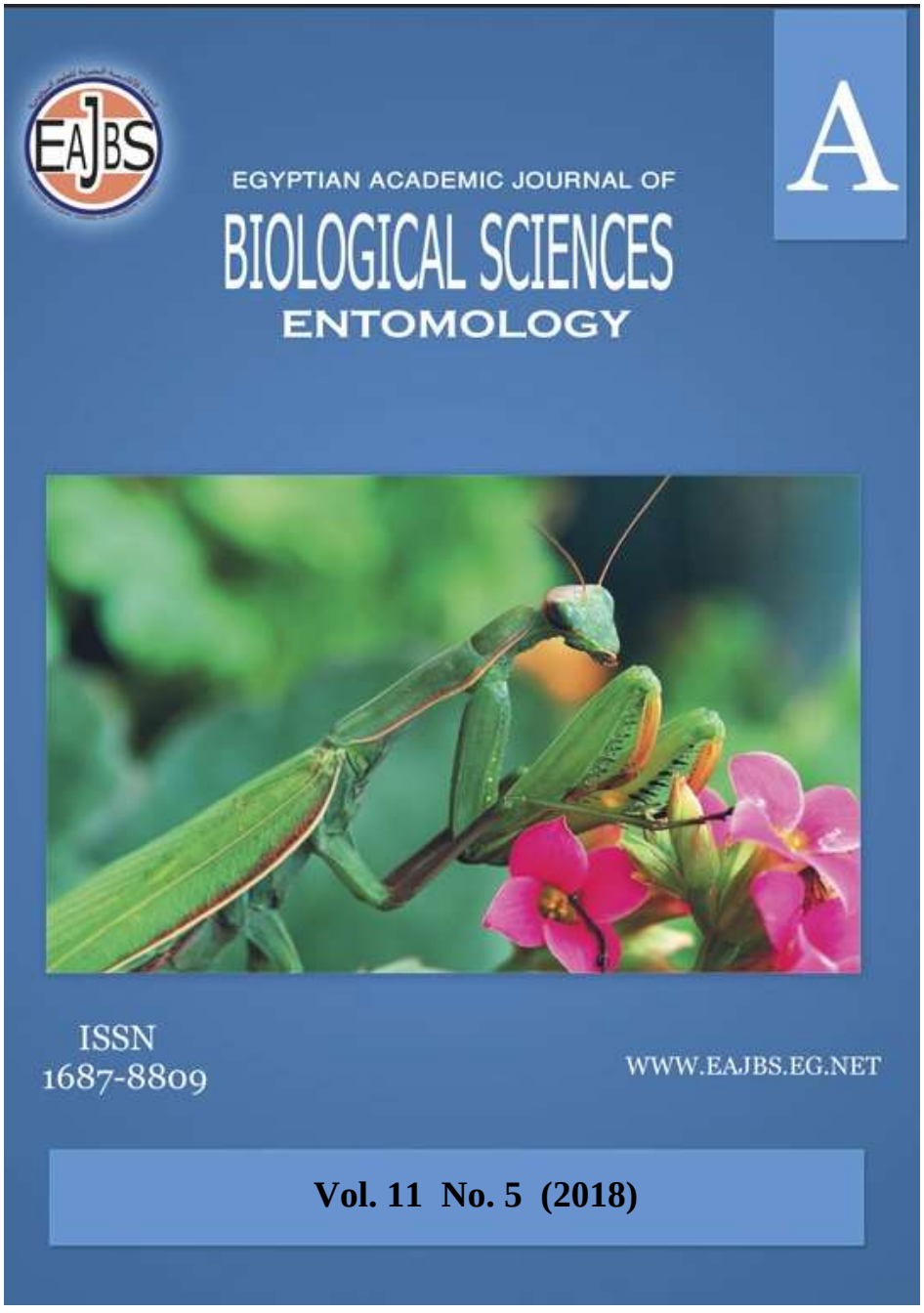

Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences, Department of Entomology, Faculty of Sciences Ain Shams University.
Entomology Journal publishes original research papers and reviews from any entomological discipline or from directly allied fields in ecology, behavioral biology, physiology, biochemistry, development, genetics, systematics, morphology, evolution, control of insects, arachnids, and general entomology.
www.eajbs.eg.net

Citation: *Egypt. Acad. J. Biolog. Sci. (A. Entomology) Vol. 11(5)pp: 149- 161 (2018)*

Egypt. Acad. J. Biolog. Sci., 11(5):149– 161 (2018)

Egyptian Academic Journal of Biological Sciences
A. Entomology

ISSN 1687- 8809
www.eajbs.eg.net

Biochemical and Molecular Studies Related to Phase Change in Gregarious and Solitarious Desert Locust, *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae)

Eman K. Mohamed[1], Ola H. Zyaan[2*], Nadia H. Ahmed[2], Noha A. Guneidy[2], Amany S. Khaled[2] and Nagi S. Badawy[1]

1. Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt.
2. Department of Entomology, Faculty of Science, Ain Shams University, Cairo, Egypt

E.Mail: ozyaan@sci.asu.edu.eg

ARTICLE INFO

Article History
Received:1/8/2018
Accepted:3/9/2018

Keywords:
Schistocerca gregaria, Protein, DNA, solitary, gregarious, transient.

ABSTRACT

Quantitative and qualitative analysis of protein and DNA were investigated in two extreme phases, gregarious and solitarious *Schistocerca gregaria*, in addition to, their transient stages. The level of protein in long-term solitarious *S. gregaria* revealed highly significant reduction when compared with the gregarious phase (P<0.05). These protein levels were 16.33±0.14 and 7.95±0.21 mg/10ml for long-term gregarious and solitarious *S. gregaria*, respectively. Data revealed 52 bands as the maximum number of protein which detected at the molecular weight (Mw) ranged from 25 to 225.4 kilodaltons (kDa). The most separated protein bands were detected in Mw ranging from 46 to 25 kDa. Likewise, quantitative analysis of DNA levels showed a significant reduction of DNA level in long-term solitarious *S. gregaria*, compared with gregarious phase (P<0.05). These DNA levels were 7.75± 0.30 and 4.4±0.28 µg/µl for long-term gregarious and solitarious *S. gregaria*, respectively. Furthermore, the fraction DNA pattern of tissue assessed that there are 15 different DNA bands detected at Mw ranging from 366 to 3484 base pair (bp). In conclusion, each phase of *S. gregaria* has a significant change in protein content and the fraction pattern of the DNA, which was clearly reflected in genetic and epigenetic differences among different phases of *S. gregaria*.

INTRODUCTION

The desert locust, *S. gregaria* is a phylogenetically heterogeneous insect group within the family Acrididae that demonstrated a distinct ability to change phases from solitary to gregarious in response to population density (Kang *et al*., 2004). Uvarov (1921) formulate the term phase to illustrate the taxonomic status of different morphological forms of migratory locusts, *Locusta migratoria* and *Locustana pardalina*, where the concept of phase polymorphism was put firstly in these species. After that, this concept extended to *S. gregaria* (Uvarov, 1966, 1977). He described *"solitaria"*, one of the two extreme phases, as isolated and relatively sedentary individuals, while "*gregaria*" is common crowded individuals and

swarming population. Undoubtedly, the local population density induces the expression of graded changes in a group of traits that include colouration, morphometry, anatomy, egg mass, food selection, nutritional physiology and reproductive physiology (Pener, 1991; Pener and Yerushalmi, 1998; Simpson *et al.*, 1999, 2005; Tanaka, 2001, 2006; Ferenz and Seidelmann, 2003; Kang *et al.*, 2004). Several investigations revealed that these changes evolved by external factors (Uvarov, 1966, 1977; Pener, 1991; Wiesel *et al*., 1996). Here, we supposed that local population density induced internal changes which may regulate and correlate the transformation processes. Consequently, we aimed to present preliminary information about levels of total proteins and DNA level, as indicators of differential internal bio-chemicals and molecular expression. Quantitative and qualitative analysis of both total protein and DNA levels were investigated in two extreme phases, gregarious and solitarious *S. gregaria*, in addition to, their transient stages.

MATERIALS AND METHODS

Experimental Insect:

The gregarious insects were reared and handled under the crowded conditions for several consecutive generations at Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza. The insects showed pronounced gregarious phase characteristics according to Hunter–Jones (1961). The solitarious phase of *S. gregaria* was derived from the gregarious stock colony. They were reared in the separated cages. Only one individual was kept in a small wooden cage (10×10×10 cm) in diameter. They were isolated from each other for several generations, five generations in this current study, under laboratory conditions. Locusts of the solitarious phase were reared singly, but at the time of the mating, they were paired. Locusts raised under these conditions showed pronounced solitarious phase characteristics (Simpson *et al.*, 1999).

Biochemical Studies:

The experimental adults were collected as the fresh molting adults from the long-term gregarious and solitarious stock colony and their intermediate stages. For biochemical analysis, five healthy fresh molting adults were collected from each generation and kept under freezing conditions at -5°C.

Quantitative Protein Analysis:

The half gram of body tissue sample was weighed and homogenized with 1ml of the extraction buffer. The homogenate was diluted by adding about 100ml of PMSF (phenylmethylsulfonyl fluoride). Samples were ground using liquid N_2 and sonicated with ice water for about 30 minutes. Samples were centrifuged for about 15 min at 12000 rpm. The soluble protein content of the supernatant was determined by the method of Lowry *et al.* (1951) using bovine albumin as standard. The concentrations of total protein of all samples were measured using spectrophotometer (UV absorbance at 280 nm).

Qualitative Protein Analysis:

Samples which were prepared to estimate the total protein levels were used in electrophoresis analysis. A part from the supernatants was withdrawn carefully using automatic pipettes and transformed to a new clear Eppendorf tube and kept frozen at -70 ^{o}C till needed. SDS-PAGE was performed in 12% acrylamide slab gel according to the method of Laemmli (1970) with some modifications (Sambrook *et al*., 1989).

Quantitative DNA Analysis:

DNA was extracted by using the gSYNC^{TM} kit. DNA concentrations were measured using spectrophotometer (UV absorbance at 260 nm).

Fraction DNA Pattern:

For molecular analysis of DNA, Polymerase Chain Reaction (PCR) amplification was carried out for extracted DNA of different phases. RAPD primer (OPA-7 5'- GAAACGGGTG -3') was used to determine the internal molecular variation between different DNA patterns. The reaction condition was performed according to Saiki *et al.* (1988), where the PCR mixture was in a total volume of 25µl contained 1µg of total DNA, 50 pmol of the primer, 0.2 mM deoxynucleoside triphosphates, 2.5 µl of the Taq polymerase enzyme, 2.5 µl of 10 X enzyme buffer and 2.5 µl $MgCl_2$.

The amplification reaction was performed in the following settings: DNA denatured firstly at 94°C/5min then for 1min, after which for 50°C/2min (base annealing temperature of first three cycles). Finally, extension or synthesis of new strands was at 72°C/3min, and was continued until the base annealing temperature reached the final condition of 49 °C and the samples were stored at 4°C. Under the final conditions, the amplification was continued for 30 cycles. The amplification products were analyzed on 1% agarose gel electrophoresis was performed according to Sambrook *et al*. (1989).

Similarity index (SI) and genetic distance (Gd) values were used to compare patterns within as well as between generations. SI reflects the extent of band sharing and calculated as: $SI = 2\ N_{ab} / (N_a + N_b)$ and $Gd = 1 - SI$. Where N_{ab} is the number of bands common to individuals a and b. N_a and N_b are the total numbers of bands in the individual a and b, respectively. The value produced by this index ranges from zero, respecting no bands sharing, to one, respecting complete identity (Nei and Li, 1979).

Statistical Analysis:

The data were subjected to statistical analysis using a software SPSS (2005) test program. The significance of the main effects was determined by ONE-way analysis of variance (ANOVA) and followed by *post-hoc* analysis using LSD-test. The significance of various generations was evaluated at $P < 0.05$.

RESULTS

1. Quantitative Analysis of Protein

1.1. Protein Levels:

Protein levels were illustrated in table (1). Data showed a significant reduction in protein levels between each phase and their transient generations ($P<0.05$).

Table 1: Protein levels of the two extreme phases of *S. gregaria* and its transient isolated generations.

No. of generation	$_{mg}$ Protein/10_{ml} sample solution
	Mean± S.E
Long-term gregarious	16.33 ± 0.14^{a}
1st isolated generation	12.86 ± 0.19^{b}
2nd isolated generation	7.89 ± 0.16^{c}
3rd isolated generation	5.75 ± 0.10^{d}
4th isolated generation	6.66 ± 0.19^{e}
5th isolated generation (long-term solitarious)	7.95 ± 0.21^{c}

*Means bearing different letters within the column are significantly different (P<0.05) ANOVA, LSD test

In long-term gregarious *S. gregaria*, protein content was about 16.33±0.14 mg protein/10ml. However, protein content was about 7.95±0.21 mg protein/10ml in long-term isolated individuals (the 5th isolated generation). This content decreased gradually and became the most minimum level at the 3rd isolated generation.

1.2. Qualitative Analysis of Protein:

SDS patterns banding of soluble tissue protein of long-term gregarious, solitarious, and transient isolated generations were illustrated in figure (1) and table (2). According to relative frequency (Rf), a maximum detected protein bands were 52 bands. These bands detected at molecular weight ranged from 25 to 225.4 kDa and Rf (0.744-0.035). The total protein bands were divided into 8, 11, 8, 9, 8 and 12, respectively, with long-term gregarious, 1st, 2nd, 3rd, 4th isolated generations and long-term isolated *S. gregaria*, 5th isolated generation. The maximum number of protein bands, detected in lane 1 (1st isolated generation) and lane 5 (long-term isolated generation), was 11 and 12, respectively.

However, the minimum number of protein bands was detected in lane 0 (long-term gregarious *S. gregaria*), lane 2 (2nd isolated generation) and lane 4 (4th isolated generation) and was 8 bands for each previous generations. Furthermore, the SDS protein pattern revealed each one of the two extreme phases and their transient generations with some unique bands. The extracted protein of long-term gregarious individuals resolved into 8 bands. These bands observed in no. 6, 13, 20, 28, 33, 41, 46 and 50 and their Rf values were ranging from 0.08-0.748. Also, their Mw was 165.72- 30.292 kDa. In between these previous bands, there are six unique bands, bands no. 6, 20, 33, 41, 46 and 50. In contrast, bands no. 13 and 28 detected in other isolated–generations. A band no. 13 detected also in both of 1st and 3rd isolated–generations as well as band 28 detected in both of 2nd and 3rd isolated– generations.

Densitometry scanning of SDS revealed that the highest concentration of protein, in long-term gregarious *S. gregaria*, was detected in a band no. 13 (Mw 98.497 kDa), where the protein concentration of this band was 26.93 %. However, band no. 27, with Mw 60.539 kDa, was the highest concentration in 1st isolated generation and amounted 19.24 %. On the other hand, in the 2nd isolated generation, the highest protein concentration was detected in the band no. 28 and amounted 26.10 % with Mw 59.894 kDa. In contrast, in the 3rd isolated generation, the highest concentration of protein, which was amounted 20.36%, was detected in the band no. 1 (Mw 225.4 kDa). In case of 4th isolated generation, the highest concentration of protein was detected in the band no. 19 amounted 24.82 % with Mw of 80.898 kDa. Furthermore, band no. 35, in the long-term solitarious generation, had the highest concentration (17.51 %) with Mw 48.503 kDa.

Generally, data in the table (2) concluded that the isolation condition of *S. gregaria* induced appearance and disappearance or sometimes creation new protein bands of the different isolated generations.

2. Quantitative Analysis DNA:

In long-term gregarious *S. gregaria*, the DNA content had the highest concentration, which was about 7.75±0.30µg/µL. In contrast, in long-term solitarious *S. gregaria* DNA content was about 4.4±0.28µg/µL (table 3). Furthermore, there was a successive significant reduction (P<0.05) between different generations. In transient isolated generations, DNA contents were 2.65±0.19, 1.63± 0.11, 1.25± 0.13, and 2.25±0.14µg/µl respectively, with 1st, 2nd, 3rd, and 4th isolated generation.

Table 2: Molecular weight (Mw) and % amount of fractionation protein pattern of crowd-reared (long- term gregarious), isolated-reared (long-term solitarious) *S. gregaria*, and transients.

No. of Bands	Rf	Mw	Marker (Mw)	%amount						
				Marker	Lane(0)	Lane(1)	Lane(2)	Lane(3)	Lane(4)	Lane(5)
1	0.035	225.4	225.4	6.77				20.36		
2	0.04	217.83							19.78	
3	0.045	210.51								7.68
4	0.06	190	190	18.46		9.21				
5	0.07	177.45								6.76
6	0.08	165.72			24.66					
7	0.09	154.78					5.69			
8	0.1	144.55								5.63
9	0.11	135	135	4.85				10.44		
10	0.119	126.29				6.52				
11	0.129	118.14							7.27	4.30
12	0.132	100	100	9.78			12.69			
13	0.157	98.497			26.93	10.16		12.36		
14	0.167	95.559								5.41
15	0.171	94.123							8.65	
16	0.19	88.592				17.86				
17	0.195	87.261								13.58
18	0.205	84.658						15.15		
19	0.219	80.898					11.76		24.82	
20	0.243	75	75	4.09	11.83					
21	0.257	72.629				9.92				
22	0.267	71.089						11.86		
23	0.271	70.332					14.75			
24	0.276	69.583								9.43
25	0.286	68.108							11.02	
26	0.31	64.557								2.43
27	0.338	60.539				19.24				
28	0.343	59.894			9.77		26.10	13.61		
29	0.357	58	58	5.68						12.53
30	0.362	57.617							16.47	
31	0.386	55.74								12.58
32	0.4	54.644						4.87		
33	0.471	49.476			16.50					
34	0.476	49.15				4.75	13.70			
35	0.486	48.503								17.51
36	0.49	48.183						7.59		
37	0.495	47.865							5.95	
38	---	46	46	7.93	----	---	----	-----	-----	-----
39	0.548	44.009					5.08			
40	0.552	43.621				3.89				
41	0.61	39.225			1.79					
42	0.614	38.879				3.05				
43	0.624	38.197								2.14
44	0.629	37.861					12.23			
45	0.652	36.222							6.03	
46	0.657	35.902			4.91					
47	0.671	34.962						3.76		
48	0.695	33.448				6.81				
49	-----	32	32	20.08	-----	-----	-----	----	-----	-----
50	0.748	30.292			3.61					
51	0.776	28.675				8.59				
52	0.744	25	25	22.34	-----	-----	-----	-----	-----	-----
Sum of % amount in lane				100	100	100	100	100	100	100
Total no. of bands				9	8	11	8	9	8	12

Table 3: DNA levels of long-term gregarious, solitarious *S. gregaria*, and transient isolated generations.

No. of generation	Conc. of DNA (µg/µl)
	Mean± S.E
Long-term gregarious	7.75± 0.30[a]
1st isolated generation	2.65±0.19[b]
2nd isolated generation	1.63± 0.11[c]
3rd isolated generation	1.25± 0.13[c]
4th isolated generation	2.25±0.14[b]
Long-term solitarious (5th isolated generation)	4.4±0.28[d]

*Means bearing different letters within the column are significantly different (P<0.05) ANOVA, LSD test

2.1. Qualitative analysis of DNA:

Changes in DNA profile in two extreme phases and their transient generations were shown in the table (4) and figure (2). The generated RAPD profile revealed differences among long-term gregarious, solitarious, and transient isolated

generations with visible changes in the number and size of amplified DNA fragments. The amplification pattern of RAPD- PCR of the genomic DNA revealed 35 different fragments. The fragments ranged from 366 bp (band No 15) to 3484 bp (band No 1). Bands 4 and 6 were common in all samples with Rf values 0.58 and 0.64, respectively. The Long-term gregarious sample was identified by the presence of 2 unique bands (366 and 860 bp). The sample of 4th isolated generation was recognized by one unique band with molecular weight 828 bp. On the other hand, five unique bands were distinguished sample of long-term solitarious with molecular weight 548, 797, 1263, 2328, and 3484 bp.

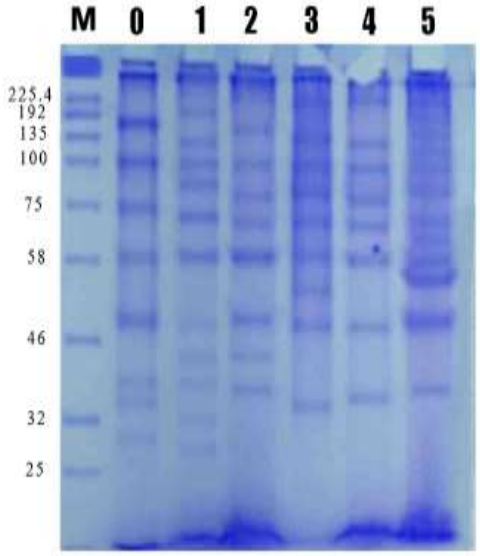

Fig. 1: SDS electrophoretic pattern of protein tissue extracted of adults from crowd-reared (long-term gregarious), isolated-reared (long-term solitarious) *S. gregaria*, and transient generation. Where, M: Marker, 0:Long-term gregarious *S. gregaria*, 1: 1st isolated generation, 2: 2nd isolated generation, 3: 3rd isolated generation, 4: 4th isolated generation, and 5: Long-term solitarious *S. gregaria*.

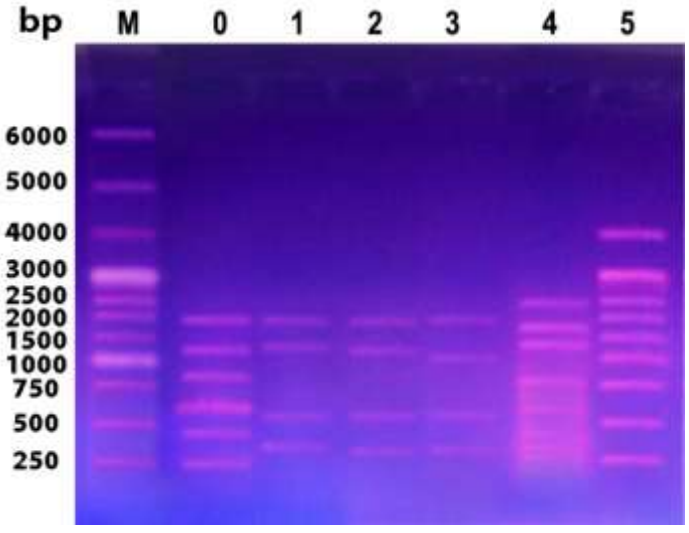

Fig. 2: Agarose gel electrophoresis showing RAPD profiles obtained by PCR-amplification of the different phase of *S. gregaria* using the primer OPA-7. Where, M: Marker, 0: Long-term gregarious *S. gregaria*, 1: 1st isolated generation, 2: 2nd isolated generation, 3: 3rd isolated generation, 4: 4th isolated generation, 5: Long-term solitarious *S. gregaria*.

Table 4: Molecular weight (Mw) of fractionation DNA pattern of crowd-reared (long-term gregarious), isolated-reared (long-term solitarious) *S. gregaria*, and transient generation.

Rows	Rf	Molecular weight (Mw)					
		Long-term gregarious	1st isolated generation	2nd isolated generation	3rd isolated generation	4th isolated generation	Long-term solitarious
r_1	0.40						3484
r_2	0.48						2328
r_3	0.54					1768	1811
r_{4*}	0.58	1498	1498	1484	1498	1401	1526
r_5	0.61						1263
r_{6*}	0.64	1111	1154	1106	1035	1181	1039
r_7	0.67	860					
r_8	0.70					828	
r_9	0.71						797
r_{10}	0.77	635	583	583	586	623	
r_{11}	0.97						548
r_{12}	0.81	491				491	
r_{13}	0.85		434	414	420	424	
r_{14}	0.87					377	382
r_{15}	0.89	366					

* Common band

Similarity index (SI) and genetic distance (Gd) values among long-term gregarious, 1st isolated generation, 2nd isolated generation, 3rd isolated generation, 4th isolated generation, and long-term solitarious samples using primer OPA-7 were recorded in the table (5). The similarity index values showed the major drop from 0.30, 0.30, 0.30, 0.29, to 0.13 between long-term gregarious sample and samples 1st isolated generation, 2nd isolated generation, 3rd isolated generation, 4th isolated generation, and long-term solitarious, respectively. This finding suggests major changes in DNA structure and sequence with the change from gregarious to solitarious phase.

Table (5): Similarity index (SI) and genetic distance (Gd) among long-term gregarious, 1st isolated generation, 2nd isolated generation, 3rd isolated generation, 4th isolated generation, and long-term solitarious samples using primer OPA-7.

	SI						
	Sample	Long-term gregarious	1st isolated generation	2nd isolated generation	3rd isolated generation	4th isolated generation	Long-term solitarious
Gd	Long-term gregarious	-	0.30	0.30	0.30	0.29	0.13
	1st isolated generation	0.70	-	0.50	0.50	0.33	0.15
	2nd isolated generation	0.70	0.50	-	0.50	0.33	0.15
	3rd isolated generation	0.70	0.50	0.50	-	0.33	0.15
	4th isolated generation	0.71	0.67	0.67	0.67	-	0.24
	Long-term solitarious	0.87	0.85	0.85	0.85	0.76	-

DISCUSSION

Carlisle *et al.* (1987) assessed that protein synthesis is necessary for the maintenance of body growth and reproduction. Also, he illustrated many factors, which have been implicated in the control of protein synthesis, enhancing the variety of biological aspects of insects. Baker *et al.* (2010) reported that each type of protein has a specific biological role; consequently, this role enhanced DNA to secrete enzymes, which act as catalysts to the produced specific type of protein, whereas this produced protein was responsible for a specific biological process.

Quantitative analysis of protein concluded that the isolation condition of *S. gregaria* induced the depression of protein content of these individuals. Protein content was higher in the gregarious *S. gregaria*, compared with the long-term solitarious generation. This result was in agreement with some authors as Ott *et al.* (2012). They reported the reduction of the protein level of solitary individuals, compared with gregarious *S. gregaria*.

Also, in the present investigation, polyacrylamide gel electrophoresis of SDS was used to separate different extracted proteins. That helped in understanding the biological process and factors that happened inside the cells of living organisms, which are related to the phase polymorphism of *S. gregaria*. The obtained results showed that each generation has its own characteristic protein pattern. Distinct differences are observed when all generations are compared to each other and with long-term gregarious *S. gregaria*. These protein patterns recorded diversity between different generations, especially in their molecular weight and number of bands. A maximum increase of the soluble protein bands is observed in both 1st isolated generation and the long-term solitarious generation, as a result of an appearance of new bands. There is an appearance of new bands, disappearance of some bands and reappearance of bands during the isolation condition. This may be due to appearing of new peptide in extracted proteins with the disappearance of others.

Indeed, these new peptides may change the chemical and physical structure of extracted proteins and undoubtedly these peptides can also change the solubility of these proteins; therefore, there were variations in the amounts of protein extractable from the long-term gregarious *S. gregaria* and its isolated generations although the same amount of protein has been applied to the gel. These findings were in agreement with some authors as Ayali *et al.* (1996a, 1996b), Rahman *et al*. (2002) and Clynen *et al.* (2002). They reported that some pronounced differences could be detected in peptide profiles of the brain, corpora cardiac and the hemolymph of both isolated and crowd-reared locusts (*S. gregaria* and *L. migratoria*).

Furthermore, the obtained results in this investigation declared that the extracted protein bands that were detected, may be related to phase polymorphism. This was concluded as these bands disappeared from other transient phases and the long-term isolated generation. These findings agree with Hirschberger *et al.* (1999) who reported that some proteins are expressed in respect to the phase state when the hemolymph protein pattern of *S. gregaria* was analyzed using 2D–gel electrophoresis.

Rahman *et al*. (2002) showed some differences in the peptide pattern of the hemolymph of solitary and gregarious animals of *S. gregaria* by using an HPLC analysis of hemolymph extracts. They reported that some peptides and proteins associated with phase polymorphism are designated as ‘phase-related peptide’ (PRP). Moreover, Rahman *et al.* (2003a) discovered a peptide with a potential molecular

marker of phase transition as it is present in higher concentrations in the gregarious phase than in the solitarious one. The peptide level decreased with the successive generations of solitary reared animals, detected by comparing the peptide of the hemolymph of successive isolated generations. Amel *et al*. (2011) reported that peptide concentration is higher in the gregarious female's accessory glands than in the solitarious, and these results suggest a role in phase polyphenism.

Indeed, Knowledge of the expression profile of the internal genetic, related to phase change, is increasingly important in understanding biological processes. Therefore, this investigation illustrated the quantitative and qualitative analysis of DNA content, extracted from both of the two extreme phases of *S. gregaria* and isolated generations. Quantitative analysis of DNA level declared that the isolation condition of *S. gregaria* induced a significant reduction of DNA level. This level was at the maximum in the long-term gregarious *S. gregaria*, compared with the long-term 5^{th} isolated generation. This finding was in agreement with some authors as Kang *et al.* (2004) who illustrated EST (expressed sequence tag) datasets and their distributions among cDNA libraries. They reported a higher level in EST datasets in gregarious than solitarious in some parts of the body such as head, midgut, and hind-leg.

Furthermore, the analysis of DNA by agarose gel PCR amplification technique was used to separate different extracted DNA. This helped in understanding the biological process and factors that happened inside the cells of living organisms, which are related to the phase polymorphism of *S. gregaria*. The obtained results showed that each generation has its own characteristic DNA pattern. Distinct differences are observed when all generations are compared to each other, and with long-term gregarious *S. gregaria*. It has been noticed that there are 6 bands detected in long-term gregarious *S. gregaria*.

Two bands out of the 6 long-term gregarious *S. gregaria* bands were specifically from the long-term gregarious *S. gregaria* generation. In contrast, the long-term solitarious *S. gregaria* generation had 9 bands. Five bands out of these 9 detected bands were specific bands to the long-term solitarious *S. gregaria* generation. However, other bands, detected in the transient stages (1^{st}, 2^{nd}, 3^{rd}, isolated generation), disappeared from long-term gregarious and solitarious generations. Furthermore, all DNA profiles were different, especially in mobility and number of bands. The maximum increase of DNA bands is observed in the last long-term isolated generations, the 4^{th} and 5^{th} generation, as a result of the appearance of new bands.

Generally, the appearance of new bands, disappearance and reappearance of bands during the isolation condition may be due to appear of new peptides in the extracted DNA. These new peptides may be related to phase change or enhancement of phase polymorphism. The highest genetic distance 0.87, which reflects the highest degree of change in DNA structure and sequence, was recorded between the genomes of the long-term gregarious sample and long-term solitarious sample using OPA-7. These findings were in agreement with some authors as Claeys *et al.* (2003), who detected some neuroparsin precursor in the different amount in gregarious and solitarious *S. gregaria*, may be related to phase polymorphism. These neuroparsin precursors are Scg-NPP1 and Scg-NPP2, which transcripts in several parts of the body such as fat body, gut, accessory glands, gonads and brain of female and male. These transcripts are generally more abundant in solitarious than in gregarious individuals. This finding constitutes the first indication of phase-dependent transcriptional regulation of Scg-NPP1and Scg-NPP2 gene expression.

Also, Kang *et al.* (2004) generated 76,012 ESTs from the whole body and

dissected organs in the two phases of *L. migratoria* to establish the molecular mechanisms of the phase change. They identified 532 genes as phase-related by comparing 12,161 unigene clusters.

Furthermore, PCR analysis in this investigation declared that each stage (long-term gregarious, transient and long-term solitarious) had a specific genome. Franz *et al.* (1998) studied brain area-specific gene expression of gregarious *S. gregaria*, and used differential display PCR without making a comparison with solitary animals. They illustrated 7 specific different areas: one from the midbrain, 3 from the thoracic and 3 from optic lobes ganglia. Also, Rahman *et al.* (2003b) reported that there are specific solitarious genes (SSG) and gregarious specific genes (GSG). They concluded that the two identified genes can now be used as novel molecular markers for a more specific characterization of the gene regulation events involved in locust phase transformation. Ma *et al.* (2011) illustrated some critical target genes related to behavioral phase changes in *L. migratoria*. They reported a relationship between genes and behavior in phase transition. Wang and Kang (2013) reported that the phase change in locusts is a continuous, accumulative, and easily reversible process and is involved in behavioral and physiological traits in response to changes in population density. Furthermore, genes and metabolites have a critical role in phase transition in desert locust. Also, Ernst *et al.* (2015) showed the important role of DNA, which is involved in phase polymorphism of *L. migratoria* and *S. gregaria*.

In conclusion, quantitative and qualitative analysis of protein and DNA revealed that the variation of population density induced internal changes. This was responsible for a specific biological process and external changes. Therefore, phase polymorphism evolved from internal changes followed by external changes, but not vice versa. Consequently, more detailed studies of these internal changes may be considered a valuable tool for the control of these internal factors. Therefore, a new strategy to control *S. gregaria* and a new environment-friendly method can be reached.

REFERENCES

Amel, B.H.; De Loof, A.; Habib, B.H.M. and Abdelrahmen, B. (2011): Phase related differences in female's accessory glands and oocytes proteins in the desert locust, *Schistocerca gregaria*. Annals of Biological Research. 2(3): 424-430.

Ayali, A.; Golenser, E. and Pener, M. (1996a): Flight fuel related differences between solitary and gregarious locusts (*Locusta migratoria migratorioides*). Physiological Entomology. 21:1–6.

Ayali, A.; Pener, M.P. and Girardie, J. (1996b): Comparative study of neuropeptides from the corpora cardiaca of solitary and gregarious Locusta. Archives of Insect Biochemistry and Physiology. 31: 439-450.

Bakr, F.A., El-Barky, N.M., AbdElaziz, M.F., Awad, M. H. and Abd El-Halim, M.E. (2010): Effect of Chitin synthesis inhibitors (flufenoxuron) on some biological and biochemical aspects of the cotton leaf worm *Spodoptera littoralis* Bosid (Lepidoptera: Noctuidae). Egyptian Academic Journal of Biological Science. (A. Entomology). 2(2): 43- 56.

Carlisle, J.A.; Oughton, B. and Ampleford, E. (1987): Feeding causes the appearance of a factor in the haemolymph that stimulates protein synthesis. Journal of Insect Physiology. 33:493-499.

Claeys, I.; Simonet, G.; Van Loy, T.; De Loof, A. and Broeck, V. (2003): cDNA cloning and transcript distribution of two novel members of the neuroparsin family in

the desert locust, *Schistocerca gregaria.* Insect Molecular Biology. (5): 473–481.

Clynen, E.; Stubbe, D.; De Loof, A. and Schoofs, L. (2002): Peptide differential display: a novel approach for phase transition in locusts. Comparative Biochemistry & Physiology. 132(B): 107-115.

Ernst, U. R.; Matthias, B. V. H.; Depuydt, G.; Boerjan, B.; De Loof, A. and Schoofs, L. (2015): Epigenetics and locust life phase transitions. The Journal of Experimental Biology. 218: 88 – 99.

Ferenz, H.J. and Seidelmann, K. (2003): Pheromones in relation to aggregation and reproduction in desert locusts. Physiological Entomology. 28:11-18.

Franz, O.; Ro-Èder, T. and Gewecke, M. (1998): Analysis of differential gene expression in the central nervous system of *Schistocerca gregaria* by differential display PCR. Journal of Comparative Physiology A. (182): 627-633.

Hirschberger, W.S.; Sickold, S.; Dorn, A.S.; Sickold, S. and Dorn, A. (1999): Expression of phase-specific haemolymph polypeptides in a laboratory strain and field catches of *Schistocerca gregaria.* Journal of Insect Physiology. (45): 1097–1103.

Hunter–Jones, P. (1961): Rearing and breeding locusts in Laboratory. Bulletin Anti-locust Research Centre, London, 12 pp.

Kang, L.; Chen, X.Y.; Yan Z.; Liu, B.W., Zheng, W.; Rui-Qiang L.P., Wang, J. and Yu, J. (2004): The analysis of large-scale gene expression correlated to the phase changes of the migratory locust. Proceedings of the National Academy of Sciences of the United States of America. 101(50) 17611–17615.

Laemmli, U.K. (1970): Cleavage of structural proteins during assembly of head bacteriophage T. Nature. 227: 680-685.

Lowry, O.H.; Rosebrough, A.A. and Randall, R.J. (1951): Protein measurement with the folin-phenol reagent. Journal of Biology and Chemistry. 193: 265-275.

Ma, Z.; Guo W., Guo X., Wang X. and Kang L. (2011): Modulation of behavioral phase changes of the migratory locust by the catecholamine metabolic pathway. Proceedings of the National Academy of Sciences of the United States of America. 108:3882–87.

Nei, M. and Li, W.S. (1979): Mathematical Model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA, 79: 5269-5273.

Ott, S. R.; Verlinden, H.; Rogers, S. M.; Brighton, C. H.; Quah, P. S.; Vleugels, R. K.; Verdonck, R. and Broeck, J.V. (2012): Critical role for protein kinase A in the acquisition of gregarious behavior in the desert locust. Proceedings of the National Academy of Sciences of the United States of America. (109): E381-E387.

Pener, M.P. (1991): Locust phase polymorphism and its endocrine relations. Advanced in Insect Physiology. (23):1-79.

Pener, M.P. and Yerushalim, Y. (1998): The physiology of locust phase polymorphism: an update. Journal of Insect Physiology. (44): 365 – 377.

Rahman, M.M.; Baggerman, G.; Begum, M.; De Loof, A. and Breuer, M. (2003a): Purification, isolation and search for possible functions of a phase-related 6080-Da peptide from the haemolymph of the desert locust, *Schistocerca gregaria*. Physiological Entomology. 28:39–45.

Rahman, M.M.; Bosch, L.V.; Baggerman, G.; Clynen, E. and Hens K. (2002): Search for peptidic molecular markers in hemolymph of crowd- (gregarious) and isolated-reared (solitary) desert locusts, *Schistocerca gregaria*. Peptides. 23:1907–14.

Rahman, M.M.; Vandingenen, A.; Begum, M.; Breuer, M.; De Loof, A. and Huybrechts, R. (2003b): Search for phase specific genes in the brain of desert locust, *Schistocerca gregaria* (Orthoptera: Acrididae), by differential display polymerase chain reaction. Comparative Biochemistry and Physiology. 135:221–28.

Saiki, K.R.; Gelfand, H.D.; Stoffel, S.; Scharf, J.S.; Higuchi, R.; Horn, T.G.; Mullis, B.K. and Erlich, A.H. (2003): Primer-Directed Enzymatic Amplification of DNA with a Thermostable DNA Polymerase. Science. 239: 487-491.

Sambrook, J.; Fritsch, E.F. and Maniatis, T. (1989): Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, New York.

Simpson, S.J.; McCaffery, A.R. and Hagle, B.F. (1999): A behavioural analysis of phase change in the desert locust. Biological Reviews. 47(4): 461-480.

Simpson, S.J.; Sword, G.A. and De Loof, A. (2005): Advances, controversies and consensus in locust phase polyphenism research. Journal of Orthoptera Research. 14: 213-222.

Tanaka, S. (2001): Endocrine mechanisms controlling body-color polymorphism in locusts. Archives of Insect Biochemistry and Physiology. 47: 139-149.

Tanaka, S. (2006): Corazonin and locust phase polyphenism. Applied Entomology and Zoology. 41: 179-193.

Uvarov B. (1921): A revision of the genus *Locusta* L. (=Pachytylus, Fieb.) with a new theory as to the periodicity and migrations of locusts. Bulletin Entomology Research. 12: 135-163.

Uvarov, B. (1966): Grasshoppers and Locusts, vol. 1. Cambridge University Press, London, UK.

Uvarov, B. (1977): Grasshoppers and Locusts, vol. 2. Centre for Overseas Pest Research, London, UK.

Wang, X. and Kang, L. (2013): Molecular mechanisms of phase change in locusts. Annual Review of Entomology. (59): 225-244.

Wiesel, G.; Tappermann, S. and Dorn, A. (1996): Effects of juvenile hormone and juvenile hormone analogues on the phase behavior of *Schistocerca gregaria* and *Locusta migratoria*. Journal of Insect Physiology. 42: 385–395.

ARABIC SUMMERY

دراسات بيوكيميائية وجزيئية للتغير في المظهر التجمعي والمظهر الإنفرادي في الجراد الصحراوي *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae)

إيمان كامل محمد[١]، علا حلمي زيان[٢]، نادية أحمد حلمي[٢]، نهي عوني جنيدي[٢]، أماني سالم خالد[٢]، ناجي ثابت بدوي[١]
١. معهد بحوث وقاية النبات، مركز البحوث الزراعية، الجيزة، مصر.
٢. قسم علم الحشرات، كلية العلوم، جامعة عين شمس، القاهرة.

قد تم اجراء التحليل الكمي والنوعي للبروتين والحمض النووي لكلاً من المظهرين التجمعي والانفرادي فى حشرة جراد الصحراء (*Schistocerca gregaria*) بالإضافة إلى المراحل الانتقالية بينهم. و قد أظهرت النتائج انخفاض مستوى البروتينات في المظهرالأنفرادي طويل المدي انخفاضاً ذو معنوية عالية مقارنة بالمظهر التجمعي طويل المدي. حيث كانت هذه المستويات حوالي 16,33 مجم/10مل للمظهر التجمعي و٧،٩٥ مجم/١٠مل للمظهر الأنفرادي علي التوالي. وقد أظهر الفصل الكهربي للأنماط البروتينية عدد 52 حزمة بروتينية مع وزن جزيئي تراوح بين 225,4 و ٢٥ كيلودالتون. وقد ظهر اكبر عدد من الحزم البروتينية المميزة بين المظهرين بين الوزن الجزيئي 46 و 25 كيلودالتون. وبالمثل أظهر التحليل الكمي لمستويات الحمض النووي انخفاضًا ملحوظًا في كل من المظهرين المتطرفين والأجيال الأنتقالية بينهم. وقد أظهرالتحليل الكمي لمستويات الحمض النووي انخفاضًا ملحوظًا في مستوى الحمض النووي في المظهر الأنفرادي مقارنة بالمظهر التجمعي. حيث كانت مستويات الحمض النووي ٧,٧٥ و ٤,٤ ميكروجرام / ميكرولتر للمظهر التجمعي و للمظهر الأنفرادي علي التوالي. كما أظهرالفصل الكهربي للأنماط الجزيئية للحمض النووي عدد ١٥ حزمة مع وزن جزيئي تراوح بين ٣٦٦- ٣٤٨٤ زوج قاعدي. مما سبق يتضح أن نظرية تعدد الأشكال التى تظهر في الجراد الصحراوي لا تعتمد علي التغير المظهري فقط بل تنعكس بوضوح علي المستوي البيوكيميائي والجزيئي حيث ملاحظة نمط بروتيني و تركيب جزئي خاص لكل مظهر فى الجراد الصحراوي (*Schistocerca gregaria*)