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Biochemical study of *Bovicola limbatus* (Mallophaga). A) Determination of different proteic fractions in immature stages. B) Proteic changes during ontogenesis[1])

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Bovicola limbatus Gervais, 1844 has a simple metamorphosis during its ontogenesis with such stages as egg, 3 immature ones (L1, L2 and L3) and the sexually different adults (Benítez-Rodríguez et al. 1986).

In a foregoing taxonomic research (Muñoz-Parra et al. 1988) the authors studied the electrophoregrams of males and females of this species, separating the total proteins by means of SDS-PAGE and grounding the determination of their R (mb), Rx, Rf and interval of mW.

In this work the electrophoretic development and characterization of total proteins of the immature stages of this species have been carried out. The proteic change during the ontogenic development of *B. limbatus* (from L1 to adult males and females) is also studied.

Previously, other authors have applied electrophoretic technics in order to study proteic changes of other insect-groups. According to Warren & Breland (1969) the sexual dimorphism is shown with the existence of a band, which mobility differs depending on the sex and possibly can appear in the larval stage. The authors also observed changes in the bands concerning the different larval stages. Depending on the species, the youngest stages posess a bigger number of bands than the next ones, although the contrary can also happen in other species.

Igbokwe & Downe (1977) analyzed the soluble proteins of 3 strais of *Aedes aegypti,* going until the sex differentiation. In a posterior work (1978) these authors mentioned that the females show more contrast and electrophoretic diversity than the males. Ravanasiddaiah & Chowdaih (1982) also use the discoidal electrophoresis in order to observe the distribution of the total proteins during the development of *Anopheles stephensi.* He noticed an increase of the number of proteic bands, from eggs to the IV larval stage, due to the high metabolical activity during the larval development, which decrease in the pupe and adult stage; therefore, the number of proteic bands decrease to half during the latter stages as a consequence of the metabolic loss.

Material and Methods. Specimens L1, L2 and L3 of *B. limbatus* were separated and kept at − 20 °C in individual groups, depending on its stage of development.

Consecutively, the number of lice we started with, their total weight, total proteins obtained and proteins used in each case are offered.

	Nr. lice	Total weight (µg)	Total proteins (µg)	Used proteins (µg)
L1	70	6,300	13.2	5
L2	60	6,400	15.0	5
L3	60	7,600	24.0	5

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Tab. 1. Analysis of electromobility of distinct proteins fractions in the first immature stage of *Bovicola limbatus*

	1	2	3	4	5	6	7	8	9	10
R (mb)	3.5	5.0	7.0	9.5	11.5	14.0	17.0	19.0	26.5	45.0
% R (mb)	7.7	11.1	15.5	21.1	25.5	31.1	37.7	42.2	58.9	100.0
R-%R (mb)	1.0	1.4	2.0	2.5	3.9	4.0	4.9	5.4	7.7	12.9
Rx**	0.16	0.22	0.31	0.42	0.51	0.62	0.76	0.84	1.18	2.00
Rx*	0.04	0.06	0.08	0.11	0.13	0.16	0.20	0.22	0.31	0.52
Rf	0.04	0.06	0.08	0.11	0.13	0.16	0.19	0.21	0.30	0.51
mW	← over 66,000 daltons →								← 66,000– → 45,000 d	← 45,000– → 24,000 d

Rx** Taking as reference bovine albumin
Rx* Taking as reference lysozyme

The data concerning adult lice, as well as the criterions of characterization of different proteic bands and the electrophoretic development of males and females of this species are mentioned by MUÑOZ-PARRA et al. (1988).

The methodology, proposed by LAEMMLI (1970) and completed in our laboratory by PEREZ et al. (1982), was used by the authors after its adaptation to our material.

In order to make the study of proteic changes during the development of the species easier, the authors introduce and propose a new key word: topos, i.e. a proper position of a certain proteic band in the electrophoretic development, which is determined by its mW. Each topos possesses a Rf, a R (mb) and a characteristic Rx, which at the same time makes it different from the next topoi. The topos has got the pecularity to exist without the appearance of the proteic band.

This concept only is valid in case of comparing two or more electrophoregrams. So, when comparing two electrophoretic developments, depending on whether the topoi are occupied or not, 4 different types are found:
++ bands are shown in the same topos for both electrophoregrams.
−+ or +− one proteic band appears in a electrophoregram and absence in the other.
−− absence of proteic bands in both electrophoregrams.

Results and Discussion

A) Electrophoretic development and characterization of total proteins in immature stages of *B. limbatus*. In the case of L1 we obtained 10 bands; L2 18 bands and L3 14 bands. The R (mb) for the stages L1 oscillated between 3.5 mm and 45.0 mm for band 10. The Rf oscillated between 0.04 mm and 0.51 mm (Tab. 1). Concerning L2, the R (mb) for the first band was 10.0 mm and for band 18, 79.5 mm[1]) (Tab. 2). With regard to the immature stages L3, the R (mb) for the first band was 7.5 mm and for band 14 it was 71.0 mm. The Rf oscillated between 0.08 mm and 0.80 mm (Tab. 3).

When a study was carried out concerning the distribution of the total number of bands with relation to its mW (Tab. 4), it was observed that the greatest number of bands for the 3 stages was higher than 66,000 d. With regard to the stages, for L1 nearly all the bands appeared to be in this part (80%), while for L2 the number of bands was proportionally divided from 66,000 to 24,000 d. It also observed that the number of proteins decrease remarkably lower than 24,000 d, whereas none band in the 3 stages is found under 14,300 d.

As one may observe, L1 possesses less bands (10) than L2 (18 bands) and L3 (14 bands).

Comparing these results with those of the adult stages (MUÑOZ-PARRA et al. 1988) (Fig. 1), where for the males 15 bands were found and for the females 22, it is noticed that not only the types of proteins change during the ontogenesis of the species, but also their number.

B) Proteic changes during the ontogenesis of *B. limbatus*. In order to estimate the proteic similarities and differencies that could happen during the ontogenesis of a species, we used the absolute mobility R (mb) in the different proteic bands.

[1]) The Rf oscillated between 0.11 mm and 0.89 mm.

Tab. 2. Analysis of electromobility of distinct proteins fractions in the second immature stage of *Bovicola limbatus*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
R (mb)	10.0	13.5	15.0	17.0	18.0	20.0	22.0	25.0	27.0	29.0	31.5	34.0	40.5	43.0	48.0	50.0	76.5	79.5
% R (mb)	12.5	16.9	18.8	21.3	22.6	25.1	27.6	31.4	33.9	36.4	39.6	42.7	50.9	54.0	60.3	62.8	96.2	100.0
R-% R (mb)	1.0	1.4	1.5	1.7	1.8	2.0	2.2	2.5	2.7	2.9	3.2	3.4	4.0	4.3	4.8	5.0	7.6	7.9
Rx**	0.44	0.60	0.67	0.76	0.80	0.89	0.98	1.11	1.20	1.29	1.40	1.51	1.80	1.91	2.13	2.22	3.40	5.53
Rx*	0.12	0.16	0.17	0.20	0.21	0.23	0.26	0.29	0.31	0.34	0.37	0.40	0.47	0.50	0.56	0.58	0.89	0.92
Rf	0.11	0.15	0.17	0.19	0.20	0.22	0.25	0.28	0.30	0.33	0.35	0.38	0.46	0.48	0.54	0.56	0.86	0.89
mW	← over 66,000 daltons →							← 66,000–45,000 daltons →					← 45,000–24,000 d →			← 24,000–14,000 d →		

Rx** Taking as reference bovine albumin
Rx* Taking as reference lysozyme

Tab. 3. Analysis of electromobility of distinct proteins fractions in the third immature stage of *Bovicola limbatus*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
R (mb)	7.5	12.5	15.0	16.5	21.0	22.5	40.5	44.0	46.0	59.5	63.5	66.0	68.5	71.0
% R (mb)	10.5	17.6	21.1	23.2	29.5	31.6	57.0	61.9	64.7	83.8	89.4	92.9	96.4	100.0
R-% R (mb)	1.0	1.7	2.0	2.2	2.8	3.0	5.4	5.9	6.1	7.9	8.5	8.8	9.1	9.5
Rx**	0.33	0.56	0.67	0.73	0.93	1.00	1.80	1.96	2.04	2.64	2.82	2.93	3.04	3.16
Rx*	0.09	0.15	0.17	0.19	0.24	0.26	0.47	0.51	0.53	0.69	0.74	0.77	0.80	0.83
Rf	0.08	0.14	0.17	0.19	0.24	0.25	0.46	0.49	0.52	0.67	0.71	0.74	0.77	0.80
mW	← over 66,000 daltons →					← 66,000–45,000 d →			← 45,000–24,000 d →				← 24,000– → 14,300 d	

Rx** Taking as reference bovine albumin
Rx* Taking as reference lysozyme

Tab. 4. Percentage and number of protein bands in function of mW in immature stages of *Bovicola limbatus*

	L 1	L 2	L 3	Nr. total bands
Nr. of bands with mW superior to 66,000 daltons	8 (80.00%)	7 (38.90%)	6 (42.80%)	21
Nr. of bands with mW understood amongst 66,000–45,000 d.	1 (10.00%)	5 (27.80%)	1 (7.14%)	7
Nr. of bands with mW understood amongst 45,000–24,000 d.	1 (10.00%)	4 (22.22%)	6 (42.80%)	11
Nr. of bands with mW understood amongst 24,000–14,300 d.	–	2 (11.11%)	1 (7.14%)	3
Total	10	18	14	42

Tab. 5. Arrangement of protein in the topoi during the development of *B. limbatus*

Topos	Male	Female	L 1	L 2	L 3
1		+	+		
2		+	+		
3	+		+		
4	+	+			+
5			+		
6		+		+	
7			+		
8	+	+	+	+	+
9		+		+	+
10	+		+	+	+
11			+	+	
12	+	+		+	
13			+	+	+
14		+		+	+
15	+			+	
16		+	+	+	
17	+				
18		+		+	
19	+	+		+	
20		+		+	
21					
22		+		+	+
23	+			+	
24	+	+			+
25	+		+	+	+
26	+	+		+	
27		+			
28					
29		+			+
30		+			
31					+
32		+			+
33	+	+			
34					+
35		+			
36	+				
37		+			+
38	+			+	
39					
40				+	

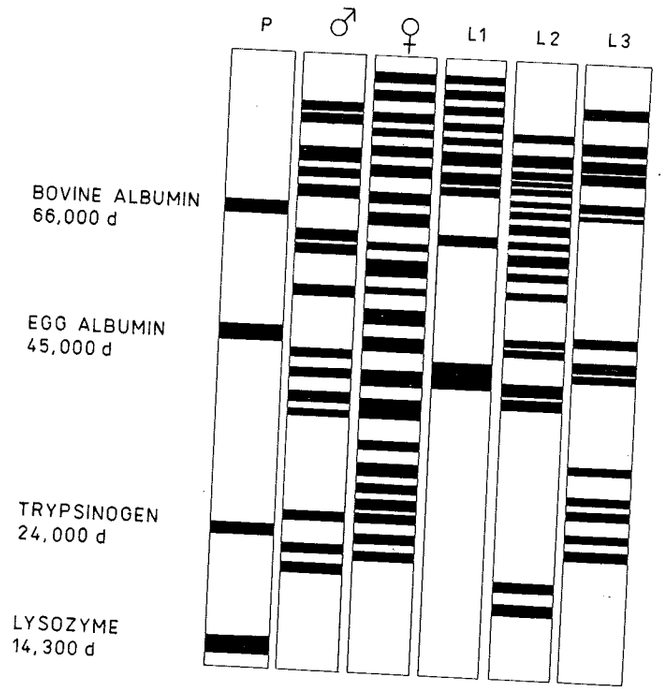

Fig. 1. Comparison of total proteins in adult and larval stages of *Bovicola limbatus*.

Tab. 5 shows the position of the proteic bands of the different topoi in L1, L2, L3, males and females of *B. limbatus*. It is remarkable that some of these bands appear in a determined stage occupying a determined topos during the ontogenesis and dissappear in another stage during the same biological process.

The topoi 8, 12 and 24 are occupied by a protein during the parasite development, as a reason for which it seems that these 3 proteins are very constant in this species.

The protein 8 for L1, 6 for L2, 5 for L3, 5 for the males and 7 for the females, that occupies topos 12, move 19 mm, 20 mm, 21 mm, 20 mm and 21 mm, respectively and could correspond to a protein called "insect albumin" by WHITTAKER & WEST (1962), which could be situated between 20 and 30 mm from the origin.

The topoi 3, 9, 10, 15 and 19 are all occupied by proteins in 3 specific stages of the development: 3 (male, L1, L3); 9 (females, L2, L3); 10 (male, L1, L2); 15 (male, L1, L2); 19 (male, female, L2). Moreover, the topoi 10 and 15 have a common protein during the same stage of development.

In this way it also could be possible to determine the proteins which are peculiar to a developmental stage. Therefore, topos 5 is occupied by a peculiar protein of stage L1; topoi 11, 39 and 40 by proteins of L2; topoi 28, 31 and 34 by proteins of L3; the topoi 16, 20, 27, 29, 30, 35 and 37 would be occupied by peculiar proteins of females and finally the male characteristic protein would occupy topos 38. The latter could be the "sex proteins" of this species (WARREN & BRELAND 1969).

The topoi 4, 26 and 33, only occupied by proteins in case of electrophoregrams of adults, would therefore contain only peculiar proteins of mature specimens. On the other hand, the topoi 7 (L1, L3); 13 (L2, L3); 21 (L2, L3) would be occupied by peculiar proteins of immature stages.

Finally, the topoi 1 (female, L1); 2 (female, L1); 6 (female, L2); 14 (female, L2); 18 (female, L2); 22 (female, L2); 32 (female, L3) have got the same protein for the immature stages and the

females of *B. limbatus,* while the same phenomenon is observed in topoi 17 (male, L2); 23 (male, L3); 25 (male, L2) and 36 (male, L3) with regard to the male of this species.

Zusammenfassung: Biochemische Studie über Bovicola limbatus. A) Bestimmung verschiedener Eiweißfraktionen bei unreifen Stadien. B) Eiweißveränderungen während der Ontogenese. – Es wurden die Proteinfraktionen und die Gesamt-Proteine der Larvenstadien sowie die Protein-Veränderungen während der Ontogenese untersucht. Das „Insekten-Albumin" sowie die „Sexualproteine" wurden ebenfalls determiniert. Die Autoren schlagen einen neuen Terminus vor, um die Protein-Veränderungen während der Entwicklung zu präzisieren: Topos. Das ist die eindeutige Position einer Protein-Bande während der elektrophoretischen Entwicklung, die mittels des mW bestimmt wird.

Резюме. Биохимическое изучение *Bovicola limbatus.* А) Определение белковых фракций у незрелых стадий. Б) Изменение белков во время онтогенеза. – Были исследованы белковые фракции и общий белок личиночных стадии, а также изменения белков во бремя онтогенеза. Кроме того были определены «инсект-альбумин» и «сексулиротены». Авторы предложили новое понятие – топос – для уточнения изменений белков во время развития Топос – это точная позиция белковой банды во время электрофоретического развития, определяемого с помощи мВ.

Summary. A study is carried out concerning electrophoretic development and determination of total proteins in immature stages of *Bovicola limbatus* as well as the proteic changes during its development. The so-called "insect albumin" and "sex-proteins" are also determined. In order to make the study of proteic changes during the biological development of a species easier, the authors introduce and propose a new "key-word": Topos, i.e. proper position of a certain proteic band in the electrophoretic development and which is determined by its mW.

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