

Effects of no mating on the female adults and embryonic development of *Schistocerca gregaria* (Orthoptera: Acrididae)

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The newly emerged adult females of *Schistocerca gregaria* were separated and kept in the rearing conditions without males to examine the detailed developmental characteristic of the no mating females and carefully observed the developmental progress of non-fertilised eggs laid by virgin females. The results showed that the females kept without males survived in average 43 d, i.e. 58% of the life span of mated controls, and laid correspondingly fewer egg pods. The size of egg batches, the weight of eggs, and their content of ecdysteroids were normal but the hatch rate was reduced to 18.27%. Blocks of early embryogenesis (58.43%) and hatching failures (23.29%) were the major causes of death. Moreover, most of the hatched insects died during the postembryonic development and only a few females reached the adult stage. All insects that developed until the penultimate or last larval instar or reached the adult stage were females.

virgin, life span, ecdysone, embryogenesis, locusts, parthenogenesis

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The primary purpose of mating in most insects is the transfer of sperm and secretions of the male accessory glands in to females for procreation. Mating has been shown to directly affect female life span, egg production rate, female fertility, etc. [1]. Frequency of mating depends on the life styles of insects. The desert locust, *Schistocerca gregaria*, is a disaster pest in the world. It has two morphisms: solitary and gregarious. Since solitary locusts live alone and avoid others, chances to find a suitable mate are less. So what kind of developmental fate and characteristic are there for no mating females and the eggs laid by them? No detailed reports can answer the question until now.

In our established gregarious culture of *S. gregaria*, where females had 110–130 ovarioles and deposited up to 6 egg pods, with 45–55 eggs each of which about 16% did not develop because of un-fertilization [2,3]. In this study, we used these locust line and artificial controlled their mating

behavior to determine the developmental fate and characteristics of virgin females that do not find a mate and therefore produce unfertilized eggs and the developmental progress of non-fertilised eggs laid by them. This will be helpful to understand the characteristics of solitary locust more detailed and to find a new way or method to control the pest efficiently in the future.

1 Materials and methods

Gregarious culture of the desert locust, *Schistocerca gregaria* (Forskål), was maintained at 30°C and 12 h photoperiod as described by Li et al. [4]. Newly emerged adult females were removed from the rearing cage into a separate container. Cages with adult locusts (both sexes or only females) were provided with plastic tubes (10 cm long and 5 cm in diameter) filled with sterile moist sand, where the females deposited their eggs. Tubes with the egg pods were

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removed from the breeding cages and covered with a polypropylene foil to retain high humidity. They were kept in dark in a thermostat until the hatching time and were inspected.

To examine the embryos, eggs were soaked in 3% sodium hypochlorite until the eggshell became transparent and then rinsed in distilled water. Developmental stage of embryos was assessed according to Shulov and Pender [5] and Tyrer [6]. Ecdysteroids were quantified in the egg homogenates as described previously [7], using antiserum 85-B/L2 from Dr. P. Maróy. [23,24-³H] ecdysone (New England Nuclear, specific activity 3.3 Tbq/mmol) was used as ligand and 20-hydroxyecdysone (20E) as a reference standard. The ecdysteroid content was expressed in ng 20E equivalents per egg.

All the statistical analyses were performed using SuperANOVA [8]. Data was reported as mean \pm S.E.

2 Results

2.1 Developmental characteristic of non-mated virgin females

Data in Table 1 demonstrated that mating had positive effect on female survival and fertility. Life span of females reared without males was 60% shorter than those with males. Similarly, hatch rate was significantly reduced in eggs laid by un-fertilized females (Table 1), while the number of eggs per pod, egg weight, and ecdysteroid content were similar between the two groups. The oviposition patterns of the fertilised and non-fertilised females were initially similar followed by a reduction in the number of deposited egg pods in the non-fertilised females which may be due to their shorter life (Figure 1).

2.2 Developmental process of the eggs from non-mated females

Development of individual eggs differed considerably. In some of the egg pods, no embryo completed development to hatching (0% hatch rate), while in some as many as 73% of them hatched successfully (Table 2). Dissections of the

non-developing eggs revealed that their development ceased at various stages of embryogenesis (data not shown). Death also occurred frequently at the time of hatching: some larvae failed to escape from the eggshell, while others perished when attempting to shed off the embryonic exuvia. Most of the eclosed larvae died in different larval instars and only very few (about 0.23%) managed to develop until the adult stage. All insects that developed until the penultimate or last larval instar or reached the adult stage were females.

3 Discussion

3.1 Low egg production rate in virgin females

The effect of mating that results in increased female egg production rate, has been reported in many insects, such as *Requena verticalis* [9], *Drosophila melanogaster* [10], *Coelopa frigida* [11], *Phthorimaea operculella* [12] and *Chrysochus cobaltinus* [13]. It has been reasoned that stimulation of female egg production by mating usually resulted from one or more of three factors: the act of mating per se, presence of sperm and transfer of accessory substances [1]. In this study, there was a marginal increase in the number of egg pods laid by mated females (5.6)

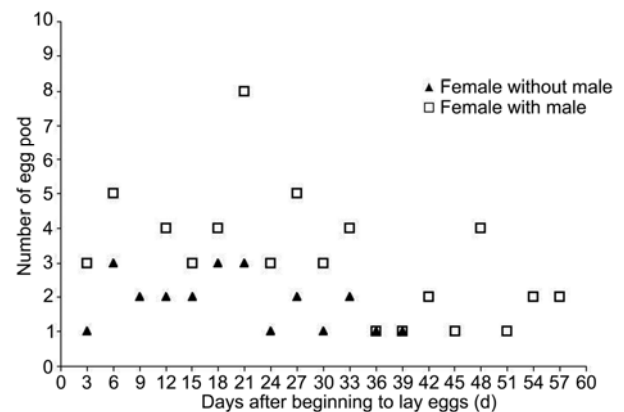


Figure 1 Numbers of egg pods deposited during the entire lifetime by ten females kept alone (solid triangles) and by ten females kept with equal number of males (empty squares).

Table 1 Performance (mean \pm S.E.) of females reared with or without males from the first day after emergence ^{a)}

Parameter	Only females, no males	Females and males
Insect number	10♀	10♀ + 10♂
Total egg pods/female	3	5.6
Mislaid egg pods/female	0.375	2
Eggs per one pod	44.60 \pm 3.23a	49.03 \pm 2.12a
Weight of fresh egg in mg	9.60 \pm 0.11a	9.51 \pm 0.12a
Hatch rate (%)	18.07 \pm 5.96a	79.15 \pm 3.71b*
Life span (days)	43.13 \pm 2.86a	74.40 \pm 2.96b*
Number of ovarioles/female	111.30 \pm 2.19a	121.90 \pm 4.47a
Ecdysteroid content (ng/egg)	101.01 \pm 10.46	129.71 \pm 12.64#

a) * $P < 0.0001$, extremely significant. # $P = 0.0823$, marginally significant. The different alphabet after the number means significant. Mislaid egg pods mean that the pods were not laid in the tube provided for female adults to lay eggs. Ecdysteroid content was assayed on day 2 after egg laying.

Table 2 Development of 15 egg pods from females reared without males^{a)}

Egg pod	No. of egg	% Early death	% Pharate death	% Hatch	% 1 Instar	% 2 Instar	% 3 Instar	% 4 Instar	% 5 Instar	% Adult	Sex
1	33	75.76	12.12	12.12	3.03	0	0	0	0	0	
2	17	76.47	11.76	11.76	23.44	6.25	6.25	6.25	1.56	0	♀
3	47	48.94	12.77	38.30							
4	44	9.09	68.18	22.73	22.73	4.55	0	0	0	0	
5	57	54.39	38.60	7.02	7.02	0	0	0	0	0	
6	52	19.23	7.69	73.08	32.22	7.78	3.33	2.22	2.22	0	♀
7	38	26.32	63.16	10.53							
8	63	19.05	15.87	65.08	25.40	11.11	9.52	9.52	7.94	1.59	♀
9	32	75	15.63	9.38	4.90	2.94	2.94	2.94	2.94	0.98	♀
10	38	94.74	5.26	0							
11	32	62.5	31.25	6.25							
12	49	100	0	0	0	0	0	0	0	0	
13	25	64	36	0	0	0	0	0	0	0	
14	39	97.44	2.56	0	0	0	0	0	0	0	
15	28	53.57	28.57	17.86	17.86	0	0	0	0	0	
Mean± S.E.	39.6± 3.23	58.43±7.62	23.29±5.40	18.27±5.95	12.42±3.64	2.97±1.19	2.00±0.98	1.90±0.97	1.33±0.74	0.23±0.16	

a) The data about larval stages were combined together in some egg pods because they were laid in the same tube, such as Nos. 2 and 3; Nos. 6 and 7; Nos. 9, 10 and 11; "Early death" means that the egg died at various stages of embryogenesis before hatching; "Pharate death" means that the egg died at the time of hatching.

versus those laid by virgin females (3) of *S. gregaria*. In addition increase in the number of eggs per pod between mated females (49.03) and virgin (44.6) was also obvious. However, we did observed an increase in the life span of mated females by about 31 d which would allow more time for egg maturation and the number of egg laid by mated females than virgin females, that could contribute to an increase in female egg production.

3.2 Reduced female life span in virgin females

The no mating female life span was 43.13 d, much less than mating female life span, 74.40 d, in *S. gregaria*. This phenomenon was also reported in many species, for example, *Gryllus veletis* [14], *Pieris napi* [15]. One of reasons to increase mating female life span is the large number of accessory substances transferred to females with the male ejaculate [14,15]. While the solitary virgin desert locusts could not get any accessory substances from males, this may be cause them had a relatively shorter longevity.

3.3 Low egg hatch rate and parthenogenesis

The hatch rate of no mating female's eggs was 18.07%, while that of mating female's eggs was 74.40%. Although the hatch rate of no mating female's eggs was low, some eggs indeed can develop to 5 instar larvae or adults, it showed that parthenogenesis happened in our gregarious culture of *S. gregaria*.

Parthenogenesis has been found in almost all insect orders except Odonata and a few small orders [16]. In the case of facultative parthenogenesis, which occurs in several spe-

cies of locusts [17,18] and various other insects, the eggs can develop with or without fertilisation. Facultative parthenogenesis was recently studied in detail in the cockroach *Nauphoeta cinerea* by Corley et al. [19]. It was found that only a few females had the ability to switch from making eggs that do not develop without fertilisation to the production of zygotes that develop in the absence of sperm. The development of non-fertilised eggs was usually interrupted shortly after the initiation of embryogenesis, presumably as a consequence of the duplication of chromosomes that carried recessive lethal mutations. The homozygosity of the maternal chromosome set, which is typical for the automictic (meiotic) parthenogenesis, is probably the major cause of developmental disturbances. Abnormal deposition of the maternal and lack of the paternal determinants is another probably source of developmental defects.

The observations on *N. cinerea* are in good agreement with our results. We found that the developmental capacities of eggs laid by virgin females differed considerably. No embryo completed development to hatching in some of the egg pods (0% hatch), while in others more than 70% of them successfully hatched (Table 2). Developmental block occurred mostly at a very early stage, as expected in the case of severe genetic defects. The rate of embryonic development did not seem to be altered until the stage when it came to a halt. The death was secondary and occurred in some cases long after the controls had hatched. Other insects died at hatching or during larval development, often as a consequence of ecdysial failures. These insects probably perished because certain functions controlled by the mutated genes could not be properly exercised.

A few daughters of virgin females survived in our ex-

periments until the adult stage and were probably able to establish parthenogenetic line as described by Schmidt [17]. Their survival apparently depended on the elimination of recessively lethal genes whose effect had been overcome in the original bisexual population by the wild alleles provided by the sperm. Only exceptionally may crossing-over recombination during oocyte meiosis yield a genome deprived of the life-threatening mutations and this explains the paucity of successful progeny development in the first parthenogenetic generation. Enormous variation in the developmental fate of eggs from different egg pods can be explained by differences in the genetic load of individual females. Duplication of the maternal haploid genome included the X chromosome and thereby determined exclusively female sex of the progeny.

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