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Role of male - female stimulation in the biology of the migratory locust *Locusta migratoria migratorioides* (Reiche & Fairmaire) (Orthoptera, Acrididae)

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Abstract: Laboratory experiments were conducted at 25° (± 2° C), 10 : 24 light hours, to assess the influence of male stimulation upon females of the migratory locust, *Locusta migratoria migratorioides*, gregary phase. Three different treatments were performed: Mated females (A); virgin, isolated from males (B); virgin, separated from males by a perforated wall, through which olfactory, visual and acoustical stimulation was possible (C). Longevity of mated females decreased by 2 to 4 days in comparison with virgin ones. There were no significant differences for the total number of eggs and of egg batches, laid by the females of all types of experiments. However, the number of both infertile eggs, and of larvae that started developing, but did not hatch, was significantly lower for the mated females, than for the virgin ones. Concomitantly, the mean number of viable larvae produced by mated females reached 15.7 larvae / egg batch, while a maximum of 2.3 larvae / egg batch originated from virgin ones. Experiments are in progress, aiming at the analysis of volatile compounds emitted by males, having pheromonal activity. The role of semiochemicals in locust population management is briefly discussed.

Papel de la estimulación macho-hembra en la biología de la langosta migratoria *Locusta migratoria migratorioides* (Reiche & Fairmaire) (Orthoptera, Acrididae)

Resumen: Se realizaron experimentos de laboratorio, bajo condiciones controladas, para determinar la influencia de la estimulación producida por los machos, sobre las hembras de la langosta migratoria *Locusta migratoria migratorioides*, fase gregaria. Se estimó el papel de la estimulación olfativa, visual e acústica, bajo tres tratamientos diferentes: A) Hembras apareadas B) Hembras virgenes y aisladas de machos C) Hembras virgenes, pero recibiendo estimulación olfativa, visual e acústica de machos. La longevidad de las hembras apareadas disminuyó de 2 a 4 días, en comparación con la de las hembras virgenes. No se registraron diferencias significativas en el número total de huevos, ni de paquetes de huevos, puestos por todas las hembras. Sin embargo, tanto el número de huevos infértiles, como de larvas que iniciaron, pero no completaron su desarrollo, fué significativamente más bajo para las hembras apareadas que para las virgenes. Estos experimentos fueron complementados con el análisis de sustancias volátiles emitidas por los machos, con acción feromonal. Se discute brevemente el papel de las substancias semioquímicas en la gestión de poblaciones de langostas.

INTRODUCTION

The migratory locust, *Locusta migratoria migratorioides* (Reiche & Fairmaire, 1850), remains as one of the most devastating pests, particularly in Africa. Research is in progress regarding the search for efficient means of locust monitoring and control (e.g. PAIVA & MARTINHO, 1992; WILPS & NASSEH, 1994). Behavioural chemicals play a decisive role in the life-cycle of Acrididae (e.g. DORN et al., 1994; FERENZ et al., 1994), and might become, in the near future, an important tool in population management schemes for species forming aggregates.

The males of some Acridid species produce pheromones of the primer type (WILSON, 1963), that act upon female physiology (e.g. LOHER, 1960; NORRIS, 1970; SCHMIDT & OTHMAN, 1994). In *Aiolopus thalassinus* (Fabr.), a male pheromone has been demonstrated to stimulate oviposition and egg pod formation through the activity of the corpora allata. This substance regulates the biosynthesis of juvenile hormone-III, which interacts with yolk uptake, and protein synthesis, in the oocytes (SCHMIDT & OTHMAN, 1993).

For *Schistocerca gregaria* (Forskal), male presence has since long been known to stimulate female oviposition (NORRIS, 1970). Recently, a volatile substance has been isolated from sexually mature males, which was neither present in the females nor in the nymphs, that strongly influences fecundity parameters and female longevity (SCHMIDT & ALBÜTZ,1994).

For the migratory locust, the mechanisms of olfactory communication and the effect of semiochemicals upon adult physiology are not yet known. This study aimed at the clarification of the role of male proximity, upon the biology and behaviour of females of *L. migratoria migratorioides.* Namely, if visual, olfactory and acoustical stimuli, emitted by males, would be able to influence the duration of the life-cycle, and / or fecundity and oviposition parameters, similarly to what has been reported for *S. gregaria* and *A. thalassinus.*

METHODOLOGY

Laboratory experiments were conducted under controlled conditions: T°= 25(± 2)° C; RH = 40(±20)%; photoperiod = 10:24 light hours. A culture of *L. migratoria migratorioides* was started in 1992, with locusts supplied by the Department of Zoology-Entomology, University of Hannover. The locusts were reared in cages containing females and males, the sex ratio being 1:1, as described by SCHMIDT (1986). All insects used thus originated from sexual reproduction, and had moulted to the adult stage within the previous 48 hours. Inside each cage, containers with moist sand were placed for oviposition. The eggs ob-

tained were incubated at 24°C. The locusts were fed daily, the diet consisting of germinated wheat from an hydroponic culture, and cereal flocks (Miluvite-multicereais).

Females were kept under different physiological conditions, either copulated (A), virgin and isolated from males (B), or virgin but receiving male stimulation (C). Eight replicates of each type of experiment were performed, by placing in separate cages the following insects: A - 10 females + 5 males. B - 10 virgin females, kept in isolation from males. C - 10 virgin females, separated from 10 males by a perforated transparent wall, through which visual, acoustic and olfactory stimulation could take place.

Observations were performed daily, until the last locust died. Since this study aimed at the obtention of population parameters, the statistical analysis performed was based on mean values, calculated for a batch of 10 females. Obviously in each experiment, the females died at different times. However, fecundity parameters were always calculated by dividing the number of egg pods (or eggs, or nymphs) obtained by 10, irrespective of the number of females that were still alive in the cage. The parameters quantified were: duration of the pre-oviposition period, defined as the number of days elapsing between the first day of the experiment, and the deposition of the first egg-pod, by any female in the cage; duration of the oviposition period, that is the interval between the oviposition of the first and the last egg-pod in each cage; duration of the post-oviposition period, referring to the interval between the oviposition of the last egg-pod and the death of the last female.

Longevity, that is the total number of days lived by each locust, was also recorded. A value for the mean longevity was calculated for each experiment, by adding the number of days lived by each female in a cage, and dividing by 10. For this reason, the parameter longevity cannot be obtained by adding the values referring to the duration of the pre-oviposition, ovipositon and post-oviposition periods. Additionally, the longevity of the longest lived locusts, in each type of experiment, was registered separately, and the mean calculated.

The statistical analysis could not be performed by ANOVA, due to heteroscedascity of the variances, which was detected using a Bartlett test (SOKAL & ROHLF, 1981). A non-parametric Kruskal-Wallis test was used instead, and Wilcoxon tests were further performed to assess differences between treatments (SOKAL & ROHLF, 1981).

RESULTS

Table 1 shows the mean values, and standard deviations, obtained for the duration of the pre-oviposition, oviposition and post-oviposition periods (co-

lumns I-III), as well as for the longevity in each type of experiment (columns IV-V). A significant difference was observed for the post-oviposition period (column III), which lasted 2,5 times longer in the virgin stimulated females than in either the mated, or the virgin isolated ones (Kruskal-Wallis test $p<0.07$; Wilcoxon test $p<0.09$). Mean longevity was, on average, 2 to 4 days shorter for mated females than for virgin ones (column IV). Male longevity (type A experiments) was not significantly different from that of the females, although it lasted on average, 2-6 days longer. Maximum longevity (column V) again indicates that virgin females lived slightly longer than mated ones. On the contrary, the longest lived males in each experiment had a life-span 8 to10 days shorter than the correspondent females.

Table 1

Mean values and standard deviations for the duration of the pre-oviposition, oviposition and post-oviposition periods (Columns I - III), and for adult longevity (Columns IV-V), of *L. migratoria migratorioides*, in three types of experiments: A - Mated females; B - Virgin females isolated from males; C - Virgin females stimulated by males, n = 80 (8 replicates X 10 locusts). ø, ¤ denote differences detected by the Kruskal-Wallis and Wilcoxon tests, respectively at p<0.07, and p<0.09 (Sokal and Rohlf, 1981).

TYPE OF EXPERIMENT		PERIOD (DAYS)			LONGEVITY (DAYS)	
		I Pre - oviposition	II Oviposition	III Post - oviposition	IV All locusts in each experiment	V Last locust to die in each experiment
A						
10 ♀♀+ 5 ♂♂	$\bar{x} \pm s$	10.8±6.3	75.9±12.9	9.4 ø±6.9	♀♀ 76.1±22.0	♀♀ 107.6±9.6
					♂♂ 82.2±26.7	♂♂ 99.1±21.2
B	$\bar{x} \pm s$	8.5±10.2	83.4±25.0	8.0 ø±12.0	♀♀ 78.4±30.1	♀♀ 109.6±19.6
10 ♀♀(virgin isolated)						
C	$\bar{x} \pm s$	7.6±8.2	76.4±15.0	22.4 ¤±14.4	♀♀ 80.6±27.7	♀♀ 110.8±13.9
10 ♀♀(virgin stimulated)						

Table 2 presents the mean values for fecundity parameters, and rates of survival of nymphs. There were no significant differences for either the number of egg pods (columns I-II), or the total number of eggs/ viable egg pod (column III), laid by the females of all types of experiments. However, both the number of infertile eggs (column IV), and of the nymphs that started developing, but did not hatch (column V), were significantly lower for the mated females, than for the virgin ones, either stimulated or isolated (Wilcoxon test, $p < 0.05$). In consequence, a significantly larger number of viable nymphs/egg pod, was produced by the mated females, in comparison with both types of virgin females (column VI).

Table 2

Mean values and standard deviations for fecundity parameters of females, and survival of nymphs of *L. migratoria migratorioides*, in three types of experiments: A - Mated females; B - Virgin females isolated from males; C - Virgin females stimulated by males, 8 replicates X 10 locusts / experiment. ø, ¤,¢ denote differences detected by the Kruskal-Wallis and Wilcoxon tests, respectively at p<0.01 and p<0.05 (Sokal and Rohlf, 1981).

TYPE OF EXPERIMENT	PERIOD (DAYS)			LONGEVITY (DAYS)		
	I N° total egg pods/ ♀	II N° viable egg pods/ ♀	III Total N° eggs /viable egg pod	IV N° eggs not developed/ viable egg pod	V N° Nymphs fully developed/ viable egg pod	N° viable nymphs / viable egg pod
A $\bar{x} \pm s$ 10 ♀♀ + 5 ♂♂	4.1±1.1	3.1±1.2	30.3 ±9.7	12.3 ø±12.3	2.7 ø±4.6	15.4 ø±14.1
B $\bar{x} \pm s$ 10 ♀♀ (virgin isolated)	3.3±1.6	3.0±1.5	29.1 ±9.1	23.1 ¤±10.0	3.8 ¤±4.6	2.2 ¤±3.7
C $\bar{x} \pm s$ 10 ♀♀ (virgin stimulated)	3.0±0.9	2.4±0.9	28.9 ±8.3	23.3 ¤±10.4	4.2 ¤±6.1	1.4 ¢±3.21

Figure 1 represents the pattern of oviposition, and the evolution of fecundity and survival parameters observed in one typical experiment only (not mean values), of each type performed. A general tendency was observed, regarding the chronology of oviposition: mated females laid over 64% of the viable egg-pods up to the 5th week, while virgin isolated females produced only 30%, and virgin stimulated females about 40% of egg-pods, during the same period.

DISCUSSION

Mated females of *S. gregaria* produced a larger number of egg pods than virgin ones, both under conditions of total isolation from males, or when stimulated by them (SCHMIDT & ALBÜTZ,1994). Such results do not agree with the present experiments performed with *L. migratoria migratorioides*, since no differences were observed for this parameter. A set of experiments is presently being conducted, to determine if additional male stimulation of virgin females, through anthenal contact, would influence fecundity parameters. Comparable experiments with *A. thalassinus*, showed that fecundity was influenced by a male produced pheromone, independently from mating (SCHMIDT & OTHMAN, 1994).

The number of nymphs emerged from eggs laid by mated females was larger than that produced by virgin females, a feature which has been consignated in the literature (e.g. UVAROV, 1966) and is in accordance with recent work ca-

rried out with *A.thalassinus* (SCHMIDT & OTHMAN, 1993) and *S. gregaria* (SCHMIDT & ALBÜTZ, 1994).

Virgin females of Acrididae normally live longer than mated ones (e.g. SCHMIDT & OTHMAN, 1994), as observed in the present study. This has been attributed to a delay in ovarian maturation (e.g. UVAROV, 1966). Our results do not support such an explanation, since no significant differences were detected between the pre-oviposition periods of virgin and mated females. However, due to individual physiological variations, oocyte volume (which was not measured), is a better indicator of JH level of activity, and thus of vitellogenesis progression, than age after eclosion (SCHMIDT & OSMAN, 1990). Nevertheless, for mated, virgin isolated, and virgin stimulated females, typical patterns of oviposition were observed - Figure 1. This general trend, showing that virgin females normally have their oviposition peak 2 to 3 weeks later than mated ones, agrees with similar findings for *A. thalassinus* (SCHMIDT & OTHMAN, 1994).

The clarification of the role of the nervous system of locusts, in pheromone production is in progress. Pheromone biosynthesis activating neuropeptides (PBANs) have been isolated from the brain of several species, and shown to stimulate pheromone biosynthesis (e.g. RAINA & KEMPE, 1990; SRENG et al.,1990; SCHOOFS *et al.,* 1991). Pheromones emitted by males, which act upon female hormone titters, and concomitantly stimulate egg production, have been detected in *A. thalassinus* (SCHMIDT & OTHMAN, 1994) and *S. gregaria* (FRANCKE & SCHMIDT, 1994; SCHMIDT & ALBUTZ, 1994).

For *L. migratoria*, a comparative analysis of the volatile compounds emitted by nymphs, males and females, has been initiated. So far, several substances were detected which are mainly produced by one stage, or sex (FRANCKE, 1995; MATEUS et al., in prep.). Sexually mature males produce benzylcyanid, which was neither detected in the immature stages, nor in the females. The role of this substance in the process of olfactory communication in *L. migratoria migratorioides* remains to be elucidated.

Given the ecological constraints shaping the bio-ecology of the migratory locust, an efficient control of this pest can only be achieved within a broad scheme of integrated pest management. However, taking into account recent findings (eg. FRANCKE & SCHMIDT, 1994), it is generally accepted that semiochemicals should, in the near future, play a role in the monitoring and control of locusts' populations.

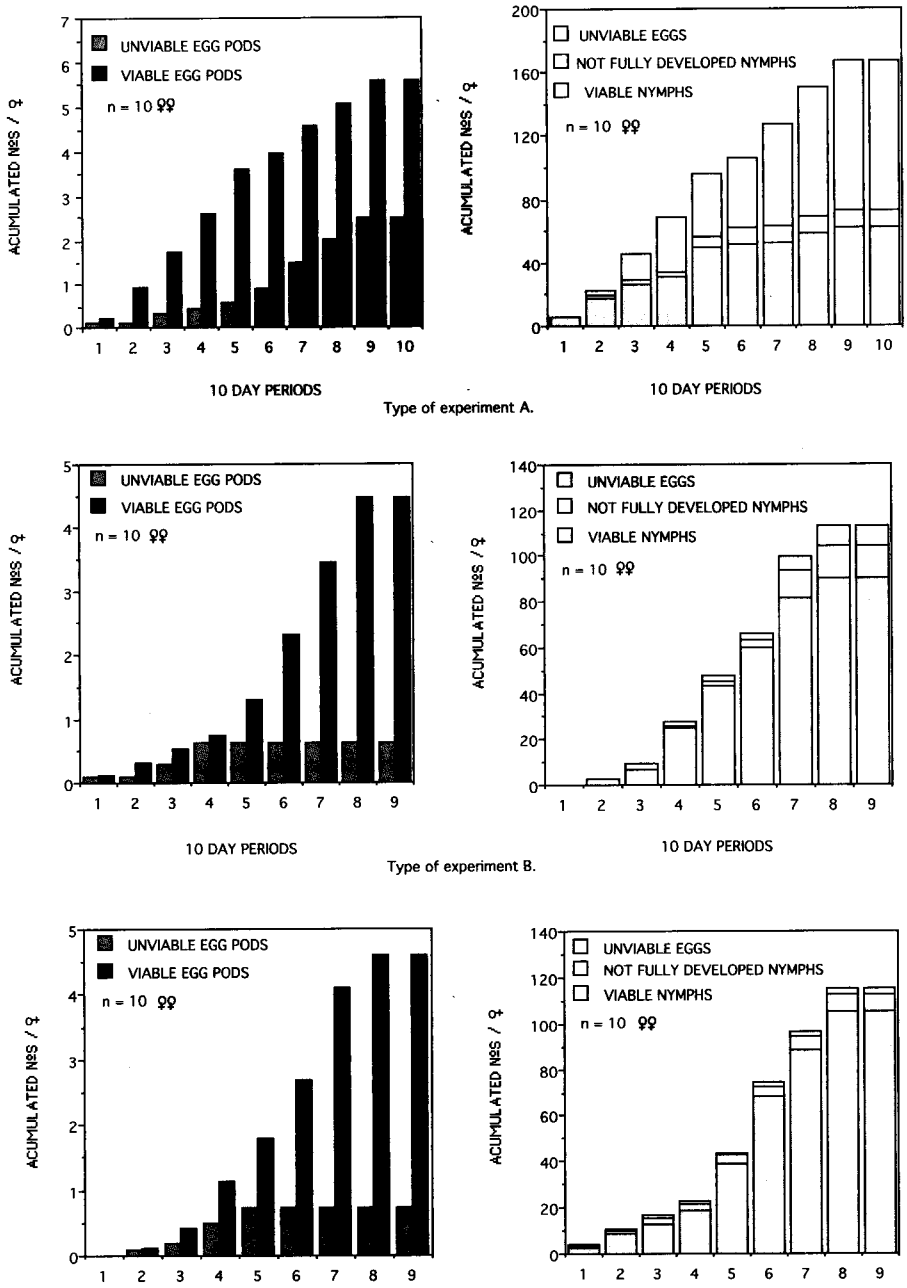

Figure 1. Chronology of the pattern of oviposition, and evolution of fecundity and survival parameters for *L. migratoria migratorioides*, observed in one typical experiment (not mean values) of the following types: A - Mated females; B - Virgin females isolated from males; C - Virgin females stimulated by males.

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