OCCURRENCE OF OVARIOLAR DILATATIONS IN NULLIPAROUS MOSQUITOES: *CULEX NIGRIPALPUS*[1]

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ABSTRACT. Ovarian dissections were performed on laboratory reared, nulliparous, sugar-fed *Culex nigripalpus* Theobald females and characterized for parity using both dilatation and tracheation methods. In all females dissected the follicles were characterized as either nulliparous or 'resorbed' 4 days after emergence, but 1 week after emergence 16% to 50% of the females dissected had developed 1 aberrant dilatation, and within the next 2 weeks more than 90% of the females had 1 to 3 aberrant dilatations indicative of the parous condition. In a comparison of the females showing 1–3 aberrant dilatations, the ovarian tracheoles showed some partially uncoiled skeins indicative of the nulliparous condition. Thus no correlation was found between ovarian tracheoles and dilatations in determining parity in *Cx. nigripalpus*. A comparison with other *Culex* mosquitoes suggests that ovarian tracheation is more reliable than dilatation method for a determination of parity in *Culex* species.

Determination of the physiological age of female mosquitoes, based on the number of ovariolar dilatations, has been extensively used as a method for studying both their rate of survival and their potential for pathogen transmission. The knowledge of such characteristics is valuable in assessing a mosquito's epidemiological danger and the impact of any control measures (Detinova 1968, Garrett-Jones 1970, Macdonald 1952, 1957, Nájera 1974). This age grading technique has been widely used by researchers working with anopheline mosquitoes (Detinova 1962, 1968, Gillies 1958, Hitchcock 1968, Spencer 1979), however, questions have arisen about the validity of this technique in some *Aedes* and most *Culex* mosquitoes (Bellamy & Corbet 1973, 1974, Kay 1979, Mitchell et al. 1980, Nelson 1964, Provost 1969, Rosay 1969, Service 1976). Rosay (1969) reported finding aberrant dilatations in *Culex pipiens quinquefasciatus*, *Cx. tarsalis* and *Culiseta inornata* specimens that had never fed on blood and were known to be anautogenous. Ovarian dissections from these 3 species after maintenance on only apples or raisins, revealed granular-appearing follicles with no structure of nurse cells or oocytes. She noted that obvious resorption was taking place and new follicles were being formed by the germarium. She concluded, therefore, exceptions to the general rule existed, since the number of dilatations and the number of completed ovarian cycles did not always correlate in these *Culex* species. Bellamy and Corbet (1973, 1974) observed that in autogenous *Cx. tarsalis*, in addition to normal dilatations which formed on the pedicel of each ovariole after an oocyte had been shed, aberrant dilatations also formed on the ovarioles if the primary follicle failed to mature and became degenerated or resorbed.

Another commonly used method for age determination is called ovarian tracheation. In this method the terminations of the fine tracheoles covering the ovaries are observed. The tracheoles are tightly coiled into 'skeins' in females that are nulliparous and have never developed oocytes as compared with females that are parous and have stretched and uncoiled tracheoles due to egg development and oviposition (Detinova 1962). Kardos and Bellamy (1961) and Burdick and Kardos (1963) observed ovarian tracheoles in

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autogenous *Cx. tarsalis,* where a few but not all skeins were uncoiled and concluded this was due to either autogeny or an incomplete blood meal. Recently, Kay (1979) observed a few *Cx. annulirostris* that had intermediately coiled skeins and were parous when dissected for dilatations. In general, therefore, with increasing physiological age there seems to be a decreasing agreement between the number of dilatations and the number of ovipositions in these *Culex* species.

Recently, Nayar and Knight (1981) observed the presence of morphologically distinct nulliparous, parous and functional 'resorbed' (as opposed to degenerating) ovarian follicles in blood-seeking, anautogenous female *Cx. nigripalpus* collected from the field. They also demonstrated the presence of 'resorbed follicles' in 2 to 7 day-old, sucrose-fed females, when either deprived of a blood meal after emergence or fed a small blood meal. They further found that at elevated temperatures, 3% to 10% of the females developed some autogenous eggs. Additional dissections of older sugar-fed *Cx. nigripalpus* produced a large number of females that had ovarioles with 1 to 3 aberrant dilatations. Since these females were never fed blood or provided with oviposition sites, they should have exhibited nulliparous characteristics.

The present study was designed to confirm these observations and further investigate the appearance of aberrant dilatations in nulliparous *Cx. nigripalpus* females. A comparison of these dilatations with ovarian tracheation in nulliparous females was also made.

MATERIALS AND METHODS

Colonized *Cx. nigripalpus* and F_1 progeny from field collected females were reared in the laboratory under standardized conditions (Nayar 1968). After emergence, approximately 300 adults of both the colony and the F_1 generation were maintained separately on a 10% sucrose solution in 45 cm^3 cages at 27°C and RH 75%. In these cages the adults were free to mate even though F_1 females were rarely inseminated, but the females were neither fed blood nor allowed to lay eggs.

In experiment 1, 30 females were removed from each group at specified intervals, their ovaries were dissected into physiological saline, and parity was determined by the dilatation method of Polovodova (cf. Detinova 1962). The follicles were characterized after Christophers (1911) as modified by Mer (1936).

In experiment 2, 10 females were removed from each group at each time interval, and their ovaries were dissected as above. However, one ovary was allowed to air-dry on a marked slide for determination of parity by the ovariole tracheation method and the other was used to determine parity by the dilatation method.

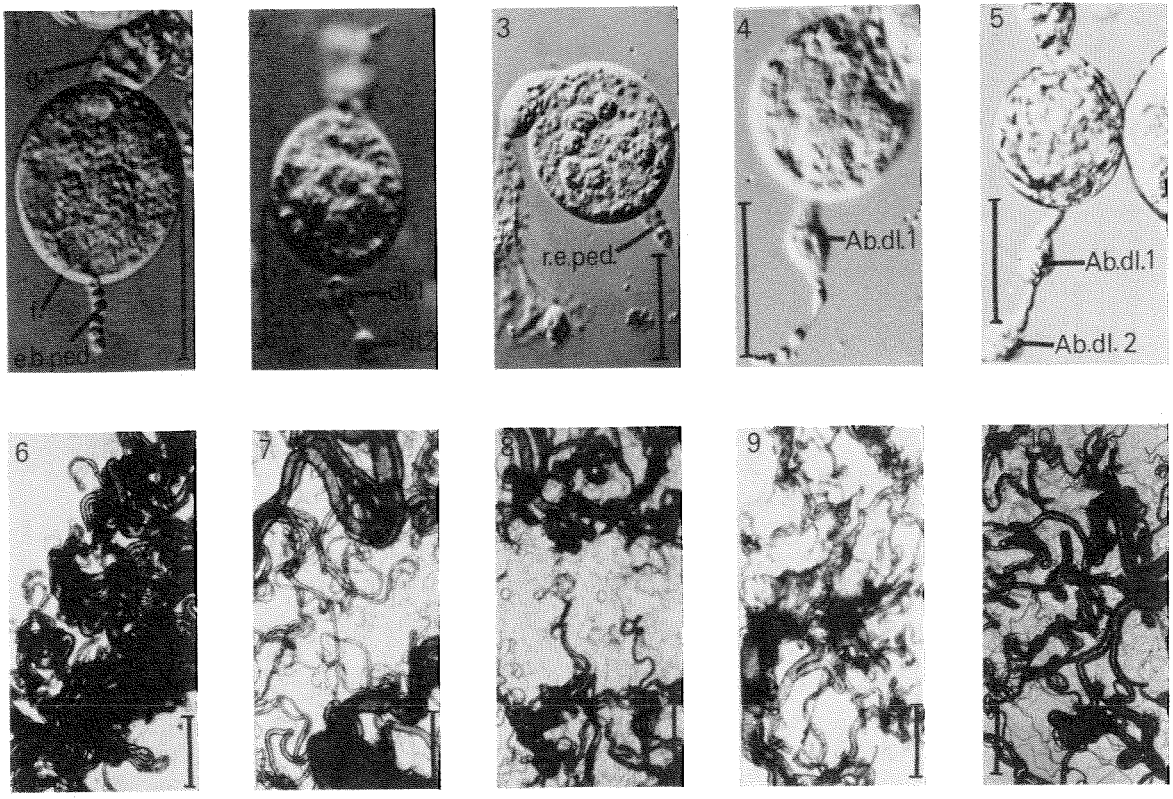
Both experiments were repeated at least once.

RESULTS AND DISCUSSION

EXPERIMENT 1. Dissections of females of both colony and F_1 generation showed follicles in stages I–II, IIR and II, which represented nulliparous (Fig. 1), 'resorbed' (Fig. 3) and gonoactive stage II (Table 1). The timing of these stages varied little between the 2 groups; however, any slight discrepancies in the appearance of the different stages could be due to variations in development between either the groups or the individual females.

Determinations of parity by the dilatation method in both groups of females produced similar results (Table 1). All the follicles were either nulliparous (Fig. 1) or 'resorbed' (Fig. 3) 4 days after emergence, but by 7 days between 16% and 50% of the females had ovaries with 1 dilatation (Fig. 4). During the next 2 weeks most of the females exhibited between 1 to 3 dilatations (Fig. 5).

Thus, if the history of these experimental groups had not been known, these females would have been classified as 1 to 3 parous (Fig. 2), whereas in fact, they were nulliparous. Similar results



Figs. 1–10. Comparison of dilatation (Figs. 1–5) and tracheole (Figs. 6–10) methods to show normal and aberrant ovarian development in *Culex nigripalpus*. Photographs were made of freshly dissected material using high-dry Nomarski interference contrast microscopy (Figs. 1–5) and of dried preparations with direct light transmission microscopy (Figs. 6–10). Fig. (1) Nulliparous follicle with evenly beaded pedicel (e.b.ped) and Fig. (6) corresponding ovary with tightly coiled tracheole skeins. Fig. (2) Parous follicle with 2 dilatations (dl. 1 and dl. 2) and Fig. (7) Corresponding ovary with completely uncoiled tracheoles. Fig. (3) "Resorbed nulliparous follicle" with enlarged beaded pedicel (r.e. ped.) and Fig. (8) Corresponding ovary with intermediate stage of uncoiled tracheoles. Figs. (4 and 5) Nulliparous follicles with 1 and 2 aberrant dilatations (Ab. dl. 1 and dl. 2) respectively, and Figs. (9 and 10) Corresponding ovaries with intermediate stages of uncoiled tracheoles. g -germarium; f - follicle. Scale line represent 50 μ.

Table 1. Ovarian development and parity of *Culex nigripalpus* maintained on 10% sucrose at 27°C and RH 75% (Experiment 1). Thirty females were dissected in each treatment and characterized by the dilatation method.

Days after emergence	Stages of ovarian development: I–II	IIR	II	II	II	II
	Parity, number of females (percentage)/stage: N*	R*	N-R & dl.1	dl.1	dl.1 & dl.2	dl.2 & dl.3
	F_1 generation females					
4	12 (40.0)	18 (60.0)	—	—	—	—
7	9 (30.0)	16 (53.3)	5 (16.7)	—	—	—
10	—	—	3 (10.0)	21 (70.0)	6 (20.0)	—
13	—	—	—	7 (23.3)	9 (30.0)	14 (46.7)
16	—	—	—	—	—	30 (100.0)
19	2 (6.7)	—	—	—	—	28 (93.3)
22	—	—	—	2 (6.7)	—	28 (93.3)
	Colony females					
4	30 (100.0)	—	—	—	—	—
7	5 (16.7)	10 (33.3)	15 (50.0)	—	—	—
10	5 (16.7)	12 (40.0)	6 (20.0)	7 (23.3)	—	—
13	5 (16.7)	3 (10.0)	—	21 (70.0)	—	1 (3.3)
16	5 (16.7)	2 (6.7)	1 (3.3)	12 (40.0)	3 (10.0)	7 (23.3)
19	—	—	1 (3.3)	25 (83.3)	3 (10.0)	1 (3.3)
22	2 (6.7)	5 (16.7)	—	14 (46.7)	6 (20.0)	3 (10.0)

* N = Nulliparous oocytes; R = Resorbed oocytes; N-R and ld1 = Nulliparous oocytes and oocytes with 1 dilatation present in the same female; dl.1-dl.3 = 1–3 dilatations.

were reported by Rosay (1969) in anautogenous *Cx. p. quinquefasciatus*, where 2–3 weeks after emergence 14% of the dissected females possessed a single dilatation, by 3–4 weeks 94% were in this condition, and after 4–5 weeks 3% already had 2 dilatations. She also observed that there was no enlargement of the glandular oviduct as would occur during normal oogenesis nor was there any uncoiling of the ovarian tracheae. She did not find any spontaneous activity that resulted in aberrant dilatations in the *Aedes* or *Anopheles* species she studied, nor was this ever seen in the hibernating females of either *Culex* or *Anopheles* species. A disagreement between numbers of dilatations observed and numbers of ovipositions was also observed in *Cx. pipiens pallens* and *Ae. albopictus* (Liu 1965).

No females in this experiment exhibited autogenous egg development. However, in an earlier study 3% to 10% of the *Cx. nigripalpus* females developed autogenous eggs 4 to 7 days after emergence (Nayar and Knight 1981). It could be argued that since there is a tendency for autogenous egg development in a small percentage of *Cx. nigripalpus* females and the occurrence of 'resorbed' follicles in a large percentage of them, it is possible that the appearance of dilatations in nulliparous females may be associated with autogenous egg development. However, it seems that the presence of aberrant dilatations in anautogenous *Culex* sp. and *Culiseta* sp. females that have never had a blood meal, results from a continuous formation of follicles by the germarium (Rosay 1969). This condition is different from that in *Anopheles* and most *Aedes*, where the formation of follicles by the germarium ceases after the primary follicle reaches stage II, until a blood meal stimulates the development of the primary follicle.

Ovarian development is known to be controlled by brain hormones (Lea 1970). Even in the absence of a blood meal, both the corpora allata (CA) and the medial

neurosecretory cell (MNC) hormones are present in the circulation of autogenous sugar-fed *Ae. taeniorhynchus* Wiedemann females, whereas in anautogenous *Ae. taeniorhynchus* females, the hormone from the MNC system does not circulate during sugar-feeding. Ovarian development in *Cx. nigripalpus* suggests that a very small titer of the MNC hormone is probably circulating during sugar-feeding, and initially results in 'resorbed' follicles. However, after 5 to 7 days of sugar-feeding, the level of this hormone circulating in the haemocoele has sufficiently increased so that abortive oogenesis (cf. Detinova 1962) occurs, resulting in the formation of a dilatation, i.e. the oocytes initiate development due to the presence of a small amount of MNC hormone, but because of a lack of stored or available proteinaceous reserves, this oocyte first becomes 'resorbed' and later degenerates and forms an aberrant dilatation.

EXPERIMENT 2. Comparing the ovarian tracheoles and the dilatations from the same female showed no correlation between these 2 methods (Table 2). Both colony and F_1 females were identical with only minor variations. The ovarian tracheoles exhibited both the nulliparous condition, where the terminations of the fine tracheoles covering the ovaries are tightly coiled into skeins (Fig. 6), and intermediate stages in which a few but not all skeins are slightly stretched (N-STR) or partially uncoiled (N-P) (Figs. 8–10). None of the tracheoles was completely uncoiled, or represented the parous state (Fig. 7). However, when examined for parity, most of the females had 1 to 3 dilatations after the age of 7 days (Table 2, Figs. 4 and 5), which suggested that they were parous (Fig. 2). Since these di-

Table 2. Comparison of parity in *Culex nigripalpus* as determined by tracheation and dilatation methods (Experiment 2). Ten females were dissected at each time interval; one ovary was used to characterize by tracheation and the other by dilatation methods.

Days after emergence	No. in different stages					
	Tracheole method			Dilatation method		
	N*	N-str*	N-P*	N*	R*	dl.* 1–3
			F_1 generation females			
4	9	1	0	4	6	0
7	6	4	0	3	7	0
10	3	6	1	—	1	9
13	2	3	5	—	—	10
16	1	6	3	—	—	10
19	3	5	2	1	—	9
22	4	4	2	—	—	10
Total	28	29	13	8	14	48
			Colony females			
4	10	0	0	10	0	0
7	6	4	0	2	8	0
10	1	1	8	2	4	4
13	4	—	6	2	1	7
16	7	—	3	2	1	7
19	—	—	10	1	0	9
22	—	2	8	0	1	9
Total	28	7	35	19	15	36

* N = Nulliparous, N-str = Nulliparous with slightly stretched tracheoles, N-P = Nulliparous with partially uncoiled tracheoles, R = Resorbed, dl. = Dilatations.

latations occurred in the ovaries of known nulliparous females, they were referred to as aberrant dilatations, and thus proved that the appearance of such dilatations was not necessarily comparable to the condition of the ovarian tracheoles. Similar results have been reported in other *Culex* species, e.g., *Cx. p. quinquefasciatus* (Rosay 1969), *Cx. tarsalis* (Kardos and Bellamy 1961, Burdick and Kardos 1963, Mitchell et al. 1980, Nelson 1964, Rosay 1969), and *Cx. annulirostris* (Kay 1979). Therefore, it appears that age determination using dilatations is not a valid method for most *Culex* species, and that the ovarian tracheole method is more reliable.

In this regard, we concur with and here repeat the cautious statement of Bellamy and Corbet (1974), "Accordingly, we call attention to the likelihood of the presence of dilatations in such mosquitoes being misinterpreted, and caution against the unqualified acceptance of dilatations as invariable indices of the parous conditions." Similar conclusions were arrived at recently by Mitchell et al. (1980) who stated, "In light of these observations it seems advisable to thoroughly evaluate the procedure in the laboratory using females with a known blood feeding and ovulation history before application in the field." We also repeat the suggestion (Rosay 1969) that in most *Culex* species there is no quiescent stage during oogenesis, but there is repetitive and continuous formation of follicles by the germarium in nulliparous females.

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SNOWSHOE HARE VIRUS INFECTIONS IN CANADIAN ARCTIC MOSQUITOES DURING 1980[1]

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ABSTRACT. Snowshoe hare (SSH) virus (California encephalitis group) was isolated from 7 of 162 pools comprising 7472 unengorged adult female mosquitoes of 3 species which were collected throughout the Yukon Territory between 9 June and 30 July 1980. All isolates were achieved from *Aedes communis* mosquitoes, with isolations ranging from 1:78 to 1:483 mosquitoes collected at southern Yukon locations (61°N) and 1:182 to 1:382 at northern Yukon locations (66°N, 137 to 138°W) along the recently constructed Dempster Highway.

INTRODUCTION

Attempts to define the ecology of two Bunyaviruses, snowshoe hare (SSH) virus (California encephalitis group) and Northway (NOR) virus (Bunyamwera group) have been undertaken annually since 1971 throughout portions of the western Canadian arctic which are accessible by road (McLean et al. 1975, 1979a). These investigations were stimulated by the isolation of these 2 mosquito-borne arboviruses in adjacent portions of Alaska during 1970 (Ritter and Feltz 1974). This report confirms the enzootic prevalence of SSH virus at intensively studied foci at southern and northern locations in the Yukon Territory, and identifies a new focus of infection along the northern extension of the Dempster Highway which was first opened to private automobiles during autumn 1978.

METHODS

Unengorged adult female mosquitoes were collected by hand aspirators at 13 locations in the Yukon Territory at latitudes 60 to 67°N from 9 June to 30 July 1980, and at Inuvik, Northwest Territories (69°N, 135°W) on 22 July 1980.

Mosquitoes were sealed immediately in glass tubes and stored at −70°C in styrofoam containers with dry ice, in which they were transported by air to Vancouver. They were held frozen at

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