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Re-examination of the roles of environmental factors in the control of body-color polyphenism in solitarious nymphs of the desert locust *Schistocerca gregaria* with special reference to substrate color and humidity

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ABSTRACT

This study re-examines the effects of environmental factors including substrate color, humidity, food quality, light intensity and temperature on the green–brown polyphenism, black patterning and background body color of solitarious (isolated-reared) nymphs of *Schistocerca gregaria*. All individuals reared in yellow–green or yellow containers became green morphs, whereas those reared in white, ivory-colored, blue, grey, brown, zinc-colored and black containers produced brown morphs in similar proportions. The intensity of black patterns was negatively correlated with the brightness of the substrate color of the containers. Humidity, which previous studies claimed controls green–brown polyphenism in this species, exerted no significant influence on either the green–brown polyphenism or the black patterning. Food quality also had little effect on body color. High temperature tended to inhibit darkening. The background body color on the thorax was greatly influenced by the substrate color of rearing containers and a close correlation was found between these two variables, indicating that, in contrast to what has been suggested by others, this species exhibits homochromy to match the body color to the substrate color of its habitat. Similar responses were observed in another strain, although some quantitative differences occurred between the two strains examined. Based on these results, a new model explaining the control of body-color polyphenism in this locust is proposed and the ecological significance of black patterns in solitarious nymphs is discussed.

1. Introduction

Intra-specific variation in body coloration is widespread among insects (Fuzeau-Beaesch, 1985). This phenomenon is particularly common in grasshoppers and locusts (Faure, 1932; Uvarov, 1966, 1977; Rowell, 1971; Dearn, 1990; Pener, 1991; Pener and Yerushalmi, 1998; Pener and Simpson, 2009). It is determined genetically in some species, whereas it is mainly controlled environmentally in other species. The former is often referred to as polymorphism and the latter as polyphenism (Dingle, 1996), although even in the latter case the phenotypic expression is not entirely independent of genetic control.

The migratory locust, *Locusta migratoria* L., is one of the most intensively studied species. The expression of body coloration in late-instar nymphs of this locust depends on three types of polyphenism: phase-color polyphenism, green–brown color polyphenism and homochromy (Faure, 1932; Rowell, 1971). At high population densities, nymphs exhibit phase-color polyphenism making them all look similar, developing black patterns with a dirty-orange color background. At low population densities, nymphs show 'green–brown' color polyphenism depending on humidity. They become green morphs at high humidity, whereas at low humidity they are brown morphs and show homochromy, which is the third form of color polyphenism in this species (Rowell, 1971; Pener, 1991; Pener and Yerushalmi, 1998; Pener and Simpson, 2009). Homochromic coloration is an adaption to match the substrate color of the surrounding habitat.

The desert locust, *Schistocerca gregaria* Forskål, is another locust that has received much attention with regard to the environmental control of the variation in its nymphal body color (Nickerson, 1956; Stower, 1959; Ellis, 1959a,b, 1963; Lester et al., 2005). This locust exhibits a remarkable polyphenism in hatchling body color that is determined by the population density to which the female parent is exposed as an adult (Hunter-Jones, 1958). Recently, it has been demonstrated that hatchling body size and color are pre-determined in the ovarioles of the mother in response to crowding conditions perceived at a specific stage during the reproductive cycle (Tanaka and Maeno, 2006, 2008, 2010; Maeno and Tanaka, 2008, 2010; Maeno et al., 2011). Another body-color variation is observed from the 2nd instar onward. According to a

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widely accepted model (Rowell, 1971; Pener, 1991; Pener and Simpson, 2009), the desert locust displays green–brown polyphenism and phase polyphenism, but does not exhibit homochromy. The green–brown polyphenism is observed only among solitarious nymphs (at low population densities) and controlled by humidity: nymphs turn green at high humidity, whereas they adopt the brown form at low humidity, as observed in *L. migratoria* (Faure, 1932) and *Schistocerca vaga* (Rowell and Cannis, 1971). The phase-color polyphenism is controlled by population density, and nymphs at high population densities develop intense black patterns with a whitish, orange or yellow background color depending on the developmental stage. In this locust, therefore, little attention has been paid to the variation of substrate color in the rearing containers and variation of light intensity in studies of body-color polyphenism (e.g. Ellis, 1959b; Lester et al., 2005).

During a field excursion to Mauritania, West Africa, Tanaka et al. (2010) observed solitarious desert locust nymphs and encountered interesting situations that motivated us to re-examine the widely accepted model of body-color polyphenism in this locust (Pener and Simpson, 2009). That is, green nymphs of the desert locust were common in habitats where relative humidity was considerably low. Hunter-Jones (1962) is the first to present empirical data supporting the importance of humidity in the induction of green color in the desert locust. However, in his experiment, the grass provided under dry (arid) conditions quickly evaporated and assumed the color of hay, whereas the grass stayed green under humid conditions. Although other explanations may also be possible, there is a possibility that humidity influences the nymphal body color indirectly by affecting the color of the grass or the background color experienced by the locusts. At another survey site in Mauritania, Tanaka et al. (2010) encountered solitarious nymphs with black patterns that looked fairly inconspicuous against the background of the habitat or bushes unless the locusts moved. Such individuals are often considered to be locusts in a transient phase, as they change from solitarious to gregarious or vice versa (Nickerson, 1956; Stower, 1959; Lester et al., 2005). In this study, we carried out a series of experiments to examine the effects of the substrate color of rearing containers, light intensity, humidity, food quality and temperature on the body color of solitarious (isolated-reared) desert locust nymphs. This paper describes the results of these experiments, proposes a new model to explain the environmental control of body-color polyphenism and discusses the ecological significance of body-color polyphenism in the desert locust.

2. Materials and methods

2.1. Insects and rearing methods

All experiments were carried out with a locust colony established with several hundred eggs sent from Prof. H. Ferenz' laboratory in 2008, unless otherwise stated. This strain was originally collected in Niger in 2004 (Tanaka and Maeno, 2010). In one experiment, an Ethiopian strain was used to examine possible intra-specific differences in the control of body-color polyphenism. This strain has been previously described (Maeno and Tanaka, 2007). All experiments were carried out with hatchlings obtained from a solitarious line in which locusts had been reared in isolation for 2–5 generations except for a short period for mating during the adult stage according to the method previously described (Tanaka and Maeno, 2006). Most hatchlings were green with few or no dark spots, but a few individuals developed conspicuous black patterns. Our standard method involved handling hatchlings as follows: newly hatched green nymphs were reared individually in Petri dishes (diameter, 9 cm; height, 1.5 cm) during the first stadium. The wall of each dish was covered with yellow–green vinyl tape so that the locust inside was visually isolated from individuals in other dishes. These dishes were placed on yellow–green paper. Pieces of cut grass leaf (1–2 cm long; *Bromus catharticus*) were supplied as food every day.

2.2. Effects of substrate color

Upon ecdysis to the second stadium, only green nymphs without black patterns were reared in transparent plastic containers (volume, 340 cm^3) individually for experiments. The inside wall of each container was lined with a piece of color paper (3 × 2.5 cm; Joyful Honda Co., Tokyo, Japan) and the container had a transparent lid with 10 holes (diameter, 2 mm each) for ventilation. In this study, a total of 11 different colors were tested, as will be described later. The containers housing locusts were placed on a piece of paper of the same color as the wall, so that they were surrounded by the same color or the same substrate color except for the perforated lid. Locusts were supplied with pieces of grass leaf (ca. 2 cm in length each) every day. The containers were cleaned every other day, and care was taken not to touch the locusts with hands directly by transferring each nymph into a plastic bag (30 × 35 cm) while the containers were cleaned. The temperature in the rearing rooms was kept at 31 ± 1 °C and relative humidity fluctuated between 6% and 35% (readings were recorded with an automatic recorder; Ondotori, Tokyo, Japan). The room was illuminated by daylight fluorescent lamps (FL15D; Toshiba Co., Tokyo) and the light intensity on the table was about 9.86 W/m^2. In some experiments nymphs were exposed to a lower intensity of light or to complete darkness, all at the same constant temperature.

2.3. Effects of high temperature

To test the effects of a high temperature, nymphs were reared in white, yellow–green or black containers at 38 ± 1 °C and LD 12:12 h in an incubator (Fuji Medical Science Co., Chiba, Japan). Mean relative humidity inside cups was 29% (readings were recorded with an automatic recorder; Ondotori, Tokyo, Japan). The light intensity of daylight fluorescent tubes was approximately 5.01 W/m^2 on the upper shelf and 2.06 W/m^2 on the lower shelf. Locusts kept on these shelves were switched every day to equalize the light conditions experienced. In this experiment, food was changed twice a day during the light period and the containers were cleaned every day.

2.4. Scoring of body color

The three types of body-color polyphenism were quantified 2 or 3 days after ecdysis to the last nymphal stadium. For the body-color polyphenism, the "green morph" included all individuals that were dark green, green or slightly green, whereas the brown hoppers were not necessarily brown but assumed various non-green colors.

In a second type of polyphenism, nymphs were divided into 5 grades based on the intensity of the black patterning on the head, thorax and hind legs (Fig. 1); grade 1, the head and thorax with no black spots; grade 2, the thorax and legs with slightly dark spots; grade 3, the thorax and legs with clear black patterns; grade 4, the thorax and legs with intensive black patterns and the vertex with black marking; grade 5, the thorax and vertex with intensive black patterns that extend to the area below and posterior to the compound eyes. Throughout this study, we encountered no individuals kept in isolation that developed black markings on the head before the pronotum was intensively covered with black patterns. Statistical comparisons between different treatments were