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Is juvenile hormone involved in the maternal regulation of egg size and progeny characteristics in the desert locust?

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ABSTRACT

The role of juvenile hormone (JH) in the maternal regulation of progeny characteristics was examined in the desert locust, *Schistocerca gregaria*. Female adults of this species are known to produce smaller but more eggs when reared in isolation than do those reared in a group. Eggs laid by isolated females develop green hatchlings typical of solitarious forms, whereas those laid by the latter produce black hatchlings typical of gregarious forms. Topical application of a juvenile hormone analog (JHA), fenoxycarb, or implantation of corpora allata (CA) taken from the migratory locust, *Locusta migratoria*, caused crowded *S. gregaria* females to deposit smaller eggs, but did not have a significant effect on the number of eggs per egg pod except at high doses of JHA. The production of smaller eggs by treated and untreated crowded females was closely associated with earlier deposition of the egg pods and shorter oviposition intervals. However, neither JHA application nor CA implantation influenced the progeny characteristics in actively reproducing aged females under crowded conditions, while untreated control females started producing smaller and more eggs upon transfer to isolated conditions. These results may suggest that JH is not directly involved in the maternal regulation of phase-dependent progeny characteristics.

1. Introduction

Adult locusts modify their progeny characteristics in response to the population density (Faure, 1932; Uvarov, 1966; Pener, 1991). In the desert locust, *Schistocerca gregaria* Forskål, solitarious adults typically produce small, green hatchlings, whereas gregarious adults produce large, black hatchlings. Gregarious hatchlings are attracted to one another and more actively move than solitarious hatchlings (Ellis, 1962; Uvarov, 1966). The characteristics of hatchlings influence their body coloration at a late nymphal stage and their morphometrics as adults, as well as other fitness-related developmental and reproductive characteristics (Hunter-Jones, 1958; Maeno and Tanaka, 2007, 2008a).

The endocrine mechanisms controlling reproduction have received much attention in locusts (for reviews, see Dale and Tobe, 1990; Pener, 1991; Pener and Yerushalmi, 1998; Verlinden et al., 2009), but little is known about the hormonal control of progeny characteristics such as egg size, numbers of eggs and hatchling body color. Most research to date has focused on the role of juvenile hormone (JH), which is biosynthesized and released by the corpora allata (CA) and plays a pivotal role in reproduction and metamorphosis in locusts and other insects. It has been often reported that the higher fecundity and smaller eggs observed in solitarious versus gregarious forms are induced by an elevated JH titer of the mother in the migratory locust, *Locusta migratoria*, and *S. gregaria* (Cassier, 1965; Cassier and Papillon, 1968; Lauga, 1977; Dale and Tobe, 1990; Pener, 1991; Islam, 1995; Schneider et al., 1995; Applebaum et al., 1997; Maeno and Tanaka, 2009). Thus, JH is often considered to have a solitarizing effect. However, this observation has not been supported consistently by other studies (e.g. Injeyan et al., 1981; Schneider et al., 1995) and the importance of JH in phase polyphenism is still open to question (Pener, 1991; Pener and Yerushalmi, 1998).

Progeny characteristics such as hatchling body size and color are pre-determined in the ovaries of the mother in *S. gregaria* (Tanaka and Maeno, 2006, 2008; Maeno and Tanaka, 2009). Recently, it was discovered that hatchling body size and coloration are influenced by the reproductive cycles: the first egg pods produced by gregarious (crowd-reared) locusts contain substantial numbers of small eggs that give rise to green hatchlings typical of solitarious (isolated-reared) forms, whereas those deposited after the first egg pod predominantly produce black hatchlings typical of gregarious forms (Maeno and Tanaka, 2008b). Most previous studies investigating the effects of JH on progeny characteristics did not consider the variation in progeny characteristics related to the reproductive cycle. It is thus possible that the production of small eggs and/or green hatchlings after JH application or CA implantation was related to the variation in reproductive cycles.

The main purpose of the present study was to examine the above possibility. Two alternative hypotheses were tested with

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S. gregaria: (1) JH exerts a solitarizing effect on the maternal regulation of progeny characteristics and (2) JH affects physiological events related to reproductive cycles, but is not directly involved in the maternal regulation. In this study, female adults were treated with JH analogs or implanted with extra CA and the effects of these treatments on progeny characteristics were observed. Crowd-reared female adults start producing smaller and more eggs upon transfer to isolated conditions (Maeno and Tanaka, 2008b). The present study also used this response in order to determine if JH exerted a solitarizing effect on reproductive and progeny characteristics when applied to crowded female adults.

2. Materials and methods

2.1. Insects and rearing conditions

The *S. gregaria* colony and rearing method used have been described previously (Tanaka and Yagi, 1997; Maeno and Tanaka, 2004). All experiments were carried out with 5th and 6th solitarious generations and with >40th gregarious generations. A laboratory colony of *L. migratoria*, originally collected on Iheya Island, Okinawa, Japan (Yamagishi and Tanaka, 2009) was maintained at 30 °C. Sexually mature female adults were sacrificed in order to collect corpora allata for transplantation.

2.2. Egg pod collection and measurements of egg size and number of eggs

Egg pod collection and measurements of egg size and number were conducted using the method of Maeno and Tanaka (2008b). Briefly, crowd-reared females were removed from a large cage (42 cm × 22 cm × 42 cm) and groups of 20 individuals were kept in medium-sized cages (28 cm × 18 cm × 30 cm) for the first 10 days. They were separated and held individually in small cages (28 cm × 15 cm × 28 cm) with two sexually mature males to obtain egg pods from individual females at 31 ± 1 °C. In *S. gregaria*, pairing of a female with a single male induces crowding effects on the progeny that are as strong as when females are reared with many males (Hunter-Jones, 1958; Maeno and Tanaka, 2008b). Females treated in various ways had free access to sand in cups (diameter, 9 cm; height, 5 cm) for oviposition. Deposited egg pods were collected every day and incubated at 31 ± 1 °C. Egg length was measured 2 days after oviposition and the number of eggs of each egg pod was also counted at that time. A total of 10 eggs were randomly chosen from each egg pod for measurements.

2.3. Scoring of hatchling body color

The body color of hatchlings was observed 6–12 h after hatching. Hatchling body color in *S. gregaria* is not influenced by the crowding conditions experienced during the embryonic stage and first nymphal stadium (Tanaka and Maeno, 2006). Nymphs were divided into five hatchling color groups (HCGs) based on the method of Maeno and Tanaka (2007): body color is green without dark spots in HCG 1, increasingly darker in HCGs 2–4 and almost entirely black in HCG 5.

2.4. Treatments of JH

Various doses of a juvenile hormone analog, fenoxycarb (JHA, Sigma Co., Tokyo, Japan), ranging from 2.5 to 250 μg dissolved in 5 μl acetone were topically applied onto the dorsal surface of the abdomen of female locusts to examine the effects on the progeny characteristics including egg size and number as well as hatchling body coloration according to the methods of Maeno and Tanaka (2009). Progeny characteristics are influenced by the mother's age at oviposition (Maeno and Tanaka, 2008b), and thus two experiments were carried out with newly emerged adults and aged adults, respectively. In the first experiment, various doses of JHA dissolved in 5 μl acetone were applied topically to crowded females at days 1, 4 and 7 after adult emergence. The same amount of acetone alone was applied to other individuals as controls. A maximum of three egg pods were collected from each female. In the other experiment, females laying eggs actively under crowded conditions (ca. 25 days old) were transferred to small cages individually with 2 sexually active males. Each female was allowed to deposit one egg pod before being treated with JHA. On the day of the first oviposition after being separated individually and maintained with two males, females were treated with 50–1000 μg of fenoxycarb, 200–800 μg of another JHA, methoprene (AccuStandard, CT, USA), or acetone alone as controls. Some control females were isolated by removing the two males from the cage after being treated with acetone. All females were treated again as above on the day that the second egg pod was deposited after the first treatment.

2.5. Implantation of CA

Two pairs of corpora allata (CA) removed from sexually matured gregarious females of *L. migratoria* were implanted into crowed females of *S. gregaria* using the methods of Tanaka (1993) on the same time schedule as for JHA-treatments. The synthetic activity of JH by isolated CA of *L. migratoria* is high (Okuda et al., 1996) and JH activity of implanted CA was confirmed to have a green-color-inducing effect on desert locust nymphs (Maeno and Tanaka, 2009). Sham-operated control females were treated in the same way as CA-implanted females except that CA were not implanted.

3. Results

3.1. Effects of JHA applications and CA implantation during the early adult stage

Adult rearing density influenced reproductive and progeny characteristics including egg size, number and hatchling body coloration (Fig. 1). Isolated females produced significantly smaller eggs than did crowded adults (Scheffé's test; $P < 0.05$; Fig. 1A). Topical application of high doses (≥75 μg) of JHA (fenoxycarb) caused crowded females to lay significantly smaller eggs than acetone-treated females (Scheffé's test; $P < 0.05$; Fig. 1A). Eggs laid by females implanted with extra CA were larger than those deposited by isolated control females, but significantly smaller than those laid by crowded control females (Scheffé's test; $P < 0.05$; Fig. 1A).

Isolated females produced significantly more eggs per pod than did crowded females (Scheffé's test; $P < 0.05$; Fig. 1B). The mean number of eggs per pod was not significantly influenced by either JHA applications or CA implantation ($P > 0.05$ each; Fig. 1B), except for the females treated with 250 μg of JHA ($P < 0.05$; Fig. 1B).

Egg pods laid by isolated females produced a high proportion (70.6%) of green hatchlings (HCG 1), whereas those laid by acetone-treated crowded control females produced only 9.6% (Fig. 1C). The difference between these two groups was statistically significant (Scheffé's test; $P < 0.05$). In the JHA-treated groups, few nymphs hatched, but many nymphs did at lower doses, although the proportion of green hatchlings was not significantly different from that for crowded controls (Scheffé's test after arcsine transformation of data; $P > 0.05$). On the other hand, the proportion of green hatchlings was significantly higher in egg pods laid by CA-implanted crowd-reared females than by crowded controls (Scheffé's test; $P < 0.05$).