Hormonal control of body-color polymorphism in *Locusta migratoria*: interaction between [His\(^7\)]-corazonin and juvenile hormone

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Received 7 January 2000; accepted 18 April 2000

Abstract

The effects of injection of [His\(^7\)]-corazonin and juvenile hormone (JH) III on the body color in *L. migratoria* were investigated using albino and normal (pigmented) nymphs. Most albino nymphs turned green in the fourth instar if injected with JH III during the last 2 days of the previous instar. When albino third instar nymphs injected with 10 pmol of [His\(^7\)]-corazonin on different days were treated with 100 \(\mu\)g of JH III on day 3.5, they developed various body colors in the following nymphal instar: those injected with [His\(^7\)]-corazonin during the first 2 days developed very dark green or black color, whereas some of those injected after this period turned green and their legs and ventral side of the body were variously pigmented, the coloration being similar to green solitary individuals often found in the field. Field-collected brown solitary nymphs injected with 1 nmol of [His\(^7\)]-corazonin and kept individually, turned reddish without any black spots in the following nymphal instar when the ecdysis occurred within 1 day after injection. Injection of [His\(^7\)]-corazonin 2 days before the following ecdysis induced black patterns on an orange background color, the coloration characteristic of gregarious forms. Similar injections into field-collected green solitary nymphs also induced black patterns but the rest of their body remained green. These results may indicate that the temporal changes in the hemolymph titers of [His\(^7\)]-corazonin and JH play an important role in the control of body-color polymorphism in this locust.

Keywords: Body-color polymorphism; [His\(^7\)]-corazonin; Juvenile hormone; *Locusta migratoria*; Albinism; Phase polymorphism

1. Introduction

The migratory locust, *Locusta migratoria*, displays body-color polymorphism in response to various factors (Faure, 1932; Uvarov 1966, 1977). This polymorphism is particularly conspicuous during the nymphal stage and the environmental relations have been studied intensively. Population density is one of the most important factors influencing body color. Nymphs at low density (solitary form) show cryptic coloration, either green or different yellow, brown, reddish or black colors whereas those at high density (gregarious form) show black patterns with an orange background color (Fuzeau-Braesch, 1985; Pener, 1991). This response to population density is not an all-or-none type, and various intermediate body colors appear at an intermediate density or when nymphs are transferred from a high (or low) density to a low (or high) density during the nymphal stage, although it may take several days before changes occur. Humidity and the background color are also important in the control of body color in solitary nymphs of this locust.

It is well known that juvenile hormone (JH) induces the green body color in locusts. Implantation of extra corpora allata, the glands producing JH, or injection of synthetic JH or JH analogs stimulates crowded (gregarious) nymphs of *L. migratoria* to turn green (Joly and Joly, 1954; Joly and Meyer, 1970; Némec, 1971). From this phenomenon, one may assume that JH controls the body-color polymorphism in locusts: nymphs with a high hemolymph JH titer become green and those at a low titer develop the gregarious body coloration, e.g. Nijhout and Wheeler (1982). However, this view was criticized by Pener et al. (1992) who found that green isolated nymphs lost green color after being alla-
tectomized with precocene III but did not develop the gregarious body coloration (black patterns with orange background color). From this result, Pener et al. (1992) concluded that JH is not a major factor in the control of body color polymorphism in locusts.

Using an albino strain of *L. migratoria*, it was found that the central nervous system and the corpuscardiacum of normal (pigmented) individuals of this species contain a factor inducing dark body color (Tanaka, 1993). When albino nymphs were implanted with corpora cardiaca taken from normal nymphs, some of them developed a body coloration characteristic of gregarious forms. Interestingly, a similar factor inducing dark color in this albino locust was also found in the brain and the corpuscardiacum of another locust, *Schistocerca gregaria* (Tanaka and Yagi, 1997). Recently, we succeeded in isolating these peptides and found that they were the same (Tawfik et al., 1999). This peptide consists of 11 amino acids with a molecular weight of 1369 and is identical to [His7]-corazonin, the peptide first isolated from another acrid *S. americana* without known function (Veenstra, 1991). By injecting this peptide, we have succeeded in inducing the gregarious dark body color in both *L. migratoria* and *S. gregaria* even under solitary (isolated) conditions (Tawfik et al., 1999). More recently, this peptide was shown to induce various other colors in *L. migratoria*, depending on the dose and the timing of injection, and some albino nymphs injected with this peptide looked similar to black, brown or reddish-colored solitary nymphs sometimes found in the field (Tanaka, 2000). Therefore, this peptide appears to be an important hormonal factor responsible for the control of body-color polymorphism in locusts.

In the present study, I examined the interacting effects of [His7]-corazonin and JH III on body color using the albino strain of *L. migratoria*. As mentioned above, JH induces green color in this species. In most studies, the effect of JH on the induction of green color was examined with normal individuals (e.g. Joly and Joly, 1954; Staal and De Wilde, 1962; Némec, 1971; Pener et al., 1992). Therefore, the results from such studies were interpreted without considering the presence or absence of [His7]-corazonin. Albino nymphs treated with JH analogs develop green color. However, they look different from normal solitary green individuals because the ventral side of the body and some portions of the legs in the former also become green or remain whitish, whereas those in the latter are variously pigmented (Tanaka, 1993; Hasegawa and Tanaka, 1994). Here, I report that both [His7]-corazonin and JH are important in the control of gregarious and solitary body color in *L. migratoria*.

In one experiment, field-collected solitary nymphs were treated with [His7]-corazonin to examine how injection of this peptide would influence the expression of body color in green and brown solitary locusts. As a result, the gregarious coloration could be induced in brown solitary individuals kept under isolated conditions, but not in green ones. Based on these results, a hypothesis for the hormonal mechanism controlling the body-color polymorphism in *L. migratoria* is proposed.

2. Materials and methods

2.1. Insects

The albino strain of *L. migratoria* used was the same as described previously (Tanaka, 1993; Hasegawa and Tanaka, 1994). Groups of ca. 100 nymphs were reared in wood-framed cages (22 cm × 42 cm × 42 cm) covered with nylon mesh at 30°C, RH 30–50% and LD 16:8 h (Hakomori and Tanaka, 1992; Tanaka et al., 1993) and supplied with sorghum leaves or orchard grass as food. Upon ecdysis to the 3rd instar, they were divided into subgroups of 10–20 individuals and each subgroup was held in a small cage (15 cm × 28 cm × 28 cm) for experiments. Care was taken not to give too many leaves in order to keep the humidity inside the cage low (<60% RH), though no green individuals appeared among those reared as groups in the albino strain.

Late instar nymphs were collected in grassland along the Kokai River near Tsukuba, Japan in August, 1999, and reared individually in small cages. Judging from the convex shape of the pronotal crest and body color, they were apparently solitary nymphs. Their body color varied greatly among individuals. Non-green individuals were kept in small cages as above, and green ones were also reared in the same way except that the humidity inside the cage was kept high (75–80% RH) by giving more leaves, placing wet tissue paper in a Petri dish on the floor and covering the cages with a plastic sheet. Under such conditions, untreated non-green individuals remained non-green and green individuals green during the rest of nymphal development.

2.2. Injection of hormones

[His7]-corazonin was synthesized by Yamazaki Co. (Tokyo, Japan) and JH III purchased from Fulka (Buchs, Switzerland). Both hormones were mixed with rape-seed oil (Hayashi Chemical, Tokyo, Japan) and 2 μl of the hormone samples containing various doses were injected into each nymph with a micro syringe (Ito, Shizuoka, Japan). JH III was weighed and mixed with rape-seed oil to obtain the desired concentrations by assuming that 1 mg of JH III was 1 μl in volume. The purity of the hormone was about 80%, and adjustment was made to compensate for this value.

Some of the field-collected solitary nymphs were injected with 1 nmol of [His7]-corazonin and reared individually as above to examine the effect of the peptide on body color.
2.3. Assays

The effects of the injection of the hormones were assessed by the visible color 2 days after ecdysis to the next nymphal instar. Nymphs injected in the third instar with JH III alone either remained unchanged (whitish) or turned green after ecdysis to the fourth instar. Their body color was scored according to Hasegawa and Tanaka (1994): 0, no green spot; 1, slightly greenish; 2, greenish; 3, green area widely spread over the body surface. Effects recorded in the fifth instar nymphs were classified as positive (greenish) or negative (whitish with no green spot). Treatments with [His\(^7\)]-corazonin and JH III induced different body colors and patterns, and they were categorized into 5 groups, as will be shown later. All non-green body colors except for black were pooled as “others” because they were apparently induced by [His\(^7\)]-corazonin without any significant influence by the injected JH.

Field-collected solitary nymphs injected with [His\(^7\)]-corazonin were photographed 2 days after ecdysis to the next (penultimate or ultimate) nymphal instar and their body color was compared to the untreated counterparts.

3. Results

3.1. Effect of JH III

The green-color inducing effect of JH III was tested first by injecting the hormone (100 \(\mu\)g) into albino nymphs at different times during the third instar and checking their body color 2 days after ecdysis to the following instar. None of the nymphs injected with JH III on day 0 or 1 of the third instar turned green after ecdysis to the fourth instar (Fig. 1). Green individuals appeared among those injected on day 2 onward and all individuals injected on day 4 turned green after the following ecdysis. All individuals ecdysed to the fourth instar either on day 4 or 5, with a mean duration of 4.8 days (\(N=93\)) in this experiment. JH III exerted no significant influence on nymphal development (ANOVA; \(P>0.05\)). No mortality was caused at this dose. The relationship between the timing of JH injection and that of the following ecdysis showed that all green individuals occurred only when the ecdysis took place within 2 days after injection (data not shown). All individuals were reared to the fifth nymphal instar and their body color was examined 2 days after the last ecdysis. In this case, their response was recorded as positive (green) or negative (white). Those which had been in grade 0 or whitish in the fourth instar were whitish in the fifth instar and those which had been greenish remained green, although the green color faded slightly in the fifth instar (data not shown). Basically the same results were obtained in another series of experiments with 300 \(\mu\)g JH III (data not shown); however, this dose caused 60% mortality when applied on day 0 and <10% mortality at later treatments (Initial \(N=15–20\) each). This high dose also delayed the time to the following ecdysis by about 1 day in nymphs injected on day 0 or 1.

Fig. 2 shows the dose relations in albino nymphs injected with various doses of JH III on day 2.5 or 3.5 of the third instar. Individuals with green color appeared at all JH doses administered on day 3.5. Mean color grade increased in a dose-dependent fashion, and the proportion of green individuals including those of grades 1–3 was 0, 7.1, 12.3, 47.1, 92.9, 94.1 and 100% at doses of 0, 0.01, 0.1, 1, 10, 100 and 300 \(\mu\)g, respectively. When injected on day 2.5, the maximal mean color
grade, which was obtained with 100 μg, was only 1.8. No green individual occurred at a dose lower than 1 μg, and the proportion of green individuals including those of grades 1–3 was 6.7, 26.7, 70.0 and 68.4% at doses of 1, 10, 100 and 300 μg, respectively.

3.2. Interaction between JH and [His<sup>7</sup>]-corazonin

To disclose possible interacting effects of JH and [His<sup>7</sup>]-corazonin, albino nymphs were injected with 10 pmol of [His<sup>7</sup>]-corazonin on different days of the third instar and 100 μg of JH III on day 3.5 of the same instar. Typical body colors manifested 2 days after ecdysis to the fourth instar are shown in Fig. 3, and the frequencies of individuals with various colors are summarized in Table 1. Most nymphs (89%; N=19) injected with JH III alone turned green (Fig. 3B) and the rest remained white (Fig. 3A), as expected from the above experiment. In all groups injected with 10 pmol of [His<sup>7</sup>]-corazonin and JH III, at least some individuals developed a greenish color. Injection of [His<sup>7</sup>]-corazonin on days 0–2 produced only blackish green color, and some individuals became completely black (Table 1). At least half of those injected on days 2–4 turned green with a black, brown or reddish color on the lower part of the body. These individuals (III in Fig. 3) looked similar to normal green solitary nymphs found in the field. A few individuals injected with [His<sup>7</sup>]-corazonin on day 3 or 4 showed a mixture of green and reddish colors (Table 1; IV in Fig. 3). The category “others” included individuals with gregarious body coloration as well as uniformly reddish color. Because they did not show any flush of green, their body color was apparently induced by [His<sup>7</sup>]-corazonin and little influenced by JH III. Similar results were obtained when higher doses of [His<sup>7</sup>]-corazonin (100 pmol) and JH III (300 μg) were applied (Table 2). In this case, no individual showing type-IV-body color was obtained. The three individuals categorized in “others” were all reddish without any black spot.

Fig. 3. Photographs showing typical body colors expressed by albino nymphs of <i>L. migratoria</i>. A, untreated; B, treated with JH III; I–IV, treated with [His<sup>7</sup>]-corazonin on different days and JH III on day 3.5 of the previous (third) instar. The photographs on the right show the ventral view of each individual.
Table 1
Frequencies of fourth instar nymphs with various body colors after injection of [His7]-corazonin (10 pmol) and JH III (100 μg) during the third instar in albino L. migratoria

<table>
<thead>
<tr>
<th>Day of [His7]-corazonin injection during third nymphal instar</th>
<th>Number of nymphs in body color type</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>13</td>
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<td>2</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

a All nymphs injected with [His7]-corazonin were injected with JH III on day 3.5 of the third instar and their body color was checked 2 days after the following ecdysis.

b For body-color types, see Fig. 3.

Table 2
Frequencies of fourth instar nymphs with various body colors after injection of [His7]-corazonin (100 pmol) and JH III (300 μg) during the third instar in albino L. migratoria

<table>
<thead>
<tr>
<th>Day of [His7]-corazonin injection during third nymphal instar</th>
<th>Number of nymphs in body color type</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>0</td>
<td>6</td>
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<tr>
<td>1</td>
<td>1</td>
<td>13</td>
</tr>
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<td>2</td>
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<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

a All nymphs injected with [His7]-corazonin were injected with JH III on day 3.5 of the third instar and their body color was checked 2 days after the following ecdysis.

b For body-color types, see Fig. 3.

JH III injected in albino nymphs at the beginning of the third instar did not influence the body color in the following instar (Fig. 1), but it was not excluded that JH modified the responses to [His7]-corazonin injected later. To test this possibility, third instar albino nymphs injected with either 100 μg of JH III or oil alone on day 0 were injected with 10 pmol of [His7]-corazonin on day 3, and their body color was examined 2 days after ecysis to the following (fourth) instar. Injection of [His7]-corazonin into day-3 albino nymphs was known to induce a high proportion of individuals with black patterns and orange color (Tanaka, 2000). The proportion of such individuals in the oil-injected and JH III-injected groups before treatment with [His7]-corazonin was 100 and 93%, respectively (N=14 each). Neither green color nor developmental abnormalities were induced in the two groups. The results indicate that the presence of JH III at the beginning of the third nymphal instar had little or no influence on the dark-color inducing effect of [His7]-corazonin injected later.

3.3. Effect of [His7]-corazonin on the field-collected solitary nymphs

The above results indicated that both [His7]-corazonin and JH were important in the expression of various body colors in albino L. migratoria. To examine how injection of [His7]-corazonin would modify the expression of body color in a normal strain of this species, field-collected solitary nymphs were injected with 1 nmol of [His7]-corazonin and reared individually at 30°C until 2 days after the following ecysis. The nymphs used were either green or brown, and the dark-colored non-green individuals were excluded. The green and brown groups will be considered separately. Fig. 4 illustrates typical body colorations at the end of the observations. Table 3 summarizes the frequencies of various body coloration types obtained from the green group. All untreated nymphs remained green (G1; Table 3). Nymphs ecysing in 2 days after injection of [His7]-corazonin all developed black patterns on the green background of
Fig. 4. Photographs showing various body colors expressed by field-collected green (right, G1–6) and brown (left, B1a–5) solitary nymphs of *L. migratoria* after injection of [His<sup>7</sup>]-corazonin. Nymphs injected were kept individually until the second day of the following instar when the photographs were taken.

Thorax (G2 and G3). Those which ecdysed 3–5 days after the injection showed black spots all over the body (G4, 5 and 6) and their general body color was darker the later the ecdysis occurred. From the three individuals that ecdysed 6 days after injection, one was fairly dark (G6), one had only a few black spots (G4), and one appeared as an untreated individual (G1).

In the brown group, all untreated individuals remained brown after ecdysis to the following instar (Table 4), though some were slightly lighter (B1a) than others (B1b). The nymphs that ecdysed one day after the injection of [His<sup>7</sup>]-corazonin were either uniformly reddish (B2) or dark brown with very dark wing-pads (B3). Out of 6 individuals which ecdysed 2 days after injection, 3 developed black patterns on an orange background (B4), i.e. a coloration characteristic for the gregarious nymphs. All individuals which ecdysed 3–6 days after injection were black with white spots on the hind legs (B5). These results indicated that [His<sup>7</sup>]-corazonin induced reddish color, black patterns and black body color, depending on the time of injection relative to the following ecdysis, and that the gregarious body color appeared only among brown nymphs.

4. Discussion

The present results indicate that both JH and [His<sup>7</sup>]-corazonin are important in the control of body-color
polymorphism in *L. migratoria*. In the albino strain used in this study, albinism is caused by the deficiency of the dark-color inducing neuropeptide [His$^7$]-corazonin (Tanaka, 1993; Tawfik et al., 1999). Altered profiles of some biogenic monoamines in the brain and corpus cardiacum (Tanaka and Takeda, 1997) seem to be the only other difference from a normal (pigmented) strain. Although crowded albino nymphs are whitish, isolated ones develop green color under humid conditions (Tanaka, 1993), as reported for other albino strains of this species (Vierdier, 1965; Fuzeau-Braesch, 1985). Therefore, the hormonal system controlling nymphal development and induction of green color appears intact in this albino strain.

Relationships between various hormonal treatments and body colors in albino nymphs of *L. migratoria* (Tanaka 1993, 1996 and the present study) are summarized and compared with the coloration of untreated individuals in Fig. 5. Albino individuals treated with JH III in the second half of a nymphal instar develop green color after the following ecdysis. In this case, however, the ventral side of the body is either greenish (Fig. 5B) or whitish as in some untreated individuals (Fig. 5A). In normal solitary green nymphs, that portion of the body is variously pigmented; such body coloration can be induced in albinos only when both JH and [His$^7$]-corazonin are injected in the second half of the previous instar (Fig. 5C). Without treatment with JH, [His$^7$]-corazonin injected into albino locusts at the beginning of a nymphal instar induces various homochrome body colors. For example, albino nymphs injected with a high dose (e.g. 100 pmol; Tanaka, 2000) turn completely black (Fig. 5D) in the following nymphal instar, whereas those injected with a lower dose may develop a black, brown, purple color (Fig. 5E; Tanaka, 2000). When albino nymphs are injected with [His$^7$]-corazonin shortly before ecdysis, they become reddish without any black spots in the fourth instar (Fig. 5F). In this case, little or no [His$^7$]-corazonin should be present during the early stage of the third instar. [His$^7$]-corazonin injected into albino nymphs shortly after the mid stage of an instar induces black patterns with orange background color, the body coloration characteristic of normal gregarious individuals (Fig. 5G). If nymphs are injected with [His$^7$]-

### Table 3

Frequencies of nymphs with various body colors after injection of [His$^7$]-corazonin in field-collected green solitary nymphs of *L. migratoria*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of nymphs in body color type $^a$</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G1</td>
<td>G2</td>
</tr>
<tr>
<td>Untreated</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>2$^b$</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
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<td>4</td>
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</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

$^a$ For body-color types, see Fig. 4.

$^b$ Time of ecdysis to the following nymphal instar after injection.

### Table 4

Frequencies of nymphs with various body colors after injection of [His$^7$]-corazonin in field-collected brown solitary nymphs of *L. migratoria*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body color type $^a$</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B1a</td>
<td>B1b</td>
</tr>
<tr>
<td>Untreated</td>
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</tr>
<tr>
<td>1$^b$</td>
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<td>0</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
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</tr>
<tr>
<td>4</td>
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<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

$^a$ For body-color types, see Fig. 4.

$^b$ Time of ecdysis to the following nymphal instar after injection.

$^c$ Nymphs were either untreated or injected with 1 nmol of [His$^7$]-corazonin on the day of collection and reared individually at a high relative humidity (75–80%) until 2 days after the following ecdysis at 30°C.
corazonin twice, first at the beginning and then shortly after the middle of an instar, they turn black and depending on the dose no orange color is expressed (Tanaka, 2000). Thus, for the gregarious body coloration to be expressed, a high titer of [His\textsuperscript{7}]-corazonin should not be present during the early stage of the instar. Because albino nymphs respond to varying doses of JH and [His\textsuperscript{7}]-corazonin by developing body colorations similar to those found in the field, it seems reasonable to propose that changes in the hemolymph titers of these hormones control the pigmentation changes of locusts under various environmental conditions.

Results obtained with the field-collected normal nymphs, particularly brown nymphs, were consistent with those on the albino nymphs, providing a support for the above hypothesis. [His\textsuperscript{7}]-corazonin caused brown solitary nymphs in spite of their continuous isolation, to develop body coloration looking like gregarious individuals. The induction of the gregarious body coloration under isolated conditions in a normal strain of *L.*
migratoria is reported for the first time. Black patterns also appeared in green solitary nymphs similarly injected with [His<sup>7</sup>]-corazonin, but the lower part of the body remained green (G2 and G3 in Fig. 4). This type of body coloration is sometimes observed when several green solitary individuals taken from the field are put together in a small cage for a week or so (Tanaka, S., unpublished observation). In the fifth nymphal instar of the albino strain, this color pattern occurred when the green fourth instar nymphs produced with an injection of JH III (100 µg) during the third instar, were injected with another dose of JH III (100 µg) and [His<sup>7</sup>]-corazonin (10 pmol) 2 days before ecdysis to the fifth instar (Tanaka, S., unpublished observation).

Little information is available on the mode of action of [His<sup>7</sup>]-corazonin. One interesting aspect of this peptide is that a high dark-color inducing activity is manifested only when the peptide is mixed with oil, and little activity can be found when it is dissolved in an aqueous solution (Tawfik et al., 1999). This phenomenon was first noticed by Tanaka and Pener (1994) who found that the dark-color inducing factor in L. migratoria was a heat-stable neuropeptide soluble in methanol or in saline and located in the corpora cardiaca and the brain. They discovered that methanol extracts of corpora cardiaca taken from normal locusts were highly effective in inducing dark color in albino nymphs of L. migratoria, but no activity was detected when the extracts were dissolved in aqueous solution and injected. The same was found for the methanol and water extracts of the cricket corpora cardiaca which contain [Arg<sup>7</sup>]-corazonin (Hua et al., 2000); this neuropeptide induces dark color in albino nymphs of L. migratoria (Tanaka, 1996) and in the normal nymphs of S. gregaria (Tanaka and Yagi, 1997; Tanaka, 1998) only when applied in oil. Several other researchers reported dramatic reductions in threshold doses of neuropeptides when oil was used as a carrier (Nachman et al., 1995; Girardie et al., 1996; Janssen et al., 1998). It has been suggested that a peptide, which may otherwise be quickly degraded or diffuses through the hemolymph, is released from the oil droplets slowly and its long-lasting presence is important in eliciting the physiological function in insects (Tanaka and Pener, 1994; Tanaka, 1996; Janssen et al., 1998; Tawfik et al., 1999).

The temporal variation in the sensitivity to JH treatment has been reported for L. migratoria (Joly and Meyer, 1970; Némec et al., 1970). In the present study, JH III was effective in inducing green color in albino fourth instar nymphs only when injected during the second half of the previous instar. My unpublished data indicate that the same is true for other nymphal instars of the albino strain, as well as for a normal strain of L. migratoria and for another locust, Schistocerca gregaria. In a previous study using JH analogs methoprene and pyriproxyfen, green color was induced when nymphs of L. migratoria were treated at the beginning of the fourth instar (Hasegawa and Tanaka, 1994). The difference in results is probably related to the fact that Hasegawa and Tanaka (1994) applied JH analogs topically whereas JH III was injected in the present study. JH analogs probably remain biologically active much longer than JH III, which is a natural hormone of locusts (Okuda et al., 1996; Okuda and Tanaka, 1997; Botens et al., 1997) and can be degraded quickly by hemolymph esterases (Okuda et al., 1996). Also, some nymphs topically treated with pyriproxyfen turned green even before ecdysis, as also observed for S. gregaria treated with other JH analogs (Roussel and Perron, 1974). In the present study, green color was never manifested before the JH-III-injected nymphs eclosed to the following instar. Thus, it is possible that there is a difference in the mode of action between pyriproxyfen and JH III.

A dramatic change in the green-color inducing effect of JH III before and after nymphal ecdysis poses an interesting question: how does this change occur? In the present study, some individuals were injected with JH III within 1 h after ecdysis to the third instar, but none of them turned green. On the other hand, some individuals underwent ecdysis within 2 or 3 h after injection, and they turned green. One possible explanation is that there is stage-specific appearance and disappearance of a JH receptor regulating the synthesis and/or incorporation of green pigments by the epidermis. Such a temporal change in the sensitivity to JH for the induction of green color seems to make sense, because it would provide a mechanism to control metamorphosis by the same hormone without inducing green color, as can be envisaged easily from the presence of non-green nymphs. In this case, however, one might have to assume the presence of another JH receptor responsible for the control of metamorphosis, because JH is also supposed to work on the epidermis directly to determine the fate of new cuticle (Riddiford, 1994). Botens et al. (1997) reported that solitary (isolated) nymphs of L. migratoria had significantly higher hemolymph titers of JH III than gregarious (crowded) nymphs in the middle of an instar, but unfortunately no description was given about the relationship between the body color and JH titers. In the present study, injection of 100 µg of JH III on day 0 of the third instar did not affect the dark-color inducing effect of [His<sup>7</sup>]-corazonin injected later, indicating that a relatively high titer of JH can be present during the early stage of an instar without affecting the body color in the following instar.

The present study indicated the important role of JH and [His<sup>7</sup>]-corazonin in the control of body-color polymorphism in this locust. Determination of titers of these hormones during the nymphal stage would provide information to examine the hypothesis proposed in the present study.
Acknowledgements

I thank Toyomi Kotaki and Masahiko Watanabe for discussion and encouragement, and Noriko Kemmochi, Sumi Ogawa and Hiroko Ikeda for laboratory assistance. Thanks are also due to Toru Shimizu for helping me with drawing Fig. 5. The two anonymous reviewers improved the manuscript greatly.

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