THE UNIVERSITY OF MANITOBA

THE BIOLOGY AND SYSTEMATICS OF *AEDES CAMPESTRIS* DYAR AND KNAB (DIPTERA:CULICIDAE) AND RELATED SPECIES IN MANITOBA AND SASKATCHEWAN

by

PENSOOK TAUTHONG

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ABSTRACT

Voltinism was determined in various populations of *Aedes campestris* Dyar and Knab from different locations. The Winnipeg population was 100% multivoltine, whereas the Churchill population was 64% multivoltine, 36% univoltine. Saskatoon and Estevan populations were 99% multivoltine, 1% univoltine. A laboratory colony of *A. campestris* was successfully maintained in a 120-x 120-x 210-cm cage for 3 generations. The optimum temperature for development and survival of *A. campestris* in the laboratory was 23°C. The time required for larval and pupal development was 9 and 3 days respectively at 23°C. Ovarian development of blood-fed females was also studied at 23°C, and females were able to complete follicular development within 3-4 days. Variation in autogeny was observed in populations from different geographic regions. In the Churchill population, 45% of the females were able to develop some autogenous eggs 15-20 days after emergence. In *A. campestris* from Saskatoon, 13.9% of the females developed a few autogenous eggs. There was no significant difference between the number of eggs laid by females fed 1, 2, or 3 blood meals in the Saskatoon or Estevan populations. The Churchill females fed 3 blood meals produced more eggs than those fed 1 blood meal.

At 23°C the critical period for induction of egg diapause in the Saskatoon population occurred between 13L:11D and 14L:10D. Egg diapause in the Saskatoon population was obtained after 10-14 days of exposure to a short photoperiod (<14L:10D). The greatest percentage of diapause occurred after 30 days. Long photoperiod (16L:8D) terminated egg diapause at 23°C in the Churchill population and the percentage of

termination increased significantly at 30°C.

The larval and adult stages of *A. campestris* and the related species, *A. dorsalis* (Meigen) and *A. mediolineata* Ludlow, are described. This study confirmed the separate species status of *A. melanimon* from *A. dorsalis* and reveals an older name than *A. melanimon*, namely *mediolineata* Ludlow 1907 which was previously a synonym of *A. dorsalis*. The key for species and the comparisons of various characters of first and fourth instar larvae, and male and female adults are listed to point out the reliable characters for separating each species. The distribution of the three species in North America is illustrated in a map.

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INTRODUCTION

Aedes campestris Dyar and Knab, and the closely related mosquitoes viz. *Aedes dorsalis* (Meigen) and *Aedes mediolineata* Ludlow (= *A. melanimon* Dyar.), are considered pests of man and animals throughout the Canadian West and the central and western United States. In both Canada and the United States, *A. campestris* and *A. dorsalis* are known as spring species; the females are pests in late spring and early summer. *A. dorsalis* and *A. campestris* have been found carrying the virus of western equine encephalitis (WEE) in Canada (McLintock *et al.*, 1970) and the United States (Ferguson, 1954). In the United States, *A. mediolineata* has been found carrying the WEE (Ferguson, 1954), St. Louis Encephalitis Virus (Reeves *et al.*, 1962), and probably California Encephalitis Virus (Richards, 1956). The time of peak abundance of *A. mediolineata* in Canada is not known, but in the United States it is known to breed in inland irrigated pastures and other irrigated crops throughout the summer (Telford, 1958; Chapman, 1960).

The geographical distribution in North America of these three species is probably fairly similar. Of the three, the most widely recognized species is *A. dorsalis*. It is recorded from 6 provinces in Canada and from 32 states in the United States (Carpenter and LaCasse, 1955; Richards *et al.*, 1956; Silverly, 1972). *A. campestris* is recorded from 7 provinces and 17 states (Carpenter and LaCasse, 1955; Chapman, 1966). *A. mediolineata* is recorded under the name of *A. melanimon* from 2 provinces, Alberta and Saskatchewan, and 12 states (Richards, 1956; Richards *et al.*, 1956; Burgess, 1957; Holmberg and Trofimenkoff, 1968; Gjullin and Eddy, 1972). From the present study, we know *A. mediolineata*

also occurs in British Columbia.

A. dorsalis (Meigen) 1830 was the first of these three species to be described. *A. campestris* Dyar and Knab 1907 was described later, and is recognized by culicidologists to be different from *dorsalis* in several important ways. *A. mediolineata* Ludlow 1907 is also different from *dorsalis*, and some of these differences were listed by Dyar (1924). The characters used in keys for the separation of these three species are inadequate for clear separation of larvae or adults. A reexamination of these characters has been long overdue, and an attempt is made here to emphasize the reliable characters and to delineate each species as completely as possible. Collections of larvae and adults of *A. campestris* and *A. dorsalis* were made in various locations in Manitoba and Saskatchewan. Specimens of all three species were obtained from the Canadian National Collection in Ottawa, from the United States National Museum, and from the personal collection of several researchers in both countries.

Although *A. campestris* is a common species in North America, especially in Saskatchewan, details of its biology and behaviour are not well understood. The colonization and maintenance of a laboratory colony has not previously been reported. This dissertation describes a technique developed for the successful colonization of *A. campestris*. The anatomical studies of larvae and adults of *A. campestris*, in comparison to larvae and adults of the related species *A. dorsalis* and *A. mediolineata*, are also presented. The characteristics of both instar I larvae and instar IV larvae are illustrated. Various aspects of the biology of *A. campestris* were studied. Laboratory experiments were conducted on the rate of larval development at different temperatures, adult female fecundity, autogeny in different populations, egg-follicle development,

and factors that induce and terminate egg diapause.

PERTINENT LITERAUTRE

Mosquitoes of Western Canada

The study of the mosquitoes of Western Canada was begun as early as 1866 by Lord (see Curtis, 1967). Later in 1922 Dyar published his paper on the mosquitoes of Canada after his visit to British Columbia (Dyar, 1922). The work of Hearle (1926) on the mosquitoes of the lower Fraser Valley and Curtis (1967) on the mosquitoes of British Columbia served as the basis for ecological studies and mosquito control in British Columbia.

The study of the mosquitoes of Saskatchewan began in 1907 when Knab (1908) published his observations on seven species of mosquitoes from Oxbow, Saskatchewan. His report included an extensive account of the bionomics of *Aedes* species, especially *A. spencerii* (Theobald). Another brief report on Saskatchewan mosquitoes was provided by Cameron (1918). Hearle (1929) published on the biology of mosquitoes of the Canadian prairies in which the life history of *Aedes flavescens* Müller was described.

An urgent need for further study of the prairie mosquitoes occurred following the 1941 outbreak of equine encephalitis in Saskatchewan. Rempel prepared a guide to the mosquito larvae of western Canada (1950), and subsequently a guide to the adults of Saskatchewan (1953), in which he illustrated the important taxonomic characters, and included the species descriptions and a brief note on their biology and distribution.

Mosquitoes of Manitoba were first studied by Knab (1908), who reported 8 species from Winnipeg. McLintock (1944) reported 22 species from Winnipeg, and published on their seasonal distribution, and relative abundance. Studies on biting flies at Churchill, Manitoba were carried

out by Twinn *et al.* (1948), and Hocking *et al.* (1950).

Systematics of *A. campestris* and related species

The adult male and female of *A. campestris* were first described by Dyar and Knab (1907). Rempel (1950, 1953) and Carpenter and LaCasse (1955) also described the adults and the fourth-instar larva of this species. The first-instar larva of *A. melanimon* has been described by Dodge (1966). Bohart (1954), Price (1960), and Dodge (1966) prepared keys for the identification of the first-instar larvae of *A. dorsalis* and other common mosquitoes of North America, but *A. campestris* was not included.

Prior to 1955, *A. melanimon* had been considered by some authors to be a "race" of *A. dorsalis* (Freeborn, 1926) and by others a synonym of *A. dorsalis* (Matheson, 1929; Freeborn and Bohart, 1951). More recently *A. melanimon* has been treated as a separate species (Barr, 1955; Carpenter and LaCasse, 1955; Bohart, 1956; and Richards, 1956). The present study confirms the separate species status of *A. melanimon*, and reveals an older name than *melanimon* for this species, namely *mediolineata* Ludlow 1907, previously a synonym of *A. dorsalis*.

Characters that separate *A. campestris*, *A. dorsalis* and *A. mediolineata* have been noted by several workers. Generally, *A. campestris* is reported to be larger and darker than *A. dorsalis*. Although quite similar, the male terminalia of *A. campestris* can be separated from *A. dorsalis*. The tarsal claws are reported to be the most reliable character for separating the females (Rempel, 1953; Vockeroth, 1954; Richards, 1956; Barr, 1958; Harmston and Lawson, 1967). Vockeroth (1954) stated that the claw of the metathoracic leg of the male adult could be used for identification. Although the wing scale

pattern on vein R_{4+5} has been one of the characters used for identification by several authors (Matheson, 1929; McLintock, 1944; Rempel, 1953; Chapman, 1966; Carpenter and LaCasse, 1955; Harmston and Lawson, 1967), Barr (1958) and Ross (1947) do not consider this to be a reliable character.

The identification of *Aedes* eggs based on the chorionic pattern, and the shape and size of eggs has been reported by many authors (Horsfall and Craig, 1956; Craig and Horsfall, 1958, 1960; Ross and Horsfall, 1965; Myers, 1967; Kalpage and Brust, 1968; Horsfall *et al.*, 1970; Horsfall and Voorhees, 1972 etc.). Myers (1967) described the egg size, shape, and chorionic pattern of *A. campestris*, *A. dorsalis*, and *A. melanimon* as well as 20 other species from California and Nevada. The chorionic detail of the egg of *A. campestris* is very close to that of *A. dorsalis* and *A. melanimon*. The size of an *A. melanimon* egg is relatively small (498-597 μ in length) compared to *A. dorsalis* (660 - 759 μ) and *A. campestris* (597-763 μ) (Myers, 1967). The size of eggs varies considerably within a species.

According to Kalpage and Brust (1968) there are 2 types of *A. dorsalis* eggs in populations in Manitoba; the length of eggs was 553-643 μ for one race and 655-732 μ for the other, and the length of *A. campestris* eggs was 681-822 μ. They suggested that there are two forms of adults, or two races in *A. dorsalis*. It is possible that one of their races of *A. dorsalis* could be *A. mediolineata*, however, this has not been confirmed.

Biology of *A. campestris*

The biology of mosquitoes common to Western Canada can be found in

Hearle (1929), Rempel (1950, 1953), Carpenter and LaCasse (1955), Horsfall (1955), Barr (1958), Clements (1963), and Happold (1965).

Prior to the present study, the following was known about *Aedes campestris*. Larvae may be found around Winnipeg from May 10 - June 15 in temporary and semipermanent pools formed by melting snow or heavy rains (McLintock, 1944). The pools occur in open areas, often near refuse dumps, and the water temperature during larval development may vary from 12-30°C. At Churchill, Manitoba, Twinn *et al.* (1948) found early instar larvae of *A. campestris* in shallow pools among marsh grasses and dwarf willow and birch on June 18. Larvae also occurred in a burnt-over forest region which was largely open. The bottom of the pool consisted of soft black muck, with a yellow surface. The salinity of the pool was 368 ppm and the pH was 8.3. Hocking *et al.* (1950) reported the pupal period in *A. campestris* at Churchill to be 2-7 days, and a mean of 4.8. Adults were abundant from June 30-July 23. Swarming of adults was observed about 8.30 p.m., over a forest clearing at the height of 6 - 12 ft (Hocking *et al.*, 1950). In Utah, adults of *A. campestris* are known to fly as far as 10 miles (Rees, 1943). Mail (1934) reported egg laying 4 days after a blood meal, whereas Hocking *et al.* (1950) observed that females did not oviposit until 15-16 days after feeding. Fully embryonated eggs have been stored for periods up to 20 months at 0-10°C, with more than 25% survival of the embryos (Mail, 1934). In nature, eggs of *A. campestris* probably hatch before those of *A. dorsalis*, since larvae and adults of *A. campestris* usually appear first (Barr, 1958). Only one generation per year was observed by McLintock (1944), Rempel (1950), Barr (1958), and Chapman (1966).

Laboratory Colonization of *Aedes dorsalis*

Blakeslee *et al.* provided a preliminary report on the colonization of *A. dorsalis* in a 180-x 120-x 210-cm cage. However, due to the limitation of large space requirements, an attempt was made to establish a strain requiring less space. The subcolony was established after 9 generations in a 120-x 60-x 60-cm cage and through 6 generations in a 60-x 60-x 60-cm cage. Egg-hatch rates of the larger-cage subcolony showed a steady increase with each generation whereas the smaller-cage subcolony showed an initially high rate, then dropped somewhat, and finally began to increase (Grimstad *et al.*, 1970). Taylor and Brust (1974) also reported the successful colonization of *A. dorsalis* and *A. vexans* (Meigen) in 120-x 120-x 210-cm cage. *A. vexans* mated more readily in the presence of *A. dorsalis*.

The Effect of Temperature on Larval Development

The effects of temperature on pre- and post- adult development of various mosquito species have been studied by Bar-Zeev (1958) in *Aedes aegypti* (Linnaeus); Brust (1967) in *A. vexans, A. nigromaculis* (Ludlow), and *Culiseta inornata* (Williston); Hanec and Brust (1967) in *Culiseta inornata;* Trpis and Horsfall (1969) in *A. sticticus* (Meigen); Trpis and Shemanchuk (1969, 1970) in *A. flavescens* and *A. vexans;* and recent work by Smelton (1973) on 8 mosquito species. None of these studies involved *A. campestris*.

Follicular Development

The stage of development of the ovaries has been classified into 5 different categories by Christophers (1911) and modified by Clements

(1963). The rate of development of the oocytes from the resting stage to maturity in a blood-fed female is controlled by several factors. One of these is temperature which acts indirectly by affecting the rate of digestion of blood, and directly on the growth of the oocyte (Clements, 1963). Another factor is genetics, which may be manifested by inter-or intra-species differences. *Anopheles gambiae* Giles oviposited 2 days after blood feeding at temperatures above 23.3° C while *Anopheles funestus* Giles required 3 days at 24.7° C (Gillies, 1953). *Anopheles vagus* Dönitz females from Assam were able to lay eggs 24 hours after taking a blood meal (Muirhead-Thomson, 1951). Females of *Anopheles maculipennis* Meigen from Stalingrad completed 12 gonotrophic cycles in 42 days in Southern Russia whereas in Moscow it took about 79 days to complete the same number of cycles (Detinova, 1962). Spielman (1957) found that ovarian development in autogenous females of *Culex pipiens* Linnaeus was suspended for 10 days or more at temperature of 4.4° C but development was resumed at normal temperature. According to Clements (1963) the gonotrophic cycles in mosquitoes varied greatly under fluctuating temperature, especially in temperate regions, but in tropical regions they were fairly constant, being only 2 days in most species.

Autogeny

The term autogeny was used by Roubaud (1929) to indicate the ability of unfed females to develop eggs. Spielman (1957) described autogeny as the absence of a developmental diapause condition of the ovary. Chapman (1962) characterized autogenous females as those that were capable of developing fully formed eggs without a blood meal or

external source of protein.

The first observation on autogeny was made on *Culex pipiens* by Theobald (1901). Roubaud (1933) found that both autogenous and anautogenous populations occurred within the same species, *Culex pipiens*. Spielman (1971) evaluated the environmental factors that influenced the relative abundance of these 2 forms in nature. He found that autogenous mosquitoes were most numerous in larval developmental sites that were enclosed, whereas anautogenous mosquitoes were most numerous from more open bodies of water. DeBoissezon (1933) suggested that autogeny in *Culex pipiens* depended on the larval diet, not on racial or genetic characteristics. The difference between these 2 populations was shown to be due to differences in the hormonal control of ovary growth (Clements, 1956; Larsen, 1958; Larsen and Bodenstein, 1959). However, in *Aedes aegypti* and *Anopheles maculipennis*, the larval nutrition is in some way connected with the appearance of autogeny in a population (Clements, 1963).

Geographical variation also influences the occurrence of autogeny in field populations. O'Meara and Evans (1973) have shown that the capacity of females to produce autogenous eggs increased from northern to southern populations of *Aedes taeniorhynchus* (Wiedemann) in Florida.

Other factors which influence autogeny in *Aedes communis* (DeGeer) have been studied by various workers. According to Beckel (1954) proteins required for autogenous ovarian development are obtained from the fat body and larval abdominal muscles of *A. communis*, whereas in the same species some populations are reported to histolyse their indirect flight muscles (Hocking, 1954). Recent work by Ellis and Brust (1973) showed that *A. communis* from Canada and the United States consisted of

3 sibling species, *A. communis*, *A. nevadensis* (Chapman and Barr), and *A churchillensis* sp. n. An autogeny survey revealed that the female adults of *A. communis* and *A. nevadensis* are normally obligatorily anautogenous whereas *A. churchillensis* are normally obligatorily autogenous.

Autogeny was also reported in *A. impiger* (Walker) and *A. nigripes* Zetterstedt (Corbet, 1964; 1967); in *A. rempeli* Vockeroth (Smith and Brust, 1970); in *A. caspius* Pallas, *A. detritus* (Haliday), *Culex modestus* Ficalbi and *C. pusillus* Macquart (Chinaev, 1964); in *A. concolor* Taylor, and *Tripteroides tasmaniensis* (Strickland) (Dobrotworsky, 1954); in *A. togoi* (Theobald) (Lien, 1960; Laurence, 1964); in *Culex tarsalis* Coquillett (Bellamy and Kardos, 1958; Moore, 1963); in *Culex peus* Speiser and *Culiseta inornata* (Williston) (Washino and Shad-del, 1969); in *Toxorhynchites* Theob. (Chapman, 1962); in *Wyeomyia smithii* (Coquillett) (Price, 1958); and also in the subfamily Anophelinae (Detinova, 1962).

Autogeny appeared to be common in *A. campestris*, but less common in *A. dorsalis* and *A. melanimon* (Chapman, 1962). The autogeny investigations were based on numbers of eggs laid by females. It took 10 days for autogenous *A. campestris* to deposit the first egg, 11 days for *A. melanimon*, and 14 to 15 days for *A. dorsalis*.

Fecundity

The number of eggs laid by a single female during one gonotrophic cycle varies greatly between species. *Aedes* species usually lay 100-150 eggs, as in *Aedes aegypti* and *A. polynesiensis* Marks (Woke *et al.*, 1956; Ingram, 1954) but *A. detritus* (Haliday) lays up to 260 (Marshall, 1938).

There are several factors that affect the number of eggs laid by mosquitoes. Jalil (1974) indicated that the factors that affect the number of eggs laid by *A. triseriatus* (Say) are blood source, blood amount, body size, body weight, mating, and age of the mosquito. More eggs were laid by females when fed on warm-blooded than on cold-blooded animals. The number of eggs laid in a batch by *A. aegypti* and *A. triseriatus* shows a positive correlation with the amount of blood ingested (Roy, 1936; Woke *et al.*, 1956; Colless and Chellapah, 1960; Jalil, 1974). However, this correlation only occurs with medium-sized and small blood meals and there is no increase in egg production after 3 mg of blood have been ingested (Clements, 1963). Meola and Lea (1972) demonstrated that when ovaries of *Aedes* spp. had retained sufficient eggs, another blood meal failed to initiate the development of more eggs.

In *A. triseriatus*, the number of eggs laid showed a positive correlation with the amount of blood ingested from 0.6 mg to 3.0 mg (Jalil, 1974) and 1 to 5 mg were required in *A. hexodontus* Dyar (Barlow, 1955). It was found that larger females of *A. hexodontus* ingested more blood than smaller females (Barlow, 1955). Females of *A. aegypti* derived from well-nourished larvae ingested more than twice as much blood as females reared under starvation conditions (Bar-Zeev, 1957). It has also been shown that the size of the female has a positive correlation with number of ovarioles in the ovaries. Colless and Chellapah (1960) found that the number of ovarioles in *A. aegypti* varied between 50 and 150. Therefore, the size of the individual female, and the conditions under which the larvae had developed can have a pronounced effect on the number of eggs laid.

Fecundity is also affected by autogeny. In Arctic mosquitoes, many follicles degenerate during autogenous ovarian development and

thus fecundity is so greatly reduced that sometimes only one egg can mature (Corbet, 1964; 1967).

Diapause

Diapause is the most highly evolved system of dormancy for overcoming cyclic, long-term, and extreme environmental conditions. The dormancy is induced well before the adversity and maintained for some time irrespective of environment. It may intervene at any of the major developmental stages of the life cycle, which is always characteristically fixed in each species (Mansingh, 1971).

All species of the genus *Aedes* are capable of arresting development at the egg stage. Diapause induction experiments measure only the results of a sequence of physiological responses which ultimately determine the response of the individual. Each individual has a different stimulus for eliciting this all or none response (DeWilde, 1962). Reactivation from diapause often requires months of extended exposure to environmental conditions such as low temperature, whereas "conditioning" may require a brief exposure to factors such as high humidity (Horsfall, 1956; Harwood and Horsfall, 1959; Clements, 1963).

In *Aedes* eggs, there are two types of diapause, namely obligatory and facultative. The multivoltine species (many generations per year) which are controlled by the environment exhibit facultative diapause. The univoltine species, which are free from environmental control exhibit an obligatory diapause. In Lepidoptera, geographical races of a species often exhibit obligatory and facultative diapause (Mansingh and Smallman, 1967). From the physiological and ecological viewpoint there is no difference between the diapausing generation of multivoltine and univoltine

species (Mansingh, 1971).

The principal stimulus for the onset of diapause is photoperiod although temperature, water, and diet may be involved. DeWilde (1962) reported that photoperiod provided the most reliable indication of seasonal changes although Lees (1956) suggested that both temperature and photoperiod provide token environmental messages, affecting the same endocrine mechanisms.

Temperature plays a major part in termination of diapause. High temperature removes the effect of short photoperiod, low temperature reduces hatching in response to long photoperiod, and prolonged exposure to low temperature terminates diapause (Lees, 1955; Beckel, 1958; de Wilde, 1962; Danilevskii, 1965; McHaffey and Harwood, 1970; Mansingh, 1971; and McHaffey, 1972).

As far as is known *A. campestris* is generally univoltine (McLintock, 1944; Rempel, 1950). However, Chapman (1966) observed that 95% of the eggs obtained from some females of the spring brood hatched after a week of "conditioning". Both Chapman (1966) and Rempel (1950) observed a second brood in the field.

Studies on embryonic diapause in *A. campestris* have not been reported to date, but studies on diapause in *A. dorsalis* have been reported by Khelevin (1958, 1959), Telford (1963), McHaffey and Harwood (1970), and Taylor (1973). Both photoperiod and temperature are important factors in regulating embryonic diapause in *A. dorsalis*.

METHODS AND MATERIALS

General Methods

Collection of Materials

The materials used for laboratory studies were collected from different locations in Manitoba and Saskatchewan. The preserved specimens of *A. melanimon* were obtained from the Canadian National Collection in Ottawa, the United States National Museum, and from the personal collections of J. McLintock (Saskatchewan), R. M. Bohart (California), T. Miura (California),and H. C. Chapman (Nevada).

The different stages of *A. campestris* and *A. dorsalis* were collected from different locations and used for laboratory colonization. Adult females were collected in early summer from areas in Manitoba and Saskatchewan that were known to have fair populations of larvae in pools or a high number of adults per mosquito trap. Adults of *A. campestris* and *A. dorsalis* were collected at Dundurn, Saskatchewan on June 25, 26, 1973; at Estevan, Saskatchewan on June 12, 1974; at Saskatoon, Saskatchewan on June 19-27, 1974; and at Churchill, Manitoba on July 15-17, 1974. The mosquitoes attempting to feed on blood were captured with an aspirator and transferred to the 25-x 25-x 150-mm, acrylic holding cages. The cages had fine-mesh nylon screen on 2 sides to allow the imagos to take blood and to oviposit through the screen. Caged female imagos were transported to the laboratory in insulated chests maintained at 10-15^{o}C, 70-85% R.H. using ice and moist towelling respectively.

Larvae were collected from their developmental sites by dipping and transferring to styrofoam food containers (300 ml) for transport to