Biochemical Systematics and Ecology 89 (2020) 104001

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Carotenoids of dragonflies, from the perspective of comparative biochemical and chemical ecological studies

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ARTICLE INFO

Keywords:
Carotenoid
Dragonfly
Food chain
Comparative biochemistry
Chemical ecology

ABSTRACT

Carotenoids of 20 species of dragonflies (including 14 species of Anisoptera and six species of Zygoptera) were investigated from the viewpoints of comparative biochemistry and chemical ecology. In larvae, β-carotene, β-cryptoxanthin, lutein, and fucoxanthin were found to be major carotenoids in both Anisoptera and Zygoptera. These carotenoids were assumed to have originated from aquatic insects, water fleas, tadpoles, and small fish, which dragonfly larvae feed on. Furthermore, β-caroten-2-ol and echinenone were also found in all species of larvae investigated. In adult dragonflies, β-carotene was found to be a major carotenoid along with lutein, zeaxanthin, β-caroten-2-ol, and echinenone in both Anisoptera and Zygoptera. On the other hand, unique carotenoids, β-zeacarotene, β,ψ-carotene (γ-carotene), torulene, β,γ-carotene, and γ,γ-carotene, were present in both Anisoptera and Zygoptera dragonflies. These carotenoids were not found in larvae. Food chain studies of dragonflies suggested that these carotenoids originated from aphids, and/or possibly from aphidophagous ladybird beetles and spiders, which dragonflies feed on. Lutein and zeaxanthin in adult dragonflies were also assumed to have originated from flying insects they feed on, such as flies, mosquitoes, butterflies, moths, and planthoppers, as well as spiders. β-Caroten-2-ol and echinenone were found in both dragonfly adults and larvae. They were assumed to be metabolites of β-carotene in dragonflies themselves. Carotenoids of dragonflies well reflect the food chain during their lifecycle.

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).

Insects are the most diverse group of animals. Therefore, carotenoids in insects show structural diversity. For example, a series of carotenoids with 2-hydroxy, 2-keto-, and 3,4-didehydro-2-keto-β-end groups are present in stick insects (Kayser, 1981a, 1981b; 1982; Matsuno et al., 1990; Davidson et al., 1991) and some species of moth (Kayser, 1975, 1976, 1979). Oxidative metabolites of luteins, philosamiaxanthin (3-hydroxy-β,ε-caroten-3′-one), fritschiellaxanthin, and papirioerythrinon, are found in several species of moths and butterflies (Kayser, 1975; Harashima et al., 1972). Astaxanthin and adonirubin were found in locust and mantis (Manuta, 1948). Fucoxanthin and fucoxanthinol were reported from larvae of mayfly and caddishfly (Matsuno et al., 1999). Several species of beetles contain phytoene, phytofluene, ζ-carotene, neurosporene, lycopene, β-zeacarotene, γ-carotene, and torulene, which are carotenoids in the torulene biosynthetic pathway (Britton et al., 1977a, 1977b). Furthermore, a series of carotenes with a unique γ-end group, such as γ,ψ-carotene, 3,4-didehydro-γ,ψ-carotene, β,γ-carotene, and γ,γ-carotene, are present in aphids (Britton et al., 1977a, 1977b).

Dragonflies are insects belonging to the order Odonata and are one of the familiar insects with symbols of courage, strength, victory, and happiness in Japan (Asahina, 1974). They are mainly divided into two suborders, Anisoptera and Zygoptera. Adults of Anisoptera dragonflies are characterized by large, multifaceted eyes, two pairs of strong, transparent wings, sometimes with colored patches, and a conspicuously elongated body. Adults of Zygoptera dragonflies have slimmer bodies, and most species fold the wings along the body when at rest. Dragonflies are carnivorous throughout their lifecycle. Larvae inhabit aquatic environments such as rice paddies, ponds, swamps, and

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https://doi.org/10.1016/j.bse.2020.104001
Received 9 December 2019; Received in revised form 24 December 2019; Accepted 5 January 2020
Available online 15 January 2020

streams and feed on aquatic insects, water fleas, tadpoles, and small fishes. After emergence, adults feed on several insects and spiders (Corbet, 1999).

It is well-known that carotenoids found in animals are either directly accumulated from feed or partly modified through metabolic reactions. Several carotenoids in animals are indicators of taxonomy and food-chain markers (Liaaen-Jensen, 1998., Maoka, 2011). Therefore, carotenoids of dragonflies were investigated from the viewpoints of comparative biochemistry and chemical ecology.

In the present paper, we describe carotenoids in the body tissue of 20 species of dragonflies, including 14 species of Anisoptera and six species of Zygoptera.

2. Materials and methods

2.1. Insects

The following adults and larvae of dragonflies were collected at Koka City, Shiga Prefecture, Kyoto City, Kyoto Prefecture, and Komono Town, Mie Prefecture in Japan during April to October.

Adult dragonflies; Lestidae; *Lestes sponsa, Lestes temporalis,* Calopterygidae; *Mnais costalis, Atrocalopteryx atrara, Calopteryx cornelia*, Coenagrionidae; *Paracercion calamorum* belonging to Zygoptera, Aeshnidae; *Sarasaeschna pryeri, Anax parthenope,* Gomphidae; *Sieboldius albardae*, *Asiagomphus pryeri, A. melaenops,* Cordulegastridae; *Anotogaster sieboldii*, Libellulidae; *Sympetrum darwinianum, S. infuscatum, S. frequens, Pantala flavescens, Lyriothemis pachygastra, Orthetrum albistylum, O. japonicum, O. melania* belonging to Anisoptera.

Larvae dragonflies; Zygoptera, Calopterygidae; *Atrocalopteryx atrara,* Coenagrionidae; *Paracercion calamorum,* Anisoptera, Cordulegastridae; *Anotogaster sieboldii*, Libellulidae; *Pantala flavescens, Lyriothemis pachygastra*, *Sympetrum frequens, Orthetrum albistylum, O. japonicum.*

Aphids, planthoppers, ladybird beetles, and spiders were also collected at Koka City, Shiga Prefecture, Kyoto City, Kyoto Prefecture, and Komono Town, Mie Prefecture in Japan during April to October.

The scientific names of dragonflies in this paper are referenced from Ozono et al. (2012).

2.2. Carotenoid extraction, analysis, isolation, and identification

The carotenoids were extracted from larvae and adults of dragonflies of each species with acetone at room temperature. They were then transferred to *n*-hexane:Et_2O (1:1, v/v) by addition of water. The *n*-hexane: Et_2O 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).

Quantitative and qualitative carotenoid analysis of dragonflies, aphids, plant hoppers, beetles, and spiders were carried out using our routine method using LC/PDA/MS (Maoka, 2016).

In the case of adult *Sympetrum frequens* and *Pantala flavescens* individual carotenoids were isolated by column chromatography followed by preparative HPLC and identified from UV-VIS, ESI TOF MS, ^{1}H NMR, and circular dichroism (CD) spectral data.

The UV-VIS spectra were recorded with a Hitachi U-2001 spectrophotometer (Hitach Field Navigator, Tokyo, Japan) in Et_2O. The ^{1}H NMR (500 MHz) spectrum was measured with a Varian UNITY INOVA 500 spectrometer (Varian Corporation, Palo Alto, California USA) in $CDCl_3$ with TMS as an internal standard. The CD spectrum was recorded in Et_2O at room temperature with a Jasco J-500C spectropolarimeter (JASCO Corporation, Hachioji, Tokyo, Japan). Preparative HPLC was performed with a Hitachi L-6000 intelligent pump and an L-4250 UV-VIS detector (Hitach Field Navigator, Tokyo, Japan) set at 450 nm. The column used was a 250 × 10 mm i.d., 5 μm Cosmosil $5C_{18}$-MS-II ODS (Nacalai Tesque, Kyoto, Japan) with $CHCl_3$:MeOH (25:75, v/v) as a solvent at a flow rate of 2.0 mL/min.

About 3000 specimens adult *Sympetrum frequens* were extracted with acetone at room temperature. They were then transferred to *n*-hexane: Et_2O (1:1, v/v) by addition of water. The *n*-hexane: Et_2O phase was washed with water and dehydrated on anhydrous sodium sulphate and evaporated. The red residue was separated by silica gel chromatography using *n*-hexane, Et_2O, and acetone as eluting solvents.

Carotenoids eluted with *n*-hexane was further separated by preparative ODS HPLC to afford β-carotene, (6′*S*)-β,γ-carotene, β-zeacarotene, β,ψ-carotene (γ-carotene), torulene, and γ,γ-carotene. Carotenoids eluted with *n*-hexane: Et_2O (1:1) from silica gel chromatography were further separated by ODS HPLC to afford echinenoone, β-cryptoxanthin, β-caroten-2-ol. Carotenoids eluted with ether from silica gel chromatography were further separated by ODS HPLC to afford lutein and zeaxanthin. Carotenoids eluted with acetone from silica gel chromatography were further separated by ODS HPLC afforded to myxol.

Individual carotenoids in aphid *Acyrthosiphon pisum* were separated as the same manner.

The following 12 carotenoids, β-carotene, (6′*S*)-β,γ-carotene, γ,γ-carotene, β-zeacarotene, β,ψ-carotene (γ-carotene), torulene, echinenone, β-caroten-2-ol, β-cryptoxanthin, lutein, zeaxanthin, and myxol, were identified in adult *Sympetrum frequens*. Fucoxanthin was identified in larva. These spectral data were described in supplementary information. Details of spectral data of (6′*S*)-β,γ-carotene and β-zeacarotene have not been reported (Britton et al., 2004). Therefore, spectral data of (6′*S*)-β,γ-carotene and β-zeacarotene were described here.

(6′*S*)-β,γ-Carotene: ESI TOF MS (*m/z*) 536.4382 [M^+] $C_{40}H_{56}$, Calcd for 536.4404; UV–Vis (Et_2O) 412, 442, 471 nm; ^{1}H NMR δ (in $CDCl_3$ at 500 MHz) 0.82 (3H, s, H-16′), 0.90 (3H, s, H-17′), 1.03 (6H, s, H-16,17), 1.33 (2H, dd, J = 14, 7 Hz, H-2′), ~1.46 (2H, m, H-2), ~1.63 (2H, m, H-3), 1.72 (3H, s, H-18), 1.95 (3H, s, H-19′), 1.97 (9H, s, H-19, 20, 20′), 2.02 (2H, t, J = 7 Hz, H-4), 2.07 (2H, m, H-3′), 2.29 (2H, m, H-4′), 2.51 (1H, d, J = 9.5 Hz, H-6′), 4.57 (1H, br. s, H-18′), 4.73 (1H, br. s, H-18′), 5.84 (1H, dd, J = 15.5, 9.5 Hz, H-7′), 6.12 (1H, d, J = 11.5 Hz, H-10′), 6.13 (1H, d, J = 15.5 Hz, H-8′), 6.14 (1H, d, J = 16 Hz, H-8), 6.14 (1H, d, J = 10 Hz, H-10), 6.15 (1H, d, J = 16 Hz, H-7), 6.24 (2H, m, H-14, 14′), 6.33 (1H, d, J = 15.5 Hz, H-12′), 6.35 (1H, d, J = 15.5 Hz, H-12), 6.62 (1H, dd, J = 15.5, 11.5 Hz, H-11′), 6.63 (2H, m, H-15, 15′), 6.65 (1H, dd, J = 15.5, 11.5 Hz, H-11′); CD (in Et_2O): λ nm (Δε) 216 (+3.0), 230 (0), 238 (−4.8), 249 (0), 270 (+4.6), 225 (0), 340 (−1.8), 375 (−0.2). These spectral data were in agreement with published data (Arpin et al., 1971; Britton et al., 2004).

β-Zeacarotene: ESI TOF MS (*m/z*) 538.4552 [M^+] $C_{40}H_{58}$, Calcd for 538.4539; UV–Vis (Et_2O) 400, 426, 451 nm; ^{1}H NMR δ (in $CDCl_3$ at 500 MHz) 1.03 (6H, s, H-16,17), ~1.46 (2H, m, H-2), ~1.63 (2H, m, H-3), 1.60 (3H, s, H-18′), 1.61 (3H, s, H-17′), 1.68 (3H, s, H-16′), 1.72 (3H, s, H-18), 1.82 (3H, s, H-19′), 1.97 (9H, s, H-19, 20, 20′), 2.02 (2H, t, J = 7 Hz, H-4), ~2.12 (8H, m, H-3′,4′,7′, 8′), 5.11 (2H, m, H-2′, 6′), 5.95 (1H, d, J = 11 Hz, H-10′), 6.14 (1H, d, J = 16 Hz, H-8), 6.14 (1H, d, J = 10 Hz, H-10), 6.15 (1H, d, J = 16 Hz, H-7), 6.23 (1H, d, J = 15.5 Hz, H-12′), 6.25 (2H, m, H-14, 14′), 6.35 (1H, d, J = 15.5 Hz, H-12), 6.50 (1H, dd, J = 15.5, 11.5 Hz, H-11′), 6.63 (2H, m, H-15, 15′), 6.64 (1H, dd, J = 15.5, 11.5 Hz, H-11’). These spectral data were in agreement with published data (Britton et al., 2004).

3. Results and discussion

3.1. Carotenoids of dragonfly larvae

Carotenoid contents and compositions in eight species of dragonfly larvae including six species of Anisoptera and two species of Zygoptera are shown in Table 1. Carotenoid compositions of dragonfly larvae were similar to each other. β-Carotene was found to be the major carotenoid along with fucoxanthin, lutein, and zeaxanthin. β-Caroten-2-ol, β-cryptoxanthin, and echinenone were also found in all species of larvae investigated. Dragonfly larvae inhabit aquatic environments and feed

Table 1
Carotenoids in eight species of dragonfly larvae.

Suborder	Zygoptera		Anisoptera					
Family	Calopterygidae	Coenagrionidae	Cordulegastridae	Libellulidae				
Genus	*Atrocalopteryx*	*Paracercion*	*Anotogaster*	*Pantala*	*Lyriothemis*	*Sympetrum*	*Orthetrum*	*Orthetrum*
Species	*atrara*	*calamorum*	*sieboldii*	*flavescen*	*pachygastra*	*frequens*	*albistylum*	*japonicum*
Total Carotenoid (μg/g)	2.2	3.75	2.44	10.5	0.8	0.66	2.8	0.9
(μg/specimen)	20.2	0.33	4.23	2.2	2.3	3.75	0.5	2.2
Composition	%	%	%	%	%	%	%	%
β,β-Carotene	30.8	27.0	10.2	20.6	38.2	37.8	18.6	37.2
β,γ-Carotene								
β-Zeacarotene								
β,ψ-Carotene								
γ,γ-Carotene								
Torulene								
Echinenone	2.2	6.5	1.6	8.8	11.5	1.8	5.6	11.6
β-Caroten-2-ol	10.2	1.2	0.5	8.2	8.2	2.2	7.2	9.2
β-Cryptoxanthin	7.1	20.2	1.5	35.2	10.2	6.2	38.5	11.2
Zeaxanthin	8.2	27.2	+	6.0	1.2	+	5.8	0.9
Lutein	11.5	2.4	34.3	+	10.5	15.2	+	11.5
Fucoxanthin	24.4	+	46.8	+	16.5	30.1	+	15.5
Myxol		+		11.4			14.9	
Unidentifiied	5.6	15.5	5.1	9.8	3.7	6.7	9.4	2.9

on aquatic insects, water fleas, tadpoles, and small fishes. In freshwater environments, herbivorous aquatic insects ingest β-carotene, lutein, zeaxanthin, fucoxanthin, from micro algae such as diatoms, green algae, blue green algae, and cyanobacteria (Matsuno et al., 1999). Dragonfly larvae appeared to accumulate these carotenoids from herbivorous aquatic insects and small aquatic animals. Myxol was identified in two species of larvae (*Pantala flavescens* and *Orthetrum albistylum*), accounting for about 10% of the total carotenoids. Myxoxanthophyll (myxol chinovoside) is a characteristic carotenoid in cyanobacteria (blue green algae) (Britton et al., 2004). Therefore, myxol might be derived from myxoxanthophyll by hydrolysis of glycoside bonds in animals through the food-chain. It was reported that several insects could convert β-carotene to β-caroten-2-ol and echinenone (Kayser, 1981a, 1981b; 1982, 1984). Therefore, β-caroten-2-ol and echinenone may be oxidative metabolites of β-carotene in dragonflies.

3.2. Carotenoids of adults dragonflies

Carotenoids of 20 species of adult dragonflies (including 14 species of Anisoptera and six species of Zygoptera) are shown in Table 2 (The lists of dragonfly species in Table 2 were presented in the order of discussion). In adult dragonflies, β-carotene was found to be a major carotenoid along with lutein, zeaxanthin, β-caroten-2-ol, and echinenone in both Anisoptera and Zygoptera. Unique carotenoids, β-zeacarotene, β,ψ-carotene (γ-carotene), torulene, β,γ-carotene, and γ,γ-carotene, were present in adults of both Anisoptera and Zygoptera dragonflies. These carotenoids were not found in larvae. β-Zeacarotene, β,ψ-carotene (γ-carotene), and torulene are carotenoids belonging to torulene biosynthetic pathways (Britton et al., 2004). β,γ-Carotene and γ,γ-carotene are carotenoids with a unique γ-end group (terminal methylene group in the C-5 end group), and have only been reported from aphids, beetles that feed on aphids (Britton et al., 1977a, 1977b), and discomycete fungus *Caloscyoha fulgens* (Arpin et al., 1971). Furthermore, myxol was found in several species of adults dragonflies. In *Pantala flavescens*, myxol comprised 23.4% of the total carotenoids. Recently, myxol was isolated from several species of bacteria belonging to Flavobacteriaceae (Yokoyama and Miki, 1995; Teramoto et al., 2003; Shindo et al., 2006). Therefore, myxol in adult dragonflies may have originated from associated bacteria in these dragonflies. On the other hand, fucoxanthin, which was present as one of the major carotenoids in larvae of dragonflies, was not found in adult dragonflies.

Dragonflies belonging to the genera *Sympetum* and *Pantala* have a red or orange body color. On the other hand, dragonflies belonging to the genera *Orthetrum* and *Asiagomphus* have a pale blue or yellowish-green body color. However, these carotenoid compositions were mostly similar to each other. Therefore, it was revealed that carotenoids in dragonflies did not directly reflect their body color. A red and yellow body color of adult dragonflies was identified to be ommochrome pigments (Futashasi et al., 2012). Characteristic carotenoids present in dragonflies are shown Fig. 1.

3.3. Change in carotenoid composition in Sympetrum frequens through the lifecycle

In Japan, *S. frequens* (akatonbo, the “red dragonfly”) is a common species distributed throughout Japan. Its larvae live in the rice fields and feed on small aquatic animals. After emergence, these dragonflies migrate to high mountains where they feed on several flying insects and grow until descending to rice fields for breeding (Asahina, 1974). Fig. 2 shows changes in the carotenoid content during the lifecycle in *S. frequens*. As described previously, β,β-carotene, echinenone, β-caroten-2-ol, β-cryptoxanthin, zeaxanthin, lutein, and fucoxanthin were present in larvae. After emergence, dragonflies do not ingest food for a few days. During this period, the carotenoid content of *S. frequens* markedly decreases. In teneral adults, the total carotenoid content was decreased to 1/5 of that found in larvae. Furthermore, fucoxanthin, one of the major carotenoids in larvae, completely disappeared in the teneral adults. Therefore, it was assumed that fucoxanthin, a characteristic carotenoid in diatoms, remained within the digestive tract of the dragonfly larvae and was not absorbed in their body. The carotenoid content of adults increased with their growth. After migration to high mountain areas, unique carotenoids, β-zeacarotene, β,ψ-carotene (γ-carotene), torulene, β,γ-carotene, and γ,γ-carotene, were found in *S. frequens.* The contents of these carotenoids increased during the adult growth period. Therefore, β-zeacarotene, β,ψ-carotene (γ-carotene), torulene, β,γ-carotene, and γ,γ-carotene were considered to have originated from dietary insects during the adult stage.

Table 2
Carotenoids in 20 species of adult dragonflies.

Suborder	Anisoptera									
Family	Libellulidae								Aeshnidae	
Genus	*Sympetrum*			*Pantala*	*Lyriothemis*	*Orthetrum*			*Sarasaeschna*	*Anax*
Species	*darwinianum*	*frequens*	*infuscatum*	*flavescens*	*pachygastra*	*albistylum*	*japonicum*	*melania*	*pryeri*	*parthenope*
Total carotenoid (μg/g)	19.5	18.1	11.2	23.5	1.7	14.4	7.1	9.2	8.3	14.0
(μg/specimen)	3.8	4.29	4.4	4.2	5.8	2.7	1.98	3.6	4.1	12.7
Composition (%)										
β,β-Carotene	39.7	33.4	41.6	20.9	46.0	29.3	52.4	62.1	45.2	75.3
β,ε-Carotene					2.9			4.4		
β,γ-Carotene	25.9	16.1	8.1	7.1	1.1	5.8	1.8	5.1	9.2	1.9
β-Zeacarotene	13.9	10.1	9.7	2.2	13.0	2.3	4.7	1.7	4.2	1.6
β,ψ-Carotene (γ-Carotene)	2.2	7.3	6.7	7.2		3.6		0.7	1.6	3.6
γ,γ-Carotene	1.5	1.3	0.4	1.0	1.0	1.0	5.6		+	+
Torulene				5.3	3.6		5.7		+	+
Echinenone	2.6	3.5	3.7	6.7	5.4	1.7	2.9	0.6	3.9	2.6
β-Caroten-2-ol	2.2	4.2	4.4	3.8	10.9	4.2	9.9	1.1	2.5	1.2
β-Cryptoxanthin	4.0	5.6	5.0	11.8	0.5	10.1	2.4	2.7	6.5	9.3
Zeaxanthin	1.1	2.6	3.4	5.2	9.4	29.2	7.6	1.5	2.1	0.6
Lutein	2.3	4.0	8.6	2.2		1.0		11.5	18.9	2.6
Myxol	1.4	6.2	1.2	23.4		3.5		1.4		
Unidentifieds	3.2	5.7	7.2	3.2	6.2	8.3	7.0	7.2	5.9	1.3

Suborder					Zygoptera					
Family	Gomphidae			Cordulegastridae	Lestidae		Calopterygidae			Coenagrionidae
Genus	*Sieboldius*		*Asiagomphus*	*Anotogaster*	*Lestes*		*Mnais*	*Atrocalopteryx*	*Calopteryx*	*Paracercion*
Species	*albardae*	*pryeri*	*melaenops*	*sieboldii*	*sponsa*	*temporalis*	*costalis*	*atrara*	*cornelia*	*calamorum*
Total carotenoid (μg/g)	14.0	12.6	5.1	3.2	7.1	3.6	3.1	1.2	10.7	14.0
(μg/specimen)	26.2	4.3	2.6	3.8	0.62	0.33	0.7	0.16	0.23	0.58
Composition (%)										
β,β-Carotene	61.2	38.1	30.0	70.0	40	43.2	30.6	48.4	35.7	31.9
β,ε-Carotene										
β,γ-Carotene	2.7	4.2	0.8	3.8	4.2	2.6	+	2.3	8.1	0.9
β-Zeacarotene	1.3	0.6	+	+	4.1	2.5	+	1.9	3.9	0.7
β,ψ-Carotene (γ-Carotene)	1.2	1.6	+	+	3.3	2.1	+	1.1	12.5	0.7
γ,γ-Carotene	+	+	+	+	+	0.1	+	+		0.2
Torulene	+	+	+	+	+	3.2	+	0.9		
Echinenone	1.6	7.0	1.1	1.2	2.3	2.3	3.8	8.1	5.7	4.5
β-Caroten-2-ol	0.8	1.2	2.0	1.0	2	7.5	13.7	13.2	4.9	2.2
β-Cryptoxanthin	7.2	21.0	5.5	4.1	8.8	10.2	13.2	15.8	4.5	23.9
Zeaxanthin	1.3	4.5	0.8	2.1	2	4.6	1.0	1.2		1.6
Lutein	19.2	20.9	54.8	13.9	25.6	5.9	19.5	5.3	5.8	24.2
Myxol				0.5						
Unidentifieds	3.5	0.9	5.0	3.4	7.7	15.8	18.2	1.8	18.9	9.2

3.4. Change of carotenoid composition in various body parts of *Sympetrum frequens*

Table 3 shows the carotenoid content and composition in the head, thorax, abdomen, feces, and eggs of *S. frequens*. The carotenoid content and composition of the head, thorax, and abdomen were similar to each other. On the other hand, feces contained about 50 times more carotenoid than the head, thorax, and abdomen. Furthermore, levels of β-zeacarotene, β,ψ-carotene (γ-carotene), and β,γ-carotene were higher than in other parts. The red color of feces in *S. frequens* was due to the presence of carotenoids. This clearly suggested that these carotenoids originated from their diet. Carotenoids were also found in eggs. Carotenoid compositions of eggs were also similar to those of adults. Therefore, carotenoids were translocated to eggs non-selectively from the female dragonfly.

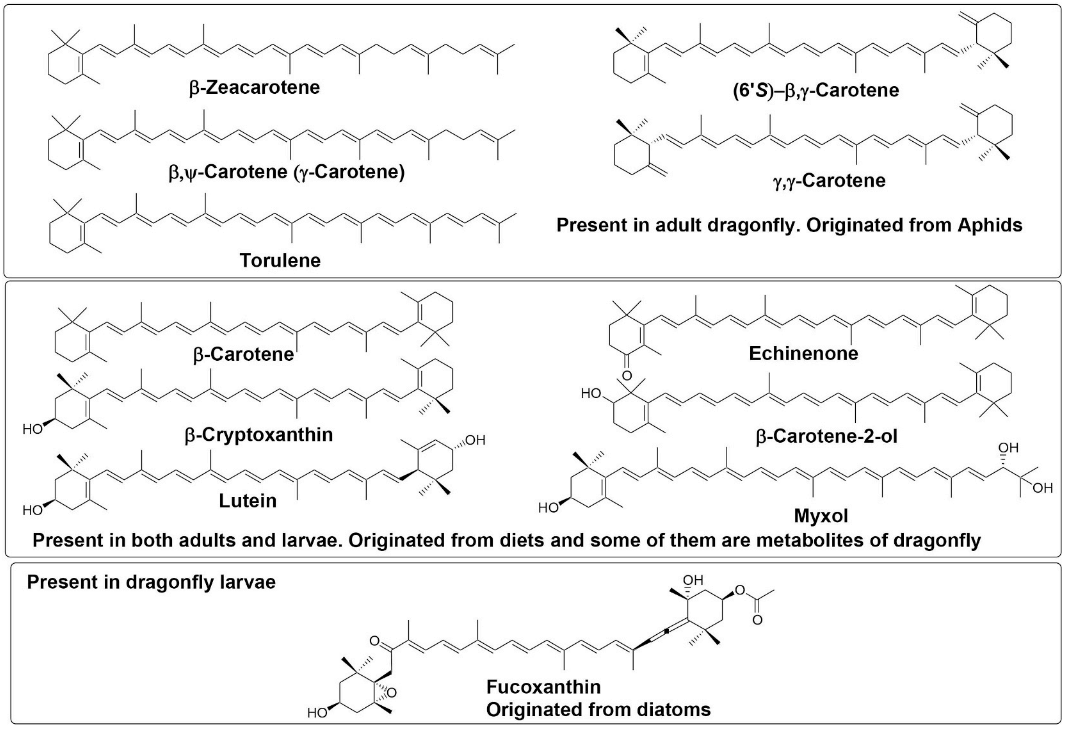

Fig. 1. Characteristic carotenoids in adults and larvae of dragonflies.

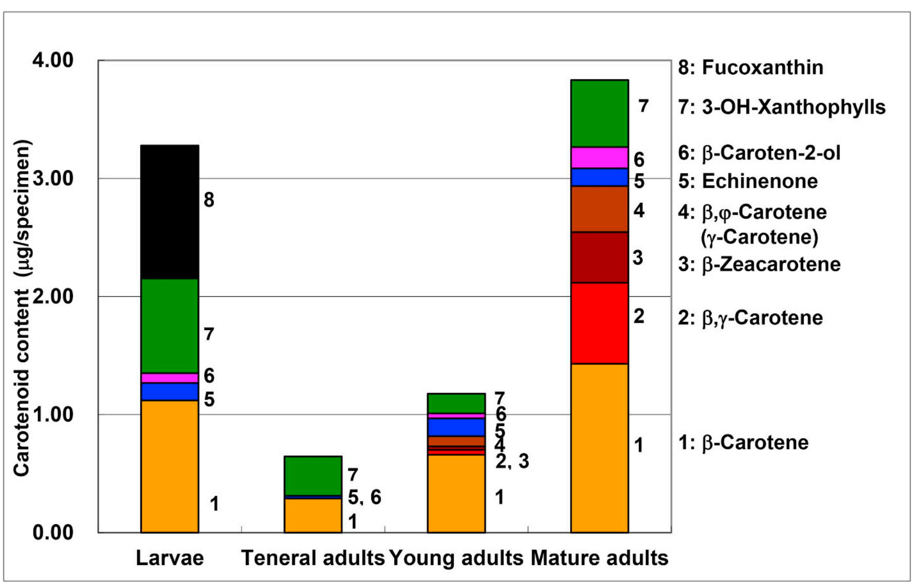

Fig. 2. Changes in individual carotenoid contents of *Sympetrum frequens* during the lifecycle.
Foot note 3-OH-xanthophylls: β-Cryptoxanthin, Lutein, Zeaxanthin.
Larvae; collected in rice paddy in June, Teneral adults; collected at around rice paddy in June, Young adults; collected at Mt Gozaisyo in July, Mature adults; collected at around rice paddy in October.

3.5. *Origins of carotenoid torulene biosynthetic pathway and with a γ-end group of dragonflies*

In order to reveal the origin of β-zeacarotene, β,ψ-carotene (γ-carotene), and torulene belonging to carotenoids in the torulene biosynthetic pathway (Goodwin, 1980) and β,γ-carotene and γ,γ-carotene with a γ-end group, carotenoids in flying insects, such as flies, mosquitoes, butterflies, moths, aphids, planthoppers, and beetles, supposed to be potential foods for dragonflies, were investigated. β-Carotene, β-cryptoxanthin, lutein, zeaxanthin, which originate from higher plants, were found in flies, mosquitoes, butterflies, moths, plant hoppers, etc. On the other hand, β-zeacarotene, β,ψ-carotene (γ-carotene), torulene, β,γ-carotene, and γ,γ-carotene were found in aphids and a ladybird, *Coccinella septenpunctata*, as shown in Table 4. The species *C. septenpunctata* feeds exclusively on aphids. Spiders are also known to feed on aphids. Thus, carotenoids in the torulene biosynthetic pathway and with a γ-end group may be present in these animals through the food chain.

Table 3
Carotenoid content and composition in head, thorax, abdomen, feces, and eggs of *Sympetrum frequens*.

Parts	Head	Thorax	Abdomen	Feces	Egg
Total Carotenoid μg/g	9.4	5.4	10.5	590	0.68
Total Carotenoid μg/specimen	0.34	0.7	0.6	4.9	0.003
Composition	%	%	%	%	%
β,β-carotene	56.8	46.1	52.8	14.0	46.9
β,γ-carotene	6.8	9.2	9.5	9.6	4.4
β-zeacarotene	2.7	9.0	4.8	16.2	3.4
β,ψ-carotene	2.3	9.0	11.5	34.0	11.9
γ,γ-carotene	1.0	1.1	1.1	4.6	0.8
torulene	0.5	1.0	1.1	5.7	0.8
echinenone	3.5	3.4	2.5	1.9	4.3
β-caroten-2-ol	3.4	4.1	3.0	3.9	6.2
β-cryptoxanthin	7.1	5.4	4.9	1.9	8.7
Unidentified	3.7	2.4	2.8	5.5	1.2
zeaxanthin	2.0	0.8	1.1	0.4	0.8
lutein	4.9	5.5	3.4	0.4	3.0
Others	5.4	3.2	1.6	1.9	7.5

Aphids are an important food of insects such as dragonflies and are called aeroplankton (Speight et al., 2008). Therefore, it was suggested that carotenoids in the torulene biosynthetic pathway and those with a γ-end group in adults dragonflies originate from aphids and also possibly from aphidophagous ladybird beetles and spiders which dragonflies prey on.

The pea aphid displays red, yellow, green, and blue-green color polymorphisms (Brown. 1975). Red and yellow body color pigments in the aphid are carotenoids (Britton et al., 1977a, 1977b), while blue-green pigments are polycyclic quinones (Brown. 1975; Tsuchida et al., 2010). β-Zeacarotene, β,ψ-carotene (γ-carotene), torulene, β,γ-carotene, and γ,γ-carotene were identified as major carotenoids in aphids (Britton et al., 1977a, 1977b). These carotenoids are known to be characteristic carotenoids in fungi (Liaaen-Jensen, 1998). Therefore, they are considered to have originated from endocymbiotics microorganisms such as fungi (Liaaen-Jensen, 1998). Recent investigations revealed that aphids synthesized carotenoid themselves by carotenoid synthetic genes, which were horizontally transferred from fungi to aphids. Namely, the aphid genome itself encodes multiple enzymes for carotenoid biosynthesis. Red colored aphids are known to have carotenoid desaturase genes and synthesize torulene from phytoene independently (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). Therefore, aphids make their own carotenoids and quinones by horizontal transfer genes from fungi and symbiotic bacteria for coloration, depending on the environmental signals. At present, our research group is conducting functional analysis of carotenoid synthetic genes in aphids.

Figs. 1 and 2 show carotenoids in both larvae and adults of dragonflies. β-Zeacarotene, β,ψ-carotene (γ-carotene), torulene, β,γ-carotene, and γ,γ-carotene, which were synthesized by aphids, were accumulated from aphids directly or beetles and spiders through the food chain. β-Carotene, β-cryptoxanthin, and lutein, which originated from algae and higher plants, were accumulated from dietary insects. β-Caroten-2-ol and echinenone were found in both larval and adult dragonflies. These carotenoids were considered to be oxidative metabolites of β-carotene in the dragonflies. Myxol might be derived from myxoxanthophyll, which originates from blue-green algae, through the food chain or from an associated bacterium belonging to Flavobacteriaceae. Fucoxanthin was found only in the larvae. This carotenoid originated from diatoms.

In conclusion, carotenoids of dragonflies well reflect the food chain during their lifecycle.

Acknowledgements

We thanks Mr. Katsuyuki Ino for collection of *Anax partheonope* in Koka City.

Table 4
Carotenoids in insects and spiders.

Order	Ephemeroptera	Orthoptera	Hemiptera	Hemiptera	Coleoptera	Diptera	Trichoptera	Lepidoptera	Araneae	
Family	Ephemeridae	Acridoidea	Aphidoidea	Pentatomidae	Coccinellidae	Chironomidae	Stenopsychidae	Papilionidae	Oxyopidae	Nephilidae
β,β-carotene	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	+ +
β,γ-carotene	–	–	+ +	–	+ +	–	–		+ +	+ +
β-zeacarotene	–	–	+	–	+	–	–		+	+
β,ψ-carotene	–	–	+	–	+	–	–		+	+
γ,γ-carotene	–	–	+	–	+	–	–		+	+
torulene	–	–	+	–	+	–	–		+	+
β-caroten-2-ol	–	–	–	–		–				
β-cryptoxanthin	–	–	–	–		–			+	+
zeaxanthin	+	+	–	+		+			+	+
lutein	+	+	–	+		+			+	+
echinenone	–	+	–	–		+	+	+ +	+	+
Canthaxanthin	–	+	–	–		+	+	+ +	+	+
Astaxanthin	–	+	–	–	–	–	–	+ +	+	+
Fucoxanthin	*	–	–	–		–	*		+	+
myxol		–	–	–		–	*			

+ + + >20%.
+ + >10%.
Examined species, Aphidoidea; *Acyrthosiphon pisum*, *Brevicoryne brassicae*, *Megoura crassicauda*, *Uroleucon formosanum* Pentatomidae; *Hecalus prasinus*, *Nephotettix cincticeps*、Coccinellidae; *Coccinella spetempunctata*, *Propylea japonica*, Acridoidea; *Locusta migratoria*, Chironomidae; *Chironomus* sp.
Ephemeroptera; Ephemera strigata, Trichoptera; Stenopsyche marmorata, Lepidoptera; Papilio xuthus, Araneae; Oxyopes sertatus, Nephila clavata.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bse.2020.104001.

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