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Original Research Article

The Incidence of the Protozoa *Malamoeba locustae* and *Gregarina granhami* in three Acridian Communities during Summer Month

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The incidence of *Gregarina garnhami* and *Malamoeba locustae* was investigated during summer 2012 among three wild grasshopper communities. Pooling the data of the three communities, it was found that about 38.5% of the grasshopper species hosted either *G. garnhami* or *M. locustae*, and 15.28% of the individuals hosted one of them. The absence of such protozoa from *Chrotogonus lugubris* was attributed to an acquired immune response associated with feeding preference. It was assumed that these parasites are among exogenous density-dependent key factors regulating the grasshopper populations, and hence play a role in community stability in the wild acridid communities. The grasshopper communities were investigated at three study sites. The first site, Abu-Rauwâsh, is a countryside of Giza governorate. The second and third sites, Wadi El-Natroun and Kom Osheem, are located about 100 kilometers to the north and the south of the governorate; respectively. The first community consisted of 8 species; the most abundant was *Heteracris littoralis* (relative abundance 29.14%). The percentage of *G. garnhami* in the community was 8.47% while *M. locustae* 27.45% of the population corresponding to 8.0% of the entire community. The second community consisted of 12 species; the most abundant was *H. littoralis* (relative abundance 22.49%). The percentage of *G. garnhami* in the community was 6.89%, while *M. locustae* was recorded in 14.39% of *H. littoralis* population corresponding to 3.23% of the entire community. The third community consisted of 10 species; the most abundant was Pyrgomorpha. conica (relative abundance 20.08%). The percentage of *G. garnhami* in the community was 13.04%; it was recorded in *H. littoralis*, *P. conica* and, *P. cognata*; again, *M. locustae* was recorded only in *H. littoralis* (28.47% of the population), corresponding to 5.66% of the entire community.

Keywords: Malamoeb, Gregarina, grasshopper, Orthoptera, Occupancy

INTRODUCTION

Parasitism, and other symbiotic activities are among the principal biological processes responsible for the transport and storage of material and energy, and the interactions of the organisms engaged in these activities may provide the pathways of feedback loops controlling the population regulation and community stability. Such extensive linkage between organisms has a profound impact on the organization in ecosystems through the food web (Price, 1999; El-Shazly, 2002). El-Shazly and Shahpa (2006) analyzed the structure and host plant selection of an acridid community in Egypt and determined the diet breadth for each acridid species. In general, grasshopper community studies have recently been centered on the mechanisms driving community assembly (Rominger and Miller 2009), as well as on the analysis of the environmental factors controlling temporal and spatial

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distribution of the investigated community (Rominger *et al.*, 2009, and Zhou *et al*., 2012).

The principal pathogenic protozoa of locusts and grasshoppers are found in the groups amoeba, (Phylum: Sarcomastigophora), gregarines (Phylum: Apicomplexa), and microsporidia (Phylum: Microspora); the most extensively studied group is the last one (Streett and McGuire, 1990); primarily because of their potential as biological control agents (Abdel Rahman and Cagáň 2001).

Concerning amoebic group, *M. locustae* is the only species of the genus *Malamoeba*; this species is characterized by a wide host range (Ernest and Baker 1982, Henry *et al*., 1985), and it is responsible for amoebic diseases of grasshoppers (Streett and McGuire, 1990). In contrast, some literatures suggest that host specificity is a general feature of gregarines; for instance, Clopton and Gold (1996) suggested that *Gregarina blattarum* comprised a complex of cryptic species marked by narrow host utilization rather than a single species persisting a broad array of cockroach taxa.

The amoeba *M. locustae* has been isolated more frequently from grasshoppers in laboratory cultures than from field collected grasshoppers. The isolation of this protozoan from laboratory cultures has been documented by King and Taylor (1936), Taylor and King (1937), Prinsloo (1960), Henry (1968) and Henry and Oma (1975). Among the above authors, Taylor and King 1937 described *M. locustae* from *Melanoplus differentialis*, *M.* (*mexicanus*) *sanguinipes* and *M. femurrubrum* raised in laboratory cultures in Iowa. Likewise, *G. garnhami* was described from an artificial population of *Schistocerca gregaria* raised in the Anti-Locust Research Center, London (Canning 1956).

The incidence of protozoan parasites on grasshopper communities is probably among the key factors controlling the community structure and species abundance. However, no attention has been paid, so far for the existence of these parasites in the wild grasshoppers communities; furthermore, Clopton and Gold (1996) pointed out that nearly 80% of the gregarine species have not been reported since their original description. Consequently, the aim of the present work is to investigate and compare the occurrence of *M. locustae* and *G. garnhami* in three acridid communities found in different habitats and separated by fairly large distances.

MATERIALS AND METHODS

Study area

Species were collected from non national park or other protected area, they live naturally on wild grasses close to the cultivated field owned by natives who allowed us to collect such grasshoppers. These specimens are not endangered or protected species. Locations of our study (GPS coordinates) are listed in the manuscript: Site I, Abu-Rauwâsh (30° 2' N; 31° 5' 42'' E); Site II, Wadi El-Natroun (30° 16´ N; 30° 02´ E). Site III, Kom Osheem (29° 33' N 30° 53' E). For contact: Giza, El-Beheira and Fayom Governorates hotlines +20 2114, +20 45 3349848 and +20 84 6336577; respectively. These study sites (Fig. 1) were chosen to study the incidence of *M. locustae* and *G. garnhami* in grasshopper communities.

Site I, Abu-Rauwâsh district

A countryside of Giza governorate; it is an old land, cultivated with usual summer crops and irrigated with Nile water. The most abundant weed species in the sampling transect were *Cyperus rotundus* (L.) (Cyperaceae), *Cynodon* 52 *dactylon* (L.), *Polypogon monspliensis* (L.), *P. monspliensis* (L.) (Graminae), *Ammi majus* (L.) (Umbellifera), *Portulaca oleraceae* (L.) (Portulaceae), *Alhagi maurorum* (Medic.) (Leguminosae), and *Brassica nigra* (L.) (Crucifera).

Site II, Wadi El-Natroun district

A depression in the western desert of Egypt located about 100 kilometers to the north of Giza governorate and belonging to El-Beheira governorate. Cultivated areas of this site where reclaimed land, irrigated with underground water. The abundant wild plants in the selected sampling transect were *C. rotundus* (L.) (Cyperaceae), *C. dactylon* (L.), *Avena fatue*, *Imperata cylindrical* (L.) (Graminae), *P. oleracea* (Portulacaceae), and *Eucalyptus* sp.(Myrtacea).

Site III, Kom Osheem district

A reclaimed land cultivated mainly with olives and figs, and irrigated with Nile water; it is located about 100 kilometers to the south of Giza governorate and belonging to Fayom governorate. Beside the common weeds, *C. rotundus* (L.) (Cyperaceae), *C. dactylon* (L.), *Imperata cylindrical* (L.) (Graminae), other wild plants including *P. oleraceae* (L.) (Portulaceae), *Alhagi maurorum* (Medic.) (Leguminosae), *Artiplex* sp. (Amaranthaceae) and *Schanginia baccata* (Chenopodiaceae) are common in this site.

Sampling

For each study site, one sampling transect about 2 meter width and 3000 meter length was designated. Care was taken to select the transects close to the borders of the cultivated fields where naturally growing plants are abundant. Because natural vegetation in Egypt is heterogeneous, patchy and fragmentary, care was taken to designate sampling transects with the most possible dense plant cover. The data were collected between June and August 2012.

The number of sampling points varied in the three sites according to the presence of low vegetation and grass patches; it ranged between 18 (in Wadi El-Natroun), and 27 sampling points (in Abu-Rauwâsh). The area of each sampling point was about 2×5 m along which grasshoppers were swept for 200 times using pest-net (net diameter 30 cm). The data of each sampling point is the average of 3 monthly samplings.

Examination

Collected insects were transferred to the laboratory. They were cut off at the at the fourth abdominal segment; then gut content, fat body and Malpighian tubes were crushed on a glass slide by a cover with a drop of distilled water and were examined by Leica microscope for seeking the microorganisms which were identified and photographed.

RESULTS AND DISCUSSION

Identification of the protozoa

The gregarine species in our study was considered as *Gregarina garnhami* (Fig. 2); while the amoebic one was considered as *Malamoeba locustae* (Fig.3) *G. garnhami* were identified according to Canning (1956) and Valigurova and Koudela (2008) based on the morphology of the free spores in the gut of the collected grasshoppers; on the other hand, *M*. *locustae* is the only species in the genus *Malameba* that is responsible for the amoebic disease of grasshoppers (Street and Mc Gure 1990). It was originally described by King and Taylor (1934) from Malpighian tubules of several *Melanoplus* species.

GRASSHOPPER COMMUNITIES

Site I (Abu-Rauwâsh):

A total of 8 grasshopper species and 1050 Individuals were recorded during the sampling period; the most abundant species was *H. littoralis* (relative abundance 29.14%); while the African migratory locust, *Locusta migratoria* (solitary phase) was the least abundant one (relative abundance 2.28%) (Table 1). Most populations exhibited peaks between July and August; except the native species, *A. aegyptium*, which was peaked in September (Fig.4). Both *M. locustae* and *G. garnhami* were recorded in *H. littoralis*, the former species was recorded in 27.25% of the collected specimens, corresponding to 8.0% of the community and the second in 4.28%.

However, the coexistence of the two parasites in the same individual was not observed; corresponding to 7.01 and 8.47% of the total community, respectively. *G. garnhami* was recorded in 10.21% of *A. aegyptium* population (1.81% of the community). Thus, we can state that 25% of the species, and 16.47% of the community were attacked by a protozoan species. Both *M. locustae* and *G. garnhami* were recorded in a wild population of *H. littoralis* for the first time. The former species, *G. garnhami*, was originally described from specimens of *S. gregaria, L. migratoria* and *A. aegyptim*; specimens of the latter species were sent to the author (Canning, 1956) from Egypt.

Table (1) The structure of acridian communities and the incidence of *Malamoeba locustae* (*Mal.*) and *G. garnhami* (*Gre*)

Acridian species	Number collected	Relative abundance	No. infected		% infected	
			Mal.	*Gre.*	*Mal.*	*Gre.*
Community (I) Abu-Rauwâsh						
Acrida pellucida	127	12.09	-	-	-	-
Acrotylus insubricus	181	17.24	-	-	-	-
Aiolopus thalassinus	120	11.43	-	-	-	-
Chrotogonus lugubris	65	6.02	-	-	-	-
Heteracris littoralis	306	29.14	84	45	27.45	14.70
Anacridium aegyptium	186	17.71	-	19	-	10.21
Locusta migratotia	24	2.28	-	-	-	-
Truxalis nasuta	41	3.90	-	-	-	-
∑ 8	**∑ 1050**		**∑ 84**	**∑ 89**		
Community (II) Wadi El-Natroun						
Acrida pellucida	61	5.06	-	-	-	-
Acrotylus insubricus	73	6.06	-	-	-	-
Aiolopus thalassinus	51	4.23	-	-	-	-
Chrotogonus lugubris	241	20.00	-	-	-	-
Heteracris littoralis	271	22.49	39	39	14.44	14.44
Platypterna sp.	25	2.07	-	-	-	-
Pyrgomorpha conica	240	19.92	-	10	-	4.17
Pyrgomorpha cognata	60	9.98	-	17	-	28.33
Sphingonotus savignyi	24	1.99	-	17	-	70.83
Sphingonotus rubescens	62	5.16	-	-	-	-
Tropidopola longicornis	51	4.23	-	-	-	-
Truxalis nasuta	46	3..82	-	-	-	-
∑ 12	**∑ 1205**		**∑ 39**	**∑ 83**		
Community (III) Kom Osheem						
Acrida pellucida	132	9.11	-	-	-	-
Acrotylus insubricus	270	18.63	-	-	-	-
Aiolopus thalassinus	177	12.21	-	-	-	-
Chrotogonus lugubris	62	4.28	-	-	-	-
Heteracris littoralis	288	19.87	82	38	28.47	13.19
Platypterna	52	3.59	-	-	-	-
Pyrgomorpha conica	291	20.08	-	96	-	32.99
Pyrgomorpha cognata	66	4.55	-	17	-	25.76
Sphingonotus savignyi	52	3.59	-	-	-	-
Truxalis nasuta	59	4.07	-	-	-	-
∑ 10	**∑ 1449**		**∑ 82**	**∑ 189**		

Table (2) The overall incidence of *Malamoeba locustae* (*Mal.*) and *Gregarina granhami* (*Gre.*) in grasshopper communities.

Acridian species	Collective percent infection in the three communities*					
	On the species level			On the communities level		
	Mal.	*Gre.*	*Mal. + Gre.*	*Mal.*	*Gre.*	*Mal. + Gre.*
Acrida pellucid	-	-	-	-	-	-
Acrotylus insubricus	-	-	-	-	-	-
Aiolopus thalassinus	-	-	-	-	-	-
Anacridium aegyptium	-	10.21	10.21	-	0.51	0.51
Chrotogonus lugubris	-	-	-	-	-	-
Heteracris littoralis	23.70	14.10	38.15	5.53	3.29	8.91
Locusta migratotia	-	-	-	-	-	-
Platypterna sp.	-	-	-	-	-	-
Pyrgomorpha conica	-	19.96	19.96	-	2.86	2.86
Pyrgomorpha cognate	-	26.98	26.98	-	0.92	0.92
Sphingonotus savignyi	-	70.83	70.83	-	0.46	0.46
Sphingonotus rubescens	-	-	-	-	-	-
Tropidopola longicornis	-	-	-	-	-	-
Truxalis nasuta	-	-	-	-	-	-

*based on summation of data given in table (1)

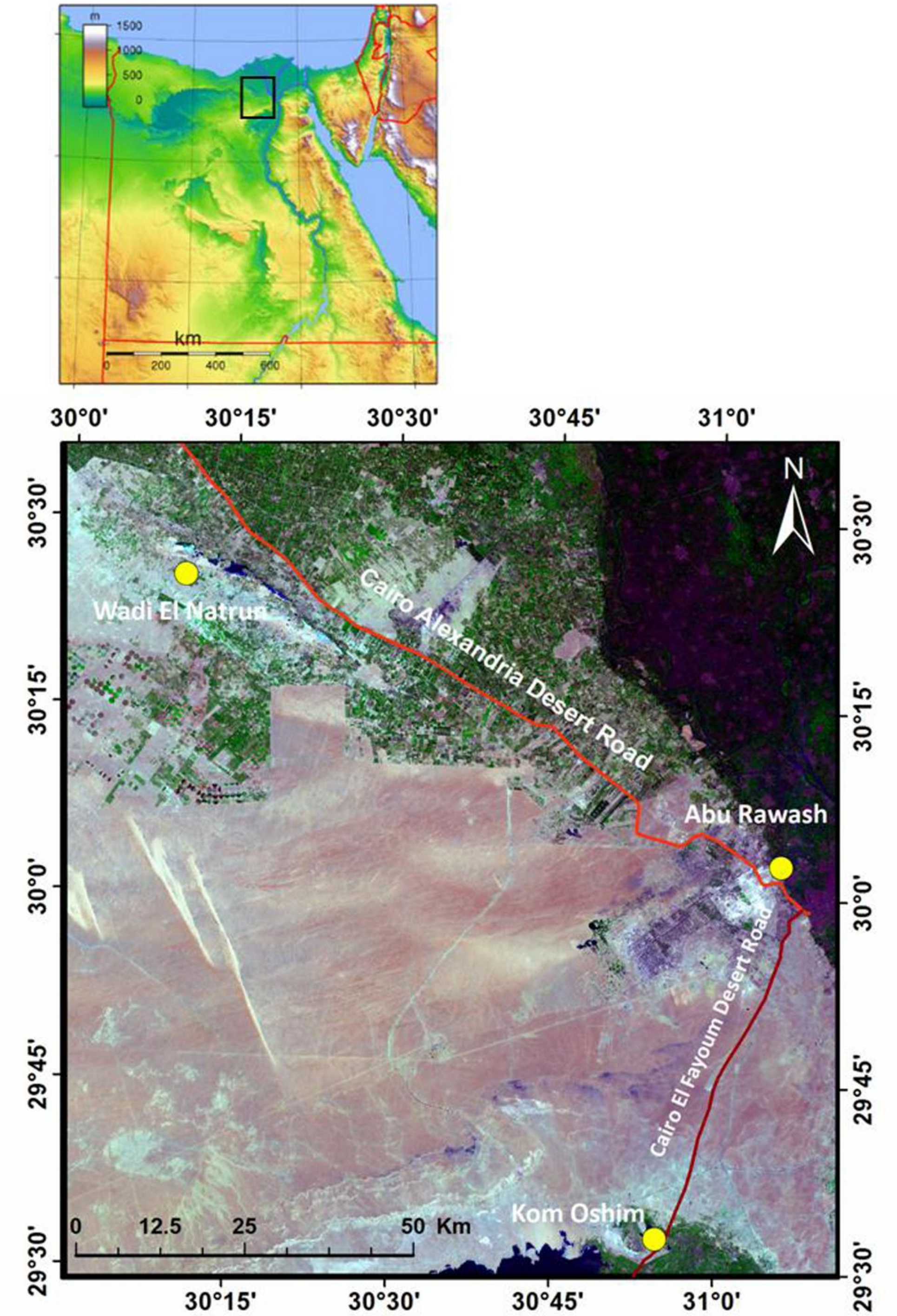

Fig. 1: Map of Egypt showing the three different localities.

Fig. 2. *Gregarina garnhami*

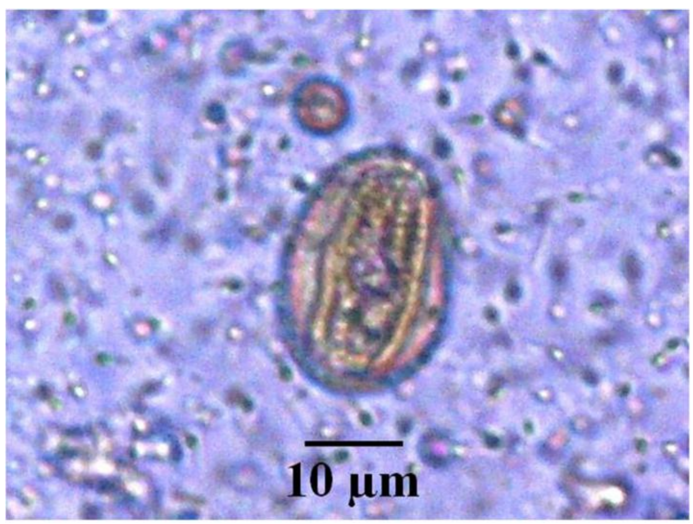

Fig. 3. *Malamoeba locustae*

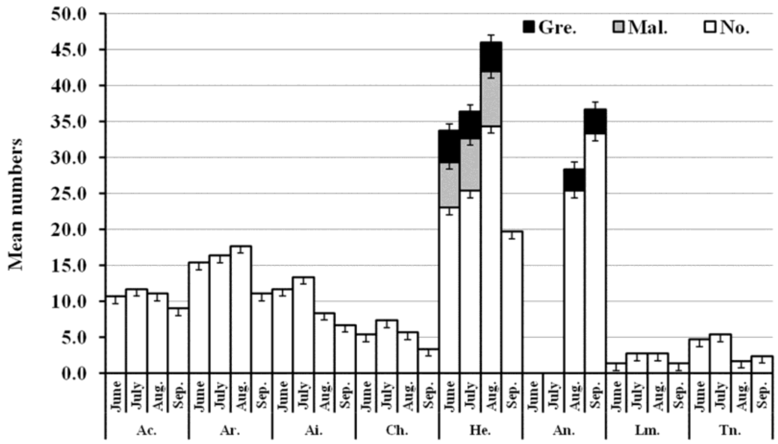

Fig. 4: Mean number of grasshoppers (No.) infected with *Malameoba locustae* (Mal.) and *Gregarina granhami* (Gre.) in Abu-Rauwâsh during summer months. Ac., *Acrida pellucida*; Ai., *Aiolopus thalassinus*; An., *Anacridium aegyptium*; Ar., *Acrotylus insubricus*; Ch., *Chrotogonus lugubris*; He., *Heteracris littoralis*; Lm., *Locusta migratotia*; Tn., *Truxalis nasuta*.

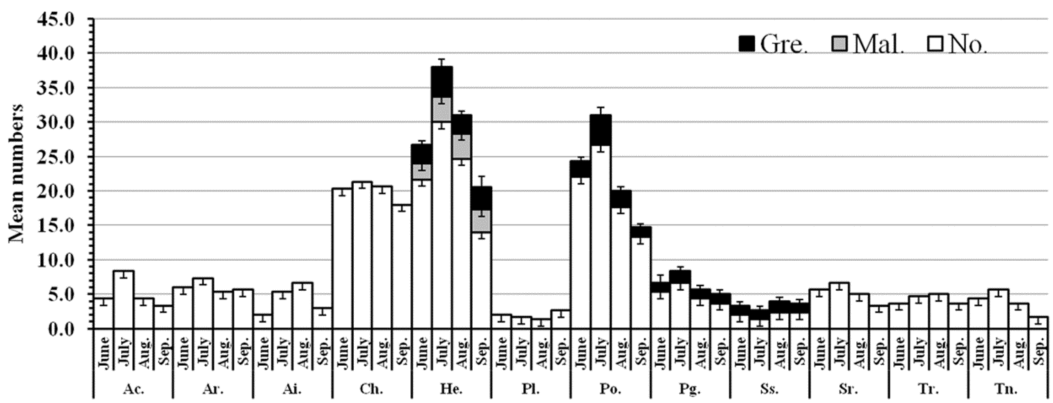

Fig. 5: Mean numbers of grasshoppers (No.) infected with *Malameoba locustae* (Mal.) and *Gregarina granhami* (Gre.) in Wadi El-Natroon during summer months. Ac., *Acrida pellucida*; Ai., *Aiolopus thalassinus*; Ar., *Acrotylus insubricus*; Ch., *Chrotogonus lugubris*; He., *Heteracris littoralis*; Pl., *Platypterna sp.*; Pg., *Pyrgomorpha conica*; Po., *Pyrgomorpha cognata*; Sr., *Sphingonotus rubescens*; Ss., *Sphingonotus savignyi*; Tr., *Tropidopola longicornis*; Tn., *Truxalis nasuta*.

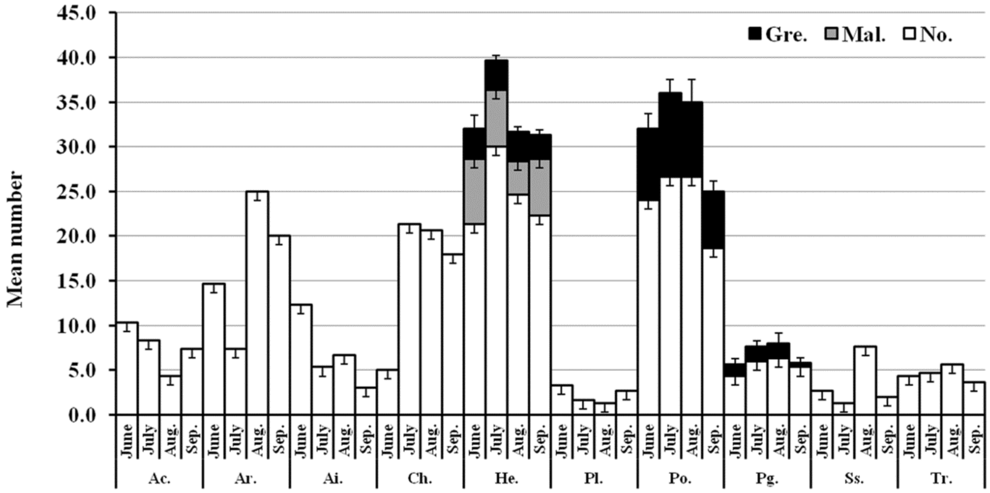

Fig. 6: Mean number of grasshoppers (No.) infected with *Malameoba locustae* (Mal.) and *Gregarina granhami* (Gre.) in Kom Osheem during summer months. Ac., *Acrida pellucida*; Ai., *Aiolopus thalassinus*; Ar., *Acrotylus insubricus*; Ch., *Chrotogonus lugubris*; He., *Heteracris littoralis*; Pl., *Platypterna sp.*; Pg., *Pyrgomorpha conica*; Po., *Pyrgomorpha cognata*; Ss., *Sphingonotus savignyi*; Tr., *Tropidopola longicornis*.

SITE II: Wadi El-Natroun

The grasshopper community in sampling area of Wadi El-Natroun consisted of 12 species, four of them were parasitized with different levels of infections (Fig.5).This site seemed to be more rich in species than the other two sites, where 1205 specimens representing 12 species were collected (Table 1); this could attributed to the loose nature of the sandy soil as well as the presence of heavy wild grass cover at the sampling site. Our field observations showed that many acridid species are connected to grasslands of every type from dry to relatively wet. The acridids that found in cultivated areas are usually connected to grass habitats along the borders of the fields. In a study on the ecology of *Acrida pellucid* in Egypt, Hafez and Ibrahim (1958) pointed out that adaptation of the genus *Acrida* to grasslands is an ancient one and that the evolution of the genus took place when grassland not only existed, but flourished, possibly during the Pliocene to the Pleistocene period.

A rapid glance at (Fig 5) is sufficient to show that the most abundant species was *H. littoralis* followed by *C. lugubris*, while the least abundant one was *S savignyi*; the three species were represented by 22.49, 20 and 1.99% of the total number of specimens collected, respectively.

As in site I, *H. littoralis* was the only species infected by both *M. locustae* and *G. garnhami*; three other species were infected by the gregarine parasite, namely *P. conica* , *P. cognata and S. savignyi*. The population of the last species, *S. savignyi*; had the heaviest infection where more than 70% of the sampled individuals hosted the gregarine parasite (Table1).

Site III: Kom Osheem

As shown in (Fig.6), the multispecies community in this site consisted of ten acridid species, with population peaked in midsummer (July-august); three species (*H. littoralis* , *P. conica* and *P. cognata*) hosted the protozoa. These species were represented by 1449 specimens. About 56.5% of the sample belonged to *P. conica, A. insubricus and H. littoralis* (Table 1). Poor representation of some species, e.g. *S. savignyi* in the collected sample could be attributed to innate or environmental factors such as reproductive capacity, seasonal history, phrenology, availability of preferred food in the sampling area, suitability of soil for oviposition of a particular specie etc. However, some species such as *H. littorlis* seemed to acquire a fair plasticity, having no definite preference for a particular habitat.

The overall incidence of the parasites on the three communities

The overall incidence of *M. locustae* (*Mal.*) and *G. granhami* (*Gre.*) in the three investigated grasshopper communities is given in (Table 2). Thirteen species were sampled from the three study sites; five species (about 38.5%) can host *G. granhami*, while *M. locustae w*as found only in *H. littoralis*.

Gregarina free species could be resistant to the parasites. Thus *C. lugubris* is clean from such protozoa as it was collected from field several times since 1990 till now (personal observation); this could be attributed to an acquired immune response associated with feeding preference. In summer, this species feeds on garden purslane (*P. oleracea*: Portulacaceae) (Ibrahim, 1971) which contains flavonoids that has anti-protozoal activity (Upadhyay and Ahmad, 2012; Wang *et al.*, 2013). The susceptibility level of the host varied considerably among different acridid species, the more susceptible species was *S. savignyi* ; more than 70% of the sampled individuals were infected with *G. garnhami* (Table 2), and this may explain the low population level of the specie in the communities (Table1).

Horizontal transmission via oral ingestion is the only reported route of amoebic disease infection in insects (Streett and Mc Guire, 1990). Among the conditions which contribute to the pathogenesis may be the absence of food and the tendency of grasshoppers and locusts to cannibalize upon dead or moribund individuals (Bidochka and khachatourians, 1991). Indeed cannibalization of hanged individuals during molting has repeatedly been observed in grasshopper laboratory colonies (personal observations). This suggests that these parasites are among exogenous density–dependent key factors regulating the grasshopper populations, and hence play a role in the stability of wild acridid communities.

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