

## **An electrophoretic study of protein patterns in the fat body and haemolymph of the solitary form of the desert locust *Schistocerca gregaria* (Foraskal).**

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**Abstract:** The polyacrylamide gel electrophoresis (PAGE) was performed on the fat body and haemolymph of the 5<sup>th</sup> nymph instar as well as three different phases of adult males and females namely; newly emerged, yellow and non yellow males (mature and non-mature), before and after oviposition females of the desert locust *Schistocerca gregaria*. The total number of protein bands detected in the fat body and haemolymph were 44, 37 respectively. The concentration percentage of these protein bands, the timing of band appearance and disappearance, the sex-specific proteins and the major protein bands for the fat body and haemolymph were detected. The protein metabolism was studied and discussed by observing and comparing the different protein patterns of the fat body tissues and the haemolymph fluid of the desert locust.

**Key words:** *Schistocerca gregaria*, Electrophoretic study, Fat body, Haemolymph, Solitary form.

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### **I. Introduction**

Locust swarms cause great damage to grassland, agriculture crops and various other plants. This damage in the invasion territories of the desert locust, *Sch. gregaria* (Orthoptera :Acrididae) is not only confined to Africa, India and Asia Minor, but also affected other areas of the earth infested by other species of locusts. The desert locust is a significant pest in its solitarious phase, in the recession areas, but gregarious phase becomes the major pest when forming swarms stimulated by the rain and improved vegetation (Notle, 1974). The biochemical composition of haemolymph is highly variable among the insect species at different developmental stages (Florkin and Jeuniaux 1974, Thomath and Nair 2011). Since metabolic activities have been reported to be different at different developmental stages in an insect, the fluctuation in the metabolites could indicate rhythmic changes that occur in the biochemistry of insect haemolymph. This could be related to the rhythm of insecticide susceptibility occurring in insects and hence may be of importance in the study of the mode of action of insecticides on the mechanism of resistance of this pest. The total quantity of protein in the blood varies in the course of development. These proteins are usually classified as storage proteins, lipid transport proteins, vitellogenesis, enzymes, proteinase inhibitors, chromo proteins and a range of different proteins that are involved in various immune responses in insects.

Most proteins in an insect body, have been reported to be synthesized in the fat body released into the haemolymph. Locust fat body sustains and assists the formation of the giant locust swarms with perfect flight performance synthesizes, stores and mobilizes energy-rich substrate that are used to overcome periods of starvation to power flight and /or to fuel egg production (Lorenz 2009). Investigation the protein patterns of the fat body of hoppers and adults may help in understanding the biochemical and physiological changes occurred in the solitary form of *Sch. gregaria* as well as opening new lines in research concerning the locust phase polymorphism.

### **II. Materials and methods**

#### **2.1. Insect culture:**

The stock colony of *Sch. gregaria* was maintained in the entomology laboratory, Faculty of Science, Cairo University according to Ibrahim (1971). The samples were taken from the haemolymph and fat body of the 5<sup>th</sup> nymph instar and of male and female adults at three different phases i.e., newly emerged, before oviposition and after oviposition females, yellow and non-yellow males (mature and non-mature). The tissue samples were weighed, homogenized and then centrifuged at 6000r.p.m. at 4°C. The supernatant was diluted 1:1 (vol :vol) with 5% sucrose solution in preparation of electrophoretic techniques.

#### **2.2. Electrophoretic technique:**

Electrophoresis was done on 1.5 mm vertical slab polyacrylamid gel (Sturdiel slap gel electrophoresis unite SE 400) at 60 mA. until the tracking dye (bromophenol blue) migrate to the end of the gel. The running gel

(6%) was made with 1.5 M tris buffer (pH 8.8), The stacking gel (4%) was made with 0.5 M tris buffer (Turunen and Chippendale 1977) diluted to the ratio 1:3 (V/V) with double distilled water. The temperature of the gel was maintained at 7°C by refrigerating the gel chamber during run. The supernatant used for direct analysis was diluted 1:1 (V/V) with 5% sucrose solution. 20µl of the diluted supernatant was loaded in each lane. The general protein bands was demonstrated by incubating the gel for one hour in stain solution (0.125% Coomassie Brilliant blue COOB, in 50% ethanol and 10% acetic acid) and destained the same solvent for maximum constant. Densitometric scanning was made to estimate the relative concentration of the identified bands using Epson GT 8000 scanner at 57-nm wavelength.

### 2.3. Relative mobility:

The total length of the separating gel, the distance travelled by the marker dye in the separating gel and the various distances migrated by the different protein fractions were measured. The relative mobility/relative fraction values of each band was calculated as follows;

$$\text{Relative mobility (Rm)} = \frac{\text{Distance travelled by the protein fraction}}{\text{Distance travelled by the marker dye}}$$

According to Abd – Elsamie and Aly (2012), bands that had a relative concentrations of 15% or over were considered of relatively high concentration (RHC), while that of 5% or below were considered of relatively low concentrations (RLC). The values between the two extremes (over 5% and below 15%) represent moderate concentration (RMC).

## III. Results and Discussion

Protein patterns of fat body and haemolymph of *Sch. gregaria* show both qualitative and quantitative changes during the nymph-adult transformation. A total of 37 and 26 bands with different Rm values were detected in the fat body and the haemolymph respectively.

### 3.1 The 5<sup>th</sup> nymph instar fat body:

Table(1) and Fig. (1) show that the fat body tissues of the 5<sup>th</sup> nymph instar contained 25 bands. These bands were classified into two main groups, bands No.1, 3, 5-8, 11-13&15 have RMC. And bands No. 16-22, 25, 26, 28-30, 32, 33&37 have RLC.

### 3.2. Adult males fat body (N<sub>1</sub>, B<sub>1</sub>, A<sub>1</sub>):

Table(1) and Fig. (1) demonstrate that the fat body tissues of N<sub>1</sub>, B<sub>1</sub> & A<sub>1</sub> male instars, amounted to 22, 19 & 24 bands respectively, Similarly to the nymph instar, the newly emerged males (N<sub>1</sub>) reveal that 10 bands (No. 1, 3, 5, 7, 8, 11, 13, 15, 16 & 37) have RMC. 12 bands (No. 18-23, 28-31, 34 & 36) have RLC. Meanwhile, 7 bands (No. 6, 12, 17, 25, 26, 32 & 33) disappeared & 4 bands appeared (23, 31, 34 & 36) as compared to nymph instar.

In case of B<sub>1</sub> individuals, 13 bands (1-4, 6-8, 10, 11, 13, 15, 17 & 37) show RMC whereas, only 6 bands (22, 28-30, 32 & 34) show RLC. Five bands appeared (No. 2, 4 & 5) and seven disappeared (No. 5, 16, 18-21 & 36) as compared to N<sub>1</sub> individuals.

For A<sub>1</sub> individuals, 12 bands (No. 1, 2, 4, 5, 7-9, 11-13, 15 & 37) show RMC while, 12 bands (No. 17, 18, 21-23, 26, 28-30, 32, 34 & 36) show RLC. Bands No. 3, 6, 10, 21 & 35) disappeared while bands No. 5, 9, 12, 18 & 26 appeared. as compared to N<sub>1</sub> group. Generally, the concentration of the last protein band (37) was duplicated in all male instars as compared to the nymph instar. Also protein band No. 2 was detected only in the fat body tissues of locust males so it is considered as a male fat body specific protein.

### 3.3. Adult female fat body (N<sub>2</sub>, B<sub>2</sub>, A<sub>2</sub>):

Table(1) and Fig. (1) show that the fat body tissues of N<sub>2</sub>, B<sub>2</sub> & A<sub>2</sub> female instars, contained 27, 21 & 30 protein bands respectively. Generally, protein concentrations in the fat body of N<sub>2</sub> and B<sub>2</sub> individuals have the same trend and similar to the males of the same developmental instars. However, three new bands (5, 9 & 32) appeared in B<sub>2</sub> and 9 bands disappeared (12, 19, 20, 22, 24, 26, 31, 33 & 34) as compared to N<sub>2</sub>. In case of A<sub>2</sub> individuals, the protein concentrations of fat body obviously fluctuated along the A<sub>2</sub> lane run. Also a higher number of bands appeared (9- bands) compared to those disappeared (3-bands).

Four protein bands number 24, 25, 27 & 35 were detected only in the female instar therefore, they considered as a female fat body specific protein. In addition, 10 protein bands (No. 1, 7, 8, 11, 13, 15, 28-30) were detected in the fat body tissues of all investigated phases (5<sup>th</sup> nymph instar as well as adult male and female instars) so, they considered as a major fat body protein bands in *Sch. gregaria*.

### **3.4. The 5<sup>th</sup>. nymph instar haemolymph:**

Table (2) and Fig. (2) demonstrate that the haemolymph fluid in the 5<sup>th</sup>. nymph instar contained 20 bands most of them were of RLC except 3 bands No. 3 with RMC, and No.13 & 22 with RHC. In addition, 2 bands (No. 1&7) were detected only in the 5<sup>th</sup>. nymph instar.

### **3.5 Adult males haemolymph(N<sub>1</sub>,B<sub>1</sub>A<sub>1</sub>):**

Table (2) and fig.(2) show that the native protein bands of the haemolymph of N<sub>1</sub>,B<sub>1</sub>&A<sub>1</sub> male instars, were 16, 14&19 bands respectively. For N<sub>1</sub> individuals, bands No. 2,3,5,9,12 &13 have RMC. Bands No. 15,16,18-21,23 &24 have RLC. However, 2 bands (No.13&22) have RHC. Five bands (No. 1, 6, 7, 19 &17) were disappeared and only one band (No. 19) was appeared as compared to the nymph instar.

For B<sub>1</sub> individuals, 8 bands(2, 3, 5, 8, 9, 11, 12 & 22) have RMC. While Five bands (No.15, 20, 23 - 25) have RLC. Two new bands were appeared (8 &25) whereas, 4 bands were disappeared as compared to N<sub>1</sub>.

For A<sub>1</sub> individuals, 13 bands (No.3, 4, 6, 8, 11, 12, 14,15,17, 21, 24-26) have RLC. Four bands (No.5, 9, 20 &23) have RML and only 2 bands (No. 13 &22) have RHC. Six bands (No. 4, 6, 14, 17, 21 &26) were appeared whereas only one band ( No. 2 ) was disappeared as compared to B<sub>1</sub>.

Also 3 bands (No. 18, 19 &26) were detected only in the haemolymph of the male instars.

### **3.6. Adult females haemolymph(N<sub>1</sub>,B<sub>1</sub>A<sub>1</sub>):**

Table (2) and Fig. (2) show that the haemolymph fluid of N<sub>2</sub>,B<sub>2</sub>&A<sub>2</sub> female instars, contained 18, 14 & 13 protein bands respectively.

Protein concentrations of the haemolymph of N<sub>2</sub> revealed that 12 bands (No. 3, 4, 6, 8, 12, 14, 16, 17, 21 &23-25) have RLC. Four bands (No.5, 9, 11 & 20) have RMC and only 2 bands (No. 13 &21) have RHC. Seven bands (No. 3, 4, 7, 10, 15, 18 &25) were disappeared and only 3 bands (No. 4, 8 &14) were appeared as compared to the nymph instar.

In case of B<sub>2</sub> individuals, 5 bands (No. 2, 6, 14, 20 & 24) have RLC. Eight bands (No. 3, 5, 8, 9, 11, 12, 15 &22) have RMC. And only one band (No. 13) has RHC. Six bands (No. 4, 16, 17, 21, 23 &25) were disappeared and two bands (No. 2 &15) were appeared as compared to N<sub>2</sub> group.

In case of A<sub>2</sub>, 8 bands (No. 3, 5, 6, 9, 12-15) have RMC. Four bands ( No. 10, 20, 22, &25) have RLC. And only one band ( No. 11) has RHC. Three bands (No. 2, 8 &24) were disappeared and only one band (25) was appeared as compared to B<sub>2</sub>.

In addition, one protein band (No. 10) was only detected in the female instars, so it is considered as a female haemolymph specific protein. Also, 7 protein bands( No.5, 9, 11-13, 20 &22) were detected in the haemolymph of all investigated locust phases therefore, they considered as a major haemolymph protein bands in *Sch.gregaria*.

Odhiambo (1967), suggested that little synthetic machinery takes place in the mature fat body of the male *Sch. gregaria* at the time the locust attain sexual maturity and is maintained throughout the remaining life. This scanty development of locust fat body in the mature insect may be related to the low feeding activity evident at that time, implying an inadequate circulation of nutrients in the haemolymph. Also he found that there was a rapid increase in total lipids after adult emergence and during early development of adult male reaching a peak at sexual maturity; thereafter there was no increase in lipid content and there was a gradual depletion from the chief energy reserves for locomotion.

Lorenz (2009) and Hill et al.(1968), mentioned that, during the first 10 days of adult life the dry weight of the female desert locust increases by 112 percent. This increase is strongly due to the somatic growth as well as an increase in the content of the haemolymph. During this period of somatic growth the native protein as well as conjugated proteins (glycoprotein, lipoprotein & glycolipoprotein ) accumulate in the fat body and haemolymph. The ovarian growth only begins towards the end of the somatic growth.

The results obtained in the course of our study are in agreement with these findings since, the concentration percentages as well as the total number of protein bands of the fat body of either males or females reach a peak at the mature stage.

McCormick and Scott (1966) reported that a total of 19 distinct haemolymph protein fractions has been demonstrated during the developmental stages of *Locust migratoria*. On the whole the band pattern is fairly constant throughout the locust's development until the locust become sexually mature.

This is also hold true for the haemolymph of mature males (19 bands) however, in case of mature females these figure decreased to 13 band.

This is may be due to the beginning of vitellogenesis and oogenesis.

Our results are in accordance with the findings of Orabi and Ali (1999), who reported that the decline occurred in the protein bands of fat body and haemolymph of the onion fly of the last larval instar and the newly emerged males were due to the histolysis of nymph tissues and histogenesis of newly emerged adult tissues.

Elliott and Gillott (1979) working on the migratory grasshopper *M.sanguinipes* found that 12 protein bands were detected in the fat body of which 7 had relative mobilities identical to those detected in the ovary. Similarly, 8 of 16 protein bands detected in the haemolymph of the vitellogenic females had electrophoretically identical counterparts in the ovary. The authors suggested that most yolk proteins are synthesized in the fat body and accumulate in the haemolymph.

These findings may explain the presence of female specific proteins in the fat body and haemolymph of *Sch. gregaria*.

Thomas and Nair (2011) reported that the variation of protein fractions during the different developmental stages of *R.ferrugineus* haemolymph apparently indicates both the synthesis and breakdown of the specific proteins. Also, Abd-ElSamie and Aly (2012) mentioned that the esterase isozymes detected in the fat body of the desert locust are involved in the catabolism of juvenile hormone, pesticides and other compounds, resulting directly in both the synthesis and breakdown of specific proteins.

**Table(1):Comparative analysis of relative mobility (Rm) and % of the protein bands density detected in fat bodies of nymph and adult males and females of *Sch. gregaria***

and No.	Rm value	% Protein bands						
		Nymph	Male			Female		
			N <sub>1</sub>	B <sub>1</sub>	A <sub>1</sub>	N <sub>2</sub>	B <sub>2</sub>	A <sub>2</sub>
1	0.030	6	7	10	10	6	6	6
2	0.048	-	-	7	7	-	-	-
3	0.056	5	6	6	-	5	5	6
4	0.074	-	-	7	7	6	6	-
5	0.087	5	7	-	7	-	5	-
6	0.11	5	-	5	-	5	6	4
7	0.14	5	6	8	6	5	5	4
8	0.18	6	7	7	6	6	5	5
9	0.20	-	-	-	5	-	6	4
10	0.21	-	-	5	-	5	6	-
11	0.23	6	7	5	5	6	8	5
12	0.27	6	-	-	5	6	-	-
13	0.30	6	6	5	6	6	7	4
14	0.33	-	-	-	-	-	-	3
15	0.35	5	5	5	5	5	6	4
16	0.39	4	5	-	-	-	-	3
17	0.41	4	-	5	4	4	4	3
18	0.44	4	4	-	4	3	4	3
19	0.46	3	3	-	-	2	-	3
20	0.48	3	3	-	-	2	-	-
21	0.50	3	3	-	3	2	3	3
22	0.53	3	3	4	3	3	-	3
23	0.55	-	3	-	3	-	-	2
24	0.59	-	-	-	-	3	-	2
25	0.62	2	-	-	-	2	2	2
26	0.63	2	-	-	2	2	-	2
27	0.65	-	-	-	-	-	-	3
28	0.66	3	3	3	2	2	3	2
29	0.69	2	3	3	2	2	2	3
30	0.72	4	4	3	3	2	3	4
31	0.77	-	3	-	-	2	-	2
32	0.81	2	-	3	2	-	2	-
33	0.84	2	-	-	-	2	-	3
34	0.89	-	3	3	3	1	-	2
35	0.91	-	-	-	-	-	-	2
36	0.94	-	3	-	3	-	-	3
37	0.97	3	6	6	6	5	6	5
Total		25	22	19	24	27	21	30

**Rm : relative mobility.**

**N<sub>1</sub>: newly emerged males .N<sub>2</sub> :newly emerged Females .**

**B<sub>1</sub>: males before maturation.B<sub>2</sub> :females before oviposition.**

**A<sub>1</sub>: males aftermaturation. A<sub>2</sub>:females after oviposition.**

Table(2): Comparative analysis of relative mobility (Rm) and % of the protein bands density detected in the haemolymph of nymph and adult males and females of *Sch. gregaria*

and No.	Rm value	% Protein bands						
		nymph	Male			Female		
			N <sub>1</sub>	B <sub>1</sub>	A <sub>1</sub>	N <sub>2</sub>	B <sub>2</sub>	A <sub>2</sub>
1	0.05	3	-	-	-	-	-	-
2	0.06	4	5	3	-	-	2	-
3	0.11	8	6	8	4	1	6	6
4	0.12	-	-	-	1	1	-	-
5	0.13	4	7	10	6	6	6	6
6	0.15	2	-	-	1	3	2	7
7	0.16	1	-	-	-	-	-	-
8	0.18	-	-	11	3	3	5	-
9	0.20	3	6	10	7	5	6	8
10	0.23	1	-	-	-	-	-	3
11	0.26	2	6	13	4	7	11	19
12	0.31	3	5	8	3	3	13	8
13	0.40	30	22	18	21	16	21	14
14	0.46	-	-	-	2	3	3	13
15	0.52	3	4	3	2	-	6	9
16	0.54	3	2	-	-	2	-	-
17	0.58	2	-	-	3	3	-	-
18	0.68	1	3	-	-	-	-	-
19	0.70	-	3	-	-	-	-	-
20	0.76	4	4	2	6	14	5	1
21	0.85	2	3	-	2	3	-	-
22	0.87	19	16	8	18	18	11	4
23	0.89	3	4	2	9	4	-	-
24	0.90	2	4	2	4	4	3	-
25	0.92	-	-	2	2	4	-	2
26	0.94	-	-	-	2	-	-	-
Total	0.65	20	16	14	19	18	14	13

Rm : relative mobility.

N<sub>1</sub> :newly emerged males. N<sub>2</sub> :newly emerged Females .

B<sub>1</sub> : males before maturation.

B<sub>2</sub> :females before oviposition.

A<sub>1</sub> :males after maturation.

A<sub>2</sub> :females After oviposition

Nymph N<sub>1</sub>B<sub>1</sub>A<sub>1</sub> N<sub>2</sub>B<sub>2</sub>A<sub>2</sub>Nymph N<sub>1</sub>B<sub>1</sub>A<sub>1</sub>N<sub>2</sub>B<sub>2</sub>A<sub>2</sub>

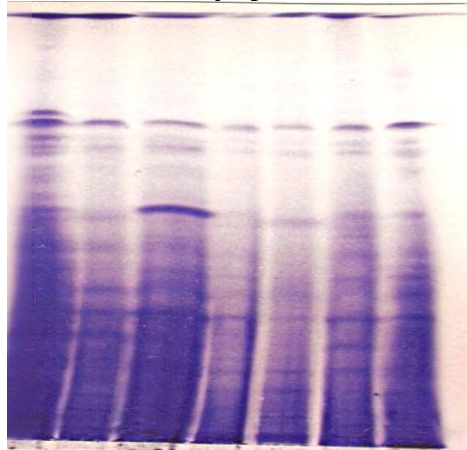


Fig. (1) :Electropherogram of native protein patterns detected in the fat body tissues of nymph and adult males and females of

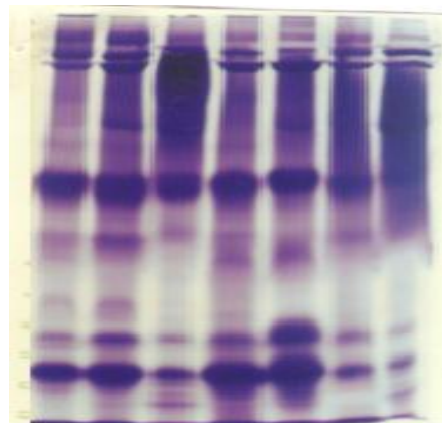


Fig.(2): Electropherogram of native protein patterns detected in the haemolymph of nymph and adult males and females

Sch. gregaria.of Sch. gregaria  
List of abbreviations as in table (1).

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