Bulletin of Entomological Research

cambridge.org/ber

Juvenile hormone identification in the cabbage bug *Eurydema rugosa*

Aya Kodama[1,*], Keiji Matsumoto[1,*], Tetsuro Shinada[2] and Shin G. Goto[1]

[1]Department of Biology and Geosciences, Graduate School of Science, Osaka City University, Osaka, Japan and [2]Department of Material Science, Graduate School of Science, Osaka City University, Osaka, Japan

Research Paper

*These authors equally contributed to this study.

Cite this article: Kodama A, Matsumoto K, Shinada T, Goto SG (2023). Juvenile hormone identification in the cabbage bug *Eurydema rugosa*. *Bulletin of Entomological Research* **113**, 293–298. https://doi.org/10.1017/S0007485321000158

Received: 3 November 2020
Revised: 14 January 2021
Accepted: 1 February 2021
First published online: 8 March 2023

Keywords:
Cabbage bug; diapause termination; Heteroptera; juvenile hormones; juvenilizing activity

Author for correspondence:
Shin G. Goto,
Email: shingoto@omu.ac.jp

Abstract

Juvenile hormone (JH) plays a pivotal role in almost every aspect of insect development and reproduction. The chemical structure of the JH in heteropteran species has long remained elusive until methyl (2*R*,3*S*,10*R*)-2,3;10,11-bisepoxyfarnesoate, commonly named as juvenile hormone III skipped bisepoxide ($JHSB_3$), was isolated from *Plautia stali* (Hemiptera: Heteroptera: Pentatomidae). Recently, several groups reported the presence of $JHSB_3$ in other heteropteran species. However, most of the studies paid no attention to the determination of the relative and absolute structure of the JH. In this study, we investigated the JH of the cabbage bug *Eurydema rugosa* (Hemiptera: Heteroptera: Pentatomidae), known as a pest for wild and cultivated crucifers. $JHSB_3$ was detected in the hexane extract from the corpus allatum (CA) product using a chiral ultraperformance liquid chromatography-tandem mass spectrometer (UPLC-MS/MS) which can inform the absolute stereochemistry of the JH. Its stereoisomers were not detected. Topical application of the synthetic $JHSB_3$ to the last instar nymphs inhibited their metamorphosis and induced nymphal-type colouration of the dorsal abdomen in a dose-dependent manner. Additionally, the topical application of $JHSB_3$ effectively terminated summer and winter diapauses in females. These results indicate that the JH of *E. rugosa* is $JHSB_3$. Although individuals in summer and winter diapauses are physiologically distinct in *E. rugosa*, the results suggest that the physiological differences between these diapauses are based, not on the responsiveness to JH, but on the processes governing activation of the CA or on its upstream cascades.

Introduction

Juvenile hormone (JH), synthesized at and released from the corpus allatum (CA), an endocrine gland, plays a key role in almost every aspect of insect development and reproduction (Nijhout, 1994; Goodman and Cusson, 2012; Jindra *et al.*, 2013, 2015; Roy *et al.*, 2018; Santos *et al.*, 2019). The JH structure was first determined from the cecropia moth *Hyalophora cecropia*, by Röller *et al.* (1967). The *cecropia* JH is now referred to as JH I, as thereafter, various JHs such as JH 0, JH II, JH III, and JHB_3 were identified from various insect orders (Jindra *et al.*, 2013).

The Heteroptera (true bugs), a suborder of Hemiptera, represent over 45,000 described species and are part of the successful radiation of hemimetabolous insects (Weirauch and Schuh, 2011; Foottit and Adler, 2017). Although the JH of heteropteran species has long been controversial (Bowers *et al.*, 1983; Baker *et al.*, 1988; Numata *et al.*, 1992; Kotaki, 1993; 1996; Miyawaki *et al.*, 2006; Teal *et al.*, 2014), Kotaki *et al.* (2009, 2011) identified methyl (2*R*,3*S*,10*R*)-2,3;10,11-bisepoxyfarnesoate as a novel JH in *Plautia stali* with a chiral GC-MS analysis and named it juvenile hormone III skipped bisepoxide ($JHSB_3$). After those landmark studies, the presence of $JHSB_3$ was investigated in several heteropteran species, i.e., *Pyrrhocoris apterus* (Hejnikova *et al.*, 2016), *Dipetalogaster maxima* and *Oncopeltus fasciatus* (Ramirez *et al.*, 2020), *Riptortus pedestris* (Lee *et al.*, 2019), and *Rhodnius prolixus* (Villalobos-Sambucaro *et al.*, 2020). The analysis was performed by high-performance liquid chromatography-mass mass analysis (HPLC-MS/MS) with a C18 reverse-phase column. The presence of $JHSB_3$ was proposed by the identification of the retention times of natural and authentic $JHSB_3$. However, there are three stereoisomers, (2*R*,3*S*,10*S*), (2*S*,3*R*,10*R*), and (2*S*,3*R*,10*S*) for $JHSB_3$, and these stereoisomers could not be separated on the C18 stationary phase (Ando *et al.*, 2020). In addition, Kotaki et al. reported the presence of not only $JHSB_3$ but also 10*S*-$JHSB_3$ in the CA product of *Halyomorpha halys*. The latter is a new JH in insects (Kotaki *et al.*, 2020). These results indicated that analytical conditions to discriminate $JHSB_3$ and its 10*S*-isomer are needed to determine the JH of heteropteran species.

The cabbage bug *Eurydema rugosa* Motschulsky (Pentatomidae) is widely distributed in East Asia and known as a pest of wild and cultivated crucifers, such as Chinese cabbage, Japanese radish, and wasabi (Tomokuni *et al.*, 1993; Schaefer and Panizzi, 2000). This species has two facultative adult diapauses, and their environmental cues are photoperiod and food

© The Author(s), 2023. Published by Cambridge University Press. This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted re-use, distribution, and reproduction in any medium, provided the original work is properly cited.

CAMBRIDGE UNIVERSITY PRESS

type (leaves or seeds). One of the diapauses is induced by feeding on seeds under long-day conditions (summer diapause), and the other is induced by short-day conditions irrespective of the food type (leaves or seeds) (winter diapause) (Numata and Yamamoto, 1990). It is generally accepted in various insect species that a lack of JH triggers adult diapause and JH biosynthesis promotes its termination (Denlinger *et al.*, 2012). In heteropterans, the significance of JH in diapause regulation was revealed by allatectomy and silencing of the genes involved in the JH-signalling cascade by RNA interference (Kotaki and Yagi, 1989; Morita and Numata, 1997; Bajgar *et al.*, 2013; Smykal *et al.*, 2014). The topical application of JH is also widely used to see its effect. However, JH analogues (JHA) or non-innate JHs have been used in such experiments with heteropterans (Kotaki and Yagi, 1989; Adams *et al.*, 2002; Miyawaki *et al.*, 2006; Cho *et al.*, 2007; Ikeno *et al.*, 2010; Bajgar *et al.*, 2013; Smykal *et al.*, 2014; Urbanová *et al.*, 2016; Penca and Hodges, 2017) simply because the heteropteran JH has long been physiochemically unidentified. Experiments with JHA advanced our knowledge on JH roles, but methoprene, one of the most well-known JHAs, is not effective in pentatomid bugs (Kotaki, 1996). Thus, we have to be careful about interpreting the JHA results. Experiments with the innate JH, $JHSB_3$, are still very limited (Kotaki *et al.*, 2011, 2020; Ando *et al.*, 2020).

In the present study, we investigated the chemical structure of the JH in *E. rugosa*, to expand our knowledge of the heteropteran JH and its role in diapause regulation. From the culture media of the CA attached with the corpora cardiaca (CC) of *E. rugosa*, the JH was physicochemically analysed by a chiral ultraperformance liquid chromatography-tandem mass spectrometer (UPLC-MS/MS) that we introduced very recently to JH research (Ando *et al.*, 2020; Kotaki *et al.*, 2020; Matsumoto *et al.*, 2020). Further, its juvenilizing activity was determined in last instar nymphs, and its effects on the termination of both the summer and winter diapauses were investigated.

Materials and methods

Insects

Adults of *E. rugosa* were collected from brown mustard *Brassica juncea* on the banks of the Yamatogawa River (34.59°N, 135.51°E), from March to April in 2018. The insects were reared in groups under long-day conditions (LD 16:8 h) at 25 ± 1 °C on seeds of rape *Sinapis alba*, with tap water. Their offspring were reared in the same manner and used in the experiments unless otherwise stated.

JH identification by UPLC-MS/MS

Fourteen of the *E. rugosa* males that were collected were utilized for the CC-CA extraction and incubation, performed according to the methods described in Matsumoto *et al.* (2013). In brief, the insects were anaesthetized on ice and immobilized with clay and were dissected in 0.9% NaCl. All the CC-CA complexes were incubated in 40 µl of minimum essential medium (with Hank's salt and L-glutamate and without sodium bicarbonate) added with 20 mM of HEPES and 5 ppm of Tween 80 and adjusted to pH 7.2 at 30 °C for 5 h in a glass tube (6 mm in diameter, 30 mm in height). After adding 60 µl of hexane, the supernatant was extracted. This hexane extraction was repeated three times and then the solvent was replaced with methanol.

The UPLC-MS/MS (ACQUITY UPLC H-Class, Xevo TQ-S micro, Waters, Milford, MA) and a chiral column (CHIRALPAK IA-U, 100 mm in length, Daicel, Tokyo, Japan) were used to compare the retention times of $JHSB_3$, its stereoisomers, JH I, JH III, and the CC-CA extract. The system operation, data acquisition, and analysis were controlled and processed by MassLynx software according to Ando *et al.* (2020). In brief, the flow rate of each sample through the chiral column was 0.4 ml min^{-1} in the solvent (15% water and 85% methanol). The column temperature was 30 °C. The mass spectrometer was operated in the positive ion mode. The tuning parameters were optimized for $JHSB_3$ and its stereoisomers: desolvation temperature 400 °C, desolvation gas flow 800 l h^{-1}, cone voltage 20 V, collision energy 10 V. For JH III, collision energy of 25 V was used. Authentic $JHSB_3$, its stereoisomers, and JH III were synthesized as described in Kotaki *et al.* (2009) and JH I was synthesized as described in Manabe *et al.* (2012). The MS/MS analysis of the authentic $JHSB_3$ showed the $[M + H]^+$ ion at *m/z* 283.2 and the $[M + Na]^+$ at *m/z* 305.3. The product ions were detected at *m/z* 42.9 and *m/z* 233.2, when ions at *m/z* 283.2 were used as a precursor, whereas no fragmentation was detected when ions at *m/z* 305.3 were used. In the present study, ions at *m/z* 283.2 and its product ions at *m/z* 233.2 were used as monitor ions for detecting $JHSB_3$ and its stereoisomers. For JH I and JH III, ions at *m/z* 294.5 and 261.3 and its product ions at *m/z* 263.3 and 43.0 were used as monitor ions, respectively.

The juvenilizing activity of $JHSB_3$

Nymphs were reared from the egg stage under short-day conditions (LD 10:14 h) at 25 ± 1 °C in a group of 30 individuals in a plastic cup (75 mm in diameter, 40 mm in depth). Nymphs that had ecdysed to the last (5th) instar stage were collected within 48 h from the ecdysis and 0.6 µl of hexane (solvent) or various concentrations of $JHSB_3$ in hexane were applied to the dorsal side of the abdomen using a 10 µl-syringe. After the application, five or fewer individuals per group were reared in individual Petri dishes (85 mm in diameter, 15 mm in depth) and maintained under the same photoperiodic and temperature conditions. After the final moult, scutellum length, forewing length, and pronotum width were measured using Nikon NIS Elements BR 3.0 software (Nikon, Tokyo, Japan) and the relative lengths of the scutellum and forewing to the pronotum width were calculated to evaluate the juvenilizing effects according to Kotaki (1996) and Kotaki *et al.* (2011). The juvenilizing effect was also assessed from the colour patterns on the dorsal abdomen. Individuals with black semi-ellipses on the yellowish background were regarded as the nymphal type, whereas individuals with yellowish spots on the black background were regarded as the adult type.

The diapause-terminating activity of $JHSB_3$

To induce summer and winter diapauses, the insects were reared under long-day conditions (LD 16:8 h) and short-day conditions (LD 10:14 h), respectively, at 25 ± 1 °C from the egg stage with rape seeds. Nymphs were reared in a group of 50 individuals in a plastic pot (145 mm in diameter, 90 mm in depth). After the final moult, adult females were reared in groups of 5 or fewer individuals per Petri dish. Thirty days after the final moult, we topically applied 2 µl of hexane (solvent) or various concentrations of $JHSB_3$ in hexane (the first application) on the females in both summer and winter diapauses and returned them to their respective conditions. Five days later, the same amount of hexane or $JHSB_3$ was applied (the second application). The diapause-terminating activity was assessed 40 days after the

https://doi.org/10.1017/S0007485321000158 Published online by Cambridge University Press

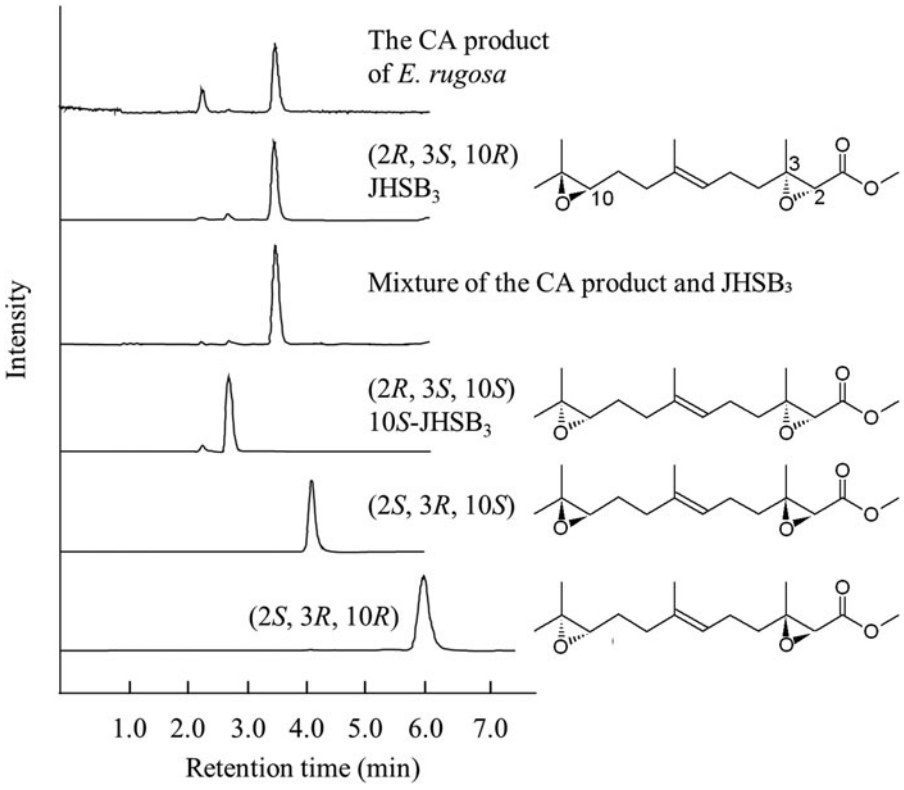

Figure 1. Chiral ultra-performance liquid chromatography-tandem mass spectrometer (UPLC-MS/MS) analyses of the corpus allatum (CA) product of *Eurydema rugosa*, juvenile hormone III skipped bisepoxide ($JHSB_3$), their mixture, and stereoisomers of $JHSB_3$. The vertical axis indicates the signal intensity for *m/z* 233.2 $[M+H]^+$. Chemical structures of $JHSB_3$ and its stereoisomers are also shown.

final moult by assessing ovarian status. Females with a yolk deposition in the oocytes were judged to be reproductive and those with no deposition were judged to be non-reproductive (diapause) according to Numata and Yamamoto (1990).

Statistical analysis

Treatment was treated as the independent variable. Relative scutellum and forewing lengths were treated as dependent variables. They were not normally distributed and were analysed with the Steel-Dwass test (Zar, 2010) to perform nonparametric multiple comparisons. The proportions were treated as dependent variables and were analysed with Tukey-type multiple comparisons for proportions (Zar, 2010).

Results

UPLC-MS/MS analysis of the CA product

UPLC-MS/MS data of $JHSB_3$, its stereoisomers, and the CA product of *E. rugosa* are depicted in fig. 1. The retention times of the main peak of the CA product (3.49 min), as well as that of co-injection of the CA product and $JHSB_3$, were identical with that of $JHSB_3$. On the other hand, the retention times of the stereoisomers were different from that of the CA product (fig. 1). In addition, peaks corresponding to JH I and JH III were undetectable in the CA product (data not shown). These results indicate that the CA of *E. rugosa* produces and secretes $JHSB_3$.

Juvenilizing effect of $JHSB_3$ on last instar nymphs

Because we detected only $JHSB_3$ in the CA product of *E. rugosa*, we topically applied $JHSB_3$ to the last instar nymphs to investigate its biological activity. After the final moult, scutellum length, forewing length, and pronotum width were measured and the relative lengths of the scutellum and forewing to the pronotum width were calculated (fig. 2A). Topical application of $JHSB_3$ inhibited metamorphosis in a dose-dependent manner. Relative lengths of the forewing and scutellum were approximately 0.60 and 0.39, respectively, in the last instar nymphs and these values were twice as small as the relative lengths in the adult females (1.37 and 0.69, respectively) (fig. 2B). Topical application of hexane (solvent) and a very small amount of $JHSB_3$ showed no juvenilizing effect. However, 0.06 µg of $JHSB_3$ significantly lowered the values (Steel-Dwass test, $P < 0.05$). These values were further lowered by 0.6 and 1.0 µg of $JHSB_3$ (Steel-Dwass test, $P < 0.05$).

The colour patterns of the dorsal abdomen in adult females and nymphs were largely different (fig. 2A). As the amount of $JHSB_3$ applied increased, the colour patterns became closer to the nymphal type (fig. 2C; Tukey-type multiple comparisons for proportions, $P < 0.05$).

Effect of $JHSB_3$ on ovarian development in females in summer and winter diapauses

None of the females developed their ovaries when the solvent or 0.002 µg of $JHSB_3$ were applied, irrespective of summer and winter diapauses (fig. 3). Small proportions of individuals developed their ovaries when they were treated with 0.02 µg of $JHSB_3$. The proportions were not statistically different between summer and winter diapauses (Tukey-type multiple comparisons for proportions, $P > 0.05$). When higher doses of $JHSB_3$ were applied, most females developed their ovaries, irrespective of the diapause type (Tukey-type multiple comparisons for proportions, $P < 0.05$). These results indicate that $JHSB_3$ effectively terminates both summer and winter diapauses.

https://doi.org/10.1017/S0007485321000158 Published online by Cambridge University Press

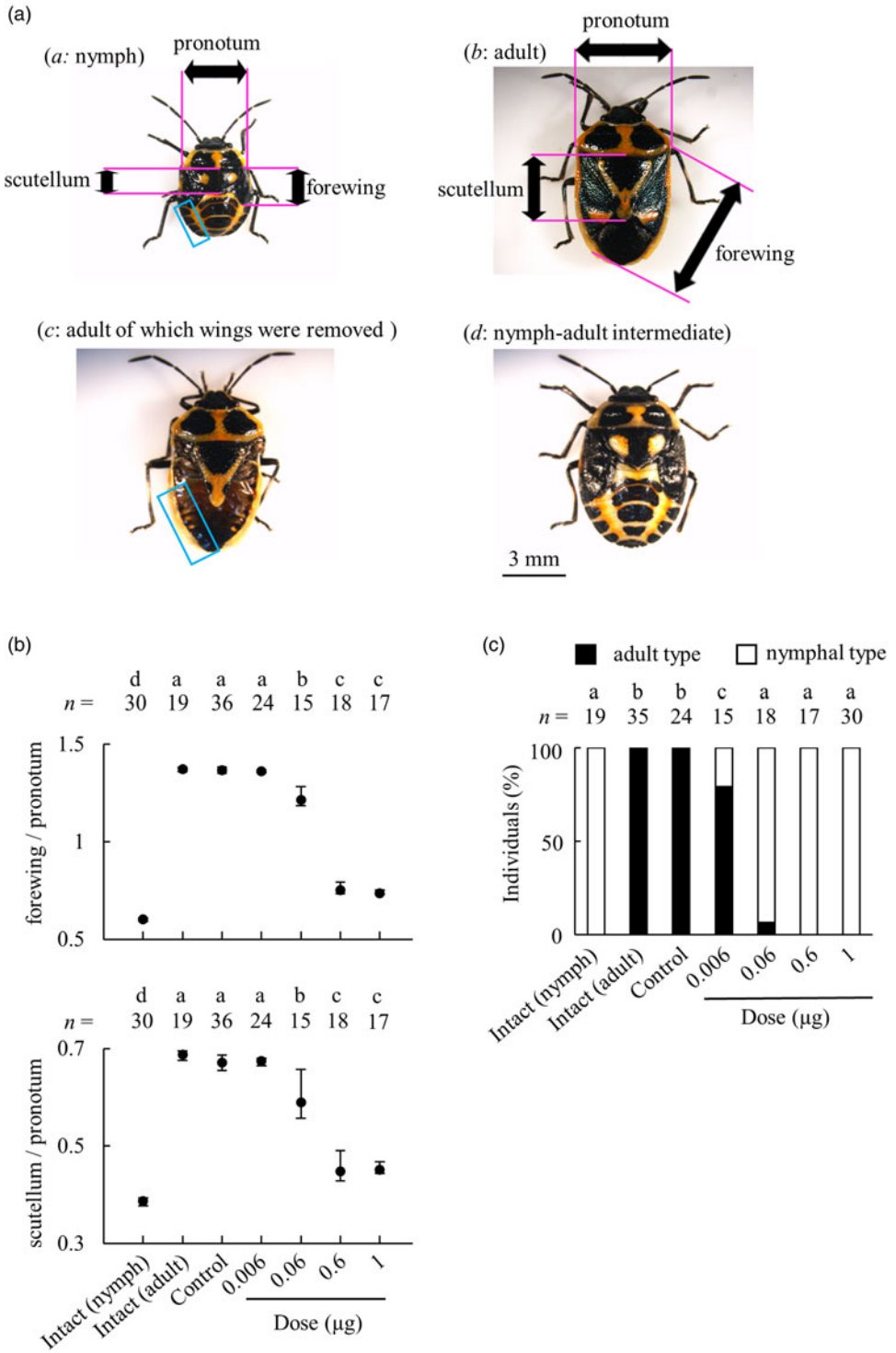

Figure 2. Juvenilizing effects of juvenile hormone III skipped bisepoxide ($JHSB_3$) in *Eurydema rugosa*. (A) Parameters for the juvenilizing activity of $JHSB_3$. A last instar nymph (*a*), an adult (*b*), an adult of which wings were removed (*c*), and a nymph-adult intermediate (*d*), obtained as a result of the application of a JH-active sample. Pronotum, forewing, and scutellum lengths indicated in *a* and *b* were measured. The colour patterns of the dorsal abdomen, shown in the framed box in *a* and *c*, were also observed. Scale bar indicates 3 mm. (B) Juvenilizing effects of $JHSB_3$ on the relative forewing (upper panel) and scutellum (lower panel) lengths in females. The solvent application to the nymph was also shown as a control. Each datum point and error bar indicate median ± interquartile range. The same letters indicate no statistical differences among the samples (Steel-Dwass test, $P > 0.05$). (C) Juvenilizing effect of $JHSB_3$ on the colour patterns of the dorsal abdomen in females. Their colour patterns were categorized into 2 types; adult and nymphal types. The solvent application to the nymph was also shown as a control. The same letters indicate no statistical differences among the samples (Tukey-type multiple comparisons for proportions, $P > 0.05$).

https://doi.org/10.1017/S0007485321000158 Published online by Cambridge University Press

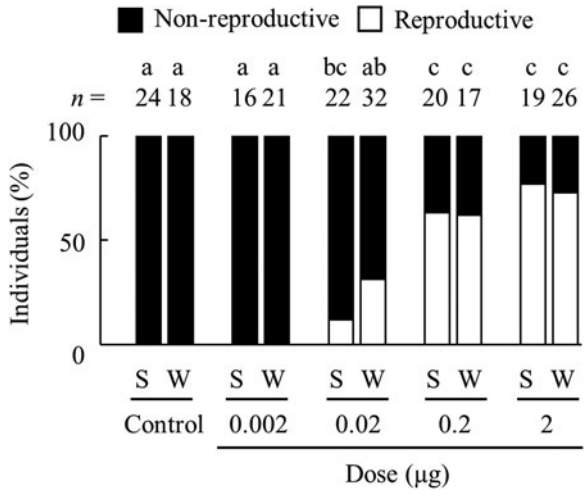

Figure 3. Effects of juvenile hormone III skipped bisepoxide ($JHSB_3$) on ovarian development in *Eurydema rugosa* females in summer (S) and winter (W) diapauses. The solvent application to the nymph was also shown as a control. The same letters indicate no statistical differences among the samples (Tukey-type multiple comparisons for proportions, $P > 0.05$).

Discussion

Our chemical analysis using the authentic $JHSB_3$ and their diastereoisomers by chiral UPLC-MS/MS clarified that *E. rugosa* synthesizes and secretes $JHSB_3$, but not its stereoisomers. Neither JH III nor JH I was detected in the CA product. The topical application of the synthetic $JHSB_3$ to the last instar nymphs inhibited their metamorphosis and induced nymphal-type colouration of the dorsal abdomen, in a dose-dependent manner. In addition, topical application of $JHSB_3$ effectively terminated summer and winter diapauses in females. It is important to note that the effective doses were comparable to those of the previous study, that determined the role of $JHSB_3$ in *P. stali* and *R. pedestris* (Kotaki *et al.*, 2011; Ando *et al.*, 2020). These data suggest that $JHSB_3$ is the JH of *E. rugosa*. Further investigations of its presence and the fluctuations of its haemolymph concentrations are required in the future study.

The present study revealed that the topical application of $JHSB_3$ effectively terminated both summer and winter diapauses. The topical application of JHA (Cho *et al.*, 2007; Bajgar *et al.*, 2013; Smykal *et al.*, 2014; Urvanová *et al.*, 2016; Penca and Hodges, 2017) and that of $JHSB_3$ (Kotaki *et al.*, 2011; Ando *et al.*, 2020) also terminated adult diapause of several heteropterans. Although we have not investigated its haemolymph concentration in *E. rugosa* females in diapause, the results are consistent with the general concept that the lack of JH triggers adult diapause and the resumption of JH biosynthesis promotes diapause termination (Denlinger *et al.*, 2012). Individuals in summer and winter diapauses are physiologically distinct in *E. rugosa*; winter diapause can be terminated by a low-temperature exposure, whereas the exposure is insufficient for terminating summer diapause (Ikeda-Kikue and Numata, 1994). The nearly identical doses of $JHSB_3$ required for terminating both summer and winter diapauses in the present study suggest that the differences in the responsiveness to the environmental factors are based, not on the responsiveness to the JH occurring during the diapause-termination process, but on the process governing activation of the CA or its upstream cascades.

As with females, *E. rugosa* males also have two facultative adult diapauses (summer and winter diapauses). Numata and Yamamoto (1990) revealed that the testis is largest in directly-developed adult males (non-diapause), smallest in males in summer diapause, and intermediate in males in winter diapause. The general cause for diapause, the lack of JH, cannot explain the size difference between diapauses. Unknown hormonal factors in addition to JH may be involved in these diapauses in *E. rugosa*. It is of interest to identify these factors and investigate the additive or synergistic effects of $JHSB_3$ and the factors on diapauses in future studies.

Acknowledgements. We thank Drs Kayoko Ikeda-Kikue and Hideharu Numata for their invaluable advice during this study. We thank Editage (www.editage.jp) for the English language editing of the manuscript submitted for publication.

References

Adams TS, Filipi PA and Yi S-X (2002) Effect of age, diet, diapause and juvenile hormone on oogenesis and the amount of vitellogenin and vitellin in the twospotted stink bug, *Perillus bioculatus* (Heteroptera: Pentatomidae). *Journal of Insect Physiology* **48**, 477–486.

Ando Y, Matsumoto K, Misaki K, Mano G, Shiga S, Numata H, Kotaki T, Shinada T and Goto SG (2020) Juvenile hormone III skipped bisepoxide, not its stereoisomers, as a juvenile hormone of the bean bug *Riptortus pedestris*. *General and Comparative Endocrinology* **289**, 113394.

Bajgar A, Jindra M and Dolezel D (2013) Autonomous regulation of the insect gut by circadian genes acting downstream of juvenile hormone signalling. *Proceedings of the National Academy of Sciences, USA* **110**, 4416–4421.

Baker FC, Tsai LW, Reuter CC and Schooley DA (1988) The absence of the significant levels of the known juvenile hormones and related compounds in the milkweed bug, *Oncopeltus fasciatus*. *Insect Biochemistry* **18**, 453–462.

Bowers WS, Marsella PA and Evans PH (1983) Identification of an hemipteran juvenile hormone: in vitro biosynthesis of JH III by *Dysdercus fasciatus*. *Journal of Experimental Zoology* **228**, 555–559.

Cho JR, Lee M, Kim HS and Boo KS (2007) Effect of the juvenile hormone analog, fenoxycarb on termination of reproductive diapause in *Scotinophara lurida* (Burmeister) (Heteroptera: Pentatomidae). *Journal of Asia-Pacific Entomology* **10**, 145–150.

Denlinger DL, Yocum GD and Rinehart JP (2012) Hormonal control of diapause. In Gilbert LI (ed.), *Insect Endocrinology*. Waltham, MA: Elsevier BV Academic Press, pp. 430–463.

Foottit RG and Adler PH (2017) *Insect Biodiversity: Science and Society*, Vol. **1**. Hoboken: John Wiley & Sons.

Goodmann WG and Cusson M (2012) The juvenile hormone. In Gilbert LI (ed.), *Insect Endocrinology*. Amsterdam: Elsevier, pp. 310–365.

Hejnikova M, Paroulek M and Hodkova M (2016) Decrease in *Methoprene tolerant* and *Taiman* expression reduces juvenile hormone effects and enhances the levels of juvenile hormone circulating in males of the linden bug *Pyrrhocoris apterus*. *Journal of Insect Physiology* **93–94**, 72–80.

Ikeda-Kikue K and Numata H (1994) Effect of low temperature on the termination of photoperiodic and food-mediated diapause in the cabbage bug, *Eurydema rugosa* Motschulsky (Heteroptera: Pentatomidae). *Applied Entomology and Zoology* **29**, 229–236.

Ikeno T, Tanaka SI, Numata H and Goto SG (2010) Photoperiodic diapause under control of circadian clock genes in an insect. *BMC Biology* **8**, 116.

Jindra M, Palli SR and Riddiford LM (2013) The juvenile hormone signalling pathway in insect development. *Annual Review of Entomology* **58**, 181–204.

Jindra M, Bellés X and Shinoda T (2015) Molecular basis of juvenile hormone signalling. *Current Opinion in Insect Science* **11**, 39–46.

Kotaki T (1993) Biosynthetic products by heteropteran corpora allata *in vitro*. *Applied Entomology and Zoology* **28**, 242–245.

Kotaki T (1996) Evidence for a new juvenile hormone in a stink bug, *Plautia stali*. *Journal of Insect Physiology* **42**, 279–286.

Kotaki T and Yagi S (1989) Hormonal control of adult diapause in the brown-winged green bug, *Plautia stali* Scott (Heteroptera: Pentatomidae). *Applied Entomology and Zoology* **24**, 42–51.

https://doi.org/10.1017/S0007485321000158 Published online by Cambridge University Press

Kotaki T, Shinada T, Kaihara K, Ohfune Y and Numata H (2009) Structure determination of a new juvenile hormone from a heteropteran insect. *Organic Letters* **11**, 5234–5237.

Kotaki T, Shinada T, Kaihara K, Ohfune Y and Numata H (2011) Biological activities of juvenile hormone III skipped bisepoxide in last instar nymphs and adults of a stink bug, *Plautia stali*. *Journal of Insect Physiology* **57**, 147–152.

Kotaki T, Matsumoto K, Kaihara K, Ando Y, Misaki K and Shinada T (2020) A stereoisomer of $JHSB_3$ with 10*S*-configuration, 10*S*-$JHSB_3$, biosynthesized by the corpus allatum of the brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Pentatomidae). *Applied Entomology and Zoology* **55**, 223–229.

Lee J, Kim C-H, Jang HA, Kim JK, Kotaki T, Shinoda T, Shinada T, Yoo JW and Lee BL (2019) *Burkholderia* gut symbiont modulates titer of specific juvenile hormone in the bean bug *Riptortus pedestris*. *Developmental & Comparative Immunology* **99**, 1033399.

Manabe A, Ohfune Y and Shinada T (2012) Stereoselective total syntheses of insect juvenile hormones JH 0 and JH I. *Synlett: Accounts and Rapid Communications in Synthetic Organic Chemistry* **23**, 1213–1216.

Matsumoto K, Numata H and Shiga S (2013) Role of the brain in photoperiodic regulation of juvenile hormone biosynthesis in the brown-winged green bug, *Plautia stali*. *Journal of Insect Physiology* **59**, 387–393.

Matsumoto K, Yasuno Y, Yasuda K, Hayashi T, Goto SG and Shinada T (2020) Structure determination of juvenile hormone from Chagas disease vectors, *Rhodnius prolixus* and *Triatoma infestans*. *Chemistry Letters* **49**, 538–541.

Miyawaki R, Tanaka SI and Numata H (2006) Role of juvenile hormone in the control of summer diapause in adult *Poecilocoris lewisi* (Heteroptera: Scutelleridae). *Formosan Entomology* **26**, 1–10.

Morita A and Numata H (1997) Role of the neuroendocrine complex in the control of adult diapause in the bean bug, *Riptortus clavatus*. *Archives of Insect Biochemistry and Physiology* **35**, 347–355.

Nijhout HF (1994) *Insect Hormones*. Princeton: Princeton University Press.

Numata H and Yamamoto K (1990) Feeding on seeds induces diapause in the cabbage bug, *Eurydema rogosa*. *Entomologia* **57**, 281–184.

Numata H, Numata A, Takahashi C, Nakagawa Y, Iwatani K, Takahashi S, Miura K and Chinzei Y (1992) Juvenile hormone I is the principal juvenile hormone in a hemipteran insect, *Riptortus clavatus*. *Experientia* **48**, 606–610.

Penca C and Hodges AC (2017) Pyriproxyfen treatment terminates *Halyomorpha halys* reproductive diapause, with an indirect mortality effect on its egg parasitoid *Trissolcus japonicus*. *Journal of Pest Science* **90**, 1269–1276.

Ramirez CE, Nouzova M, Michalkova V, Fernandez-Lima F and Noriega FG (2020) Common structural features facilitate the simultaneous identification and quantification of the most common juvenile hormones by liquid chromatography-tandem mass spectrometry. *Insect Biochemistry and Molecular Biology* **116**, 103287.

Röller H, Dahm DH, Sweeley CC and Trost BM (1967) The structure of the juvenile hormone. *Angewandte Chemie International Edition* **6**, 179–180.

Roy S, Saha TT, Zou Z and Raikhel AS (2018) Regulatory pathways controlling female insect reproduction. *Annual Review of Entomology* **63**, 489–511.

Santos CG, Humann FC and Harftfelder K (2019) Juvenile hormone signalling in insect oogenesis. *Current Opinion in Insect Science* **31**, 43–48.

Schaefer CW and Panizzi AR (2000) *Heteroptera of Economic Importance*. Boca Raton: CRC Press.

Smykal V, Bajgar A, Provaznik J, Fexova S, Buricova M, Takaki K, Hodkova M, Jindra M and Dolezel D (2014) Juvenile hormone signaling during reproduction and development of the linden bug, *Pyrrhocoris apterus*. *Insect Biochemistry and Molecular Biology* **45**, 69–76.

Teal PEA, Jones D, Jones G, Torto B, Nyasembe V, Borgemeister C, Alborn HT, Kaplan F, Boucias D and Lietze VU (2014) Identification of methyl farnesoate from the hemolymph of insects. *Journal of Natural Products* **77**, 402–405.

Tomokuni M, Yasunaga T, Takai M, Yamashita I, Kawamura M and Kawasawa T (1993) *Terrestrial Heteropterans: A Field Guide to Japanese Bugs*. Tokyo: Zenkoku Noson Kyoiku Kyoukai.

Urbanová V, Bazalová O, Vaněčková H and Dolezel D (2016) Photoperiod regulates growth of male accessory glands through juvenile hormone signaling in the linden bug, *Pyrrhocoris apterus*. *Insect Biochemistry and Molecular Biology* **70**, 184–190.

Villalobos-Sambucaro MJ, Nouzova M, Ramirez CE, Alzugaray ME, Fernandez-Lima F, Ronderos JR and Noriega FG (2020) The juvenile hormone described in *Rhodnius prolixus* by Wigglesworth is juvenile hormone III skipped bisepoxide. *Scientific Reports* **10**, 3091.

Weirauch C and Schuh RT (2011) Systematics and evolution of Heteroptera: 25 years of progress. *Annual Review of Entomology* **56**, 487–510.

Zar JH (2010) *Biostatistical Analysis*, 5th Edn. Upper Saddle River: Prentice Hall.

https://doi.org/10.1017/S0007485321000158 Published online by Cambridge University Press