The Cost and Benefice of Multiple Mating in Desert Locust Schistocerca gregaria

Dushimirimana Severin

Abstract: A better understanding the evolution of female desert locust multiple mating role is key to understand locust phase polymorphism and locust outbreaks. In this study, we analyse the cost and the benefice of multiple mating in desert locust Schistocerca gregaria. We demonstrated that female desert locust fecundity and fertility is not affected by multiple copulation. We found that multiple mating affect female lifespan where we observed an increase in mortality in females mated with more than one male comparatively to females mated with a single male. We found that survival of offspring from females mated with two, three, four males remain higher and our results show that about 90% of larvae reach at imago stage while only 74% of larvae reach at imago stage in females mated with single male. The sense of this result could be male-derived nutrients which are incorporated in female eggs thereafter in hatchlings. Multiple mating in S. gregaria acts as hormonal factor which controlling egg size and eggs contain which acted on offspring condition with a really impact on survival.

Keywords: desert locust, multiple mating, Schistocerca gregaria, offspring.

INTRODUCTION

For millennia, farmers have been confronted with swarms of locusts capable of spectacular migrations and which represent major threats to their crops. More than 1200 species of locusts are known in the world, of which 500 species have economic consequences in agriculture. Twenty species are among crop pests especially in Africa [1].

The main species are the desert locust (Schistocerca gregaria), the migratory locust (Locusta migratoria), the nomadic locust or red locust (Nomadacris septemfasciata), the tree locust (Anacridium melanorhodon), the Mato Grosso locust (Rhamatocerus schistoceroides) and the Brazilian locust (Chromacris speciosa) [2]. These species have a common characteristic: phase transformation from a solitary phase to a gregarious phase which is responsible for the invasion during which bands of larvae evolve in swarms causing considerable damage. These species have also the possibility of presenting varied and reversible aspects according to living conditions. This polymorphism is essentially linked to the conditions of the social environment of each species [3]: in the presence or isolated from its congeners [4]. The main triggering factor is the density of individuals [5].

Among these species, our study focused on desert locust S. gregaria, a polyphagia specie which consumes its own weight of fresh food per day [6]. Normally solitary, these locusts from the Saharan and sub-Saharan regions reproduce when certain ecological and meteorological conditions are met [7, 8], to become gigantic swarms which attack all types of crops [9]. These swarms migrate to desert and semi-desert countries in Africa and Asia causing enormous economic consequences.

The females of S. gregaria have multiple mating with various males. The benefits for female of multiple mating in Orthoptera have been shown to have many functions as prevent the females from sperm depletion [10], increasing the rate of oviposition [11-13] or provide material incorporated into eggs [14, 15]. However, until now, adaptive significance of polyandry is poorly understood in S. gregaria.

In their study on behavioural analysis of phase change, Simpson et al. [16] have concluded that female locusts can predict the probability that their offspring will emerge into a high-density population, and alter the development of their embryos accordingly through a gregarizing agent added to the foam that surrounds the eggs at laying.
Recently, it was discovered that progeny characteristics such as hatching body size and color are pre-determined in the ovaries of the mother in *S. gregaria* [17-19].

In desert locusts, it has been shown that males presence (with or without mating) increased female activity, which stimulates development and oogenesis [20, 21] and increases clutch size [22].

In this study, we analysed the cost and the benefice of multiple mating in desert locust *S. gregaria*. Concretely the effects of multiple mating on fecundity and fertility are analysed. Paternal and maternal investments of multiple mating are examined as females and offspring longevity.

**MATERIALS and METHODS**

*Insect rearing*

Mass rearing of gregarious *S. gregaria* was conduct in cages (dimensions: 50X60X80 cm) containing 100 to 200 locusts per cage. Rearing temperatures were 35°C ± 2 °C (12h day) and 28°C± 2°C (12h night) with a relative humidity of 60%. Locusts were fed on fresh cabbage leaves and rolled oats. Mature females and mature males were collected in cages few days before sexual maturation and separated by sex to ensure virgin status.

*Mating test*

Mature females were placed individually with mature males in experiment cage for mating. These cages were observed until the male disengaged his genitalia and males were then removed to prevent remating. To ensure that mated females were inseminated, only females that copulated for more than 1h (according to our previous results: Dushimirimana *et al.* [23] were taken. Among these females (n= 63) have been placed in cage to determine the number of eggs (fecundity) and the number of nymphs (fertility) for each female until the females died. Plastic cups filled with clean moist sand were placed in cages to collect egg pods. Egg pods collected were incubated at 30°C. The once mated remaining females were marked with paint for the remating tests.

Marked mated female were placed with a new virgin male 24 hours after the first mating. After the second copulation, females were allowed to lay egg till death. Third and fourth rematings were conducted in the same way. A group of females (n= 47) was allowed to mate and stay with a group of four males. Fecundity and fertility were examined.

Polyandrous females, used in remating test, were monitored every day and dead individuals were counted for longevity. Survival of offspring produced by multiple mating or single mating was also determined until imaginal moult. Offspring were maintained in the same conditions of rearing as explained above and dead individuals were counted.

**RESULTS**

In our laboratory experiences, we tested the cost and the gain for a female *S. gregaria* to accept the mating with several males. Five treatments have been analysed during this survey: a female mated with only one male, two, three and four males. We also analysed a female remained with four males to test the influence of the presence of a male.

**Fecundity**

There is no significant difference in fecundity among females mated with single male or multiple mated. Indeed, during the first clutch, the mean fecundity is 66.5±12.6 eggs in females mated with a single male. 63.8±9.6 eggs in females mated with two males, 57±7.2 eggs in females mated with three males, 62.8±11.1 eggs in females mated with four males 63±6.5 eggs in females with four males (One-way Analysis of Variance P=0.43) Table 1.

In second clutch, the mean fecundity is 64±6.6 eggs in females mated with a single male, 53 ±6.5 eggs in females mated with two males, 61±10.5 eggs in females mated with three males, 53 ±15.1 eggs in females mated with four males 55.8 ± 3.8 eggs in females with four males ( One-way Analysis of Variance P =0.49) Table 1.

In third clutch, the mean fecundity is 58.5±7.7eggs in females mated with a single male, 54.5± 3.5 eggs in females mated with two males, 56 ±2.8 eggs in females mated with three males, 49 ±7 eggs in females mated with four males 47.5± 6.3 eggs in females with four males ( One-way Analysis of Variance P =0.38 ) Table 1.

| Treatment | First clutch | Second clutch | third clutch |
|-----------|-------------|--------------|-------------|
| Female mated with a single male | 66.5±12.6 | 64±6.6 | 58.5± 7.7 |
| Female mated with two males | 63.8±9.6 | 53 ±6.5 | 54.5± 3.5 |
| Female mated with three males | 57±7.2 | 61±10.5 | 56 ±2.8 |
| Female mated with four males | 62.8±11.1 | 53 ±15.1 | 49 ±7 |
| Female &4 males | 63±6.5 | 55.8 ± 3.8 | 47.5± 6.3 |
| One-way Analysis of Variance | P | 0.4301 | 0.4995 | 0.3871 |
Fertility
In three clutches, we found also no significant difference among treatment (Table 2). There is no significant difference in fertility among females mated with single male or multiple mated. Indeed, during the first clutch, the fertility is 90.2 ± 2.6 larvae in females mated with a single male, 91.7 ± 4.6 larvae in females mated with two males, 88.6 ± 5.2 larvae in females mated with three males, 90.7 ± 7.1 larvae in females mated with four males 88.3 ± 3.5 larvae in females mated with five males (One-way Analysis of Variance P = 0.78) Table 2.

In second clutch, the mean fertility is 85.4 ± 2.6 larvae in females mated with a single male, 92.6 ± 4.5 larvae in females mated with two males, 88.9 ± 10.5 larvae in females mated with three males, 87.8 ± 8.1 larvae in females mated with four males 89.3 ± 4.8 larvae in females mated with four males (One-way Analysis of Variance P = 0.77) Table 2.

In third clutch, the mean fertility is 80.2 ± 2.7 larvae in females mated with a single male, 83.4 ± 6.5 larvae in females mated with two males, 79.2 ± 6.2 larvae in females mated with three males, 89.5 ± 6.3 larvae in females mated with four males (One-way Analysis of Variance P = 0.6) Table 2.

Table 2: Fertility (hatchling rate %)

| Treatment               | First clutch | Second clutch | Third clutch |
|-------------------------|--------------|---------------|--------------|
| Fertility (hatchling rate %) | 90.2 ± 2.6   | 85.4 ± 2.6    | 80.2 ± 2.7   |
| Single mated            |              |               |              |
| 2 Mated                 | 91.7 ± 4.6   | 82.6 ± 4.5    | 83.4 ± 6.5   |
| 3 Mated                 | 88.6 ± 5.2   | 88.9 ± 10.5   | 81.3 ± 2.8   |
| 4 Mated                 | 90.7 ± 7.1   | 87.8 ± 8.1    | 79.2 ± 6.2   |
| Female & 4 males        | 88.3 ± 3.5   | 89.3 ± 4.8    | 89.5 ± 6.3   |
| One-way Analysis of Variance | 0.7830      | 0.7707        | 0.6089       |

Female's longevity
Our results show a difference between the five treatments (With a log-Lank test, chi-square: 36.18 df: 4 P: 0.0056) (Fig.1). We found that single mated females survival is 62% in 40 days of observation while those mated with two males died, with a survival of 18% in 40 days. Mortality increase since 10 days with females mated with more than one male. There is no survival at 40 days in groups of females which remain with 4 males or mated with 4 males (Figure 1).

Offspring survival
Our results show that survival of offspring from females mated with a single male is about 30 days and then begun to die. Mortality increase and only 74% of larvae reach at imago stage. The survival of offspring from females mated with two, three, four males remained higher and our results show that about 90% of larvae reach at imago stage (Figure 2).
**DISCUSSION**

A better understanding the evolution of female desert locust multiple mating role is therefore key to understand locust phase polyphenism and locust outbreaks. The mechanism underlying the phase polyphenism in desert locust have been studied since many years and various aspects of this phenomenon has been clarified [24, 1, 25, 26, 27, 3, 28], but desert locust multiple mating have entail little attention in the reproduction strategies of this specie. Polyandry is known to be fitness successful strategies [29, 30, 31, 32]. In this paper, we experimentally varied the rate of copulation with different males in gregarious desert locust. We analysed female desert locust fecundity and fertility, females and offspring survival in order to understand the cost and beneice of desert locust multiple mating.

Our results suggest that multiple mating is not associated with fitness benefits. We have demonstrated that female desert locust fecundity and fertility is not affected by multiple copulation. We found that multiple mating affect female lifespan where we observed an increase in mortality in females mated with more than one male comparatively to females mated with a single male. Tetsuhiro et al. [33] have demonstrated that multiple mating reduces longevity of females, our results show that it is also the case in *S. gregaria*. We found that survival of offspring from females mated with two, three, four males remain higher and our results show that about 90% of larvae reach at imago stage while only 74% of larvae reach at imago stage in females mated with single male.

The sense of this result could be male-derived nutrients which are incorporated in female eggs thereafter in hatchlings as shown in other species [11, 34, 35]. Our idea is sustained by the research on the function of egg yolk [36, 37].

Even though multiple mating in orthopteran species has been explained in terms of direct effect as to maximize fitness: fecundity and fertility enhancement, increase female lifespan and offspring survival [11, 38, 12, 13, 39, 29, 40], this study demonstrate that generalization cannot be made. Multiple mating has another explanation in *S. gregaria*. We found an increase of offspring survival from females mated with many males which survive longer than those from females mated with one male. We can conclude that multiple mating in *S gregaria* acts as hormonal factor which controlling  eggs contain which acted on offspring condition with a really impact on survival.

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