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The trans-generational phase accumulation in the desert locust: Morphometric changes and extra molting

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ABSTRACT

To understand the underlying trans-generational phase accumulation, a classical morphometric characteristic, the *F*/*C* ratio (*F*, hind femur length; *C*, maximum head width), of adult desert locusts (*Schistocerca gregaria*) was monitored over eight consecutive generations. Adult *F*/*C* ratios, which are larger in solitarious locusts than in gregarious ones, were negatively correlated to the darkness of body color at hatching. Two successive generations were required for a complete shift from the gregarious (crowd-reared) to the solitarious (isolated-reared) phase and vice versa in the laboratory. That is (1) female adults needed to be exposed to crowded (or isolated) conditions so that their hatchlings would become large (or small) and dark (or green) in color, and (2) the hatchlings then needed to be exposed to crowded (or isolated) conditions for their entire nymphal stage. Solitarious locusts exhibited extra molting that influenced the *F*/*C* ratio in the adult stage, but did not exert significant influences on the trans-generational changes in this trait because the incidence was low. The incidence of extra molting was negatively correlated with nymphal survival rates. The morphometric trans-generational changes may be explained without assuming any accumulating internal factor.

1. Introduction

The desert locust often undergoes outbreaks and causes serious damage to agricultural crops. The last major outbreak that occurred in 2003–2004 affected as many as 26 countries, covering 20% of the entire land surface of the earth (FAO, 2005).

One of the most unique features of locusts is that they display a phenomenon called phase polyphenism in which various morphological, physiological, biochemical and behavioral traits change continuously in response to the population density (Uvarov, 1921; Faure, 1932; Ellis, 1959; Albrecht et al., 1959a,b; Hunter-Jones, 1958). Individuals in low-density populations, the solitarious phase, are characterized by a light-colored body, small egg size, a relatively small ratio of wing length to hind femur length and sedentary and solitary behaviors. Individuals in high-density populations, the gregarious phase, have a dark body color, large egg size, a large ratio of wing length to hind femur length, and migratory and gregarious behaviors (Uvarov, 1921, 1966; Faure, 1932; Dale and Tobe, 1990; Pener, 1991; Pener and Yerushalmi, 1998; Tanaka, 2006). Locusts shift from one phase to another in response to changes in local population density, but they usually go through a transient phase because the phase shift is not completed in one generation (Uvarov, 1966). Because some of the phase-dependent characteristics are directly associated with population growth (Maeno and Tanaka, 2008a), it is important to understand the process of phase change and phase polyphenism in locusts.

Whereas maternal effects alone can explain some phase characteristics such as hatchling body size, hatchling body coloration and ovariole numbers in locusts (Albrecht et al., 1959a,b; Hunter-Jones, 1958), other characteristics such as morphometric traits, behavior, and concentrations of some biochemical compounds have been suggested to change over a few to several generations in a phenomenon called phase accumulation (Uvarov, 1966; De Loof et al., 2006). However, experiments to determine the occurrence of phase accumulation are not always conclusive due to technical limitations. In *Schistocerca gregaria,* for example, the trans-generational accumulation of morphometric traits and peptidic molecules was proposed, but the correlation appears to have been mainly due to large values in one of the generations tested (Fig. 2; Rahman et al., 2002). Behavioral phase accumulation frequently cited (Simpson et al., 1999; Simpson and Sword, 2009) was asserted in the absence of statistical analysis (Roessingh et al., 1993). Data on the trans-generational accumulation of amino acids and biogenic compounds (Rogers et al., 2004) were based on pooled samples collected from different individuals over several days of a

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developmental stage. Pooled samples can produce substantial errors because the concentrations of such compounds fluctuate greatly on a daily basis, as has been known in the migratory locust, *Locusta migratoria* (Tanaka and Takeda, 1997). Circadian variation of serotonin contents in the brain and hemolymph of a cricket, *Acheta domesticus*, have been observed (Muszynska-Pytel and Cymborowski, 1978). In another cricket, the hemolymph juvenile hormone titer exhibits a large diurnal cycle (Zhao and Zera, 2004). To examine trans-generational changes in biochemical compounds, these phenomena need to be taken into account. Small-scale experiments carried out with successive solitarious (isolated-reared) generations may also result in difficulty in distinguishing generational effects from those of unintentional selection on the phase characteristics examined. Probably the best-known case for the occurrence of phase accumulation in the desert locust may be the one cited by Uvarov (1966) and Chapman (1976) in which the morphometric (*F*/*C*) ratio of hind femur length (*F*) to head width (*C*) changed across generations. However, that information was based on an unpublished study by Hunter-Jones, and neither the details of the experimental procedure nor the data have been published.

It has often been noted that the morphometric ratios typically observed in solitarious or gregarious populations cannot be obtained in the laboratory (Faure, 1932; Hunter-Jones, 1958). While there is no doubt that locust morphometric traits are mainly controlled by local population density or crowding conditions, other environmental factors such as temperature and humidity also modify these traits (Gunn and Hunter-Jones, 1952; Stower et al., 1960; Dudley, 1964; Uvarov, 1966). Thus, under different sets of environmental conditions, locusts are likely to show different morphometric ratios even under the same crowding conditions. However, under any set of conditions, a stable value should be reached after a certain number of generations. In the present study, locusts reaching a stable *F*/*C* value under isolated or crowded conditions were assumed to have attained the solitarious or gregarious phase.

The endocrine control of phase-dependent morphometric characteristics has received much attention (Dale and Tobe, 1990; Pener, 1991; Pener and Yerushalmi, 1998; Tanaka, 2006). It has been suggested that [His^7]-corazonin is responsible not only for the control of phase-dependent melanization (Tawfik et al., 1999; Tanaka, 2001; Maeno and Tanaka, 2009) and morphogenesis of antennal sensilla (Yamamoto-Kihara et al., 2004; Maeno and Tanaka, 2004) but also for the *F*/*C* ratio (Hoste et al., 2002; Tanaka et al., 2002; Maeno et al., 2004) in two species of locust.

In the present study, the *F*/*C* ratio was used to determine if phase accumulation occurs in the desert locust under laboratory conditions. Because solitarious locusts often exhibit extra molting, the effects of extra molting on adult *F*/*C* ratios in individuals and in successive generations were also examined. This paper reports that the morphometric phase accumulation occurs in *S. gregaria* and is completed in either direction in two generations. We further demonstrate that a complete phase shift may be possible in one generation under certain conditions.

2. Materials and methods

2.1. Insects

The colony of *S. gregaria* used has been described previously (Tanaka and Yagi, 1997). Before this study was conducted, locusts had been maintained for more than 40 generations at 30–32 °C in large wood-framed cages (42 cm × 22 cm × 42 cm). Each cage was covered with nylon screening except for the wood floor and the front sliding door, which was composed of a transparent acrylic plate. Locusts were fed cut grass inserted in water jars, cabbage leaves and wheat bran. Grass was changed every 1 or 2 days. Two kinds of grass, orchard grass and *Bromus catharticus*, were grown in crop fields by the Field Management Department of the National Institute of Agrobiological Sciences at Ohwashi, and cabbage was purchased. Orchard grass was mainly used, but it was not available from mid-December to mid-February, during which *B. catharticus* was used. Locusts were allowed to lay egg pods in moist sand held in plastic cups (vol. 380 ml), and egg pods were incubated at 30–32 °C.

2.2. Handling of locusts and rearing methods

To examine the effect of rearing density on phase characteristics, hatchlings obtained from the crowd-reared colony were reared either under crowded conditions (C line) or in isolation (I line) in a well-ventilated room (30–32 °C). In the C line, about 100 hatchlings were reared in a large cage, and the egg pods that were collected were handled as above. More than 100 individuals can be reared in a cage of this size, but mortality increases in the last nymphal stadium and shortly after adult emergence, presumably due to overcrowding. In the I line, 200–300 hatchlings were individually reared in each generation in small cages (22 cm × 12 cm × 28 cm) and maintained in isolation throughout the adult stage except for a short period (<48 h) for mating. Each small cage had a front slide door made of an acrylic plate, two sides covered with white acryl board and the other side and top covered with nylon screening. Most hatchlings of the C line were black in color, while those of the I line were green. However, both lines contained small proportions of hatchlings with unexpected body color, particularly in the first egg pods (Maeno and Tanaka, 2008b). Hatchlings were usually obtained from egg pods after the first oviposition, but hatchlings were chosen at random for successive generations to avoid selection for a particular body-color type. In the I line, all nymphs were checked every day for molting, and the number of nymphal stadia was determined for each individual. Morphological measurements were made a week after adult emergence in each generation of both lines; maximum head width (*C*) and hind femur length (*F*) were measured with a pair of digital callipers for each individual to determine the classical morphometric ratio of *F*/*C*.

2.3. Effects of hatchling characteristics on F/C ratios

To examine the effect of hatchling body weight on the adult *F*/*C* ratio, more than 2000 hatchlings obtained from isolated-reared females were weighed within 6 h of hatching and reared in isolation. These constituted the I line in the above observations. Nymphs were checked every day for molting, and the number of nymphal stadia was recorded for each individual. The *F*/*C* ratio was also determined as above. This experiment was carried out over a period of 2 years.

Female adults change the size of their eggs or hatchlings in response to a shift in rearing density during the adult stage (Maeno and Tanaka, 2008b). In another experiment, hatchlings of various body sizes were obtained by exposing crowd-reared females to isolation or isolated-reared females to crowding by pairing with a male adult before oviposition. In *S. gregaria*, pairing of a female with a single male induces crowding effects that are as strong as rearing her with many individuals (Hunter-Jones, 1958; Maeno and Tanaka, 2007). Nymphs (>6 h after hatching) were sorted out by color into five hatchling color groups (HCGs 1–5; Maeno and Tanaka, 2007). Hatchlings in HCG 1 were green, as typically observed in solitarious forms, and those in HCG 5 were almost completely black, as observed in gregarious forms. Individuals in HCGs 2–4 were intermediate in color. To test the relationships between hatchling color and body weight, hatchlings categorised into different color grades were weighed using a Mettler A201 balance. Others were reared either in isolation or in groups as described above to determine their *F*/*C* ratio after adult emergence.