# OVARIAN CYCLES AND LONGEVITY IN SOME UNIVOLTINE *AEDES* SPECIES IN THE ROCKY MOUNTAINS OF WESTERN UNITED STATES[1]

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INTRODUCTION. A knowledge of ovarian cycles in mosquitoes is of great significance in life history studies. The mosquito control worker should know as much as possible about the reproductive potential and longevity of the species he is concerned with. This information is of particular importance in disease transmission studies. A mosquito must feed at least twice in order to acquire a pathogen and also serve as its vector. The critical importance of knowing the age structure and refeeding habits of vector populations has been stressed recently by Reeves (1965).

Although many techniques have been used to determine mosquito longevity and oviposition cycles, the most promising are those that were developed by Russian workers following World War II. These were first brought to the attention of American workers by Gillies (1958) who summarized the Russian literature on the subject.

Perhaps of greatest significance was the discovery by Polovodova (1949) that when an egg passes from the ovariole into the oviduct it leaves a small sac-like dilatation at the site in the pedicel where the egg was originally formed (Fig. 1E, 1). If additional eggs are deposited, each also leaves a dilatation anterior to the dilatation of the previous egg (Fig. 1 I). Thus a dilatation is left to represent each oviposition cycle with the most posterior dilatation representing the original oviposition and the most anterior the most recent. Examination of ovarioles to determine the number of dilatations was referred to as Polovodova's method by Gillies (op. cit.). Polovodova's work was extended and her technique refined by Detinova (1949, 1962). Detinova's technique is now generally used for this type of ovarian study.

Mosquitoes which are known to oviposit more than once during a single season are referred to as multivoltine species. Females of many genera fit this category as do numerous species of the genus *Aedes*. However, there are considerable numbers of northern *Aedes* species which are univoltine and produce but a single larval brood each season. Comparatively little is known about the reproductive potential of these species. Nielsen (1959) reported on the seasonal distribution and longevity of 12 univoltine *Aedes* species of Rocky Mountain snow mosquitoes. His observations indicated an average longevity of four to six weeks for these species with a range of two to eight weeks. He also stated that the females apparently died shortly after the first oviposition. Rosay (in litt.) however, called our attention to the fact that females of some univoltine *Aedes* species collected in the mountains of California which she had examined by the Detinova technique had oviposited several times. We, therefore, set out in the present study to determine the frequency of oviposition in univoltine *Aedes* species by using this dissection technique of ovarian examination. For certain species we also attempted to determine longevity of the females and the effective biting period. The following is a preliminary report of our findings.

MATERIALS AND METHODS. Twelve hundred and seventy-seven specimens representing 18 species of univoltine species occuring in the Rocky Mountains of the western United States were examined.

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Collections were made in the states of Idaho, Utah and Wyoming. Two of the species studied, *Aedes campestris* D. and K. and *Aedes canadensis* (Theo.) are known to be multivoltine under favorable conditions and two others, *Aedes cinereus* (Meigen) and *Aedes spencerii* (Theobald) are suspected by some workers of being capable of producing more than a single seasonal brood. However, under the ecological conditions in which they occur in the western United States these species are generally univoltine.

Mosquitoes examined by the Detinova technique must be kept alive until the time of dissection. Therefore, all specimens were collected in the field with a sucking tube and stored in pint cardboard

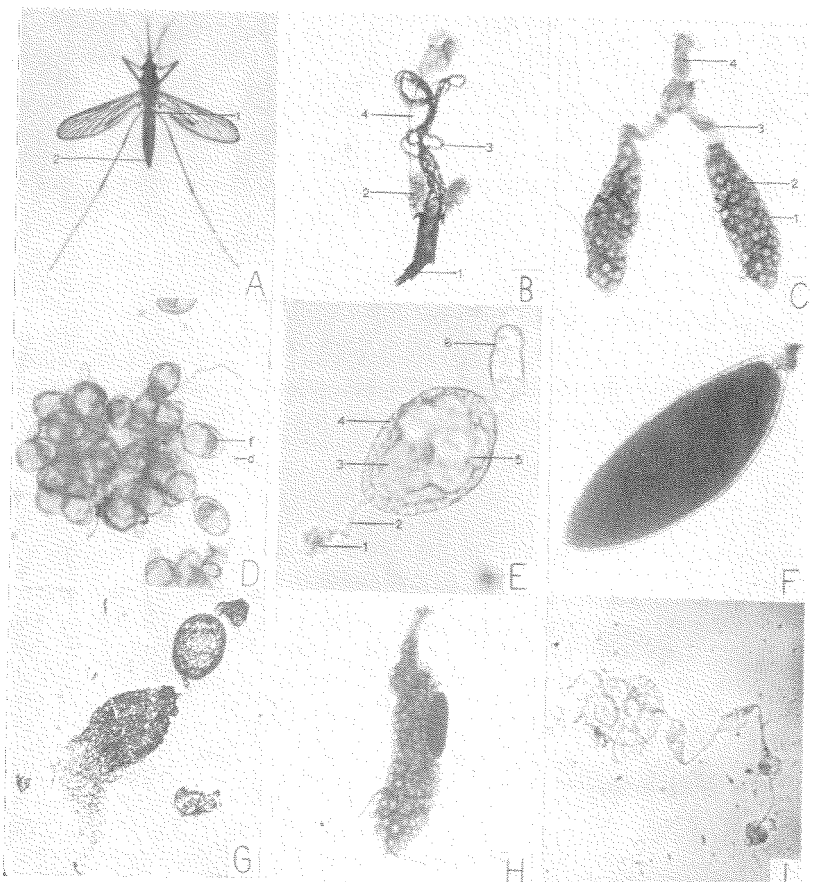

FIG. 1.—Photographs of *Aedes fitchii,* A-H; *Aedes cataphylla,* I. A, female mosquito in position for ovarian dissection; 1, 2, sites of pin placement. B, terminal segments removed with internal organs attached; 1, terminal segment; 2, ovary; 3, malpighian tubules; 4, gut. C, isolated ovaries; 1, ovary; 2, ovariole; 3, lateral oviduct; 4, common oviduct. D, group of separated ovarioles; d, dilatation; f, follicle in Stage II. E, individual ovariole; 1, dilatation; 2, pedicel; 3, oocyte; 4, follicular epithelium; 5, nurse cell; 6, germarium. F, follicle in Stage V. G, ovariole with pedicel distended at site where follicle has just released egg during oviposition. This site shrinks to form dilatation. H, ovary with one follicle which did not release egg at previous oviposition. I, ovariole showing 4 dilatations.

containers with a cheesecloth covering for a lid. A small hole was made in the side of the container in which the end of the sucking tube could be inserted to add or remove specimens. An ordinary No. 3 cork was used to close the opening. While in the field specimens were carried in an ice chest about one-third full of cubed or crushed ice. Specimens could be kept alive in this manner for at least 48 hours provided the temperature remained at about 40° F.

When ready for dissection the specimen was lightly anesthetized with chloroform. It was first identified and then placed on its dorsum on a glass slide with abdomen resting on a drop of normal saline solution. The specimen was then observed under a stereoscopic microscope at 20X–30X magnification. With the female lying as illustrated in Fig. 1A an insect pin secured the specimen at site (1) on the upper abdomen. Another pin was placed at site (2) on next to last abdominal segment. Pressure was exerted on the latter pin and it was slowly pulled posteriorly. With this operation the last two abdominal segments were pulled free along with the gut and female reproductive organs (Fig. 1 B). This latter operation works poorly or fails unless the specimen is alive during the procedure. Therefore, great care is necessary in the application of the anesthetic.

The ovaries (Fig. 1B, 2) were then carefully separated from the surrounding structures with insect pins. In a careful dissection the ovaries and oviducts now appeared as shown in Fig. 1 C. To examine the individual ovarioles the outer ovarian membrane was torn in several places with a minuten pin and the ovarioles teased out into small clusters (Fig. 1 D). The ovarioles were then teased out individually and examined. Each ovariole (Fig. 1 E) characteristically showed a proximal germarium (6) followed posteriorly by a developing follicle (3–5), the pedicel (2) and dilatations (1) if present. Proper orientation is vital lest the germarium be mistaken for a dilatation. Magnification should now be at least 125X–150X. Great care should be taken to prevent drying out. Additional saline solution should be added if necessary. The ovarioles were examined not only for dilatations, but also for stage of follicular development. During the gonotrophic cycle the developing follicle passes through five stages:

Stage 1. Follicle is spherical or slightly oval and without yolk granules. One oocyte and seven nurse cells are present.

Stage II. Follicle is oval with yolk granules occupying less than one-half of the follicle (Fig. 1D, E).

Stage III. Follicle becomes slightly elongated with yolk granules occupying more than one-half ot the follicle.

Stage IV. Follicle becomes distinctly elongate with yolk granules occupying almost all of the follicle.

Stage V. Follicle contains fully formed egg with visible chorionic structures. Eggs are ready for oviposition (Fig. 1F).

This system of classification is essentially that devised by Christophers (1911). The stages are generally easily recognizable. If doubt exists, the stage selected is the more advanced.

When dilatations are present they are always posterior to the developing follicle on the pedicel leading to the internal oviduct. The dilatations may be pigmented or unpigmented. If the female has just recently oviposited, the pedicel is greatly distended at the follicular site from which the egg was released (Fig. 1G). Within 48 hours this shrinks to form the distinctive dilatations.

To determine the total number of ovipositions that had occurred in each specimen a minimum of 10–15 ovarioles were examined for dilatation.

This is necessary as not every ovariole releases an egg at each oviposition. Sometimes fully formed eggs are not released during oviposition (Fig. 1H). These retained eggs may be released at a subsequent oviposition or they may be reabsorbed. In any event such ovarioles may show fewer dilatations than others. Also, in older females some follicles may degenerate or cease to function after one or two ovipositions.

Of great value to us during the course of this study were papers by Rosay (1963, 1965) which contained excellent discussions of the age determination technique as well as detailed information about follicular changes in the ovary. The latter reference also included a comprehensive bibliography.

Our longevity studies were carried out on nine species (Table 3) during the 1963 and 1964 seasons in mountain and valley areas of northern Utah.

We were fortunate in being able to find larvae of each of these particular species in pools isolated by distances of from one-fourth to more than three miles from the nearest pools containing larvae of the same species. After the emergence of the adults we visited each of these pools once or twice each week and made collections of biting and resting females. Each was examined by the Detinova technique. Collections were continued until adults had completely disappeared from the areas. Because of the restricted flight ranges of most of these species (Nielsen, 1957) and our knowledge of the general region we felt reasonably certain that females from other larval producing areas had not invaded the collection sites.

An attempt also was made during the course of this study to bring freshly killed and preserved females in from the field for ovarian examination. Preservatives used were various concentrations of ethyl alcohol, alcohol-formalin mixtures, formalin, cellosolve, glycerin, and Bouin's fluid. Females collected in the field also were frozen in the field with dry ice and brought into the laboratory to thaw out. All of the preservatives as well as the freezing technique rendered the specimens unsuitable for examination by the Detinova technique.

RESULTS AND DISCUSSION. It is apparent from the results of this study that all of the 18 species examined were capable of passing through at least one complete gonotrophic cycle and feeding again (Table 1), and thus have the potential of serving as vectors of disease. Of the females listed in Table 2 a total of 1148 was collected while attempting to bite. The remainder, 129, were collected resting in the vegetation or while ovipositing. It is important to note that every species

TABLE 1.—Results of ovarian cycle studies on 18 western univoltine *Aedes* species. Each dilatation represents one complete gonotrophic cycle.

| Species | Number of females examined | Dilatation number and number of females with each | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 |
| *Aedes campestris* | 63 | 30 | 23 | .. | 10 | .. | .. |
| *Aedes canadensis* | 11 | 2 | 9 | .. | .. | .. | .. |
| *Aedes cataphylla* | 51 | 13 | 23 | 6 | 3 | 5 | 1 |
| *Aedes cinereus* | 21 | 10 | 11 | .. | .. | .. | .. |
| *Aedes communis* | 197 | 99 | 72 | 26 | .. | .. | .. |
| *Aedes diantaeus* | 10 | .. | 10 | .. | .. | .. | .. |
| *Aedes excrucians* | 97 | 25 | 55 | 17 | .. | .. | .. |
| *Aedes fitchii* | 123 | 32 | 53 | 13 | 12 | 13 | .. |
| *Aedes flavescens* | 20 | 11 | 9 | .. | .. | .. | .. |
| *Aedes hexodontus* | 250 | 97 | 81 | 56 | 16 | .. | .. |
| *Aedes impiger* | 98 | 59 | 30 | 8 | 1 | .. | .. |
| *Aedes implicatus* | 10 | .. | 10 | .. | .. | .. | .. |
| *Aedes increpitus* | 208 | 43 | 102 | 35 | 19 | 9 | .. |
| *Aedes intradens* | 2 | .. | 2 | .. | .. | .. | .. |
| *Aedes niphadopsis* | 38 | 15 | 23 | .. | .. | .. | .. |
| *Aedes pullatus* | 46 | 32 | 14 | .. | .. | .. | .. |
| *Aedes punctor* | 5 | .. | 3 | 2 | .. | .. | .. |
| *Aedes spencerii* | 27 | 16 | 11 | .. | .. | .. | .. |
| Total Number | 1,277 | 484 | 541 | 163 | 61 | 27 | 1 |

TABLE 2.—Follicular stage of development of 18 species of western univoltine *Aedes* species collected while biting or while resting in vegetation.

| | Follicular stage of development of biting females and number in each | | | | | Follicular stage of development of resting females and number in each | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | I | II | III | IV | V |
| *Aedes campestris* | 37 | 23 | .. | .. | .. | 1 | .. | .. | .. | 2 |
| *Aedes canadensis* | 3 | 8 | .. | .. | .. | .. | .. | .. | .. | .. |
| *Aedes cataphylla* | 29 | 10 | 2 | .. | .. | .. | .. | .. | .. | 10 |
| *Aedes cinereus* | 5 | 14 | 2 | .. | .. | .. | .. | .. | .. | .. |
| *Aedes communis* | 70 | 100 | 21 | .. | .. | 1 | 3 | .. | 1 | 1 |
| *Aedes diantaeus* | 3 | 7 | .. | .. | .. | .. | .. | .. | .. | .. |
| *Aedes excrucians* | 44 | 26 | 5 | 1 | .. | 13 | .. | .. | .. | 8 |
| *Aedes fitchii* | 75 | 39 | .. | .. | .. | 4 | .. | .. | .. | 5 |
| *Aedes flavescens* | 2 | 12 | 3 | .. | .. | 3 | .. | .. | .. | .. |
| *Aedes hexodontus* | 135 | 66 | 19 | .. | .. | 13 | .. | .. | 2 | 15 |
| *Aedes impiger* | 28 | 40 | 4 | .. | .. | 7 | .. | .. | .. | 19 |
| *Aedes implicatus* | .. | 9 | 1 | .. | .. | .. | .. | .. | .. | .. |
| *Aedes increpitus* | 101 | 72 | 35 | .. | .. | .. | .. | .. | .. | .. |
| *Aedes intrudens* | 2 | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| *Aedes niphadopsis* | 27 | 1 | .. | .. | .. | 4 | 3 | .. | .. | 3 |
| *Aedes pullatus* | 4 | 25 | 7 | .. | .. | 7 | 2 | .. | .. | 1 |
| *Aedes punctor* | 4 | 1 | .. | .. | .. | .. | .. | .. | .. | .. |
| *Aedes spencerii* | 16 | 10 | .. | .. | .. | .. | .. | .. | .. | 1 |
| Total Number | 585 | 463 | 99 | 1 | .. | 53 | 8 | .. | 3 | 65 |

was represented by biting females that had at least one dilatation.

Of the total examined 38 percent contained no dilatations and 62 percent contained one or more. Of those containing dilatations 68 percent contained one, 21 percent two, 8 percent three, 3 percent four, and one female (*Aedes cataphylla*) had five.

The figures are somewhat misleading as they do not take into consideration the season of the year. Most of the specimens without dilatations were collected early in the season before the first oviposition had occurred. Also as the season progressed the number of dilatations naturally increased as multiple ovipositions occurred only in the older females. One cannot conclude, however, that a large number of females survive to oviposit repeatedly. Our records show that as the season progressed fewer biting females were present in all collection areas and the time required to collect a study sample was greatly increased.

Our data indicate that the greatest reduction in numbers of females occurs after the first oviposition and that an additional reduction occurs after each subsequent oviposition. The females which remain late in the season and which have the greatest number of dilatations represent only a very small fraction of the total number produced. Thus the high mortality rate is due to a combination of natural causes such as unfavorable environmental conditions, predation, parasitism, disease and oviposition itself. We believe the latter to be one of the principal death contributing factors. On numerous occasions we have found dead females at oviposition sites; some of these still contained many unlaid eggs.

We were also interested in the stage of follicular development present in the captured females. Christophers (1960:677) stated that Stage I was seen only in newly emerged females and that in the absence of a blood meal development of the follicle did not proceed beyond Stage II.

Rosay (1965) in her ovarian studies with *Aedes nigromaculis* and *Culex p.*

TABLE 3.—Longevity and ovarian cycle data on nine western *Aedes* species for 1963 and 1964

| Species | Year | Date first biting females collected | Date last biting females collected | Total biting period (days) | Max. No. ovarian cycles |
|---|---|---|---|---|---|
| *Aedes communis* | 1963 | VII–17–63 | VIII–13–63 | 27 | 2 |
| | 1964 | VII–20–64 | VIII–16–64 | 27 | 2 |
| *Aedes campestris* | 1964 | V–10–64 | VII–21–64 | 72 | 3 |
| *Aedes cataphylla* | 1964 | VI–10–64 | VII–19–64 | 40 | 5 |
| *Aedes excrucians* | 1963 | VII–22–63 | IX–8–63 | 48 | 2 |
| | 1964 | VII–25–64 | IX–10–64 | 47 | 2 |
| *Aedes fitchii* | 1964 | VII–5–64 | VIII–27–64 | 53 | 4 |
| *Aedes hexodontus* | 1963 | VII–8–63 | VIII–28–63 | 51 | 3 |
| | 1964 | VII–15–64 | VIII–17–64 | 46 | 3 |
| *Aedes impiger* | 1964 | VII–13–63 | VIII–15–63 | 33 | 3 |
| | 1964 | VII–15–64 | VIII–8–64 | 24 | 2 |
| *Aedes increpitus* | 1963 | VII–4–63 | VIII–28–63 | 55 | 4 |
| | 1964 | VII–5–64 | VIII–27–64 | 53 | 4 |
| *Aedes niphadopsis* | 1964 | V–10–64 | VII–10–63 | 61 | 1 |

*quinquefasciatus* reported that in these species the follicles did not develop beyond Stage II until they had fed on blood. The results of our studies shown in Table 2 are in general agreement with these data, but certain discrepancies do occur. Ninety percent (1148) of the females were collected from our persons, with the great majority actively attempting to bite. Of this number 91 per cent (1048) were in Stage I or II of follicular development; 51 percent (585) were in the first, 40 percent (463) in the second. Almost 9 percent (99) were in Stage III and one female was in Stage IV.

Some of the females in Stage III or IV may have been attracted to our persons without inclination to bite and it is possible that some of those assigned to Stage III may actually have been in very late Stage II. However, in lieu of the large number involved we believe that most, if not all, of those which we assigned to Stage III were actually attempting a blood feeding.

The number of females in Stage III represented a significant number of the total and involved 10 of the 18 species. In every instance the number represented at least 5 percent of the total biting collection. In three species, *A. flavescens, A. increpitus* and *A. pullatus,* the percentage of biting females in Stage III was 18, 17 and 19 respectively. Some of the females may have obtained a previous partial blood feeding which permitted development to Stage III. It is well known that female mosquitoes are often disturbed before feeding is completed, and that this does not abate their urge to bite again. We also noted that 42 of the females showing Stage III development were nulliparous. This suggests the possibility of autogenous development. Chapman (1962) reported that 12 *Aedes* species were known to be capable of autogeny. They included five of the species in the present study, *A. campestris, A. communis, A. flavescens, A. niphadopsis* and *A. punctor.* Two of these, *A. communis* and *A. flavescens,* had a significant percent of biting females showing late Stage III follicular development.

We plan to continue our studies to pursue the possibility that some females of univoltine species may be capable of autogeny in the absence of a blood feeding, but that they may still continue to seek a blood feeding until at least as late as Stage III.

An analysis of the 129 females collected while resting in vegetation revealed that 91 percent (118) were in either Stage I or V; 41 percent (53) were in Stage I, 50

percent (65) in Stage V. Our data indicate that those in Stage I were either newly emerged females or females that had just completed an oviposition. Those in Stage V were almost all taken in areas where active oviposition was occurring.

The remaining females (11) representing 8.5% of the total were either in Stage II or Stage IV. No resting females were taken in Stage III. This supports our contention that those in Stage III are still actively seeking a blood feeding.

Table 3 shows the results of our longevity studies on nine species. All of these species were first observed in the larval stage. Pools were carefully watched until emergence of adults was completed. The period from the time emergence began until it was completed was extremely variable and usually required at least a week. In certain instances when cold or stormy weather prevailed the period was extended to two weeks or more. Biting did not begin for several days after the emergence of the first females. In most instances this was a period of at least five to seven days. Thus the females were probably at least one week old on the date that biting was first recorded. The date of the last biting record was also recorded. The total period from the first biting date to the last represents the nuisance period and also would be the significant period during which the female could acquire and transmit a pathogen. As noted above, it does not represent the true age of the oldest females.

On five species, *A. communis, A. excrucians, A. hexodontus, A. impiger* and *A. increpitus,* we have data for both 1963 and 1964. For each there is a remarkable correlation between the two years in the length of the biting period and the maximum number of ovarian cycles. This suggests the possibility that genetic factors are involved. In terms of longevity it appears that two species, *A. communis* and *A. impiger,* are particularly short-lived with an effective biting period of only about one month. This was still sufficient time for two ovarian cycles to occur in the former species and three in the latter. *Aedes cataphylla* proved to be most prolific with a maximum of 5 ovarian cycles in only 40 days. Most of the other species showed two to four ovarian cycles over a six to eight week period. The data on *A. niphadopsis* is interesting in that only one ovarian cycle was completed in a maximum period of 61 days. We believe this is due to the unusual habits of this species. After emergence both males and females move from the valleys into surrounding foothill areas where mating swarms occur. The females become further dispersed in search of blood feedings, but eventually return to the valleys to oviposit. Our data indicate that these activities require a period of from three to six weeks.

We are now attempting to determine the amount of time required by these univoltine species to complete each stage of follicular development. It is extremely variable and is very difficult to determine precisely for field specimens. It appears to differ with the species and also is influenced by temperature, availability of food and probably other factors.

SUMMARY. The gonotrophic cycles of 1277 females representing 18 univoltine *Aedes* species occurring in the western United States were studied. Each female was examined by the Detinova technique of ovarian examination to determine the number of follicular dilatations and the stage of follicular development present. Details are included on techniques used. Results showed that some biting females of every species survived at least one oviposition; nine of the species had completed at least two, and six at least three. The maximum was five in *Aedes cataphylla.* Thus all species examined are potentially capable of acquiring and transmitting pathogens.

Data also are presented on stages of follicular development in both biting and resting females, with evidence to show that biting can continue until at least Stage III of follicular development.

Longevity data are included on nine species. The maximum life span of fe-

males, varying with species, ranged from about five to eight weeks with an effective biting period of about one week less.

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*References Cited*

BEKLEMISHEV, V. N., DETINOVA, T. S., and POLOVODOVA, V. P. 1959. Determination of physiological age in anophelines and of age distribution in anopheline populations in the U.S.S.R. Bull. World Health Organ. 21:223–32.

CHAPMAN, H. C. 1962. A survey for autogeny in some Nevada mosquitoes. Mosquito News 22:134–136.

CHRISTOPHERS, S. R. 1911. The development of the egg follicle in anophelines. Paludism 2:73–88.

———. 1960. *Aedes aegypti* (L.). The Yellow Fever Mosquito: Its Life History, Bionomics, and Structure. London and New York: Cambridge University Press. 739 pp.

DETINOVA, T. S. 1949. Physiological changes in the ovaries of female *Anopheles maculipennis*. Med. Parasit. and Parasitic Disease (Moscow) 18:410.

———. 1962. Age-grouping methods in Diptera of medical importance. World Health Organization, Geneva, Switzerland. 216 pp.

GILLIES, M. T. 1958. A review of some recent Russian publications on the technique of age determination in *Anopheles*. Trop. Disease Bull. 55:713–21.

NIELSEN, L. T. 1957. Notes on the flight ranges of Rocky Mountain mosquitoes of the genus *Aedes*. Proc. Utah Acad. Arts, Science, Letters, 34:27–29.

———. 1959. Seasonal distribution and longevity of Rocky Mountain snow mosquitoes of the genus *Aedes*. Proc. Utah Acad. Arts, Science, Letters 36:83–87.

POLOVODOVA, V. P. 1949. Determination of the physiological age of female *Anopheles* by the number of gonotrophic cycles completed. Med. Parasit. and Parasitic Disease (Moscow) 18:352–355.

REEVES, W. C. 1965. Ecology of mosquitoes in relation to arboviruses. Ann. Rev. Entomol. 10:25–46.

ROSAY, B. 1963. Age determination techniques in adult mosquitoes. Outline for CMCA Entomology Committee Seminar (Mimeo.). 11 pp.

———. 1965. Anatomical indicators for assessing age of mosquitoes (Diptera-Culicidae): Follicular changes in the ovaries (in manuscript).