Journal of Insect Physiology 58 (2012) 718–725

Contents lists available at SciVerse ScienceDirect

Journal of Insect Physiology

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

Phase-dependent locomotor activity in first-stadium nymphs of the desert locust, *Schistocerca gregaria*: Effects of parental and progeny rearing density

Ken-ichi Harano [a,*], Seiji Tanaka [a], Yasuhiko Watari [b], Osamu Saito [b]

[a] *Locust Research Laboratory, National Institute of Agro-biological Sciences at Ohwashi, Tsukuba, Ibaraki 305-8634, Japan*
[b] *Laboratory of Biotechnology, Faculty of Education, Ashiya University, Hyogo 659-8511, Japan*

ARTICLE INFO

Article history:
Received 20 January 2012
Received in revised form 20 February 2012
Accepted 20 February 2012
Available online 28 February 2012

Keywords:
Body coloration
Body size
Desert locust
Parental effect
Phase polymorphism

ABSTRACT

This study examined the effects of parental and progeny rearing density on locomotor activity of 1st-stadium nymphs of the desert locust, *Schistocerca gregaria*, using an actograph. Progeny obtained from solitarious (isolated-reared) or gregarious (crowd-reared) locusts were reared in isolation or in a group of 30 nymphs. Crowding after hatching had a slight influence on mean activity shortly after the start of measurements, but no clear effect was detected until day 2, when maximum activity during the 6–24 h of observation was significantly higher than that of the nymphs kept in isolation. On the other hand, the effects of parental rearing density on locomotor activity manifested at all ages examined (0–2 days old). Progeny of gregarious locusts showed consistently higher activity than those of solitarious locusts. In newly hatched nymphs, the effect of parental rearing density was explained by variation in body size at hatching, one of the phase-dependent characteristics. Hatchling body color was also correlated with locomotor activity and body weight. Similar levels of locomotor activity were exhibited when green, solitarious and black, gregarious nymphs were similar in body weight. These results suggested that parental rearing density indirectly influences locomotor activity in the progeny shortly after hatching by affecting their body size as eggs or hatchlings.

1. Introduction

Phase polyphenism is one of the most remarkable phenomena in locusts. Their behavior, morphology and body coloration are considerably different between locusts occurring at low and high population densities (Uvarov, 1966, 1977). For example, locusts found at low population densities (solitarious locusts) are sedentary and have a tendency to avoid conspecific locusts, whereas those observed at high population densities (gregarious locusts) often form a large swarm or marching bands and migrate over long distances. These traits change continuously depending on the local population density and various intermediate forms are present between the two extreme forms, gregarious and solitarious locusts.

Some phase-dependent characteristics are affected not only by the local population density after hatching but also by the population density perceived by their female parents (Faure, 1932; Hunter-Jones, 1958). In the desert locust, *Schistocerca gregaria*, crowding stimuli perceived by the antennae of adult females affect body size and color of their progeny (Maeno et al., 2011). In a series of studies Ellis (1951, 1959a, 1964) demonstrated the effects of parental rearing density on progeny behavior in another locust species, the migratory locust, *Locusta migratoria*. Ellis noticed that nymphs derived from gregarious adults were more active in marching at the 4th nymphal stadium than those from solitarious adults even when reared under the same conditions after hatching. However, Ellis found that the rearing density after hatching had a larger impact on marching behavior than that experienced by the parents in this locust species, suggesting that the parental effects are partly masked by the effects of progeny rearing density. Therefore, parental rearing density is expected to be more important in controlling behavior for younger nymphs. To investigate the role of parental density in the control of progeny behavior, Islam et al. (1994a,b) observed *S. gregaria* 1st-stadium nymphs. They analyzed total behavior of 1st-stadium nymphs by integrating more than 10 behavioral parameters using logistic regression analysis, and they concluded that rearing density of parents, especially density at oviposition, as well as rearing density of the nymphs themselves affects nymphal behavior in the same way.

Locomotor behavior is another phase-dependent trait. Ellis (1962) observed 4th stadium nymphs of *L. migratoria* and *S. gregaria* in the laboratory and reported that the distance walked, time spent in walking and speed of walking were all greater in crowd-reared nymphs than in isolated-reared nymphs in both species. Hoste et al. (2006) focused on general locomotor activity by developing a simple bioassay and addressed the effect of parental and

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* Corresponding author. Present address: Honeybee Science Research Center, Tamagawa University, Machida, Tokyo 194-8610, Japan. Tel./fax: +81 42 739 8249.
E-mail addresses: kharano@lab.tamagawa.ac.jp, hacchiken@hotmail.com (K. Harano).

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http://dx.doi.org/10.1016/j.jinsphys.2012.02.007

progeny rearing density on behavior in *S. gregaria* 1st-stadium nymphs. In their studies, each test nymph was placed in a small square arena surrounded by walls and the number of contacts of the locust with the walls was recorded. Hoste et al. (2006) demonstrated that locomotor activity is enhanced by crowding after hatching relative to nymphs kept in isolation but the effect was more pronounced when their parents were reared in a group than in isolation.

Harano et al. (2009, 2011) monitored locomotor activity for 24 h in 1st-stadium nymphs of *L. migratoria* using an actograph, and found that (1) nymphs greatly change their activity levels depending on the time elapsed after the beginning of measurements and (2) the parental effect is found only later in the measurement period. This study suggested the necessity of long-term monitoring to understand the controls on locust behavior. Most previous studies have analyzed behavior for only a short period: 400 s in Islam et al. (1994a,b), 10 min in Bouaïchi et al. (1995) and 60 min in Hoste et al. (2006). The present study investigated the influence of parental and progeny rearing density on locomotor activity in *S. gregaria* hatchlings by monitoring them for 24 h or more using an actograph system.

S. gregaria hatchlings show considerable variation in body color. Gregarious hatchlings are typically black and solitarious hatchlings green, and various intermediate colorations are found in hatchlings depending on the rearing density of their parents. The relationship between hatchling behavior and body coloration is controversial in this locust. Islam et al. (1994b) and McCaffery et al. (1998) found a significant correlation between the two traits, whereas Bouaïchi et al. (1995) and Hägele et al. (2000) reported no linkage between them. A recent review article by Pener and Simpson (2009) indicated that behavior and body coloration are controlled independently.

A series of studies reported by Simpson and his co-workers (McCaffery et al., 1998; Hägele et al., 2000; Miller et al., 2008) suggested that a gregarizing factor is contained in the egg foam plug deposited by gregarious adult females. They claimed that this factor is water-soluble and can be washed off from deposited eggs. Interestingly, simply separating eggs can also remove this factor. Separated gregarious eggs produced green hatchlings that behaved like solitarious forms, whereas eggs deposited by solitarious adult females produced dark-colored hatchlings that behaved like gregarious nymphs when water extracts from egg foam plugs deposited by gregarious females was applied (McCaffery et al., 1998). However, recent studies by Tanaka and Maeno (2006, 2008, 2010) demonstrated that separating and washing eggs was not effective in inducing green color in gregarious eggs that were thought to give rise to black hatchlings, and suggested that hatchling body color is pre-determined in the ovarioles of the female parent based on the correlation between hatchling body color and size. Hatchling body size is a direct function of egg size, which is determined in the ovarioles. It has been known that gregarious hatchlings are larger in size and darker in color than solitarious hatchlings (Hunter-Jones, 1958). This relationship appears to be extremely difficult to explain using the foam plug factor as the determinant of hatchling body color because it has been assumed to work after oviposition (McCaffery et al., 1998) or after ovulation (Simpson and Miller, 2007). Few studies have investigated the relationship between hatchling behavior and body size in locusts. In the present study, we examined how body size would influence locomotor activity in *S. gregaria* hatchlings.

2. Materials and methods

2.1. Insects

A laboratory colony of the desert locust, *S. gregaria* derived originally from a Niger population (Tanaka and Maeno, 2010) was used to obtain 1st-stadium nymphs. These nymphs were reared in a group of 100 + locusts in a large cage (42 × 22 × 42 cm) for about 50 generations or in isolation in small cages (28 × 15 × 28 cm) for more than 2 generations, as previously described (Maeno and Tanaka, 2010). The number of crowded locusts per cage was about 100 at oviposition. All locusts were fed leaves of orchard grass, cabbage and wheat bran and maintained at 31 °C with a light–dark (LD) cycle of 16:8 h. Moist sand in a plastic cup (diameter, 10 cm; height, 6 cm) was provided for oviposition. The cup with egg pod(s) was transferred to another room at 30 °C under continuous light (LL) conditions to avoid possible influences of light–dark cycles on progeny activity (Harano et al., 2009).

2.2. Effects of parental and progeny rearing density

To examine the effects of parental and progeny rearing density on locomotor activity, 1st-stadium nymphs were obtained from gregarious and solitarious lines (referred to as G and S nymphs, respectively) and reared under crowded or isolated conditions as will be described below. G and S nymphs reared in a group were termed Gg or Sg nymphs and those reared in isolation Gi or Si nymphs, respectively. Their locomotor activities were measured at day 0, 1 and 2 from hatching.

Within 4 h after hatching, nymphs (0 day old) were split into three groups. Some were subjected to an actograph for measurements of locomotor activity. The remainder were reared in plastic containers (diameter, 10 cm; height, 6 cm) individually or in a group of 30 nymphs following the method of Harano et al. (2011) developed for *L. migratoria*. Each container had a lid with a hole (2 cm in diameter) covered with screen mesh. Nymphs were given leaves of *Bromus catharticus* inserted into a wet block of floral foam (1 × 1 × 2 cm; Oasis Deluxe; Smithers-Oasis Japan Co. Ltd., Tokyo, Japan) as food and were kept under crowded or isolated conditions for 1 or 2 days. In individual rearing, each rearing container was separated by a piece of white cardboard for visual isolation, although no special measure was made for olfactory isolation. Fresh grass was given every day. To avoid possible inter-pod variation, nymphs obtained from the same egg pods were divided into two groups and reared in a group or in isolation. All nymphs were maintained at 30 °C under continuous light (LL) conditions until used. To examine the effects of crowding during a short period after hatching, some eggs were removed from egg pods 2 days before the expected time of hatching and individually placed on a piece of wet tissue paper in small Petri dishes (diameter, 6 cm; height, 1.5 cm). The remaining eggs in the pod were placed on wet tissue paper in a group of more than 30 eggs in a 9-cm Petri dish. Nymphs hatched from isolated eggs were kept for 4–6 h in their dishes after hatching whereas nymphs hatched from grouped eggs were transferred to a plastic bag (30 × 40 cm) within 2 h and kept for 4 h in a group of more than 30 nymphs. After each treatment, the locomotor activity was measured by actograph. This experiment was performed with both G and S nymphs. All G nymphs used in this experiment were almost completely black and categorized in hatching color grade (HCG) 5 according to the grades previously described (Maeno and Tanaka, 2009) and S nymphs were green with little or no black patterns (HCG 1).

To investigate the relationship between locomotor activity and body size and color, hatchlings were weighed to the nearest 0.1 mg using an electric balance and their body color was graded on a scale from 1 to 5 (Maeno and Tanaka, 2009) before measurements of locomotor activity. Egg pods produced by solitarious or gregarious adults sometimes produce a mixture of green and black hatchlings (Maeno and Tanaka, 2009). In some experiments, hatchlings from such egg pods were used to examine the relationship between body color and behavior. The relationship between body