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ORIGINAL ARTICLE

The association of the development of the internal reproductive organs of male desert locusts, *Schistocerca gregaria* (Orthoptera: Acrididae), with age, phase and the effect of exposure to pheromones

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Abstract. The regulation of the development of the male reproductive organs in insects is still an open question. Although the desert locust, *Schistocerca gregaria*, has been extensively examined, there is little information on the effects of phase and pheromones on the development of the male reproductive organs. This study clarified the association of these two factors with reproductive development (length or width of each organ) of the testis, testicular follicles, accessory glands, and seminal vesicles in this locust. The width of the follicles and width and length of the accessory gland mass are significantly associated with phase (solitary or gregarious). Developmen of all reproductive organs is age dependent as these organs developed in the adult stage. The development of follicles and accessory glands (width) in males in the first two weeks of adulthood was promoted by exposing them to pheromones from mature adults, but not from nymphs. These results indicate the incidence of male reproductive development is associated with phase and affected by pheromones.

INTRODUCTION

The desert locust, *Schistocerca gregaria* (Forskål) (Orthoptera: Acrididae), which is distributed from Africa to Western Asia, is a notorious pest of agriculture. This locust is characterised by distinct phases, long-distance migration and changes in group behaviour (Pener, 1991; Ignell et al., 2001; Breuer et al., 2003; Tanaka, 2005; Buhl et al., 2006; Pener & Simpson, 2009; Ernst et al., 2015). In addition to these ecological, physiological, and behavioural features, this locust is excellent experimental material because it is large and easy to breed. Thus, it is well studied. For example, male reproduction in this locust has been examined in terms of mating and sexual behaviour (Pener, 1967; Inayatullah et al., 1994; Seidelmann & Ferenz, 2002; Golov et al., 2018), testis development (Tobback et al., 2011, van Wielendaele et al., 2013), sperm storage (Norris, 1954; Hiroyoshi et al., 2021a), spermatogenesis (Das et al., 1965; Cantacuzène et al., 1972; Coggins, 1973; Jones, 1978; Bakr et al., 2010), sperm transfer (Pickford & Padgham, 1973; Boerjan et al., 2012; Dushimirimana et al., 2012) and accessory glands (Odhiambo, 1969, 1970; Avruch & Tobe, 1978; Dhadialla et al., 1986; Claeys et al., 2005; van Lommel et al., 2022).

Desert locusts show density-dependent phase changes in morphology, colouration, reproduction, development, physiology, cytology, behaviour, and ecology (Pener, 1991). The extremes of the phase polymorphism are refered to "solitary" and "gregarious", and the intermediates as "transients". It is known that sexual maturation in solitary desert locusts is delayed compared to that in gregarious ones (Pener & Simpson, 2009). To characterize the phase of a locust, colour, morphology and behaviour are frequently used (Dirsh, 1951; Breuer et al., 2003). For example, the F (length of the hind femur) / C (maximum width of the head) ratio is greater in solitary than in gregarious locusts (Pener, 1991). Furthermore, yellowing of the integument is characteristic of mature gregarious locusts.

The development of *S. gregaria* is regulated by various factors, i.e., age, maternal effects, paternal effects, pheromones, tactile or visual stimuli, hormones, population density, and phase variation are the biotic factors and temperature, humidity, and diet the abiotic factors (Ellis et al., 1965;

Richard & El-Mangoury, 1968; Tawfik et al., 1997, 2000; Hassanali et al., 2005; Simpson & Miller, 2007; Maeno & Tanaka, 2011; Tanaka & Nishide, 2012; Sugawara & Tanaka, 2018; Sugawara et al., 2018). Desert locusts produce pheromones that regulate sexual maturation. Mature adult males of this locust produce pheromones that accelerates the development of young, immature adults, inducing copulation or yellowing of integument of males earlier than in the controls (Norris, 1952, 1954, 1964; Loher 1960; Amerasinghe 1978a, b; Assad et al., 1997), whereas the presence of nymphs retards the maturation of young adults (Pener, 1991; Assad et al., 1997), which require almost one month of sexual development before mating (Norris, 1954, 1964; Norris & Pener, 1965; Richard & El-Mangoury, 1968). The main components of the sexual maturation pheromones seem to be phenylacetonitrile (P.A.N.) or benzylcyanide, which are produced by gregarious males but not solitary locusts (Ferenz & Seidelmann, 2003; Pflüger & Bräunig, 2021). The responses to these pheromones synchronize population development.

For the desert locust it is unclear, how the development of male reproductive organs is affected by phase and pheromones. The present study determined the associastions between the development of male internal reproductive organs, adult age, phase and exposure to pheromones.

MATERIALS AND METHODS

Insects

Schistocerca gregaria used in the experiments were reared in an insectary at the International Centre of Insect Physiology and Ecology (I.C.I.P.E.) in Nairobi, Kenya, under gregarious conditions, except for locusts in the solitary phase, which were reared using the procedures described by Rai et al. (1997), except for the rearing temperatures. In brief, locusts (300–400) of both sexes were bred in aluminum cages (50 × 50 × 50 cm). After ecdysis to the adult stage, locusts were collected from the stock colonies and were transferred to other rooms maintained at 30 ± 2°C (phase research) or 32 ± 2°C (pheromonal research) and a photoperiod of 12L : 12D. Adult males were kept in aluminum cages (15.5 × 15.5 × 31 cm height) and fed daily on a diet of wheat bran and wheat seedlings. Solitary locusts from an isolated line were reared individually in aluminum cages (10 cm × 12 cm × 14 cm height) throughout their life except during the egg stage, well separated from the rearing room with gregarious phase locusts.

Phase

As mentioned above, to compare the development of internal reproductive organs of solitary and gregarious males, solitary virgin male locusts were individually reared. In contrast, after becoming adult five gregarious virgin male locusts were collectively reared in aluminum cages (15.5 cm × 15.5 cm × 31 cm height) at 30 ± 2°C. Locusts were dissected at 0, 1, 2, or 4 weeks after becoming adult. The sample size was ten to twenty on each occassison.

Pheromone

To investigate the effect of pheromones on the development of the internal reproductive organs in males, five newly moulted immature gregarious virgin adults (referred to as recipients) were exposed to five mature (yellow) gregarious virgin adult males or five male gregarious nymphs (4^{th} or 5^{th}) (referred to as donors) in two-chamber aluminum cages (15.5 cm × 15.5 cm × 31 cm height) with a sliding glass front at 32 ± 2°C, which allowed the insects to smell the donors (no visual or tactile contact possible). The donors were kept in the top part of the cage and, the recipients in the bottom part so that they could more easily perceive the volatiles emitted by the locusts in the upper chamber. There was an aluminum barrier with small holes between the top and bottom parts of the cages. When a donor died during an experiment, it was replaced in order to keep the number constant. When a donor nymph moulted to an adult, it was replaced by another nymph. The recipients were dissected 1, 2, or 4 weeks latter and sample size rangned from eleven to fifteen on each occassion.

Dissection

The internal reproductive system of male desert locusts consists of a large testis, a pair of vasa deferentia, a pair of accessory glands, a pair of seminal vesicles, and an ejaculatory duct. The accessory glands each consist of 15 glands and a seminal vesicle, which is long and slender and folded approximately 30 times (Hiroyoshi et al., 2021a). The size of each reproductive organ and tissue was measured as follows. Locusts were dissected in 0.86% NaCl solution, and the reproductive system removed and placed in Petri dishes (diameter 9 cm) filled with saline. The sizes (maximum length and/or maximum width) of testis, testicular follicles, accessory reproductive glands mass and seminal vesicle mass were measured with the aid of a micrometer (Nikon Instruments stage micrometer Type-A MBM11100) under a stereo-microscope. We first measured the length and width of the testis and then separated the individual testicular follicles, whose number exceeds 100 (Szöllösi, 1982). Five testicular follicles were randomly chosen to be measured after removing the fat body surrounding the testicular follicles. The largest and smallest measurements were discarded, and the remaining three averaged. To assess the development of accessory glands, the length and width of the right side of the accessory glands were measured. After removing accessory glands, the width of the remaining seminal vesicle was measured.

Sexual maturation

The external changes in the colour of males were determined using the classification of Norris (1954) and Amerasinghe (1978a). Briefly, the body colours of locusts ranged from stage 1 (immature, pink) to stage 4 (mature, yellow). The colour of the locusts exposed to each pheromone for four weeks was classified in this way.

Statistics

To compare the differences in the sizes of each reproductive organ associated with different phases, ages, and their interactions, data were analysed using a general linear model. Non-significant interactions were omitted. If necessary, a Tukey-Kramer test was used as a post-hoc test to compare the results for different ages. To satisfy normality and homoscedasticity the results were Box-Cox transformed prior to the regression analysis.

Sexual maturity (i.e., stage 1 to 4) at each age in the pheromone experiments was analysed using ordinary logistic regression analysis. Sexual maturity was not determined in phase tests.

To compare the differences in sizes of the reproductive organs associated with the two pheromones, the same statistical methods as mentioned above were used. The number of individuals used in the experiments is shown in each figure.

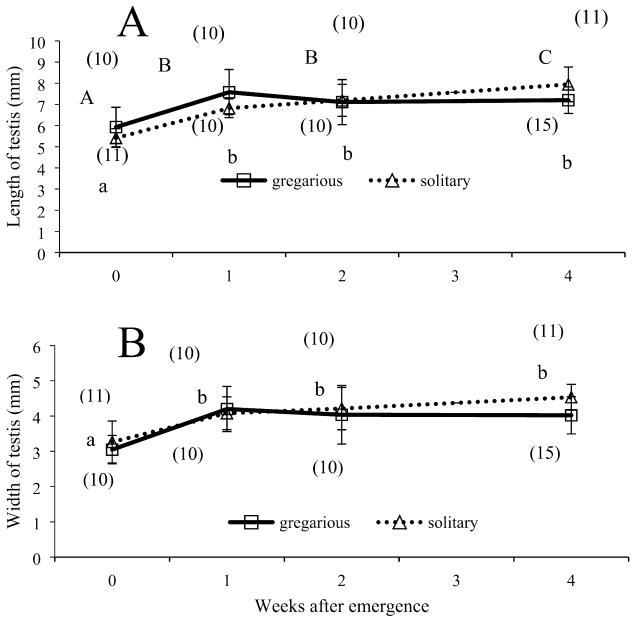

Fig. 1. The association between testis length (A) and width (B), and phase and age of adult desert locust *Schistocerca gregaria*. A simple main effect test was used to compare the values for the solitary and gregarious phases to determine if there is a significant difference for each age, because there was a significant difference in interaction (phase × age) (A). Since there was no significant difference in the interaction (phase × age), testis width on each day was compared using the Tukey-Kramer test (B). Different letters (upper = gregarious, lower = solitary) indicate significant differences at $P < 0.05 / 2$ (A) or 0.05 (B).

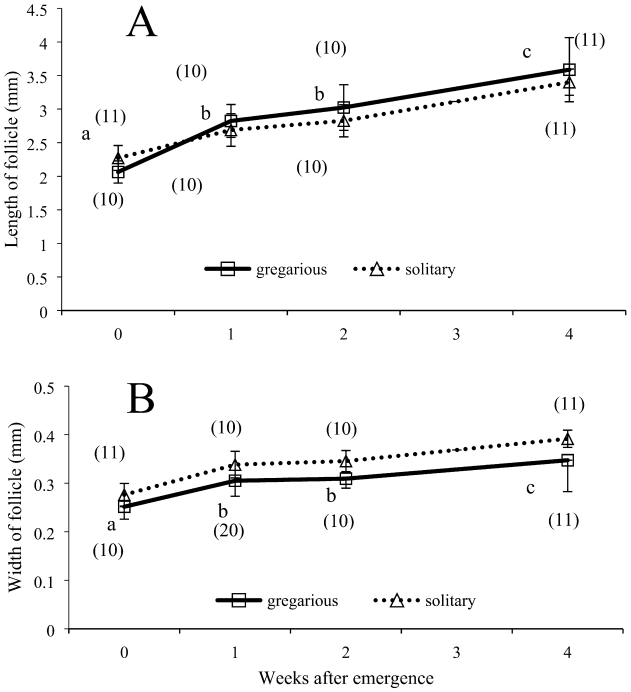

Fig. 2. The associations between testicular follicle length (A) and width (B) and the phase and age of adult desert locust, *Schistocerca gregaria*. Tukey-Kramer Test was used to compare whether the values differed significantly with age. Different letters indicate significant differences at each age, $P < 0.05$.

RESULTS

The association between phase and the development of male reproductive organs

(a) Testis

The associations between phase and the development of testis, testicular follicles, accessory glands, and seminal vesicle were recorded in male desert locusts. First, testis size was recorded. In both solitary and gregarious phase locusts, testis length increased during the first week after adult emergence (Fig. 1A). The length of the testis in solitary locusts was longer than in gregarious ones 1 week after emergence, whereas the results were opposite 4 weeks after emergence. In summary, age ($df = 3$, $F = 22.73$, $P = 0.0134$; error, $df = 79$) and interaction (phase × age: $df = 3$, $F = 3.80$, $P < 0001$) were significantly associated with testis length, whereas phase ($df = 1$, $F = 0.39$, $P = 0.5361$; error, $df = 79$) was not. Next, the width of the testis, irrespective of phase, also increased during the first week after emergence (Fig. 1B). Together with the above results for testis length, this result indicates that testis size enlarged slightly after adult emergence. Finally, phase and interaction were not associated with testis width, whereas age was significantly so (phase, $df = 1$, $F = 2.54$, $P = 0.1153$; age, $df = 3$, $F = 17.75$, $P < 0001$; interaction, $df = 3$, $F = 1.17$, $P = 0.3267$; error, $df = 79$), indicating that the testis width increases with adult age.

(b) Follicle

The testis of a locust consists of several testicular follicle cells. It is likely that follicular cell size is related to the progression in spermiogenesis. Therefore, we measured the size of testicular follicles. The results indicate that the length of follicles was significantly associated with age, but not phase and interaction (age, $df = 3$, $F = 81.53$, $P < 0001$; phase, $df = 1$, $F = 1.59$, $P = 0.2114$; interaction, $df = 3$, $F = 2.47$, $P = 0.0681$; error, $df = 75$) (Fig. 2A). These results indicate that follicles increased in length in both solitary and gregarious adult locusts with age. On the other hand, the width of the follicles is significantly associated with age and phase, but not the interaction (age, $df = 3$, $F = 37.84$, $P < 0001$; phase, $df = 1$, $F = 23.22$, $P < 0.0001$; interaction, $df = 3$, $F = 0.33$, $P = 0.8042$; error, $df = 75$) (Fig. 2B). The width of the follicles of solitary locusts tended to be greater than that of gregarious ones. The width of the follicle in adults in both phases increases with age.

(c) Accessory gland

Development of the accessory glands mass was recorded (Fig. 3). First, the length of the accessory glands is associated with age and phase, but not interaction (age, $df = 3$, $F = 41.66$, $P < 0.0001$; phase, $df = 1$, $F = 11.06$, $P = 0.0013$; interaction, $df = 3$, $F = 1.63$, $P = 0.1877$; error, $df = 84$). The length of the accessory gland in solitary locusts tended to be longer than that in gregarious locusts 1, 2, and 4 weeks after becoming adult. Next, the association between the width of the accessory gland and phase was recorded.

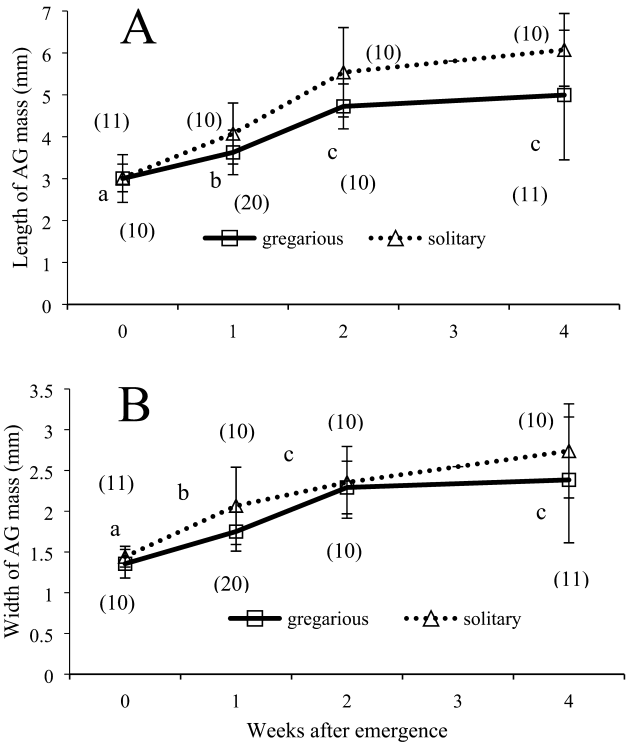

Fig. 3. The associations between the length (A) and width (B) of the accessory glands (A.G.) and the phase and age of adult desert locust, *Schistocerca gregaria*. Tukey-Kramer Test was used to compare whether the values differed significantly with age. Different letters indicate significant differences at each age, $P < 0.05$.

The width of the accessory gland was significantly associated witih age and phase, but not the interaction (age, $df = 3$, $F = 30.20$, $P < 0.0001$; phase, $df = 1$, $F = 5.08$, $P = 0.0268$; interaction, $df = 3$, $F = 0.69$, $P = 0.5636$; error, $df = 84$). The results for the accessory gland (length and width) indicate that it increased in size with adult age, with that of length remarkable so in solitary locusts.

(d) Seminal vesicle

Finally, the association of the development of the seminal vesicle with phase was recorded (Fig. 4). The width of the seminal vesicle was significantly associated with adult age, but not phase and interaction (age, $df = 3$, $F = 33.41$, $P < 0.0001$; phase, $df = 1$, $F = 3.93$, $P = 0.0508$; interaction, $df = 3$, $F = 1.08$, $P = 0.3607$; error, $df = 83$). The width of the seminal vesicle mass in solitary locusts was greater than in 2 and 4 weeks old adult gregarious locusts, and increased with adult age.

Sexual maturity

In the first two weeks, the sexual maturity of recipient locusts exposed to pheromones from mature adults tended to be more advanced than those exposed to nymphal pheromones (Fig. 5). After that, it was similar in the next two weeks. The maximum power parameter analysis revealed significant differences in the sexual maturity of gregarious locusts exposed to pheromones from mature adults and nymphs (pheromone, $df = 1$, $G = 11.32$, $P = 0.0008$; age, $df = 2$, $G = 61.75$, $P < 0.0001$; interaction of pheromone versus age, $df = 2$, $G = 6.21$, $P = 0.0449$).

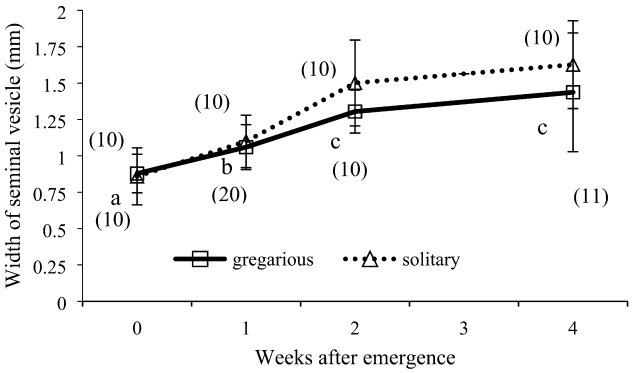

Fig. 4. The association between the width of the seminal vesicles and the phase and age of adult desert locust, *Schistocerca gregaria*. Tukey-Kramer Test was used to compare whether the values differed significantly with age. Different letters indicate significant differences at each age, $P < 0.05$.

Effects of pheromones on the development of male reproductive organs

(a) Testis

There was an association between testis length and the developmental stage (nymph or mature adult) of the pheromone source ($df = 1$, $F = 17.05$, $P < 0.0001$; error, $df = 75$), but not age ($df = 2$, $F = 4.26$, $P = 0.1062$; error, $df = 75$) or interaction ($df = 2$, $F = 0.76$, $P = 0.4730$; error, $df = 75$) (Fig. 6A). The length of the testis of recipients exposed to the odour of nymphs was greater than those exposed to that of mature adults. On the other hand, there were significant associations between interaction (pheromone source × age) ($df = 2$, $F = 3.47$, $P = 0.0362$; error, $df = 75$) and testis

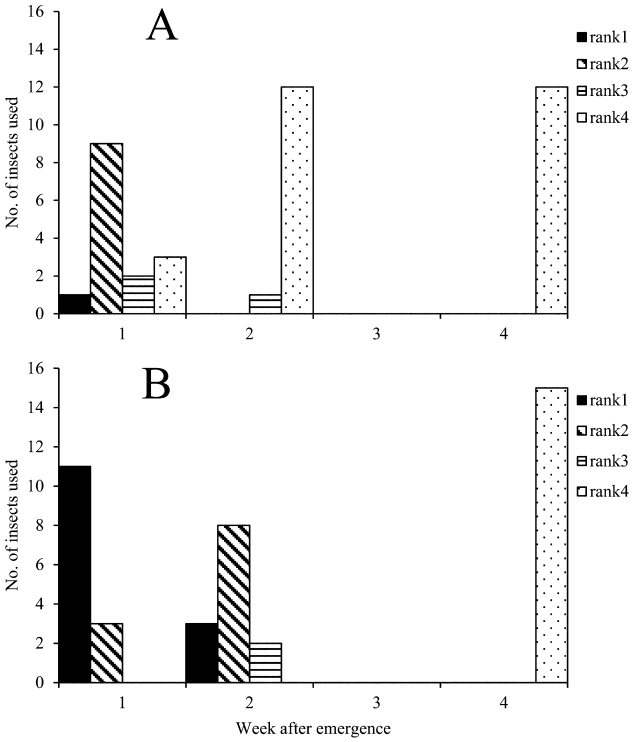

Fig. 5. Comparison of the sexual maturity of adult males of the desert locust, *Schistocerca gregaria*, exposed to pheromones from mature adult males (A) and nymphs (B).

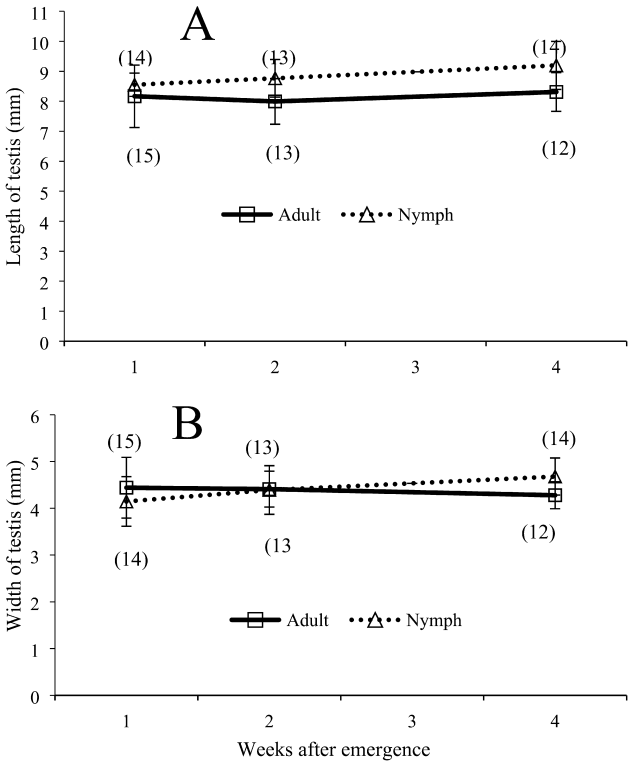

Fig. 6. The length (A) and width (B) of the testis of adult desert locust, *Schistocerca gregaria*, at different ages when exposed to pheromones produced by mature adult males (Adult) and nymphs (Nymph).

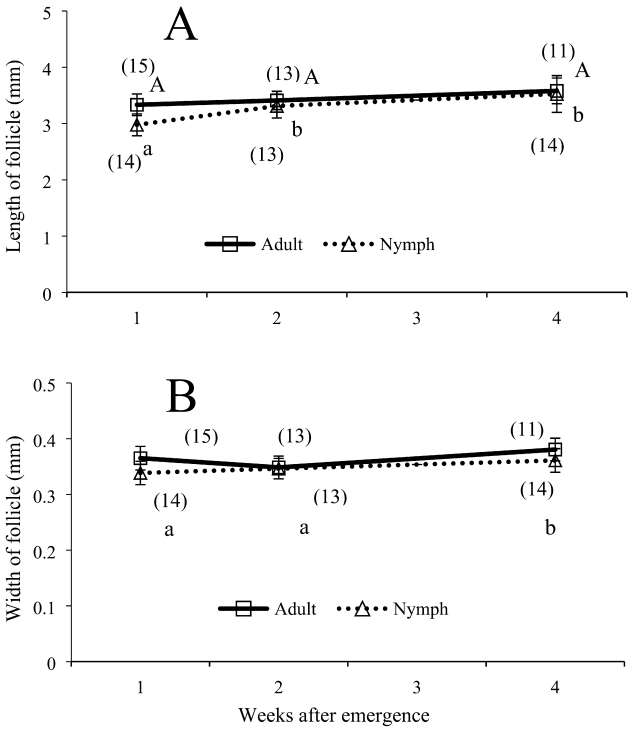

Fig. 7. The length (A) and width (B) of the follicles of adult males of the desert locust, *Schistocerca gregaria*, exposed to pheromones from mature adult males (Adult) and nymphs (Nymph). A simple main effect test was used to compare the values for the developmental stages of the pheromone source (adult or nymph) in order to determine if they differed significantly with age, because there was a significant difference in the interaction (source × age) (A). Different letters (upper = adult, lower = nymph) indicate significant differences between ages, at $P < 0.05/2$. Tukey-Kramer Test was used to compare whether the values differed significantly with age (B). Different letters indicate significant differences between ages, at $P < 0.05$.

width, but not with the developmental stage of pheromone source ($df = 1$, $F = 0.06$, $P = 0.8118$; error, $df = 75$) and age ($df = 2$, $F = 1.14$, $P = 0.3255$; error, $df = 75$) (Fig. 6B), although the testis width results were very variable (smaller, similar or larger) for 1, 2 and 4 weeks old adults, respectively.

(b) Follicle

In terms of the length of testicular follicles, both developmental stages of pheromone source ($df = 1$, $F = 10.72$, $P = 0.0016$; error, $df = 74$), age ($df = 2$, $F = 20.78$, $P < 0.0001$; error, $df = 74$) and interaction ($df = 2$, $F = 3.39$, $P = 0.0364$; error, $df = 74$) significantly affected length (Fig. 7A). The follicles in both types of recipients, especially those exposed to the pheromone from nymphs, increased in length with adult age to some degree. There were significant associations between the width of testicular follicles, the stage of development of the pheromone source ($df = 1$, $F = 12.20$, $P = 0.0008$; error, $df = 74$) and age ($df = 2$, $F = 9.22$, $P = 0.0003$; error, $df = 74$), but not the interaction ($df = 2$, $F = 2.56$, $P = 0.0839$; error, $df = 74$) (Fig. 7B). The width of follicles of recipients exposed to the pheromone from mature adults tended to be greater or similar to that of those exposed to pheromone from nymphs.

(c) Accessory gland

There were no significant associations between the length of the accessory glands and the stage of development of the pheromone source ($df = 1$, $F = 0.32$, $P = 0.5742$; error, $df = 75$) and interaction ($df = 2$, $F = 1.82$, $P = 0.1684$; error, $df = 75$), but significant associations with age ($df = 2$, $F = 26.05$, $P < 0.0001$; error, $df = 75$) (Fig. 8A). Although there was no significant association between the width of the accessory glands and the interaction ($df = 2$, $F = 1.29$, $P = 0.2814$; error, $df = 75$), there was with the stage of development of the pheromone source ($df = 1$, $F = 5.13$, $P = 0.0264$; error, $df = 75$) and age ($df = 2$, $F = 19.02$, $P < 0.0001$; error, $df = 75$) (Fig. 8B). The width of the accessory gland of recipients exposed to pheromones from mature adults was greater than that of males exposed to pheromones from nymphs 1 and 2 weeks after emergence. Exposure to the pheromone of either of the donors (nymphs or mature adults) resulted in an increase in the width of the accessory gland with adult age.

(d) Seminal vesicle

Its width was only significantly associated with age ($df = 2$, $F = 30.49$, $P < 0.0001$; error, $df = 74$) (Fig. 9). This indicates that the width of this organ increases with adult age and that the stage of development of the pheromone source did not affect its development.

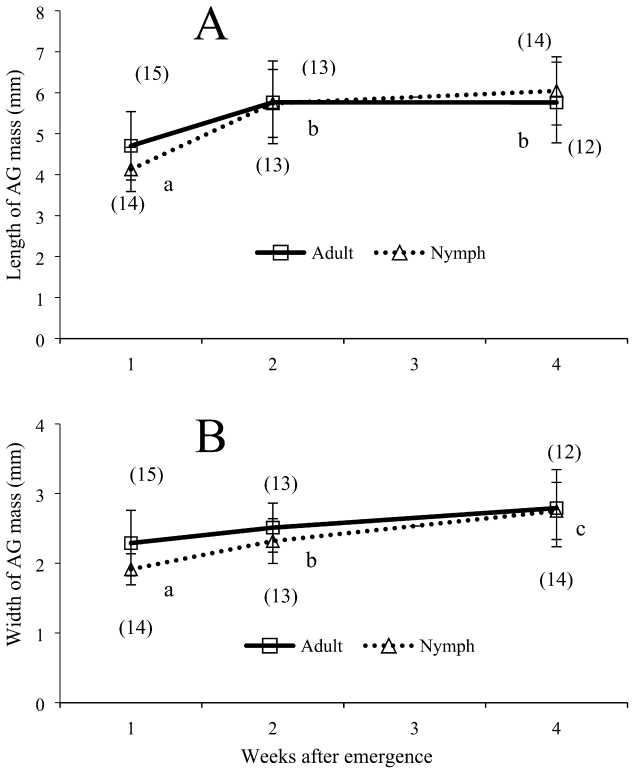

Fig. 8. The length (A) and width (B) of the accessory glands (A.G.) of adult desert locust, *Schistocerca gregaria*, exposed to pheromones from mature adult males (Adult) and nymphs (Nymph) of this locust. Tukey-Kramer Test was used to compare whether the values differed significantly with age. Different letters indicate significant differences between ages, at $P < 0.05$.

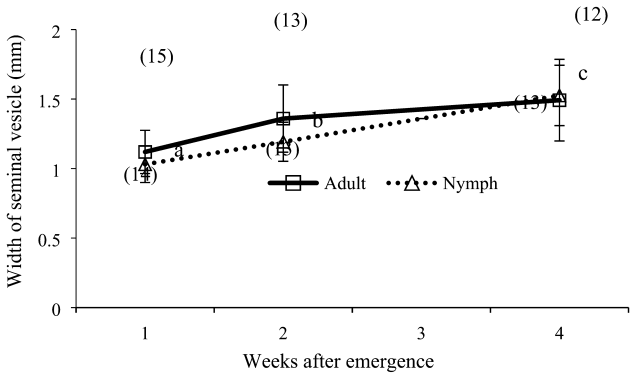

Fig. 9. The width of the seminal vesicle of adult males of the desert locust *Schistocerca gregaria*, exposed to pheromones produced by mature adult males (Adult) and nymphs (Nymph) of this locust. Tukey-Kramer Test was used to compare whether the values differed significantly with age. Different letters indicate significant differences between ages, at $P < 0.05$.

DISCUSSION

(a) Phase and the development of reproductive organs

This study determined the association between the development of the reproductive organs in the male desert locusts, adult age, phase and exposure to pheromones. Surprisingly, this has not been attempted previously. Contrary to a previous report (Norris, 1954) on sexual maturation of the reproductive organs of adult males, it occurred earlier in the solitary than the gregarious phase in this study (Figs 2–4). However, the present study did not investigate the mating behaviour to keep the virgin status of males. In particular, there were differences in the development of the accessory glands of male locusts. Various substances synthesized or taken up from the haemolymph by accessory glands are used for producing the spermatophorse that are passed to females during mating. Although unknown in the desert locust, the migratory grasshopper *Melanoplus sanguinipes* (F.) offer females several spermatophores during a single mating (Pickford & Gillott, 1971, 1972). In the desert locust, the increase in length and width of the accessory glands after the onset of adulthood could be closely related to the elongation of the entire accessory glands and growth in its content with sexual maturity. In addition, the length of the accessory glands is longer in solitary than gregarious locust, which is probably due to the difference in rate of sexual maturation, the former are smaller based on their head width. In other words, solitary locusts may appear to mature sexually faster in terms of the development of male internal reproductive organs than gregarious ones. However, sperm accumulation in the seminal vesicle of gregarious locusts occurs earlier than in solitary locusts (Hiroyoshi et al., 2021a). In general, under laboratory conditions, gregarious locusts mate earlier than the solitary ones (Norris, 1954), and thus the present results are somewhat difficult to understand. A possibility is that solitary locusts mate more often than gregarious ones, or produce relatively large spermatophores in terms of their body size. However, we have no evidence to support these assumptions, which need to be tested in the future.

(b) Testis development in terms of spermiogenesis and sperm movement

The testis in adult desert locusts grew for at least for a week, regardless of phase, and had similar values 4th weeks later. The testicular follicular cells that make up the testis also continued to grow in adulthood. This is strongly supported by the fact that spermiogenesis is initiated and mainly occurs shortly after the onset of adulthood (Hamilton, 1936; Hiroyoshi et al., 2021a). In Lepidoptera, where spermatogenesis is not active during the adult stage, testis size decreases from the middle or late pupal stage to the adult stage (Omura, 1936; Chandhury & Raun, 1966; Chase & Gillilalnd Jr., 1972; Scheepens & Wysoki, 1985; Sridevi et al., 1989; Hoque, 1992; Hiroyoshi, 2000; Hiroyoshi et al., 2021b). On the other hand, in some Coleoptera, Diptera, and Hemiptera, in which spermatogenesis occurs during the adult stage, testis size increases intially and then remains constant (Nilakhe & Earle, 1976; Kotaki & Yagi, 1989; Hiroyoshi & Moriya, 1999; Hiroyoshi et al., 2016) or continues to increase with age (Ascerno et al., 1978; Ward & Simmons, 1991). In the desert locust, it is reported that sperm moves from the testis to the seminal vesicles via the vasa deferentia for at least four weeks after onset of adulthood (Hiroyoshi et al., 2021a). Therefore, the development of accessory glands, seminal vesicles, and testis in this locust could be closely associated with sexual maturity.

(c) Effects of pheromones on the development of testis

The length, but not the width, was significantly associated with the stage of development of the pheromone source (Figs 6A, B). The length of the testis of locusts exposed to pheromones from nymphs tended to be longer than that of locusts exposed to those from mature adults. The length of the follicular cells was significantly associated with the stage of development of pheromone source, age, and interaction (Fig. 7A). It is likely that the size of the follicular cells is related to the degree of sperm cell elongation. The length of the follicular cells tended to be longer in individuals exposed to pheromones from mature adults than in those exposed to those from nymphs. In contrast the results were the opposite for length of testis. The effect of age may have been weaker due to the lack of results for week 0, but the follicular cells seemed to grow in adult locusts irrespective of the stage of development of the pheromone source. The width of the follicular cells was greater in the locusts exposed to pheromones from mature adults than in those exposed to those from nymphs (Fig. 7B). The above results possibly indicate that pheromones affect the development of the testis. However, as the results for testis and follicular cell development are not similar, no conclusion can be drawn at this time.

(d) Effects of pheromones on the development of the accessory gland

The mass of the accessory glands (length and width) in adults increased with age (Fig. 8A, B). There was a significant association of the width of the accessory gland and the stage of development of the pheromone source. Although it is unclear whether the length of the accessory gland increases in adult desert locust, it is reported that the elongated seminal vesicles that are located next to the accessory gland also increase in length with age (Hiroyoshi et al., 2021a). With sexual maturity, the length and width of the accessory glands are likely to increase with increasing content. One week after becoming adult, the width of the accessory glands was greater in locusts exposed to pheromones from mature adults than in those exposed to those from nymphs. The results for the 4th week of maturity are ambiguous, but nevertheless the associations with age, stage of development of pheromone source, and interaction were significant. This might contradict the results in other reports (Norris, 1964; Richards & Mangonry, 1968; Amersinghe, 1978a), which indicate that pheromones from mature males promote sexual maturity and those from nymphs delay it. This was possibly due to some of the locusts exposed to pheromones from nymphs maturing sexually probably because the pheromone source was contaminated by one of the nymphs maturings and inducing the reproductive development of other individuals in the same cage.

(e) Effects of pheromones on the development of the seminal vesicle

It is likely that in the early stages of sexual maturation, pheromones affected the development of accessory glands. The storage of sperm in the seminal vesicles is dependent on the stage of development of the pheromone source (Hiroyoshi et al., 2021a), but not the development of the seminal vesicles (width of the mass) (Fig. 9). Although the seminal vesicles increase in length with age (Hiroyoshi et al., 2021a), it is possible that the width of the seminal vesicle may not be associated with the affect of pheromones. Interestingly, in *M. sanguinipes*, protein synthesis in the accessory glands and seminal vesicles (Gillott & Venkatesh,1985; Couche & Gillott,1987) is another mechanism that needs to be considered. This might be why the results for seminal vesicles differed from those for accessory glands.

(f) Conclusion

In conclusion, the results of this study indicate that the development of the internal reproductive organs of males is associated with phase and exposure to pheromones in early adult life. These two factors are known to strongly influence sexual maturation in the desert locust (Pener & Simpson, 2009), and at least the development of the accessory glands that produce spermatophores seems to be associated with phase and pheromones. In addition, the increase in the size of the testis and accessory glands during adult life supports active spermatogenesis and spermatophore production.

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