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ORIGINAL ARTICLE

Effects of the juvenile hormone mimic NC-184 on the development of the reproductive organs and mating behaviour of nymphs of the desert locust, *Schistocerca gregaria* (Orthoptera: Acrididae)

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Key words. Orthoptera, Acrididae, *Schistocerca gregaria*, juvenile hormone mimic, moulting, reproductive organs, oogenesis, spermatheca

Abstract. The insect growth regulator NC-184, a juvenile hormone mimic, prevents moulting to the adult stage in the desert locust, *Schistocerca gregaria* (Forskål) (Orthoptera: Acrididae). Male nymphs treated in the penultimate or final nymphal instar with NC-184 exhibit precocious mating behaviour in the final instar. We examined whether this chemical affects the development of the internal reproductive organs of crowded nymphs. In treated males, both accessory glands and seminal vesicles were underdeveloped, and no sperm was found in the seminal vesicle, whereas these organs in control individuals had greatly increased in size 10 days after treatment, when all the insects had moulted to adults. Testis size in treated males was similar to that in controls, regardless of their smaller body size due to the inhibition of moulting. Oogenesis and development of spermatheca in females treated with NC-184 continued to some degree, but no eggs matured, unlike what occurred in the control. In conclusion, treatment of *S. gregaria* nymphs with NC-184 resulted in changes in the reproductive organs in both sexes.

INTRODUCTION

The desert locust *Schistocerca gregaria* Forskål (Orthoptera: Acrididae) exhibits remarkable phase polyphenism, i.e. gregarious and solitary phases that differ considerably in behaviour, physiology and morphogenesis (Applebaum et al., 1997; Rogers et al., 2014). These phases are determined by crowding, chemotactic and olfactory cues, tactile and visual stimuli and maternal effects, and this insect is able to transform reversibly between the two phases (Hassanali et al., 2005). The process of trans-generational changes involves various changes in behaviour, body colour, morphology, physiology and biochemistry (Maeno & Tanaka, 2012). The neuropeptide [HIS^7]-corazonin changes the body coloration but not the behaviour of *S. gregaria*, so probably it is not the only factor involved in phase transition (Breuer et al., 2003). Phenylacetonitrile, a major component of the aggregation and maturation accelerating pheromones emitted by gregarious locusts, elicits stage-dependent aggregation behaviour in gregarious desert locusts (Torto et al., 1994, 1996; Pener & Yerushalmi, 1998; Ignell et al., 1999). The behavioural response to adult aggregation pheromone is age and juvenile hormone dependent (Ignell et al., 2001).

The desert locust undergoes marked behavioural and morphological changes when they start swarming, which have been extensively studied for many years (Pener &

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Simpson, 2009; Ernst et al., 2015). Most typical aspects of the phase transition from solitary to gregarious are the changes in body size and colour (Pener & Simpson, 2009). Solitary locusts are bigger and more cryptically coloured than the gregarious individuals (Ernst et al., 2015). Tanaka (2005) reports that corazonin (neuropeptide) influences body colour and body shape in the African migratory locusts, *Locusta migratoria migratoroides* and *S. gregaria*, and suggests that corazonin has an important role in the control of phase-related characters in locusts. Juvenile hormone (JH) also induces the greenish body colour characteristic of solitary locusts (Hoste et al., 2002). Hoste et al. (2002) confirm that corazonin affects the morphometrical (F/C ratio; F = length of the hind femur and C = maximum width of the head) phase change.

Mating behaviour of gregarious males of *S. gregaria* depends entirely on the corpora allata (CA) and juvenile hormone (Pener & Simpson, 2009). Similarly, in the grasshopper *Melanoplus sanguinipes* Stål and Egyptian locust, *Anacridium aegyptium* L.*,* male sexual behaviour is dependent upon an active CA (Cheeseman & Gillott, 1990; Greenfiled & Pener, 1992). Juvenile hormone mimic (JHM) (pyriproxyfen) enhances homosexual behaviour, when adult males of the African migratory locust *L. migratoria* are deprived of females (Guershon et al., 2012). Interestingly, phenyl acetonitrile released only by gregarious adult males of the desert locust is a courtship-inhibiting pheromone (Seidelmann & Ferenz, 2002). Similarly, enhancement of pheromone release and mating in males of the Caribbean fruit fly, *Anastrepha suspense* (Loew), are induced by the topical application of JH and JHM (methoprene or fenoxycarb) (Teal et al., 2000). *Drosophila melanogaster* Meigen also shows JH-dependent male mating behaviour (Wijesekera et al., 2016). On the other hand, in the South American fruit fly, *Anastrepha frateculus* (Wiedemann), treatment with methoprene accelerate sexual maturation of males, but not the enhancement of mating in males close to sexual maturation, suggesting methoprene might act as a pheromone (Bachmann et al., 2017). In the Asian comma butterfly, *Polygonia aureum* L., transplantation of active corpora allata into adult butterflies in diapause does not induce males to copulate (Endo, 1973), although methoprene or active corpora allata promotes reproductive development in both sexes (Hiroyoshi et al., 2017). Apparently, JH is irrelevant to mating in this case. Therefore, the involvement of JH in mating behaviour may differ in different species of insects.

The presence of mature males of *S. gregaria* accelerates the maturation of young adults, whereas that of young males retards the maturation of young adults (Pener, 1991; Assad et al., 1997). The maturation accelerating effect exerted by mature males on young adults is due to a CA-dependent production of a maturation accelerating pheromone (Loher, 1961; Norris, 1962; Amerasinghe, 1978). The male mating behaviour of the desert locust is also affected by hormones produced by the CA (Pener, 1991). Allatectomy of crowded adult locusts leads to no or reduced incidence of mating behaviour (Loher, 1961; Pener, 1967a, b), reduction in accessory reproductive gland development (Loher, 1961; Odhiambo 1966; Szopa, 1981), sexual receptivity in males (Strong & Amerasigne, 1977) and oogenesis (Pener, 1967a) in females. However, implantation of an active CA into allatectomized locusts restores reproductive development (Pener, 1967b). Application of JH or JHM also has similar effects to implantation of an active CA (Pener & Lazarovici, 1979; Schneider et al., 1995). These studies reveal an important role of JH in the control of reproduction in this locust.

NC-184 is a 3(2H) pyridazinone derivative and exhibits JH-like activity, such as the inhibition of ecdysis in various species belonging to 11 orders (Miyake & Ogura, 1992). NC-184 is an insect growth regulator (IGR) and a JHM in several species of insects (Miyake & Ogura, 1992). Interestingly, treatment with NC-184 provokes precocious mating behaviour in crowded final (5^{th}) instar nymphs of the male desert locust when administered to penultimate or final instar nymphs (Yagi, 1996; Yagi et al., unpubl. data).

However, this behaviour does not lead to coitus and transfer of spermatophores into the female's genital organs. Instead, a male mounts the female and bends its abdomen towards that of the female. However, it is not known whether this behaviour initiates the activation of sexual maturity. If treated males become reproductively mature, the development of their internal reproductive organs, such as testes (occurrence of spermatogenesis), accessory glands (secretion of spermatophoral substances) and seminal vesicles (sperm storage organ) would follow, because males have to transfer sperm and spermatophores produced by these organs to females during copulation.

As juvenile hormone mimics have been used to control pest species this study was undertaken to clarify whether the internal reproductive organs of males are affected by treatment with NC-184. In addition, the effect of NC-184 on the reproduction of females was also recorded.

MATERIALS AND METHODS

Study organism

S. gregaria used in the experiments were reared in an insectary at the International Centre of Insect Physiology and Ecology (ICIPE) under gregarious conditions using procedures described by Rai et al. (1997). In brief, locusts (300–400) of both sexes were kept in aluminium cages (50 × 50 × 50 cm). After ecdysis to the final nymphal stadium, they were collected from the stock colonies and transferred to another room maintained at 30 ± 2°C and a photoperiod of 12L : 12D. Nymphs treated with NC-184 were separately reared in a different room from controls to avoid contamination with NC-184 and pheromones. Approximately 20 insects of each sex were separately kept in aluminium cages (15.5 × 15.5 × 31 cm) and fed daily on a diet of wheat bran and wheat seedlings.

Experimental protocol

On day 0 of the 5th stadium, nymphs of each sex were treated with 4 µg of JHM, NC-184, donated by Nissan Chemical Co. Ltd., at a concentration of 4 µg/µL diluted in acetone. Controls (non-treated groups) were not treated with any chemicals. NC-184 was dissolved in pure acetone and topically applied onto the dorsum of the 1st segment of the abdomen of nymphs using a mi-

cro-syringe (Hamilton, 705-N). Since application of acetone did not induce precocious mating behaviour in a preliminary experiment, we did not use acetone as a control in this study. Treated locusts and corresponding controls were dissected 2, 5, 10 and 20 days after treatment to determine the morphological changes in the internal reproductive organs. Control locusts were also examined on the day of the treatment. Since control nymphs took approximately 7 days to moult into adults, all the controls dissected on days 10 and 20 were adults. On the other hand, none of the nymphs treated with NC-184 moulted into adults and remained as nymphs throughout the experimental period.

Locusts were dissected in 0.86% NaCl solution and the reproductive system was removed. In males, the sizes of testis, testicular follicles, accessory reproductive glands and seminal vesicle were measured with the aid of a micrometre (Nikon Instruments stage micrometre Type A MBM11100) under a stereo-microscope. We first examined the length and width of the testis and then separated the individual testicular follicles. After removal of the fat body surrounding the testicular follicles, 5 testicular follicles were randomly selected to be measured. Desert locust males have a pair of accessory gland complexes, each of which consists of 15 accessory glands and a seminal vesicle, which are folded several times (Odhiambo, 1969). To assess the development of accessory glands, the length and width of the right side of the accessory glands complex were measured. After removal of the accessory glands, the width of the remaining seminal vesicle mass was measured.

In females, the length of the spermatheca and terminal oocytes and the degree of ovarian development were recorded. Three large terminal oocytes per ovary were selected and their length was measured with an optic micrometre under a stereo-microscope. Stages of oogenesis were divided into three categories according to the occurrence of vitellogenesis and mature eggs. The appearance of yellow coloration in the oocytes was a criterion for determining the status of vitellogenesis. All measurement of reproductive organs in both sexes were done at ×20 to ×100 magnifications under a microscope.

Statistical analyses

All data recorded in this study, except that for ovarian development, were compared using the Mann Whitney *U*-test of the results for treated and control locusts on each day after treatment with NC-184 with a significance level of 0.05. Ovarian development (three grades) was analysed by ordinary logistic regression analysis to estimate the effect of treatment, days after treatment and the interaction. All statistical analysis was performed using JMP version 11.2 (SAS Institute, 2012).

RESULTS

Effects of NC-184 on testis development

We investigated the effects of NC-184 on the development of testis of *S. gregaria* (Fig. 1A and B). Although the testis size in both treated and control males increased with age, the developmental pattern differed slightly. The sample size ranged from 9 to 27 for each of the measurements. Both the length and width of testis in treated males were significantly smaller than those in controls until day 5 after treatment, but from day 10 to day 20 there were no significant differences in the sizes (Table 1). These results indicate that NC-184 suppressed the development of the testis during the first few days after treatment, but its effect was temporary. We observed abnormal spermatogenesis and testis membrane in treated males.

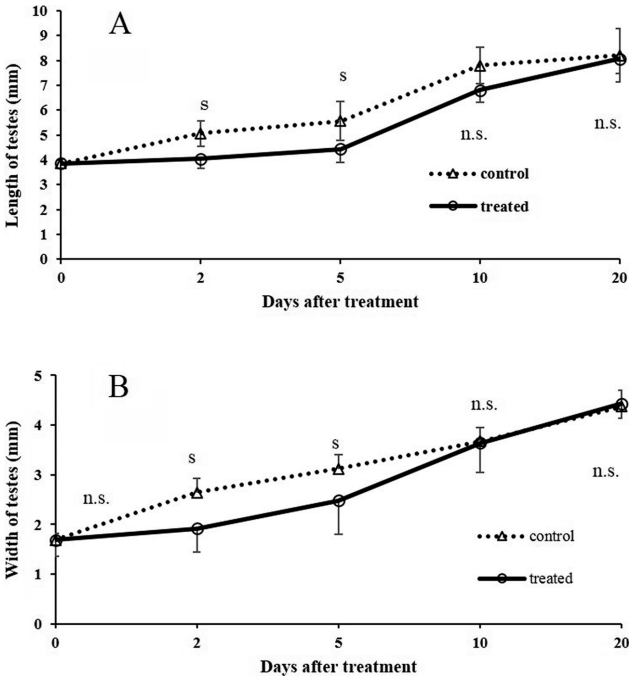

Fig. 1. Changes in the length (A) and width (B) of the testes recorded during the 5th instar of *S. gregaria* treated with JHM (NC-184). The sample sizes ranged from 9 to 27 (A and B) for the different measurements. Each point and the vertical bar indicate the mean and SD, respectively. S – significant; n.s. – not significant. Significant difference at $p < 0.05$ level. Control locusts reached the adult stage by day 10, whereas none of those treated with NC-184 became adults.

To monitor whether the inside of the testis develops or not, we investigated the effects of NC-184 on the development of testicular follicles. The sample size ranged from 6 to 27 for each measurement. Fig. 2A and B shows that the testicular follicles of both treated and control males also increased in size with age, as the testis grew. Although the length of testicular follicles in treated males was not significantly different from those of controls at any age, there were significant differences in the widths of the testicular follicles (Table 2).

Effects of NC-184 on male accessory glands

The effect of NC-184 on the development of the male accessory glands in *S. gregaria* was determined (Fig. 3A and

Table 1. Changes in the length and width of the testis during the 5th instar of *S. gregaria* after the administration of NC-184.

Changes in the length and width of the testis	U1-value	U2-value	W-value	P
Length (mm)				
Day 2	224.5	9.5	54.5	<0.0001
Day 5	110.0	16.0	61.0	0.0020
Day 10	36.5	62.5	81.5	0.3312
Day 20	50.0	50.0	105.0	1.0000
Width (mm)				
Day 2	200.5	33.5	78.5	0.0009
Day 5	104.0	22.0	67.0	0.0086
Day 10	56.0	43.0	101.0	0.6556
Day 20	123.0	147.0	202.0	0.6812

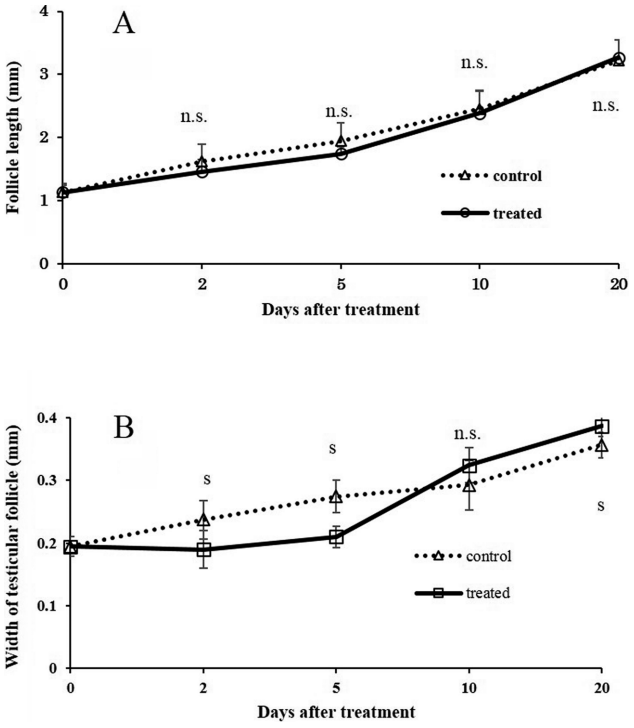

Fig. 2. Changes in the length (A) and width (B) of the testicular follicles recorded during the 5th instar of *S. gregaria* treated with JHM (NC-184). The sample sizes ranged from 7 to 27 (A) and 6 to 27 (B) for the different measurements, respectively. Each point and the vertical bar indicate the mean and SD, respectively. S – significant; n.s. – not significant. Significant difference was set at $p < 0.05$ level. Control locusts reached the adult stage by day 10, whereas none of those treated with NC-184 became adults.

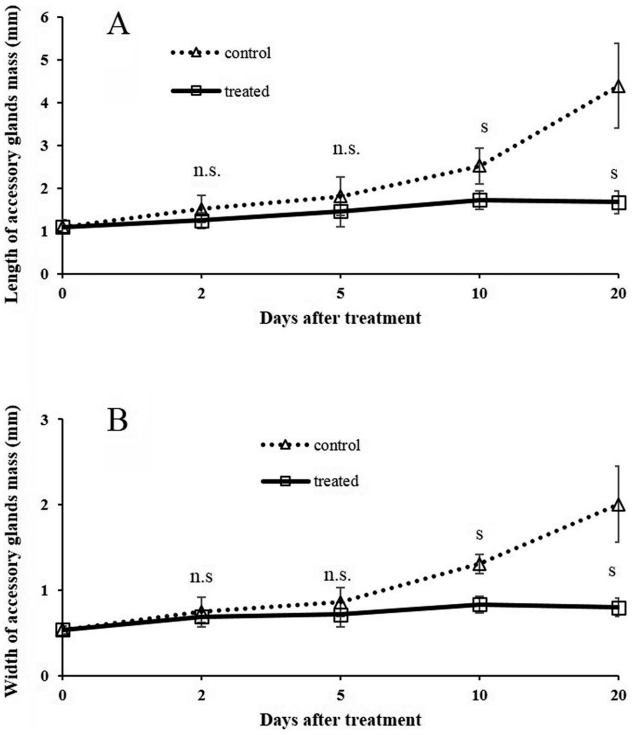

Fig. 3. Changes in the length (A) and width (B) of the accessory gland complex recorded during the 5th instar of *S. gregaria* treated with JHM (NC-184). The sample sizes ranged from 9 to 27 (A and B) for the different measurements. Each point and the vertical bar indicate the mean and SD, respectively. S – significant; n.s. – not significant. Significant difference was set at $p < 0.05$ level. Control locusts reached the adult stage by day 10, whereas none of those treated with NC-184 reached the adult stage.

B). The sample size ranged from 8 to 27 for each measurement. The length of the accessory gland complex in treated males tended to be shorter than that in controls at each age. The differences were particularly marked in 20-day-old locusts (Fig. 3A). The width of the accessory gland complex in treated nymphs was significantly broader than that in 10-day- and 20-day-old control nymphs (Fig. 3B). No secretions in the accessory glands were observed when viewed under a microscope. It is noted that these results were quite different from those for the testis, which irrespective of treatment, enlarged with age.

Table 2. Changes in the length and width of the testicular follicles of *S. gregaria* during the 5th instar after the administration of NC-184.

Changes in the length and width of testicular follicles	U1-value	U2-value	W-value	P
Length (mm)				
Day 2	160.0	74.0	119.0	0.1097
Day 5	107.0	43.0	98.0	0.0759
Day 10	28.0	26.0	47.0	0.9546
Day 20	127.0	143.0	198.0	0.7844
Width (mm)				
Day 2	203.0	31.0	76.0	0.0006
Day 5	149.0	1.0	56.0	0.0000
Day 10	14.0	40.0	61.0	0.1447
Day 20	30.5	239.5	294.5	0.0003

Effects of NC-184 on seminal vesicle

To monitor whether the seminal vesicle develops or not, we investigated the effects of NC-184 on those of *S. gregaria* (Fig. 4). The sample size ranged from 5 to 27 for each measurement. NC-184 treatment did not have a significant effect on the seminal vesicle during at 5 days, but thereafter there was a significant decline in the rate of increase in

Table 3. Effect of NC-184 on the ovarian development of 5th instar *S. gregaria*.

Days[1] after treatment	No. of animals used	Grade of ovarian development			
		–	±	+	
Treatment					
2	10	10	0	0	n.s
5	9	1	8	0	a
10	10	0	10	0	a
20	16	0	16	0	a
Untreated controls					
0	21	21	0	0	
2	14	14	0	0	n.s
5	12	12	0	0	a
10	14	13	1	0	a
20	13	1	6	6	a

[1] NC-184 treatment was carried out on day 0 of the final nymphal stage; – no vitellogenesis, ± vitellogenesis, + formation of mature eggs; a – the difference in the distribution of grades in the treated nymphs and untreated controls was significant ($p < 0.05/4$) compared by using a *G*-test and adjusted odds ratio with Bonferroni correction.

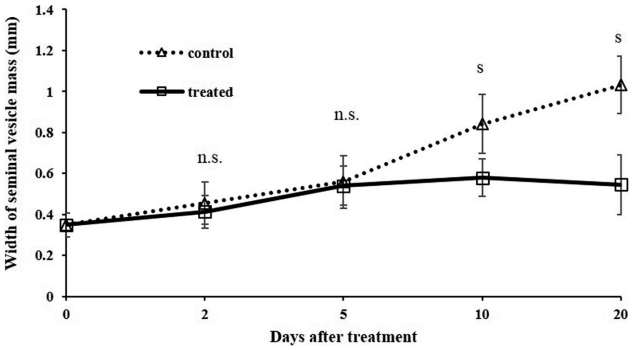

Fig. 4. Changes in the width of the seminal vesicle recorded during the 5th instar of *S. gregaria* treated with JHM (NC-184). The sample sizes ranged from 3 to 27 for the different measurements. Each point and the vertical bar indicate the mean and SD, respectively. S – significant; n.s. – not significant. Significant difference was set at $p < 0.05$ level. Control locusts reached the adult stage by day 10, whereas none of those treated with NC-184 reached adulthood.

width with time (Fig. 4). The effect on the seminal vesicle was similar in pattern to that on the accessory gland complex. The seminal vesicle of treated males did not develop and no sperm was produced.

Effects of NC-184 on spermatheca

We investigated the effects of NC-184 on the development of spermatheca of *S. gregaria* relative to whether the nymphal females accept mates or not (Fig. 5). The sample sizes ranged from 8 to 16 for each measurement. The length of spermatheca in control females increased with age and was significantly longer than that of nymphs treated with NC-184 except on day 5 (Fig. 5A), whereas the width of spermatheca in females treated with NC-184 was not significantly different from that of controls, except on day 20 (Fig. 5B).

Effects of NC-184 on oogenesis

The results of NC-184 treatment on ovarian development are summarized in Table 3. The sample size ranged from 9 to 16 for each measurement. On day 2 after treatment, there were no significant differences in oocyte lengths recorded for treated and control females (U1-Value = 50.0, U2-Value = 90.0, W-value = 145.0, P = 0.2413) (Fig. 6). However, the oocytes in treated females were significantly longer than those in controls on day 5 (U1-Value = 0.0, U2-Value = 108.0, W-value = 153.0, $P < 0.0001$) and day 10 (U1-Value = 0.0, U2-Value = 140.0, W-value = 195.0, P = 0.0000), but the reverse situation was recorded on day 20 (U1-Value = 183.8, U2-Value = 25.0, W-value = 274.0, P = 0.0005). The controls first showed an accumulation of yellow substances in the oocytes on day 10, when they were all adults. On day 20, nearly half of them had mature eggs. On the other hand, almost all the treated females showed an accumulation of yellow substances in the oocytes even on day 5. Thereafter, the percentage of individuals undergoing vitellogenesis was 100% on day 20, but no females with mature eggs were recorded. There were significant differences in the grade of ovarian development between individuals treated with NC-184 and controls (See Table 4, $P < 0.0001$).

Table 4. Results of statistical analysis of the ovarian development of *S. gregaria* hoppers treated with NC-184 and controls using ordinary logistic regression analysis.

Factor	Parameter	df	Likelihood rate χ	P-value
Treatment	1	1	30.022466	<0.0001
Days after treatment	1	1	87.630786	<0.0001
Treatment * Days after treatment	1	1	31.601641	<0.0001

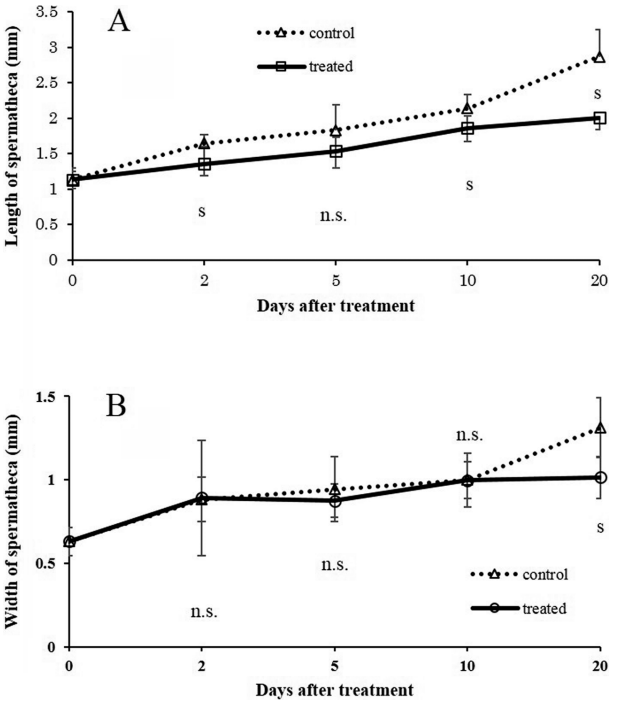

Fig. 5. Changes in the length (A) and width (B) of the spermathecae recorded during the 5th instar of *S. gregaria* treated with JHM (NC-184). The sample sizes ranged from 8 to 21 (A and B) for the different measurements. Each point and the vertical bar indicate the mean and SD, respectively. S – significant; n.s. – not significant. Significant difference was set at $p < 0.05$ level. Control locusts reached the adult stage by day 10, whereas none of those treated with NC-184 reached adulthood.

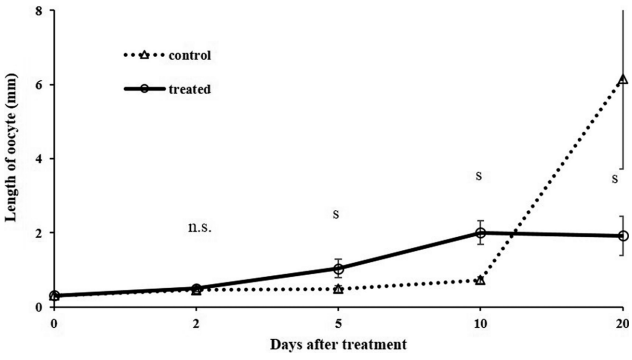

Fig. 6. Changes in the length of the oocytes recorded during the 5th instar of *S. gregaria* treated with JHM (NC-184). The sample sizes ranged from 9 to 22 for the different measurements. Each point and the vertical bar indicate the mean and SD, respectively. S – significant; n.s. – not significant. Significant difference was set at $p < 0.05$ level. Control locusts reached the adult stage by day 10, whereas none of those treated with NC-184 reached adulthood.

DISCUSSION

Effects of JH on male reproduction

JH accelerates the maturation of gregarious adults of the desert locust. Sexual maturation in both sexes of the desert locust occurs after adult emergence. In males, the sperm in the testis begin to migrate into the seminal vesicles 1 to 2 weeks after emergence (Norris, 1954). They take 1 or 2 weeks to develop the accessory reproductive glands before copulating with females, although the rate of sexual maturation differs considerably depending on day length (Norris, 1957), temperature and relative humidity (Hamilton, 1936), density (Norris, 1952, 1964) and pheromones (Amerasinghe, 1978; Pener, 1991; Mahamat et al., 1993). In the red flour beetle, *Tribolium castaneum* Herbst, JH deficiency affects male reproduction in terms of less vigorous mating behaviour and poor sperm transfer, resulting in the females being less fecund (Parthasarathy et al., 2009). Since male insects develop gonads before they are able to mate and transfer sperm and the spermatophore to females, mating activity in males could closely be related to the development of their reproductive organs. However, the present study indicates that final instar male locusts treated with NC-184 had both underdeveloped seminal vesicles and accessory glands and no sperm or spermatophoral substances were observed. This indicates that these nymphs were not reproductively mature internally. Other JHM or chemical substances also affect the physiology of male locusts. For instance, feeding them with JHM (hydroprene) results in an increase in the size of male accessory glands in the red flour beetle, *Tribolium castaneum* Herbst (Parthasarathy et al., 2009). In *S. gregaria*, application of the insect growth regulator Consult (chitin synthesis inhibitor) results in the degeneration of spermatogenesis (Bakr et al., 2010). Also in *S. gregaria* females, treatment of their eggs with the juvenile hormone analogue pyriproxyfen inhibits their embryogenesis (Vennard et al., 1998). Injection of azadirachtin, extracted from neem seeds, into last instar *L. migratoria* hoppers suppresses moulting and induces overaged nymphs to exhibit sexual mating behaviour (Shalom & Pener, 1984, 1987). This behaviour does not lead to successful mating, because their external genitalia are not as fully developed as in adults. Their mating behaviour is less vigorous than that of adults, probably because the basic motor pattern is not as fully developed as in adults (Pener & Shalom, 1987). On the contrary, precocenes chemically allatectomize *L. migratoria* (Pener et al., 1978). Third- and fourth-instar adultiform males produced by applying precocene III exhibit sexual behaviour and injections of JH intensify this behaviour in a dose-dependent manner (Shalom & Pener, 1984, 1986). However, the mating behaviour of adultiforms in earlier stadia is less vigorous than that of adultiforms in later stadia or adults.

Effects of JHM on female reproduction

In the present study, female desert locusts were also affected by JHM (NC-184). During the final nymphal stage, treated females showed a marked enlargement and increase in the yellow substances in the oocytes, which normally occurs only after adult emergence. In *L. migratoria*, application of a JH mimic induces synthesis of vitellogenin in the fat body of final instar nymphs (Dhadialla & Wyatt, 1983; Girardie et al., 1996). Injection of methoprene (JHM) promotes limited vitellogenic oocyte development in the over-aged nymphs induced by azadirachtin in *L. migratoria* (Shalom et al., 1993). Thus, it seems likely that application of NC-184 also induced synthesis and uptake of vitellogenin in hoppers of the desert locust. Because the size of the spermatheca in treated females was smaller than in the controls, however, treated females may not be able to copulate with males.

Effects of JHM on accessory glands

We found that in control male hoppers, the accessory glands were greatly enlarged on and after day 10, perhaps because of the imaginal moult on about day 7. On the other hand, treated males remained as nymphs and showed slight development of the accessory glands in the first 10 days, which was suppressed thereafter. If NC-184 treatment directly affected accessory gland development, we expected that the inhibitory effect would have appeared immediately after treatment. However, delayed inhibitory effects indicates that NC-184 affected accessory gland development indirectly, probably by inhibiting the imaginal moult.

Ecdysteroids influence the final differentiation of the accessory glands in *L. moratoria* (Gallois, 1989) and cell cycling in the mealworm beetle, *Tenebrio molitor* L. (Happ, 1992). In the red-legged grasshopper, *M. sanguinipes*, Ismail and Gillot (1993, 1995) report that synthesis of certain polypeptides in the male accessory glands is regulated by ecdysteroids. If this is also true for the desert locust, the accessory glands of hoppers treated with NC-184 may not be exposed to a high titre of ecdysteroids during the second half of the final nymphal stage of this locust (Wilson & Morgan, 1978; Baehr et al., 1979; Gande et al., 1979; Tawfik et al., 1996) and therefore did not differentiate. On the other hand, JH also stimulates the maturation of accessory glands during the adult stage as shown in *M. sanguinipes* (Friedel & Gillott, 1976; Gillott & Friedel, 1976; Venkatesh & Gillott, 1983) and *L. migratoria* (Braun & Wyatt, 1995). The present study indicates that the application of NC-184 did not affect the growth of accessory glands in the nymphal stage. It seems likely that the nymphal accessory glands had not yet acquired competence for NC-184. Development of accessory glands of this locust are promoted by JH and ecdysteroid.

Effects of JHM on testis

It was interesting that the treated males of the desert locust had approximately the same size of testis as the controls on days 10 and 20, irrespective of their smaller body size. Similar developmental patterns of the testis in treated and control males indicate that their development may be independent of the endocrinological events following the imaginal moult, which may affect the development of the accessory gland and the seminal vesicle. It is known that ecdysteroids promote spermatogenesis, whereas JHs depress it (Heming, 2003).

CONCLUSION

In the present study, application of the JHM NC-184 promoted ovarian development in female nymphs but not the development of the internal reproductive organs in males. These results might indicate that sensitivity to NC-184 differs in terms of the induction of precocious mating behaviour and maturation of reproductive organs in the sexes. Although we demonstrated that the internal sexual organs of last instar male desert locust did not develop prematurely in response to NC-184, the effect of this juvenile hormone mimic on mating behaviour is not indirect, but due perhaps to the secretion of some kind of sex hormone by the internal sexual organs. The results strongly indicate that the effects of juvenile hormone on mating behaviour are indeed direct and that the mechanisms underlying mating behaviour and development of the internal reproductive organs must be different.

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