THE EFFECT OF DAYLENGTH AND TEMPERATURE ON THE INDUCTION AND TERMINATION OF DIAPAUSE IN *AEDES ATROPALPUS* (COQUILLETT), AND FIELD AND LABORATORY STUDIES OF AUTOGENY AND HIBERNATION IN SOME MOSQUITOES FROM MANITOBA.

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ABSTRACT

THE EFFECT OF DAYLENGTH AND TEMPERATURE ON THE INDUCTION AND TERMINATION OF DIAPAUSE IN *AEDES ATROPALPUS* (COQUILLETT), AND FIELD AND LABORATORY STUDIES OF AUTOGENY AND HIBERNATION IN SOME MOSQUITOES FROM MANITOBA.

Embryonic diapause, which is determined by daylength, has been demonstrated in a multi-voltine mosquito, *Aedes atropalpus* (Coquillett). Experiments conducted under controlled temperature and photoperiod show that short-days, 8 hours to 14 hours light per 24 hour day, induce diapause in the autogenous Belleville strain of *A. atropalpus*. The effect of long photoperiods is shown to be independent of low temperatures, therefore indicating that in nature the deposition of diapausing eggs in autumn is due to the influence of the shorter days experienced by the mosquitoes. Experiments also show that the sensitive stages for light reception are the fourth larval instar, pupa and adult.

Two strains of *A. atropalpus* were studied, one being the autogenous strain from Belleville, Ontario (44°N latitude) and the other being the anautogenous strain from Austin, Texas (30°N latitude). A latitudinal difference in critical photoperiod for the two populations is noted. The critical photoperiod for the Austin strain lies between $12\frac{1}{2}$ -13 hrs. light per day and for the Belleville strain it lies between 14-$14\frac{1}{2}$ hrs. light per day.

A light interruption around eight hours in a diapause inducing scotophase causes the Belleville strain *A.atropalpus* females to produce non-diapause eggs and it is proposed that the 'lights-off' signal triggers some endogenous secretory rhythm with an 8-hour periodicity.

A temperature of 30°C terminates diapause sooner than a temperature of 20°C. A long photoperiod (16L:8D) also brings about the termination of diapause. Results of an experiment to determine where the photoreceptors lie in the embryo are presented.

The effects of photoperiod and diet on fecundity in autogenous *A.atropalpus* are discussed. It is seen that photoperiod has a direct influence on fecundity and an optimum egg production was reached at 16L:8D photoperiod. More eggs were also produced when the larvae were fed a protein rich diet.

The effect of photoperiod and temperature on the biting activity and ovarian development of anautogenous *Culiseta inornata* (Williston) showed that this species exhibits the phenomenon of "gonotrophic concordancy".

Studies on the autogenous ovarian development in *Aedes* species at Churchill, Manitoba (58°N) showed that four species, *A. communis* (Degeer), *A. nigripes* (Zett.) *A. impiger* (Walker) and *A. campestris* (Dyar & Knab) are autogenous.

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CHAPTER I

INTRODUCTION

A majority of the mosquitoes in North America belong to the genus *Aedes* Meigen. The aedine species which have only a single generation per year (univoltine) have an obligatory diapause in the egg stage, i.e. the eggs do not hatch even though they are laid in summer when conditions are suitable for hatching. These eggs will remain in diapause and will hatch only after a period of cold conditioning. Multi-voltine species of *Aedes* like *A. vexans* (Meigen), *A. atropalpus* (Coq.) etc. lay eggs in summer which hatch within a few days after embryogenesis is complete, provided the oviposition sites are inundated and other conditions for hatching are favourable. Until quite recently, it was thought that overwintering eggs of multi-voltine *Aedes* were not in a state of diapause, and would hatch anytime conditions of temperature and moisture were favourable. It now appears that there are exceptions to this, and the case of *A. atropalpus* is one of these exceptions.

While maintaining a culture of the Belleville strain of *A. atropalpus* in our laboratory in early 1967, for studies on autogeny, it was surprising to find that several batches of eggs failed to hatch even when they were subjected to a hatching stimulus, although these eggs

contained live embryos. In earlier studies Kappus (1964) had reported egg diapause in *A. triseriatus* (Say) and Vinogradova (1965) had described egg diapause in *A. togoi* (Theobald) induced by short photoperiods on the female parent. Since my work on *A. atropalpus* was begun, Anderson (1968) has reported on the influence of temperature and photoperiod in a Connecticut strain of *A. atropalpus*, and found that diapause is induced under certain conditions. My work was done independently of Anderson's and in many cases my experiments have duplicated what he has done, and confirm his findings. In other cases my work extends into areas not investigated by Anderson.

It is apparent from the earlier experimental studies of species of Lepidoptera (eg. *Bombyx mori* L) that have an embryonic diapause in the seasonal cycle, that the factors of light and temperature play a significant role in determining whether the eggs should be of the diapause type or should develop without interruption (Kogure 1933). Hence I undertook this investigation to determine whether photoperiod and/or temperature influences the autogenous Belleville strain of *A. atropalpus* to lay diapause eggs. Kappus and Venard (1967) rightly stated that "the induction of facultative diapause in culicid eggs has been little studied, and it may well be that upon investigation the eggs of a number of culicid species will prove to be photoperiod sensitive."

Photoperiod is the most reliable of all environmental signals used by insects for forecasting seasonal changes and is the one most frequently used for controlling diapause. Generally, diapause is considered as a suppressed state of development in the life cycle which ensures survival through unfavourable seasons. Beck (1968), Danilevskii (1965), Adkisson (1964), to mention but a few workers, have shown that most insects enter a period of diapause as daylengths in autumn decrease to a value critical for the particular species in a given latitude. In the temperate regions the insects remain in diapause during the winter months. Generally, as the daylengths increase during the subsequent spring, diapause is terminated and the emergence is timed to coincide with the availability of food and ideal conditions for growth and development.

In the insects exhibiting a facultative diapause, the ambient conditions, during some earlier stage(s) of development of the species concerned, would determine whether the genetic response to facultative diapause will actually be triggered or not. Since diapause is induced to enable the insect species to survive a period of adverse environmental conditions, it is possible that multi-voltine species have some special mechanism which ensures the synchronization of the different developmental stages with the appropriate environmental conditions.

The purpose of the present research was to determine what factors induce diapause in the embryos in autogenous *A. atropalpus* and if daylength was involved to determine the critical daylength that causes diapause in the strain of *A. atropalpus* from Belleville, Ontario (44°N latitude), and also to determine the developmental stage that is most sensitive to the rhythm of light.

A knowledge of the critical photoperiod would enable us to appreciate the reasons for the geographical distribution of strains of the same species. In order to study this phenomenon a southern strain of *A. atropalpus* from Austin, Texas (30°N latitude) was used. Latitudinal differences in photoperiodic response have been reported by Vinogradova (1960) for *Anopheles maculipennis messeae* Falleroni and *Anopheles hyrcanus* (Pallas) and by Depner (1966) for populations of *Anopheles freeborni* Aitken from Washington and California and by Anderson (1968) in populations of *A. atropalpus*.

Other influences of photoperiod, viz, its effects on fecundity in autogenous *A. atropalpus* (Belleville strain) and its effects on anautogenous *Culiseta inornata* (Williston) with particular reference to its influence on the biting activity and ovarian development in blood fed females at different temperatures have also been investigated.

Studies in the Arctic and sub-Artic have indicated the possibility that some species from these northern

latitudes are able to develop their eggs autogenously (Corbet 1967, Hocking 1952, Beckel 1954). This undoubtedly has a certain adaptive value, because if the search for a blood meal is difficult due to the scarcity of vertebrate hosts in these regions, then autogenous egg development would ensure the survival of the species. Therefore studies to determine the extent of autogeny in *Aedes* species in Churchill, Manitoba (58°N), were undertaken in the summers of 1967 and 1968.

The research reported in this dissertation was conducted in the laboratory of the Department of Entomology, University of Manitoba, with the field studies on *Aedes* in northern Manitoba being conducted at Fort Churchill, Manitoba, Canada.

CHAPTER II

REVIEW OF THE LITERATURE

DIAPAUSE

Diapause is defined as a suppression of development, which in a given species would occur even if the insects were kept under optimum conditions. A period of relatively adverse conditions of a definite character, for example, chilling or drought is necessary before development can continue. This suppressed state is brought about by certain physiological reactions which are genetically controlled. Although the phenomenon of diapause in insects was known in the early part of this century, it has been only in the past two decades that an interest has been shown by insect physiologists and ecologists. By their research we are able not only to appreciate better some of the intricate factors influencing the induction and termination of diapause, but also to have a better understanding of the seasonal and geographical distribution of insects. In recent years numerous comprehensive reviews of the literature on diapause in insects and mites have been produced, notably by Andrewartha (1952), Hinton (1953, 1957), Lees (1955, 1956), Harvey (1962), de Wilde (1962), Beck (1963, 1968), and Danilevskii (1965).

The first experimental demonstration of this phenomenon we now refer to as diapause goes as far back as 1869 when Duclaux observed that eggs of *Bombyx mori* (Linnaeus) failed to hatch at room temperature and they eventually died. But if these eggs were first chilled for forty days in an ice box he noticed that they hatched successfully when returned to warm temperatures. Wheeler (1893) was the first to use the term "diapause" to describe a stage in the embryogenesis of the grasshopper *Xiphidium ensiferum* wherein the embryo remained stationary during the interval between two active stages - anatrepsis and katatrepsis - in blastokinesis. Subsequently Henneguy (1904) used it not to describe a stage of morphogenesis (or embryogenesis) but to a condition of arrested growth whether it was in the developing insect or in the adult. Immediately following this ecologists used the term loosely to include any form of arrested development like a cold or heat torpid condition which is a dormant state being a direct response to deleterious physical forces. Recognizing the need for a distinction Shelford (1929) preferred to restrict the meaning of diapause to those cases where the activity or development of an insect is arrested 'spontaneously'. When its activity or development is temporarily inhibited by unfavourable environmental factors it is referred to as being 'quiescent'. This distinction as proposed by Shelford has undoubted utility both in ecology and physiology,

but at times insufficient knowledge would make it difficult to make a clear cut distinction between the two phenomena (Lees 1955).

Diapause is of two types, either it is obligatory or facultative. In those insects which exhibit an obligatory diapause all individuals in every generation undergo a period of suppressed development even though the environmental conditions are suitable for growth and reproduction. In these cases the induction of diapause is entirely independent of any stimulus from the insect's external environment. These insects have one generation per year and are referred to as univoltine species. In other words, one could say that diapause in a univoltine species is due to certain internal mechanisms which are genetically controlled (Beck 1968).

In those insects which exhibit a facultative diapause, dormancy is conditional. In an insect that develops slowly from egg to adult to egg, there may only be a single generation per year. Where an insect develops rapidly, there may be one or more generations where no individuals enter diapause. In the multivoltine species the suppressed growth stage is almost always influenced by the external environment, viz. daylength, or daylength plus temperature, or temperature plus diet, etc. The more recent studies by Beck (1963) with the European corn borer, *Ostrinia nubilalis* Hbn., and Adkisson *et al* (1963) with the

Pink bollworm *Pectinophora gossypiella* (Saunders), both of which exhibit a facultative diapause, clearly indicate that diapause sets in very much earlier than the onset of unfavourable temperatures and other environmental conditions.

Even in species where univoltinism is known to be rigid, recent studies by Mansingh and Smallman (1966, 1967) have demonstrated that in the two univoltine saturniids *Hyalophora cecropia* (L.), and *Antheraea polyphemus* Cramer, daylength regulated the induction, prevention, maintenance, and termination of diapause. Mansingh and Smallman have rightly questioned the validity of the generally accepted distinction between obligatory and facultative diapause.

Diapause occurs in any given stage of the life cycle - egg, larva, prepupa, pupa, or adult, but the stage in which it occurs is quite distinct for each species. In the egg stage, the embryo could enter diapause at (1) an early stage of development, as in *Gryllulus commodus* Walker (Browning 1952) or (2) when the embryo is half grown as in *Melanoplus differentialis* Thomas (Bodine 1929) or (3) when it is fully grown as in the eggs of aedine mosquitoes (Beckel 1958, Horsfall *et al* 1958). Larval or nymphal diapause generally occurs towards the end of the stadium, very often in the last instar eg. the codling moth, *Carpocapsa pomonella* L (Peterson and Hamner 1968) and the mosquito *Aedes triseriatus* (Kappus and Venard 1967). There are instances

of diapause in earlier larval instars as observed in the pitcher plant mosquito, *Wyeomyia smithii* (Coq.) (Jenner 1951 & S. M. Smith pers. comm.). Pupal and imaginal diapause is also common, the former more often reported among the Lepidoptera and the latter more prevalent among the Coleoptera and Diptera. During the diapause period there are certain gradual processes of physiological development taking place within the organism which Andrewartha (1952) has referred to as "diapause development". The latter is an essential pre-requisite for the termination of diapause and resumption of growth. The exact nature of diapause development is still unresolved; but from recent studies by Williams (1946, 1952, 1956), Cloutier *et al* (1962) and Van der Kloot (1955) it would be safe to assume that diapause development is intimately connected with the production of certain hormones which prepare the organism for active morphogenesis on the onset of favourable environmental conditions.

The intensity of diapause is variable depending to a marked extent on the conditions required for the completion of "diapause development". It is a concept which is difficult to define and would depend on the criteria used for its measurement. The duration and stability of diapause would vary among insect species and even among certain individuals of a given species. Andrewartha (1952) suggested that the duration of the diapause stage could be

taken as one measure of its intensity and the treatment for completion of diapause development as another. The studies by Beck and Hanec (1960) with diapausing larvae of *Ostrinia nubilalis* and Schneiderman and Williams (1953) with the diapausing pupae of *Hyalophora cecropia* have shown that more pertinent physiological data could be obtained for measuring the intensity of diapause. During the diapause state, metabolism is suppressed (measured by the rate of oxygen consumption), and there is a suppression of the developmental rate. Intensity of diapause is generally considered to be inversely proportional to the rates of oxygen consumption and development.

Prior to discovering the importance of photoperiod in regulating seasonal cycles of insect development, several workers considered diet, temperature and moisture as the external stimuli solely responsible for the induction of diapause. Strelnikov (1936) reported that larvae of *Loxostege sticticalis* (L) entered diapause because they fed on dry food material or on plants with an increased nutritive value. Until a few years ago the onset of diapause in the pink bollworm, *Pectinophora gossypiella*, was believed to be caused by the changing composition of the diet (Squire 1937, 1940). The pink bollworm diapauses as an instar IV larva, and Squire advanced the theory that diapause was induced when larvae fed on mature cotton bolls. Mature cotton bolls have a high oil content, and subsequently

diapause was associated with a high oil content diet. However, the recent studies by Bull and Adkisson (1960) and Adkisson (1961) showed that the larval diet had only a token influence since diapause could be prevented in these larvae by rearing them under long day photoperiods regardless of the diet. An interesting observation they made was that once the photoperiod became suitable for the induction of diapause then the greatest response occurred in those populations which were reared on diets having a high oil content. This clearly proves that the effect of the lipid content of the diet was not of primary importance. From their laboratory and field studies Bull and Adkisson (1960) concluded that diapause in natural populations of the bollworm was not caused by a single factor, but by a complex interaction of several factors. Food also plays an important role in the induction of diapause in the potato beetle, *Leptinotarsa decemlineata* Say (Faber 1949, de Wilde *et al*, 1959). Jeremy (1956 - cited by de Wilde 1959) observed that when beetles were reared at intermediate photoperiods the incidence of diapause increased when the beetles ate senescent leaves.

Of the several external stimuli responsible directly of indirectly for inducing diapause in insects, photoperiodism (i.e. the relative lenght of day and night) is one of the chief environmental factors regulating diapause. Temperature cannot be discounted as it too plays an important role.

INFLUENCE OF PHOTOPERIOD AND TEMPERATURE ON DIAPAUSE AND HIBERNATION

(1) Lepidoptera, Coleoptera and Diptera (excluding Culicoidea).

Since the literature on photoperiodic induction of diapause is now voluminous I thought it prudent to review briefly only some of the more pertinent examples in a few orders of insects.

It was after the discovery of photoperiodism in plants by Garner and Allard in 1920 that biologists began to realize its role in influencing the seasonal development in insects. Marcovitch (1923, 1924) was the first to observe that daylength determined the occurrence of sexual forms in aphids. The classical work of Kogure (1933) illustrated the effect of photoperiod in bringing about embryonic diapause in the commerical silkworm, *Bombyx mori*. This extensive work also clarified the relative effect of photoperiodism, temperature, and light quality. His results showed that egg diapause in the bi-voltine races of *Bombyx mori* was due to long days and high temperatures. Since then, diapause has been shown to be daylength dependent in hundreds of insects.

In insects that react to changes in daylength two main types of photoperiodic responses are observed: (a) the long-day type, and (b) the short-day type. In the long-day type of development, relatively long daylengths tend to favour the continuous growth and reproduction of

the species. When such a species develops under shorter daylengths, i.e. below the critical daylength value, it is induced to enter diapause. The long-day type of photoperiodic reaction is characteristic of multivoltine species in temperate climates with a facultative winter diapause. In the short-day type of development, continuous growth and reproduction is observed only under short day conditions. Long daylengths induce diapause. The critical daylength referred to in the statements above is a measure of the response of a population to the daily photoperiod and may be defined as the point at which the response curve changes from a high incidence to a low incidence of diapause (Beck 1968). It has been associated with latitude (reviews by Danilevskii 1965; Beck 1968) and it may be influenced by temperature (Beck and Apple 1961).

The noctuid moth *Acronycta rumicis* L. is typical of a large majority of species which show a marked response to photoperiod. Danilevskii (1965) observed that at 27-28°C, if the larva was exposed to a short photoperiod (range 6 to 15 hrs.) virtually every individual entered diapause in the pupal stage. As the daily illumination was extended to 17 hrs. the incidence of diapause fell gradually, and under continuous illumination the development was uninterrupted. Other examples in Lepidoptera, where diapause induction is caused by short daylengths, include (a) the tussor silk moth, *Antheraea pernyii* Guer at 22°C (Tanaka 1950), (b) the agrotid

moth, *Diataraxia oleracea* L at 24°C (Way & Hopkins 1950), (c) the pink bollworm, *Pectinophora gossypiella* (Adkisson *et al* 1963), (d) the codling moth, *Carpocapsa pomonella* (Peterson & Hamner 1968), and (e) the viceroy butterfly, *Limenitis archippus* Cramer (Clark *et al* 1969). Certain long-day insects, the European corn borer *Ostrinia nubilalis* (Beck 1962), and the oriental fruit moth, *Grapholitha molesta* (Busck) (Dickson 1949) which tend to enter diapause under relatively short daylengths, have shown a very characteristic feature when reared under laboratory conditions, viz, under extremely short day conditions or total darkness they have a tendency towards a non-diapause development. These insects show two well defined critical daylengths and they fall into Beck's Type III group (Beck 1968) which show a "short-day - long-day" response to photoperiod. In the 'long-day' insects a low ambient temperature would tend to increase the threshold of critical daylength so that diapause would tend to occur at longer daylengths. A higher ambient temperature would have a reverse effect. In the current literature only a few examples showing a short-day response are documented (see Danilevskii 1965), the most noteworthy being *Bombyx mori* (Kogure 1933). In the short-day species a relatively high ambient temperature will tend to promote diapause induction.

The effects of photoperiod in Coleoptera has been demonstrated by several workers, and the work of de Wilde (1953) is especially noteworthy. He demonstrated that a short photoperiod was a significant factor in initiating diapause in the adult Colorado potato beetle, *Leptinotarsa decemlineata*. Other examples of diapause in Coleoptera, include the work of McMullen (1967), who reported that in *Coccinella novemnotata* Herbst lower temperatures and a reduced quantity of diet given to adult females increased the effectiveness of short and long photoperiods for inducing diapause. Hodek and Cerkasov (1961) showed that short photoperiods and low temperatures induced diapause in *Coccinella septempunctata* L.

Amongst the Diptera, Cragg and Cole (1952) reported that eggs obtained from wild caught females of *Lucilia sericata* (Mg.) in late summer produced a very high percentage of diapausing larvae even when they were reared in the laboratory under conditions favouring normal development. They were of the opinion that diapause in these species was of maternal origin. Fraser and Smith (1963), and Ring (1967) came to similar conclusions while maintaining cultures of *Lucilia caesar* L. Depner (1961) working with the horn fly *Siphona irritans* (L), reported that photoperiod, acting on the adult of the previous generation or on the developing unlaid egg, was probably responsible for the predisposition of larvae to enter diapause.

(2) Diptera: Culicoidea (Culicidae, Ceratopogonidae and Chironomidae).

(a) Embryonic diapause.

Among the mosquitoes diapause occurs in the embryo, larva or adult depending on the species and their geographical distribution. There are no reports on diapausing pupae. Late embryonic diapause is characteristic of the culicine genera - *Aedes*, *Psorophora* and *Haemagogus* (Clements 1963). In mosquito eggs it is essential to distinguish between embryos in diapause and those that are quiescent. Diapause eggs of *Aedes* will not hatch even though they are flooded during the summer, autumn or winter, until they have experienced a period of cold conditioning. Horsfall and Fowler (1961) observed that with *Aedes stimulans* (Walker) the optimum hatch was obtained when eggs were exposed to a cold period (4°C) for 90 days following a pre-cold period of 120 days of warm temperature (24°C). No hatch was obtained if they were exposed to only a cold period or only a warm period. Beckel (1958) reported that in *A. hexodontus* Dyar, eggs hatched only after cold conditioning. Eggs of *A. abserratus* (Felt & Young), in addition to warm summer temperatures, need at least two months at 2-4°C (Brust & Kalpage 1967). Telford (1958) observed that the univoltine *A. squamiger* (Coq.), which is a marsh breeder in California, entered an obligatory egg diapause in summer. His opinion was that induction of diapause was genetically determined whereas its termination was

brought on by low autumn temperatures. In his laboratory experiments, diapause was terminated only after eggs were subjected to temperatures below 8°C. Distinct from eggs that are in an obligatory diapause, are the fully embryonated eggs of *Aedes aegypti* (L). These remain quiescent in the absence of water and hatch immediately when flooded (Clements 1963).

The univoltine *Aedes* in temperate regions, regardless of their North-South distribution, enter an obligatory diapause, in the egg stage, which is genetically determined and appears to be independent of external environmental conditions. On the other hand in some diapausing multivoltine species of mosquitoes, diapause is governed by external factors, chiefly photoperiod and temperature. Baker (1935) observed that eggs laid in autumn by a strain of *A. triseriatus* obtained from tree holes in the vicinity of Ithaca, N.Y., failed to hatch. After five weeks of constant illumination to one group of eggs, diapause was terminated. Another group of eggs was kept under a short daylength for the same period of time, and only 2 eggs hatched. Vinogradova (1965), in experiments with *A. togoi* has shown that the photoperiod experienced by the female parent determined the type of egg laid. Under short day conditions (LD 12:12) most females laid diapausing eggs whereas under long day conditions (LD 16:8), and continuous illumination, the eggs developed without

interruption. Furthermore, under short day conditions more diapausing eggs were obtained at a lower temperature (15°C) than at a higher temperature (18°C). These experiments illustrate the combined effect of short day length and low temperature as being factors conducive to diapause induction. Short photoperiods during the developmental stages cause *Aedes albopictus* (Skuse) to lay diapausing eggs (Ren-lai 1966). Short photoperiods experienced by 4th instar larvae, pupae and adults produced the greatest percentage of diapause eggs in *A. atropalpus* (Anderson 1968). *A. nigromaculis* (Ludlow) and *A. dorsalis* (Meigen) enter a facultative diapause in the egg stage and in this way they survive the winter. In both species low temperature plays a major role in diapause development and termination (Telford 1963).

Hurlbut (1938) reported that in *Anopheles walkeri* Theobald, eggs that are laid in the fall enter diapause, but not eggs laid in summer. The diapausing eggs hatch only after a period of reactivation such as chilling (Matheson & Hurlbut 1937).

(b) Embryonic and larval diapause.

Khelevin (1958) reported that embryonic diapause in *Aedes caspius dorsalis* Mg. was determined by the temperature prevailing during the final stages of the development of the embryo. He observed that eggs laid by

the first generation females did not enter an embryonic diapause. He also found that diapause could not be induced in eggs by the action of low temperatures on the females completing their gonotrophic cycle, or by keeping the eggs at low temperature. However, eggs of the second and third generations produced diapausing larvae or diapausing eggs depending on the temperature. Although Khelevin is of the opinion that temperature was the factor inducing diapause in the autumn eggs, I think he overlooked the possibility that photoperiod may have had a significant influence on the induction of diapause in eggs laid by the females of the fall generation.

Kappus (1964), Kappus and Venard (1967) in a detailed study of diapause in *Aedes triseriatus* eggs reported that diapause in the egg stage is governed by the photoperiodic treatment of the eggs. The photoperiodic treatment of the adults had no effect on the induction of diapause in the eggs laid by them. When the temperature was reduced from 27°C to 18°C under short-day conditions the percentage of diapausing eggs was increased. In these experiments it was the Ohio strain of *A. triseriatus* that diapaused only as eggs whereas the strain from a more southern latitude, viz. the Alabama strain, diapaused as either 4th instar larvae or eggs.

(c) Larval diapause.

A few culicidae overwinter in the larval stage. Love and Whelchel (1955), working with a strain of *Aedes triseriatus* from South Western Georgia, showed that larvae which were obtained from eggs subjected to short photoperiods entered diapause (in the 4th instar) if the larvae too were reared under short photoperiods. The diapausing larvae pupated only when the photoperiod was lengthened. Similar results were obtained by Vinogradova (1967) with *A. triseriatus* in Russia. Wright (1966) observed that a temperature of 32°C terminated diapause in larvae of *A. triseriatus*. A low temperature of 10°C was best for the maintenance of larval diapause for over 16 weeks. He also found that the critical photoperiod which induced diapause in this species was between 13.30 - 13.45 hrs. of light per 24 hour cycle.

In the temperate zones there are some species of *Anopheles* which diapause as larvae. Roubaud and Colas-Belcour (1933) observed that daylength influences the life cycle of a tree hole mosquito, *Anopheles plumbeus* Stephens. This is a multi-voltine species and larvae that are subjected to spring and summer daylengths have an uninterrupted development. But eggs laid in September - October give rise to larvae which diapause as instar IV larvae and need at least 135 days to pupate at room temperature. Vinogradova (1962) made similar observations on this species in her work

in the U.S.S.R. Her results also indicate that instar II and III larvae are sensitive to changes in photoperiod. Baker (1935) observed that when collections were made from tree holes in autumn there were a large number of *Anopheles barberi* Coquillett overwintering as instar II larvae, and pupated only in the spring under the influence of longer photoperiods.

Vinogradova (1960) working with *Anopheles pulcherrimus* Theobald in Russia showed that a majority of the larvae reared at 18°C on a short photoperiod diapause as 3rd and 4th instar larvae. At a higher rearing temperature (28°C) the larvae did not enter diapause. These results tend to confirm earlier field observations of Trofimov (1942) who reported that *A. pulcherrimus* overwintered as 3rd instar larvae in his studies conducted in south eastern Azerbaijan in 1937-1938. *Anopheles claviger* (Meigen) diapauses as a 2nd instar larva (Kennedy, cited by Andrewartha 1952), with diapause being induced by short days; long days terminated diapause.

In populations of *Anopheles bifurcatus* L obtained from the northern part of its range (Luge, U.S.S.R., 59°N), the duration of larval diapause depends on the light rhythm and the induction of diapause in fourth instar larvae is associated with reduced daylength (Khodukin & Lisova 1953), Vinogradova 1963). Temperatures of 11° - 15°C appeared to be the optimum for the expression of short daylengths. At

a slightly higher temperature, 18°C, and diapause inducing short days, there was a sharp increase in the percentage of pupation. Populations of *A. bifurcatus* in the extreme south (South Italy and Israel), do not enter a diapause stage.

In the genus *Orthopodomyia* three species which show a larval diapause have been recorded. Tate (1932) observed that larvae of *O. pulchripalpis* (Rondani) which were collected in the field in autumn and brought to the laboratory, failed to pupate. Baker (1935) reported that *O. signifera* (Coq.) overwintered as larvae in tree-holes and pupated only in the spring under the influence of longer photoperiods. Chapman (1964) states that cold temperatures appear to be the principal factor which kept larvae of *O. californica* Bohart in diapause. Daylight did not seem to be important in terminating diapause in these larvae which breed in tree holes in cottonwood, willow and oak.

Hayes and Maxfield (1967) observed that larvae of *Culiseta melanura* (Coq.), collected in October from breeding sites in swamps near Taunton, Massachusetts, were in diapause. In earlier studies, Wallis (1962) showed that feeding 5% liver concentrate solutions stimulated pupation among 50% of the overwintering *C. melanura* Subsequent experiments showed that both feeding the larvae and extending the photoperiod to 20 hrs. terminated the larval

diapause in 80% or more of the larvae treated (Hayes & Maxfield 1967).

Chapman (1959) reported that in surveys of pools in Nevada he observed *Culiseta inornata* (Williston) larvae from November to early spring. He found that *C. inornata* is able to overwinter both as late instar larvae and adults in the southern latitudes. Hanec & Brust (1967) reported *C. inornata* overwintered only as adults in the northern latitudes (Manitoba, Canada). Chapman (1959) also observed overwintering larval populations of *Culex erythrothorax* Dyar.

Short photoperiods induce the larvae of the giant mosquito *Toxorhynchites rutilus* (Coq.) to enter diapause (Jenner & McCrary 1968). Chiba (1968) in his studies with a culicine *Armigeras subalbatus* (Coq.) observed that fall populations of this species diapause as instar IV larvae. Diapause was terminated in laboratory experiments when larvae were subjected to long daylight regimes (LD 16:8). Larvae of *Wyeomyia smithii* which breeds in the water contained in the leaves of the pitcher plant *Sarracenia purpurea* have been shown to enter a larval diapause in the fall and this diapause was terminated by long photoperiods, the critical photoperiod lying between LD 15:9 and 16 1/2: 7 1/2 (Jenner 1951). Experiments with *W. smithii* larvae collected from pitcher plants in the vicinity of Kenora, Ontario, showed that the critical photoperiod for terminating diapause was

between 14 1/2 - 15 hr. light per 24 hr. day (Smith, pers. comm.).

Baker (1935) reports that diapausing larvae of *Culicoides guttipennis* (Coq.) a ceratopogonid midge found in tree-hole water, terminated diapause when subjected to long daylengths.

The midge *Metriocnemus knabi* Coq., which develops in the pitcher plant, overwinters as mature larvae and development resumes only on the onset of favourable conditions in spring (Paris & Jenner 1959). Short photoperiods (8 - 12 hrs.) induced, while long photoperiods (more than 13 hrs.) terminated diapause in *Metriocnemus*. Engelmann and Shappirio (1965) reported that in the Chironomid, *Chironomus tentans* Fabr. short days favoured and long days terminated diapause.

(d) Adult diapause or hibernation.

Adult diapause in mosquitoes has been reported in several *Anopheles* of the temperate regions. It is only the adult female that hibernates and is generally induced by short photoperiods on the fall generations of these multivoltine species. Diapause in adult female mosquitoes has been reported in *Anopheles labranchiae atroparvus* Van Thiel (Swellengrebel 1929; de Buck and Swellengrebel 1934). Also in *An. sacharovi* Favre (Mer 1931), *An. hyrcanus* (Pallas), *An. superpictus* Grassi and *An. maculipennis messeae* Falleroni

(Vinogradova 1960), *An. psuedopunctipennis franciscanus* McCracken (Chapman 1961), and *An. freeborni* Aitken (Chapman 1961, Depner & Harwood 1966). Vinogradova (1958) showed that the adult diapause in *An. maculipennis messeae* was determined by the short autumnal photoperiods experienced by the 3rd and 4th instar larvae and pupae.

Adult diapause is in fact a reproductive diapause because development of ovarian follicles is halted. Swellengrebel (1929) coined terms to explain the difference between those females which in natural populations would not blood feed and as a result not develop eggs from those that would take a few blood meals but still not develop their ovaries. The former behaviour was termed '*concordance gono-trophique* ' and the latter '*dissociation gonotrophique*'. Swellengrebel 1929, de Buck & Swellengrebel (1934) observed that natural populations of *Anopheles labranchiae atroparvus* exhibited 'gonotrophic dissociation', whereas *An. maculipennis messeae*showed 'gonotrophic concordancy'. The fact that the ovaries of *An. l. atroparvus* do not develop after blood meals taken in winter indicates that the sequence of events which normally follows a blood meal is inhibited in some way. The exact explanation of this mechanism is still unknown. Detinova (1945) was of the opinion that failure of ovarian development even after a blood meal was due to failure of hormone production. She showed that the corpora allata of hibernating females of

An. maculipennis messeae differed in staining properties from actively reproducing females. The corpora allata also increased in size during diapause and diminished in spring after the first blood meal and was similar to corpora allata of the actively reproducing summer females. de Buck and Swellengrebel (1934) have quite rightly pointed out that the terms 'gonotrophic dissociation' and 'gonotrophic concordancy' describe the behaviour of the mosquito under natural conditions. But under artificial laboratory conditions and forced feeding techniques they could behave differently from the behaviour pattern in nature. In laboratory reared females of *Culex pipiens pipiens* L. reproductive diapause was expressed mainly as a result of the refusal of females to feed on blood, whereas in laboratory reared *An. maculipennis messeae* under short photoperiods entered a diapause expressed in gonotrophic dissociation (Danilevskii and Glinyanaya 1958). These workers also showed that the chief external factor responsible for the induction of diapause was shortened daylengths during the imaginal stage in *C. pipiens* whereas in *An. maculipennis messeae* it was manifested only when larvae too had developed under short-day conditions. Tate and Vincent (1936) reported that the fall species of *C. p. pipiens* do not feed on blood but instead feed on plant juices and build up fat reserves before entering hibernation. Vinogradova (1960) observed that although *C. p.*

pipiens fed on blood the ovaries still failed to develop in hibernating females.

Several reports of females of *Culiseta* and *Culex* species showing reproductive diapause are known. This phenomenon has been reported to occur in *Culiseta incidens* (Thomson), (Chapman 1966); *Culiseta annulata* (Schrank) (Chapman 1966, Service 1968); *Culiseta impatiens* (Walker) (Chapman 1966, Frohne 1953); *Culex tarsalis* Coq. (Bellamy & Reeves 1963; Chapman 1966, Kliewer *et al* 1969); *Culex tritaeniorhynchus* Giles (Newson and Blakeslee 1957); *Culex quinquefasciatus* Say (Eldridge 1966); *Culex restuans* Theobald (Wallis 1959); *Culex pipiens pallens* Coq. (Hosoi 1954); *Culex apicalis* Adams (Linam & Nielson 1966); and *Culex pipiens pipiens* (Tate & Vincent 1936), Danilevskii & Glinyanaya 1958, Service 1968).

In most of the examples cited above, the adults enter a reproductive diapause influenced by short photoperiods and low temperatures. These females showed a reluctance to take blood, but those that feed under these conditions in nature or in controlled laboratory experiments utilize the blood to build up large quantities of fat reserves (Eldridge 1963, 1966, 1968). Termination of diapause was brought on by an increasing daylength in spring and more so due to a critical threshold of heat accumulation during the hibernating period. This is why females resume blood feeding early in spring even though the temperature

during this period is lower than in autumn at the time of entering hibernation.

Short daily photoperiods increased the size of the fat body in *Culex tarsalis* in laboratory experiments of Harwood and Halfhill (1964). Kliewer *et al* (1969) concluded that a similar mechanism occurs in nature. Depner and Harwood (1966) reported a similar behaviour among two latitudinally different strains of *Anopheles freeborni*. They observed that there was a difference of one hour in the critical photoperiod between the strains. This was consistent with the expected seasonal differences in photoperiod and temperature for the two areas. Similar latitudinal responses to photoperiod have been reported for *Anopheles maculipennis messeae* and *Anopheles hyrancus* by Vinogradova (1960).

Harwood and Takata (1965) have shown that the fat reserves built by *Culex tarsalis* when exposed to hibernation inducing photoperiods and temperatures, have a greater accumulation of unsaturated fatty acids. This is in agreement with the findings of Barlow (1964) who studied fatty acids of arthropod species and drew attention to the fact that species from colder climates may have more unsaturated fatty acids than those from warmer climates which have more saturated fatty acids. The unsaturated fatty acids have a lower melting point than saturated ones and therefore it is advantageous in these temperate hibernating females because the unsaturated fatty acids could be mobilized more readily in times of cold stress.

AUTOGENY IN CULICIDAE

Only a brief account of autogeny in Culicidae will be presented here. For a more detailed account of autogeny the reader is requested to refer to the first comprehensive literature review on autogeny in biting flies, which has just been completed by my colleague Stephen Smith (1970). Reference could also be made to Downes (1958) where he deals with the feeding habits of biting flies.

A number of the blood sucking Nematocera are now known to be able to develop and lay the first batch of eggs without having to ingest a blood meal. This behaviour has been known for many years in mosquitoes. Theobald (1901), Knab (1907) reported that they observed various species of Culicidae "sucking the juices of flowers". Knab (1907) thought that most of these haematophagous females resorted to the nectar of flowers when a blood meal was not obtainable and as such served as a supplementary diet which prevented starvation. He has also noted that the pitcher plant mosquito, *Wyeomyia smithii* does not suck blood, neither did *Culex territans* Walker a species common in summer in eastern North America. He had also observed female mosquitoes of *Megarhinus septentrionalis* D & K, probing flowers for nectar. This mosquito does not attack animals because the proboscis is unfit for piercing the skin. Trembley (1947) cites the work of Sen

(1917) who reported that *Aedes albopictus* (≡ *Stegomyia scutellaris*) laid viable eggs when females fed on milk and sugar, peptone and sugar and sugar alone.

Most of the work on autogeny has been reported on studies connected with the *Culex pipiens* complex. Roubaud (1929) observed that females of certain strains of *Culex pipiens* were able to lay fertile eggs without a blood meal. He, therefore, coined the term 'autogeny' to describe certain females in this complex which had the capacity to develop and lay eggs without food of any kind. Egg formation which results from feeding on blood or an external source of protein is referred to as 'anautogeny'. Boissezon (1933) claimed that autogeny in *Culex pipiens* was not a racial characteristic, but is dependent on the larval diet. This view was disproved by Roubaud (1934), and Tate and Vincent (1936). Marshall and Staley (1935, 1936) reported for the first time that a British strain of *Culex pipiens* was both autogenous and stenogamous (i.e. able to mate in small cages). These workers also reported (1936) that laboratory reared females of *Theobaldia subochrea* Edw. exhibited these two phenomena.

Since this early realization of the phenomenon of autogeny among the biting flies, research in this field has gained a tremendous impetus and several aspects of it have been, and are being worked on. Trembley (1945, 1947) studied the biology of *Aedes atropalpus* which breeds in

rock pools along rivers and streams and concluded that it was autogenous, stenogamous, homodynamic and did not exhibit spanogamy (i.e. a decrease in the number of females with the increase in successive generations). Dobrotworksky (1954) reported that three autogenous species were found in Victoria, Australia. They were *Culex pipiens molestus* Forsk., *Aedes concolor* Taylor, and *Tripteroides tasmaniensis* Strickl. Chapman (1962) reported autogeny in ten species of mosquitoes from Nevada, U.S.A., namely, *Aedes communis* (Degeer), *A. campestris* D & K, *A. dorsalis* Meigen, *A. melanimon* Dyar, *A. nigromaculis* Ludlow, *A. niphadopsis* D & K, *A. schizopinax* Dyar, *Culiseta incidens* (Thomson), *Culex tarsalis* Coq., and *Culex erythrothorax* Dyar. Autogeny has also been reported in *Aedes togoi* (Theobald) (Lien 1960, Laurence 1964), and *Aedes taeniorhynchus* (Wied) (Lea 1964b, Lea & Lum 1959), and in *Culex peus* Speiser and *Culiseta inornata* (Williston) (Washino & Shad-del 1969). This phenomenon has also been reported in the sub-family *Anophelinae* (Detinova 1962) and in the sub-family *Culicinae*, tribe *Sabethini* viz, *Wyeomyia smithii* (Price 1958).

A few of the other important records of autogeny among *Aedes* are those from Northern Canada and the Arctic, Beckel (1954), Hocking (1954), Kalpage & Brust (in press) on *Aedes communis;* by Smith & Brust (1970) on *Aedes rempeli* Vock., and by Corbet (1964, 1967) on *Aedes nigripes* (Zett.) and *Aedes impiger* (Walker) in the high arctic. The genus

Toxorhynchites is said to be wholly autogenous (Chapman 1962). With an increasing number of species exhibiting autogeny it would be safe to assume that this phenomenon is not rare. The main reason for not observing this phenomenon in the past has been the lack of good field studies, i.e. rearing adults from field collected larvae or pupae and examining these for autogeny after a week or so.

It is now well established that autogeny is an inherited character (Spielman 1957, O'Meara & Craig 1969) and that it also depends on the ability of the larva to build large fat reserves which could be utilized for the maturation of eggs without a blood meal. Roubaud (1932) suggested that the larval muscles were broken down later in the adult and contributed to the protein and lipid reserve in the fat body. As a result of the histolysis of the larval muscles, yolk was deposited in the developing ovarian follicles. It was his opinion that the reserves from larval muscles were sufficient for only one oviposition. The fat, glycogen and nitrogen composition of pupae and adult females of autogenous *C. p. molestus* is significantly greater than these amounts in the anautogenous *C. p. pipiens* when they are reared under comparable conditions (Roubaud & Toumanoff 1930; Twohy & Rozeboom 1957; Rozeboom & Twohy 1958). However, a greater quantity of reserve food material in the autogenous form is not the sole criterion for the ability to develop eggs

without an external source of protein. Clements (1956) noted that although the fat body in *Culex p. pipiens* was smaller than that in *C. p. molestus*, it contained sufficient reserves to permit at least some eggs to develop. Clements therefore postulated that there was some other inherent ability such as the production of hormones which may help in utilizing the reserves for developing eggs autogenously. The autogenous females of *Culex tarsalis* laid fewer egg rafts and each raft contained fewer eggs than those deposited by the anautogenous females (Bellamy & Kardos 1958; Chao 1958). In Laboratory experiments with *C. tarsalis*, Kardos (1959) observed that there may be a threshold of larval nutrition which affects the development of autogenous eggs. Below this threshold autogeny may be suppressed, but there was no significant difference in expression of autogeny above certain nutritional levels. Experiments with the highly autogenous strain of *Aedes taeniorhynchus* (Wied.) showed that autogeny could be reduced in the population by altering the quality and quantity of the larval diet (Lea 1964b).

Field populations of *Culex tarsalis* in Northern California, U.S.A. show a wide seasonal change in both the percentage of autogenous females and in the number of eggs laid per female (Moore 1963). From field collected pupae the autogenous egg production was 116 eggs per female in May, and only 27 eggs per female in September. Moore

attributes this variation in autogeny to a combination of temperature, nutrition, and population density. Gaschen (1932) stated that reproduction of autogenous females is a function of temperature and larval nutrition. Harwood (1966) showed that there was a relationship between photoperiod and autogeny in *Culex tarsalis*. His experiments showed that autogeny suddenly increased with an increase in photoperiod from 8 hr. light to 10 hr. light per day and remained steady thereafter till 16 hr. light per day. But once the optimum range was exceeded longer photoperiods decresed autogeny. Knight (1951), and Mattingly (1952, 1953) claim that the percentage of autogeny is low in natural populations. Mattingly has suggested that the high frequency observed in laboratory colonies is a result of unconscious selection. It was in fact due to strong selection pressure in laboratory rearings that Lea (1964a) obtained autogenous individuals of *Aedes aegypti* from an anautogenous strain which was totally dependent on a blood meal for egg maturation.

Most of the investigations regarding autogeny in mosquitoes have centered around the physiological and biochemical aspects of the phenomenon. However there are some studies dealing with the mode of inheritance of this character. Most of these studies were done with *Culex pipiens*. Roubaud (1930) proposed that it was a monofactorial mode of inheritance and said that it was due to a single recessive

gene in the homozygous condition. Later Spielman (1957) and Laven (1967) disregarded the monofactorial hypothesis and have postulated multifactorial hypotheses. O'Meara and Craig (1969) studied the inheritance of autogeny in *Aedes atropalpus* and their back crosses to the parental strain and the F_2 progeny showed that autogeny in this species is determined by a single dominant, autosomal gene with anautogeny being recessive.

From the experimental data before us we could state that although autogeny is genetically determined (Roubaud 1930, Spielman 1957, O'Meara & Craig 1969) the mechanism promoting autogenous ovarian development is undoubtedly hormonal involving the corpora allata and the medial neurosecretory cells (Lea 1963, 1964b). Other factors such as larval nutrition, developmental temperature, photoperiod and population density (Krishnamurthy & Laven 1961) are also significant.

MOSQUITO STUDIES AT FORT CHURCHILL, MANITOBA.

A biting fly survey and an experimental laboratory field program was started in 1947 at Fort Churchill, Manitoba, Canada (58°N), to study the biology of the various species present in that area with the intention of formulating satisfactory control measures (Twinn *et al* 1948, Hocking *et al* 1950; Twinn 1950, Twinn *et al* 1950). More

specific ecological studies on *Aedes communis* in Churchill with particular reference to the behaviour of the immature stages to its environment (Haufe 1957), and the development and emergence of this species at Churchill (Haufe & Burgess 1956) were conducted subsequently. *Aedes communis* was selected for these studies because it is the most abundant species that at times occurred by itself in certain types of pools in the wooded areas. Beckel (1958) attempted to colonize the *Aedes* mosquitoes of Northern Canada. He reported that he was unable to obtain sufficient successful matings, and oviposition of fertile eggs, in cages containing adults of *A. hexodontus*, *A. nigripes*, *A. excrucians* (Walker) or *A. campestris*. *A. communis* fed only on raisin juice, and maintained in a cube cage 2 feet to a side, mated and laid fertile eggs, but the numbers were insufficient to maintain a colony. The possibility that there may be two forms of *A. communis* in the Churchill area has been proposed by Hocking *et al* (1950) based on their measurements of the ratio of proboscis length to wing length. They proposed that the small form with its habit for developing in large numbers in rather special woodland pools should be regarded as the true *Aedes communis* (Degeer) and the larger form a new species rather closer in its habits to *Aedes punctor* (Kirby).

An interesting and important question that arose in the minds of these workers was regarding the source of

food of the female mosquitoes and other northern biting flies, and whether a blood meal was always essential for developing the eggs. Several female mosquitoes, of most species at Churchill, were seen to have pollinia attached to the head near the eyes, which indicated that a large number visit the flowers of the northern orchid *Habernaria obtusata* for nectar (Twinn *et al* 1948; Hocking *et al* 1950). Recently Thien (1969a.b) (in laboratory studies) has shown that mosquitoes feed on the nectar in *Habernaria* and are agents in pollination. Corbet (1964, 1967) has observed *A. nigripes* & *A. impiger* to feed on nectar of flowers of *Dryas integrifolia* in his studies at Hazan Camp, Ellesmere Island, N.W.T. (81° 49'N). West and Jenkins (1951), in laboratory studies at Churchill, showed that *Aedes communis* when fed flowers with radioactive phosphorous, took up sufficient material, probably nectar, to become highly radioactive. Hocking (1953) concluded that northern biting flies, including mosquitoes, obtain their energy for flight from the nectar of flowers and that peaks of nectar production on the tundra and in the forest coincide with peaks of flight of the tundra and forest mosquitoes respectively. Observations made on the crop of swarming female mosquitoes indicated that they were full of nectar. All these studies lend indirect evidence to the hypothesis that some subarctic and arctic mosquitoes ingest plant juices and may be able to produce viable eggs without a blood meal.

From a study of field collected females of *A. communis* in Churchill, Manitoba, Hocking (1954) reported that this species utilizes the nitrogen from autolysis of the flight muscles to develop its eggs autogenously. Beckel (1954) on the other hand reported that females reared in the laboratory from pupae collected in the field developed and laid eggs when fed on sucrose and raisins, without utilization of the flight muscles. He suggested the possibility of the adults using fat reserves and larval muscles in the adult as possible sources of protein for developing the eggs.

CHAPTER III

MATERIALS AND METHODS

This chapter is divided into three main parts. Part 1 describes the methods that were used to select an oviposition medium and to maintain a successful culture of *Aedes atropalpus* in the laboratory. Part 2 deals with the methods and techniques that were adopted in the photoperiodic experiments in connection with (a) diapause and fecundity in *A. atropalpus* and (b) in studies dealing with the effect of photoperiod on feeding activity and ovarian development in *Culiseta inornata*. Part 3 covers the studies on autogeny that were undertaken at Fort Churchill, Manitoba, and also with experiments that were conducted with the northern species in the laboratory of the Department of Entomology, University of Manitoba, Canada.

1. INVESTIGATIONS ON OVIPOSITION MEDIA AND COLONY MAINTENANCE IN *AEDES ATROPALPUS*.

(a) Selection of oviposition media by autogenous *A. atropalpus* (Belleville Strain).

When I began to culture the autogenous *A. atropalpus* (Belleville Strain), I observed that a large number of eggs were deposited on the water in the dish containing the pupae rather than on the moist paper towelling that was

provided in a funnel for the purpose of oviposition. Therefore, a series of experiments were designed to determine which type of liquid medium was preferred for oviposition.

The test solutions used were:

(i) Larval "holding" medium. 150 late fourth instar larvae were removed from the rearing pans, washed well in two to three changes of distilled water and placed in a 250 ml. pyrex crystallizing dish containing 125 ml. distilled water (holding medium). They were allowed to remain in the water for 24 hrs. The water was filtered using "Whatmen Filter Paper" No. 1, (medium), and the filtrate used in the tests.

(ii) Emergence water. This was obtained by placing 150 pupae (washed in distilled water) in a pyrex crystallizing dish containing 125 ml. distilled water. After emergence of adults was completed, the solution was filtered to remove the pupal exuviae. The filtrate was used in the experiments.

(iii) Pupal "holding" medium. This differed from emergence water in that it was collected 24 hours after 150 pupae were placed in 125 ml. of distilled water. No adults emerged from this water.

The adults of autogenous *A. atropalpus* were reared in plastic cube cages 25 cm. to a side supplied with moisture wicks and honey. The larval rearing methods

are described in a later section. Pyrex crystallizing dishes were used to contain the oviposition test solutions. In experiments (a) to (c) of Table I, each cage contained two oviposition dishes, one containing the test solution and the other the control which was distilled water. In experiments (f) to (g) of this table, two different test solutions were used in each cage. The experiments were performed in a constant temperature (20°±1°C) room with a photoperiod of 16 hr. light per day. Under these rearing conditions, oviposition commences within 5-6days of emergence. The dishes containing the test solutions were placed in the cages on the fifth day after the females were placed in the test cages. Eggs laid in these dishes were removed each morning with the aid of a fine camel hair brush and the position of the dishes reversed daily. Any dead adults on the surface of the liquid were removed. Every two days fresh test solutions were placed in each cage.

(b) Storage of eggs.

A batch of eggs was divided randomly into four plastic petri dishes each containing approximately 200 - 300 eggs. Two of the dishes contained filter paper on which the eggs were stored, and the other two dishes contained a layer of glass wool over which was placed a piece of nylon cloth. The eggs were pipetted onto the cloth or filter paper. All four dishes were moistened with distilled

water. In addition to distilled water, two of the dishes, one of which contained a filter paper base and the other a nylon base, were sprinkled over with a solution of methyl para-hydroxybenzoate (4 gm. in 1000 ml. distilled water). All four dishes were then seeded with fungal spores by dusting a heavily infected pad over the test dishes. The fungi used in the seeding were not identified but were taken from a dish of *A. atropalpus* eggs which was very heavily infected. A point scale system was used to denote the amount of growth in each experimental dish after a given period of time. The dishes were periodically moistened and never allowed to dry out. Every month the two dishes which contained para-hydroxybenzoate were given an additional sprinkling of the solution, just enough to keep the surface moist. Any excess liquid was removed with a pipette. The dishes containing the eggs were stored in a constant temperature room ($20\pm1°C$) at a relative humidity (R.H.) of approximately 60-70%. The eggs in the dishes were near 100% R.H.

(c) Colony maintenance.

Two strains of *A. atropalpus* were cultured in the laboratory. The autogenous strain was established in our laboratory in September, 1966, from eggs received from Dr. J. A. Armstrong, formerly of the Department of Entomology, Queens University, Kingston, Ontario, Canada.

These eggs were from a strain of *A.atropalpus* which he had originally obtained from larvae breeding in rock pools in the vicinity of Belleville, Ontario (44° 15'N). The anautogenous *A. atropalpus* was obtained from Dr. George Craig's Laboratory, University of Notre Dame, Indiana, U.S.A. This strain originated from a population of wild caught females in the vicinity of Austin, Texas, U.S.A. (30° 15'N). The autogenous and anautogenous *A. atropalpus* will subsequently be referred to as the Belleville strain and Austin strain respectively.

Eggs were hatched by placing them in a hatching medium at room temperature. The hatching medium used was nutrient broth (a Difco Laboratories product) dissolved in tap water at a concentration of 1:1000 by weight. Within 3-4 hours a large percentage of the eggs hatched. Any eggs that failed to hatch within 24 hrs. were discarded. The larvae were transferred by means of a pipette to white plastic pans 21 x 30 x 8 cms. containing 350 ml. distilled water. Since the number of eggs laid by the autogenous females is determined to a certain extent on the larval nutrition, a very rich diet consisting of dog food, Fleishman's dry yeast, yeast extract, blood fibrin, blood meal and two fish foods - Tetramin E and L - referred to as the seven component (7C) diet was used. (See Appendix B for composition and proportion of diets used in various experiments.) A pinch of peat moss was also added to the water in each pan. The larval medium was changed every

second day and fresh food added. Various numbers of larvae per pan were treated and it was found that survival yielding a high percentage of healthy pupae occurred when not more than 100 larvae were reared in each pan.

Pupae were placed in pyrex crystallizing dishes 3/4 filled with distilled water. Adults emerged in a transparent plastic cube cage 25 cm. to a side. Each cage was supplied with a wet wick of paper towelling and a dish containing emergence water for oviposition. The autogenous females laid the first cycle of eggs without a blood meal. Honey pads placed in small plastic containers was the sole source of imaginal food for the Belleville strain. On the other hand the anautogenous Austin strain needed a blood meal for developing eggs. The anautogenous female adults were aspirated into small plastic feeding cages 15 x 2.5 x 2.5 cms. which had plastic mesh on two sides. Approximately 20 females were placed in each cage and fed on human blood. After two blood meals they were returned to the cube cages and provided with a pad of honey. At least two blood meals were required before a substantial number of eggs was obtained. In order to obtain non-diapausing eggs of *A. atropalpus* the laboratory stocks were reared in the constant temperature room (20°±1°C) which had a non-diapause photoperiod of 16 hr. light per day. The females oviposited on the surface of the water. To facilitate easy removal of the eggs they were wetted. Once they settled to the bottom they were easily picked up by means of a pipette. The eggs

were then stored as described earlier. To prevent hatching, the eggs were stored at 15°C for ten days, to enable them to complete embryonation, and then transferred to 10°C.

2. PHOTOPERIODIC EXPERIMENTS.

(a) Experiments on the induction of diapause in *Aedes atropalpus* (Belleville Strain).

The larvae used in these experiments were obtained from the stock culture. The diapause induction experiments, except when otherwise stated, were conducted in wooden boxes, 60 x 48 x 50 cms. constructed to provide controlled internal illumination (Fig. 1a). Some photoperiod experiments, which are mentioned later, were conducted in CreLab Type incubators (Fig. 1b). Each box or incubator was illuminated by a 15 watt-cool white daylight type fluorescent lamp wired to a timer. No crepuscular lighting was provided in these experiments.

The larvae and adults received from 40 to 100 ft-candles of light, depending on the position of the pan or cage in the light boxes, and from 30 to 180 ft-candles in the CreLab Type incubators. The light intensities were measured with a Model 756 Weston illumination meter. The variability of light intensity within the light box or incubator, did not appear to influence the results of the experiments. The minimum intensity recorded

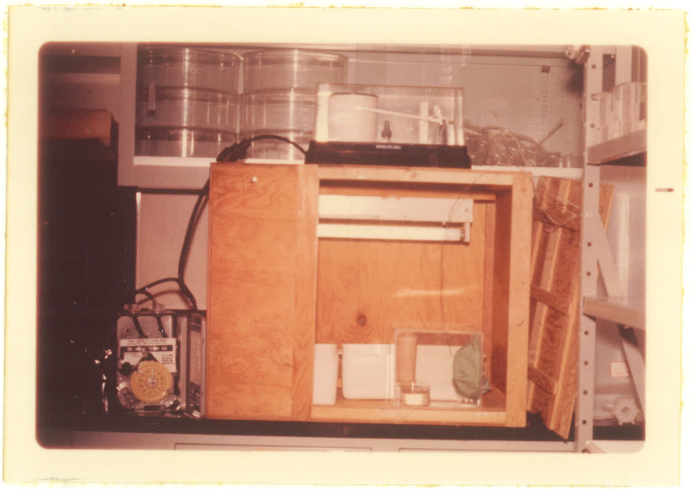
Fig. 1 Appartus used in photoperiod experiments

(a) Photoperiod controlled boxes, 60 x 40 x 50 cms., constructed of plywood and provided with a 15-watt fluorescent lamp. Temperature in this box was 23°±2°C (see 2(a)).

(b) Photoperiod and temperature controlled cabinets used in experiments with *Aedes atropalpus* 2 (a) and *Culiseta inornata* (see 2(d)).

(c) Photoperiod controlled boxes, 30 x 30 x 30 cms., constructed with the ballasts for the 8-watt fluorescent lamp fixed outside the box. This helped to dissipate the heat and the temperature inside the box was 25°±1°C. (see 2(b)).

(d) Flexible fibre optics light guides conduct light from flood-lit lamps into wooden boxes placed in a temperature controlled incubator. The light guides are used to focus a beam of light on the anterior or posterior portion of mosquito eggs arranged in a circle (see 2(b)).



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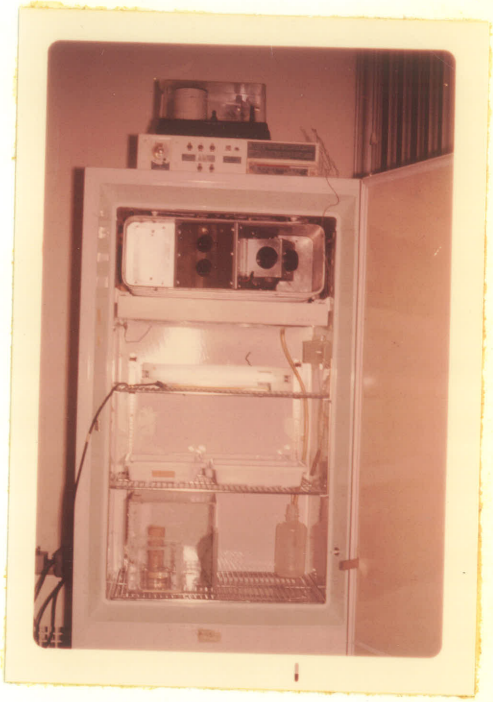

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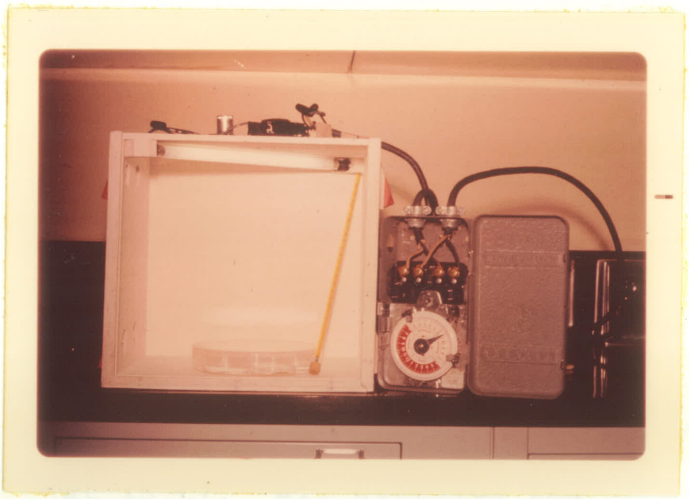
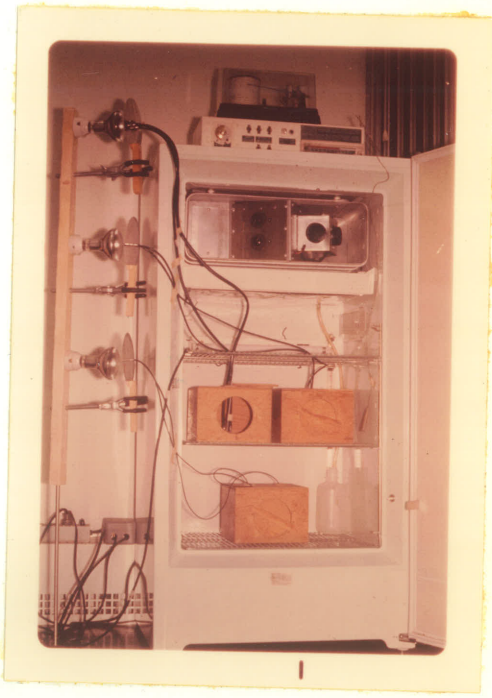


c



d

(30 ft-candles) was many times greater than the threshold of light response in photoperiod-sensitive insects reported by Lees (1955).

The temperature in each light box was recorded on a thermograph and was 23±2°C during the course of each experiment. The increase in temperature was because the ballasts for the lamps were mounted inside the boxes.

The handling of the larvae, pupae and adults was similar to that reported in the earlier section and was conducted during the light-period,i.e. during the photophase of the photoperiod cycle. In the experiment in which the box was in constant darkness, handling of the experimental material was done in the dark by using a red lamp. The eggs were removed daily, pipetted on to a piece of moist nylon cloth and stored in petri dishes. The eggs were maintained at the photoperiod provided during oviposition.

For the purpose of the experiments, diapause was considered as a resting stage of the mature embryo. Once initiated, diapause persisted even under conditions favourable for hatching. The eggs were tested to determine whether the embryos were in diapause by placing them in hatching medium (10 days after oviposition when the experiments were conducted at 23°C and 30°C, and 15 days after oviposition when the experiments were performed at 15°C). All hatching was conducted at room temperature

and the medium used was nutrient broth. The eggs were kept in the hatching medium for 24 hours. Larvae were counted the following day and unhatched eggs were dissected in physiological saline. All fully formed whitish embryos which showed signs of movement in the physiological saline were recorded as viable and in diapause, while eggs that were not embryonated or had brownish embryos and showed no signs of life were recorded as dead and were discarded.

(b) Experiments to terminate diapause in *A. atropalpus* (Belleville Strain).

Eggs used in these experiments were obtained from autogenous *A. atropalpus* (Belleville strain) reared at 23°±2°C, and a photoperiod of 8L:16D. To determine whether the diapause was intense or whether eggs would hatch within 3-4 weeks when stored at 23°C and 8L:16D, different batches of eggs were subjected to the hatching stimulus at intervals of ten days.

To determine the effect of high temperatures on diapausing embryos, eggs were divided into three batches and kept at 23°C and at a diapause sustaining photoperiod of 8L:16D for periods of 30, 60 and 90 days. At the end of each period the eggs were transferred to an incubator maintained at 30°±0.5°C and which was provided with 8L:16D photoperiod. Eggs from the 30°C incubator were placed in hatching medium, 5, 10 and 20 days after

treatment at this temperature. The number of eggs hatched were counted the following day. All unhatched eggs were dissected and only viable eggs were recorded.

To determine the effect of two different photoperiods on eggs in diapause, different batches of eggs were subjected to (a) a short photoperiod of 8L:16D and (b) a long photoperiod of 16L:8D. The photoperiod boxes (30 x 30 x 30 cms.) used here were made of plywood and the ballasts for the fluorescent lights were placed outside the box (Fig. 1c). This helped to dissipate the heat produced by the ballasts. At intervals of 10, 30, 60,and 90 days, 50 eggs from each treatment were placed in the hatching medium. The larvae and unhatched eggs were recorded the next day and the unhatched eggs were dissected as previously described. Only viable eggs were counted.

Another experiment was set up to determine, by a different method, whether diapausing embryos could perceive light and, if so, whether the site of the photoreception was situated at the anterior end or posterior end of the embryo. For this purpose diapause *A. atropalpus* eggs (i.e. eggs from 8 hr. light treatments which failed to hatch after 10 days) were arranged in circles of three different diameters, approximately 3mm, 5mm, and 12 mm, equal in diameter to the circle of light focussed on the paper when light tubes were placed a few millimeters above the surface. The eggs were arranged on moist filter

paper in small plastic petri dishes. The dishes were placed in wooden boxes (23 cms x 23 cms x 18 cms) into which the "Flexible Fibre Optics Light Guides" (An American Opticals product) were led and held vertically above the circle of eggs. Some of these circles had eggs with the anterior poles pointing towards the centre and others had the posterior poles pointing towards the centre. A very narrow strip of plasticine was used to cover the distal half of the egg. In this manner the light affected only the proximal portion of the egg. The eggs were arranged with the dorsal surface facing upwards. It was easy to cover the eggs with plasticine by working under a stereomicroscope. The temperature of the incubator was maintained at 23°±1°C. The fibre optic tubes were led out of the incubator to three light sources maintained on a stand outside the incubator (Fig. 1d). The light sources were wired to timers which were set to provide 8L:16D and 16L:8D. The eggs were taken out for hatching 30, 60 and 90 days after light treatment.

(c) Effect of photoperiod on fecundity in autogenous *A. atropalpus* (Belleville Strain).

In these experiments different photoperiods were tested to determine their effect on fecundity. The photoperiod treatment was applied to larvae, pupae, and adults. Rearing techniques were similar to those mentioned earlier, but adults were handled differently. Females from each

photoperiod treatment, were removed from the emergence cages following mating (2 - 3 days old) and placed singly in small cylindrical cages of 1.6 cm acrylic tubing (1.5 mm wall, 1.27 cm. long). The top end of each cylindrical cage was covered with fine mesh nylon cloth and the bottom end was left open. The circular cage was set on an oviposition pad, which consisted of a circular piece of moist paper towelling the same diameter as the cage. The oviposition pads were kept moist by using cheesecloth pads and moist wicks. A piece of cotton, soaked in honey, was placed on the nylon mesh of each circular cage. The adults in the single-female-cages were then returned to their respective photoperiod treatments. After 10 days the females were removed from the light boxes, anaesthetized with ether, and dissected in physiological saline under a compound stereomicroscope. The number of eggs left in each ovary were counted and added to the number laid.

(d) Effect of photoperiod on biting activity and ovarian development in *Culiseta inornata*.

A laboratory colony of *Culiseta inornata* was established from adults collected in the summer of 1968 in the vicinity of the Glenlea Research Station, Winnipeg, Manitoba. The laboratory colony was maintained in the constant temperature room (20±1°C) under a photoperiod of 16L:8D.

For the experiments in this study, *Culiseta inornata* was maintained through all the developmental stages in incubators held at a constant temperature and photoperiod depending on the treatment. The different temperatures used were 10°C, 15°C, 20°C, 25°C and 30°C. The photoperiods used were 8L:16D, 12L:12D, and 16L:8D. The illumination in each incubator was provided by a 40 watt fluorescent lamp and the photoperiod was controlled by 24 hr. timers. The relative humidity in the incubators was approximately 60%. The larval rearing procedures were similar to those outlined for *A. atropalpus*. As the adults found it difficult to emerge at 10°C, the pupae (after 48 hrs.) were placed in a 20° or 15°C incubator with a similar photoperiod until the adults emerged. Upon emergence these were returned to the 10°C incubator. The adults were blood fed 5 days after emergence. This was done by placing the adults in 15 x 2.5 x 2.5 cm feeding cages and placing the cages on my arm for a period of 1/2 - 1 hr. At the end of the feeding trial period, the mosquitoes were examined for the presence of blood in the stomach. A female was recorded as "fed" if even a trace of blood was visible in the abdomen. Very often unaided visual examination was sufficient, but in some instances, expecially when the female has had only a small blood meal, she was examined under a stereomicroscope for confirmation. After blood feeding the females were returned to their respective incubators

and offered honey. After all the blood in the abdomen had been digested, the females were dissected in physiological saline and the stage of ovarian development noted.

In all the photoperiod experiments, the light and dark cycle was 24 hrs. in duration. In the results and discussion, photoperiods are therefore designated in terms of their light (L) and dark (D) régimes, i.e. 12L: 12D designates a photoperiod having a 12 hour light (photophase) and a 12 hour dark (scotophase) per 24 hour day.

3. STUDIES ON AUTOGENY IN *AEDES* SPECIES AT CHURCHILL, MANITOBA.

First to fourth instar larvae and pupae were collected from pools in the Camp Nanuk area, 7 miles south-east of Fort Churchill, and the Goose Creek area approximately 13 miles south-west of Fort Churchill (58°N). The pools in the Camp Nanuk site were of the completely exposed type which is common on the tundra. This tundra site was bounded by a lake on the south and south-east sides and a forested area commencing on the south-west side. (See sketch, Appendix C1.)

Larvae were collected from the pools with dippers, and excess water was removed by straining through a fine mesh copper sieve. The larvae were concentrated into small cardboard cartons, using a separate carton for each pool. The temperature and pH of the water, and

temperatures of the air and the water at each pool were recorded. The cartons containing larvae were placed in styrofoam coolers for transport to the laboratory at Fort Churchill. In the laboratory, larvae from each pool were placed in white plastic pans 21 x 30 x 8 cms approximately 100 per pan in 350 ml distilled water, or in clear plastic dishes (15 cm in diameter) each containing 50 larvae per 100 ml distilled water. A pinch of peat moss was added to the larval rearing medium. Various larval diets were used (See Appendix Bii) depending upon the experiment. The larval rearing medium was changed, and larvae were fed, every other day. When the larvae reached the fourth instar stage they were separated to species using the key to the fourth instar larvae of North America by Carpenter & LaCasse (1955). They were then grouped according to species, diet and pool. The pupae were placed in clean distilled water in glass crystallizing dishes which were in turn placed in the emergence cages. Only the female pupae were used. The adults on emergence were transferred onto trays of moist cheese cloth and placed in individual cages on a circular pad of paper towelling. Small strips of cheese cloth dipped in honey were placed on top of each cage. In certain instances the females were offered only water.

At different intervals of time the females were anaesthetized and dissected in physiological saline and

the ovaries examined under a stereo-microscope. The females that could not be dissected at Churchill were placed in small stoppered glass vials (one per vial) and frozen. They were transported from Churchill to Winnipeg, packed in dry ice, and then transferred to the freezer in the laboratory at Winnipeg. In this manner the females were maintained in good condition for examination of the ovaries at a latter date. For dissection of the frozen material, the females were removed from the freezer, allowed to thaw for 10 minutes, then wetted with a little detergent and immersed in physiological saline for dissection.

The stage of development of the oocytes was classified according to Macan's (1950) and Clement's (1963) modification of the scheme proposed by Christopher's (1911). In Macan's modified scheme, Christopher's stages II and III are each sub-divided into 'early', 'middle', and 'late' which are referred to as IIe, II and III, etc. In the scheme shown below I have sub-divided stage IIb into IIb (early), IIb (middle), and IIb (late). The follicular stages are:

Stage Ia - The follicle is spherical and consists of 8 cells of similar appearance. The oocyte is not differentiated from the nurse cells.

Ib - The follicle consists of an oocyte and 7 nurse cells.

Stage IIa - A few yolk granules present around the oocyte nucleus. Visible only under phase contrast with a high power objective.

Stage IIb(e) - A few coarse yolk granules appear around the oocyte nucleus seen as a small plaque at a magnification of 40x.

IIb(m) - Yolk covers entire oocyte. Yolk occupies much less than 1/2 the follicle.

IIb(l) - The yolk occupies 1/2 the follicle.

As stage IIb is reached without a blood meal in the anautogenous forms it is commonly referred to as the resting stage.

Stage IIIa - The yolk occupies between half to two-thirds of the follicle.

IIIb - The yolk occupies between two-thirds to three-quarters of the follicle.

Stage IVa - The follicle starts to elongate; the yolk occupies about nine-tenths of the follicle.

IVb - The follicle assumes the shape of the mature egg.

Stage V - The mature egg with chorionic covering.

Wild caught females were taken at Camp Nanuk and Goose Creek. The females were collected as they alighted on the collector and were transferred into 15 x 2.5 x 2.5 cm. feeding cages with the aid of an aspirator. The transportation of adults from field to laboratory, blood feeding of adults in the laboratory, rearing of blood fed adults to obtain eggs, and the transportation of eggs and adults to Winnipeg were similar to those described earlier by Kalpagé and Brust (1968).

All eggs from Churchill, Manitoba, were stored at 20°C for 3 months, at 2°C for 2 months and at -10°C till required for hatching. The eggs were removed from

the freezer and kept at 10°C for 24 hours before hatching. They were then placed in a hatching medium for 24 hours at 15°C. Larvae were reared at 15°C in pans containing 1000 ml. distilled water, sufficient peat moss and larval food. The maximum larval survival and pupation was obtained when the medium received continuous mild aeration. This was accomplished by placing a fine glass capillary at the end of a rubber tubing which was attached to a controlled compressed air source. This prevented the formation of a pellicle on the surface of the water which otherwise contributed to very high mortality of larvae. Pupae were placed in distilled water in dishes and placed in emergence cages. The female adults on emergence were transferred to circular single female cages. The females were fed on strips of cotton or cheese-cloth soaked in honey.

Experiments were performed to find the effect of photoperiod on autogeny in *Aedes campestris* from Churchill. The hatching of eggs, rearing of larvae, pupae and adults was similar to that described for *Aedes atropalpus*. The females on emergence were placed in individual cages and were later dissected at known age. The stage of development of the ovarian follicles was classified according to the classification referred to earlier.

CHAPTER IV

RESULTS

1. SELECTION OF OVIPOSITION MEDIUM BY AUTOGENOUS *A. ATROPALPUS* (BELLEVILLE STRAIN) AND STORAGE OF EGGS.

(a) OOviposition stimuli.

The results are given in Table I and Appendix A. The results of experiments a-c indicate that *A. atropalpus* selected between two laying dishes. In experiment (a) emergence water received more eggs than distilled water and statistically this was highly significant. In experiments (b) and (c) the females laid a large number of eggs in the larval and pupal holding media respectively and showed a preference for these over distilled water. For a statistical analysis of the data a Student 't' Test was done for each experiment.

In experiments (d), (e) and (f) the adults were given a choice between two dishes containing solutions in which different developmental stages had been present. These experiments show that the two dishes in each of these experiments contained the required ovipositional stimulus or stimuli and the adults did not show any special preference for either of the two dishes. In order to determine whether there was a possibility of sex being responsible for the stimulus, experiment (g) was performed. In this experiment the adults had a choice between male emergence water and female emergence water. The results were not significantly different and it is concluded that the females do not discriminate between male and female samples.

TABLE I

The number of eggs laid by autogenous *Aedes atropalpus* (Belleville strain) in different test solutions. Experiments were conducted at 20°C and 16L:8D photoperiod.

Experiment No.	Dish A*	Dish B*	Total No. Eggs in A	Total No. Eggs in B	Student 't' test
(a)	EW	DW	6717	2315	Significant at 99% level
(b)	LM	DW	2213	740	Significant at 95% level
(c)	PM	DW	2072	812	"
(d)	LM	EW	2912	1662	Not significant at 95% level
(e)	LM	PM	1985	784	"
(f)	EW	PM	1497	1189	"
(g)	Female EW	Male EW	3034	2145	"

* See methods 1a.

EW - Emergence water

LM - Larval holding medium

PM - Pupal holding medium

DW - Distilled water

(b) Storage conditions for eggs.

A preliminary study was undertaken to determine a method of storing *A. atropalpus* eggs devoid of fungal growth. Telford (1963) used various concentrations of phenol solutions. Concentrations sufficient to prevent fungal growth were toxic to the eggs and non-toxic solutions were ineffective. He also used dimethyl dithiocarbamate which gave good results. Judson (1960) reported that Roccal in water at 1:1000 dilution inhibited fungal growth in *Aedes* eggs during 2-8 weeks storage periods under conditions of high humidity. Meola (1964) reported he used non-chemical inert substrates like glass, nylon and saran. In a personal communication Dr. R. Meola suggested I try a chemical called methyl para-hydroxybenzoate. The results of a preliminary study using this compound on *A. atropalpus* eggs are shown in Table II.

The dishes containing the eggs were stored in a constant temperature room (20°±1°C), and the eggs were kept moist, near 100% R.H. These results indicate that a satisfactory method of storing eggs at present would be to place the eggs on an inert material like glass wool, and nylon cloth and treat the eggs with a solution of methyl parahydroxybenzoate (4 gm/1000 ml. distilled water) at least once a month. Prior to storage, eggs should be examined in order to remove from the surface any debris, like pieces of insect legs, wings or broken infertile eggs because these are ideal substrates for the growth of fungi.

TABLE II

A preliminary study to determine a satisfactory method of storing *Aedes* eggs free from fungal growth at 20°C and under moist conditions (near 100% R.H.).

Treatment	Time (weeks) after treatment and amount of fungal growth+ 2	4	13	26
1. Eggs stored on moist filter paper. No fungicide* added.	1	2	3	4
2. Eggs stored on moist filter paper. Fungicide added.	0	1	2	2
3. Eggs stored on moist nylon cloth over glass wool. No fungicide added.	0	0	1	2
4. Eggs stored on moist nylon cloth over glass wool. Fungicide added.	0	0	1**	0

* Para-hydroxybenzoate (4 gm./1000 ml. dis. water).

** Fungal mycelia present on a few broken infertile eggs.

+scale: 0 - No fungus.
1 - Trace of fungal growth in localised spots.
2 - Light fungal growth on eggs.
3 - Moderate fungal growth.
4 - Heavy fungal growth.

2. INFLUENCE OF PHOTOPERIOD AND TEMPERATURE ON DIAPAUSE INDUCTION IN *A. ATROPALPUS*.

(a) Combined effect of photoperiod and temperature in the autogenous *Aedes atropalpus* (Belleville strain).

The photoperiods used in this experiment were 8L:16D; 12L:12D; and 16L:8D at 15°C, 23°C and 30°C. The experiments at 15°C and 30°C were conducted in standard BOD incubators where the temperature fluctuation was ±0.5°C. The experiments at 23°C were conducted in photoperiod boxes constructed of plywood where the temperature fluctuation was ±2°C. Experiments to be described later, on the induction of diapause, were also conducted in these boxes. The other two temperatures selected, viz. 15°C and 30°C, were near the minimum and maximum rearing temperatures for *A. atropalpus*. At 30°C growth was very rapid and survival good whereas at 15°C growth was very slow. At 15°C there was a high mortality in all stages, and only a few eggs were laid.

The results in Table III show that photoperiod had a marked influence on the induction of diapause. At 16L:8D, and all combinations of temperatures, only a very small percentage (<3%) of eggs remained in diapause. At 8 hr. and 12 hr. photophases, a very high percentage (99%) of diapause eggs were obtained at 15°C and 23°C. However at a short photophase (8 or 12 hr.) and a high rearing temperature (30°C) 95% of the eggs were in a non-diapause condition similar to those laid under the influence of a long photoperiod. These results are in agreement with those

TABLE III

The combined effect of temperature and photoperiod on the induction of embryonic diapause in autogenous *Aedes atropalpus* (Belleville strain).

Photoperiod	Temperature(°C)	No. Replicates	Total No. Viable Eggs Laid	No. Hatched	No. Eggs in Diapause	% Diapause
8L:16D	15	3	584	4	580	99.3
	23	2	1987	4	1983	99.8
	30	2	2014	1906	108	5.4
12L:12D	15	3	647	7	640	98.9
	23	2	1814	6	1808	99.7
	30	2	2134	2032	102	4.7
16L:8D	15	3	703	687	16	2.3
	23	2	1906	1891	15	0.8
	30	2	2208	2200	8	0.4

obtained by Anderson (1968) for the Connecticut strain of autogenous *A. atropalpus*. From these results it could be concluded that diapause in *A. atropalpus* is under photoperiodic control, but the effect of this stimulus under the conditions of these experiments could only be measured in temperature regimes lower than 30°C.

(b) Effect of different photoperiods, at a constant temperature, in autogenous *A. atropalpus* (Belleville strain).

Experiments were conducted in wooden photoperiod boxes at a temperature of 23°±2°C. The purpose of this experiment was to determine what range of photoperiods caused the induction of embryonic diapause. Photoperiods ranged from total darkness, through combinations of light and dark cycles to continuous illumination.

The results, as shown in Table IV and in Fig. 2, demonstrated that a high percentage of embryonic diapause is induced only between 7L:17D and 14L:10D. At photoperiods less than 6 1/2L per 24 hour cycle, and in complete darkness, the percentage of diapause eggs was very low, similar in effect to long photoperiods of 15L and more.

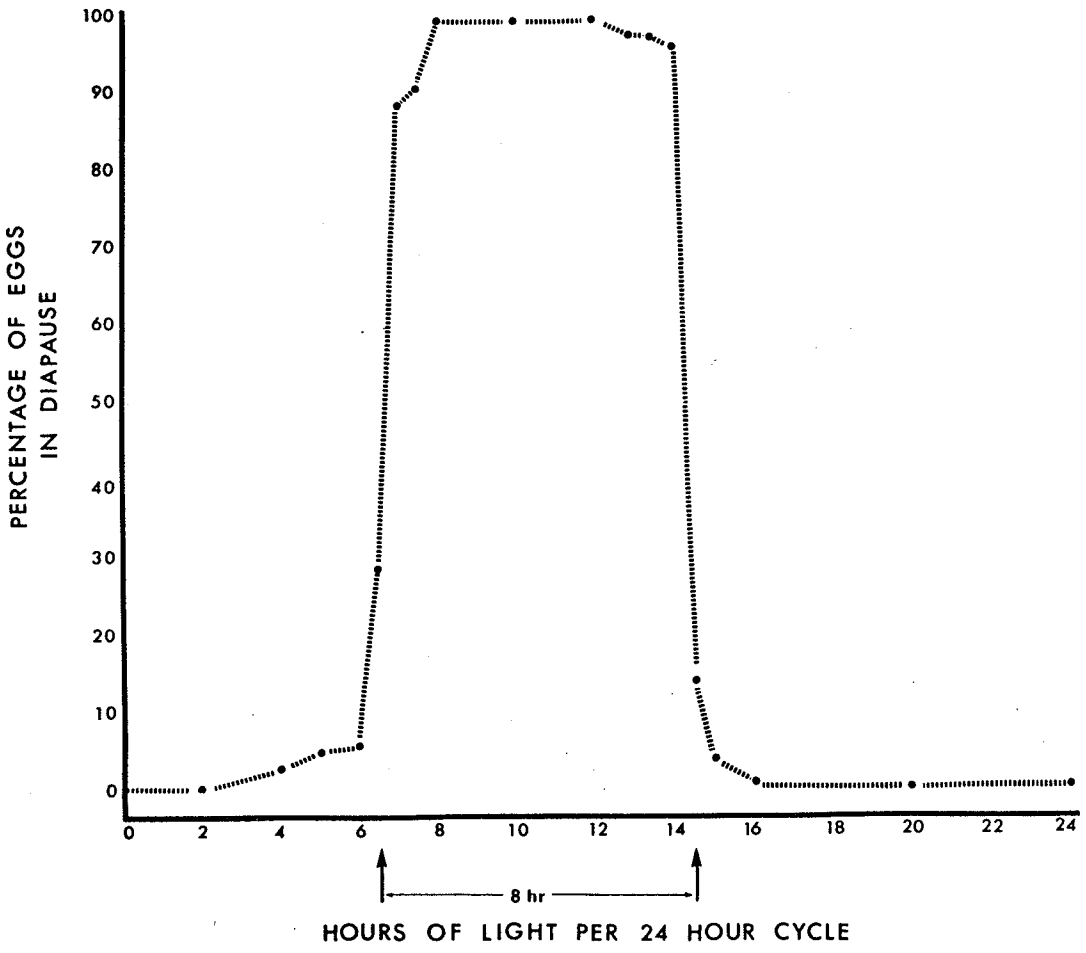
Photoperiods of less than 8L per 24 hr. cycle do not occur in nature during summer, and therefore, they do not have any ecological significance. However, short photoperiods may be useful in understanding certain physiological processes. At the photoperiods which are of ecological

TABLE IV

Effect of different photoperiods on the induction of diapause in autogenous *Aedes atropalpus* (Belleville strain) reared at 23°±2°C.

Photoperiod L:D	No. Replicates	Total No. Viable Eggs	No. Eggs Hatched	No. Viable Eggs Unhatched	% Diapause
0:24	2	1908	1897	11	0.6
2:22	2	1614	1611	3	0.2
4:20	2	988	955	33	3.5
5:19	2	1317	1242	75	5.7
6:18	2	1763	1656	107	6.1
$6\frac{1}{2}:17\frac{1}{2}$	2	1485	903	582	39.2
7:17	2	1894	222	1672	88.3
$7\frac{1}{2}:16\frac{1}{2}$	2	1004	95	909	90.5
8:16	3	2413	5	2408	99.8
10:14	2	1131	4	1127	99.6
12:12	3	2106	5	2101	99.8
13:11	2	941	25	916	97.3
$13\frac{1}{2}:10\frac{1}{2}$	2	1210	27	1183	97.8
14:10	2	3057	114	2943	96.3
$14\frac{1}{2}:9\frac{1}{2}$	2	968	825	143	14.8
15:9	2	1913	1821	92	4.8
16:8	3	3817	3772	45	1.2
20:4	2	2439	2420	19	0.8
24:0	2	2814	2775	39	1.4

Fig. 2 The effect of photoperiod on the incidence of embryonic diapause in the autogenous (Belleville) strain of *Aedes atropalpus*, reared at 23°±2°C.


PERCENTAGE OF EGGS IN DIAPAUSE
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
20
22
24
8 hr
HOURS OF LIGHT PER 24 HOUR CYCLE

significance, the critical photoperiod lies between 14L:10D and 14 1/2L:9 1/2D (Fig. 3), since at 14L, 96.3% of the embryos were in diapause whereas with an additional increase of 1/2 hr. light period the percentage of diapausing eggs was less than 5%. Considering the entire range of photoperiods it is noted that there is an interval of 8 hours between the points of critical photoperiod (Fig. 2).

(c) Effects of different photoperiods at a constant temperature in the southern anautogenous *A. atropalpus* (Austin strain).

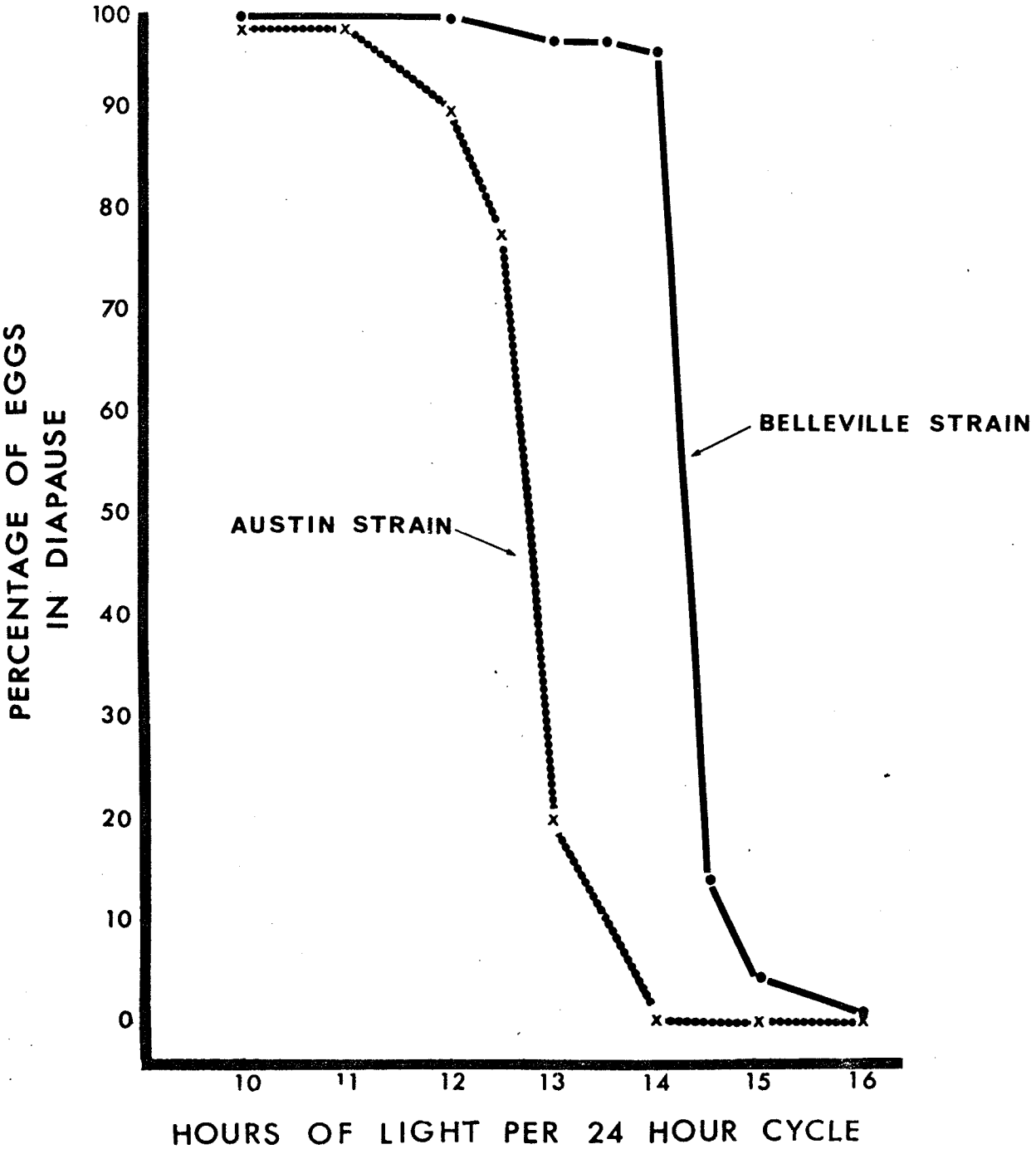
Having determined the critical photoperiod for the Belleville strain, the following experiments were performed to find out what the critical photoperiod is for a southern strain of *A. atropalpus*. Photoperiod experiments with mosquitoes by Vinogradova (1960), Depner and Harwood (1966), Kappus and Venard (1967), and with the Lepidoptera by Danilevskii (1965) have shown that there is a latitudinal difference in the critical photoperiod. To determine the critical photoperiod of the Austin strain, experiments were conducted in the photoperiod boxes (23°±2°C) at photoperiods ranging from 10L:14D to 16L:8D. Results are shown in Table V and Fig. 3. It is quite evident that here too, short photophases induce diapause and long photophases terminate diapause. The data show that the critical photoperiod for Austin strain lies between 12 1/2L and 13L per 24 hrs. cycle.

TABLE V

Effect of photoperiods on the induction of diapause in a southern anautogenous *Aedes atropalpus* (Austin strain) reared at 23°±2°C.

Photoperiod L:D	No. Replicates	Total No. Viable Eggs	No. Eggs Hatched	No. Viable Eggs Unhatched	% Diapause
10:14	2	1310	25	1285	98.1
11:13	2	1164	16	1148	98.6
12:12	2	1081	104	977	90.4
$12\frac{1}{2}:11\frac{1}{2}$	2	1194	259	935	78.3
13:11	2	1010	802	208	20.6
14:10	2	1276	1266	10	0.8
15:9	2	987	985	2	0.2
16:8	2	1412	1404	8	0.6

Fig. 3 The effect of photoperiod on the incidence of embryonic diapause in the autogenous Belleville strain of *Aedes atropalpus* and in the anautogenous Austin strain of *A. atropalpus*, reared at 23°±2°C.


100
90
80
70
60
50
40
30
20
10
0
PERCENTAGE OF EGGS IN DIAPAUSE
BELLEVILLE STRAIN
AUSTIN STRAIN
10
11
12
13
14
15
16
HOURS OF LIGHT PER 24 HOUR CYCLE

These results are similar to the observations made by Anderson (1968), with the exception that at 13L:11D he obtained 55.9% diapause.

(d) Effect of photoperiod on different stadia of autogenous *A. atropalpus* (Belleville strain).

In experiments pertaining to photoperiodic induction of diapause, several workers have found that a certain stage or instar in the developmental cycle of the insect is sensitive to changes in photoperiod. The following series of experiments were designed to determine if any particular instar or stage of *A. atropalpus* was more sensitive to long or short photoperiods, than another instar or stage. Two photoperiods were used 8L:16D and 16L:8D. *A. atropalpus* was transferred from short photoperiods to long photoperiods and vice versa during specific stages of the developmental cycle. Transferring of the different stages, from one photoperiod to another,took place during the photophase. The results are shown in Table VI, and are in agreement with those observed by Anderson (1968). From these experiments it could be concluded that the sensitivity to the photoperiod begins during the fourth larval instar and is also perceived by the pupa and adult. It is only when these three stages experience short daylengths that they give rise to adults which lay diapause eggs.

TABLE VI

Effect of long-day (16L:8D) and short-day (8L:16D) photoperiods applied to the developmental stages of *Aedes atropalpus* (Belleville strain) reared at 23°±2° C., on the production of embryonic diapause.

	LARVAL INSTARS I	II	III	IV	PUPA	ADULT	No. replicates	Total no. of viable eggs	No. eggs hatched	No. unhatched viable eggs	% eggs in diapause
a							2	1734	1710	24	1.4
b							2	1486	1468	18	1.2
c							2	2014	1845	169	8.4
d							2	1021	37	984	96.4
e							2	964	2	962	99.8
f							2	1312	8	1304	99.4
g							2	1427	6	1421	99.6
h							2	1009	915	94	9.4
i							2	1137	1112	25	2.2

Note: Stippled areas denote short photoperiods (8L:16D) and non-stippled areas denote long photoperiods (16L:8D).

(e) Effect of light interruptions in scotophase on diapause induction in autogenous *A. atropalpus* (Belleville strain).

Beck (1962) showed that the duration of the scotophase was more critical than the photophase duration in the induction of diapause in the larvae of the European corn borer. Several workers have suggested that if the photoperiodic induction of diapause involves the measurement of time then it must primarily be the duration of the scotophase that determines whether or not diapause is induced. As a result of this the scotophase has been critically analysed with regard to its importance in diapause and its possible role in time relationships. In these types of experiments the typical procedure is for the scotophase to be interrupted by short periods of light at different intervals.

The photoperiods used were 8L:16D and 12L:12D both of which were diapause inducing photoperiods (See Table IV). The scotophases of 16 hr. and 12 hr. were interrupted with 1 hr. light periods at definite intervals of time, as shown in Tables VII and VIII and Figs. 4 and 5 respectively. When the 16 hr. scotophase was interrupted by a light period of 1 hour, the incidence of diapause eggs was reduced but the amount of reduction depended upon the time at which the light break occurred. During the 16 hr. scotophase the lowest percentage of diapause eggs was obtained when the light breaks occurred 7, 8 and 9 hours after

TABLE VII

Effects of 1-hr. light-breaks, during a 16 hr. scotophase, on the incidence of embryonic diapause in *Aedes atropalpus* (Belleville strain) reared at 23°±2°C.

Schedule	Total No. Viable Eggs	No. eggs Hatched	No. Viable Eggs In Diapause	%Diapause
A	1650	3	1647	99.80
B	1861	7	1854	99.62
C	2455	219	2236	91.08
D	1945	492	1453	74.70
E	1149	597	552	48.04
F	2012	1246	766	38.07
G	1287	1065	222	20.85
H	2349	2217	132	5.62
I	3521	3318	203	5.76
J	2550	2393	157	6.16
K	1730	425	1305	75.43
L	2534	50	2484	98.03
M	1679	4	1675	99.76
N	1383	4	1379	99.71
O	1496	6	1490	99.60

Note: Conditions A to O are shown in Fig. 4.

Fig. 4 Effect of 1-hr. light breaks made during a 16-hr. scotophase on the incidence of embryonic diapause in *Aedes atropalpus* (Belleville strain).

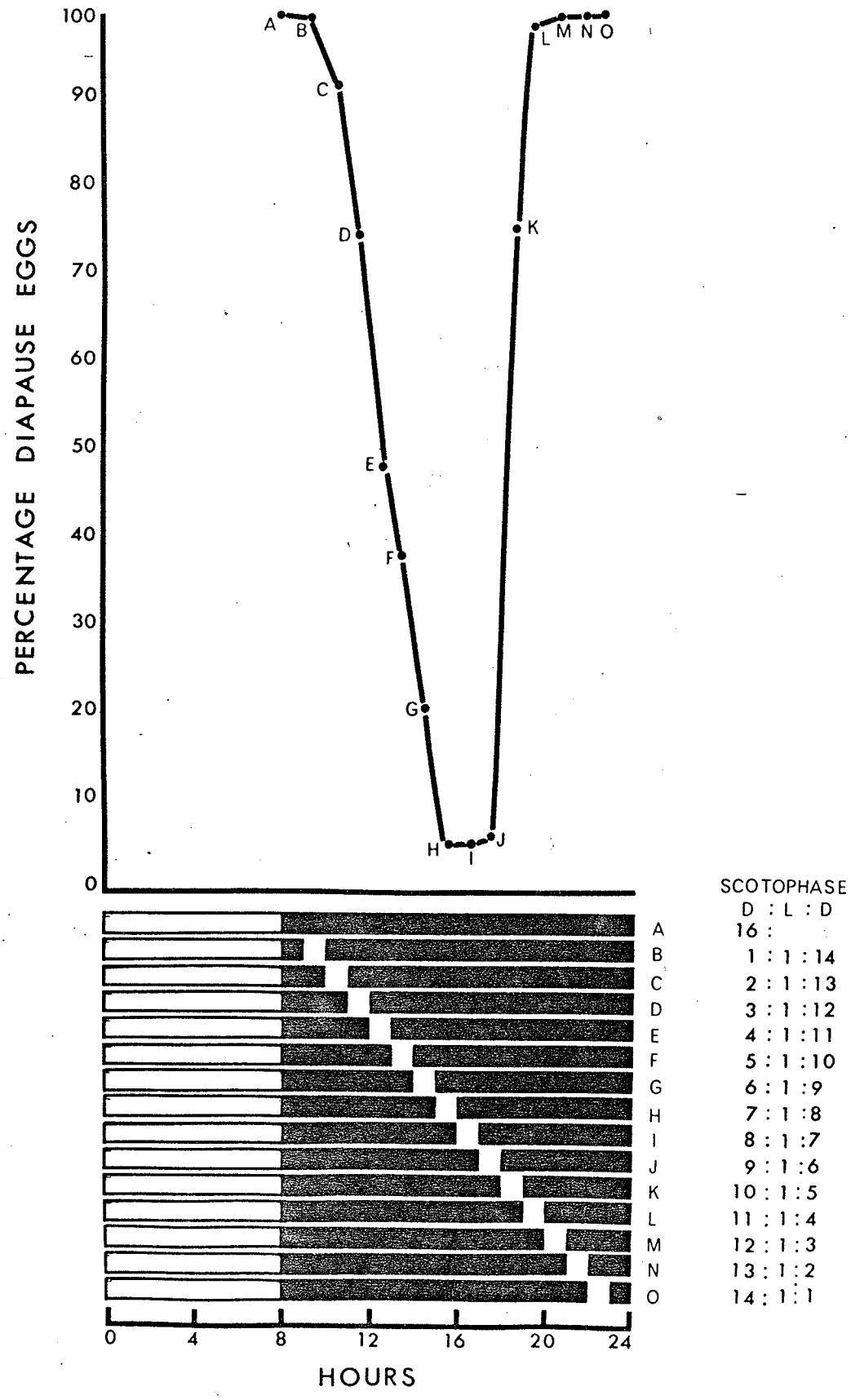

PERCENTAGE DIAPAUSE EGGS
100
90
80
70
60
50
40
30
20
10
0
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
0
4
8
12
16
20
24
HOURS
SCOTOPHASE
D : L : D
A 16 :
B 1 : 1 : 14
C 2 : 1 : 13
D 3 : 1 : 12
E 4 : 1 : 11
F 5 : 1 : 10
G 6 : 1 : 9
H 7 : 1 : 8
I 8 : 1 : 7
J 9 : 1 : 6
K 10 : 1 : 5
L 11 : 1 : 4
M 12 : 1 : 3
N 13 : 1 : 2
O 14 : 1 : 1

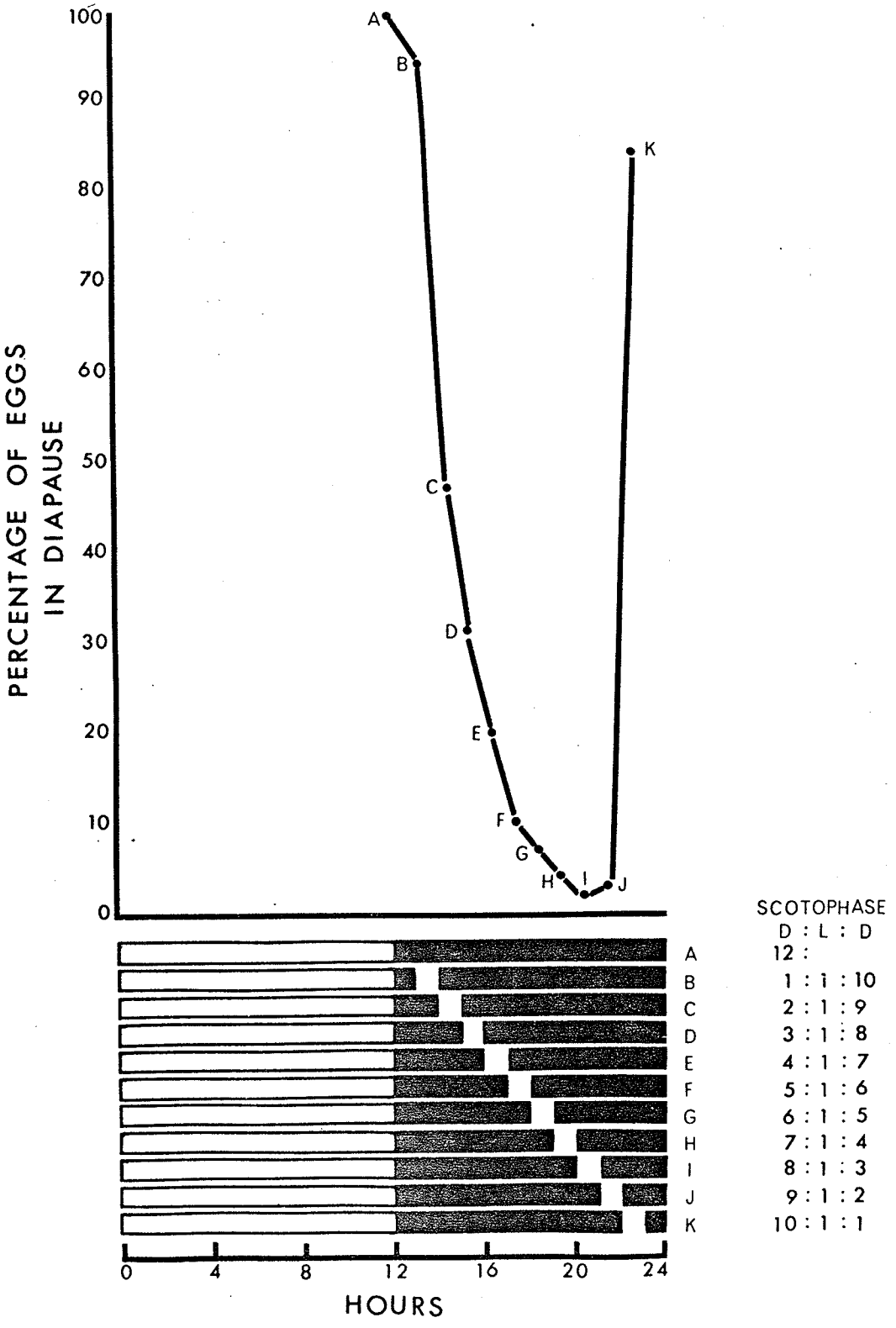
TABLE VIII

Effects of 1-hr. light-breaks, during a 12 hr. scotophase on the incidence of embryonic diapause in *Aedes atropalpus* (Belleville strain), reared at 23°±2°C.

Schedule	Total No. Viable Eggs	No. Eggs Hatched	No. Viable Eggs In Diapause	% Diapause
A	974	8	966	99.17
B	1041	62	979	94.04
C	1276	668	608	47.65
D	1531	1043	488	31.87
E	1132	901	231	20.41
F	2107	1883	224	10.63
G	1786	1650	136	7.61
H	1479	1419	60	4.06
I	1204	1173	31	2.57
J	2349	2273	76	3.24
K	1967	309	1658	84.30

Note: Conditions A to K are shown in Fig. 5.

Fig. 5 Effects of 1-hr. light breaks made during a 12-hr. scotophase on the incidence of embryonic diapause in *Aedes atropalpus* (Belleville strain).


PERCENTAGE OF EGGS IN DIAPAUSE
100
90
80
70
60
50
40
30
20
10
0
A
B
C
D
E
F
G
H
I
J
K
0
4
8
12
16
20
24
HOURS
SCOTOPHASE
D : L : D
A 12 :
B 1 : 1 : 10
C 2 : 1 : 9
D 3 : 1 : 8
E 4 : 1 : 7
F 5 : 1 : 6
G 6 : 1 : 5
H 7 : 1 : 4
I 8 : 1 : 3
J 9 : 1 : 2
K 10 : 1 : 1

the dark period commenced (See schedules H, I, J in Fig. 4). When the 12 hr. scotophase was interrupted by 1 hr. light intervals, the lowest percentage of diapause eggs was again obtained when the light breaks occurred 7, 8 and 9 hours after the "lights-off" signal (schedules H, I, J in Fig. 5). Experiments were also performed where the 16 hr. and 12 hr. scotophases were interrupted with 2 hr. light periods. Similar results were obtained and the data is presented in Appendices D and E.

3. TERMINATION OF DIAPAUSE IN AUTOGENOUS *A. ATROPALPUS* (BELLEVILLE STRAIN).

The Belleville strain of *A. atropalpus*, when reared under short day photoperiods at 23°C produced diapause eggs which remained in diapause for a period of 3 months. Table IX shows that only 39 out of 462 eggs hatched after 90 days. This indicates that "diapause development" (Andrewartha 1952) at 23°C is very slow. It also indicates that for the termination of diapause, the *A. atropalpus* eggs have to be activated by subjecting them to some other treatment.

It was demonstrated earlier that when *A. atropalpus* (Belleville strain) was reared at a high temperature 30°C, and a short photoperiod (8L:16D or 12L:12D), females laid mainly non-diapause eggs (Table III). An experiment was

TABLE IX

Effect of the hatching stimulus on diapause eggs of *Aedes atropalpus* (Belleville strain) at 10 day intervals from the day of oviposition. The eggs were maintained at a photoperiod of 8L:16D and a temperature of 23°C.

Days following Oviposition	No. Hatched	Total No. Viable Eggs	% Hatch
10	0	405	0
20	6	488	1.0
30	4	321	1.2
40	6	231	2.5
50	5	211	2.4
60	4	176	2.2
70	8	204	3.8
80	15	287	5.2
90	39	462	8.4

therefore designed (Table X) to determine the effect of this high temperature on the termination of embryonic diapause in *A. atropalpus*. The results summarized in Table X indicate that the longer they were maintained at a diapause sustaining temperature (23°C), the shorter the time required at a diapause terminating temperature (30°C) to enable the eggs to hatch. When kept at 23°C for 30 days, only 15% hatched after 5 days treatment at 30°C, and approximately 90% hatched after 20 days at 30°C. On the other hand, eggs maintained under oviposition conditions (8L:16D, 23°C) for 90 days showed a very high percentage (76%) hatch after only 5 days at 30°C, and after 10 days at 30°C nearly all the eggs hatched.

Kappus and Venard (1967) have shown that the termination of diapause in *Aedes triseriatus* eggs is influenced by long photoperiods. In order to determine whether long photoperiods influenced the termination of diapause in the Belleville strain of autogenous *A. atropalpus*, an experiment was performed in which two groups of diapause eggs were subjected to short and long photoperiods respectively. The temperature in the photoperiod boxes was 25°±1°C. The results of this experiment are given in Table XI and indicate that a long photoperiod (16L:8D) caused 29.2% and 52.2% of the eggs to hatch after they had been treated at this photoperiod for 60 and 90 days respectively, whereas only 2% and 6% of the eggs hatched at 8L:16D after a similar period of time.

TABLE X

Effect of a high temperature (30°C) on the termination of diapause in *Aedes atropalpus* (Belleville strain) eggs. The eggs were maintained at a diapause sustaining photoperiod of 8L:16D.

No. Days at 23°C & 8L:16D	No. Days at 30°C & 8L:16D	No. Eggs Hatched	Total No. Viable Eggs	% Hatch
30	5	33	217	15.2
30	10	98	378	25.8
30	20	196	211	92.7
60	5	196	326	60.1
60	10	162	220	72.7
60	20	315	355	88.7
90	5	149	194	76.8
90	10	143	146	97.9
90	20	162	164	99.1

TABLE XI

Effect of photoperiod on the termination of diapause in eggs of autogenous *Aedes atropalpus* (Belleville strain) at 25°C.

Duration of Treatment (Days)	Length of Photoperiod					
	8L:16D			16L:8D		
	No. Hatch	Total No. Viable Eggs	% Hatch	No. Hatch	Total No. Viable Eggs	% Hatch
10	0	50	0	0	50	0
30	1	50	2	5	50	10
60	1	50	2	14	48	29.2
90	3	50	6	24	46	52.2

Lees (1964), in a series of experiments using light conducting plastic filaments, was able to focus a narrow beam of light on different portions of the body of the aphid *Megoura viciae* Buckton. He postulated that the photoreceptors lie beneath the cuticle within the protocerebrum. With this type of experiment in mind, I proposed to carry out a preliminary examination to determine whether there was a particular section of the mosquito embryo that was able to perceive differences in photoperiod. In one batch of eggs the beam of light from the "Fibre Optics Light Guides" was focussed on the anterior, dorsal portion of the egg and in the other batch the posterior half was illuminated. The results of these preliminary observations are shown in Table XII. It is possible that diapause eggs are sensitive to a long photoperiod and the region of light perception lies in the anterior dorsal region of the embryo. A serious problem encountered in this experiment was that many eggs had stuck to the plasticine and were not in contact with the moist surface. This resulted in dehydration, and only a small number of eggs were suitable for hatching.

TABLE XII

Effect of photoperiod in terminating diapause when *Aedes atropalpus* (Belleville strain) eggs were exposed to a localized illumination provided by "Fibre Glass Optics Light Guides" at 23°±1°C. When the anterior portion of the eggs was illuminated, the posterior part was covered with opaque plasticine. The reciprocal experiment was carried out simultaneously.

	No. Eggs Hatching per Total No. Viable Eggs at			
	8L:16D		16L:8D	
Duration of Treatment (Days)	Anterior End Exposed	Posterior End Exposed	Anterior End Exposed	Posterior End Exposed
30	- *	-	1/10	-
60	1/12	0/8	3/10	0/10
90	1/15	-	6/13 **	0/8

* eggs dried out

** 2 eggs hatched during treatment. The other 4 eggs hatched after eggs were placed in hatching medium.

4. EFFECT OF PHOTOPERIOD AND DIET ON FECUNDITY IN AUTOGENOUS *A. ATROPALPUS* (BELLEVILLE STRAIN).

(a) Effect of photoperiod on fecundity.

In this experiment the different photoperiods ranged from 8L:16D to continuous illumination. All experiments were performed in photoperiod boxes at a temperature of 23°±2°C. The larvae were fed a rich diet (7C) every other day, and the adults were offered honey. The adults were dissected when they were 10 days of age, and the number of eggs developed plus those laid per female were counted. The results of the experiment are summarized in Table XIII. At short photoperiods (8L:16D) the mean number of mature eggs plus stage V follicles per female was approximately 107. The fecundity increased with photoperiod, and the greatest number of eggs per female was obtained at 16L:8D. With any further increase in photoperiod there was a slight decrease in the fecundity, although the females at 20L and 24L were on the average slightly larger than the ones at 16L, as determined by wing measurements. Christophers (1960) showed that wing length in *Aedes aegypti* is correlated with weight, and Laurence (1964) is of the opinion that there is a direct relationship between the size of the female and the length of the radial sector vein in the wing of adult mosquitoes. The data obtained in this experiment would indicate that the number of eggs developed within the female is not solely dependent on the size of the female, but another factor such as the photoperiod it experiences plays even a greater role in the development of ovarian follicles.

TABLE XIII

Effect of photoperiod on the fecundity of autogenous *Aedes atropalpus* (Belleville strain) reared at 23°±2°C.

Photoperiod L:D	No. of Females Dissected	Eggs per Female Mean±S.E	Range Min-Max	Wing Measurement Rs vein (μ)
8:16	30	107.1±2	87-127	832
12:12	30	114.4±3	93-140	877
14:10	21	122.7±4	91-156	890
15:9	11	142.2±5	106-162	909
16:8	24	157.2±2	126-195	896
20:4	23	150.4±3	121-176	928
24:0	10	147.7±3	133-162	1028

(b) Effect of diet on fecundity.

These experiments were conducted in the constant temperature room (20°±1°C) which had a photoperiod of 16L: 8D. Larvae were reared in white plastic pans (21 cms x 30 cms x 8cms) and fed daily. A different diet was used in each treatment (See Appendix B). Samples from each development stage (larva-adult) were taken and oven dried at 60°C. Dry weight of the material was determined by weighing on a micro-balance. Five females from each diet treatment were placed in individual cages and fed on honey. They were dissected on the tenth day and the number of eggs laid and retained by each female was recorded and the mean calculated. The results of these experiments are shown in Table XIV.

The Belleville strain of autogenous *A. atropalpus* gained the most weight and developed the largest number of eggs (mean 174) when fed a rich diet composed of dog food, brewer's yeast, blood fibrin, blood meal, yeast extract, and two fish foods, Tetramin E and L (referred to as 7C diet in Table XIV). The least number of eggs was obtained when the larvae were fed a diet containing only blood meal. Under a constant photoperiod there was also a direct correlation between the weight of the females and the number of eggs developed. Larvae fed on rich diets (7C or 5C) were on the average heavier than those fed on a single component like Brewer's yeast or blood meal. A rich diet

TABLE XIV

Effect of diet on the size and fecundity of *Aedes atropalpus* (Belleville strain) reared at 20°C, and 16L:8D photoperiod.

Diet*	Mean Weights (mg.)					Mean No.+ Eggs Per Female	Percentage Adult Emergence
	Larvae (instar IV)	Pupae ♂♂	Pupae ♀♀	Adults ♂♂	Adults ♀♀		
DF	1.383(19)	0.60 (24)	1.146(20)	0.463(20)	1.049(20)	130.0	77
BY	0.934(5)	0.514(14)	0.667(10)	0.245(21)	0.484(5)	114.0	69
BM	0.956(8)	0.404(10)	0.594(6)	0.265(13)	0.475(5)	107.8	63
$FF_{(E)}$+$FF_{(L)}$	1.111(10)	0.571(15)	0.948(19)	0.310(13)	0.698(13)	119.0	68
DF + BY	1.326(19)	0.703(15)	1.449(16)	0.529(42)	1.121(16)	141.0	81
DF + BM	1.224(20)	0.609(15)	1.174(22)	0.490(20)	0.976(20)	121.0	79
DF + 2FF	1.500(20)	0.710(15)	1.386(17)	0.577(22)	1.220(20)	151.0	72
5C	1.641(12)	0.708(9)	1.546(10)	0.486(20)	1.293(19)	155.8	93
7C	1.662(20)	0.740(20)	1.685(20)	0.556(11)	1.347(17)	174.0	92

* For composition of diet refer Appendix B1. DF = dog food; BY = Brewer's yeast; BM = blood meal; $FF_{(E)}$ = fish food Tetramin E; $FF_{(L)}$ = fish food Tetramin L;
\+ 5C = 5 component diet; 7C = 7 component diet.

mean of 5 individuals.

Note: The figures in parentheses denote sample size.

not only gave rise to larger and heavier adults and more eggs per female, but also contributed to better survival. On a 5C or 7C diet, more than 90% emerged while with the poorer diets, brewer's yeast, blood meal, or fish foods, the percentage emergence was 69, 63, and 68 respectively.

5. EFFECT OF PHOTOPERIOD AND TEMPERATURE ON BITING ACTIVITY AND OVARIAN DEVELOPMENT IN ANAUTOGENOUS *CULISETA INORNATA*.

It is known that photoperiod influences the blood feeding activity in *Culex pipiens* under laboratory conditions (Tate and Vincent 1936, Hosoi 1954, Danilevskii and Glinyanaya 1958). Eldridge (1963) observed a similar phenomenon in laboratory reared *Culex tritaeniorhynchus*. Further work by Eldridge (1966, 1968) demonstrated that gonotrophic dissociation in *Culex pipiens* was dependent not only on rearing these mosquitoes at low temperatures and short photoperiods, but it is also dependent on maintaining low temperatures after feeding.

Since *C. inornata* hibernates as an adult in Winnipeg, (Hanec & Brust 1967) I wanted to determine what effect combinations of photoperiod and temperature would have on blood feeding activity and ovarian development in the females. Five temperatures, 10°, 15°, 20°, 25°, 30°C and three photoperiods, 8L:16D, 12L:12D, and 16L:8D were used in all combinations as shown in Table XV. All stages,

TABLE XV

Effect of different combinations of temperature and photoperiod on the feeding activity and ovarian development of anautogenous *Culiseta inornata*.

Temp.°C.*	Photoperiod L:D	No. Replicate	Feeding Activity of Females			State of Ovaries of Blood Fed Females After Digestion of Blood Meal			
			Total No.	No. Blood Fed	% Blood Fed	Ovarian Stage #	Stage	No. Eggs per Gravid Female Mean±S.E.	Range (Min. Max.)
10°	8:16	3	28	5	17.8	1	IIb		
						4	V	----77.3±10	(33-152)
	12:12	3	36	8	22	1	IIb-IIIa		
						1	IV		
						6	V	----109.1±12	(51-144)
	16:8	2	31	10	30	3	IIIa		
						7	V	----141.6±12	(91-176)
15°	8:16	3	27	6	22	2	IIb-IIIa		
						4	V	----102.0±15	(72-147)
	12:12	3	18	5	28	1	IIb		
						4	V	----123.8±20	(68-181)
	16:8	3	24	12	50	1	IIb		
						11	V	----170±8	(132-214)

cont.....

TABLE XV (cont.)

Temp. °C*	Photoperiod L:D	No. Replicate	Feeding Activity of Females: Total No.	No. Blood Fed	% Blood Fed	State of Ovaries of Blood Fed Females After Digestion of Blood Meal: Ovarian Stage #	Stage	No. Eggs per Gravid Female: Mean±S.E.	Range (Min. Max.)
20°	8:16	2	25	8	32	1	IIb		
						1	IIb-IIIa		
						6	V----	104.2±6	(84-132)
	12:12	2	25	9	36	1	IIb		
						2	IV		
						6	V----	122±10	(70-147)
	16:8	2	25	15	60	15	V----	174.7±8	(103-230)
25°	8:16	3	20	8	40	1	IIb		
						3	IIIb		
						4	V----	140±12	(71-154)
	12:12	2	25	15	60	4	IIIa		
						11	V----	131.5±10	(44-163)
	16:8	2	20	16	80	2	IIIb		
						14	V----	177±12	(98-184)
30°	8:16	3	20	14	70	2	IIa-IIIb		
						12	V----	133.3±11	(73-241)
	12:12	3	20	15	75	15	V----	152.9±6	(98-186)
	16:8	3	20	20	100	20	V----	192.0±13	(142-263)

* Air temperature in incubator.

from larva to adult, were maintained at the temperature of the experiment. The results indicate that with a combination of low temperature and short photoperiod, the percentage of females feeding was lower than in those that were incubated at high temperatures and long photoperiods. Under a short photoperiod (8L:16D) and the highest temperature tested (30°C) the percentage of females feeding was not very different from that of mosquitoes kept under a long photoperiod at 20° & 25°C. At temperatures of 10°, 15°, 20° and 25°C the females reared under a photoperiod of 8L:16D were more reluctant to bite than those reared under a long photoperiod. Those reared under the short photoperiod of 12L:12D showed a low percentage of biting females for 10°, 15° and 20°C, but at 25°C, 60% fed.

The last two columns in Table XV give the stage of development of the ovaries of the blood fed females and the mean number of eggs in those females that developed their ovarian follicles to stage V. It appears that females reared at a photoperiod of 16L:8D developed a larger number of eggs than those at 8L:16D. There was also an increase in the number of eggs with an increase in temperature. These two factors undoubtedly influence the number of eggs formed. From these experiments it is possible to conclude that photoperiod and temperature influence both the feeding activity and ovarian development in *C. inornata*. It is also apparent that *C. inornata* did not exhibit the phenomenon of gonotrophic dissociation.

6. STUDIES ON AUTOGENY IN *AEDES* SPECIES AT CHURCHILL, MANITOBA (58°N).

Hocking *et al* (1950) suggested the possibility of species in Northern Canada being able to develop their eggs autogenously. Hocking (1954) was of the opinion that autolysis of the flight muscles in *A. communis* provided a source of protein for the development of the eggs. Beckel (1954), on the other hand, observed that *A. communis* which laid eggs when fed on sucrose or raisins, had not autolysed the flight muscles. I undertook a study at Churchill, Manitoba, in spring and summer of 1967 and 1968 to determine which species at Churchill were autogenous and also to clarify the controversy on flight muscle autolysis and egg development in *A. communis*.

Two main study sites were selected (a) Camp Nanuk, a tundra habitat where the two predominant species were *A. nigripes* and *A. impiger* and (b) Goose Creek, a semi-forested location where attention was focussed on *A. communis* and *A. campestris*. Small numbers of other species, viz, *A. excrucians*, *A. punctor* (Kirby), *A. pionips* Dyar and *A. fitchii* (Felt & Young) present in the wooded area were also examined for autogenous egg development.

(a) Tundra species.

In 1967 I began my studies at Churchill on June 5. Most of the larval collections were made from pools C3, C4 and C5 (See Appendix C1 for sketch). These are open grassy

pools, the pH of the water ranged from 6.0 - 6.5 and the temperature of the water on June 5, 1967, was 5° - 6°C. Pool #C3 contained mostly *A. impiger*. Pools C4 and C5 had about equal numbers of *A. impiger* and *A. nigripes*. On June 5, these pools contained mostly late instar III and instar IV larvae. These pools were shallow and they dried out by the 12th of June. The larvae were fed a protein rich diet (7C) in the laboratory. Only a small number of *A.nigripes* larvae were available for study in 1967 and of these 90% of the adult females showed an autogenous development of the ovaries when they were maintained only on honey. An examination of 9 adults, which were less than 2 hours old, showed that about 14% of the follicles contained a deposition of yolk granules around the oocyte. This would indicate that yolk deposition in certain autogenous *A. nigripes* takes place in the late pupal period or very early in adult life. The yolk granules in *A. nigripes* were of a characteristic bright yellow colour in contrast to the other species which had white or yellowish-white yolk granules.

Of the 70 *A. impiger* females fed on honey, one female on the 12th day laid 16 eggs and an examination of the ovaries showed that the other follicles had retrogressed. Six females from this batch had ovarian follicles developed to stage IIIb on day 20. The remainder of these adults had the follicles developed only up to stage IIa even though

Fig. 6 *A. nigripes* and *A. impiger* habitats at Camp Nanuk, Churchill, Manitoba. (Tundra habitat)

(a) Pool #C3. A shallow pool in which *A. impiger* was the most predominant species. There was a copious growth of moss at the bottom of this pool.

(b) Pool #C20. Also a shallow pool in which *A. impiger* was more predominant than *A. nigripes*. Vegetation in the pool was mostly grass and moss.

(c) Pool #C21. Pool deeper than above. *A. nigripes* was the most abundant in this pool. Larvae were found browsing along the sides and among the tufts of grass.

(d) Pool #C32. A deep pool (about 15-20" at centre) contained only *A. nigripes* larvae. The water in the pool was clear and with very little bottom vegetation.

(Photographs (a), (b), and (c) were taken on 24th May, 1968, and (d) was taken on 20th June, 1968. For location of these pools, see Sketch - Appendix C1.)

a

b

c

d

some were dissected 25 days after emergence. None of the *A. impiger* adults, fed 25% sucrose, developed their ovarian follicles beyond stage IIa. On raisins, 2 out of 40 adults examined at 15 days had developed the follicles beyond the resting stage and were in stage IIIa.

Only 8 *A. hexodontus* females, reared from larvae, obtained from pool #C5, were available for examination of autogeny. None developed their follicles beyond IIb when adults were supplied with honey.

As a result of the limited information in 1967, I visited Churchill early in the spring of 1968 (May 15th, 1968) before the snow melted. On May 20th the snow first melted in pools C3, C4, C20, and C21. The water temperature in these pools varied from 11° - 14°C. By the 24th of May, the snow had melted in nearly all the pools at Camp Nanuk. (Fig. 6).

Larvae were collected beginning May 20. Most of the *A. impiger* larvae were obtained from pools C3, C4, C5 and C20 (Appendix C1). *A. nigripes* was found to be the main species inhabiting pools C21 and C32 (Fig. 6(c) and 6(d), Appendix C1). The water in these two pools was clear and the larvae were often seen browsing along the edges and among the tufts of grass. Instar I and II larvae from these pools were fed a protein rich diet (7C) in the laboratory. The adults were supplied with honey only. The females were dissected at different periods of time and the stage of

development of the ovarian follicles recorded. I wanted to compare the field-reared *A. nigripes* with the laboratory reared individuals. For this purpose *A. nigripes* pupae from C21 and C32 were collected and allowed to emerge in the laboratory. Adults were handled in the same way as the previous group, and dissected after varying periods of time. The results of these observations are summarized in Table XVI.

Preliminary studies on *A. nigripes* indicated that the ovarian follicles of anautogenous females never developed beyond late stage IIb, when females were offered carbohydrates but no blood. Ovarian follicles of autogenous females developed to stage V within a period of 10 - 20 days when held at 15° - 20°C. Therefore all females that developed their follicles beyond stage IIb (resting stage) were considered as being able to develop their ovarian follicles autogenously. The results indicate that there is no difference between laboratory-reared and field-reared *A. nigripes*. It is also observed that only about 25% of the ovarian follicles develop autogenously to mature eggs in *A. nigripes* and only 9% of the follicles matured in the three *A. impiger* that developed eggs autogenously. A summary of the dissections performed in 1968 are shown in Table XVIII, indicate that approximately 82% of *A. nigripes* and 6% of *A. impiger* at Camp Nanuk, Churchill, are autogenous.

TABLE XVI

Ovarian development in *Aedes nigripes* (Zett.) and *Aedes impiger* (Walker) from Camp Nanuk, Churchill, Manitoba. Adults were offered honey. Laboratory temperature approximately 20°C. (Study conducted in Spring-Summer, 1968).

Species	Larval Diet		Stage of ovarian follicles IIa	IIb(e)**	IIb(m)**	IIb(l)**	IIIa	IIIb	IV	V
A. nigripes (Laboratory reared)	7C*	No. individuals	-	11	1	5	4	3	4	53$^{\theta}$
		Mean no. follicles	-	-	124.5	-	114.5	83.6	73.5	31.2±2 (Range 6-59 eggs)
		Age of individuals	-	10-15 days			10-20 days		12 days	5-20 days
A. nigripes$^{\Delta}$ (Field reared)	-	No. individuals	-	1	-	3	-	-	3	35$^{\theta}$
		Mean no. follicles		132.0			-	-	70.0	34.1±2 (Range 9-57 eggs)
		Age of individuals		15 days		15 days	-	-	2-5 days	6-18 days
A. impiger (Laboratory reared)	7C	No. individuals	33	17	40	27	2	2	1	3$^{\theta}$
		Mean no. follicles	96.4		74.1		47.2		23	9.0 (Range 7-12 eggs)
		Age of individuals	7-20 days	8-25 days			All these individuals 20-25 days old.			10-25 days
				Resting stage of anautogenous females.						

* See Appendix Bii for composition.
Δ *A. nigripes* pupae collected from pools C21 and C32 (see sketch 1, Appendix E).
θ Laid eggs.
** IIb(e)=IIb early; IIb(m)=IIb middle; IIb(l)=IIb late (see methods, Section III, p.57).

(b) Woodland species.

A. communis was the most abundant species in the immature stages, and in fact the only species to be found easily in pure or nearly pure colonies in a number of pools. Most of my study was confined to specimens collected from pool #C2 (Fig. 7 & Appendix C2) in the Goose Creek locality. The pool was located in the clearing about 500 yards south of the Power house which is at the end of the Goose Creek Road. The vegetation in the pool is mainly grass and sedges. The pH of the water in the pool varied from 4.5 - 5.5. Larvae of *A. communis* were also collected from another open grassy pool in the vicinity of Farnsworth Lake. The vegetation around *A. communis* habitats is characteristic of the transition between the tundra and boreal forest. The dominant vegetation is black spruce.

In a preliminary examination of *A. communis* in the spring of 1967, about 95% of the adults maintained on honey developed their ovarian follicles beyond the resting stage, with several individuals developing fully mature eggs in 10 days.

In the spring of 1968, as the snow in this pool began to thaw (May 25), several hundred 1st instar larvae were found in pockets among the tufts of grass. The temperature of the water near the surface was 3°C and there was still a thick layer of ice at the bottom of the pool. First instar larvae collected were fed different diets. The adults

Fig. 7 *A. communis* habitat at Goose Creek, Churchill, Manitoba. (Semi-forested habitat).

(a) Pool #C2 in which *A. communis* was the most abundant. Over 90% of the *A. communis* from this pool were autogenous. Photograph taken on 28th May 1968.

(b) Same pool as seen in mid-June, covered by a thick growth of grass. Larvae and pupae were found in small pockets of water among the tufts of grass.

(For location of this pool, see Sketch - Appendix C2.)

a

b

were provided with honey as the sole source of imaginal food. Adults were dissected from 10 - 25 days of age. There was no appreciable difference in the number of eggs developing in adults when larvae were fed different diets. The larvae reared in the laboratory on the richest diet (7C) developed approximately 34 eggs. This was similar to the number of eggs (mean 33) developed by females obtained from larvae allowed to grow in their natural environment (Table XVII). The results also show that adults obtained from larvae reared on rich diets and from field reared specimens, developed only about 33% of their follicles to stage V. Table XVIII shows that 94.3% of the *A. communis* population in Churchill was autogenous.

Several *A. communis* females, reared on different diets in the laboratory, and females from field collected pupae were preserved at different ages and microtome sections examined. Also sections of females which laid eggs were examined. None of the serial sections showed any evidence of autolysis of the flight muscles. My observations confirm those reported earlier by Beckel (1954).

Around mid-July 1967, while collecting adult mosquitoes, I observed a few *A. campestris* adults which had pollinia of *Habernaria* spp. stuck to the head. This meant that *A. campestris* had visited this plant for nectar. Hence I wanted to investigate whether *A. campestris* developed eggs autogenously. Several wild caught females of *A. campestris*

TABLE XVII

Ovarian development in laboratory and field reared *Aedes communis* (Degeer) from Churchill, Manitoba (58°N). Adults maintained only on honey at approximately 20°C.

	No. of individuals in the different stages of ovarian development								Mean	
Larval Diet*	IIa	IIb(e)**	IIb(m)**	IIb(l)**	IIIa	IIIb	IV	V	No. Eggs	Range
1. Dog food + Brewer's Yeast	-	3	-	-	-	-	3	16	21.9±1.6	15-45
2. Dog food + two fish foods	-	-	-	2	-	1	-	3	32.3±3.2	16-52
3. Brewer's Yeast + Two fish foods	-	-	-	-	-	-	-	14	24.5±2.0	11-38
4. 4 Component diet	-	-	-	-	-	-	2	19	22.4±1.5	11-34
5. 6 Component diet	-	3 (94)	-	3 (86)	3 (74.6)	4 (77.5)	13 (38.6)	91	29.9±0.2	11-53
6. 7 Component diet	-	1 (96.2)	-	-	1 (80)	4 (76.8)	6 (41.8)	19	34.4±1.8	21-57
7. Field Reared	-	1 (96) (resting stage in anauto-genous females)	-	-	-	-	2 (49.0)	15	32.9±3	13-55

* See Appendix Bii for composition of diets.
Fourth instar larvae collected from pool C2 (See sketch 2, Appendix E). Only larval that pupated within 24 hours were taken.

** IIb(e)=IIb early; IIb(m)=IIb middle; IIb(l)=IIb late (See Methods, Section III, p.57.)
Note: Numbers in parentheses refer to mean number of follicles.

TABLE XVIII

Summary of studies showing percentage autogeny in four species of *Aedes* at Churchill, Manitoba.

Species	No. females dissected	No. females with ovarian follicles in stages IIa - IIb	No. females with ovarian follicles in stages III - V	% females developing follicles autogenously
A. communis[1]	229	13	216	94.3
A. nigripes[2]	123	21	102	82.9
A. impiger[2]	125	117	8	6.4
A. campestris[3]	47	27	20	42.6

1. Summary of Table XVII.
2. Summary of Table XVI.
3. Wild caught females supplied only honey. Observations made in summer, 1967.

were placed in single cages and provided with honey as a source of carbohydrate food. Ten females were dissected on the day collected, and all were found to be nulliparous. The females were dissected after 10 days, and of 47 adults, 20 developed their ovaries beyond the resting stage i.e. 42.6% were autogenous (Table XVIII).

In mid-July, 1968, just prior to leaving Churchill I collected 30 *A. campestris* adults in the field. These were blood fed to obtain eggs, in order to investigate the phenomenon of autogeny in laboratory reared *A. campestris*. Of the 24 females that laid eggs after a blood meal, 6 adults laid over 100 eggs per female, with one individual laying as many as 200 eggs. These eggs were kept at 20°C for 3 months, and then placed in the cold. Prior to placing eggs at 2°C for cold conditioning, each batch of eggs was placed separately in vials containing hatching medium. 78 eggs hatched without any cold treatment and the larvae were healthy. These larvae were fed a protein rich diet, and reared at 20°C, and 16L:8D photoperiod. Adults were offered only honey. Six out of sixteen (37.5%) females developed their ovarian follicles autogenously (Table XIX).

The unhatched *A. campestris* eggs from 20°C were placed in the cold for conditioning (3 months at 2°C). After cold conditioning the eggs were hatched and two experiments were performed with the larvae. In the first experiment, larvae were fed on different diets at 20°C, 16L:8D. The

TABLE XIX

Autogenous egg development in multivoltine *A. campestris*, from Churchill, Manitoba, reared at 20°C. and 16L:8D photoperiod. (Larvae obtained from eggs which hatched at 20°C prior to cold conditioning, equivalent to second generation.)

Age at dissection	No. dissected	Stage of ovarian follicles IIa	IIb	III	IV	V	% Autogeny
10-12 days	3		2			1 (50)	
20 days	13		8	4		1 (20)	
Total	16		10	4		2	37.5

Note: Figures in parentheses indicate number of eggs developed.

results of this experiment are shown in Table XX. It is observed that 8 out of 12 adults obtained from larvae fed a 6C diet, and 10 out of 14 adults obtained from larvae fed a 7C diet developed their ovaries autogenously. Only 4 out of 15 adults obtained from larvae on a less nutritious diet (dog food and two fish foods) developed their ovaries autogenously. A total record of all the adults examined in this experiment shows that 30 out of 62 adults (or 48.3%) were autogenous.

In the second experiment, *A. campestris* larvae obtained from conditioned eggs, were fed a 7C diet and treated at two different photoperiods. Results of this experiment are given in Table XXI. When reared at a short photoperiod (12L:12D) 76.9% of the adults were autogenous while under a long photoperiod (16L:8D) only 43.3% were autogenous.

A. excrucians, *A. fitchii*, *A. pionips* and *A. punctor* from the forested area in Churchill were tested for autogenous development of the ovaries. *A. pionips* developed its follicles to late stage IIb, whereas most of the other species seem to rest in early stage IIb, i.e. with the ovarian follicles with a few yolk granules around the oocyte.

TABLE XX

Effect of different larval diets on autogenous ovarian development in univoltine *Aedes campestris* from Churchill, Manitoba, reared at 20°C, and 16L:8D photoperiod.

Diet*	No. Females Dissected	Stages of ovarian development				Mean No. Eggs	% Auto-geny	Range
		IIa	IIb	III	IV V			
Dog food + Blood meal	6		5		1	7		-
Dog food + 2 fish foods	15		11	3	1	37		-
Blood meal + 2 fish foods	4		4					
Dog food, blood meal and 2 fish foods	11		4	3	4	9		(7-10)
6 Component diet	12		4	2	6	28.6		(17-52)
7 Component diet	14		4	5	5	24		(14-30)
Total	62		32	13	17		48.3	

* For composition of diet see Appendix Bii.

TABLE XXI

Effect of photoperiod on autogenous ovarian development in univoltine *Aedes campestris* from Churchill, Manitoba, reared at a constant temperature of 20°C.

Photoperiod L : D	No. Females Dissected	Stages of ovarian development							Mean No. Eggs	Range	% Autogeny
		IIa	IIb(e)	IIb(l)	IIIa	IIIb	IV	V			
12 : 12	13			3	3			7	21.7±2	10-40	76.9
16 : 8	60		34		10	4		12	20.8±2	9-39	43.3

CHAPTER V

DISCUSSION

PART 1 - SELECTION OF OVIPOSITION MEDIUM BY AUTOGENOUS *A. ATROPALPUS* (BELLEVILLE STRAIN), AND STORAGE OF EGGS.

From the results of the experiments summarized in Table I it is evident that *A. atropalpus* females showed a preference for water which contained the developing stages of the species. The females responded to the filtrate, so it appears that the response is to some chemical(s), which is soluble in water. Hudson and McLintock (1967) reported that a certain chemical factor associated with the developing stages of *Culex tarsalis* influenced the selection of an oviposition site by the females of this species. They postulated that the response was primarily due to a non-volatile material present in the water rather than to an odour emanating from it. Dethier *et al* (1960) suggest that there are three possibilities of how a chemical stimulus could act, (i) as an arrestant which may stop or slow the insect as it contacts the material, (ii) as an oviposition deterrent which inhibits oviposition or (iii) as an oviposition stimulant which induces the insect to oviposit on it. Since eggs were laid in all the experimental dishes the second possibility is excluded. Several times I have noticed females settle on

the surface of the water and if the chemical acted as an arrestant, then it is probable that the insect would have remained on the surface at first contact. But since at times they were seen to fly away after contact with the water surface for a few seconds, it is possible that the chemical did not act as an arrestant. On the other hand since the majority of eggs were laid between the dusk and dawn period, it is likely that the oviposition behaviour is brought on by dusk and the females then respond to the ovipositional stimulant.

If female *A. atropalpus* respond to a non-volatile stimulant, the response could have been initiated by contact chemoreceptors present on the legs and mouth parts of the female. Chemoreceptor hairs, which can detect appropriate concentrations of different sugars, have been found in the tarsi, labella and ligula of *Culiseta inornata* and *Aedes dorsalis* by Owen (1963) and Feir *et al* (1961). If on the other hand the ovipositional stimulant was volatile then certain setae on the antennae which serve as olfactory organs (Steward and Atwood 1963) could detect the odour.

The natural habitats of *A. atropalpus* in temperate regions are rock pools in the vicinity of streams (Dyar 1903, Hedeen 1953) and in tropical regions they have been found to breed in vases, cans, etc. (Kumm, Komp & Ruiz 1940). These breeding places are rather small enclosed areas. The preference for such habitats would undoubtedly be reinforced

by an ovipositional stimulant in the water in which they developed as shown in my experiments.

Since *Aedes* eggs are placed on a moist medium until they embryonate, and kept slightly moist thereafter to prevent dehydration, it has been an ideal environment for the development of fungi which grow abundantly on moist cellulose subsrates. My studies indicate that a satisfactory method of storing *Aedes* eggs free from fungus is to place eggs on inert material, nylon cloth over glass wool, and sprinkle a saturated solution of parahydroxybenzoate at least once a month. This solution has no harmful effects on the eggs.

PART 2 - PHOTOPERIODIC EFFECTS ON DIAPAUSE INDUCTION IN AUTOGENOUS *AEDES ATROPALPUS* (BELLEVILLE STRAIN).

In their recent reviews, Lees (1955), de Wilde (1962), Beck (1968) and Danilevskii (1965) have stressed the importance of daylength as one of the chief factors which governs the seasonal cycles of many insects. A large number of insect species could survive the adverse conditions by entering into a state of embryonic diapause. The role of photoperiod in the induction of embryonic diapause has been demonstrated in only a relatively small number of species (Beck 1968). Kappus (1964) and Kappus & Venard (1967) made elaborate studies on the influence of

photoperiod on the induction and termination of embryonic diapause in *Aedes triseriatus*. Vinogradova (1965) reported on the influence of photoperiod on embryonic diapause in *Aedes togoi*. Anderson (1968) reported on the influence of photoperiod in the induction of diapause in *Aedes atropalpus* strains from Texas, and Connecticut, U.S.A. The experiments reported herein, pertaining to the influence of photoperiod and temperature on the induction and termination of diapause in the autogenous *A. atropalpus*, were begun in the winter of 1966/67 and conducted independently and concurrently to that reported by Anderson on the Connecticut strain of *A. atropalpus*.

From Tables III and IV, and Fig. 2, it can be seen that at constant temperatures of 15°C and 23°C and at photoperiods of 8L:16D to 12L:12D, the Belleville strain of *A. atropalpus* produces diapause eggs. The reason I say they are in diapause, is that they do not hatch when subjected to a hatching stimulus, when kept at 23°C for periods up to 90 days (Table IX). Very warm temperatures (30°C) or longer photoperiods (16L:8D) prepare the eggs for hatching (Tables X and XI). The Austin strain of *A. atropalpus* would lay diapause eggs only when reared at photoperiods of less than 13 hr. light per day.

Results of experiments (Table III) on the Belleville strain of autogenous *A. atropalpus* show that diapause was averted almost entirely at 30°C regardless of the

photoperiod regime. These results also indicate that the induction of diapause in *A. atropalpus* (Belleville strain) was under photoperiodic control, but the effect of the stimulus could only be measured at temperatures lower than 30°C.

During the late summer and autumn when daylength hours are conducive to the production of females which lay diapause eggs, high temperatures (30°C) would rarely be obtained even for a few hours per day. Therefore the suppression of the effect of short photoperiods by high temperatures has no ecological significance. At present it may only be suggested that it could be of physiological interest. Once the nature of the "factor" that induces diapause in these insects is known, it could well be that this factor, under short daylengths, is a compound which is chemically thermolabile and in some way changed or broken down at a temperature of 30°C, hence causing the females to lay non-diapause eggs. Data in Table III also indicate that the long photoperiod is independent of low temperatures and that even at 15°C non-diapause eggs are laid. Hence it is clear that the ability of females to lay diapause eggs in autumn is due solely to the influence of the shorter daylengths, in late August and early September, which the fourth instar larvae, pupae and adults experience.

Many investigators have demonstrated that, in general, the photoperiodically sensitive period directly precedes the stage associated with diapause. For example, in the

larval diapause of *A. triseriatus*, which is associated with the fourth instar, it is the preceding instars that are sensitive (Love & Whelchel 1955). In the imaginal diapause of *Anopheles* and *Culex*, the late instars of the larvae are the most sensitive (Vinogradova 1960). Vinogradova (1965) showed that in *A. togoi* (a species which undergoes embryonic diapause) it was the photoperiod conditions during the imaginal life which played a significant role in determining the type of egg laid by the mosquitoes. Therefore, also in *A. togoi*, the sensitive stage directly precedes the diapause stage. The data presented in Table VI shows that the type of eggs laid by the autogenous Belleville strain of *A. atropalpus* was determined by the photoperiods experienced during the fourth larval instar, pupa and adult. Anderson (1968) further showed that by crossing long-day females with short-day males and vice versa, the induction of embryonic diapause in the Connecticut strain of *A. atropalpus* was strictly maternal. In *Bombyx mori* (Kogure 1933) and the aphid, *Megoura viciae* (Lees 1959) the interval between the sensitive and responsive (diapausing) stages is practically an entire generation. In *A. atropalpus*, although the stages sensitive to photoperiod are farther removed from the responsive stage than in many mosquitoes, they are not as far removed as in *Bombyx mori* and *Megoura viciae*.

According to Beck (1968), to determine the effects of photoperiod on the induction of diapause, experimental

insects are usually reared for the entire life cycle under controlled conditions of light, temperature, and diet. The results are most frequently measured in terms of the percentage incidence of diapause within the experimental population. The effect of different photoperiods on diapause incidence is plotted as a diapause induction response curve as in Fig. 2. The experimental results in Table IV and Fig. 2 show that the Belleville strain of *A. atropalpus* conforms to Beck's (1968) Type III diapause induction response curve which shows two well defined critical photoperiods. He terms this type of reaction a "short-day - long-day response". In this type of response diapause is induced by only a relatively narrow range of daylengths, which for the Belleville strain is around 6 1/2 hr. - 14 1/2 hrs. light per 24 hr. cycle. This curve shows that neither continuous illumination nor continuous darkness induces diapause. This is in contrast to (1) the long-day response curve of insects like *Leptinotarsa decemlineata* (de Wilde 1958), *Pectinophora gossypiella* (Adkisson *et al* 1963) in which constant darkness promotes the induction of diapause and continuous illumination does not; and (2) the short-day response curve where the converse to (1) applies as seen in the silkworm *Bombyx mori* (Kogure 1933). Daylengths shorter than 8 hrs. are never encountered by insects in their natural environments during the growing season. Very short daylengths would be experienced only in the high Arctic, but the very low temperatures during this period

would be a limiting factor in the distribution and survival of insects in such regions. In *A. atropalpus*, and other temperate zones insects exhibiting the Type III diapause induction response curve, the portion of the curve less than 8 hours is of no apparent ecological significance, but it could be of interest from a physiological and theoretical point of view. The greatest percentage of diapause eggs (> 90%) was laid when the populations of the Belleville strain were reared at photoperiods ranging from 8-14 hrs. light (or 16 hrs. and 10 hrs. darkness respectively). The data in Table IV also indicates that at photoperiods of 14 1/2 hr. light or longer in a 24 hr. cycle, females lay a very high percentage of non-diapausing eggs. This is the upper limit of the critical photoperiod for this strain of *A. atropalpus*.

The critical photoperiod is defined as the one at which the transition from a long- to a short-day effect occurs. In most studies in insect photoperiodism, the critical photoperiod is determined by rearing the insects in sufficiently large numbers for a population response to be obtained, when maintained under well defined temperature and light regimes. Certain workers consider the 50% response point on the population response curve to represent the critical photoperiod. In the experiments reported herein, I have taken the critical photoperiod to lie between the two consecutive photoperiods one of which induces a high percentage of diapause eggs and the other a very low

percentage of diapause eggs. This interval has been calculated to within a difference of a half hour (Table IV). The critical photoperiod for the autogenous Belleville strain lies between 14 and 14.5 hrs. of light per day and that for the Austin strain is between 12.5 and 13 hrs. of light per day (Fig. 3). The difference in the critical photoperiod among strains is due to the fact that insect species may consist of many geographical strains each of which exhibits a characteristic critical photoperiod (Danilevskii 1965). As a result each strain is able to enter diapause at the most appropriate time of the season in the latitude in which it lives. One of the best documented examples of this phenomenon is found in the work of Danilevskii (1965) with *Acronycta rumicis* in latitudes between 40° and 60°N. The geographical strains of *A. rumicis* living in these regions show significant differences in the critical photoperiod for inducing diapause. Depner and Harwood (1966) showed that there were differences in the photoperiodic responses of strains of *An. freeborni* from Washington and California. Similarly Kappus and Venard (1967) showed variations in the photoperiod requirements of the Ohio and Alabama strains of *A. triseriatus*.

In order to make the laboratory observations of the critical photoperiod of the Belleville and Austin strains of *A. atropalpus* more meaningful, it is best to relate the laboratory data to the daylength conditions in nature. For this

purpose the daylengths inclusive of civil twilight for Belleville, Ontario, (44°15'N) and Austin, Texas (30°20'N) are compiled and listed in Appendix F. The curves depicting these daylengths are shown in Appendix G. From this it is possible to postulate that in the field the larvae of the Belleville strain, developing in the last week of August and September, are subjected to daylengths of 14.5 hrs. and less and hence would give rise to females which lay diapause eggs. If daylength had no influence on the induction of diapause, then these late summer and fall mosquitoes would lay non-diapause eggs which would hatch and give rise to larvae that would only perish as a result of the cold temperatures in late September - early October. It is in order to avoid such catastrophic conditions that organisms have evolved the ability to distinguish long and short daylengths and hence be able to utilize this information as clues to impending seasonal changes. In this way they are able to undergo the appropriate physiological changes necessary for winter survival.

The experiments reported here on diapause induction were studied under controlled conditions in which the insects were exposed to constant rather than gradually changing photoperiods. de Wilde (1962) stated that nearly all insects studied responded to absolute daylengths rather than to changes. However, Danilevskii (1965) is of the opinion that although the role of the absolute duration of

daylength in governing diapause in insects has been fully proved, it does not exclude an effect due to photoperiodic changes from a long- to a short-day or vice versa. Corbet (1956) was the first to suggest that diapause was regulated by changes in the duration of the photoperiod rather than the absolute duration of daylength. He was of the opinion that the regulating factor was not absolute daylengths but the process of its lengthening within the limits of definite products. Bunning (1964) suggested that for organisms to orient their behaviour with respect to the proper season, they must be able to determine whether a particular day-length is, within a certain period of time, increasing or decreasing. Recently Tauber and Tauber (1970) reported that *Chrysopa carnea* responds to changing daylengths. When the insects experienced decreasing daylengths, though still greater than the critical photoperiod, they entered diapause. Conversely those which experienced increasing photoperiods, even less than the critical photoperiod did not diapause or if they were in diapause they terminated diapause. The work of Wellso and Adkisson (1966) explains how the bollworm may distinguish autumn from spring. However, the nature of the mechanism governing this reaction is unknown. Most of the workers on the photoperiodic induction of diapause have used absolute daylengths. Although in the present studies no experiments were done with gradually changing daylengths (light periods) it would be safe, after studying most of the recent

investigations, to surmise that insects actually measure changes in the daylength because this is how it happens in nature. Investigations on these lines using gradually changing daylengths should be undertaken in the future. The advantage of using absolute daylengths is that it may provide some clues to the physiological mechanisms that govern the photoperiodic induction of diapause.

Much valuable information concerning the light and dark phase reactions which control flowering in many plants has been obtained by interrupting an effective dark period by short light exposures (Borthwick *et al* 1960). Such experiments have established that flowering is governed essentially by the dark period. Beck (1962) is also of the opinion that the duration of the scotophase is more critical than the photophase duration in the induction of diapause in the European corn borer. Bunning and Joerrens (1960) showed that the cabbage worm *Pieris brassicae* enters a pupal diapause when reared under short photoperiods and the incidence of diapause was reduced when the scotophase was interrupted by a short period of light of 2 hours and the amount of the reduction depended on the time at which this light occurred. In two separate experiments using scotophases of 16 hrs. and 12 hrs. duration the diapause was prevented when the light interruption occurred each day at about 16 hrs. after the beginning of the photophase. They interpreted these results to suggest that diapause induction

involves the effect of photoperiod on endogenous circadian rhythms, with the circadian time being measured from the onset of the photophase.

Results of my experiments (Figs. 4 and 5) show that the light interruptions of 1 hr. during the 16 hr. and 12 hr. scotophases of a 24-hr. photoperiod cycle caused a reduction in the incidence of diapause in the Belleville strain of *A. atropalpus*. The maximum inhibition of diapause is obtained when the light interruption occurs 7-9 hrs. (around 8 hrs.) from the onset of the dark phase. These results are somewhat similar to those of Bunning (1960) in that only one maximum response was obtained, but the response he obtained with the cabbage worm was associated with the onset of the light phase. My data is typical of the responses of most insects to light interruptions during the night inasmuch as one dip is found in the curves (Danilevskii 1965; Minis 1965; Peterson & Hamner 1968). However Adkisson (1964) working on the larval diapause in the bollworm shows two maxima, one of which he associated with dawn and the other with dusk. When he changed the duration of the photophase the two sensitive periods changed relative to each other. Therefore he concluded that both the 'lights-on' signal and the 'lights'off' signal were involved in the time measuring aspects of diapause induction.

In an attempt to explain the biological effects of light interruptions, Bunning (1960) postulated that

diapause induction in the cabbage worm was prevented when such light breaks during the scotophase coincided with the time of the endogenous cycle at which the insect's light sensitivity was at a maximum. For the cabbage worm the maximum light sensitivity occurred at about 16 hrs., after the beginning of the photophase. The data obtained from my experiments with *A. atropalpus* (Belleville strain) indicates that the maximum light sensitivity for this species occurs around 8 hours after the onset of the scotophase. This response may indicate an "endogenous rhythm of sensitivity" to light. In order to obtain a better understanding of any possible rhythm of sensitivity, future work on light interruptions will have to be designed in which *A. atropalpus* would be subjected to abnormal light : dark cycles with short light interruptions in the dark period. Each cycle should contain a short photophase and the length of the cycle being determined by the length of the dark period. In this way it may be possible to obtain an undulating curve which would then indicate the nature of an endogenous rhythm of sensitivity, whether it be circadian or sub-circadian with an 8 hour periodicity. Photoperiodically controlled metabolic and endocrine rhythms have been observed in several insect groups (Beck 1968) and several of these rhythms were found to display non-circadian periodicities of 12, 8, or 6 hrs.

At present I could only propose a hypothesis that in *A. atropalpus* the 'lights-off' signal appears to trigger

some endogenous secretory rhythm with an 8-hr. periodicity. The fact that in both experiments (Figs. 4 & 5) a light interruption around 8 hours in a diapause inducing scotophase causes the Belleville *A. atropalpus* females to produce non-diapause eggs would indicate that when the short light break coincides with the time of a secretory activity rhythm, it could either (a) suppress the secretion of the material or inactivate it. If this was a 'diapause hormone' then its suppression or inactivation would give rise to non-diapause eggs; or (b) increase the secretory activity. If the secretion is a hormone which promotes continuous development then its presence in concentrations above a certain threshold would inevitably give rise to non-diapause eggs.

Kind (1968), in studies of embryonic diapause in *Orgyia antigua*, observed that the secretion of the sub-oesophaegeal neurosecretory cells played a deciding role in diapause regulation. Visible changes in the cells appeared in 'diapause' females prior to emergence from the pupa and remained until completion of egg laying. In a study of diapause in the chrysomelid beetle *Galeruca tanaceti* L. based on histological observations Siew (1965) concluded that diapause, ovarian maturation etc. are controlled by different levels of hormonal activity and diapause was sustained by a low level of activity of the neurosecretory cells of the brain. These two examples indicate that a certain type of internal secretory activity in the

particular individual would determine its ability to induce diapause or not.

It is my opinion that with *A. atropalpus* more detailed physiological experiments complemented by histological examinations should be undertaken to determine the exact nature of the 8 hour rhythmic activity, its source and target organs.

PART 3 - TERMINATION OF DIAPAUSE

Aedes atropalpus, being a multivoltine species, has a facultative diapause induced by short daylengths. The diapause in the embryonic state can only be terminated if the eggs are subjected to some reactivation process. When maintained under diapause inducing conditions i.e. short photoperiods, 8L:16D and 23°C a very high percentage of the eggs, 91.6%, remained in diapause even after 90 days (Table IX). This indicates that the "diapause developmental processes" (Andrewartha 1952) were taking place only very slowly, if at all. It is during the period of diapause development that certain physiological processes take place within the insect in preparation for the resumption of morphogenesis. Results of experiments summarized in Table X suggest that an elevation of temperature to 30°C accelerates the processes of diapause development and culminates in the physiological processes which cause the termination

of diapause. It is possible that temperature could affect the relative rates of synthesis of materials that are required for the resumption of growth and development.

It appears that diapausing eggs of multivoltine species are different in their internal chemical organisation from eggs of univoltine *Aedes* which have an obligatory diapause. In the latter, as presently known, diapause has not been terminated unless they were reactivated by cold conditioning. However, low temperatures are not always required for termination of diapause as shown by Lloyd (1920) with *Diataraxia oleracea,* a multi-voltine lepidopteran.

Results in Table XI indicate that the exposure of diapause eggs to long day photophases at 25°C results in the termination of diapause in 52.2% of the individuals after 90 days in contrast to only 6% when exposed to short daylengths. The fact that long photoperiods can terminate diapause has also been shown by Oliver (1969) when he exposed unchilled pupae of *Papilio polyxenes* to long day photophases and obtained adult emergence in most individuals. Earlier, Williams & Adkisson (1964) reported that a 16-hr. photophase was most effective in terminating the diapause in unchilled pupae of *Antheraea pernyii* at 25°C.

In nature, however, the diapausing *A. atropalpus* eggs would be subjected to cold temperature. As Hayes *et al* (1968) suggested it is possible that the cold period may have some effect on the permeability of the brain membranes

and tissues which would change the diffusion rates of biologically active materials. With increasing daylengths in the spring, termination processes may involve the release of a hormone from the brain under long day conditions as suggested by Lees (1963). Therefore, as a result of cold influencing the permeability of the brain membranes and tissues and the subsequent secretion of the hormone triggered by lengthening daylengths, the ultimate effect of these two factors is to synchronize the emergence of adults in the spring.

Lees (1964) using micro-illuminators was able to show that the site of photoreception in the aphid *Megoura viciae* was in the dorsum of the head slightly anterior of centre. From the results of my preliminary study (Table XII) using "Flexible Fibre Optics" light guides it is noted that long day conditions promoted the termination of diapause when they acted on the anterior end (head). By contrast exposure of the posterior end was without any detectable effect. Although my results are not adequately documented, it is possible that the photo-receptors lie beneath the chorion in the region of the embryonic brain. Earlier to Lees' observation Shakhbazov (1961) showed that pupae of *A. pernyii* had a transparent "facial" cuticle which overlies the pupal brain and subsequent experiments by Williams & Adkisson (1964) showed that this transparent portion was the main light pathway to the brain.

Lees (1955) suggested that the neurosecretory cells of the protocerebrum provide the link which co-ordinates the internal physiology of the insect with the external environment. Siew (1965) is of the opinion that it is this link that permits whatever mechanism, that governs diapause, to function as a timing device synchronizing the periods of dormancy and active growth with the seasonal changes of the environment.

In the *A. atropalpus* experiment the intensity of the light emitted by the three light guides of diameter 2 mm, 3 mm and 7 mm was 8, 26, and 84 ft.-candles respectively. The amount of light entering the egg is not known, but it is likely that very little would be required. Wright (1966) showed that diapausing larvae of *A. triseriatus* were sensitive to an intensity as low as 0.0016 ft.-candle. Prokopy (1968) showed that a light intensity of 32 lux (approx. 2.976 ft.-c) on the skin surface of the apple would have an intensity of 1.2 lux (0.1116 ft.-c) at the pulp-core interface where the apple maggots, *Rhagoletis pomonella* (Wash.), feed. These apple maggots were able to perceive this light within the apple. Dickson (1949) reported that an intensity of 32 lux at the skin surface of an apple was sufficient to induce diapause in the larvae of the oriental fruit moth, *Grapholitha molesta,* developing inside the apple. It is possible, therefore, that the mosquito embryo within the egg also perceives light, as the results of the experiment (Table XII) indicate.

PART 4 - EFFECT OF PHOTOPERIOD AND DIET ON FECUNDITY IN AUTOGENOUS *AEDES ATROPALPUS* (BELLEVILLE STRAIN).

(a) Effect of photoperiod.

Moore (1966) observed that the frequency of autogeny in *Culex tarsalis* (California strain) was not significantly related to the three different photoperiods of 8, 10 and 16 hours used in his experiments. On the other hand, Harwood (1966) reported that the Washington strain of *C. tarsalis* showed a relationship between the expression of autogeny and photoperiod. The percentage autogeny in the population increased with an increase in photoperiod till an optimum was reached. Individuals reared under photoperiods greater than 16 hr. expressed a low percentage of autogeny. He also showed that *C. tarsalis,* subjected to long photoperiods, produced a larger number of mature oocytes per autogenous female.

The Belleville strain of *A. atropalpus* expresses an obligatory autogeny. Results of experiments, summarized in Table XIII show that photoperiod has a direct influence on the fecundity of the autogenous Belleville strain of *A. atropalpus,* similar to the observations made by Harwood (1966) with *C. tarsalis*. Autogenous *atropalpus* females, reared at short photoperiods (8L:16D), produced the least number of eggs (mean 107 eggs). The number of eggs per female increased with an increase in photoperiod until an optimum was reached at 16L:8D. Beyond this photoperiod there was a decrease in the number of eggs, although the

females reared at 20L and 24L were larger in size (as determined by measuring the radial sector vein, Rs, of the wing) than those reared at 16L. The direct relationship of size to photoperiod would indicate that most of the larval feeding was during the photophase, and larger adults resulted under long light periods. The maturation of oocytes is under hormonal regulation (Lea 1963) and it is possible that the hormonal secretions in the adult autogenous *atropalpus* is regulated by photoperiod. If this is the case, the optimal secretory activity may occur at 16L: 8D, causing the maturation of the largest number of oocytes at this photoperiod.

(b) Effect of diet.

Although autogeny is genetically determined the number of eggs which develop autogenously will also be determined by the larval nutrition and subsequent fat reserves of the adults. Results summarized in Table XIV indicate that with a very rich diet (7C) individuals with more reserves were obtained, as expressed by their dry weights. These individuals developed more eggs (mean 174) than the individuals reared on a poor larval diet like blood meal. This diet gave rise to females with an average of 107 eggs. In these experiments larvae fed on a 7C diet gave rise to females developing on the average 174 eggs whereas in the earlier experiment (Table XIII) at 16L the females developed on the average only

157 eggs. This difference may be attributed to a difference in experimental conditions. In the above experiment, Table XIV, larvae were fed daily at 20°C while in the 'photoperiod experiment' (refer Part 4a) larvae were fed every other day and the experiments conducted at 23°±2°C. Both the quantity and quality of larval food influences the number of eggs developing autogenously. Kardos (1959) reported that in *C. tarsalis* the frequency of autogeny and the average number of eggs can be increased by increasing the total quantity of larval food. Lea (1964b), with *A. taeniorhynchus*, showed that with the larger quantity of food rich in protein he was able to obtain a larger proportion of autogenous individuals. My results show that although the Belleville strain of *A. atropalpus* has an obligate autogeny, the number of eggs developing within a female could differ by as much as 67 eggs between adults obtained from larvae on poor diets, versus rich diets.

PART 5 - EFFECT OF PHOTOPERIOD AND TEMPERATURE ON BITING ACTIVITY AND OVARIAN DEVELOPMENT IN *CULISETA INORNATA* (WINNIPEG STRAIN).

Anopheline and some Culicine females in the temperate regions overwinter as adults. During this reproductive diapause the ovarian follicles do not develop beyond the resting stage. Workers in recent years have been experimenting with factors which induce hibernation in adult

mosquitoes. Attempts have also been made to find out if hibernating females would engorge if offered a blood source. A knowledge of the latter is of epidemiological importance, because if hibernating mosquitoes (those that are vectors of disease) take an infective blood meal in the fall, they could serve as reservoirs. Another important feature is to find out whether hibernating females, which engorge, develop their ovaries or whether they show "gonotrophic dissociation" as defined by Swellengrebel (1929). If the latter happens then nulliparous females collected in spring may be vectors of disease. Danilevskii and Glinyanaya (1958) and Vinogradova (1960) observed that with *C. pipiens* short daylengths inhibit blood feeding and initiate hibernation behaviour. In recent studies Eldridge (1966, 1968) reported that blood-fed females of *C. pipiens* showed gonotrophic dissociation when incubated at low temperatures with short photoperiods and held at low temperatures after feeding. On the other hand, Tate and Vincent (1936) showed that hibernating females of *C. pipiens* were induced to engorge with prolonged illumination and they oviposited readily after a single blood meal. In *Culiseta annulata* (Schrank), it was found that temperature was the factor influencing the degree of ovarian development following a blood meal (Service 1968). At 13.5°C, complete ovarian development occurred in most blood fed females; at 10.4°C ovarian development proceeded to IIb-IIIb, and at temperatures below 10.4°C the ovaries developed only to IIb.

The results summarized in Table XV indicate that some females of *Culiseta inornata* that fed at low temperatures (10°C) and short photoperiods (8L:16D) developed their ovarian follicles to stage V. These mosquitoes exhibit "gonotrophic concordance" (Swellengrebel 1929). At 10°C and 15°C, and short photophases (8L and 12L) the blood feeding activity of the females was very low, and less than 30% engorged. At higher temperatures a greater percentage of the mosquitoes took blood. Also, those reared at a long photoperiod fed more readily than those reared at a short photoperiod. Vinogradova (1958) reported that the attacking activity of *C. pipiens* females increased directly with increases in the photophase used during the rearing of the adults. She further observed that, in contrast to *Anopheles maculipennis messeae*, all the females of *C. pipiens* that took blood matured their ovarian follicles and she concluded that the difference between the active individuals and those entering diapause was expressed only in the differing degrees of aggressiveness. My observation that *C. inornata* engorged under conditions inducing hibernation (short photophases and low temperatures) is similar to that reported by Hsoi (1954) where he observed that some field collected females of *Culex pipiens pallens* sucked blood even in winter months. Feeding began as soon as the cage was placed on the human skin and some gorged at the second or third feeding test. Marshall (1938) observed that *C. annulata* adults take blood meals

both before and at intervals during hibernation.

The experiments with *C. inornata* were performed at 10°, 15°, 20°, and 30°C combined with three photoperiods of 8L:16D, 12L:12D, and 16L:8D. The temperatures recorded indicate the air temperatures in the incubator. The temperature of the larval rearing medium would have been about 2-3° less. This would account for the fact that although there was high mortality at 10°C and 30°C, some adults emerged at these temperatures. Brust (1967), Hanec & Brust (1967) have reported that the upper lethal limit for *C. inornata* was 29°C when the larvae were reared at a constant bath temperature of 29°C.

The laboratory observations may not give a correct explanation of the feeding behaviour of *C. inornata* which might be expected in natural populations. In the laboratory, *C. inornata* were induced to engorge by placing the cage between the arms for a period of time, an almost forced feeding condition. In nature, in autumn, the shorter daylengths and decreasing temperatures may cause the activity of the adults to decrease and thus they do not actively search for a blood meal. The fact that they go into hibernation without blood may be related to decreased flight activity, which in turn separates them from a blood source, and not to an inhibition to feeding when in close proximity to the host. More experiments are needed to sort out these factors.

Photoperiod and temperature have a direct relationship

to the number of eggs developed. At 30°C and 8L the mean number of eggs developed was 133.3 whereas at 10°C and 8L they had only a mean number of 77.3 eggs. It is also observed that under the same temperature conditions the long day females laid more eggs than females reared at short photophases. In this respect it is similar to my observation reported earlier for autogenous *A. atropalpus* (Belleville strain). The reasons for *C. inornata* developing more eggs under these conditions are similar to those I proposed for autogenous *A. atropalpus* reared under long photoperiods. In addition *C. inornata,* under long photoperiods and high temperatures, were more aggressive and took more blood, thereby having more proteinaceous food material for the development of more eggs. The wide range in the egg number per female shows that the quantity of blood engorged by each female differed considerably. Earlier workers (Roy 1936, Woke *et al* 1956) have shown a positive correlation between the amount of blood ingested and the number of eggs produced in *A. aegypti*. The small quantities of blood ingested would also account for some engorged females being able to develop their ovarian follicles to only stage III or IV.

I propose that in the future a study should be undertaken to determine whether field populations of *C. inornata* collected in autumn contain some females which will engorge and develop their ovaries. Hibernating sites should be examined to find whether there are any blood fed adults

in nature. It is possible that if some females feed in autumn they could digest their blood meals in niches where temperatures favour egg maturation. It is unlikely that a parous female could overwinter, although there has not been an adequate study made of overwintering populations of this species.

With a wide variation in the behaviour of hibernating adults it is only possible to speculate that the physiological mechanisms of hibernating adults differ considerably depending on the species. Some hibernating species have no inclination to feed, others feed but show gonotrophic dissociation, while still others feed under laboratory conditions and show gonotrophic concordance.

PART 6 - STUDIES ON AUTOGENY IN *AEDES* SPECIES AT CHURCHILL, MANITOBA.

Of the species studied at Churchill, four aedine species viz, *A. nigripes*, *A. impiger*, *A. communis*, and *A. campestris* exhibited autogenous ovarian development. Of these, *A. communis* was never caught as an adult coming to bite. Hence it could be stated that *A. communis* in Churchill exhibited obligatory autogeny, developing its first cycle of eggs without a blood (human) meal. The other three species were observed to be attracted to man indicating that they go in search of a blood meal. Failing to obtain an external

source of protein to develop eggs these species have the ability of developing some of their follicles autogenously.

Studies conducted with specimens collected at Camp Nanuk, Churchill, a tundra location, indicate that of the tundra species 83% *A. nigripes* showed autogenous ovarian development while only a small percentage (6%) of *A. impiger* were autogenous (Table XVIII). Smith (1970) reported a similar pattern in autogenous ovarian development from populations of *A. nigripes* and *A. impiger* collected at Baker Lake, N.W.T. Only 8 individuals of *A. hexodontus* were obtained from larvae collected in the field, and none of these developed autogenous eggs when the adults were maintained on honey.

The larvae of *A. nigripes* reared in the laboratory on a rich protein diet (7C) produced adults which developed (autogenously) a mean of 31 eggs. They were similar to adults of *A. nigripes* obtained from larvae reared in their natural environment which developed (autogenously) 34 eggs (Table XVI). It would appear that the artificial rich diet was of similar nutritive value, at least from the point of being able to form yolk, to the food material the larvae obtained from their habitat. It appears that *A. nigripes* has the potential to develop only about 60 eggs autogenously, because the number of eggs developed ranged from 6-59 when adults were obtained from laboratory reared larvae and from 9-57 when the adults were obtained from field reared larvae. In contrast to this some blood fed *A. nigripes* have laid around 120 eggs.

Observations made earlier by Hocking *et al* (1950), Hocking (1952; 1954) and Beckel (1954) indicated that *A. communis* in Churchill was able to develop eggs without a blood meal. My studies at Churchill with laboratory and field reared *A. communis* confirm their observations; and further indicate that about 94% of this species is autogenous and 6% are anautogenous. In *A. communis* as well, the adults obtained from larvae reared on a protein rich diet in the laboratory developed approximately the same number of eggs (34 eggs) autogenously as adults obtained from larvae which fed in their natural environments till pupation (Table XVII).

Gross dissection of several *A. communis* females and also examination of sectional material show no sign of reduction or histological abnormality in the flight muscles of the specimens examined. My observations confirm those made earlier by Beckel (1954) when he rebutted the theory advanced by Hocking (1952; 1954). Hocking reported in these papers that he had observed the progressive autolysis of flight muscles of field collected specimens believed to be *A. communis* from Churchill, Manitoba. It was Hocking's opinion that autolysis of these muscles provide a source of protein for the development of eggs. Although it is possible, I doubt very much whether the physiological mechanisms for producing proteinaceous and lipid yolk for the development of eggs is by causing an autolysis of the flight

muscles. It would be an exception rather than the rule. Roubaud (1932) and Beckel (1954) suggest that the larval muscles in newly emerged adults disappear rapidly. The larval muscles which had been carried through the pupal stage are broken down and changed into protein and lipid reserves in the fat body which is later utilized for yolk deposition in the ovaries. However Beckel (1954) has observed that in adults of *A. hexodontus* too, there is histolysis of the larval muscles, but it does not contribute to egg development. Hence it is obvious that the mechanisms by which adults develop autogenous eggs is more involved than a mere histolysis of tissue. The important factor in the manifestation of autogeny in autogenous species is the amount of reserves carried over from the larval period, as was demonstrated for *Culex pipiens* by Clements (1956), *A. taeniorhynchus* (Lea 1964b) and *A. togoi* (Laurence 1964). However, Clements (1956) has also remarked that anautogenous *C. pipiens* has sufficient quantities of reserves to develop some eggs, but is still unable to do so until it has had a blood meal. The exact physiological mechanisms which cause autogenous egg development are not completely solved at present. However, the work of Larsen & Bodenstein (1959), Clements (1956) and Lea (1963) indicate that certain endocrine mechanisms are involved in which the corpora allata play a prominent role.

Whatever mechanism operates it is observed that the females of *A. nigripes, A. impiger* and *A. communis*

develop a few eggs autogenously by depositing the yolk granules only in a small number of follicles. In doing so they withdraw yolk from a large number of follicles. On examination of these ovaries a large number of follicles are seen to be retrogressing. This fact is shown in my data (Tables XVI and XVII) because laboratory reared *A. nigripes*, *A. impiger* and *A. communis* originally had 124, 96 and 96 follicles in stage II, but developed only 31, 9, and 34 eggs respectively.

A. campestris is an abundant woodland species in Churchill, seen flying about mid-July. Studies in 1967 showed that 42.6% (Table XVIII) of the wild caught adults examined developed eggs autogenously when females were maintained in the laboratory solely on honey. *A. campestris* females fed readily on blood if they had access to a host. But in the absence of a blood meal they were able to develop a few eggs, indicating that they were facultatively autogenous.

When *A. campestris* larvae were reared on different diets, a large number of adults obtained from rich larval diets, 6 out of 12 adults from a 6 component larval diet and 5 out of 14 adults from a 7 component larval diet, developed eggs autogenously. In contrast only 1 out of 6 adults obtained from larvae reared on dog food and blood meal and 1 out of 15 adults obtained from larvae fed dog food and two fish foods (Tetramin E and L) developed autogenous eggs.

When *A. campestris* eggs, prior to cold treatment, were placed in hatching medium, few eggs hatched. These may be considered to be equivalent to a second generation of *A. campestris* larvae developing in summer. When these larvae were reared in the laboratory at 20°C, and long photoperiods 16L:8D (≡summer daylengths) only 37.5% were autogenous (Table XIX).

In an experiment in which *A. campestris* larvae were reared at 20°C and two different photoperiods, 76.9% reared on a short photoperiod (12L:12D) developed autogenous eggs and only 43.3% reared on a long photoperiod exhibited autogeny. From these observations and the results in Tables XVIII and XIX I would say that only a small percentage of *A. campestris* adults ($< 50\%$) exhibit autogeny in early summer. And it could mean that a larger percentage (77%) of the adults developing towards the end of summer or early fall, would exhibit facultative autogeny, possibly as a result of hosts being more scarce in the fall than in summer. If this were true it would appear that photoperiod could be a factor determining facultative autogenous development of the ovaries, and it could be stated that with *A. campestris* short photoperiods would give rise to a larger percentage of autogenous individuals than long photoperiods. However, for a more accurate interpretation of these laboratory observations, further detailed field studies would have to be undertaken at Churchill, Manitoba. Populations of

campestris from early summer and others from late summer or fall would have to be tested for autogeny.

From these studies it is apparent that in certain northern mosquitoes, autogeny is often facultative and is of an adaptive value. If the search for a blood meal is difficult due to the scarcity of suitable vertebrate hosts, autogenous egg development ensures the survival of the species.

CHAPTER VI

SUMMARY

1. Females of the autogenous *A. atropalpus* (Belleville strain) showed a preference for water which contained the developing stages of this species for egg deposition. They selected water from which adults had emerged in preference to the larval holding medium or pupal holding medium.

2. A satisfactory method of storing eggs free from fungus is to place eggs on a moist, inert material like nylon cloth over glass wool. Use a saturated solution of methyl parahydroxybenzoate as the fungicide.

3. The Belleville strain of *A. atropalpus* shows a high incidence of diapause at constant temperatures of 15°C and 23°C and short photoperiods of 8L:16D and 12L:12D. High temperatures (30°C) or long photoperiods (16L:8D) do not produce diapause eggs. The combined effect of photoperiod and temperature indicate that diapause in the Belleville strain of *A. atropalpus* is under photoperiodic control, but the effect of the stimulus could only be measured at temperatures less than 30°C.

4. Very short photoperiods, less than $7\frac{1}{2}$ hours per day, do not induce diapause in *A. atropalpus*. Adult females respond to very short photoperiods (at 23±2°C) in the same way as to very long photoperiods ($> 14\frac{1}{2}$ hrs. per day).

5. The critical photoperiod for the autogenous *A. atropalpus* from Belleville, Ontario (44°N) lies between 14L: 10D and $14\frac{1}{2}$L:$9\frac{1}{2}$D and the critical photoperiod for the anautogenous *A. atropalpus* from Austin, Texas (30°N) lies between $12\frac{1}{2}$L and 13L. The difference in the critical photoperiod among strains is due to the fact that the species are of a different geographical origin. As a result of this difference in critical photoperiods each strain could enter diapause at the most appropriate time of the season in the latitude in which it exists.

6. Sensitivity to photoperiod in the Belleville strain of *A. atropalpus* begins during the fourth larval instar, and is also perceived by the pupa and adult.

7. Interruption of a 16-hr. scotophase and a 12-hr. scotophase with light breaks of 1 hour duration show that the lowest percentage of diapause eggs in the Belleville strain was obtained when the light break occurred around 8 hrs. (7-9 hrs.) from the onset of darkness. This indicates that the maximum light sensitivity for *A. atropalpus* (Belleville strain) occurs around 8 hrs. after

the onset of the scotophase.

8. Diapause eggs, if maintained under diapause sustaining conditions (8L:16D and 23°C), remain in diapause for a long time. An elevation of the temperature to 30°C terminates diapause rapidly. This may indicate that the 'diapause developmental' processes (Andrewartha 1952) are accelerated at a high temperature.

9. At a temperature of 25°C long photoperiods (16L:8D) lead to diapause termination.

10. Preliminary studies with "light guides" indicate that it may be possible for embryos within the egg to perceive light and the region of this light perception is possibly in the anterior dorsal region of the embryo.

11. Photoperiod has a direct influence on the fecundity of autogenous *A. atropalpus*. The number of eggs per female increased with an increase in photoperiod until an optimum was reached at 16L:8D. Beyond this photoperiod there was a decrease in egg development, although females reared at longer photoperiods (20L & 24L) were larger in size.

12. Although autogeny is genetically controlled, a very rich larval diet gives rise to individuals which laid a larger number of eggs than those reared from larvae fed on a poor diet.

13. Under short photoperiods and low temperatures, laboratory experiments indicate that *Culiseta inornata* exhibits gonotrophic concordance. The blood feeding activity was low at low temperatures and short photoperiods, and at higher temperatures a greater percentage fed on blood. Photoperiod and temperature have a direct relationship to the number of eggs developed. Under the same temperature conditions the long day females laid more eggs than the short day females. It is possible that they engorged more at long photoperiods and high temperatures.

14. Studies at Churchill, Manitoba (58°N latitude) indicate that four of the northern *Aedes* species are able to develop their eggs autogenously. They are *A. nigripes*, *A. impiger*, *A. communis* and *A. campestris*. Over 90% of *A. communis* are autogenous, while 83% of *A. nigripes*, 6% of *A. impiger* and 42% of *A. campestris* are autogenous.

15. The histological sections of autogenous *A. communis* indicated that none of the females had autolysed their flight muscles as was proposed by Hocking (1954). My results confirm those reported by Beckel (1954).

16. Photoperiod experiments at 20°C, with *A. campestris* indicate that at a short photoperiod of 12L:12D, 77% of the individuals show autogeny while at a long photoperiod

only about 43% express autogeny. I have proposed that it is possible that in the field a higher percentage of the late summer or fall individuals of *A. campestris* would show autogeny. However, this would have to be verified by studying natural populations at Churchill, Manitoba.

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APPENDICES

APPENDIX A

Data of experiments designed to determine which oviposition medium was preferred by autogenous *Aedes atropalpus* (Belleville strain).

Experiment: (a) Emergence water vs Distilled water			(b) Larval holding media vs Distilled water			(c) Pupal holding media vs Distilled water		
Oviposition Day	Dish A EW*	Dish B DW	Oviposition Day	Dish A LM	Dish B DW	Oviposition Day	Dish A PM	Dish B DW
1	1374	522	1	174	138	1	249	235
2	458	103	2	348	107	2	553	162
3	395	194	3	648	243	3	487	106
4	653	127	4	459	37	4	212	144
5	867	314	5	239	134	5	103	47
6	678	265	6	151	31	6	197	70
7	873	293	7	198	50	7	43	8
8	562	187				8	20	7
9	391	112		2213	740	9	58	9
10	277	123				10	54	16
11	216	75				11	96	8
	6717	2315					2072	812

Experiment: (d) Larval holding medium vs Emergence water

Oviposition Day	Dish A LM	Dish B EW
1	701	617
2	577	486
3	1141	261
4	439	170
5	54	128
	2912	1662

(e) Larval holding midium vs Pupal holding media

Oviposition Day	Dish A LM	Dish B PM
1	137	30
2	434	361
3	719	102
4	215	225
5	396	23
6	32	26
7	45	16
8	7	1
	1985	784

(f) Emergence water vs Pupal holding medium

Oviposition Day	Dish A EW	Dish B PM
1	429	293
2	244	396
3	728	412
4	32	0
5	48	0
6	0	23
7	16	65
	1497	1189

(g) Female Emergence water vs Male Emergence water

Oviposition Day	Dish A EW	Dish B EW
1	31	28
2	854	283
3	347	134
4	336	332
5	491	376
6	355	245
7	398	174
8	60	223
9	55	186
10	82	143
11	25	21
	3034	2145

- -

* EW - Emergence water
LM - Larval holding medium
PM - Pupal holding medium
DW - Distilled water

APPENDIX B(i)

The composition of the larval diet used in the culture of, and photoperiod experiments with, *Aedes atropalpus* and *Culiseta inornata*.

7 Component Diet (7C)

		mg. (approx.)
DF	- Dog food (Gaines Gravy Train)	25
BY	- Brewer's Yeast (Fleischmann's)	10
BM	- Blood Meal (Swift of Canada Product)	10
BF	- Blood Fibrin (Nutritional Biochem. Corp.)	10
YE	- Yeast Extract (Difco Laboratories)	10
$FF_{(E)}$	- Tetramin Fish Food E (Tetra Kraft Werke Product)	10
$FF_{(L)}$	- Tetramin Fish Food L (Tetra Kraft Werke Product)	10

Larval diets used in experiments summarized in Table XIV.

1.	DF	- Dog food	- 25 mg.
2.	BY	- Brewer's Yeast	- 25 mg.
3.	BM	- Blood Meal	- 25 mg.
4.	$FF_{(E)}+FF_{(L)}$	- Fish $Food_{(E)}$ + Fish $Food_{(L)}$	- 10 mg. each
5.	DF + BY	- Dog food + Brewer's Yeast	- 25 mg. + 10 mg.
6.	DF + BM	- Dog food + Blood Meal	- 25 mg. + 10 mg.
7.	DF + 2FF	- Dog food + 2 Fish Foods	- 25 mg. + 10 mg. each
8.	5C	- 5 Component diet	- as listed below*
9.	7C	- 7 Component diet	- as listed above

* 5C = DF + BY + BM + $FF_{(E)}$ + $FF_{(L)}$ - 25 mg. of dog food and 10 mg. each of the other components.

APPENDIX B(ii)

Larval diets used in experiments summarized in Table XVII.

1. DF + BY - Dog food + Brewer's Yeast - 25 mg. + 10 mg.
2. DF + 2FF - Dog food + Fish Food(E) + Fish Food(L) - 25 mg. + 10 mg. + mg.
3. BY + 2FF - Brewer's Yeast + Fish Food(E) + Fish Food(L) - 25 mg. + 10 mg. + 10 mg.
4. 4C - 4 Component diet*
5. 6C - 6 Component diet**
6. 7C - 7 Component diet (See Appendix B(i))

Larval diets used in experiments summarized in Table XX.

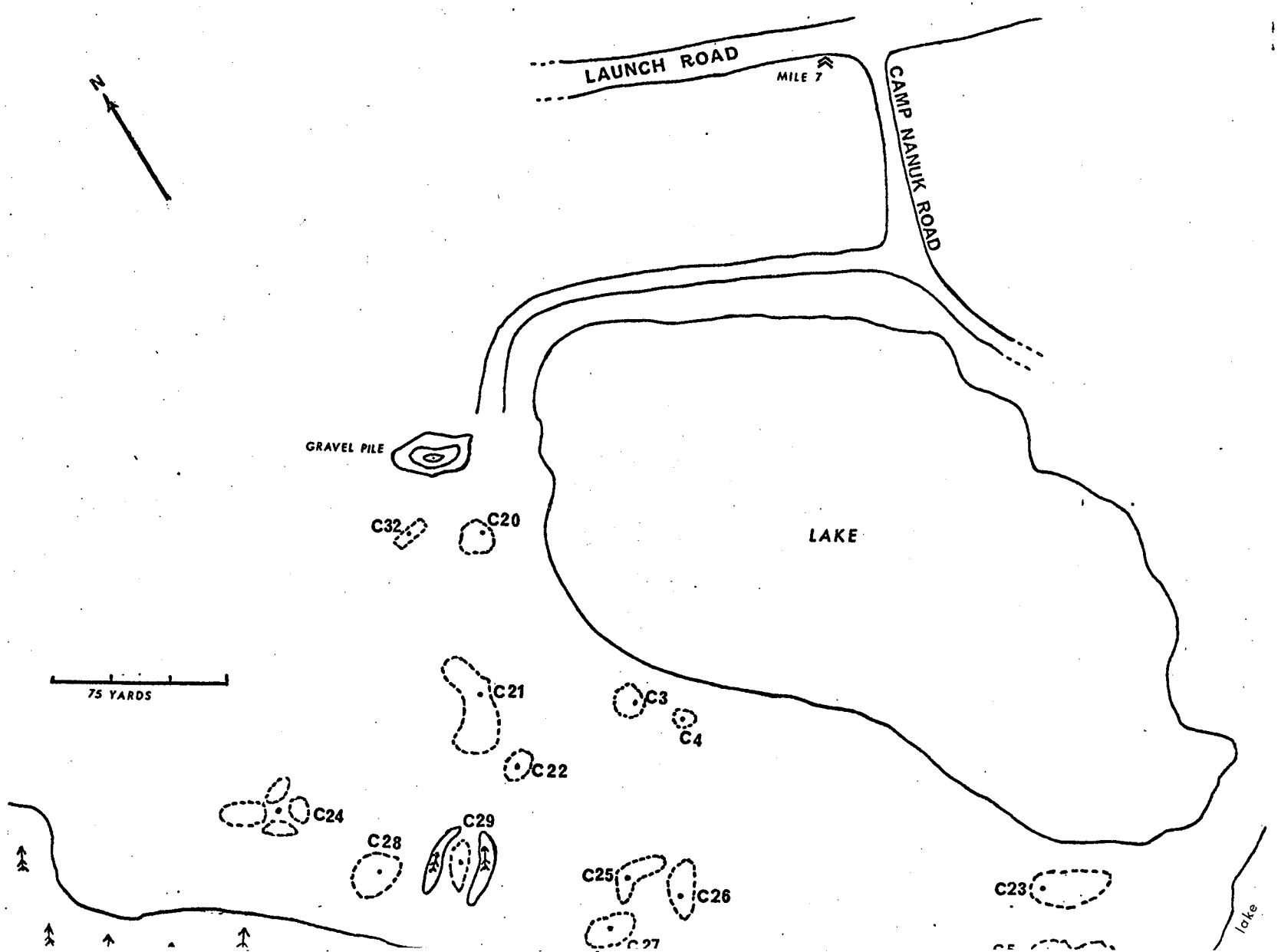
1. Dog food + Blood Meal - 25 mg. + 10 mg.
2. Dog food + 2 Fish Foods (E+L) - 25 mg. + 10 mg. each of fish foods
3. Blood Meal + 2 Fish Foods (E+L) - 25 mg. + 10 mg. each of fish foods
4. Dog food + Blood Meal + 2 Fish Foods (E+L) - 25 mg. + 10 mg. + 10 mg. each of fish foods
5. 6 Component diet**
6. 7 Component diet. See Appendix B(i).

* 4 Component diet = Dog food + Brewer's Yeast + Blood Meal + Blood Febrin - 25 mg. of dog food and 10 mg. each of the other components.

** 6 Component diet = Dog food + Brewer's Yeast + Blood Meal + Blood Fibrin + Fish Food(E) + Fish Food(L) - 25 mg. of dog food and 10 mg. each of the other components.

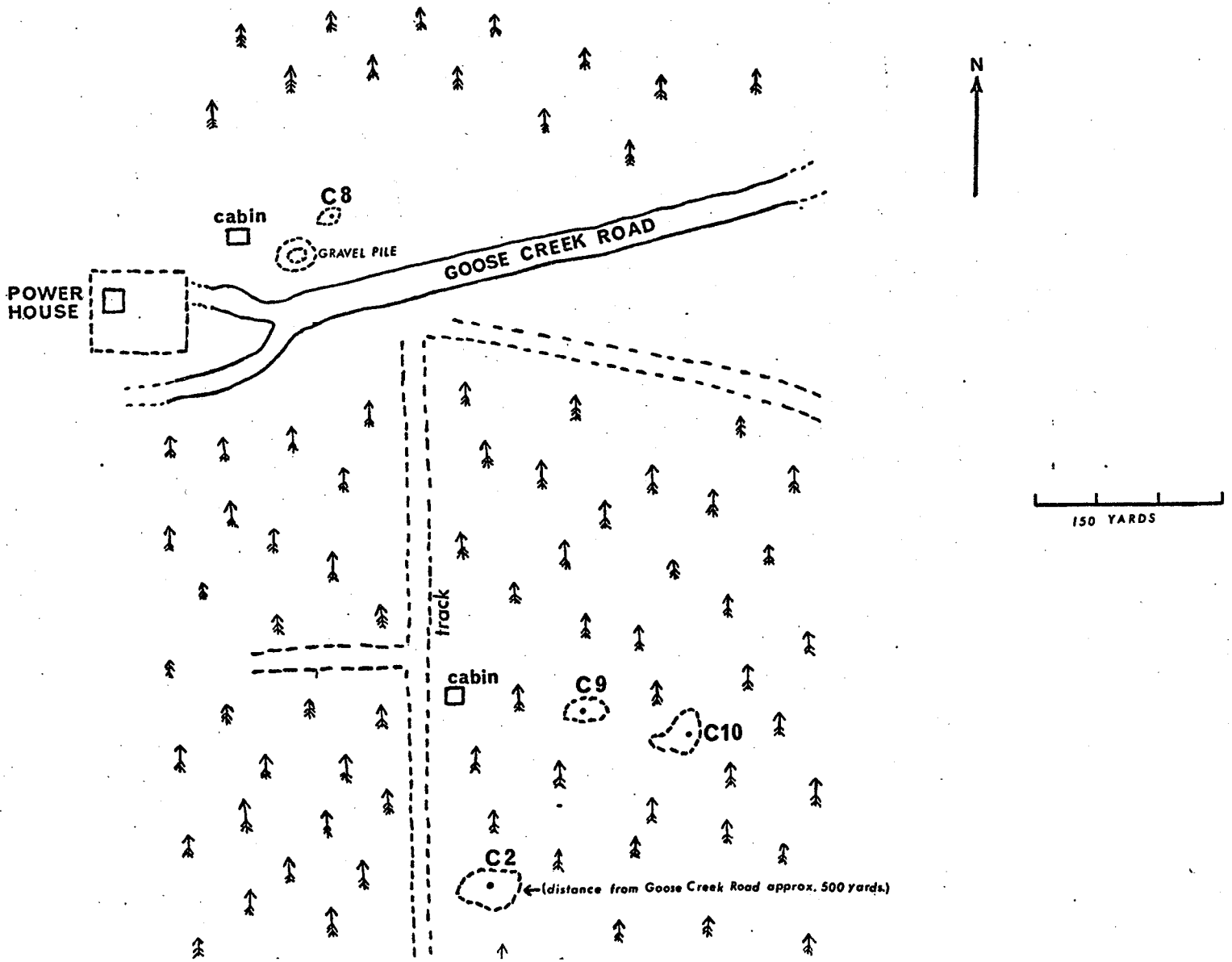
APPENDIX C (1)

Sketch-map illustrating the location of pools at Camp Nanuk, Churchill, Manitoba, from which *Aedes nigripes*, *A. impiger* and *A. hexodontus* larvae were collected.


LAUNCH ROAD
MILE 7
CAMP NANUK ROAD
N
GRAVEL PILE
C32
C20
LAKE
75 YARDS
C21
C3
C4
C22
C24
C29
C28
C25
C26
C23
C27
lake

APPENDIX C (2)

Sketch-map illustrating the location of pool, C2, at Goose Creek, Churchill, Manitoba, from which *A. communis* larvae were collected.


N
C8
cabin
GRAVEL PILE
GOOSE CREEK ROAD
POWER HOUSE
track
cabin
C9
C10
C2
(distance from Goose Creek Road approx. 500 yards.)
150 YARDS

APPENDIX D

Effect of 2-hr. light-breaks, during a 16 hr. scotophase, on the incidence of embryonic diapause in *Aedes atropalpus* (Belleville strain) reared at 23°±2°C.

Schedule	Total no. viable eggs	No. eggs hatched	No. viable eggs in diapause	% diapause
8L:16D	1208	2	1206	99.83
:1D 2L 13D	1394	7	1387	99.50
:2D 2L 12D	954	13	941	98.60
:3D 2L 11D	768	323	445	57.94
:4D 2L 10D	2502	1908	594	23.74
:5D 2L 9D	3133	2466	667	21.30
:6D 2L 8D	1383	1126	257	18.58
:7D 2L 7D	2733	2466	267	9.77
:8D 2L 6D	1365	1335	30	2.41
:9D 2L 5D	1148	1131	17	1.50
:10D 2L 4D	1241	535	706	56.89
:11D 2L 3D	748	35	713	95.32
:12D 2L 2D	1349	35	1314	97.40

APPENDIX E

Effect of 2-hr. light breaks, during a 12-hr. scotophase, on the incidence of embryonic diapause in *Aedes atropalpus* (Belleville strain), reared at 23°±2°C.

Schedule	Total no. viable eggs	No. eggs hatched	No. viable eggs in diapause	% diapause
12L:12D	719	2	717	99.72
:2D 2L 8D	3351	2556	795	23.72
:4D 2L 6D	2501	2258	243	9.72
:6D 2L 4D	1649	1559	90	5.46
:8D 2L 2D	1843	1779	64	3.47
:10D 2L	1440	144	1296	90.07

APPENDIX F

Duration of daylight inclusive of civil twilight for Austin, Texas (30°-20'N) and Belleville, Ontario (44°-15'N).

LATITUDE 30°N - (Austin, Texas, U.S.A.)

Day of Month	Jan. h. m.	Feb. h. m.	Mar. h. m.	Apr. h. m.	May h. m.	June h. m.	July h. m.	Aug. h. m.	Sept. h. m.	Oct. h. m.	Nov. h. m.	Dec. h. m.
1	10.41	11.11	11.57	12.53	13.45	13.84	14.30	14.00	13.10	12.17	11.24	10.48
17	10.53	11.46	12.26	13.22	14.08	14.32	14.19	13.36	12.42	11.49	11.01	10.40
25	11.02	11.49	12.40	13.35	14.17	14.32	14.09	13.23	12.27	11.35	10.54	10.39

LATITUDE 44°N - (Belleville, Ontario, Canada)

1	9.31	10.23	11.39	13.14	14.43	15.51	16.02	15.09	13.44	12.14	10.45	9.41
17	9.51	11.05	12.28	14.02	15.23	16.05	15.42	14.27	12.55	11.26	10.06	9.17
25	10.06	11.28	12.52	14.26	15.39	16.05	15.26	14.04	12.32	11.04	9.51	9.27

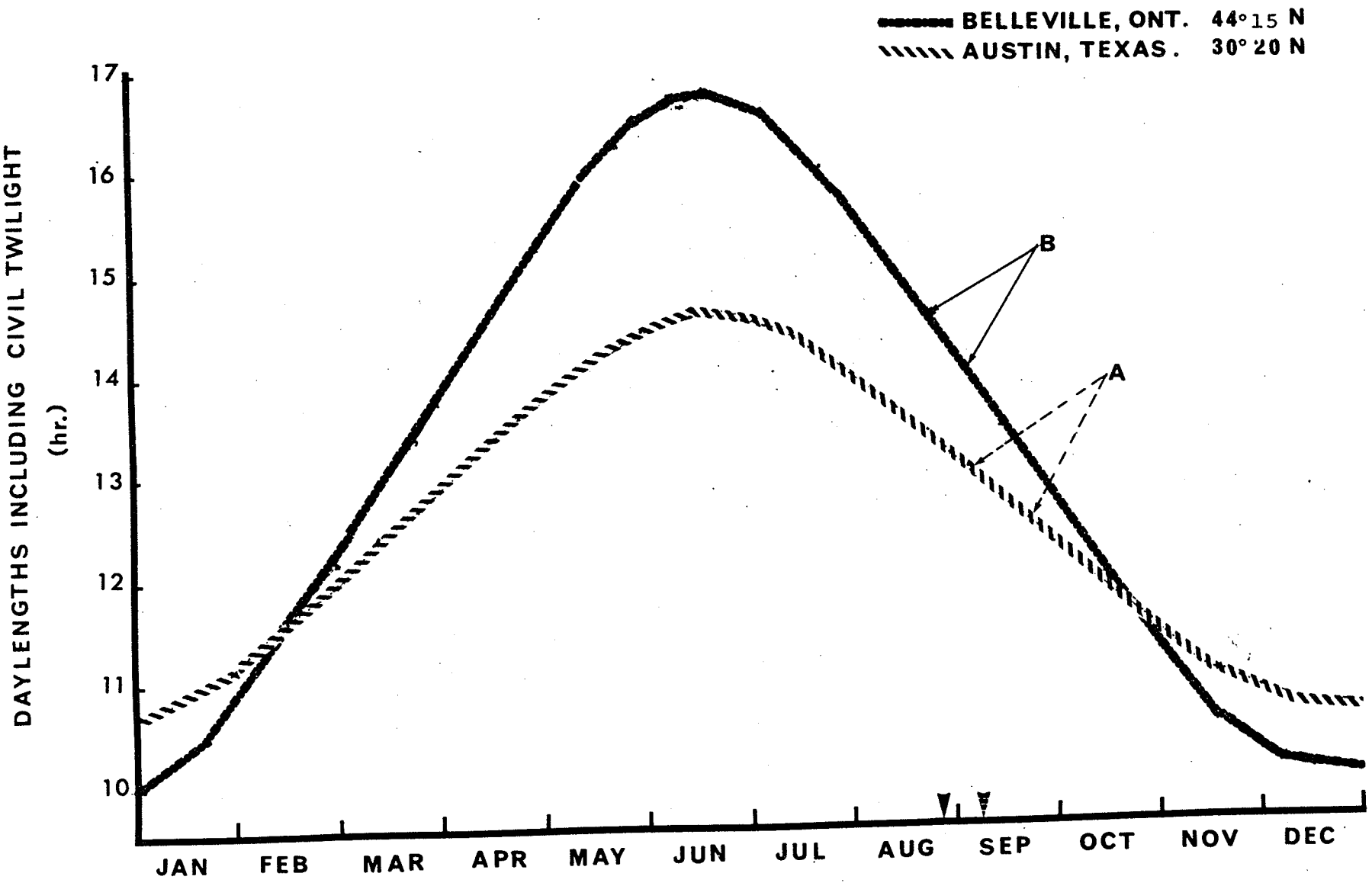
Compiled from Smithsonian Meterological Tables, Smithsonian Miscallaneous Collection 114. (R.J. List, 1951).

APPENDIX G

Daylength curves for Austin, Texas (30° 20'N) and Belleville, Ontario (44° 15'N). Data taken from the Smithsonian Meterological Tables, Smithsonian Miscellaneous Collection 114.

A - The critical photoperiod for the anautogenous Austin strain of *A. atropalpus* lies between 12.5-13 hr. light per day.

B - The critical photoperiod for the autogenous Belleville strain of *A. atropalpus* lies between 14-14$\frac{1}{2}$ hr. light per day.


BELLEVILLE, ONT. 44°15 N
AUSTIN, TEXAS. 30° 20 N
DAYLENGTHS INCLUDING CIVIL TWILIGHT (hr.)
17
16
15
14
13
12
11
10
B
A
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC