International Journal of Entomology Vol. 27, no. 3: 280–288 10 September 1985

OOCYTE DEVELOPMENT AND FECUNDITY IN *MEGASELIA SCALARIS* (PHORIDAE: DIPTERA)

D.B. Benner[1]

Abstract. The literature contains conflicting reports on the length of the fertility period and the number of eggs laid by *Megaselia* females. Eggs are laid in clusters, suggesting a synchronous maturation of oocytes. Oocyte development pattern was examined in young and aged females. In young females, oocytes mature at 32–34 h following eclosion. The rate may be more rapid in mature females. In both young and older females, each ovariole contained only 1 mature oocyte. The next stage was a pre-yolk egg chamber. Evidence suggests that this egg chamber will not mature until essentially all of the mature oocytes have entered the oviducts. Oocytes mature in synchrony, and females are capable of producing large numbers of eggs over extended periods.

Megaselia scalaris is a carrier of disease-producing organisms (James & Harwood 1970) because it lives on refuse and decaying waste material, and it is a cause of myiasis in animals and man (James 1947, Haider 1956). Basic studies on the ecology and development of the fly have been reported by Patton (1922), Semenza (1953), El-Miniawi & Moustafa (1965), Robinson (1971), Prawirodisastro & Benjamin (1979), and Trumble & Pienkowski (1979).

Reports in the literature on the level of fertility in *Megaselia* females are contradictory. Reports of numbers of eggs produced and the length of the female's reproductive life vary considerably. Mainx (1964) noted that females lay eggs immediately after copulation but are not fertile later in life. Tumrasvin et al. (1977) observed only 2 oviposition periods within the 7-day life span of their females; the total number of eggs laid varied from 9 to 26 per female. El-Miniawi & Moustafa (1965), on the other hand, found that females laid eggs up to 32 days of age and that even dead flies contained mature oocytes. They reported a mean fecundity of 391 eggs per female; each female laid eggs during a mean of 10 egg-laying periods and oviposition began when females were 38 h of age. Prawirodisastro & Benjamin (1979) observed a mean fertility period of 25.7 days (max. of 36) with a mean egg production of 665 per female. Their females laid eggs for 16 days and egg laying began at a mean of 1.9 days (45.6 h) of age.

All of these authors note that females tend to deposit eggs in clusters. El-Miniawi & Moustafa (1965) and Prawirodisastro & Benjamin (1979) showed that eggs are not deposited evenly on consecutive days, the peak of egg production being at 17–18 days. The fluctuating oviposition periods and the cluster deposition suggests that *Megaselia scalaris* females produce eggs in synchronized clusters as reported for *Coch-*

1. Department of Biological Sciences, East Tennessee State University, Johnson City, Tennessee 37614, USA.

lomyia hominivorax (LaChance & Leverich 1962), *Rhodnius prolixus* (Pratt & Davey 1972a, Huebner & Davey 1973), and *Musca domestica* (Adams et al. 1968, Adams 1970) rather than in a continuous progression as occurs in *Drosophila* (King & Wolfsberg 1957, King 1970).

The purpose of this study was to examine the basic stages of oocyte development and to determine when the oocyte matures. Older females were examined to determine whether oogenesis is synchronous or continuous, since it is possible that laying eggs in clusters is a behavioral character of the fly rather than a manifestation of the oogenesis process, as it appears to be in bot flies, which deposit eggs at scattered sites to reduce predation and increase exposure to hosts (Catts 1982).

METHODS AND MATERIALS

Flies were obtained from cultures maintained at East Tennessee State University. Females were collected either from pupal cases when the development appeared to be nearly complete or immediately after eclosion (±0.5 h). The eclosed flies did not have their wings inflated so were less than 1 h old (see El-Miniawi & Moustafa 1965). Uneclosed flies were examined as soon as they were removed from the pupal case, but eclosed females were aged in yeasted 8-dram shell vials containing a cornmeal-yeast-corn syrup-agar medium at 25 °C. Ovaries were dissected in Ringer's solution and examined unstained under phase contrast optics or were stained with aceto-orcein. Pole-cap cells were removed and counted from ovaries fixed in 22.5% acetic acid. Oocytes were judged to be mature when they had lost the pole-cap cells and were filled with yolk. Haematoxylin-stained sections were prepared using the methods of King et al. (1965).

The oocyte stage in older females was determined from females that were stored for 6 days. At this age females were laying unfertilized eggs. Some females were dissected and examined on the 6th day. Others were mated and permitted to lay eggs for 4 more days. The 10-day-old flies were then dissected and examined.

RESULTS

Table 1 summarizes the egg chamber sizes and oocyte stages observed in uneclosed and young females. Uneclosed and newly eclosed flies have a germarium and 1 maturing egg chamber per ovariole (Fig. 1A). The germarium has 2 distinct regions (A and B in Table 1) (Fig. 1A). Region A is the most proximal tip and undergoes extensive contraction in fresh material. The terminal egg chamber (region C in Table 1) contains approximately 15 visible nurse cells and a trace of yolk. At 12 h the egg chamber contains a maximum of 33% yolk (percentage of the length). By 24 h, following eclosion, there are 2 egg chambers. The terminal one (region D in Table 1, formerly region C of the 12-h stage) contains the maturing oocyte. At this stage the oocyte contains about 50% yolk (percentage of length), has become elongate, and the nurse cells are forming a polar cap (Fig. 1B). A few females have mature-appearing

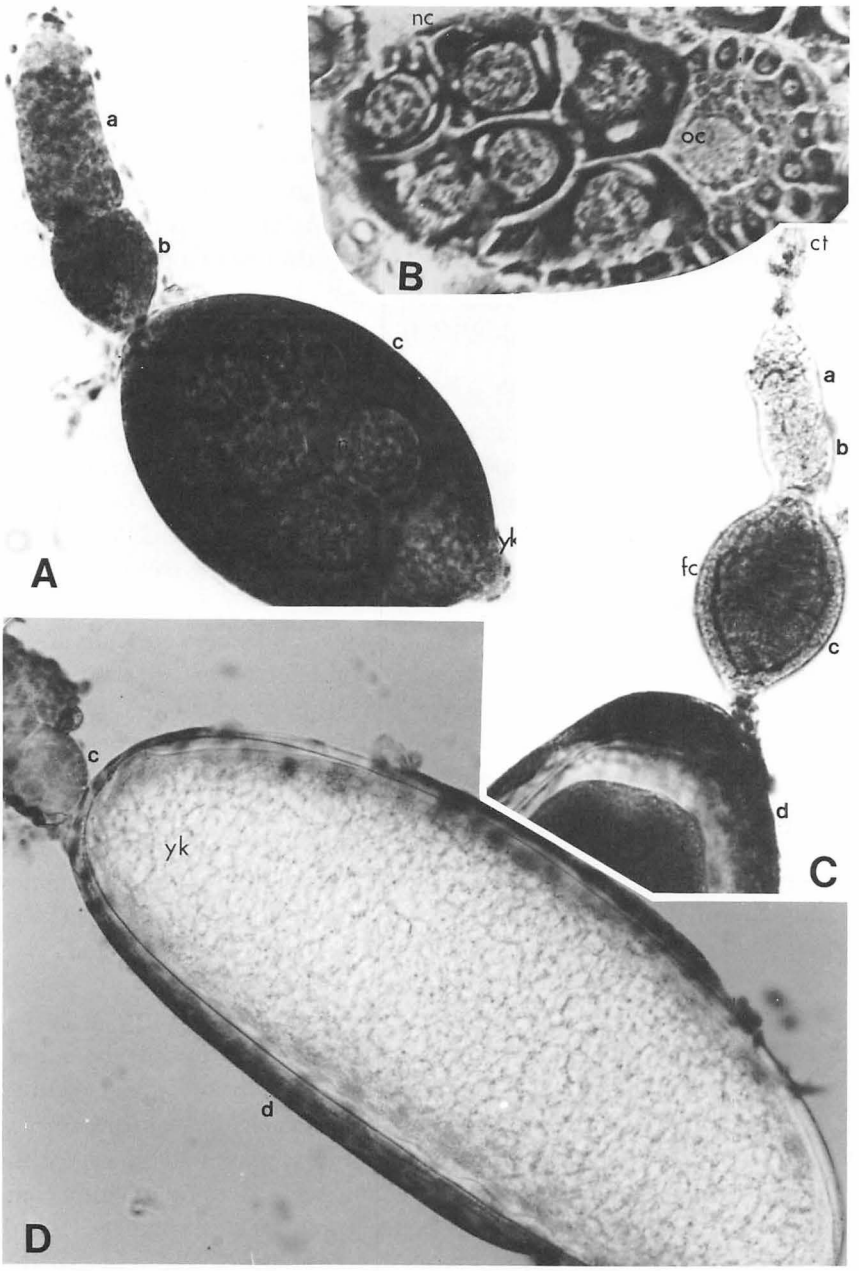

FIG. 1. Stages of egg chamber–oocyte development. **A.** 12-h ovariole, showing germarium stages (a and b) and developing egg chamber (c). In b and c the nurse cells can be seen, and in the distal region of c a small deposit of yolk (yk) is present. **B.** Section of a maturing oocyte (age unknown). The nurse cells (nc) are being pushed to one end to form a polar cap as yolk accumulates. The

TABLE 1. Sizes of regions of the ovarioles at selected stages.

STAGE	REGION*							
	A	a	B	b	C	c	D	d
Uneclosed	0.9 (10)	0.5 (10)	0.6 (10)	0.7 (10)	1.5 (10)	1.0 (10)		
Newly eclosed	0.9 (5)	0.5 (5)	0.8 (5)	0.6 (5)	2.0 (6)	1.3 (6)		
12 h	1.1 (7)	0.5 (7)	1.0 (9)	0.7 (9)	3.3 (11)	2.1 (11)		
24 h	0.9 (3)	0.5 (3)	0.5 (3)	0.5 (3)	1.1 (10)	0.8 (10)	5.5 (10)	2.9 (10)
32 h	0.8 (6)	0.5 (6)	0.6 (6)	0.4 (6)	1.2 (14)	0.9 (14)	10.4 (14)	4.1 (14)
48 h	0.8 (14)	0.5 (14)	0.8 (14)	0.6 (14)	2.0 (14)	1.4 (14)	9.1 (14)	3.5 (14)

* Regions **A** and **B** represent subdivisions of the germarium. Regions **C** and **D** are egg chamber–oocyte stages. Upper case letters are the lengths, lower case letters are the greatest diameters. The units are ocular micrometer units (1 unit = 0.07 mm). The numbers of measurements are given in parentheses.

oocytes as early as 29 h following eclosion. By 32 h the majority of females have oocytes that appear to be fully developed; they have become completely filled with yolk, have lost the pole-cap cells, and are covered with a chorion that has the characteristic filaments (El-Miniawi & Moustafa 1965) (Fig. 1C, D). By 34 h all of the females have mature oocytes. The proximal egg chamber remains the same size (see Table 1). This growth is summarized in Fig. 2, where it can be seen that the oocyte length approximately doubles every 12 h for 24 h. It more than doubles again in the next 8 h.

The 24-h oocyte is approximately ½ filled with yolk, which has begun to displace the nurse cells toward the pole to form a pole-cap. Dissection of the cap cells from 10 oocytes produced 12–15 cells ($\bar{x} = 14$). The oocyte is enclosed in a common membrane with the earlier stage egg chambers.

The number of mature oocytes in each ovary and in each female varies greatly from individual to individual. The number of oocytes observed in 41 ovaries from 21 mature females was 4–44 ($\bar{x} = 27$). The total number of mature oocytes present in 20 females, from which both ovaries were recovered, was 12–77. There was never more than 1 mature oocyte present in an ovariole, and the next egg chamber in each ovariole contained little or no visible yolk.

The oocyte patterns for virgin 6-day-old and mated 10-day-old females are summarized in Table 2. All of the oocytes within any female appeared to be in the same general stage of development. When mature oocytes were present, the majority of the next stage egg chambers contained little or no yolk. When the most advanced stage was not mature there was a tendency to find intermediate stages, but it appears

←

oocyte nucleus (oc) is visible below the nurse cells. **C.** 32-h ovariole with a mature oocyte. The connective tissue (ct) is visible at the proximal end of the germarium. Follicle cells (fc) can be seen lining the egg chamber (c). **D.** Mature oocyte from a 32-h-old female. The oocyte is filled with yolk and is surrounded by a chorion. The next egg chamber (c) is visible at the proximal end.

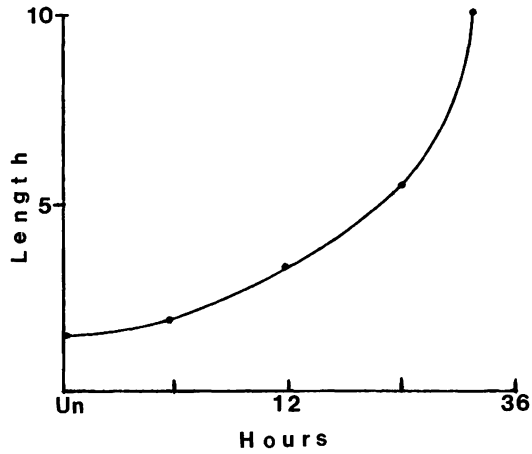

FIG. 2. Mean oocyte lengths at each of the sample periods. Un = uneclosed. Length in micrometer units (1 unit = 0.07 mm).

that this is only a chance event, probably because the maturation process proceeds rapidly once begun. These results are consistent with the oocytes developing in synchrony.

In 4 of the females with mature oocytes there were only 3 to 8 oocytes present. In the 2 females with only 3 mature oocytes, the next egg chambers contained oocytes with ⅓ and ½ yolk. When 5–8 mature oocytes were present, the next stage egg chamber had a small trace of yolk. Four females is too small a sample on which to base a conclusion, but it appears that emptying the majority of the ovarioles triggers the maturation of the next egg chambers and that 5 eggs is enough to repress this response while 3 is not.

The egg chamber is surrounded by follicle cells (Fig. 1C). In young females (0–12+ h) there is a high level of mitotic activity in these cells (Fig. 3). Cells were observed in almost every mitotic stage. Similar activity is seen in the distal region of the germarium, although fewer figures were observed. In the egg chamber the activity decreased from a mean of 2.5 metaphase–anaphase figures per chamber (37 in 15 egg chambers) in newly eclosed flies to 1.1 (20 in 18 egg chambers) in 12-h-old females. This period of mitotic activity occurs as the follicle is growing in preparation for the accumulation of yolk. The activity in the distal germarium may indicate growth of the region that will become the next egg chamber.

DISCUSSION

Megaselia scalaris females have typical dipteran polytropic meroistic type ovaries in which nurse cells are included within the same follicular epithelium as the oocyte (Bonhag 1958, Mahowald & Kambysellis 1980). The present study indicates that 4 oogonium divisions occur resulting in 16 cells: 15 nurse cells and the oocyte. Each

TABLE 2. Summary of oocyte stages present in 6-day-old virgin and 10-day-old mated females and the state of the proximal egg chamber as compared to the distal oocyte within each female.

AGE (DAYS)	STATE OF DISTAL OOCYTE*	NO. ♀♀ WITH PROXIMAL EGG CHAMBER WITH				TOTAL
		NO TRACE YOLK	¼ YOLK	½ YOLK	¾ YOLK	
6	Mature	15	3			18
	Near mature	0				0
	Not mature	1			2	3
	No ovaries	2				2
	Total					23
10	Mature	19	5	4		28
	Near mature	3				3
	Not mature	11	3	1	1	16
	No ovaries	5				5
	Total					52

* **Mature** oocytes contain no pole-cap cells. **Near mature** oocytes retained terminal pole-cap cells and may lack a full amount of yolk. **Not mature** indicates little yolk and large nurse cells.

ovariole is enclosed within a thin membrane that surrounds all of the developmental stages. The oocyte is retained within this membrane until it enters the oviduct. The ovarioles of each ovary are held together by a gossamer-like membrane similar to the peritoneal sheath observed in *Drosophila* (Mahowald & Kambysellis 1980). These connective fibers are associated with a network of tracheoles that enter at the region of the germarium. This complex covers the proximal ½ to ¾ of the ovary. The individual ovarioles have a suspensory ligament that binds the proximal germarium to the support tissue. Since each ovariole contains only 3 developmental stages, the ovary has a flattened appearance (see Semenza 1953) in contrast to the more cone-shaped appearance of ovaries in *Drosophila,* where oocytes may be in any one of 14 stages of development (Miller 1950, King 1970). A thin fatty tissue layer covers the proximal surface of the ovary.

The germarium has 2 regions that can be recognized at low power magnification. The terminal region appears to be similar to the 16-cell clusters designated as region 3 of the *Drosophila* germarium by Mahowald & Strassheim (1970). Further study is required to determine what specific activities occur in each area of the *Megaselia* germarium. Vitellogenesis begins before the females are 12 h of age, and, as with other insects (Hagedor & Kunkel 1979), once yolk accumulation begins the oocyte matures quickly. Studies are now in progress to determine what changes occur in egg chamber–oocyte organization during the maturation process.

Oocyte development in *Megaselia scalaris* is synchronized so that all ovarioles contain egg chambers that are at the same general stage of development at any time. This is similar to the pattern observed in *Musca domestica* (Adams et al. 1968). In *Rhodnius prolixus* (Pratt & Davey 1972b) eggs develop in waves following blood meals, but the ovarioles are not synchronized as in *Musca.* Eggs are probably laid as they mature,

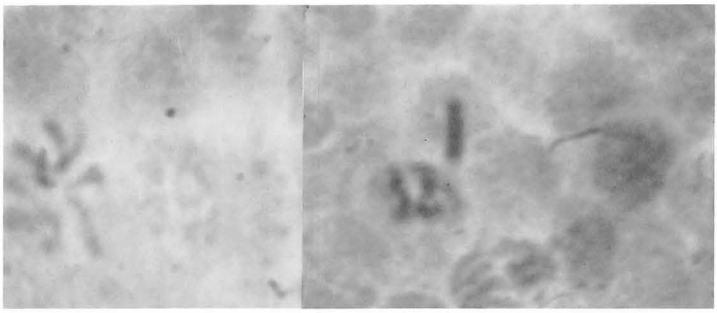

FIG. 3. Mitotic figures in egg chamber follicle cells.

which accounts for the clusters of eggs observed by Mainx (1964), El-Miniawi & Moustafa (1965), Tumrasvin et al. (1977), and Prawirodisastro & Benjamin (1979). The first eggs mature at 32–34 h after eclosion. Oviposition may begin by females as young as 38 h of age (El-Miniawi & Moustafa 1965), so in young flies additional time may be required for other reproductive organs to mature. The rate of development and maturation is influenced by temperature (Prawirodisastro & Benjamin 1979, Trumble & Pienkowski 1979), and at 15 °C fecundity is decreased and the number of egg-laying-days is reduced (Prawirodisastro & Benjamin 1979). However, there is no evidence that the reported differences in egg-laying patterns are temperature related. The results reported here and those of Prawirodisastro & Benjamin (1979) were obtained from flies reared at 25 °C, and both are very similar to the results of El-Miniawi & Moustafa (1965), who conducted their studies at 27 °C. The results of El-Miniawi & Moustafa are very different from those of Tumrasvin et al. (1977), who also reared flies at 27 °C. The strain of flies or culture conditions, other than temperature, may be responsible for these differences.

It is not known how long it takes for oocytes to mature in older females, but based on 16 egg-laying-days in a 25.7-day fertility period (Prawirodisastro & Benjamin 1979) 15 recovery periods would occupy approximately 10 days. This would result in recovery periods of approximately 16 h, which is less than ½ the time that it takes for the first oocytes to mature and females to lay their first eggs. This suggests that oocyte maturation may occur more rapidly in mature flies. In young females the greatest increment of oocyte elongation occurs in the final 8 h. During this time the oocyte more than doubles in size, so it is very possible that, in mature females where all of the systems are already mature and active, the egg chamber could become a mature oocyte in a much shorter period of time.

The results presented here indicate that the immature egg chambers will not mature as long as some number of mature oocytes remain in the ovary. It is not known what minimal number is necessary to maintain this state or what mechanism of control is involved. In *Musca domestica* (Adams et al. 1968, Adams 1970) and in *Rhodnius prolixus* (Pratt & Davey 1972a, 1972b) oocyte development is inhibited by products of the

ovaries or mature oocytes. A similar hormonal regulation is likely in *Megaselia.* A few flies were observed to have developed the next group of oocytes when mature oocytes were present, but the mature oocytes may have been in the oviduct rather than in the ovariole. Virgin females lay unfertilized eggs, so mating is not required to stimulate oviposition. Mating may influence the rate at which the eggs are laid as it does in *Rhodnius prolixus* (Pratt & Davey 1972b) and in *Drosophila* (Boulétreau-Merle 1973), since in a small sample of virgin females oviposition was not observed until the 3rd day (unpubl. observations) compared to egg laying beginning on the 2nd day in mated flies (El-Miniawi & Moustafa 1965, Prawirodisastro & Benjamin 1979, unpubl. observations).

The data presented here support the observations of El-Miniawi & Moustafa (1965) and Prawirodisastro & Benjamin (1979) that *Megaselia* females may remain fertile for an extended period, that fecundity can be rather high, and that egg production is not uniform because the eggs mature in synchronized clusters.

Acknowledgment. I would like to thank Debra Boone for making her histological materials available.

LITERATURE CITED

Adams, T.S. 1970. Ovarian regulation of the corpus allatum in the housefly *Musca domestica. J. Insect Physiol.* **14:** 349–60.

Adams, T.S., A.M. Hintz & J.G. Pomonis. 1968. Oostatic hormone production in houseflies *Musca domestica* with developing ovaries. *J. Insect Physiol.* **14:** 983–93.

Bonhag, P.F. 1958. Ovarian structure and vitellogenesis in insects. *Ann. Rev. Entomol.* **3:** 137–60.

Boulétreau-Merle, J. 1973. Fonctionnement ovarien comparé des femelles vierges et des femelles insémineés de *Drosophila melanogaster. Ann. Soc. Entomol. Fr.* **9:** 181–91.

Catts, E.P. 1982. Biology of New World bot flies: Cuterebridae. *Ann. Rev. Entomol.* **27:** 313–38.

El-Miniawi, S.F. & M.A. Moustafa. 1965. On the biology of *Megaselia scalaris* Loew (Diptera: Phoridae). *Bull. Soc. Entomol. Egypte* **49:** 89–91.

Hagedor, H.H. & J.G. Kunkel. 1979. Vitellogenin and vitellin in insects. *Ann. Rev. Entomol.* **24:** 475–505.

Haider, S.R. 1956. Description of the male genitalia of *Megaselia scalaris* (Loew) an intestinal myiasis producing fly (Diptera: Phoridae). *Pak. J. Health* **6:** 191–92.

Huebner, E. & K.G. Davey. 1973. An antigonadotropin from the ovaries of the insect *Rhodnius prolixus* Stål. *Can. J. Zool.* **51:** 113–20.

James, M.T. 1947. *The flies that cause myiasis in man.* U.S. Dept. Agric. Misc. Publ. No. 631. 160 p.

James, M.T. & R.F. Harwood. 1970. *Herms's medical entomology.* 6th ed. Collier-Macmillan Ltd., London. 263 p.

King, R.C. 1970. *Ovarian development in* Drosophila melanogaster. Academic Press, New York, London, San Francisco.

King, R.C., Ann C. Rubinson & R.F. Smith. 1956. Oogenesis in adult *Drosophila melanogaster. Growth* **20:** 121–57.

King, R.C. & M.F. Wolfsberg. 1957. Oogenesis in adult *Drosophila melanogaster.* VI. A comparison of oogenesis among *Drosophila melanogaster, virilis, pseudoobscura* and *gibberosa. Growth* **21:** 281–85.

LaChance, L.E. & A.P. Leverich. 1962. Radiosensitivity of developing reproductive cells in female *Cochliomyia hominivorax. Genetics* **47:** 741–53.

Mahowald, A.P. & M.P. Kambysellis. 1980. Oogenesis, p. 141–224. In: Ashburner, M. & T.R.F. Wright, eds., *The genetics and biology of* Drosophila. Vol. 2d. Academic Press, New York, London, San Francisco.

Mahowald, A.P. & J.M. Strassheim. 1970. Intracellular migration of centrioles in the germarium of *Drosophila melanogaster.* An electron microscope study. *J. Cell Biol.* **45:** 306–20.

Mainx, F. 1964. The genetics of *Megaselia scalaris* (Loew) (Phoridae): a new type of sex determination in Diptera. *Am. Nat.* **98:** 415–30.

Miller, A. 1950. The internal anatomy and histology of the imago of *Drosophila melanogaster*, p. 420–534. In: M. Demerec, ed., *Biology of* Drosophila. John Wiley & Sons, New York.

Patton, W.S. 1922. Notes on some Indian Apiochaetae. *Indian J. Med. Res.* **9:** 683–91.

Pratt, G.E. & K.G. Davey. 1972a. The corpus allatum and oogenesis in *Rhodnius prolixus* (Stål). I. The effects of allatectomy. *J. Exp. Biol.* **56:** 201–14.

1972b. The corpus allatum and oogenesis in *Rhodnius prolixus* (Stål). III. The effects of mating. *J. Exp. Biol.* **56:** 223–37.

Prawirodisastro, M. & D.M. Benjamin. 1979. Laboratory study on the biology and ecology of *Megaselia scalaris* (Diptera: Phoridae). *J. Med. Entomol.* **16:** 317–20.

Robinson, W.H. 1971. Old and new biologies of *Megaselia scalaris* (Dipt.: Phoridae). *Stud. Entomol.* **14:** 1–14.

Semenza, L. 1953. *Apiochaeta xanthina* Speiser. Contributo alla consecenza morfologia e del biologico. *Rend. Ist. Lomb. Sci. Lett. Cl. Sci. Math. Nat.* **86:** 320–30.

Trumble, J.T. & R.L. Pienkowski. 1979. Development and survival of *Megaselia scalaris* (Diptera: Phoridae) at selected temperature and photoperiods. *Proc. Entomol. Soc. Wash.* **81:** 207–10.

Tumrasvin, Watanasak, Supat Sucharit & Samran Vutikes. 1977. Studies on the life history of *Megaselia scalaris* (Loew) in Thailand. *Southeast. J. Trop. Med. Pub. Health* **8:** 74–76.