Journal of Insect Physiology 57 (2011) 74–82

Contents lists available at ScienceDirect

Journal of Insect Physiology

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

Tactile stimuli perceived by the antennae cause the isolated females to produce gregarious offspring in the desert locust, *Schistocerca gregaria*

Koutaro Maeno, Seiji Tanaka *, Ken-ichi Harano

Locust Research Laboratory, National Institute of Agrobiological Sciences at Ohwashi (NIASO), Tsukuba, Ibaraki 305-8634, Japan

ARTICLE INFO

Article history:
Received 18 August 2010
Received in revised form 24 September 2010
Accepted 24 September 2010

Keywords:
Crowding
Density-dependent phase polyphenism
Maternal effect
Schistocerca gregaria
Tactile stimulus

ABSTRACT

Maternal determination of progeny body size and coloration in the desert locust, *Schistocerca gregaria*, depends on the crowding conditions experienced during the short sensitive period that occurs two to six days before the deposition of the egg pod. Solitarious (isolated-reared) females produce relatively small eggs that yield solitarious green hatchlings but, females that are exposed to crowded conditions during the sensitive period, produce larger eggs that yield the dark-colored hatchlings characteristic of gregarious forms. The present study aimed to determine the stimuli influencing the maternal determination of progeny characteristics as well as the site at which such stimuli are perceived. By exposing isolated female adults to various combinations of visual, olfactory and tactile stimuli from a crowd of other adults, we found that no crowding effects could be elicited without tactile stimulation. Coating of various body surfaces with nail polish followed by exposure to crowding stimulation suggested that female adults perceive crowding stimuli with their antennae. This finding was supported by another experiment in which the antennae were either removed or covered with wax before the isolated females were exposed to crowded conditions. Neither serotonin nor an antagonist of its receptor affected the density-dependent maternal determination of progeny characteristics when injected into isolated or crowded female adults.

1. Introduction

Crowding is the primary factor that induces gregarization in locusts. The stimuli responsible for this phase change have received much attention (Uvarov, 1966, 1977; Pener, 1991; Applebaum and Heifetz, 1999; Hassanali et al., 2005; Pener and Simpson, 2009). Many studies investigating the sensory stimuli that induce gregarization have focused on nymphal behavior (Ellis, 1959, 1963; Ellis and Pearce, 1962; Roessingh et al., 1998), presumably because behavioral change occurs relatively rapidly as compared to other phase-related traits such as body color and morphometric ratios. However, there is considerable confusion regarding the stimuli associated with crowding that are actually responsible for the induction of behavioral gregarization as well as the site at which suggested stimuli are perceived. In *Locusta migratoria* and *Schistocerca gregaria*, tactile stimulation has been proposed to be the main stimulus causing solitarious nymphs to display gregarious behavior (Ellis, 1959, 1963; Roessingh et al., 1998; Simpson et al., 2001; Rogers et al., 2003; Lester et al., 2005). According to Ellis (1959), gregarious behavior can be induced in *S. gregaria* and *L. migratoria* after solitarious nymphs are touched repeatedly with metal wires for 7 h, suggesting that mechanical stimulation is involved. The same conclusion was repeatedly reported for nymphs of the former species by Roessingh et al. (1998) and Rogers et al. (2003), who used rolling paper balls or paint brushes to apply the mechanical stimulation. On the other hand, it has been reported that gregarious behavior can be induced without a tactile stimulus in *S. gregaria* if the solitarious nymphs receive visual and olfactory stimuli from gregarious individuals (Roessingh et al., 1998; Lester et al., 2005). However, this observation contrasts a pioneer study in the same species by Ellis (1959), who demonstrated that social gregarization was not increased after solitarious nymphs were exposed to a combination of visual, olfactory and auditory stimuli. A series of intensive studies by Heifetz et al. (1996, 1997, 1998) suggested that contact chemicals such as hydrocarbons can induce behavioral gregarization without mechanical stimuli in *S. gregaria*.

Corresponding information for the adult stage is limited. However, phase changes during the adult stage are important to study because upsurges in locust populations leading to outbreaks may begin when solitarious adults gather and interact locally and subsequently produce offspring characteristic of gregarious forms (Tanaka et al., 2010). In 2009, upsurges in *S. gregaria* populations were observed in Mauritania, where numerous adults were found aggregating and laying eggs in groups, as is typically observed in gregarious adults. It appears that they had grown at a low

* Corresponding author. Tel.: +81 29 838 6110; fax: +81 29 838 6110.
E-mail address: stanaka@affrc.go.jp (S. Tanaka).

0022-1910/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jinsphys.2010.09.009

population density but aggregated after adult emergence. Eggs produced by those aggregating adults were larger than those produced by solitarious adults. In some areas, numerous hatchlings were found aggregating on vegetation, and some were indistinguishable in body color from gregarious hatchlings. Hunter-Jones (1958) demonstrated that solitarious females reproducing parthenogenetically switched from being green-hatchling producers to dark-hatchling producers after being kept with other conspecific female adults. Therefore, neither mating nor male-specific stimuli are necessary for the induction of gregarious offspring. Maeno and Tanaka (2008b) found that rearing with *L. migratoria* males causes solitarious *S. gregaria* females to produce offspring characteristic of gregarious forms, indicating that the two species share common properties that can serve as a crowding cue for *S. gregaria* female adults.

Recently, it was demonstrated that isolated-reared female adults of *S. gregaria* rapidly respond to crowding by increasing egg size in the next oviposition (Maeno and Tanaka, 2008b). Egg size and hatchling body coloration are highly correlated; the larger the egg the darker the body color (Tanaka and Maeno, 2008, 2010; Maeno and Tanaka, 2009a). Maternal determination of progeny characteristics depends on a short stage in each reproductive cycle during which female adults are sensitive to crowding conditions (Maeno and Tanaka, 2010). Their response to crowding can be easily quantified by measuring the length of eggs deposited after various treatments or by determining the proportion of green hatchlings. In the present study, we used these methods to evaluate various sensory stimuli including tactile, olfactory and visual cues, that might be involved in the maternal determination of progeny characteristics and to determine the body surface site involved in the perception of such stimuli in *S. gregaria*.

Maternal determination of progeny characteristics plays an important role in the epigenetic-like transmission of crowding conditions or phase across generations in *S. gregaria* (Hunter-Jones, 1958; Maeno and Tanaka, 2008a, 2009a, 2010). Recently, a model was proposed to explain the hormonal mechanism controlling the maternal determination of progeny body size and coloration in *S. gregaria* (Tanaka and Maeno, 2010). According to this model, a hypothetical hormonal factor is released in response to the maternal crowding to control egg size by affecting the ovarioles. Based on changes in 'total behavior', which consists of many behavioral traits, the neuromodulator, serotonin (5HT) was suggested to induce behavioral gregarization in final stadium nymphs of this locust (Anstey et al., 2009), although the exact mechanism by which it causes changes in behavior and other phase-related traits is unknown. In the present study, we examined the possible involvement of serotonin in processes ranging from the perception of crowding stimuli to the determination of progeny body size and coloration.

2. Materials and methods

2.1. Insects and rearing conditions

The *S. gregaria* laboratory colony and rearing method have been described previously (Maeno and Tanaka, 2010). Locusts used for experiments were obtained from strains reared as groups of approximately 100 individuals in large cages (42 cm × 22 cm × 42 cm) for more than 50 generations or in isolation in small cages (28 cm × 15 cm × 28 cm) (except for short periods to allow mating during the adult stage) for two or more generations. In the present study, test females were allowed to lay at least two egg pods before being used for experiments because the first egg pod tends to contain a mixture of green and black hatchlings (Maeno and Tanaka, 2008b). All locusts and eggs were maintained at 31 ± 1 °C with a light-dark (LD) cycle of 16:8 h (lights-on at 0800 and lights-off at 2400).

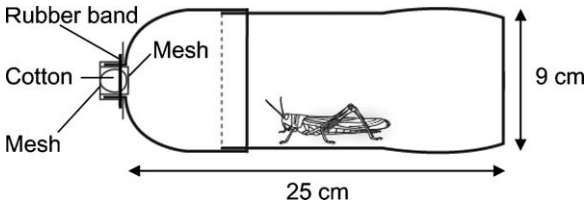

Fig. 1. A plastic container used to expose locusts to various stimuli.

2.2. Determination of effective stimuli

To identify the stimuli that induce a gregarizing effect on progeny characteristics, individually maintained, mated female adults were exposed to tactile, visual and/or olfactory stimuli in cylindrical transparent containers (9 cm height × 25 cm diameter; Fig. 1) for three days after the deposition of an egg pod, and then re-isolated in small cages to obtain another egg pod. The cylindrical containers were made of clean, transparent soft-drink-pet-bottles. Each bottle was cut in half and the two parts were put together after locusts and grass were housed inside. The opening (diameter, 2.1 cm) of the bottle was plugged with a loose ball of cotton covered with nylon mesh for ventilation. This plug also served as a barrier to prevent direct contact between the locust housed inside and locusts outside.

Females produce an egg pod every four days on average under the conditions used in the present study (Maeno and Tanaka, 2009b). In this locust, a period of four days consisting of two two-day periods before and after the deposition of an egg pod is the stage at which females are sensitive to crowding conditions and consequently affect progeny characteristics in the next egg pod (Maeno and Tanaka, 2010). In the present study, three-day treatments starting at the deposition of an egg pod spanned the last two days of the sensitive period for the next egg pod. Isolated females exposed to crowded conditions during the last two days of the sensitive period significantly increase egg size and reduce the proportion of green hatchlings in the next egg pod (Maeno and Tanaka, 2010).

To test the effects of tactile stimuli on progeny characteristics, each test female was housed with four sexually mature males together with a small amount of grass in a cylindrical container. In this species, pairing a female with a single male induces crowding effects on progeny equivalent to those observed when a female is housed with many males; crowding effects can be elicited even when an isolated-reared female is crowded with females (Hunter-Jones, 1958; Maeno and Tanaka, 2008b). In this case, however, the target female locusts received not only tactile but also visual and olfactory stimuli. To exclude the visual stimuli, similarly prepared females were treated as above after their compound eyes were coated with white-out and then black nail polish (Daiso Co., Tokyo, Japan). Visual and olfactory stimuli were investigated by placing test females in cylindrical containers on the floor of a large rearing cage in which about 100 crowd-reared sexually mature females and males were held with food. Olfactory stimuli were tested by pumping air from a transparent plastic bag (70 L in volume) holding a large cage containing about 100 sexually mature crowd-reared adults to another plastic bag holding cylindrical containers containing test females and food (NISSO, Chikara α2000 air pump, Co., Tokyo, Japan). The walls of these containers were perforated for better ventilation. The plastic bag holding the large cage was tied loosely with a rubber band so that air could come in as air inside the cage was pumped out. The pumped air was sent to the