Some observations of oogenesis in *Eucorethra underwoodi* Underwood (Diptera: Chaoboridae)

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FigShare paper, 05 August 2013

Abstract

Females of *Eucorethra underwoodi* Underwood (Diptera: Chaoboridae) are autogenous and exhibit marked ovarian precocity, deposition of yolk in the terminal ovarian follicles beginning during the pupal stage. The picture of ovarian staging in the young female is complex — a mix of maturing follicles, regressing follicles, and apparently healthy, partially developed follicles, perhaps in a "holding pattern", suggesting that asynchronous first-cycle oogenesis may be the norm in this species. In the ovarioles of gravid females, penultimate follicles are extensively yolked and the fatbody remains ample, suggesting the possibility of more than a single cycle of autogenous oogenesis.

Introduction

The monospecific genus *Eucorethra* is the earliest lineage of the Chaoboridae (Borkent 2012); the genus contains the single Nearctic species, *E. underwoodi* Underwood. The larvae inhabit ground pools, (often vernal pools) in the taiga and coniferous-forest regions of much of North America (Lake 1960; Borkent 1981; Rossignol 1978). Unlike other members of the family, the larvae of *E. underwoodi* prey on insects that fall onto the *surface* of the pool, as well as prey such as mosquito larvae *in* the water (Maire *et al.* 1978; Rossignol 1978; Borkent 2012).

The species has received little scientific study; the evolutionary relationships are well understood (Ogawa 2007; Borkent 2012) and there is *some* information about the behavior and ecology of the larvae (Maire *et al.* 1978; Rossignol 1978) but almost nothing is known of the biology of the adults.

The egg of *E. underwoodi* has been figured (Rossignol 1978) but the process of oogenesis is undescribed. In the laboratory, the eggs were laid singly, as in *Mochlonyx*, on wet cotton and were sticky for the first few days after oviposition, after which they were easily dislodged; Rossignol (1978) speculated that the eggs were laid on poolside vegetation and then washed into the pool by rain. However, hatching may be delayed — Lake (1960) reported that the pools he observed dried up shortly after adult emergence and were not reflooded until the autumn, but the species is also known to occur in more-permanent ground pools.

There are two notes in the literature that provide a *little* information about oogenesis in *E. underwoodi*, both derived from long-ago personal communications from me:

- "*E. underwoodi* is autogenous and apparently has synchronous ovarian development (Smith, personal communication)." (Rossignol 1978, p. 63).
- "Another dipteran *Eucorethra underwoodi* (Chaoboridae) shows penultimate follicle yolk (mosquito stage IIIa) before the terminal follicles mature (Dr. S.M. Smith, personal communication)." (Venkatesh and Morrison 1980, footnote on p. 714).

Here I provide some photographic evidence on the basis of which these personal communications were cited.

Methods

Adult female *E. underwoodi* were reared from pupae collected from vernal pools in Flin Flon and The Sandilands Provincial Forest, MB. Adults were maintained in single-female cages (for a photo of these cages, *see* Smith (2013)) and offered honey as a food source.

Females were dissected in physiological saline and ovarian development was examined under phase-contrast microscopy. Ovarian staging follows the scheme described in Watts and Smith (1978).

Observations

In the Diptera – Nematocera, the consumption of a protein meal by the adult female, aided by armed mouthparts, is plesiomorphic (Downes 1971); some groups, however, including all the Chaoboridae except *Corethrella*, have evolved reduced, unarmed and weakened mouthparts and the females do not consume protein as a prerequisite for oogenesis. Therefore, as expected, females of *E. underwoodi* are *autogenous* (i.e. the adult female does not require a protein meal for oogenesis). As well, the females exhibit *ovarian precocity* (i.e. ovarian development is initiated in the pupal stage), so that, at the time of adult emergence, substantial ovarian development has already taken place, with terminal follicles in stage IIIa–IIIb (Figs. 1–2).

The ovarian picture is one of substantial heterogeneity (Figs. 1–3), with some follicles advancing to eggs, some failing to initiate development, some regressing after some yolk deposition has occurred (Fig. 3), and many follicles possibly postponing development, as would be the case if oogenesis were asynchronous among ovarioles (Fig. 6).

Ovarian development proceeds rapidly at 20°C; by 2 d of age the terminal follicles are at stage IVa (Figs. 4–5) and mature follicles (i.e. eggs) are present by days 5–7 (Figs. 6–7). The ovaries of gravid females contained not only eggs but healthy-appearing follicles at earlier stages of development (Fig. 6).

Two females examined at 7 d of age, each honey-fed, had, respectively, 39 and 34 stage-V follicles, 35 and 26 penultimate follicles at stages IIIa–IIIb, and a moderate-to-ample brown fatbody, certainly sufficient for the maturation of many more eggs. The abundance of partially developed 1st-cycle follicles (Fig. 6) in gravid females suggests the possibility of asynchronous oogenesis and the advanced stage of the penultimate follicles in ovarioles containing eggs (Fig. 7), suggests the possibility of 2nd-cycle autogenous oogenesis.

Discussion

In the absence of information about the biology of the adults in the field, I engage in some speculation. If *E. underwoodi* is similar to other autogenous Nematocera, I would expect the females, on emergence, to seek a carbohydrate meal; in at least some Nematocera, carbohydrate feeding is known to precede mating (e.g. Smith and Gadawski 1994). In the laboratory, female *E. underwoodi* readily accepted honey and it is likely that carbohydrate feeding is essential to ensure the longevity required to mate, complete oogenesis and oviposit. In the related chaoborid *Mochlonyx cinctipes* (Coquillett), adults kept on only water had almost no energy reserves left after 24 h, whereas when maintained on sucrose, adults lived long enough for some of them to complete autogenous oogenesis within 8 d (Magnarelli and Andreadis 1987).

Following carbohydrate feeding, females would then seek a mate — likely in a swarm. Swarming of *E. underwoodi* is unknown but males of *Mochlonyx* form swarms in the crepuscular period (O'Connor 1959) — a common mating syndrome in the Nematocera, and the description of the antenna of male *E. underwoodi* (Coquillett 1903) suggests swarming as in other Nematocera. The adults of *Mochlonyx* do not disperse far from the breeding sites; O'Connor (1959) collected no adults farther than 70 feet from the pools; *E. underwoodi* likely has similar, restricted vagility, as Underwood (1903) found.

Autogenous oogenesis appears repeatedly in several families of the otherwise bloodsucking Diptera – Nematocera / Brachycera (*see* Smith 1970 for an extensive review), particularly in marginal habitats near the limits of the range of a species, in which case rapid development of the ova could be of selective advantage, or in species occupying habitats that are uncommon spatially or temporally, in which case, the restrictions on dispersal that autogeny would confer could be of considerable value.

Ovarian precocity is common among the many species of autogenous Simuliidae (Prokof'eva 1959; Smith 1970) but is much less well known in the other families of the Nematocera. In a northern strain of the Pitcher-Plant Mosquito, *Wyeomyia smithii* (Coquillett), ovarian precocity is almost identical to that exhibited by *E. underwoodi* — the females emerge with stage-IIIa follicles (Smith and Brust 1971) whereas almost all other mosquitoes emerge with follicles at stage I or earlier, even in autogenous species (e.g. *Ochlerotatus rempeli* (Vockeroth) — Smith and Brust 1970). Some females of *Leptoconops bequaerti* (Kieffer) (Diptera: Ceratopogonidae) are autogenous and emerge with follicles already in stage IVa; they complete ovarian development within 1–1.5 d following emergence (Linley 1968).

Only larger samples and longer-duration studies (and perhaps also field studies) could substantiate the possibility that ovarian development in *E. underwoodi* is asynchronous, but there are precedents among the Nematocera. Magnarelli and Andreadis (1987) showed that in *M. cinctipes*, some ($^{8}/_{24}$) sucrose-fed females developed stage-V follicles in the laboratory by 8 d of age but many other females of the same age had follicles in a variety of stages, from I to IV (their Table 4). In the mosquito *Toxorhynchites rutilus* Coquillett, the larvae of which, as in *E. underwoodi*, are predatory, oogenesis is extraordinarily precocious, more so than in *E. underwoodi*, but also asynchronous, females developing eggs over a period of time rather than as a synchronous batch (Watts and Smith 1978). Underwood (1903) and Lake (1960) reported that the larvae of *E. underwoodi* were cannibalistic in the laboratory. If, as in the larvae of *Toxorhynchites* (Corbet 1985), larvae of *E. underwoodi* are cannibalistic in the natural habitats, then asynchronous oogenesis accompanied by temporally staggered oviposition, might reduce the risk of intraspecific predation — a type of bet-hedging perhaps — a strategy that would be adaptive only if hatching occurs soon after oviposition or if egg hatch is itself asynchronous.

There is almost no information on second-cycle autogenous oogenesis in the Nematocera. Oliver (1968) showed that some species of arctic Chironomidae are able to complete 2 ovarian cycles (the second of much reduced fecundity) without any adult protein nourishment. The healthy appearance of extensively yolked penultimate follicles in *E. underwoodi* suggests that 2nd-cycle autogenous oogenesis may be normal, but requires confirmation. Similar autogenous promotion of the penultimate follicles has been observed in *Wy. smithii* (Smith and Brust 1971) and *Oc. rempeli* (Smith 1970).

Acknowledgments

This work was done while I was a doctoral student of Dr R.A. Brust, Department of Entomology, University of Manitoba, Winnipeg. I thank Dr Brust for the opportunity to have worked in a then state-of-the-art biting-fly laboratory with great facilities, modern microscopy, and supportive colleagues. Dr Brust was supported by NSERC and I received doctoral fellowships from the same organization.

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1. The quality of the online PDF of this thesis is quite wretched — in particular, the plates are utterly uninformative and largely illegible. Readers wishing to see a high-quality PDF, including color plates of the egg, may request a copy from me.

Fig. 1. The ovary of a teneral female (<2 h old) of *Eucorethra underwoodi*. Adult from Flin Flon, MB.
The most advanced follicles are at stage IIIa but some follicles have much less yolk and are probably regressing.
Photo taken 9 June 1969.

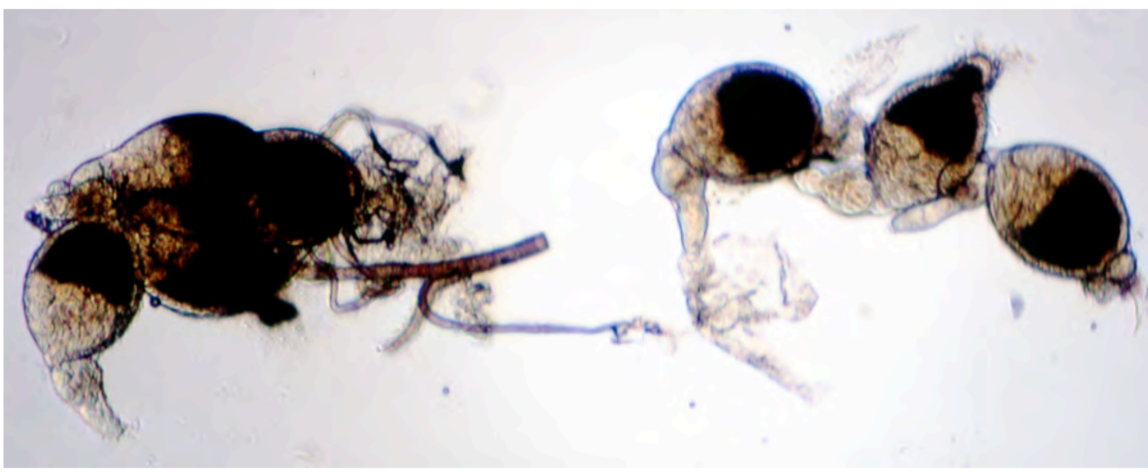

Fig. 2. Ovarioles of a teneral female)<2 h old) of *Eucorethra underwoodi*. Adult from Flin Flon, MB.
The most advanced follicles are at stage IIIa–IIIb but a few terminal follicles
are without yolk — either regressed or uninitiated.
Photo taken 9 June 1969.

Fig. 3. Ovarioles of a teneral female of *Eucorethra underwoodi*. Adult from Flin Flon, MB. The follicle at the left is regressing; the 2 follicles at the right are either in an advanced stage of regression or never did initiate development.
Photo taken 9 June 1969.

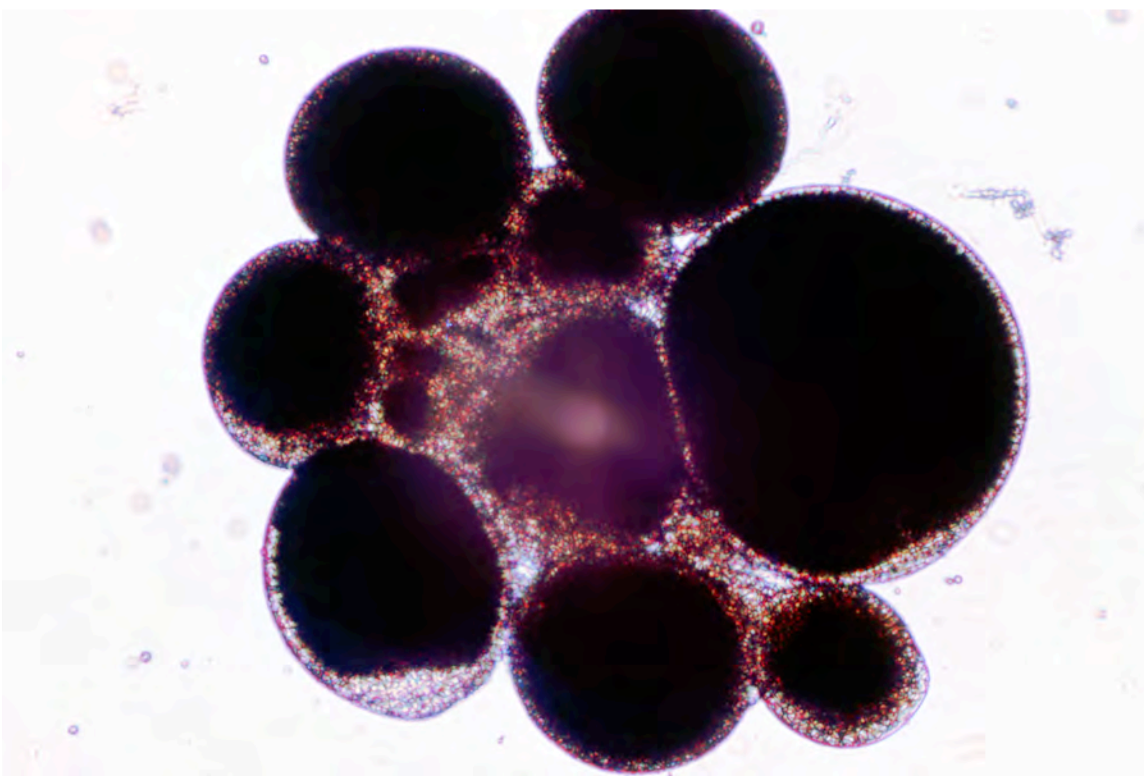

Fig. 4. *Eucorethra underwoodi*. Follicles of a 2-d-old female from Sandilands, MB. held at 20°C, 16L:8D. The most advanced follicles, of which the female had 45, are at stage IVa; ~15 regressing follicles.
Photo taken 21 June 1969. Gentian-violet stain.

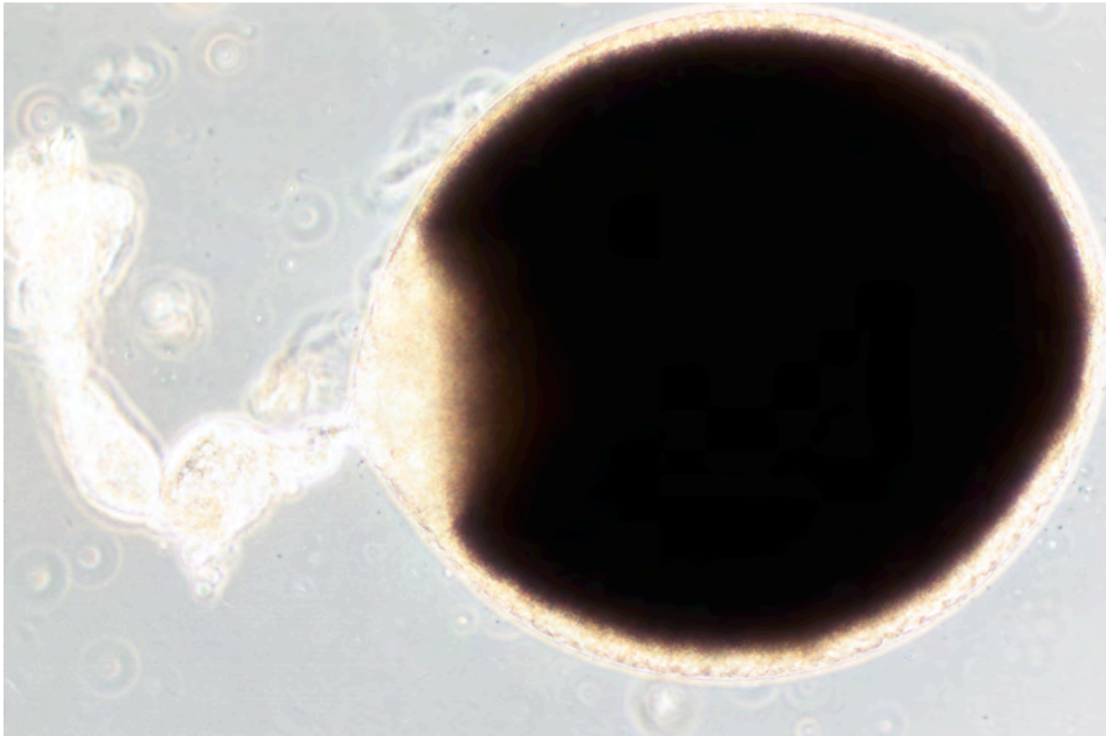

Fig. 5. *Eucorethra underwoodi*. A single ovariole of a 2-d-old female from Sandilands, MB. held at 20°C, 16L:8D. The terminal follicle is at stage IVa; no yolk yet visible in the penultimate follicle.
Photo taken 21 June 1969.

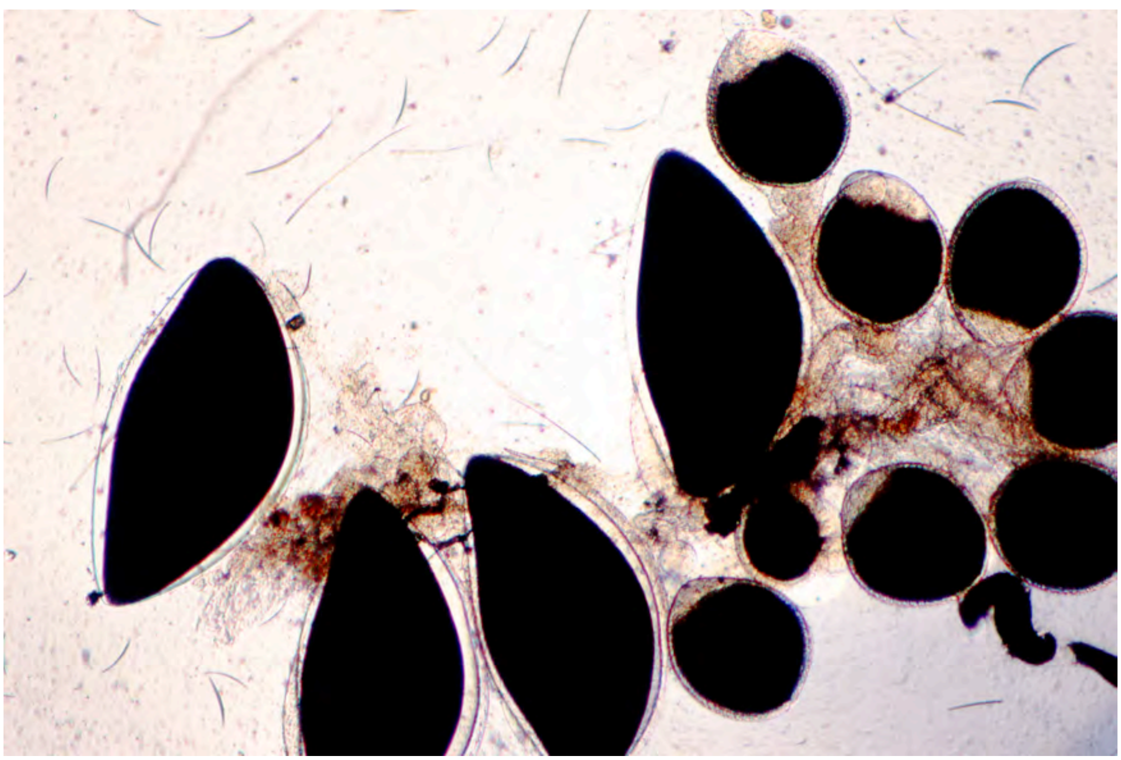

Fig 6. *Eucorethra underwoodi*. Ovarioles from a 7-d-old, honey-fed female from Sandilands, MB, held at 20°C, 16L: 8D. The female is gravid (34 stage-V follicles — chorionated eggs — were present in the ovaries) but many follicles are at earlier stages of development (26 stage-IIIa–IVb follicles).
The mature follicles are about 1.03 mm long. Photo taken 24 June 1969.

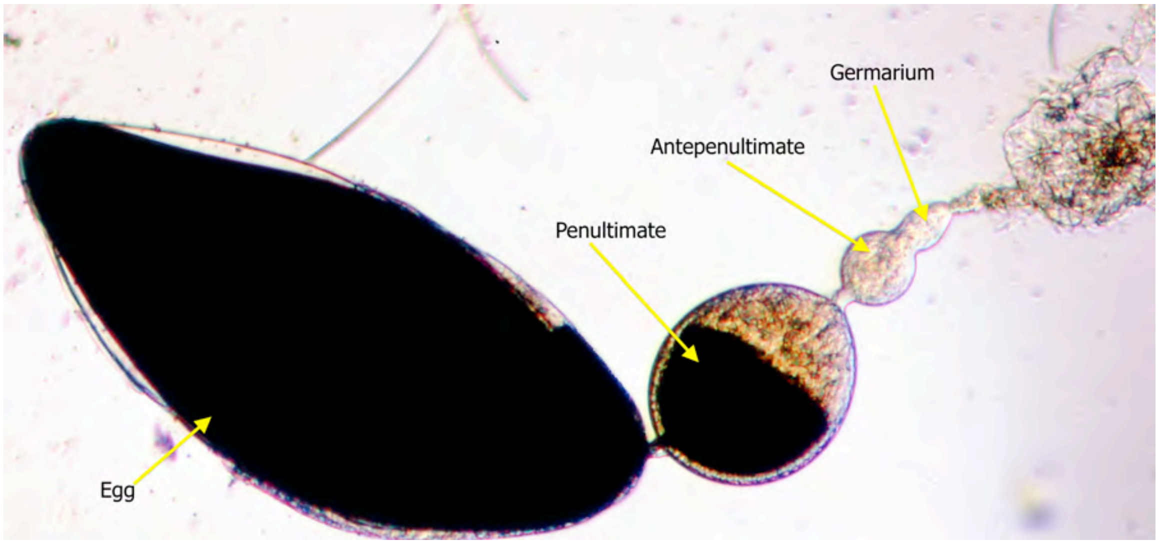

Fig. 7. *Eucorethra underwoodi*. A single ovariole from a 5-d-old female from Sandilands, MB, held at 20°C, 16L: 8D; honey-fed. The terminal follicle is at stage V (i.e. a chorionated egg), the penultimate follicle is at stage IIIa, the antepenultimate follicle is at stage N.
Photo taken 26 June 1969.