Induction of darkening by corazonins in several species of Orthoptera and their possible presence in ten insect orders

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Abstract

[His⁷]-corazonin is known to play an important role in the control of body-color polymorphism in the migratory locust, *Locusta migratoria* (Orthoptera: Acrididae). Another neuropeptide, [Arg⁷]-corazonin, which has been isolated from other insects, is also known to induce darkening in this locust. These two neuropeptides were injected into nymphs of other orthopteran species to determine if they could induce darkening in those species. The test species belonged to 4 families: Acrididae (*Acrida cinerea*, *Gastrimargus marmoratus* and *Nomadacris succincta*), Catantopidae (*Oxya yezoensis*), Pyrgomorphidae (*Atractomorpha lata*) and Tettigoniidae (*Euconocephalus pallidus*). Except for the katydid, *E. pallidus*, all species injected with 1 nmol of [His⁷]-corazonin turned dark as compared with oil-injected controls. [Arg⁷]-corazonin showed a similar effect in the two acridid species tested, but no effect in the katydid. These results suggest that [His⁷]-corazonin or a similar neuropeptide may be involved in the control of body color in the locusts and grasshoppers, but not in the katydid. When a brain and/or corpora cardiaca (CC) taken from nymphs of each test species were implanted in albino nymphs of *L. migratoria*, dark color was induced in the latter, indicating that all test orthopterans contained some factor identical or similar to [His⁷]- or [Arg⁷]-corazonin. Likewise, brain-CC complexes from 47 other species of 10 insect orders including Orthoptera, Dermaptera, Dictyoptera, Isoptera, Homoptera, Hemiptera, Odonata, Hymenoptera, Lepidoptera and Diptera induced darkening in albino locusts, whereas those from 8 species of Coleoptera all failed to do so.

Key words: *Locusta migratoria*, [His⁷]-corazonin, [Arg⁷]-corazonin, body-color polymorphism

INTRODUCTION

Body-color polymorphism is one of the most conspicuous phenomena in insects. Some species of acridids change body color in response to various environmental factors such as crowding, temperature, humidity and background color (Uvarov, 1921, 1966, 1977; Faure, 1932). The migratory locust, *Locusta migratoria*, is one such species known to express a highly complex body-color polymorphism particularly during the nymphal stage. It shows three kinds of color polymorphism: (1) homochromy which is an adaptation to the neighboring background; (2) green-brown polymorphism and (3) phase or density dependent color polymorphism (Pener, 1991).

It has long been known that juvenile hormone is involved in the control of this polymorphism, and ample data confirming its green-color inducing effect have been accumulating for *L. migratoria* (Joly

and Joly, 1954; Staal and De Wilde, 1962; Němec et al., 1970; Roussel, 1975, 1976; Fuzeau-Braesch, 1985; Couillaud et al., 1987; Hasegawa and Tanaka, 1994). Recently, a factor(s) present in the brain and the corpora cardiaca (CC) was found to induce non-green dark colors in this and another locust, *Schistocerca gregaria* (Tanaka, 1993, 1998; Tanaka and Pener, 1994; Tanaka and Yagi, 1997). Both factors were heat-stable but lost their biological activity after incubation with a proteinase (Tanaka and Pener, 1994; Tanaka and Yagi, 1997). Using an albino mutant strain of *L. migratoria*, we succeeded in determining the chemical structures of these factors from the two locusts, and found that they were identical to [His⁷]-corazonin (Tawfik et al., 1999), a neuropeptide first isolated from another acrid, *S. americana*, with unknown function (Veenstra, 1991). This neuropeptide induces various body colors in *L. migratoria* depending upon the dose and timing of its administration (Tanaka,

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2000a). By injection of this neuropeptide, the body coloration characteristic for the gregarious phase can be induced in field-collected brown nymphs under isolated conditions, but not in green ones (Tanaka, 2000b). Based on these findings, a model for the hormonal mechanism controlling the body-color polymorphism in *L. migratoria* has been proposed (Tanaka, 2000b).

The function of [His⁷]-corazonin in the control of other phase-related traits is not well understood. In *S. gregaria*, hatchlings produced by gregarious females develop black patterns, whereas those produced by solitary females are green in color (Faure, 1932; Uvarov, 1966, 1977; Pener, 1991). It has recently been reported that the population density of adult female *S. gregaria* during mating and oviposition influences the behavior and coloration of the hatchlings from egg pods laid by the females (Islam et al., 1994a, b). McCaffery et al. (1998) demonstrated that the foam plugs of egg pods from crowd-reared, gregarious adults contain a hydrophilic factor which is produced at the time of oviposition and which causes the hatchlings to develop dark color and gregarious behavior. No studies have been reported concerning the relationship between this factor and [His⁷]-corazonin, but injection of [His⁷]-corazonin into green solitary female *S. gregaria* adults had no influence on the body color of their hatchlings (Tanaka, S., unpublished observations).

It is interesting that the brain and CC from other insect taxa such as moths, crickets, cockroaches and katydids also contain some factor(s) inducing dark color in locusts (Tanaka, 1993, 1996, 1998; Tanaka and Yagi, 1997). From a cricket, *Gryllus bimaculatus* and a silkworm, *Bombyx mori*, we have isolated [Arg⁷]-corazonin as the factor responsible for this effect (Hua et al., 2000). It differs from [His⁷]-corazonin by a single amino acid: the arginine has been substituted for a histidine residue (Veenstra, 1991).

In the present study, I injected [His⁷]-corazonin and [Arg⁷]-corazonin into various other insects showing green-brown polymorphism to observe the effect of corazonin on the body color in those insects. The insects tested belong to four families of orthopterans including Acrididae, Catantopidae, Phrgomorphidae and Tettigoniidae. I also tested the effect of implantation of the brain and CC from these and other insects on the induction of dark

color in albino nymphs of *L. migratoria*. This albino strain apparently lacks [His⁷]-corazonin (Tanaka, 1993; Tawfik et al., 1999; Schoofs et al., 2000a, b). The results of the present observations indicate the possibility that [His⁷]-corazonin or a similar neuropeptide plays an important role in the green-brown polymorphism in locusts and grasshoppers, but not in the katydids, and suggest that a corazonin or corazonin-like compound(s) is widespread among many insect species.

MATERIALS AND METHODS

Insects. The dark-color inducing activity of [His⁷]- and/or [Arg⁷]-corazonin was tested in 6 species (*Acrida cinerea*, *Gastrimargus marmoratus*, *Nomadacris succincta*, *Oxya yezoensis*, *Atractomorpha lata* and *Euconocephalus pallidus*). Except for *N. succincta* and *E. pallidus*, these insects were collected as nymphs in grassland in Tsukuba, Japan in 1998, and individuals of each species were fed with *Bromus catharticus* or *Dactylis glomerata* in small wood-framed cages covered with nylon screen (15×28×28 cm) at a 16L–8D photoperiod and 30°C. Nymphs of *G. marmoratus* were kept individually after collection, because crowding is known to influence their body color (Tanaka, S., unpublished observations). Nymphs of *E. pallidus* were collected on the campus of the University of Ryukyus, Okinawa in 1999, and reared as a group. Adults of *N. succincta* were collected in Naha, Okinawa in 1998 and their progenies were reared as described previously (Tanaka and Okuda, 1996). The other insects for which the dark-color inducing activity of brain-CC complexes was tested were derived from laboratory stocks at the National Institute of Sericultural and Entomological Science (NISES) or collected in Ibaraki, Okinawa or Tokyo in 1998–2000. The albino strain of *L. migratoria* was the same as previously described (Tanaka, 1993; Hasegawa and Tanaka, 1994).

Chemicals. [His⁷]-corazonin was synthesized by Yamazaki Co. (Tokyo, Japan) and [Arg⁷]-corazonin purchased from Sigma-Aldrich Co. (Tokyo, Japan).

Injection and assays. The peptides were mixed with rape-seed oil and 2 µl of the mixed samples injected into each insect (1 nmol per animal). This dose had been known to induce darkening in normal green nymphs of *L. migratoria* (Tanaka,

2000b). Because field-collected nymphs were used immediately after collection, their age was unknown. The injected insects were returned to their rearing cages and their body color was examined 2 or 3 days after ecdysis to the following (penultimate or last) stadium. Some individuals were injected with oil alone as controls. The effect of the treatment was visually evaluated in comparison with the body color in the controls, but the method was different among species because the response varied greatly among species, as will be described later.

Injection of brain-CC extracts. To determine which organ contains the dark-color inducing activity, brains and CC removed from last stadium nymphs of *G. marmoratus* and *N. succinata* were extracted with 99.9% methanol and the dried extracts prepared according to the method of Tanaka and Pener (1994). The extracts were mixed with rape-seed oil and injected into albino nymphs (4 μ l per individual). The effect of injection was recorded based on the scoring system of Tanaka and Pener (1994): 0, whitish (no effect); 1–3, increasingly darker coloration.

Organ transplantation. Brains and/or CC were dissected out from various species and implanted into 4th stadium albino *L. migratoria* nymphs according to the method of Tanaka (1993). Each albino nymph was implanted with 1 brain-CC complex except for a few cases. For a termite, *Reticulitermes speratus*, 10 brains removed from adult workers were implanted into each albino locust, because they were extremely small. The effect of implantation was checked 4 days later, still in the same (4th) stadium and recorded as negative if the nymph remained whitish, and as positive if the nymph turned darker, i.e. $\geq$ grade 1 in the scoring system of Tanaka and Pener (1994). For most species, at least 3 complexes were tested, but only 1 or 2 were available for a few species.

RESULTS

Effects of corazonins

Gastrimargus marmoratus

My unpublished observations indicated that this species showed body-color polymorphism similar to that of *L. migratoria*: nymphs grown under isolated conditions became green, brown or reddish in color whereas those reared under crowded condi-

tions developed black patterns on an orange background color. During the present study, various body colors such as green, light green, yellow, brown, gray and beige were observed among field-collected nymphs, and some examples are shown in Fig. 1A–C. Injection of [His^7]-corazonin into green nymphs caused them to develop black spots after ecdysis to the last stadium ($N=5$; Fig. 1E and F), whereas those injected with oil remained green ($N=5$; Fig. 1D). Three light brown nymphs and one reddish-colored nymph injected with the same dose also developed black spots (not shown) and one individual turned black, like the one shown in Fig. 1F. Likewise, [Arg^7]-corazonin injected into green nymphs was also effective in inducing dark color looking like those in Fig. 1E and F ($N=3$).

Acrida cinerea

The background color of field-collected nymphs was either green or gray in color. Among 52 penultimate stadium nymphs tested, 63.5% remained green after ecdysis to the last stadium and the rest turned gray without any green flash. There was no significant difference in the proportion of green individuals between the three groups injected with [His^7]-corazonin (55.6%), [Arg^7]-corazonin (66.7%) and oil only (68.4%) (χ^2 test; $p>0.05$). However, all individuals injected with either corazonin developed dark stripes on the head and thorax and their hind-femur turned darker (Fig. 2B and C). Such stripes were not observed in the controls (Fig. 2A), although sometimes nymphs with dark stripes can be observed in the field (Tanaka, S., unpublished observations). These results indicated that corazonins did not influence the background color but induced dark stripes in this species.

Nomadacris succincta

Nymphs reared on green *B. catharticus* leaves were green, yellow or light brown in color. In the present study, only green nymphs were used. Injection of corazonins caused them to develop various body colors, which were categorized into three grades according to the color on the thorax: 0, green; 1, light brown with dark spots; 2, reddish color with dark spots, as shown in Fig. 3. All but one oil-injected controls remained green (grade 0) (mean grade=0.1; $N=10$). Out of 10 nymphs injected with [His^7]-corazonin, 8 were grade 2 and the rest grade 1 (mean grade=1.7). The front two pairs of legs of grade-2 nymphs turned completely black. All individuals injected with [Arg^7]-cora-

Fig. 1. Body color of *G. marmoratus*. A–C, field-collected nymphs; D, oil-injected nymph, E and F, [His⁷]-corazonin-injected nymphs.

Fig. 2. Body color of *A. cinerea*. A, oil-injected nymph; B and C, [His⁷]-corazonin-injected nymphs.

Fig. 3. Body color of *N. succincta*. A, oil-injected nymph (grade 0); B, [His⁷]-corazonin-injected nymph (grade 1); C, corazonin-injected nymph (grade 2).

Fig. 4. Body color of *O. yezoensis*. A and B, oil-injected nymphs; C, [His⁷]-corazonin-injected nymph.

Fig. 5. Body color of *A. lata*. A and B, field-collected nymphs; C, [His⁷]-corazonin-injected nymph.

zonin turned reddish with dark spots (mean grade=2; $N=9$).

Oxya yezoensis

Two of the oil-injected green penultimate stadium nymphs of *O. yezoensis* turned brown after ecdysis to the last stadium (Fig. 4B) and the other 7 remained green (Fig. 4A). Among 11 green nymphs injected with [His⁷]-corazonin, 5 turned brown (Fig. 4C) and looked darker than the oil-injected brown nymphs. The remaining 6 individuals injected with [His⁷]-corazonin retained the green background color with some black spots on the head and abdominal tergites (not shown). [Arg⁷]-corazonin was not tested for this species.

Atractomorpha lata

Field-collected nymphs were green (Fig. 5A), brown (Fig. 5B) or gray in color. Out of 14 green nymphs injected with [His⁷]-corazonin, 5 developed dark spots after ecdysis to the following stadium (Fig. 5C) and the rest remained green or turned brown without developing conspicuous dark spots, as in oil-injected controls ($N=15$) or untreated controls ($N=15$). [Arg⁷]-corazonin was not tested in this species.

Euconocephalus pallidus

All field-collected katydid nymphs were green. Out of 10 oil-injected nymphs, 6 remained green and the rest turned brown after ecdysis to the following (penultimate or last) stadium. Injection of either corazonin failed to induce any noticeable darkening or change in body color in this species ($N=12$ total). The number of brown individuals was 1 and 2 in those injected with [His⁷]-corazonin ($N=6$) and [Arg⁷]-corazonin ($N=6$), respectively. It appeared that the body color of this katydid was not influenced by corazonins.

Effects of extract injection and organ transplantation

Injected methanol extracts of brains and CC from *G. marmoratus* induced dark color in albino locusts (1-individual equivalent per individual; $N=8$ each). Mean grade was 1.6 and 1.4 for extracts of brains and CC, respectively. Methanol extracts from *N. succincta* showed higher activity than those from *G. marmoratus*: mean grade was 2.6 and 2.3 for extracts of brains and CC, respectively ($N=10$ each). In both species, both the brain and CC contained a factor(s) inducing dark color in albino locusts.

Brain-CC complexes taken from all 6 orthopteran species induced darkening in albino locusts when implanted. These results and those from other insects are summarized in Table 1. In the present study, a total of 53 species was tested. In all orders except for Coleoptera, every species tested contained some factor inducing dark color in albino locusts. In Coleoptera, all 8 species tested were negative. To confirm the results of this observation, two brain-CC complexes taken from a scarab beetle, *A. cuprea*, a cerambycid, *A. malasiaca* or a lady beetle, *H. axyridis* were implanted into each albino locust. The results were consistent with the above findings: no dark-color inducing activity was detected ($N=5$ each). Furthermore, brains and CC of cerambycids were extracted with methanol and injected into 4th stadium albino *L. migratoria* nymphs after being dried and mixed with oil (1 individual equivalent/locust). This experiment was done to determine if the absence of the dark-color inducing effect was due to a failure of the brains and CC to survive in the recipients. However, neither brain nor CC extract gave a positive effect, indicating that these organs contained little or no corazonin. These results suggested that [His⁷]-corazonin or a similar compound inducing dark color in albino *L. migratoria* was present in all insect orders tested including Orthoptera, Dermaptera, Dictyoptera, Isoptera, Homoptera, Hemiptera, Odonata, Hymenoptera, Lepidoptera, and Diptera, but not in Coleoptera.

DISCUSSION

As stated earlier, [His⁷]-corazonin and JH have been suggested to control the body-color polymorphism in *L. migratoria* (Tanaka, 2000a,b). Injection of JH induces green color in albino nymphs, but such individuals fail to develop a brown or orange color that is expressed on the legs and the ventral surface in normal green solitary nymphs. The coloration of the latter type can be induced in albino individuals only by injections of both JH and [His⁷]-corazonin. Various types of body coloration including the one characteristic of gregarious nymphs can also be induced in albino nymphs by injection of certain doses of the peptide at certain stages. The present results demonstrated that injection of this peptide was effective in inducing darkening in various other orthopterans except for

Table 1. A list of insect species from which the brain and/or CC were taken and implanted into albino nymphs of *L. migratoria*

Species	Stage	Activity ^a	Reference ^b	
(Orthoptera)				
Acrididae	<i>Schistocerca gregaria</i>	Nymph	1	
		Adult		
	<i>Nomadacris succincta</i>	Nymph		
	<i>Acrida cinerea</i>	Nymph		
	<i>Gastrimargus marmoratus</i>	Nymph		
	<i>Aiolopus tamulus</i>	Adult		
Catantopidae	<i>Oxya yezoensis</i>	Nymph	+	
	<i>Oxya chinensis formosana</i>	Adult		
Pyrgomorphidae	<i>Atractomorpha lata</i>	Nymph/adult	+	
Tridactylidae	<i>Tridactylus japonicus</i>	Adult	+	
Tetrigidae	<i>Tetrix japonica</i>	Adult	+	
Gryllidae	<i>Gryllus bimaculatus</i>	Adult	+	2
		Nymph		
	<i>Teleogryllus occipitalis</i>	Adult	+	2
	<i>Modicogryllus confirmatus</i>	Adult	+	2
	<i>Diestrammena apicalis</i>	Nymph	+	
Rhaphidophoridae	<i>Metrioptera hime</i>	Nymph	+	2
Tettigoniidae	<i>Conocephalus melas</i>	Adult		
	<i>Euconocephalus pallides</i>	Nymph		
	<i>Euconocephalus thunbergii</i>	Nymph/adult		
	(Dermaptera)			
Labiduridae	<i>Anisolabis maritima</i>	Adult	+	
(Dictyoptera)				
Blattidae	<i>Periplaneta japonica</i>	Nymph/adult	+	
	<i>Periplaneta americana</i>	Adult	+	
Epilampridae	<i>Opisthoplatia orientalis</i>	Adult	+	
(Isoptera)				
Rhinotermitidae	<i>Reticulitermes speratus</i>	Adult worker	+	
(Homoptera)				
Cicadidae	<i>Oncotympana maculaticollis</i>	Adult	+	
	<i>Graptopsaltria nigrofuscata</i>	Adult	+	
(Hemiptera)				
Pentatomidae	<i>Eurydema rugosum</i>	Adult	+	
	<i>Plautia crossota stali</i>	Adult	+	
Coreidae	<i>Cletus trigonus</i>	Adult	+	
Pyrrhocoridae	<i>Dysdercus cingulatus</i>	Adult	+	
(Odonata)				
Cordulegasteridae	<i>Anotogaster sieboldii</i>	Adult	+	
Libellulidae	<i>Sympetrum infuscatum</i>	Adult	+	
	<i>Sympetrum darwintanum</i>	Adult	+	
(Hymenoptera)				
Argidae	<i>Arge similis</i>	Adult	+	
Scoliidae	<i>Campsomeris schulthessi</i>	Adult	+	
Apidae	<i>Apis mellifera</i>	Adult	+	
	<i>Xylocopa appendiculata</i>	Adult	+	
	<i>Anthophora florea</i>	Adult	+	
	<i>Camponotus japonicus</i>	Adult worker	+	
Formicidae				
(Lepidoptera)				
Noctuidae	<i>Bombyx mori</i>	Pupa	+	2
		Larva	+	
	<i>Mythimna separata</i>	Larva	+	4
	<i>Spodoptera litura</i>	Larva	+	

Table 1. (Continued)

Species		Stage	Activity ^a	Reference ^b
Sphingidae	<i>Cephonodes hylas</i>	Larva	+	2
	<i>Agrius convolvuli</i>	Larva	+	
Geometridae	<i>Biston robustum</i>	Larva	+	
Pieridae	<i>Pieris rapae</i>	Adult	+	
Nymphalidae	<i>Vanessa indica</i>	Adult	+	
(Diptera)				
Syrphidae	<i>Metasyrphus nitens</i>	Adult	+	
	<i>Syrphus japonicus</i>	Adult	+	
	<i>Eristalis cerealis</i>	Adult	+	
	<i>Eristalomyia tenax</i>	Adult	+	
	<i>Megaspis zonata</i>	Adult	+	
Muscidae	<i>Musca domestica</i>	Adult	+	
(Coleoptera)				
Scarabaeidae	<i>Anomala cuprea</i>	Adult	—	
	<i>Mimela flavilabris</i>	Adult	—	
	<i>Cetonia pilifera</i>	Adult	—	
	<i>Glycyphana fulvitemma</i>	Adult	—	
Cerambycidae	<i>Anoplophora malasiaca</i>	Adult	—	
Coccinellidae	<i>Coccinella septempunctata</i>	Adult/larva	—	
	<i>Harmonia axyridis</i>	Adult	—	
Chrysomelidae	<i>Aulacophora nigripennis</i>	Adult	—	

^a +, positive; —, negative.

^b 1, Schoofs et al. (2000a); 2, Tanaka (1993); 3, Tanaka (1996); 4, Hua et al. (2000).

the katydid. Preliminary observations indicated that the nymphal body color of another katydid, *Conocephalus melas*, was also unaffected by injection of the same neuropeptide (10 pmol per individual; $N=10$; Tanaka, S., unpublished data). These results suggest the possibility that [His⁷]-corazonin or a similar peptide is involved in the control of body color in these grasshoppers, as in *L. migratoria* and *S. gregaria* (Tawfik et al., 1999; Tanaka, 2000a,b), but not in the katydids. On the other hand, a brain-CC complex from all these species including the katydid induced dark color when implanted into albino locusts. Therefore, [His⁷]-corazonin or a similar substance appears to be present in both grasshoppers and katydids.

In the present study, the brain-CC complexes from a total of 53 insect species were tested for their dark-color inducing activity in albino locusts. These and previous results summarized in Table 1 indicate that [His⁷]-corazonin or a similar compound is widespread among various orders. Out of the 11 orders examined, 10 contained species giving a positive response in the albino bioassay. In Coleoptera, none of the 8 species tested produced a positive response. This lack of activity indicates

several possibilities: 1) the implanted coleopteran brain-CC complexes did not survive in the recipients; 2) they survived, but did not release sufficient amounts of corazonin; 3) they lacked any factor inducing dark color in locusts. From each of 3 coleopteran species including *A. cuprea*, *A. malasiaca* and *H. axyridis*, two brain-CC complexes were dissected out and implanted into each albino locust, but no dark color was induced in the recipient. Methanol extracts of brains and CC from *A. malasiaca* also failed to induce dark color in locusts when injected. These results seem to favor the last possibility, although further confirmation by other means such as immunocytochemistry would be necessary before this can be concluded.

The possible presence of [His⁷]-corazonin or a similar compound in the 10 insect orders raises an interesting question as to its functional relationship to the body color in these insects. The regulation of pigment synthesis and breakdown has been investigated in several other insects (Raabe, 1989; Gäde et al., 1997). In noctuids, the melanization and reddish coloration hormone (MRCH) released from the suboesophageal ganglion (SG) causes larval cuticular melanization and is chemically character-

ized (Ogura, 1975; Matsumoto et al., 1981, 1986, 1990, 1992; Hiruma et al., 1984; Morita et al., 1988). However, implantation of even 6 SGs from the Oriental armyworm, *Mythimna separata*, failed to induce darkening in albino locusts, although the latter developed dark color when implanted with 1 brain-CC complex from the armyworm (Tanaka, 1993). It is thus likely that the armyworm factor inducing dark color in locusts is different from MRCH. In a bivoltine silkworm, *B. mori*, the brain-SG complex contains a factor controlling the number of dark scales on the wings, and the possible involvement of the diapause hormone in this phenomenon has been suggested, because this neuropeptide mimicked the brain-SG extracts (Yamanaka et al., 2000). The brain-SG complex from a pupa of *B. mori* is also known to induce dark color in albino locusts (Tanaka, 1993). Recently, the silkworm factor inducing dark color in locusts was isolated from 10,000 larval brains and identified as [Arg⁷]-corazonin (Hua et al., 2000). This peptide was first isolated from a cockroach, *Periplaneta americana*, as the most potent cardiostimulatory peptide (Veenstra, 1989) and also found in other species such as a sphingid, *Manduca sexta*, and a cockroach, *Nauphoeta cinerea* (Veenstra, 1991). However, the exact function of the peptide in these insects is yet to be fully elucidated. In *B. mori*, [Arg⁷]-corazonin affects neither pigmentation nor metamorphosis (Hua et al., 2000). Likewise, the same peptide of unknown functions was isolated from the CC of a cricket, *G. bimaculatus* (Hua et al., 2000). Bursicon induces darkening and hardening of the cuticle in a number of insects, although the chemical nature of this hormone has not been fully characterized (Gäde et al., 1997).

From an evolutionary standpoint, the present results are of great interest, because a factor(s) with a common biological activity, i.e. induction of dark color in locusts, is present in the brain and/or CC in most insect orders. Identification of the chemical structure and function of this factor(s) in these insects may provide new insights into the evolution of neuropeptides in insects.

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