

# Carotenoids of hemipteran insects, from the perspective of chemo-systematic and chemical ecological studies<sup>☆</sup>

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## ABSTRACT

Carotenoids of 47 species of insects belonging to Hemiptera, including 16 species of Sternorrhyncha (aphids and a whitefly), 11 species of Auchenorrhyncha (planthoppers, leafhoppers, and cicadas), and 20 species of Heteroptera (stink bugs, assassin bugs, water striders, water scorpions, water bugs, and backswimmers), were investigated from the viewpoints of chemo-systematic and chemical ecology. In aphids, carotenoids belonging to the torulene biosynthetic pathway such as  $\beta$ -zeacarotene,  $\beta,\psi$ -carotene, and torulene, and carotenoids with a  $\gamma$ -end group such as  $\beta,\gamma$ -carotene and  $\gamma,\gamma$ -carotene were identified. Carotenoids belonging to the torulene biosynthetic pathway and with a  $\gamma$ -end group were also present in water striders. On the other hand,  $\beta$ -carotene,  $\beta$ -cryptoxanthin, and lutein, which originated from dietary plants, were present in both stink bugs and leafhoppers. Assassin bugs also accumulated carotenoids from dietary insects. Trace amounts of carotenoids were detected in cicadas. Carotenoids of insects belonging to Hemiptera well-reflect their ecological life histories.

## 1. Introduction

Carotenoids are tetraterpene pigments that are distributed in photosynthetic bacteria, some species of archaea and fungi, algae, plants, and animals. About 850 naturally occurring carotenoids had been reported up until 2018 (Britton et al., 2004; Maoka, 2019). Photosynthetic bacteria, some species of archaea and fungi, algae, and plants can synthesize carotenoids *de novo*. However, as animals generally do not have carotenoid synthetic genes, they cannot synthesize carotenoids *de novo*. Thus, carotenoids found in animals are either directly accumulated from food or partly modified through metabolic reactions (Liaaen-Jensen, 1998; Maoka, 2011).

Insects are the most diverse group of animals and they contain various carotenoids. Most insects accumulate carotenoids that have originated from plants through the food chain (Liaaen-Jensen, 1998; Maoka, 2011; Maoka et al., 2020).

Hemiptera is an order of insects including Sternorrhyncha (aphids

and whiteflies), Auchenorrhyncha (e.g., planthoppers, leafhoppers, and cicadas), and Heteroptera (e.g., stink bugs and water striders). Most hemipteran insects feed on plant sap using their sucking and piercing mouthparts, and some of them are serious agricultural pests. On the other hand, some kinds of hemipteran insects such as assassin bugs and water striders are carnivorous and mostly prey on insects. Some of the insects belonging to Hemiptera have red, yellow, green, and blue-green body colors.

Carotenoids without a plant origin,  $\beta$ -zeacarotene,  $\beta,\psi$ -carotene (generally called  $\gamma$ -carotene), torulene,  $\beta,\gamma$ -carotene, and  $\gamma,\gamma$ -carotene, were identified in some species of aphids (Andrews et al., 1971; Britton et al., 1977a, 1977b). These carotenoids are known to be characteristic carotenoids in fungi. Therefore, they are considered to have originated from endosymbiotic microorganisms such as fungi (Liaaen-Jensen, 1998). Recent investigations revealed that aphids synthesize carotenoids themselves by carotenoid synthetic genes, which are horizontally transferred from fungi to aphids. Namely, the aphid genome itself

<sup>☆</sup> The results of functional identification of carotenoid biosynthesis genes that exist in the genome of the pea aphids *Acyrthosiphon pisum*, with recombinant *Escherichia coli* cells, and a part of carotenoid composition in aphids have been submitted to BMC Zoology by Takemura, M., Maoka, T., Koyanagi, T., Kawase, N., Nishida, R., Tsuchida, T., Hironaka, M., Ueda, T., and Misawa, N., as the title of 'Elucidation of the whole carotenoid biosynthetic pathway of aphids at the gene level and arthropodal food chain involving aphids and the red dragonfly' (submitted September 22, 2020; under review January 2021).

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encodes multiple enzymes for carotenoid biosynthesis. Red aphids have carotenoid desaturase genes and synthesize torulene from phytoene by themselves (Moran and Jarvik, 2010; Nováková and Moran, 2012; Mandrioli et al., 2016; Ding B-Y et al., 2019). On the other hand, blue-green aphids synthesize polycyclic quinones using genes of the endosymbiotic bacterium *Richettsiella* (Tsuchida et al., 2010).

However, few reports have provided an overview of carotenoids in hemipteran insects (Andrews et al., 1971; Britton et al., 1977a, 1977b). Especially, there are no reports on the carotenoids of Auchenorrhyncha and Heteroptera. In the present paper, we describe the carotenoids in 47 species of insects belonging to Hemiptera, including 16 species of Sternorrhyncha (aphids and whiteflies), 11 species of Auchenorrhyncha (planthoppers, cicadas, etc.), and 20 species of Heteroptera (stink bugs, etc.) from the viewpoints of comparative biochemistry and chemical ecology.

## 2. Materials and methods

### 2.1. Insects

The following 47 species of insects belonging to Hemiptera were collected in Koka City, Shiga Prefecture, Kyoto City, Kyoto Prefecture, and Kanazawa City, Ishikawa Prefecture in Japan during April to October. Sternorrhyncha (aphids and a whitefly, 16 species): *Shivaphis celti*, *Aphis gossypii*, *A. nerii*, *A. spiraeicola*, *Semiaphis heraclei*, *Toxoptera citricida*, *Acyrtosiphon pisum*, *Brevicoryne brassicae*, *Uroleucon formosanum*, *Uroleucon kikioensis*, *Macrosiphum euphorbiae*, *Megoura crassicauda*, *Lachnus tropicalis*, *Ceratovacuna nekoashi*, *Schlechtendalia chinensis*, and *Dialeurodes citri*; Auchenorrhyncha (planthoppers, leafhoppers, and cicadas, 11 species): *Nilaparvata lugens*, *Hecalus prasinus*, *Nephotettix cincticeps*, *Bothrogonia ferruginea*, *Platypleura kaempferi*, *Cryptotympana atrata*, *C. facialis*, *Graptopsaltria nigrofusca*, *Tanna japonensis japonensis*, *Hyalessa maculicollis*, and *Meimuna opalifera*; Heteroptera (stink bugs, assassin bugs, water striders water scorpions, water bugs, and backswimmers, 20 species): *Parastrachia japonensis*, *Poecilocoris lewisi*, *Halyomorpha halys*, *Alcimocoris japonensis*, *Plautia stali*, *Nezara antennata*, *Sastragala esakii*, *Acanthocoris sordidus*, *Haematolochea nigrorufa*, *Isyndus obscurus*, *Sirthena flavipes*, *Corythucha marmorata*, *Orius sauteri*, *Aquarius elongatus*, *A. paludum*, *Gerris laticaudinis*, *Metrorocoris histrio*, *Ranatra chinensis*, *Appasus major*, and *Notonecta triguttata*.

We identified each hemipteran specimen referencing from following bibliographies: Enju (2013), Matsumoto (2008), Miyatake et al. (1992), Saigusa et al. (2013) and Yasunaga et al. (1993).

### 2.2. Carotenoid extraction, analysis, isolation, and identification

The carotenoids were extracted from insects with acetone at room temperature. After filtration, the acetone extract was partitioned with hexane:Et<sub>2</sub>O (1:1, v/v) and water. Then carotenoids were transferred to hexane:Et<sub>2</sub>O phase. The hexane:Et<sub>2</sub>O phase was washed with water and dehydrated on anhydrous sodium sulphate. The total carotenoid amount were calculated using coefficient of E 1%cm = 2400 at λ max (Britton, 1995; Schiedt and Liaaen-Jensen, 1995).

Quantitative and qualitative carotenoid analyses were carried out using our routine method using a high performance liquid chromatography/photodiode-array detector/mass (LC/PDA/MS) system (Maoka, 2016; Maoka et al., 2020).

The LC/MS analysis of carotenoids was carried out using a Waters Xevo G2S Q TOF mass spectrometer (Waters Corporation, Milford, CT, USA) equipped with an Acquity UPLC system. The electro-spray ionization (ESI) time-of-flight (TOF) MS spectra were acquired by scanning from *m/z* 100 to 1,500 with a capillary voltage of 3.2 kV, cone voltage of 20 eV, and source temperature of 120 °C. Nitrogen was used as a nebulizing gas at a flow rate of 30 L/h. MS/MS spectra were measured with a quadrupole-TOF MS/MS instrument with argon as a collision gas at a collision energy of 20 V. UV-VIS absorption spectra were recorded

from 200 to 600 nm using a photodiode-array detector (PDA). An Acquity 1.7 μm BEH UPLC C18 (2.1 id X 100 mm) column (Waters Corporation, Milford, CT, USA) was used as a stationary phase and MeCN:H<sub>2</sub>O (85:15) - MeCN:MeOH (65:35) (linear gradient 0–15 min) as a mobile phase, at a flow rate of 0.4 mL/min for the HPLC system. Carotenoids were identified by UV-VIS, MS, and MS/MS spectral data and retention time in HPLC with comparison of authentic samples.

In the case of *Acyrtosiphon pisum*, carotenoids were isolated by silica gel column chromatography followed by preparative HPLC and identified from UV-VIS, ESI TOF MS, <sup>1</sup>H NMR, and circular dichroism (CD) spectral data as described previous literature (Maoka et al., 2020). Details of spectral data of γ,γ-carotene has not been reported (Britton et al., 2004). Therefore, spectral data of γ,γ-carotene was described here.

γ,γ-Carotene: ESI TOF MS (*m/z*) 536.4382 [M<sup>+</sup>] C<sub>40</sub>H<sub>56</sub>, Calcd for 536.4404; UV-VIS (Et<sub>2</sub>O) 419, 439, 468 nm; <sup>1</sup>H NMR δ (in CDCl<sub>3</sub> at 500 MHz) 0.82 (6H, s, H-16, 16'), 0.90 (6H, s, H-17, 17'), 1.33 (4H, dd, *J* = 14, 7 Hz, H-2, 2'), 1.95 (6H, s, H-19, 19'), 1.97 (6H, s, H-20, 20'), 2.07 (4H, m, H-3, 3'), 2.29 (4H, m, H-4, 4'), 2.51 (2H, d, *J* = 9.5 Hz, H-6, 6'), 4.57 (2H, br. s, H-18, 18'), 4.73 (2H, br. s, H-18, 18'), 5.84 (2H, dd, *J* = 15.5, 9.5 Hz, H-7, 7'), 6.12 (2H, d, *J* = 11.5 Hz, H-10, 10'), 6.13 (2H, d, *J* = 15.5 Hz, H-8, 8'), 6.24 (2H, m, H-14, 14'), 6.33 (2H, d, *J* = 15.5 Hz, H-12, 12'), 6.61 (2H, dd, *J* = 15.5, 11.5 Hz, H-11, 11'), 6.63 (2H, m, H-15, 15'). These spectral data were in agreement with published data (Arpin et al., 1971; Britton et al., 2004).

## 3. Results and discussion

Fig. 1 shows structures of carotenoids found in hemipteran insects in this study. Carotenoids in 47 species of insects belonging to Hemiptera, including 16 species of Sternorrhyncha, 11 species of Auchenorrhyncha, and 20 species of Heteroptera are shown in Table 1. Aphids contained relatively higher levels of carotenoids among hemipteran insects. Among them, *Aphis nerii* (242.1 μg/g), *A. gossypii* (192.7 μg/g), *Semiaphis heraclei* (170.0 μg/g), and *Megoura crassicauda* (133.6 μg/g) contained high levels of carotenoids. On the other hand, *Uroleucon kikioensis*, *Macrosiphum euphorbiae*, and *Ceratovacuna nekoashi* contained low levels (less than 10 μg/g) of total carotenoids. Carotenoids were not detected in *Lachnus tropicalis*. β-Carotene was found to be a major carotenoid (5.8–70.2% of total carotenoids) in many aphids. Carotenoids generated by the torulene biosynthetic pathway such as β-zeacarotene, β,ψ-carotene, and torulene (Goodwin, 1980), were also present as major carotenoids in many species of aphids. Torulene was also found in two species of aphids: *Brevicoryne brassicae* (12.8%) and *Aphis spiraeicola* (5.2%). Furthermore, carotenoids with a γ-end group (terminal methylene group in the C-5 end group, Arpin et al., 1971) such as β,γ-carotene, γ,γ-carotene, and γ,ψ-carotene, were detected in all species of aphids as major carotenoids (19.6–67.6%). Among them, β,γ-carotene was found to be a major carotenoid (14.8–54.5%) in several species of aphids. Whiteflies (Aleyrodidae) also belong to Sternorrhyncha. The citrus whitefly, *Dialeurodes citri*, contained a very low level of carotenoids (0.5 μg/g). β,ψ-Carotene was found to be a major carotenoid (more than 95% of total carotenoids) in the citrus whitefly. Carotenoid compositions in aphids were markedly different from those in Auchenorrhyncha and Heteroptera, as described later. It was reported that aphids synthesized carotenoid themselves by carotenoid synthetic genes, which were horizontally transferred from fungi to aphids. Namely, aphids synthesize torulene from phytoene by carotenoid desaturase genes (Moran and Jarvik, 2010; Nováková and Moran, 2012; Mandrioli et al., 2016; Ding B-Y et al., 2019). As described above, β,ψ-carotene was the main carotenoid in citrus whitefly, *Dialeurodes citri*. β,ψ-Carotene was not present in its host plant (*Citrus unshiu*). Sloan and Moran (2012) reported that the tobacco whitefly *B. tabaci* synthesized carotenoid by employing the genome of the obligate bacterial endosymbiont Portiera. Therefore, β,ψ-carotene in the citrus whitefly was also considered to be synthesized by endosymbiotic bacteria.

Recently, our research group conducted a functional analysis of

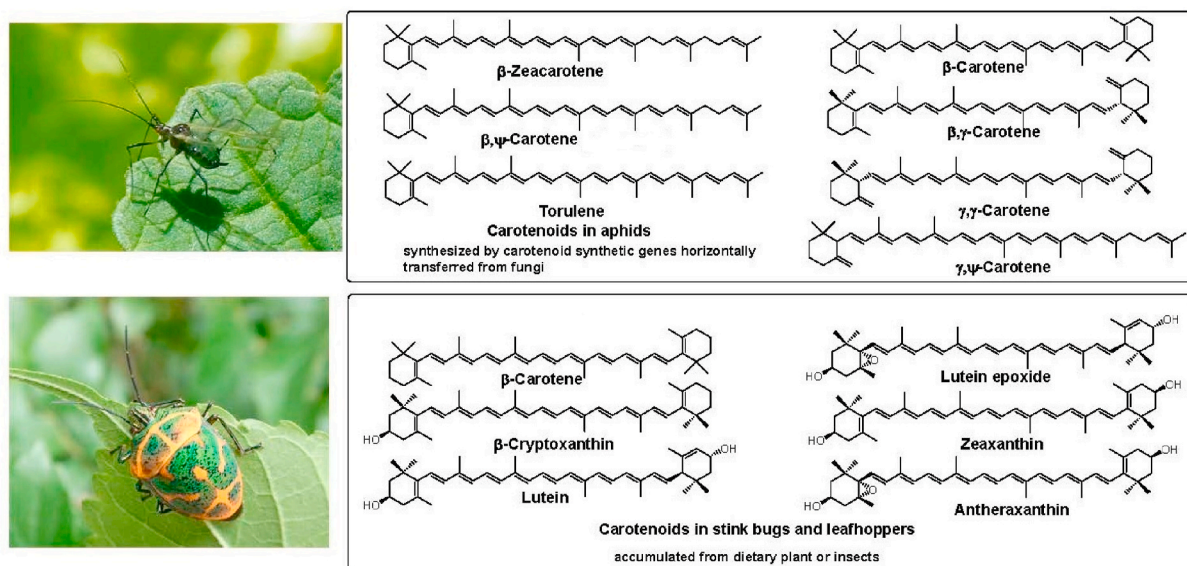


Fig. 1. Characteristic carotenoids in Hemiptera. Photos: upper, *Megoura crassicauda* (Aphididae: Sternorrhyncha); lower, *Poecilocoris lewisi* (Scutelleridae: Heteroptera).

carotenoid biosynthesis genes that existed in the genome of the pea aphid *Acyrthosiphon pisum* using recombinant *Escherichia coli* cells. As the results, we found that a series of carotenoids with the torulene biosynthetic pathway and  $\beta,\gamma$ -carotene were synthesized in *E. coli* encoded by the carotenoid synthase/carotenoid cyclase gene sequences from the aphid (Takemura et al., 2021; see footnote).

It is well-known that aphids feed on sap of phloem vessels in host plants. However, characteristic carotenoids in plants such as lutein and zeaxanthin were not detected in aphids. These results indicate that aphids do not intake carotenoids from host plants but synthesize them themselves by the carotenoid synthase/carotenoid cyclase gene horizontally transferred from fungi. Aphids are an important food of carnivorous insects and spiders and are called aeroplankton (Speight et al., 2008). Thus, carotenoids characteristic to aphids have been found in predatory dragonflies, beetles, and spiders, which potentially prey on aphids through the food chain (Maoka et al., 2020).

On the other hand, carotenoids were not found in the aphid *Lachnus tropicalis*, belonging to Lachninae. Quinones are present as the main pigments in *L. tropicalis*. In *Ceratovacuna nekoashi*, belonging to Hormaphidinae, quinones are present as the major pigments, whereas carotenoids are also present but only as minor pigments.

Contrary to aphids, carotenoid levels in Auchenorrhyncha (planthoppers, leafhoppers, and cicadas) were very low, and carotenoids produced by the torulene synthetic pathway and with a  $\gamma$ -end group were not present in these insects, as shown in Table 1. Planthoppers and leafhoppers are plant feeders that suck plant sap from grasses.  $\beta$ -Carotene,  $\beta$ -cryptoxanthin, and lutein, ingested from plants, were found to be major carotenoids in planthoppers and leafhoppers: *Nilaparvata lugens*, *Bothrogonia ferruginea*, *Hecalus prasinus*, and *Nephotettix cincticeps*. Green and yellow body colors of planthoppers and leafhoppers were not extractable with organic solvents such as acetone and ethanol. Therefore, these body colors were considered to be due to structural coloration.

A low level of carotenoids (0.1–0.02  $\mu\text{g/g}$ ) was detected in cicadas, *Graptopsaltria nigrofuscata*, *Hyalessa maculaticollis*, and *Tanna japonensis japonensis*, whereas trace amounts of carotenoids were detected in other species of cicadas (Table 1).  $\beta$ -Carotene and echinenone were found to be major carotenoids. Cicadas ingest only xylem sap, which is essentially free from carotenoids, throughout their life cycle. Therefore, carotenoids in cicadas might have originated from a symbiont, as in aphids.

$\beta$ -Carotene,  $\beta$ -cryptoxanthin, lutein, and zeaxanthin were found to be

major carotenoids in both stink bugs and leafhoppers belonging to Heteroptera (Table 1). Among them,  $\beta$ -carotene was found to be a major carotenoid (94.5–80.7%) in *Sirthena flavipes*, *Corythucha marmorata*, *Haematolochea nigrorufa*, and *Orius sauteri*. Lutein was present as a major carotenoid (74.0–40.0%) in *Nezara antennata*, *Plautia stali*, *Halyomorpha halys*, *Alcimocoris japonensis*, and *Isyndus obsurus*. Zeaxanthin (40.6%) and its epoxide, antheraxanthin (34.6%), were present as major carotenoids in *Parastrachia japonensis*. They are characteristic carotenoids in plants. Stink bugs and leafhoppers also feed on sap of phloem vessels in plants. Stink bugs and leafhoppers might intake carotenoids from host plants. *P. japonensis* displays a bright red body color. This red color could not be extracted with organic solvent such as acetone and ethanol. Therefore, it is considered to be due to structural coloration (Britton et al., 2008) (Manuta, 1948) (Seki et al., 1987).

Assassin bugs are carnivorous and prey on insects.  $\beta$ -Carotene,  $\beta$ -cryptoxanthin, echinenone, and lutein, which were found in several insects, were present in *Isyndus obscurus*. Therefore, these carotenoids are considered to be of dietary origin.

Carotenoids in water striders (Gerridae) belonging to Heteroptera are shown in Table 1. Water striders contain relatively high levels of carotenoids (35.2–23.3  $\mu\text{g/g}$ ) among hemipteran insects.  $\beta,\gamma$ -Carotene,  $\gamma,\gamma$ -carotene,  $\beta$ -zeacarotene,  $\beta,\psi$ -carotene,  $\beta$ -cryptoxanthin, and echinenone were found in water striders, *Gerris latiaabdominis*, *Aquarius elongatus*, *A. paludum*, and *Metrocoris histrio*, along with  $\beta$ -carotene as a major carotenoid (about 60%). Water striders are carnivorous and often feed on insects that fall onto the water surface.  $\beta$ -Carotene,  $\beta$ -cryptoxanthin, and echinenone, are present in several insects (Maoka et al., 2020). Therefore,  $\beta$ -cryptoxanthin, echinenone, and a part of  $\beta$ -carotene might be accumulated from prey insects that water striders have fed on. On the other hand,  $\beta,\gamma$ -carotene,  $\gamma,\gamma$ -carotene,  $\beta$ -zeacarotene,  $\beta,\psi$ -carotene, and a part of  $\beta$ -carotene might be synthesized in water striders themselves by carotenoid synthetic genes, which were horizontally transferred from fungi just as in aphids.

*Notonecta triguttata* (backswimmer), belonging to Notonectidae, is also carnivorous and feeds on small fish, tadpoles, and several insects that fall onto the water surface.  $\beta$ -Carotene was present as a major carotenoid (72.2%) along with echinenone (4.5%) and  $\beta$ -cryptoxanthin (15.2%). Carotenoids with the  $\gamma$ -end group and belonging to the torulene biosynthetic pathway were not present in this predatory species (Table 1).

Structures of carotenoids present in Hemiptera are shown in Fig. 1.

**Table 1**  
Carotenoids in 47 species of insects belonging to Hemiptera.

Suborder	Family	Species	Common name	Total Carotenoid (µg/g)	(µg/Specimen)	β-β-Carotene	β-Zeacarotene	β-ψ-Carotene	Torulene	β-γ-Carotene	γ-γ-Carotene	γ-ψ-Carotene	Echinonone	β-Cryptoxanthin	Zeaxanthin	Antheraxanthin	Lutein	Lutein epoxide	Unidentified		
Sternorrhyncha	Aphididae *1	<i>Shivaphis celti</i>		26.5		5.8	51.6	13.5		14.8		6.4							7.9		
		*2-1	<i>Aphis gossypii</i>	cotton aphid	192.7		18.7	0.5	44.6		23.6									12.6	
	*2-2	<i>A. nerii</i>	oleander aphid	242.1		18.2	2.1	11.0			54.5	11.0	2.1							1.1	
		<i>A. spiraeola</i>	Spiraea aphid	90.2		12.1	32.9	14.2	5.2		25.1	3.2	2.2							5.1	
		<i>Semiaphis heraclei</i>	celery aphid	170.0		22.1	1.8	11.2			33.7	13.1	7.5							10.6	
		<i>Toxoptera citricida</i>	citrus brown aphid	14.4		24.3	20.5	32.5			22.4									0.3	
		<i>Acyrtosiphon pisum</i>	pea aphid	39.7		70.2		2.2			15.9	3.7	1.2							6.8	
		<i>Brevicoryne brassicae</i>	cabbage aphid	52.0		20.0				12.8	33.8	10.0	12.0							11.4	
		<i>Uroleucon formosanum</i>	Formosan hairy aphid	72.7		19.4		33.5			38.8									8.3	
		<i>U. kikioensis</i>		2.8		25.8		17.6			42.2	11.6	2.3							0.5	
		<i>Macrosiphum euphorbiae</i>	potato aphid	5.3		71.1	14.2	7.3			4.9									2.5	
		<i>Megoura crassicauda</i>		133.6		19.5					48.8	4.3	13.7							13.7	
		*3	<i>Lachnus tropicalis</i>	large chestnut aphid	N.D.																
		*4	<i>Ceratovacuna nekoashi</i>		2.2		24.2		55.2		18.1	1.5									
		*5	<i>Schlechtendalia chinensis</i>	sirex parasite	12.8		20.3	30.4	28.3		1.2	3.7									3.3
		Auchenorrhyncha	Aleyrodidae	<i>Dialeurodes citri</i>	citrus white fly	0.5				>95											
			Delphacidae	<i>Nilaparvata lugens</i>	brown rice planthopper	±		±													
Cicadellidae	<i>Bothrogonia ferruginea</i>		black-tipped leafhopper	+		+									+			+			
	<i>Hecalus prasinus</i>		spoon-headed leafhopper	2.4	0.1	31.2					22.1								43.8	2.9	
Cicadidae	<i>Nephotettix cincticeps</i>		green rice leafhopper	0.1		50.2					35.5								13.5	0.8	
	<i>Graptosaltia nigrofusca</i>		large brown cicada	0.022	0.056	74.2							15.7							10.1	
	<i>Hyalessa maculaticollis</i>		robust cicada	0.15	0.11	~100															
	<i>Tanna japonensis</i>		evening cicada	0.11	0.094	80.5								5.5						14.0	
	<i>Cryptotympana facialis</i>			0.009	0.03	~100															
	<i>C. atrata</i>			±		±															
	<i>Meimuna opalifera</i>		elongate cicada	±		±															
	<i>Platypleura kaempferi</i>		Kaempfer cicada	±		±															
Heteroptera	Cydnidae		<i>Parastrachia japonensis</i>	ground bug	6.0	1.1	2.5								2.0	40.6	34.6			20.3	
	Scutelleridae		<i>Poecilocoris lewisi</i>	red-striped golden stink bug	1.3	0.5	25.5									24.5	35.5			14.5	
	Pentatomidae		<i>Halyomorpha halys</i>	brown marmorated stink bug	1.2	0.2	14.8									2.0			74.0	9.2	
			<i>Alcimocoris japonensis</i>		19.2		10.0										45.0			45.0	
	Coreidae		<i>Plautia stali</i>	Oriental stink bug	27.0	1.3	13.2									5.6	20.2			50.5	10.5
		<i>Nezara antennata</i>	green stink bug	6.3		42.2										5.6	5.4		40.6	4.4	
		<i>Sastragala esakii</i>	shieldbug	19.2		65.8									2.5	14.2			15.3	2.2	
	Coreidae	<i>Acanthocoris sordidus</i>	winter cherry bug	+		+															
		Reduviidae	<i>Haematoloeca nigrorufa</i>	assassin bug	3.4	3.4	83.1													0.9	
			<i>Isyndus obscurus</i>		5.7	1.3	33.5								1.2	15.2	9.2			40.5	0.4
	Tingidae	<i>Sirthena flavipes</i>		4.6	0.7	94.5									4.5				1.0		
		<i>Corythucha marmorata</i>	chrysanthemum lace bug	34.2		82.1										3.5			13.7	0.7	
	Anthocoridae	<i>Orius sauteri</i>	mulberry flower bug	2.1		80.7									15.4					3.9	
		Gerridae	<i>Aquarius elongatus</i>	water strider	23.3	2.6	65.5	4.3	8.1			3.2	2.6		3.1	10.1				3.1	
	<i>A. paludum</i>			26.7	1.3	58.9	2.3	2.8			11.5	5.4		4.4	12.5				2.2		
	<i>Gerris latiabdominis</i>			35.2	0.5	60.5	2.3	6.6			7.3	3.3		4.2	12.4				3.4		
	<i>Metrocoris histrio</i>			23.3		60.1	1.5	5.2			11.8	1.2		2.3	10.5				7.4		
<i>Ranatra chinensis</i>	Chinese water scorpion		9.5	4.0	95.0										4.5				0.5		
Belostomatidae	<i>Appasus major</i>	giant water bug	3.0	1.5	50.1									25.2	6.1			11.1	7.5		
Notonectidae	<i>Notonecta triguttata</i>	three-spotted backswimmer	20.9	2.1	72.2								4.5	15.2					8.1		

\*1: Drepanosiphinae, \*2-1: Aphidinae (Aphidini), \*2-2: Aphidinae (Macrosiphini), \*3: Lachninae, \*4: Hormaphidinae, \*5: Eriosomatinae.

The list of hemipteran insects in Table 1 were referenced from Hayashi et al. (2016) and Yano (2018).

As described above, aphids contained characteristic carotenoids, which were synthesized by carotenoid synthase/carotenoid cyclase genes horizontally transferred from fungi, whereas carotenoids from host plants were not detected. On the other hand, stink bugs and leafhoppers possess carotenoids, likely from host plants. Furthermore, both types of carotenoids were present in water striders.  $\beta$ -Carotene was scarcely detected in cicadas.

Carotenoids are widely distributed in insects and play important functional roles such as a photo-protector, an antioxidant, and for protective coloration (Shamim et al., 2014). Most insects acquire carotenoids from foods and accumulated them in their body (Maoka et al., 2020). Aphids are sap-feeding insects that cannot intake carotenoids from their host plants but acquire the ability to synthesize them by carotenoid synthetic genes from symbiotic fungi, as described above. On the other hand, some aphids biosynthesize quinone pigments, which play a role as a photo-protector as carotenoids (Shamim et al., 2014). Therefore, *Lachnus tropicalis* and *Ceratovacuna nekoashi*, which lack carotenoids, might not need carotenoids for such a purpose.

Carotenoid contents in planthoppers, leafhoppers, stink bugs, and assassin bugs are very low, as shown in Table 1, since the body color of these insects is due to structural coloration. Therefore, they may not need to accumulate large amounts of carotenoids.

Cicadas spend a large part of their life histories in their nymphal stage underground. Therefore, photoprotectors, such as carotenoids and quinones may not be needed in their larval stage. During adult molting, they rapidly synthesize melanins (Asano, 2013), which may play a role as a protector against sunlight instead of carotenoids or quinones.

In conclusion, carotenoids of Hemiptera well-reflect their ecological life histories.

#### Author statement

TM conceived this research and carried out carotenoid analysis. N.K., M.H. and R.N. collected insects samples and discussed the systematics and ecological history of insects.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bse.2021.104241>.

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