Comparison of reproductive traits of regular and irradiated male desert locust Schistocerca gregaria (Orthoptera: Acrididae): Evidence of last-male sperm precedence

Severin Dushimirimana[1,*], Thierry Hance[1] and David Damiens[1]

[1]Biodiversity Research Centre, Earth and Life Institute, Université Catholique de Louvain, 4-5 Place Croix du Sud 1348, Louvain-la-Neuve, Belgium

*Author for correspondence (dusev2001@yahoo.fr)

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Summary

The sterile insect technique (SIT) is increasingly used to control pest insect populations. The success of SIT control programs depends on the ability to release sterile males and on the capacity of sterile males to compete with wild males to inseminate wild females. In this study, we evaluated the mating performance of Schistocerca gregaria (Försk.) males irradiated with 4 Gray. We compared reproductive traits, such as duration of precopulation time, mating duration, quantity of sperm stored by females after copulation, number of females mated successively and postmating competition of irradiated males with non-irradiated males. Irradiated males were able to mate but the resulting number of offspring was dramatically reduced compared to the average number of offspring observed during a regular mating. During a single copulation, irradiated males transferred fewer sperm than regular males but, theoretically, this quantity is enough to fertilize all the eggs produced by a female during its reproductive life. Irradiated males also had the ability to remove sperm from a previous mating with unirraditated males. This new information on the mating strategies helps explain the post-copulation guarding behaviour of S. gregaria.

Key words: Last-male sperm precedence, Irradiation, Sperm management, Schistocerca gregaria

Introduction

Sterilised males are classically used to control pest populations through the Sterile Insect Technique (SIT), (Klassen, 2000; Dyck et al., 2005). The SIT is, "a method of pest control using area-wide inundative releases of sterile insects to reduced fertility of a field population of the same species," (International Plant Protection Convention, FAO, 2005). To efficiently reduce the potential fecundity of female populations, sterilized males must be able to copulate efficiently with several females and to compete efficiently with wild males. Therefore, the development of successful control programs requires robust studies on the reproductive traits (such as male mating ability, reproductive success and sperm transfer) of irradiated males compared to non-irradiated males. For instance, the mating success of irradiation-sterilised male tsetse flies Glossina palpalis (Opiyo, 2001) and sweetpotato weevils (Kumano et al., 2008) was evaluated before the implementation of the respective SIT programs (Calkins and Parker, 2005; Bakri et al., 2005).

Schistocerca gregaria (Försk.) (Orthoptera, Acrididae) and other locusts and grasshoppers are major insect pests in Africa, particularly in the Sahelian zone (Uvarov, 1966; Popov et al., 1991; Showler, 1995; Pener and Yerushalmi, 1998), so methods for controlling S. gregaria could have considerable value. Coggins (Coggins, 1973) irradiated male S. gregaria with 4 Gray and found that the hatching success of eggs from matings with irradiated males was only 17.9% whereas the hatching success from eggs resulting from matings with regular males was 67.5%. Coggins suggested that low doses may cause cellular abnormalities, such as cell breakdown, delay of cell division and mitochondrial disturbances.

Schistocerca females copulate several times during their lifetime, both before and after oviposition (Hunter-Jones, 1960), and significant mating competition has been observed (Hunter-Jones, 1960; Seidelmann and Ferenz, 2002); thus, the impact of irradiated males on female fecundity could be limited by a subsequent copulation with a regular male. However, if the irradiated males have the ability to remove sperm from a previous mating, then a reduction in fecundity might still occur. We tested how irradiating males with a dose of 4 Gy affects reproduction by S. gregaria. First, the effects of irradiation on male survival and fertility were compared to the results obtained by Coggins (Coggins, 1973). Male mating ability, as defined as the duration of precopulation period, mating duration, and number of sperm stored per females after copulation were assessed. The number of successive matings was also measured for both types of males. Finally, we investigated the impact of two consecutive copulations with regular and irradiated males on female fecundity.

Materials and Methods

Insect rearing

S. gregaria individuals were provided by the Laboratory of Entomology of the Zoological Institute of KUL (Leuven, Belgium). These individuals were descended from locusts originally collected in Nigeria (Hoste et al., 2002). Mass rearing of gregarious S. gregaria was conducted in cages containing 50 to 100 locusts per cage. Rearing temperatures were 35℃±2℃ (12h day) and 28℃±2℃ (12h night) with a relative humidity of 50%. Locusts were fed on fresh cabbage leaves and rolled oats. Mature females deposited eggs in pots filled with slightly moistened sterile sand. Eggs were collected once weekly and transferred to a new cage. Non-irradiated individuals used in mating experiments were collected from cages before sexual maturation and separated by sex to ensure virgin status.

Irradiation

Locusts were exposed to gamma days generated by a cobalt-60 source (Compact Cell) at the Sterigenics facility in Belgium. Insects were placed in the middle of a chamber to maximise dose uniformity within the batch.An irradiation dose of 4 Gy was applied 10 days after the imaginal moult, about 4 days before sexual maturation. The developmental time of S. gregaria is about 58 days (Duranton and Lecoq, 1990) and in our conditions, after the imaginal moult, sexual maturation takes 14 days.

Survival and fertility of irradiated males

Fifty irradiated males and 18 non-irradiated males were placed into two separate rearing cages and monitored daily for mortality. To assess the fertility of irradiated males, 18 individuals were each paired with a virgin female. If copulation occurred, females were isolated after mating in individual cages containing a sand pot for oviposition. Pots were examined daily. Numbers of offspring emerging each day per pot were recorded.

Sperm transfer

To determine the quantity of sperm transferred during a copulation, regular virgin males (n=16) and irradiated virgin males (n=16) were individually placed with a virgin female. Precopulation time (time between pair formation and copulation) was recorded. After copulation, inseminated females were dissected in a saline solution (PBS, Phosphate buffered saline, 137 mM NaCl, 2.7 mM KCl, 10 mM Sodium Phosphate dibasic, 2 mM Potassium Phosphate monobasic, pH=7.4) to count the quantity of sperm transferred per mating. The receptaculum seminis and the complete spermathecal duct were removed and put in an Eppendorf® containing 1 ml of a PBS solution. They were slightly ruptured using a pestle and homogenized by drawing the solution 10 times through a syringe. Three 20 µl-drops of the homogenised solution were deposited on a clean microscopic slide. The slides were then dried at room temperature prior to fixation in ethanol and stained 10 min in a solution of DAPI (4′,6-diamidino-2-phenylindole dihydrochloride, 2 µg/ml). The number of sperm present in the storage organs was counted using a fluorescence microscope (×200 magnification) (Bressac and Chevrier, 1998; Damiens and Boivin, 2005). To determine if mating duration influences the number of sperm stored by females, the copulation durations were recorded. Moreover, some additional pairs were interrupted 30 (n=5), 60 (n=10), 120 (n=7) and 180 min (n=6) after the beginning of copulation and females were dissected to count the amount of stored sperm.

Male mating capacity

To characterize male mating capacity of S. gregaria, regular (n=10) and irradiated (n=8) mature males were provided with a succession of mating opportunities. We presented each male with one female per day during six consecutive days. The first day, a virgin male was placed in a cage with one virgin female. After copulation, the female was removed and dissected. The following days, previously mated males were offered virgin females. Males that did not copulate after one hour were discarded and not tested the days after. Females were dissected 2 hours after being mated to analyse spermatheca content.

Consequences of female re-mating

The egg hatch rate was recorded for females that mated with regular males followed by a copulation with irradiated males one day later (RM+IM, n=18) and for females that mated with irradiated males first followed by a copulation with regular males one day latter (IM+RM, n=18). The numbers of offspring that reached adult stage under these two treatments were compared with offspring of females inseminated by one regular male alone (n=17) or one irradiated male alone (n=19).

Results

Survival and fertility of irradiated males

Clear differences between the survival of irradiated and regular males were observed (10.42±2.1 *vs* 25.2±11.0 days; Unpaired *t*-test with Welch correction; df =33, t=7.6, $P<0.001$). All regular (n=18) and irradiated (n=20) males were able to inseminate the presented females. Egg laying was normal for all females; however, females inseminated by an irradiated male had a very low hatch rate compared to regular males (3.4±2.2 *vs* 45.1±4.1 larvae, unpaired t-test with Welch correction, df=24, t=37.33, $P<0.001$).

Sperm transfer

The precopulation time was similar for pairs involving irradiated or regular males but the copulation duration appeared to last significantly longer in irradiated males (Table 1). For both treatments, the number of sperm per spermatheca increased at a constant rate with copulation duration (Fig. 1, Pearson rank correlation: $r^2=0.842$, n=54, $P<0.001$). The mean number of sperm transferred by irradiated males (15,359±8,935) was significantly lower than for regular males (24,040±6,887) (Table 1).

Male mating capacity

After sexual maturation, males were able to mate with a mean of 3.70±2.11 females for regular males and with 2.87±0.83 for irradiated males (NS, t test with Welch correction, t=1.13; df =12; P=0.28). For both treatments, males transferred the same quantity of sperm during the first two matings with a clear decrease for the following ones (Fig. 2).

Female re-mating

Results of the four treatments were significantly different (Kruskall Wallis test; KW =63.05; $P<0.001$) (Fig. 3). According to Dunn's multiple comparisons tests (Fig. 2), females that copulated with either one regular male alone or with irradiated males followed by a second mating with a regular male produced similar numbers of offspring. Interestingly, the number of offspring produced by a female first mated with a regular male and then with an irradiated male showed no significant difference from the number of offspring results from a female inseminated by one irradiated male alone (Fig. 3).

Discussion

Our study expands what is known about the mating strategy of S. gregaria. First, during copulation, S. gregaria males transfer 24,000 sperm on average and the number of sperm stored in the spermatheca increases constantly with copulation duration. Pickford and Padgham (Pickford and Padgham, 1973) showed

Table 1. Duration of precopulation and copulation and quantity of sperm transferred by regular and irradiated males during a single copulation with a virgin female.

	Regular males	Irradited males	t Test
Precopulation duration(min)	10.67±4.23	11.65±4.40	t=−0.16; df=19; P=0.55
Copulation duration(min)	235.04±50.44	274.42±66.92	t=−2,89; df=23; P<0.01
Number of sperm stored	24040.62±6887.36	15359.37±8935.74	t=3.18; df=15; P<0.01

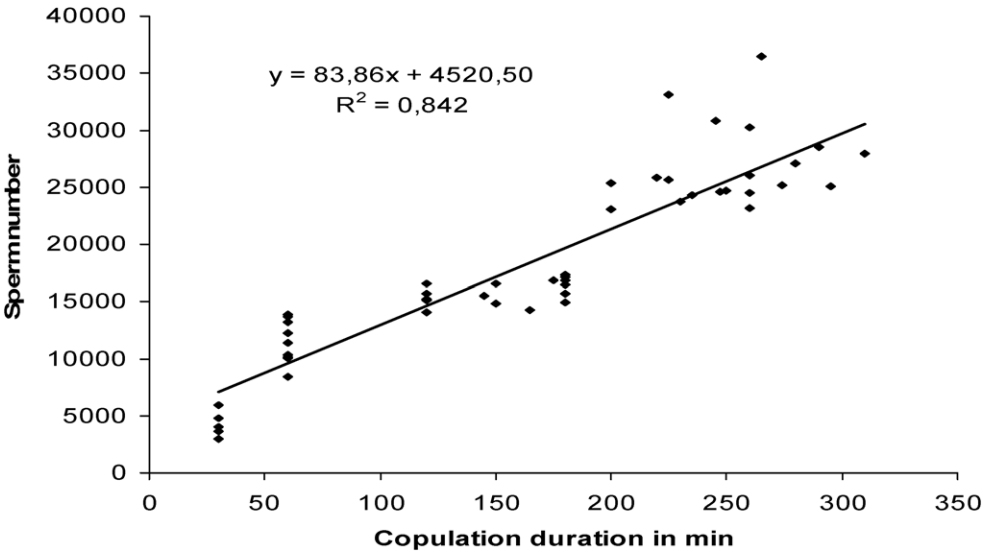

Fig. 1. Sperm number stored in female spermatheca according to the duration of Copulation.

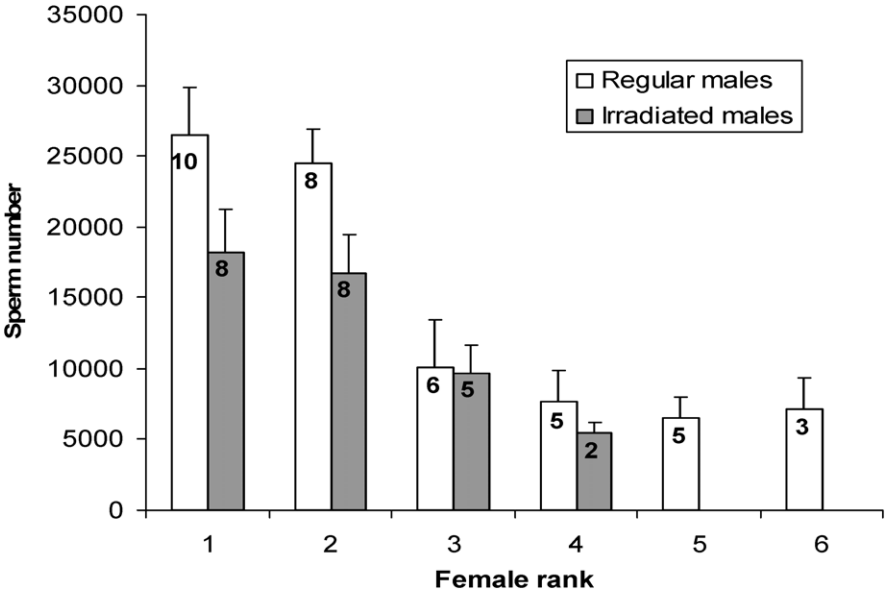

Fig. 2. Mean number of sperm stored in female spermatheca according to the female rank in a succession of mating of regular and irradiated males. Error bars are standard deviation. Numbers inside bars are sample sizes.

that during a single copulation, a male transfers from 6 to 14 spermatophores (which consists of a simple sperm-containing ampulla), and they estimated that a spermatophore was formed and ejected every 43 minutes. Spermatophores are discharged into the proximal portion of the spermathecal duct, where spermatodesms (bundles of sperm) are released and swim towards the distal portion of the spermatheca. Spermatophores are then ejected by males.

In our experiment, copulation took around 235 minutes, which suggests that around 6 spermatophores were transferred. Because ~24,000 sperm were transferred, that means each spermatophore contains about 4,000 sperm. Irradiated males obtained copulation with females as rapidly as regular males suggesting that irradiation did not affect their attractiveness to females. In our experiment, irradiated males had longer copulation duration but transferred fewer sperm (15,000) than regular males. Irradiation may damage sperm in male seminal vesicles, or it may impair a male's ability to transfer sperm. The decrease of sperm number is probably due to the destruction of secondary spermatogonia.

According to Coggins (Coggins, 1973), in S. gregaria, after a 4-Gy dose exposure, cells in interphase degenerate almost completely after one day. In other species, it is well known that irradiation induces dominant lethal mutations in the germ cells (LaChance, 1967; Proverbs, 1969; Anwar et al., 1971; Bakri et al., 2005). However, even if fewer sperm are transferred by irradiated males, the number of sperm transferred is high compared to the number of sperm theoretically needed to fertilise a female's lifetime production of eggs. Indeed, under optimal conditions in the laboratory, S. gregaria females produce a mean of 22 pods, each containing 47.6 eggs (Wang and Sehnal, 2002), which suggests that they produce a total of 1,050 eggs during their entire reproductive life.

Others studies showed reduced fecundity under different conditions. For instance, Norris (Norris, 1952) reported that females reared in the laboratory, laid 5–9 pods each of which contained 10 to 140 eggs. Popov (Popov, 1958) estimated that desert locust females from natural populations probably did not lay more than 2 or 3 pods each. Whatever the conditions, our

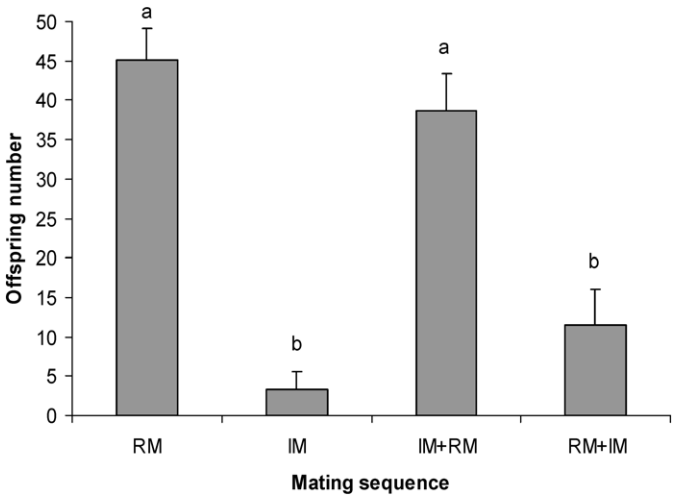

Fig. 3. Offspring production of females inseminated by a regular male alone (RM), an irradiated male alone (IM), an irradiated male then a regular male (IM+RM) and a regular male followed by an irradiated male (RM+IM). Error bars are standard deviation. Letters indicate differences between treatments determined by Dunn's multiple comparisons tests following a Kruskall Wallis.

results indicate that the number of sperm transferred by irradiated males is sufficient for the female to induce egg-laying to fertilise a female's lifetime production of eggs. Interestingly, eggs produced after mating with an irradiated male never hatched, which means a 98.5% of reduction in fecundity in our experiment.

In Schistocerca gregaria, males that mated every day transferred more sperm during the two first matings and fewer sperm during the following matings. Greater investments in the first copulations with reduced future copulation investments has been reported for other Orthoptera, such as Requena verticalis or Ephippiger ephippiger (Orthoptera: Tettigoniidae) in which males donate a nutrient rich spermatophylax (Gwynne et al., 1984). After mating, males need several days without copulating to recover and to be able to transfer a new spermatophylax (Simmons, 1993; Wedell and Ritchie, 2004). Irradiated Schistocerca males decreased sperm transfer after the second mating, which suggests that in the field, released sterile males would affect the reproductive output of at least two females.

In our experiments, S. gregaria exhibited strong last-male sperm precedence as suggested by Hunter-Jones (Hunter-Jones, 1960). Indeed, females first mated with a regular male and then remated with an irradiated male laid mostly unviable eggs. The reverse is also true, females first mated with an irradiated male and then remated with a regular male laid mostly viable eggs. These results suggest that although the impact of irradiated males on a female's fecundity could be limited by a subsequent copulation with a regular male, an irradiated male also has the ability to remove the sperm of a previous mating with a regular male. Hence, the influence of an irradiated male in a wild population could be related to his female encounter rate.

Some Orthoptera species, such as Chorthippus sp, show such last-male sperm precedence. (Reinhardt, 2000). In S.gregaria, sperm displacement is probably the mechanism involved as it has been observed in some other species such as gryllids (Ono et al., 1989) and bushcrickets (von Helversen and von Helversen, 1991). In Metaplastes ornatus, the male removes sperm from spermatheca at the beginning of the copulation by positioning the keel of its subgenital plate within female's genitalia exactly in the egg position during oviposition. The male then makes numerous back and forth keel moves to stimulate the release of stored sperm because the female wants to fertilize the "egg". Last-male sperm precedence is probably an evolutionary force that leads S. gregaria to develop post-copulatory mate guarding. The male stays on the female and guards it physically until oviposition takes place. Under crowded conditions, another mechanism occurs; a pheromone, phenylacetonitrile, is released preventing other males from attempting to court (Seidelmann et al., 2000; Seidelmann and Ferenz, 2002).

According to our results, S. gregaria male sterilized by irradiation with 4-Gy dose had the same ability to obtain copulations with females as regular males had. Despite a decrease in sperm number and an increase in mating duration time, irradiated males significantly reduced the number of offspring produced by females they inseminated. These results suggest that SIT might be effective in S. gregaria population control.

Before such SIT control programs development, more studies are needed to estimate the sterile male reproductive qualities such as the competitiveness of irradiated male with wild male in semi-field experiments. Moreover, unexpected results, such as the high mortality of irradiated S. gregaria have to be studied. Indeed, our entire population of 4Gy-irradiated males died after 17 days, probably due to non-specific damage on somatic cells (Dushimirimana et al., 2010). Coggins (Coggins, 1973) irradiated larvae with the same dose and observed no mortality of males up to 65 days after irradiation. The difference between his results and our results could be due to the ontogenetic variations in radiosensitivity. This hypothesis should be analysed further.

Finally, the feasibility of SIT techniques depends on its ability to be used. One challenge with managing populations of S. gregaria is that some zones where reproduction and gregarisation take place are inaccessible or are sites where social and political conditions make application unsafe (Showler, 2003). Moreover, S. gregaria show periodic cycles related to climate conditions. This fact combined with a large geographical range and huge dispersal capacities limit the feasibility of SIT for this species. However, the principle of focal applications on well delimited zones where gregarisation is expected could be an interesting new preventive tool.

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Competing Interests

The authors declare no competing interests.

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