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NATIVE AND DISSOCIATED PROTEIN PATTERNS OF LARVAL FOOD OF HONEY BEES *(APIS MELLIFERA CECROPIA L.)*

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SUMMARY

The changing patterns of honey bee larval food proteins were studied during larval life with polyacrylamide disc electrophoresis. When sodium dodecyl sulfate detergent was used the number of protein bands increased from 10 to 15 in worker jelly, from 8 to 10 in royal jelly and from 11 to 13 in drone jelly. Patterns of older worker and drone larval diet were more complex than those of queen larval diet. Comparing the results with a previous analysis, slight differences in protein patterns between two races of bees *(A. Mellifera cecropia* and *A. mellifera liqustica)* were found.

INTRODUCTION

Extensive knowledge on protein changes throughout larval development would be of great help in elucidating the processes which control the caste differentiation of honey bees.

The proteins of honey bee haemolymph have been examined extensively in the larval and adult stage (LIU and DIXON 1965, LENSKY 1967, GILLIAM and JACKSON 1972, BOUNIAS 1975). The enzymatic activity of haemolymph during caste development (TRIPATHI and DIXON 1968, 1969) and the protein patterns of hypopharyngeal gland (HALBERSTADT 1980) were also studied.

Comparative analyses of protein constituents of honey bee larval food showed some contradictory conceptions. HABOWSKY and SHUEL (1959) reported a pronounced fading of the protein bands in the diet of older worker larvae. PATEL *et al.* (1960) found that worker jelly of older larvae had less protein bands (spots)

than the diet of young larvae. On the other hand TOMODA *et al.* (1977) postulated that the same electrophoretic patterns were found in worker and royal jelly, while THRASYVOULOU (1982) found an increase in the number of protein bands of older worker larvae. Differences may be due to analytical techniques to the race of honey bees, to seasonal, hive or other variables.

In this study, the protein patterns of honey bee larval food collected from Greek colonies *(Apis mellifera cecropia)* were examined electrophoretically (native proteins). Subsequently the proteins were exposed to a disulfide bond reducing agent which yields individual polypeptide chains (dissociated proteins) and the new patterns were correlated to the age, caste, sex and race of the larvae receiving the food.

MATERIALS AND METHODS

Samples of honey bee larval food were collected in successive intervals over twenty developmental ages of worker, queen and drone larvae. The contents of twenty worker cells, twenty drone cells and a single queen cell were used for each sample. Appoximately 0.20 ml distilled water was thoroughly mixed with the larval food of the cells, drawn up into Pasteur pipette and transfered into vials. Samples were centrifuged for 10 minutes at 2 500 rpm (450 × G). The supernatant liquid was filtered through a 0.45 μ Millipore filter, brought to 6.0 ml with distilled water and stored in a freezer (— 20 ± 2 °C) until analyzed.

The larva of each cell was weighed and the age of each was estimated with the aid of equations for growth rates of honey bees (THRASYVOULOU and BENTON 1982).

Twenty five micrograms of protein was used for each electrophoretic gel. This amount was estimated spectrophotometrically as described by LOWRY *et al.* (1951) with lysozyme as a standard. A Canalco Model 24 polyacrylamide gel disc electrophoresis apparatus was used for separation. Power supply (Model 13014 A) was adjusted to 6 mA per electrophoretic tube.

Electrophoretic separations of native proteins were made as proposed by ORNSTEIN and DAVIES (1962). The lower or separating gel contained 7.5 % acrylamide. Beta-Alanine acetic acid buffer at a running pH 4.5 was used. Protein bands were stained with 0.04 % (W/V) Coomassie brilliant blue G-250 dye in 5 % perchloric acid as described by REISNER *et al.* (1975).

Electrophoretic separations of dissociated proteins were carried out as described by WEBER and OSBORN (1975) using 0.5 M sodium phosphate dibasic, at pH 7.0 as buffer. The lower gel was 10 % acrylamide. Each sample consisted of larval food protein, glycerol, 0.1 % bromophenol blue in buffer, and 10 % sodium dodecyl sulfate (SDS) with 1 % 2-mercaptoethanol in a ratio 7 : 1 : 1 : 1. Samples were heated in a boiling water bath for 2-5 minutes. Following separation, protein bands were stained for one hour with a solution consisting of 250 mg Coomassie brilliant blue G-250, 45 ml distilled water, 45 ml methanol and 9.2 ml glacial acetic acid.

Mobilities of polypeptide chains were plotted versus the known molecular weights of phosphorylase (100,000 M.W.), catalase (58,000 M.W), carbonic anhydrase (29,000 M.W.), myoglobin (17,200 M.W.) and lysozyme (14,300 M.W.) standards on semilogarithmic paper. The standard curve was used to estimate molecular weights of polypeptide chains from larval diets.

The samples where collected from the Aristotle University apiary during the summer of 1982 and were analyzed in the Pesticide Research Laboratory of Pennsylvania State University.

RESULTS AND DISCUSSION

Native proteins. Figure 1 gives the position and Rf-values of protein bands that characterize the diet of honey bee larvae. A code number (1-11) was assigned to each band according to its mobility. Bands with the number 1, 2, 3, 5, 6, 7, 8, were sharp and well stained by Coomassie blue dye, while bands with the number 4, 9, 10 and 11 were very faint. Only those bands that were clearly shown on the gels were recorded. Number 5 and 6 had almost equal mobility. Generally, these two bands were well separated, but in a few cases they appeared as one. The same was true with the bands numbered 7 and 8.

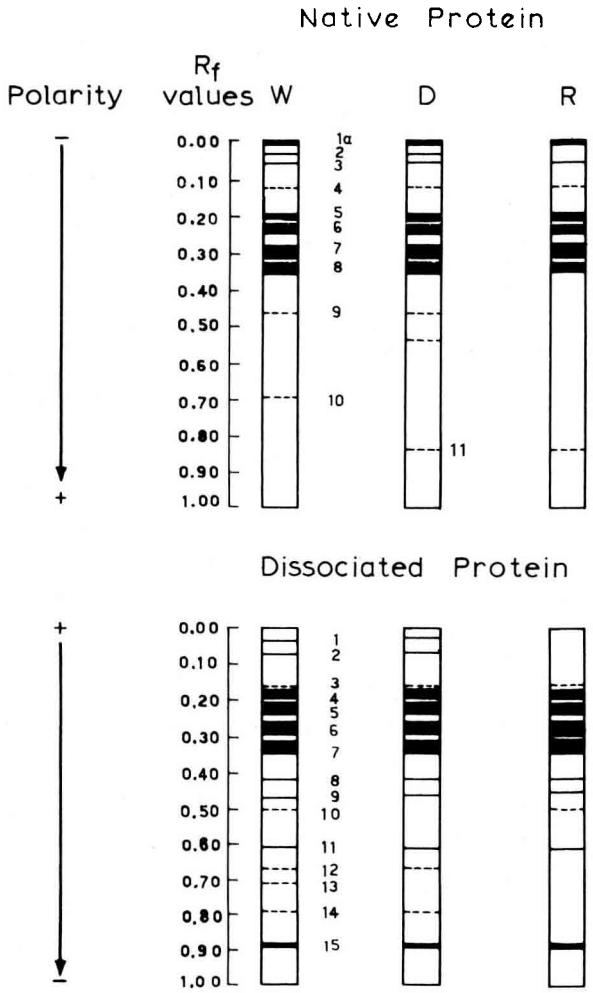

FIG. 1. — *Native and dissociated water soluble protein patterns of honey bee larval food.* W = worker jelly; D = drone jelly; R = royal jelly; α = number of protein bands.

TABL. 1. — *Electrophoretic protein bands of native proteins from larval food their Rf-values and their percent occurence in 60 samples.*

Protein Band No.	Rf value[a]	% occurrence of protein bands by age of larval development								
		6-37 hr[b]			38-73 hr[b]			74-130 hr[b]		
		W	R	D	W	R	D	W	R	D
1	0.01 ± 0.000	100	100	100	100	100	100	100	100	100
2	0.03 ± 0.009	0	—	0	40	—	20	100	—	100
3	0.07 ± 0.013	100	100	100	100	100	100	100	100	100
4	0.13 ± 0.009	100	100	100	100	100	100	100	100	100
5	0.22 ± 0.008	100	100	100	100	100	100	100	100	100
6	0.26 ± 0.023	100	100	100	100	100	100	100	100	100
7	0.34 ± 0.037	100	100	100	100	100	100	100	100	100
8	0.37 ± 0.037	100	100	100	100	100	100	100	100	100
9	0.65 ± 0.041	15	—	20	20	—	20	60	—	60
10	0.73 ± 0.069	15	—	0	20	—	0	60	10	80
11	0.86 ± 0.042	—	—	0	—	—	10	—	—	30

a. Means and standard error (p = 0.05) of twenty samples (each sample was replicated three times).
b. Percentage was based on twenty samples from each developmental period.
W = worker jelly, R = royal jelly, D = drone jelly.

Table 1 shows the Rf-values and their percentage present for each development stage. The whole period of larval development was grouped into three shorter periods for easier presentation of the results.

During the first 37 hr of larval age, seven protein bands (1, 3, 4, 5, 6, 7) were found in all the samples of worker, royal and drone jelly. The percentage occurence of the rest of the bands was either zero (2, 11) or very small (9,10). Between 38-73 hr of larval development, the number of protein bands increased in worker and drone jelly. During this period, band 2 appeared in 40 % of the worker jelly and in 20 % of drone jelly samples. The earliest this band appeared was 65 hr into worker and 71 hr into drone larval development. Later band 2 was found consistently in all the samples and band 3 became sharp and more distinct. After 74 hr worker and drone larvae are fed with a diet that showed more protein bands than before. Band 2 was found in all the samples, while the frequency in occurence of bands 9, 10 and 11 was increased. The number of protein bands remained unchanged in royal jelly during the entire feeding period.

THRASYVOULOU (1982) reported the protein patterns of honey bee larval food collected from Italian colonies. Comparing the patterns of the two races of bees *(cecropia* and *ligustica)* the following similarities were noted. In both races there was (a) an increase in the number of protein bands of worker and drone jelly as larvae developed (b) band 2 appeared only in the diet of older larvae; (c) band 3 became sharp in the diet of older larvae; (d) the number of bands in royal jelly remained

unchanged during the whole developmental period. Larval food collected from Italian bees had three consistent protein bands while larval food collected from Greek bees had seven. This was so because bands with close mobilities (5,6 and 7,8) considered as subunits were reported as single bands in Italian bees. Yet, bands numbered 3 and 4 were found in all samples of Greek bees but not in all the samples of Italian bees.

Dissociate proteins. Fifteen polypeptide chains were found in the diet of worker larvae, thirteen in the diet of drone larvae and ten in the diet of queen larvae. Their position and Rf-values are given in Figure 1. Bands numbered 2, 4, 5, 6, 7, 8, 9, 11 and 15 were well separated and stained while bands 3, 10, 12, 13 and 14 were faint. Table 2 shows the Rf-values, molecular weights and occurence of polypeptide chains. As was the case with native proteins, some bands were present in all samples of worker, drone and queen larvae (4, 5, 6, 7, 8, 9, 11). A few bands were found only in the diets of older worker and drone larvae (1 and 2) and some

TABL. 2. — *Electrophoretic protein bands of dissociated proteins from larval food their Rf-values, molecular weight, their percent occurence in 60 samples.*

Protein Band No.	Rf values[a]	Approximate[b] Molecular Wieght × 10^4	% occurrence of protein bands by age of larval development								
			6-37 hr[c]			38-73 hr[c]			74-130 hr[c]		
			W	R	D	W	R	D	W	R	D
1	0.05 ± 0.011	10.00	0	—	0	20	—	10	100	—	80
2	0.15 ± 0.070	10.00	0	—	0	30	—	10	60	—	80
3	0.18 ± 0.040	8.45	40	60	0	45	50	10	100	50	100
4	0.24 ± 0.033	7.67	100	100	100	100	100	100	100	100	100
5	0.29 ± 0.035	6.83	100	100	100	100	100	100	100	100	100
6	0.35 ± 0.035	6.05	100	100	100	100	100	100	100	100	100
7	0.40 ± 0.052	5.34	100	100	100	100	100	100	100	100	100
8	0.44 ± 0.059	4.78	100	100	100	100	100	100	100	100	100
9	0.51 ± 0.052	3.83	100	100	100	100	100	100	100	100	100
10	0.57 ± 0.017	3.19	40	20	—	40	20	—	50	50	—
11	0.62 ± 0.151	2.36	100	100	100	100	100	100	100	100	100
12	0.68 ± 0.083	1.48	20	—	—	60	—	—	80	—	—
13	0.75 ± 0.057	1.40	10	—	10	30	—	60	30	—	75
14	0.86 ± 0.059	1.40	80	—	50	60	—	60	80	—	90
15	0.92 ± 0.059	1.40	100	100	10	100	100	60	100	100	100

a. Means ans standard error (p = 0.05 %) of twenty samples (each sample was replicated three times).
b. Based on molecular weights of standard protein plot.
c. Percentage was based on twenty samples from each developmental period. W = worker jelly, R = royal jelly, D = drone jelly.

other bands were not consistent, but their occurence increased with the age of worker and drone larvae (3, 13, 14).

The protein patterns of the native system were compared to that of the dissociate system. The seven consistent native protein bands increased to eight when dissociated. There was no increase in the two bands that appeared in the diet of older worker and drone larvae (including band number 3-sharp), and the inconsistent protein bands increased from two to five. These results suggest that the majority of protein fractions of larval food of honey bees may consist of single polypeptides.

The external nutritional source may influence to a great extent the haemolymph composition of larvae (CHEN, 1966). In many insects, the ingested protein must first be catabolized into amino acids before being absorbed (HOUSE, 1974). TSAO and SHUEL (1968) showed that royal jelly proteins pass through the midgut as a mixture of polypeptides and a minor proportion as amino acids. They also found that haemolymph of worker larvae at 72 ht had more protein bands than at 60 hr. Yet they indicated that throughout larval life, queen larval had fewer haemolymph protein fractions than worker larvae. It seems that there is a relationship between the number of protein bands in larval food and the protein bands of haemolymph of worker larvae at 72 ht had more protein bands than at 60 hr. Yet complex as development of worker larvae proceeds. The diet of worker larvae had more protein bands than the diet of queen larvae.

The increase in number of protein bands in worker and drone jelly is followed by an increase in enzymatic activity in the diet (THRASYVOULOU, 1982). Enzymatic activity remains the same in queen larvae. Some of the additional bands in the diet of older worker or drone larvae may functio as enzymes. A further study of larval food proteins would be useful to understand both their morphogenetic meaning and their potential role in caste differentiation.

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RÉSUMÉ

SPECTRE DES PROTÉINES GLOBALES ET DISSOCIÉES DE LA NOURRITURE LARVAIRE DES ABEILLES (*APIS MELLIFICA CECROPIA* L.)

Les spectres protéiques de la nourriture larvaire des abeilles prélevée dans des colonies grecques *(Apis mellifica cecropia)* ont été corrélés avec l'âge, la caste, le sexe et la race des larves qui recevaient la nourriture.

Chaque échantillon comportait la nourriture larvaire de 20 cellules d'ouvrières, 20 cellules de mâle et une cellule royale. On a prélevé au total 60 échantillons au cours des stades successifs de développement des larves d'ouvrières, de mâle et de reine. Le prélèvement et le traitement des échantillons avant l'analyse ont été faits selon THRASYVOULOU (1982). On a pesé la larve de chaque cellule et estimé leur âge à l'aide des équations de taux de croissance des abeilles (THRASYVOULOU et BENTON, 1982). Les protéines totales de chaque échantillon ont été déterminées par la méthode de LOWRY *et al.* (1951) et pour chaque gel d'électrophorèse on a utilisé 25 µg de protéines. La séparation des protéines globales a été faite par électrophorèse sur disque de polyacrylamide (ORNSTEIN et DAVIES, 1962). Le gel inférieur renfermait 7,5 % d'acrylamide. L'acide acétique bêta-alanine a été utilisé comme tampon au *p*H 4,5 et les bandes ont été colorées selon la méthode décrite par REISNER *et al.* (1975). Les séparations électrophorétiques des protéines dissociées ont été faites selon la méthode décrite par WEBER et OSBORN (1975) en utilisant comme tampon au *p*H 7 le phosphate de sodium dibasique à 0,5 M et comme détergent le sulfate dodécyl de sodium (SDS).

Les larves d'ouvrières et de mâles reçoivent à partir de 74 heures une nourriture qui possède un nombre croissant de bandes protéiniques. Durant toute la période de nourrissement, les larves de reine reçoivent une nourriture dont le spectre protéique reste constant. Ces résultats concordent avec ceux obtenus à partir de la nourriture larvaire prélevée dans des colonies italiennes (THRASYVOULOU, 1982).

Lorsqu'on a utilisé le SDS, le nombre de bandes protéiniques est passé de 10 à 15 pour la gelée d'ouvrières, de 8 à 10 pour la gelée royale et de 11 à 13 pour la gelée de mâles. Ceci laisse à penser que la majorité des fractions protéiniques de la nourriture larvaire des abeilles consiste en polypeptides uniques.

ZUSAMMENFASSUNG

PROTEINMUSTER VON NATÜRLICHEN UND AUFGETRENNTEN PROTEINEN IM LARVENFUTTER DER HONIGBIENEN (*APIS MELLIFERA CECROPIA* L.)

Die Proteinmuster des aus griechischen Bienenvölkern *(Apis mellifera cecropia)* entnommenen Larvenfutters korrelieren mit dem Alter, der Kaste, dem Geschlecht und der Rasse der gefütterten Larve.

Das Larvenfutter von zwanzig Arbeiterinnenzellen, zwanzig Drohnenzellen oder einer Weiselzelle bildete eine Probe. Insgesamt wurden 60 Proben genommen in der Reihenfolge des Entwicklungsstadiums von Arbeiterinnen, Drohnen und Königinnen. Die Sammlung und Behandlung der Proben vor der Analyse erfolgte gemäß THRASYVOULOU (1982). Die Larve aus der jeweiligen Zelle wurde gewogen und ihr Alter gemäß der Gleichung der Wachstumsrate (THRASYVOULOU and BENTON 1982) bestimmt. Das Gesamtprotein jeder Probe wurde nach der Methode von LOWRY *et al.* (1951) bestimmt und 25 µg des Proteins zur Elektrophorese verwandt. Die Auftrennung des natürlichen Proteins erfolgte mit Polyacrylamid Disc Elektrophorese (DAVIS 1962). Das untere Gel enthielt 7,5 % Acrylamid. Beta-Alanin Essigsäure wurde als Puffer bei *p*H 4,5 verwendet und die Banden, wie bei REISNER *et al.* (1975) beschrieben, gefärbt. Die elektrophoretische Auftrennung der zerlegten Proteine erfolgte nach WEBER und OSBORN (1975) unter Verwendung von 0,5 M di-Natriumhydrogenphosphat bei *p*H 7.00 und Dodecylhydrogensulfat Natriumsalz (SDS) als Detergenz.

Arbeiterinnen- und Drohnenlarven werden mit einem Futter gefüttert, das bei 74 h alten Larven eine erhöhte Anzahl von Proteinbanden zeigt. Das Futter der Königinnenlarven zeigt ein unverändertes Proteinmuster während der gesamten Fütterungsperiode. Diese Ergebnisse entsprechen denen, die bei der Untersuchung des Larvenfutters von italienischen Völkern (THRASYVOULOU 1982) erzielt wurden.

Wenn SDS benutzt wurde, erhöhte sich die Anzahl der Proteinbanden von 10 auf 15 bei Arbeiterinnenfutter, von 8 auf 10 bei Weiselfutter und von 11 auf 13 bei Drohnenfutter. Dies weist darauf hin, daß die Mehrzahl der Proteinfraktionen des Larvenfutters von Honigbienen aus einfachen Polypeptiden besteht.

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