Journal of Insect Physiology 54 (2008) 1072–1080

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Maternal effects on progeny size, number and body color in the desert locust, *Schistocerca gregaria*: Density- and reproductive cycle-dependent variation

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ARTICLE INFO

Article history:
Received 25 February 2008
Received in revised form
12 April 2008
Accepted 14 April 2008

Keywords:
Density-dependent phase polyphenism
Locusts
Maternal effect
Progeny size and number
Schistocerca gregaria

ABSTRACT

The effects of rearing density and maternal age on the progeny size, number and coloration of the desert locust, *Schistocerca gregaria,* were investigated. Isolated-reared females deposited smaller, but more eggs than crowd-reared females. The former produced smaller and more eggs with age, whereas the latter showed a tendency to produce larger and fewer eggs over time. A similar tendency was also observed with virgin females, indicating that mating or the presence of males was not important. The first egg pod produced by each mated crowd-reared female contained significantly smaller and more eggs than did the subsequent egg pods. The former often produced many green hatchlings (0–100%) characteristic of solitarious forms, whereas the egg pods deposited after the first pod produced predominantly black hatchlings typical of gregarious forms. Adults were highly sensitive to a shift in rearing density and quickly modified the quality and quantity of their progeny depending on the density encountered. The number of eggs per pod was influenced not only by the mother's rearing density but also by rearing density of the grandmother. The present results demonstrated that the characteristics of progeny are influenced not only by the crowding conditions experienced by the mother and grandmother but also by the mother's reproductive cycle.

1. Introduction

Locusts exhibit density-dependent phase polyphenism in behavioral, morphological and physiological characteristics (Uvarov, 1966, 1977; Pener, 1991). For example, phase-dependent differences are observed in hatchling characteristics such as body color, size and behavior. In the desert locust, *Schistocerca gregaria* Forskål, solitarious hatchlings are typically green and small, whereas gregarious hatchlings are dark and large. The former are relatively inactive compared with the latter (Faure, 1932; Husain and Ahmad, 1936; Hunter-Jones, 1958; Stower, 1959; Uvarov, 1966). The large body size of gregarious hatchlings is likely to be adaptive at high population density, because they not only show a higher tolerance to desiccation and fasting than smaller hatchlings of solitarious forms (Albrecht and Blackith, 1960) but also grow into larger adults (Maeno and Tanaka, 2008).

Hatchling characteristics are influenced by the crowding conditions experienced by the female parent during the adult stage in *S. gregaria* (Faure, 1932; Hunter-Jones, 1958). Although the fact that solitarious hatchlings are smaller than gregarious ones is well known, the underlying mechanism controlling their size and body color has not been well understood. Based on a series of studies (Islam et al., 1994; McCaffery et al., 1998; Simpson et al., 1999; Hägele et al., 2000), the Oxford research group claimed that hatchling body color is determined after oviposition by exposure to a gregarizing factor in the egg foam in *S. gregaria*. According to their proposal, this gregarizing factor is a small (<3 kDa) hydrophilic substance secreted by gregarious female adults into foam plugs of egg pods. Because it is water soluble, it can be removed from the eggs by washing with water within 1 h of deposition, causing presumptive gregarious eggs laid by crowd-reared females to produce green hatchlings typical of solitarious forms (McCaffery et al., 1998). This factor was also assumed to induce gregarious behavior in the hatchlings (McCaffery et al., 1998; Simpson et al., 1999; Hägele et al., 2000). In a previous study, however, we re-examined the role of this pheromonal factor in the control of hatchling body color, but could not reproduce their results (Tanaka and Maeno, 2006). Instead, we reached a different conclusion that hatchling characteristics are determined prior to oviposition (Tanaka and Maeno, 2006). Simpson and Miller (2007) then modified their hypothesis by including a possibility that hatchling body coloration could be determined after ovulation in the oviduct fluids under certain conditions. They suggested that the active factor contained in the foam components would be an alkylated L-dopa analog, but its biological effects on hatchling body coloration or behavior have

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doi:10.1016/j.jinsphys.2008.04.010

not been tested (Miller et al., 2008). In a more recent study, we demonstrated that the phase-dependent difference in egg size, which is closely correlated with hatchling size and coloration, occurs in the ovary. Furthermore, we found that egg foam plugs derived from gregarious females had no dark-color-inducing effects on hatchlings of the eggs deposited by solitarious females (Tanaka and Maeno, 2008). In the present study, we present a new profile for phase-dependent variation of maternal effects on progeny characteristics in *S. gregaria*, which might explain the above discrepancy between the two research groups.

In *S. gregaria*, a mixture of green, intermediate and black hatchlings often appear from egg pods laid by either solitarious or gregarious females (Faure, 1932; Husain and Ahmad, 1936; Hunter-Jones, 1958; Bouaïchi et al., 1995; McCaffery et al., 1998; Tanaka and Maeno, 2006). It seems reasonable to assume that such a variation is caused by the duration and intensity of crowding stimuli that the female parent receives from other individuals, including her mating partner. However, we observed that adult density alone could not fully explain the variation in proportions of different hatchlings, because such a variation occurred even without changing adult density. In this study, we observed how the variations in progeny size, number and body color were brought about in relation to adult age and reproductive cycles.

While the phenomenon that adult density influences the progeny size and number in locusts is well known (Norris, 1952; Uvarov, 1966; Injeyan and Tobe, 1981; Pener, 1991), most studies have been conducted under constant density conditions by keeping locusts either in isolation or in a group. In the present study, we changed the rearing density at or after adult emergence and observed the effects on the progeny characteristics. As a result, we found that females of *S. gregaria* were highly sensitive to rearing density during the adult stage and modified the quality and quantity of their progeny rapidly. The stimulus perceived by the females appears to be neither sex- nor species specific. Furthermore, we observed that the number of eggs produced was influenced by the density experienced not only by the mother but also by the grandmother.

2. Materials and methods

2.1. Insects and rearing conditions

The *S. gregaria* colony used in the present study has been described (Tanaka and Yagi, 1997; Maeno and Tanaka, 2004). Nymphs and adults were kept in groups of approximately 100 individuals in large cages (42 × 22 × 42 cm) or isolated in small cages (28 × 15 × 28 cm) at 32 ± 1 °C, with a 16:8 h light:dark photoperiod under 40–70% relative humidity in a well-ventilated room as described (Maeno et al., 2004). They were fed fresh leaves of orchard grass and cabbage together with wheat bran. A gregarious line had been maintained for more than 20 generations, and a solitarious line was established from the gregarious colony by rearing nymphs and adults individually in small cages except for a short period for mating (Maeno and Tanaka, 2007). All experiments were carried out with third and fourth solitarious generations and with >20th gregarious generations. The *Locusta migratoria* colony used in the present study was established from adults collected on Iheya Island, Okinawa, Japan in 2006 and maintained at 30 °C and at 12:12 h light:dark photoperiod.

2.2. Effect of the number of males on progeny size

In *S. gregaria*, pairing of a female with a single male induces crowding effects on the progeny that are as strong as rearing her with many males (Hunter-Jones, 1958). To confirm this phenomenon with our strain, we determined how many males would be required for a female to perceive crowding by adding one, two or ten sexually mature males to isolated-reared females in small cages. For comparison, the effect of hetero-specific males was also tested using two *L. migratoria* males for each isolated-reared *S. gregaria* female. Each female was paired with a male for mating once (<24 h) and allowed to lay three egg pods under isolated conditions before males were introduced to her. Then, another three egg pods were collected from each female to analyze the crowding effects on the lengths of deposited eggs. As controls, some females were not added with any males and were continuously kept under isolated conditions. Egg length was measured for egg pods produced by each female using an ocular micrometer installed in a microscope 2 days after egg deposition (Tanaka and Maeno, 2008). A total of 10 eggs were randomly chosen from each egg pod and placed on a piece of moist filter paper (9 cm diameter) to avoid desiccation before egg length measurements.

2.3. Egg pod collection and measurements of egg size and egg number

Females of a gregarious line were marked individually with white paint (Pentel, EZL31-W, Japan) at adult emergence. They were kept together with males in a group of about 100 individuals in a large cage during the first 12 days of adult life. Then, females were removed from the large cage and held individually in small cages with two sexually mature males to obtain egg pods from individual females. To determine the role of males or mating on the progeny size and number, some females were reared individually both as nymphs and adults in small cages to collect eggs without mating. Twenty females reared under crowded conditions were held together in a medium-sized cage (28 × 18 × 30 cm) to maintain their virginity and egg pods produced were collected every day. Females of *S. gregaria* lay eggs without mating and some eggs develop parthenogenetically (Hamilton, 1955; Hunter-Jones, 1958). Plastic cups (diameter, 9 cm; height, 5 cm) filled with clean moist sand were placed in cages to collect egg pods. Egg pods collected during the first 2 months after adult emergence were incubated at 32 ± 1 °C. Egg length was measured 2 days after deposition as described above, and the number of eggs in each egg pod was also counted at that time. After length measurements, eggs were returned to moist sand and incubated at the same temperature until hatching.

2.4. Scoring of hatchling body color and body weight

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 or during the first nymphal stadium (Tanaka and Maeno, 2006). Nymphs were divided into five hatchling color groups (HCGs 1–5) according to Maeno and Tanaka (2007): HCG 1, green body color without dark spots; HCG 2–4, increasingly darker body color; HCG 5, almost entirely black body color. After body color was scored, hatchlings were weighed individually using an electronic balance (METTLER AT201, Japan).

2.5. Effects of a change in adult density on the progeny

To investigate the effects of a change in adult density on the progeny size, number and coloration, female locusts reared under crowded conditions as nymphs were kept either under crowded or isolated conditions after adult emergence and then exposed to a