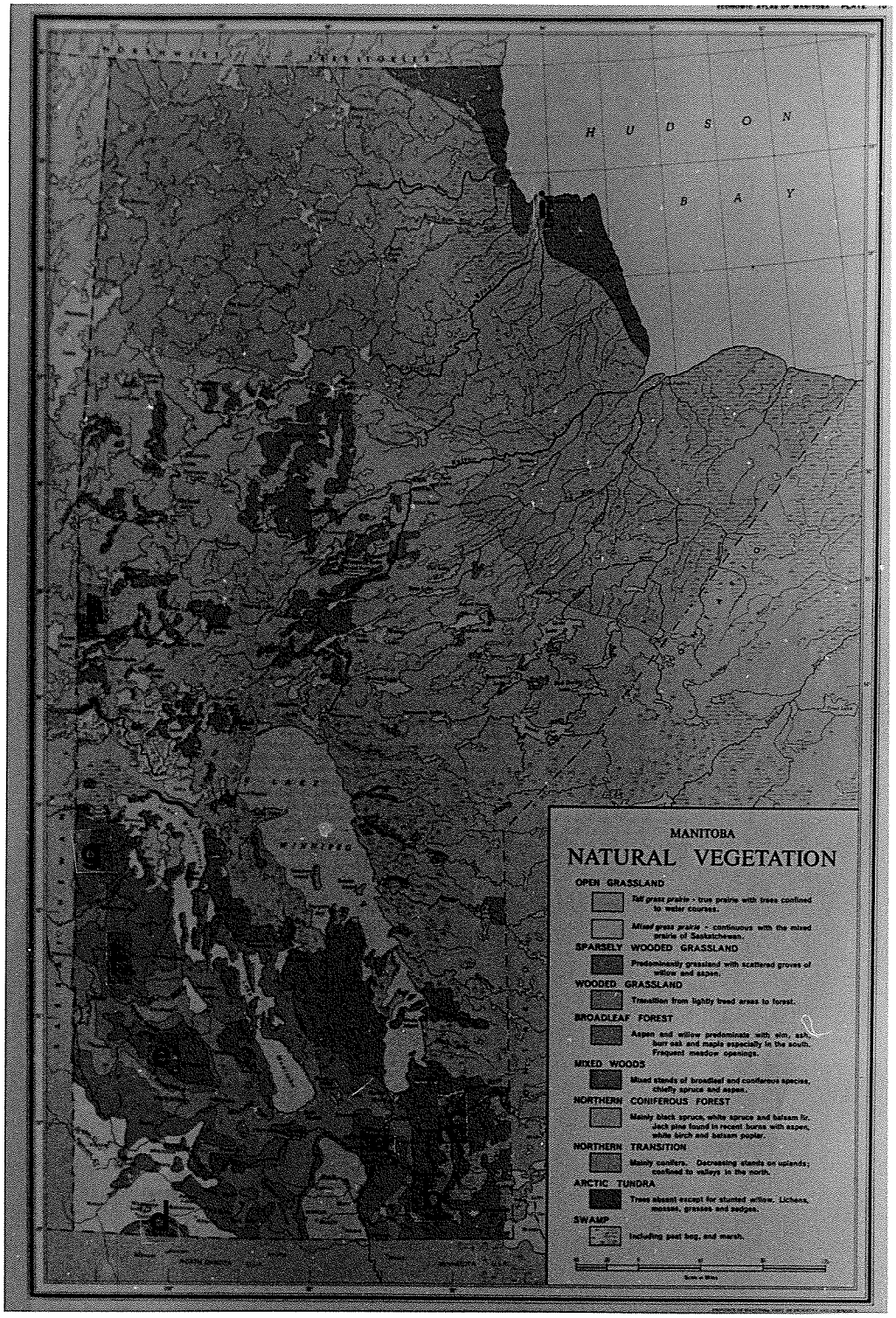

H U D S O N
B A Y
WINNIPEG
MANITOBA
NATURAL VEGETATION
OPEN GRASSLAND
Tall grass prairie - true prairie with trees confined to water courses.
Mixed grass prairie - continuous with the mixed prairie of Saskatchewan.
SPARSELY WOODED GRASSLAND
Predominantly grassland with scattered groves of willow and aspen.
WOODED GRASSLAND
Transition from lightly treed areas to forest.
BROADLEAF FOREST
Aspen and willow predominate with elm, ash, burr oak and maple especially in the south. Frequent meadow openings.
MIXED WOODS
Mixed stands of broadleaf and coniferous species, chiefly spruce and aspen.
NORTHERN CONIFEROUS FOREST
Mainly black spruce, white spruce and balsam fir. Jack pine found in recent burns with aspen, white birch and balsam poplar.
NORTHERN TRANSITION
Mainly conifers. Decreasing stands on uplands; confined to valleys in the north.
ARCTIC TUNDRA
Trees absent except for stunted willow. Lichens, mosses, grasses and sedges.
SWAMP
Including peat bog, and marsh.

Fig. 188

APPENDIX II

DISTRIBUTION OF EGG SIZE IN THREE POPULATIONS

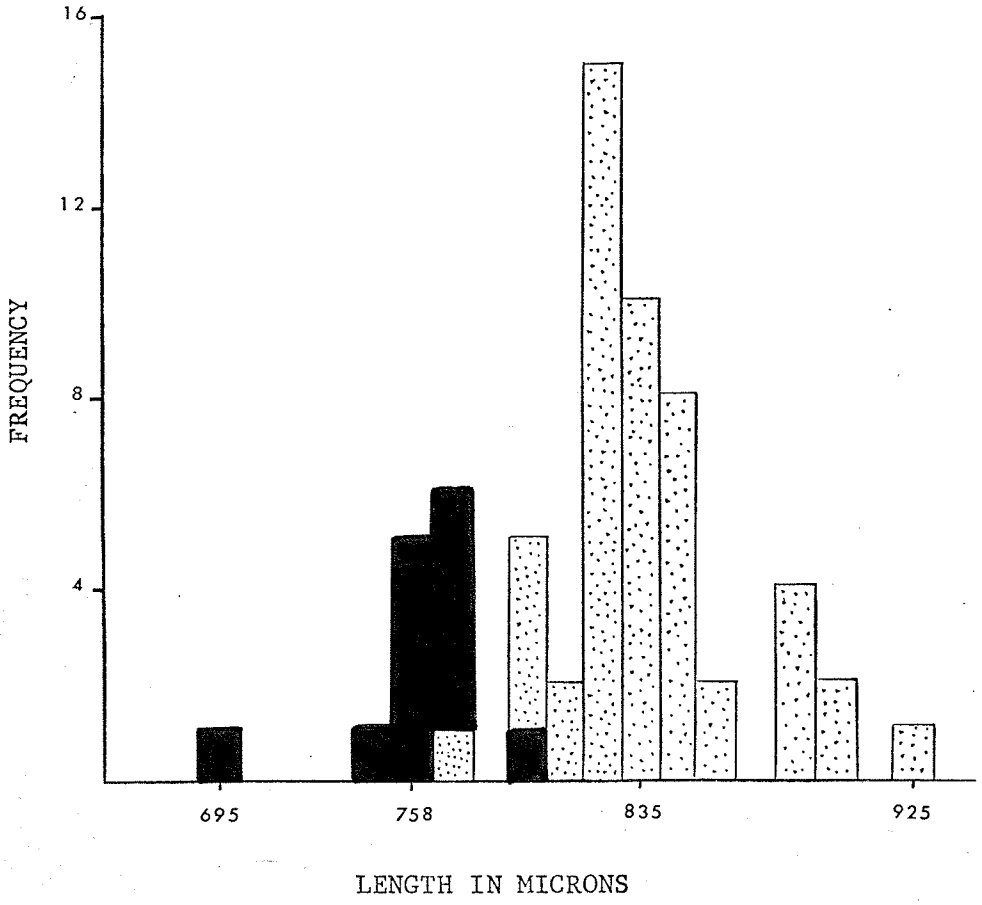
OF *AEDES CINEREUS*

Eggs with rounded anterior end.

Mean 766 microns; range 694 - 797 microns.

Eggs with tapered anterior end.

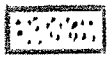
Mean 835 microns; range 771 - 925 microns.


16
12
8
4
FREQUENCY
695
758
835
925
LENGTH IN MICRONS

APPENDIX III

DISTRIBUTION OF EGG SIZE IN TWO POPULATIONS

OF AEDES COMMUNIS

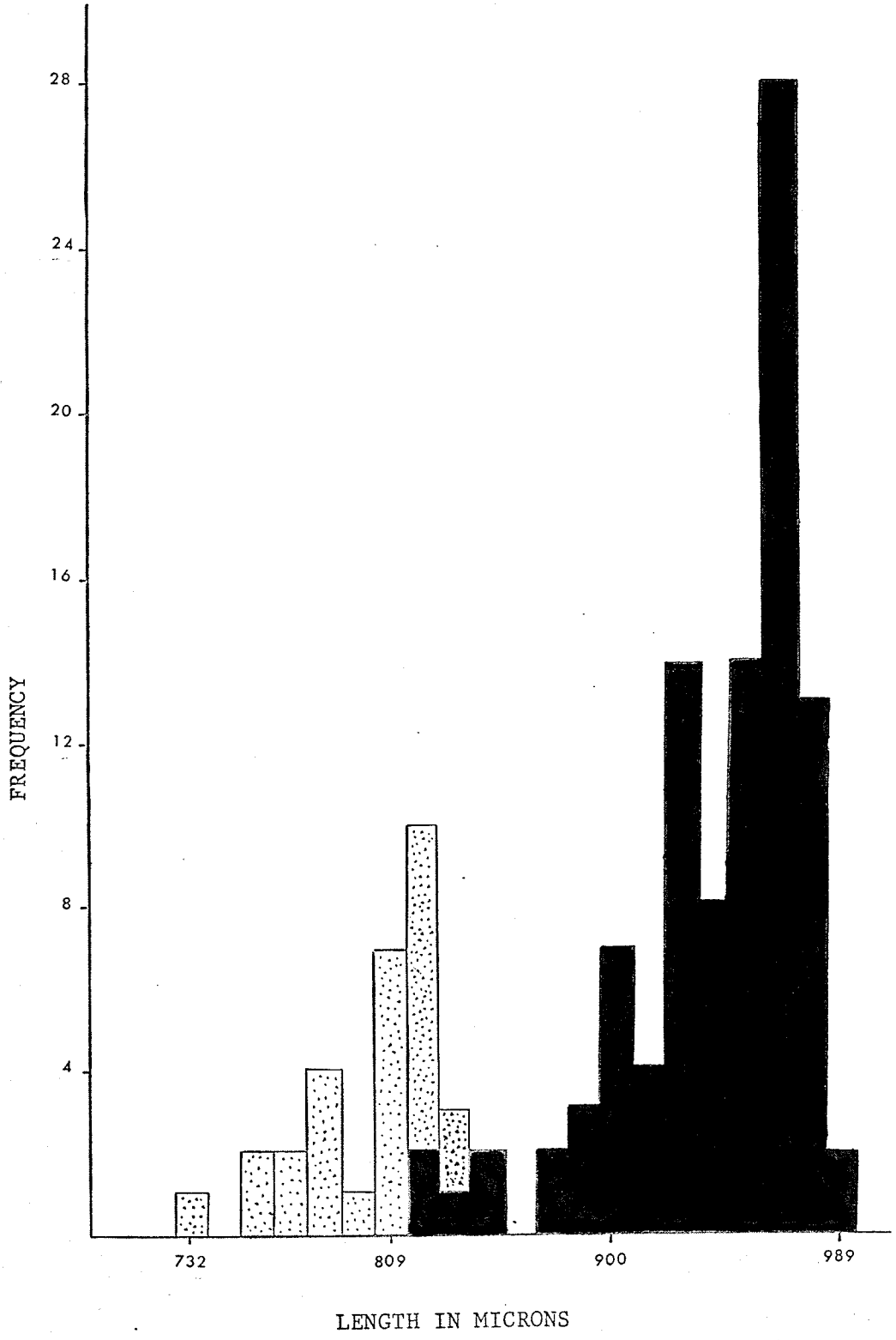


From Sandilands Forest Reserve only.

Mean length: 804 microns; range 732 - 835 microns.

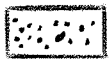
Broadly fusiform egg from Sandilands Forest Reserve, Flin Flon and Churchill.

Mean length: 940 microns; range 822 - 989 microns.


28
24
20
16
12
8
4
FREQUENCY
732
809
900
989
LENGTH IN MICRONS

APPENDIX IV

DISTRIBUTION OF EGG SIZE IN TWO POPULATIONS OF AEDES DORSALIS

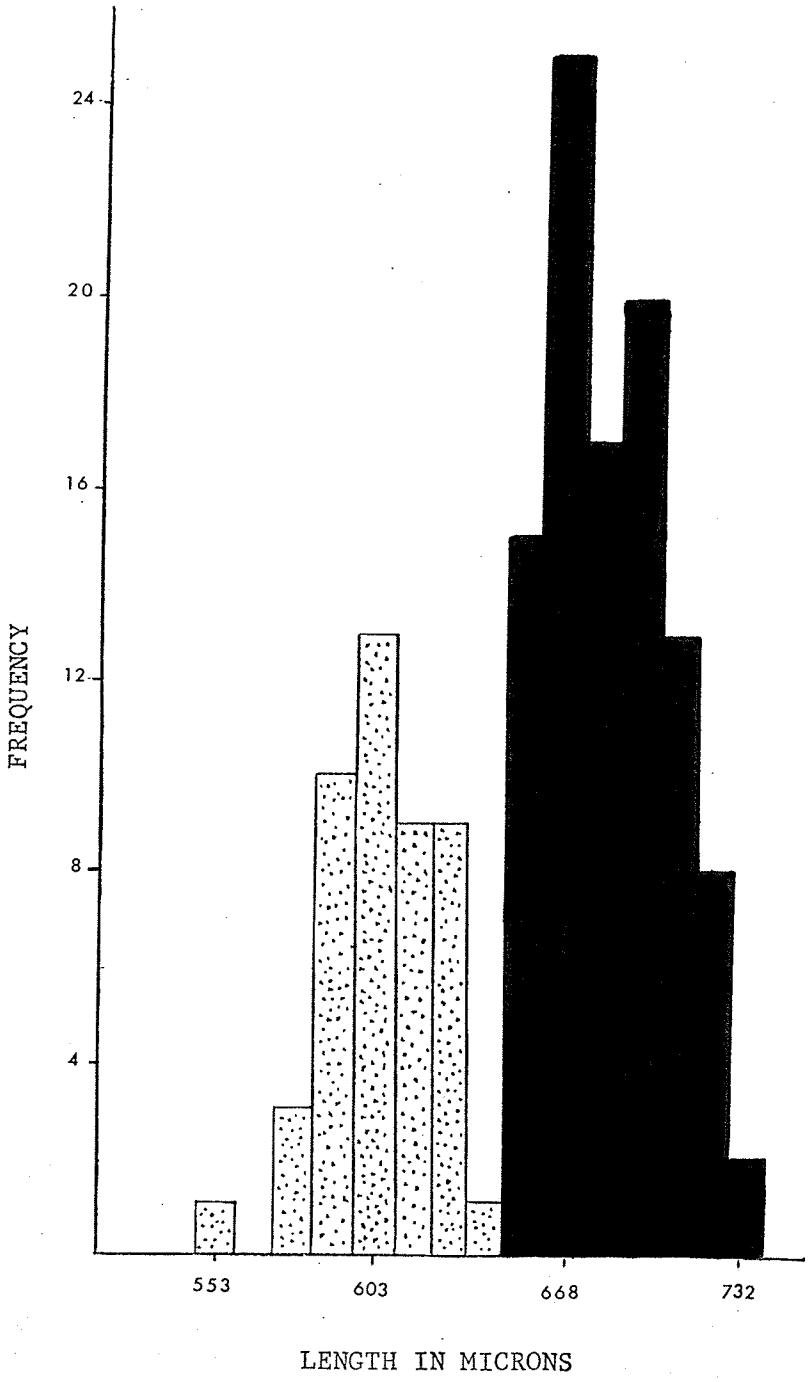


Eggs obtained from a soil sample taken near the Winnipeg Airport.

Mean 606 microns; range 553 - 643 microns.

Eggs obtained from wild caught females from Winnipeg and Sandilands Forest Reserve.

Mean 684 microns; range 655 - 732 microns.


FREQUENCY
24
20
16
12
8
4
553
603
668
732
LENGTH IN MICRONS

APPENDIX V

DISTRIBUTION OF EGG SIZE IN THREE POPULATIONS OF *AEDES HEXODONTUS*

From Churchill, Manitoba.

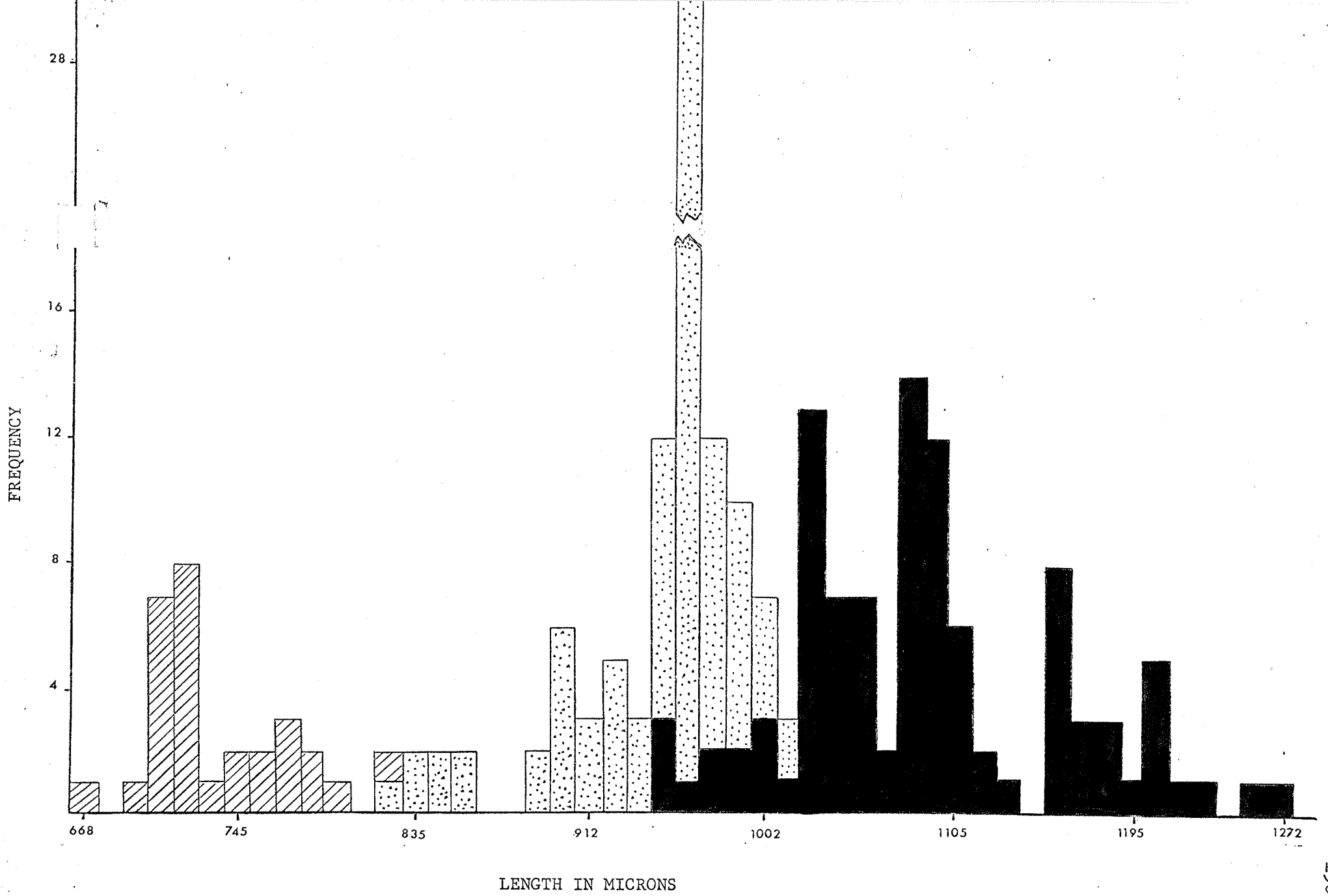
Mean 737 microns; range 668 - 822 microns.

From Churchill, Manitoba.

Mean 955 microns; range 822 - 1015 microns.

From Baker Lake, North West Territories.

Mean 1086 microns; range 951 - 1272 microns.


28
16
12
8
4
FREQUENCY
668
745
835
912
1002
1105
1195
1272
LENGTH IN MICRONS

APPENDIX VI

LIST OF BLACK LEGGED SPECIES

	A. abserratus	*	A. nigripes
*	A. cinereus		A. nigromoculis
*	A. communis	*	A. pionips
*	A. decticus	*	A. punctor
*	A. diantaeus		A. spencerii
*	A. hexodontus		A. sticticus
*	A. impiger		A. trichurus
*	A. implicatus		A. triseratus
	A. intrudens		A. vexans

* Black legged species complex in the Churchill region.