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The role of blood meal in the life of haematophagous horse flies (Diptera: Tabanidae)

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Abstract

Background and Purpose: *The aim of this study was to prove whether the most abundant species* Tabanus bromius *L., 1758 in Eastern Croatia is autogenous or anautogenous. Analysis was performed on the ovarioles in the ovaries of 25 females.*

Materials and Methods: *Periodical collections were carried out at localities close to the river Sava: Plesmo (XL 41) and Spačva (CQ 39) in June, July and August 1995. Horse flies were put in 4% formalin solution. In the laboratory female specimens were dissected in 0.9% water solution of NaCl. After dissection ovaries were removed and preserved in 4% formalin solution. Ovaries were then processed through a standard dehydration series of ethanol solutions, cleared in benzene solutions and embedded in paraffin. Sections were cut at 5 micrometers with the Reichert – Jung rotary microtome. The slices were stained following standard staining procedures.*

Results and Conclusions: *The follicles of the ovarioles were mostly in stage I and II of maturation, proving that blood meal is essential for egg maturation. Based on these results we can conclude that the* Tabanus bromius *is an anautogenous species in the studied area. Shrunk cellular remains of the follicular epithelium in ovarioles are found in the samples collected during July. A second fully finished gonotrophic cycle was not found.*

INTRODUCTION

The Tabanidae comprise some 4000 species, and include some of the largest biting flies, commonly called horse flies, deer flies and clegs *(1)*. Tabanidae are mainly diurnal and only the females take blood *(1)*. Some of these flie species have been the subject of many studies because they are known as vectors of pathogenic agents *(2)* that can be passed on to people (humans) and livestock. In recent years attention has been focussed on their gonotrophic cycle, especially in the Nearctic and Palaearctic regions. In the Nearctic region, the first report about autogeny was found on deer flies of the genus Chrysops *(3)*. Other authors *(4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19)* found that the species *Chrysops fuliginosus*, *Chrysops atlanticus*, *Chrysops hirsuticallus*, *Tabanus nigrovittatus*, etc. occur autogenously in the first gonotrophic cycle in several parts of the studied area. These species usually have a single cycle but occasionally have two or exceptionally three gonotrophic cycles. However, the species *Chrysops ater*, *Chrysops callidus*, *Hybomitra lasiophthalma*, *Tabanus quinquevittatus* and other species are anautogenous and they have one or two gonotrophic cycles. In Japan, the species *Tabanus iyoenensis*, *Tabanus chrysurus*, *Tabanus kataoi*, *Tabanus rufidens*, *Tabanus trigeminnus*, *Tabanus humilis*, *Tabanus sapporoensis* belong to the autogenous species *(20, 21)*. This problem in Europe was investigated in central European conditions in Czechoslovakia. Of 520 examined tabanid females belonging to 12 different species, all except one female of Chrysops viduatus, were autogenous in the first gonotrophic cycle *(22)*. In West Germany there were both autogenous and anautogenous females in 25 species *(23, 24, 25)*. According to this perception, only *Heptatoma pellucens* and Haematopota pluvialis are autogenous, while other species are anautogenous. In Switzerland, of the horse fly females caught 10 belong to different species *(26)*. *Hybomitra kaurii*, *Haematopota pluvialis* and *H. crassicornis* were found to be autogenous in the first gonotrophic cycle. Tabanus bromius was anautogenous *(26)*. Furthermore, in Eastern Croatia Tabanus sudeticus was anautogenous *(27)*.

The aim of this study was to prove whether the females of *Tabanus bromius* are anautogenous or autogenous in the first gonotrophic cycle in Eastern Croatia.

MATERIAL AND METHODS

The horse flies were collected on livestock, at localities close to the river Sava: Plesmo (XL 41) and Spačva (CQ 39) in June, July and August 1995. There were 25 female flies of *Tabanus bromius* that were put in 4% formalin solution. Ovaries were removed in the laboratory and preserved in the same solution. Two weeks later, the formalin fixed ovaries were water – rinsed in a 24- hour period and then processed through a standard series of ethanol solutions. The dehydrated material was first cleared in benzene solutions, and then embedded in paraffin in a paraffin bath (58 °C). The paraffin sections were cut with the Reichert – Jung rotary microtome and affixed to slides with albumin affixative. The 5 micrometer slices were stained with haematoxylin and eosin, following standard histologic staining procedures, covered with Canadian balsam and glass lid. The pictures were taken with an Opton Standard photomicroscope. The horse fly species were determined by using key *(28)*.

RESULTS

Anatomic analysis revealed that the fusiform ovaries of *Tabanus bromius* lie laterally in the abdomen and mostly extend from the 3rd to 5th abdominal segment. The suspensory ligament connects the apex of each ovary with the second abdominal segment. Each ovary consists of ovarioles. *Tabanus bromius* has meroistic polytrophic ovarioles consisting of one sheath tube with germarium, primary follicle and secondary follicle (Figure 1). Each follicle has an oocyte, as well as nurse cells enclosed within the follicular epithelium. The oviducts are short and join the common oviduct. The common oviduct turns into a sack – shaped genital chamber. The eggs pass through several stages during their maturation. In stage N, the follicle consists of eight cells not yet differentiated into nurse cells and an oocyte (Figure 1). The follicle is spherical and follicular epithelium consists of cubic cells.

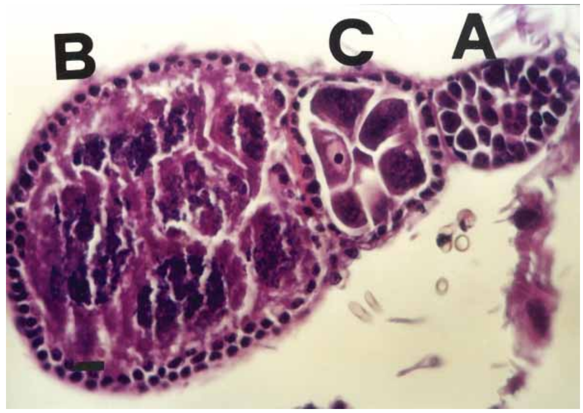

Figure 1. *Longitudinal section of an ovariole of* Tabanus bromius. *A – germarium, B – primary (terminal) follicle, C – secondary follicle, 125x.*

In stage I, the oocyte is clearly visible in the distal portion of the follicle, with the seven nurse cells lying in the proximal portion of the same follicle. In stage II, the follicle takes an oval shape, with the yolk granules appearing around the nucleus in the oocyte protoplasm (Figure 2). The oocyte increases its share within the follicular space over the nurse cells in stage III. The oocyte occupies more than 50% of the follicle. The oocyte in stage IV and V is full of yolk granules, and the nucleus is no longer visible through the mass of yolk. The nurse cells are restricted to the small proximal portion of the follicle. After ovulation oocytes migrate to the oviduct where they are fertilised. Thereafter to follow oviposition, ovarioles are characterised by a distended follicular epithelium containing the remnants of nurse cells on the distal portion. The shrunk cellular remains of the follicular epithelium on ovarioles were found on the samples collected during July, but the June and August specimens did not have any, confirming one oviposition. The follicles of the ovarioles of the *Tabanus bromius* collected in June were in stages I, II and III of maturation (Table 1), proving that blood meal is essential for egg maturation. According to these results, we can conclude that the *Tabanus bromius*

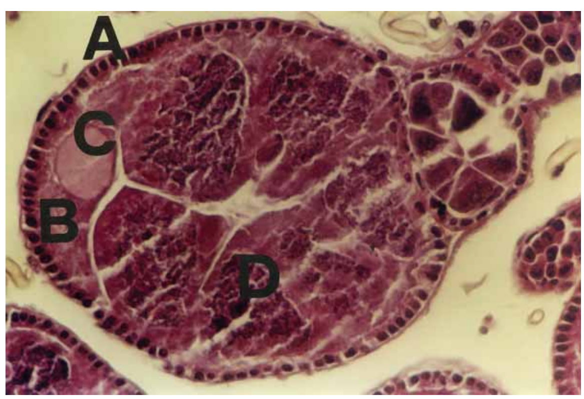

Figure 2. *Longitudinal section of an ovariole of* Tabanus bromius. *A – follicular epithelium, B – yolk, C – nucleus, D – nurse cells, 125x.*

TABLE 1

Developmental stages of egg follicles of analyzed females of the species Tabanus bromius in Croatia.

		Developmental stages of egg follicles					
Months	Specimens	I	II	III	IV	V	Cellular remains
June	10	3	6	1	–	–	–
July	10	1	2	–	–	–	7
August	5	2	3	–	–	–	–
Total	*25*	*6*	*11*	*1*	–	–	*7*

is an anautogenous species. Each female of an anautogenous species matures its first batch of eggs exclusively after taking a blood meal.

DISCUSSION

Ovarioles are meroistic politrophic, typical of most dipterans, with both an oocyte and sterile nurse cells made (produced) in the germarium. Follicles are mostly in stage II of maturation (Table 1). The presumption is that the anautogenous species have some reserve food from the larval stage which is deposited in the form of yolk in terminal oocytes. However, the blood meal is still a prerequisite for maturation of the eggs *(7)*. Not surprisingly, the specimens were collected while blood sucking the livestock. The blood meal is necessary for the completion of one egg series maturation and for the yolk deposition in the next oocytes series *(7)*. One blood meal is an energy source for 100 to 1000 eggs production *(29)*. An estimated 3.1 eggs were produced for each mg of blood ingested by the fly *(30)*. The shrunk cellular remains of the follicular epithelium on ovarioles were found on the samples collected during July (Table 1). Immediately after ovulation the ovarioles are characterised by a distended follicular epithelium containing the remnants of nurse cells on the distal portion *(31)*. The formation of the follicular remains following ovulation, oviposition is one of the basic diagnostic features used in determination of the number of gonotrophic cycles completed by female dipterans *(32, 33)*. The reproductive system status of female horse flies can affect the number of the collected specimens by different attractants and methods *(34)*. Fluctuations of some horse fly populations can be explained by the number of ovipositions *(35)*. Analysis of the female reproductive system is helpful in differentiating the anautogenous and autogenous species in the first cycle – the latter do not use blood meal for the first oviposition *(7, 20, 36)*. The July horse flies had follicular remains in the ovariole but the June and August specimens did not have any, showing that *Tabanus bromius* is an anautogenous species and proving that blood meal is essential for egg maturation. However unfavourable climatic conditions could prolong the larval period, increasing the consumption of the feeding substances for egg development, resulting in the appearance of anautogenous females *(37)*. June 1995 in Croatia was rather chilly and rainy and we assume that this is also one of the reasons for the emergence of a great number of anautogeneous females in the first gonotrophic cycle *(27)*.

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