Chemoecology (2023) 33:147–159
https://doi.org/10.1007/s00049-023-00391-1

CHEMOECOLOGY

ORIGINAL ARTICLE

Larvae of *Sasakia charonda* (Lepidoptera: Nymphalidae: Apaturinae) and three related species use oral odorants to repel ants and wasps

Taro Hayashi[1] · Kaori Holikawa[1] · Hisako Akiba[1] · Takashi A. Inoue[2,3] · Kinuko Niihara[3] · Tatsuya Fukuda[3]

Received: 29 November 2022 / Accepted: 16 August 2023 / Published online: 7 September 2023

Abstract

We incidentally discovered that the larvae of *Sasakia charonda* (Hewitson, 1863) (Lepidoptera: Nymphalidae: Apaturinae) disturbed by ants, wasps, or humans release volatile compounds orally. To identify these substances, we collected oral odorant samples directly from the mouths of *S. charonda* larvae into volatile-collecting tubes. The trapped oral odorant samples were subjected to gas chromatography–mass spectrometry (GC–MS). We confirmed the identity of 19 substances by comparing them to GC results of known standards and inferred them to mainly be alcohols and aldehydes/ketones, with main chains of 4–5 carbons. Three of the chemicals in the oral odorant samples, 2-butanol, 1-penten-3-ol, and 3-pentanone, showed a repellant effect on the ants *Pristomyrmex punctatus* (Smith, 1860) and *Formica japonica* Motschoulsky, 1866 (Hymenoptera: Formicidae). We also examined the effects of these 19 volatiles on *Polistes* spp. (Hymenoptera: Vespidae) and confirmed that some of them were effective repellants against these wasps. We propose that the substances we identified in this study can be used as defensive chemicals, analogous to the osmeterium emissions specific to Papilionidae butterflies. Furthermore, we examined the oral odorants of three related Japanese Apaturine species, *Hestina assimilis* (Linnaeus, 1758), *H. persimilis* (Westwood, 1850), and *Apatura metis* (Freyer, 1829) using the same approach. The chemical compositions of the oral odorants of *H. assimilis* and *H. persimilis* were similar to that of *S. charonda*, whereas that of *A. metis* differed. Some of the oral substances also induced a defensive response in conspecific Apaturinae larvae. We consider these substances to also act as alarm pheromones in these larvae.

Keywords Japanese Apaturinae butterflies · Oral odorants · Ants · Wasps · Alarm pheromone · Repellent

Introduction

Many animals use distinctive odorants to ward off predators. Among insects, the repellents of Pentatomidae (Hemiptera), Carabidae (Coleoptera), and Coccinellidae (Coleoptera) are particularly well known. As *Sasakia charonda* (Hewitson, 1863) (Lepidoptera: Nymphalidae: Apaturinae) is the largest nymphalid species in terms of body size in Japan and its neighboring countries, some researchers have a special interest in this species. *Sasakia charonda* has been bred since 2012 in cages in the Kashihara City Museum of Insects, Kashihara, Nara, by Taro Hayashi.

While manually transferring *S. charonda* larvae to fresh leaves of *Celtis sinensis* (Rosales: Cannabaceae), the larvae released foul-smelling volatile compounds from their mouths without emitting a fluid orally. Moreover, in response to handling, they raised their heads, opened their mandibles, and made clicking sounds, resembling "Kachi, Kachi", to threaten their predators. Furthermore, some larvae made

Communicated by Günther Raspotnig.

Taro Hayashi, Kaori Holikawa, Hisako Akiba, Takashi A. Inoue, Kinuko Niihara, and Tatsuya Fukuda have contributed equally to this work.

✉ Takashi A. Inoue
inoueatptalent@yahoo.co.jp

[1] Sasakia Charonda Research Center, Kuzumoto 367-2-1-208, Kashihara, Nara 634-0007, Japan

[2] Studio Ace Enterprise and Pretties, Shimosueyoshi 5-5-2, Tsurumi, Yokohama, Kanagawa 230-0012, Japan

[3] Department of Natural Science, Faculty of Knowledge Engineering, Tokyo City University, Tamadzutsumi, Setagaya, Tokyo 158-8557, Japan

movements as if they were trying to bite the predator. Many ants live in the same cage, and Hayashi (pers. comm.) also observed that the larvae showed similar behavior toward the ants. Specifically, *S. charonda* larvae were observed to successfully repel ant attacks by turning the opened mandible toward the ant's head (Fig. 1). Moreover, Kaori Holikawa (pers. comm.) verified the same behavior in *Hestina assimilis* (Linnaeus, 1758) and *H. persimilis* (Westwood, 1850) (Lepidoptera: Nymphalidae: Apaturinae), as well as in other Japanese Apaturinae species. Thus, we aimed to conduct a chemical analysis of the compounds released by these Apaturinae larvae. Additionally, in the field, the fourth author observed that *H. assimilis* fifth (final) instar larvae succeeded in repelling *Polistes chinensis* (Fabricius, 1793) (Hymenoptera: Vespidae) by opening their mandibles. Thus, these oral odorants may also be useful in evading attack by certain wasps. We, therefore, also aimed to confirm whether these compounds are effective deterrents to ants.

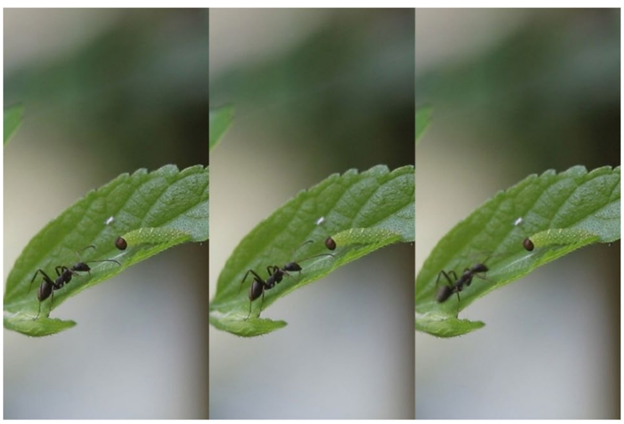

Fig. 1 A second instar larva of *Sasakia charonda* that successfully repelled an ant. **a** Ant approached the larva to capture it. **b** Larva turned the opened mandible toward the ant's head, and ant's antenna touched the larva's mandible. **c** Ant responded by retreating quickly

In addition, during oral odorant sample collection, we noticed that the behavior to emit volatiles orally was transmitted to neighboring larvae. Based on this observation, the odorants may also act as an alarm pheromone, and we examined whether these substances elicited the same effect in *S. charonda* larvae.

Materials and methods

Collection of oral odorant samples from Japanese Apaturinae larvae

The first experiments were conducted at the Sasakia charonda Research Center, Kashihara, Nara. *Sasakia charonda* larvae were reared in a 6.5 m × 5 m × 3 m (L × W × H) cage covered with a 1-mm nylon mesh net and fed with fresh *C. sinensis* leaves. We also used *S. charonda* larvae reared in the Museum of Sasakia charonda in Hokuto, Yamanashi. The sources, dates, origins, and additional information of the butterflies used in these experiments, and the origin of larvae, sampling dates, larval stages, sampling temperature (°C), individual numbers (n), and sampling durations are shown in Table 1. Oral odorant samples of the 4th–6th instar larvae of *S. charonda* in butterfly cages were collected in sampling tubes (Tenax TA, GL Sciences, Japan) using an air suction pump with an initial suction capacity of approximately 20 L/min, as shown in Fig. 2. Oral odorant samples of the 4th–5th instar larvae of *H. persimilis* and *A. metis* were transported from their origin to the power supply point, and then collected in sampling tubes using the

Table 1 Origin, collecting date, and additional information of caterpillars used

Sample	Origin	Date	Larval stage	Temp (°C)	*n*	Sampling duration (s)
S. charonda -0	Kashihara, Nara	May 19, 2019	5–6	25	10	15
S. charonda -1	Kashihara, Nara	May 19, 2019	5–6	25	10	15
S. charonda -2	Kashihara, Nara	May 21, 2020	5–6	28	10	15
S. charonda -3	Kashihara, Nara	May 21, 2020	5–6	28	10	15
S. charonda -4	Hokuto, Yamanashi	Jun 11, 2020	4–6	20	10	15
S. charonda -5	Hokuto, Yamanashi	Jun 11, 2020	4–6	20	10	15
H. assimilis -0	Aoba, Kanagawa	Apr 16, 2020	4	20	3	30
H. assimilis -1	Tsurumi, Kanagawa	Jun 29, 2020	5	27	3	60
H. persimilis -0	Aoba, Kanagawa	Apr 16, 2020	5	20	5	30
H. persimilis -3	Setagaya, Tokyo	Jul 02, 2020	5	27	1	30
A. metis -0	Minuma, Saitama	Jul 02, 2020	5	27	4	60
A. metis-1	Minuma, Saitama	Oct 20, 2020	5	22	2	30

Identification of larvae was performed using Teshirogi (2016)

Fig. 2 Collection of oral odorant samples from the sixth instar (final) larvae of *Sasakia charonda*

same air suction pump. For the collection of oral odorant samples from *H. assimilis* larvae, a manual pump was used, because the larvae of *H. assimilis* in Honshu are prohibited from being moved from their place of origin, and we could therefore not transport them to the power supply point. The collection time for each larval group is shown in Table 1. The air just above the fresh leaf surface of host plants—*C. sinensis* for *S. charonda*, *H. assimilis*, and *H. persimilis*, and *Salix* sp. (Salicaceae) for *A. metis*—was used as the control.

Analysis of volatile substances from host plant leaves

For this analysis, we collected 1.7 g of leaf material from both *C. sinensis* and *S. babylonica* var. *babylonica*. Each collected leaf was preserved in a 50 mL polyethylene tube and crushed using a medicine spoon. Then, the volatiles from leaves were trapped and extracted into a sampling tube for 30 s, as above. Trapped volatile substances were analyzed in the same way as the larval odorants.

Analysis of volatiles

Gas chromatography–mass spectrometry (GC–MS) analysis of the samples was performed in the Analyzing Center, Showa Denko Materials Techno Service, Hitachi, Japan. The oral odorant compounds were subjected to GC–MS analysis on an Agilent system consisting of a model 7890B gas chromatograph, a model 5977A mass-selective detector (EIMS, electron energy of 70 eV), and an Agilent ChemStation data system (Santa Clara, CA, USA). The GC column was a DB-VRX column (Agilent, USA) with a film thickness of 1.40 µm, length of 60 m, and internal diameter of 0.25 mm. The carrier gas was helium (He), supplied at a flow rate of 2.1 mL/min. The collected volatiles were analyzed using the thermal desorption method. The GC oven temperature program was as follows: 3 min at 40 °C, increasing 5 °C/min up to 260 °C, then 8 min at 260 °C. Sample components were identified by comparing their mass spectral-fragmentation patterns against those stored in the MS library (NIST14 database) and with reagents. The mass acquisition range is m/z 20 to 500. When we re-used these sampling tubes, they were re-initialized using the TC2-Tube Conditioner (GERSTEL K.K., Tokyo) under the following conditions: heating temperature 300 °C, heating time 2 h, purging gas N_2, 50 mL/min. These were manualized by GERSTEL HP.

Reagents

We detected 22 substances in the oral odorants of *S. charonda* larvae. Among these, we were able to obtain and purchase 19 of those reagents. We also purchased reagents detected in samples from the three other species used in the same investigation. These are shown in Table 2 and were used to confirm the substances that we detected; they were also used in the behavioral testing of ants and larvae described below.

Calculation of the retention index of the detected substances

We could not find the retention index (RI) for DB-RVX, and therefore, we calculated the RI of the substances that we detected. First, we analyzed a mixture of pentane, heptane, and analytical standard, containing C_8–C_{20}, approximately 40 mg/L each, in hexane (MERCK), to determine the retention time of these standard substances. Next, mixed reagents were purchased and analyzed in the same manner as the oral odorant samples, and the RI of these reagents was calculated. The RI of other substances was estimated from their RI values before and after detection.

For this calculation, we used the equation shown below.

$$I_A = 100 \times n + 100 \times \left(\log t_A - \log t_n\right) / \left(\log t_{n+1} - \log t_n\right),$$

where n is the carbon number of n-H-$(CH_2)_n$-H, t_n is the retention time of n-alkane, and t_A is the retention time of the substance whose RI was calculated; each retention time is located between t_{n+1} and t_n.

Effect of oral odorants on predatory ants

This experiment was performed in the Sasakia charonda Research Center, Kashihara, in Nara, and the Museum of Sasakia charonda in Hokuto, Yamanashi. The effects of the 19 confirmed substances on *Pristomyrmex punctatus* (Smith, 1860) and *Formica japonica* Motschoulsky, 1866 (Hymenoptera: Formicidae) were investigated. Except in one case in Setagaya, Tokyo, Aug 1, 2020, where the experiment was carried out in the field, these ants' nests were in butterfly

Table 2 Volatile substances detected and identified in the oral odorants of four Japanese Apaturinae species

RI	Species Detected substances	*S. c.*	*H. a.*	*H. p.*	*A. m.*	Vendor	Purity (%)
511	2-methyl-3-buten-2-ol[b]	O					
591	2,3-butanedione[a]	O	O	O		A	> 98
596	2-butanol[a]	O	O	O	O	A	> 99
600	hexane[a]			O		A	
610	2-methyl-3-buten-2-ol[a]	O	O	O		B	> 97
681	1-penten-3-ol[a]	O				A	= 95
692	1-penten-3-on	O					
697	3-pentanol[a]	O				B	> 98
700	3-pentanone[a]	O				A	98 +
713	3-hydroxy-2-butanone[a]	O		O		B	> 98
725	3-methyl-1-penten-3-ol	O					
733	3-methyl-3-buten-1-ol[a]	O				B	> 98
737	2-methyl-1-butanol[a]	O				A	not shown
773	3-methyl-2-buten-1-ol		O				
775	2,3-butanediol[a]	O		O		B	> 97
782	3-methyl-2-buten-1-ol[a]		O			B	> 98
794	2,3-butanediol[a]	O					
804	3-methyl-2-butenal[a]	O	O	O		B	> 97
806	hexanal[a]			O		A	95 +
832	4,5-dimethyl-2-hepten-3-ol	O					
841	3-hexen-1-ol[b]	O				A	≥ 95
859	?			O			
889	?	O					
891	cyclohexanol[a]	O				A	= 98
984	2-methyl-2-pentenal[b]	O				B	> 97
993	benzaldehyde[a]				O	A	98 +
997	6-methyl-5-hepten-2-one[a]		O		O	A	96 +
1116	?	O					
1123	nonanal[a]			O	O	A	95 +
1294	fdulan-2?		O	O			
1312	2-undecane				O		
1335	dihydroedulan?	O					
1336	dihydroedulan?		O	O			
1342	dihydroedulan?		O	O			
1350	edulan-1?		O	O			
1543	?		O	O			
1559	*(E)*- Geranylacetone [a,c]		O				
1796	?			O			

S. c: *Sasakia charonda*, *H. a*: *Hestina assimilis*, *H. p*: *H. persimilis*, *A. m*: *Apatura metis* (same as Table 6)

Vendor A: Wako, Fujifilm; B: Tokyo Chemical Industry Co., Ltd

?: Unidentified substances

The remaining are unconfirmed estimates because we were unable to purchase their reagents

RI retention index, *O* detected in that species

[a]Confirmed by comparing to purified reagents

[b]Not confirmed, but basic structures accurately estimated by comparing to purified reagents

[c]Reagent is a *cis–trans* mixture

cages where the ants constantly interacted with the butterfly larvae.

First, we attempted the following experiment. One side of a silicone tube was connected to the outlet of an air pump, while the other side of the tube was connected to polyethylene pipettes. Small pieces of filter paper (5 mm × 20 mm) soaked in 0.2 mL of each volatile were inserted into each pipette, to serve as a source for each volatile substance. Following that, vapor containing the substance (or without it, serving as the control) was blown onto the heads of examined individuals (hereafter, we refer to this as "Method A"). However, in this method, ants were frequently blown away by the vapor stream. Thus, we soaked a cotton swab in 0.2 mL of each substance and placed the swabs individually near the ants. We then observed whether the ants showed avoidance behavior of the swabs soaked with the sample (hereafter referred to as "Method B"). The method of assessment is described in the footnote of Table 3.

Effect of oral odorants on predatory wasps

The instrument used in this experiment is shown in Fig. 3. The suction pump was continuously active throughout the experiment to prevent the volatile substances used from persisting in the glass tube containing the *Polistes* wasps. The method of assessment is described in the footnote of Table 4.

Effect of oral odorants on the larvae of the four Apaturinae species

The oral odorants identified were presented to the four Apaturinae larvae at the Sasakia charonda Research Center, Kashihara, Nara, the Museum of Sasakia charonda in Hokuto, Yamanashi, and the Park Parada Saku, Nagano. In some cases, both Methods A and B were applied to the same individuals. We observed the behavior of the larvae in response to these substances. Almost all behavioral responses of ants, wasps, and larvae were recorded using a video camera (Standard resolution NTSC video, 720 × 480 pixels, HDR-HC7, SONY, Japan).

Results

Identified oral odorants and their retention indices

The individual substances detected in the oral odorants of the larvae of the four species are summarized in Table 2. We were able to identify the following 13 volatiles from *S. charonda*: 2,3-butanedione, 2-butanol, 2-methyl-3-buten-2-ol, 1-penten-3-ol, 3-pentanol, 3-pentanone, 3-hydroxy-2-butanone, 3-methyl-3-buten-1-ol, (±)-2-methyl-1-butanol, 2,3-butandiol, 3-methyl-2-butenal, 3-hexen-1-ol, and cyclohexanol. None of these volatiles were detected in the control air samples. The compounds, 1-penten-3-ol, 1-penten-3-on? and 3-hexen-1-ol?, were detected as volatile substances from the pulverized leaves of *Celtis sinensis* and *Salix babylonica* var. *babylonica*, and were not considered to be the important components in larval oral odorants. In contrast, 3-hexen-1-ol and 2-hexenal, which are thought to be the main components from crushed leaves, could not be detected in larval oral odorants. There seemed to be no relationship between oral odorants and leaf volatiles.

We also analyzed the oral odorants of the other three Apaturinae butterfly species in Japan (Table 2). Among these, 3-methyl-2-buten-1-ol, hexanal, benzaldehyde, 6-methyl-5-hepten-2-one, nonanal, and (*E*)-geranylacetone were confirmed using reagents. The calculated RI of each substance is shown in Table 2.

Effect of oral odorants produced by *S. charonda* on predator ants, wasps, and larvae of four Apaturinae species

Results of ant responses were obtained from ten nests of *P. punctatus* and five nests of *F. japonica* that were tested, which showed contrasting responses to different oral odorants produced by *S. charonda*, as well as temporal and/or spatial variation in their responses to a particular compound (Table 3). These ants were strongly repelled by 2,3-butanedione, 2-butanol, 2-methyl-3-buten-2-ol, 1-penten-3-ol, 3-pentanol, 3-pentanone, and 3-hydroxy-2-butanonea.

We obtained response results from 24 *Polistes jokahamae* Radoszkowski, 1887, three *P. nipponensis* Saussure, 1858, and 12 *P. chinensis* during Apr and Sep 2022 (Table 4).

The responses of the larvae of four species of Apaturinae are summarized in Table 5 (*S. charonda*) and Table 6 (other three Apaturinae species). We could obtain results from 63 *S. charonda*, 11 *H. assimilis*, 19 *H. persimilis*, and 14 *A. metis* larvae between Jun 2020 and Nov 2022.

Positive responses of the larvae to the substances were based on a specific behavior; that is, the larvae raised their heads from the host leaf and opened their mandibles toward the pipette or swab. This behavior was only displayed by individuals that were resting on a scaffold formed by themselves during the larval stage (Figs. 4, 5). All individual ants, wasps, and larvae responded almost instantly if the substances had an effect, and did not respond to only air or a dry swab (Fig. 6).

Discussion

Although *S. charonda* is popular among butterfly breeders in Japan, it is likely that oral odorants emitted by *S. charonda* and the three other Apaturinae larvae had not been

Table 3 Responses of ants to chemical samples

Species	*Pristomyrmex punctatus*											*Formica japonica*				
Origin/date Substances	a	b	c	d	e	f	g	h	i	j	l	c	e	g	k	L
2,3-Butanedione	O		O		O	o			o			O	o		O	O
2-Butanol		O	O		O				o			o		o		o
hexane													o			
2-Methyl-3-buten-2-ol			o		O				o			o		o		o
1-Penten-3-ol		o	O			o						o	o	o	o	
3-Pentanol			O		o								O	O		o o
3-Pentanone		o	O		o							o	O	o		o
3-Hydroxy-2-butanone[a]			O	O		o	o		o	o]	o			o		O
3-Methyl-3-buten-1-ol			O			o						o	o	O		
2-Methyl-1-butanol			O		o							o		o		
2,3-Butandiol[b]			o		o									o	o	
3-Methyl-2-butenal		o	O		o			o						O	O	o
Cyclohexanol Prenol Hexanal Benzaldehyde 6-Methyl-5-hepten-2-on Nonanal Geranylaceton					o	o			o					o	o	o
3-Methyl-2-buten-1-ol				o										o	O	O
Hexanal						O								o		
Benzaldehyde						O									o	O
6-Methyl-5-hepten-2-one					o											
nonanal											o			o	o	o
Geranylacetone					o									o		

a: Setagaya, Tokyo, Aug 01, 2020. n > 5. These ants co-inhabited with *H. persimilis* larvae shown in Column f of Table 6

b: Hokuto, Yamanashi, Aug 05, 2020. n > 5

c: Kashihara, Nara, Jul 27, 2020. n > 5

d: Kashihara, Nara, Apr 12, 2022. n > 5

e: Kashihara, Nara; Jul 21, 2022. n > 4

f: Hokuto, Yamanashi, Jul 29, 2022. n > 3

g – h; j: Nara, Nara, May 22, 2023. n > 4

i: Nara, Nara, May 22, 2023. n > 10

k – l: Ôsio, Kanagawa, May 28, 2023. n > 4

Ants in a, i, k, l were nested on *C. sinensis* in the field, b–f were nested on *C. sinensis* in a greenhouse containing *S. charonda*, and g and j were nested in *Salix* spp. trees in the field

[a]: may exist as a crystalline dimer, [b]: a mixture of three isomers (same as Tables 4, 5 and 6)

Because individual identification of each ant was impossible, we showed each result as follows: O: almost all examined individuals responded (> 80%), o: only some individuals responded, blank cells: no individual responded. The cells highlighted in gray show that there was no result for that combination (same as Tables 5 and 6)

Whether the response was valid or not was determined as follows: when a cotton swab soaked with each substance was brought close to the head of an ant, the individual that had stopped was still, not valid; the individual that was walking continued walking as it was, not valid; the stationary individual began to run away from the swab, valid; the walking individual changed direction and began to run away from the cotton swab, valid (Fig. 2)

Identification of ants were performed using Terayama et al. (2014)

Fig. 3 Instrument used in the experiment investigating wasp behavior. **a** Tube connected to a funnel for air intake and odorant substances. **b** Glass or PET tubes to enclose wasps. c: Tube connected to the suction pump

previously identified because breeders rear the larvae in the absence of predators. Among butterflies, Papilionidae larvae have a defensive tongue-like organ known as the osmeterium, which secretes a mixture of strong odorant substances that repel ants (Honda 1983a) and wasps (Hirose 1981). However, the emission of such defensive substances has not been reported in other butterfly families before (Fig. 7).

We could not collect oral odorants from some individuals, because they did not consider us as predators and did not open their mandibles in response to our handling of them. It is well known (at least in Japan) that Papilionidae larvae that are handled often by their breeders become accustomed to them, and, eventually, they do not extend their osmeterium in response to handling. It appears that the larvae no longer consider the breeders a threat. Thus, we assumed that Apaturinae larvae also have this learning ability. We also noticed that non-responsive individuals were in the process of walking or eating, activities during which their aggression may be reduced.

The chemical composition of the oral odorants produced by *H. assimilis* and *H. persimilis* was similar to that of *S. charonda*, but that of *A. metis* was distinct from the other three species. Despite some similarities between *S. charonda* and the two *Hestina* spp., the latter mainly contained edulan-group substances instead of low-molecular-weight alcohols and ketones. In contrast, the chemical composition of *A. metis* only contained 2-butanol and benzaldehyde, although the former compound was also found in the oral odorant samples of the other three Apaturinae larvae. These differences may occur because the larvae utilize different host plants.

Another reason might be the phylogenetic relationships of these species. However, as a phylogenetic study (Ohshima et al. 2010) on Apaturinae species did not include *S. charonda*, we cannot draw a conclusion regarding the relationship between oral odorant composition and phylogeny. We could conclude that as the control samples taken from above the host plant leaves did not contain these chemicals, they must be synthesized in the larval body de novo. The same has already been confirmed for the components of the osmeterium in Papilionidae butterfly larvae (Honda 1983b).

Considering the reactions to each substance, most of the individuals used in the experiment (ants, wasps, and Apaturinae larvae) responded to 2,3-butanedione, 2-butanol, and 3-methyl-2-buten-1-ol. Of these, 2-butanol was detected in all four Japanese Apaturinae species, and 2,3-butanedione was detected in *S. charonda*, *H. assimilis*, and *H. persimilis*. It can be proposed that these two substances play a major role in repelling predatory ants and wasps in the larvae of these four Japanese Apaturinae. However, 3-methyl-3-buten-1-ol and 2-methyl-1-butanol could only be detected in *S. charonda*. These substances were not listed in Knudsen et al. (2006). In contrast, benzaldehyde and 6-methyl-5-hepten-2-one were detected from *Salix* flowers by Füssel et al. (2007) and Tollsten and Knudsen (1992), respectively (6-methyl-5-hepten-2-one was in trace amounts). Benzaldehyde, 6-methyl-5-hepten-2-one were also not listed in previous studies by either Füssel et al. (2007) or Leme et al. (2020). However, a considerable number of individuals used in the experiment responded to 2,3-butanedione, 2-butanol, and 3-methyl-2-buten-1-ol., which may have been included in unidentified RI peaks. The results of *H. persimilis* were different from those of other species, which is likely due to the small number of experimental individuals by stage, and the small number of total examinations, necessitating a follow-up test. However, Hayashi observed that larvae of *S. charonda* were successful in repelling an attack by a small hunting spider (Araneae). Thus, these oral odorants may also be effective against smaller generalist predators.

In this study, we did not use a large number of specimens. In the case of ants, all individuals used from each institute were the same nest members, and the genetic diversity may be poor. In the case of *S. charonda*, although individuals are morphologically similar, they have been bred for many generations in the same institute, and the genetic diversity of each institute is also poor. Thus, in ants and *S. charonda*, we did not try to increase the number of individuals held at any one institute but instead increased the number of sites where we performed our experiments. In the case of the other three Apaturinae species, they are not established as experimental insects. However, we could collect several larvae of these species in the field and perform our experiments. Moreover, we collected a number of wasps in the field and successfully completed our experiments.

Table 4 Responses of wasps to chemical samples

Species	*Polistes jokahamae*										*P. nipponensis*				*P. chinensis*			
Origin/Date Examined substances	a_1	b	c	d	e	f	g	h	i	j	a_2	k	l	m	n	o	p	q
2,3-butanedione	50	100	100	100	100	100	100	100	0	60	100	100	100	100	100	100	100	100
2-butanol	0	25	0	100	100	0	75	100	100	20	100	0	100	0	0	100	0	100
hexane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2-methyl-3-buten-2-ol	0	0	0	100	100	100	25	0	0	60	0	0	0	0	0	0	0	71
1-penten-3-ol	0	0	0	100	100	100	100	100	0	60	0	100	0	0	0	0	100	86
3-pentanol	0	0	0	100	100	50	50	100	0	0	0	0	0	0	100	0	100	43
3-pentanone	100	50	0	100	100	100	100	50	0	40	50	0	0	100	0	100	100	43
3-hydroxy-2-butanone[1)]	50	50	0	100	100	100	100	100	0	100	100	100	0	0	100	100	100	100
3-methyl-3-buten-1-ol	100	0	0	100	100	100	75	100	0	60	0	100	100	0	100	100	0	86
2-methyl-1-butanol	0	25	100	100	100	100	75	100	0	0	100	0	100	0	100	0	100	57
2,3-butandiol [2)]	0	0	0	0	100	50	25	100	0	0	0	100	0	0	0	100	0	57
3-methyl-2-butenal	50	50	100	0	100	100	100	100	0	100	100	100	100	100	100	100	100	86
cyclohexanol	0	25	0	0	100	50	100	100	100	40	100	100	100	0	100	0	0	57
3-methyl-2-buten-1-ol	0	50	0	0	100	100	100	100	0	80	100	0	100	0	0	100	0	71
hexanal	0	100	0	0	100	100	100	100	0	80	100	100	0	0	100	100	100	71
benzaldehyde	0	100	100	100	0	100	75	100	0	100	100	100	100	0	100	100	100	57
6-methyl-5-hepten-2-one	100	25	100	0	50	100	100	100	0	100	100	100	100	0	100	100	0	57
nonanal	0	0	0	0	0	100	100	100	0	0	0	0	0	0	0	0	0	71
geranylacetone	0	0	0	0	0	100	50	100	0	0	0	0	0	100	0	100	100	43

a_1: Kashihara, Nara, Apr 12–13, 2022. n = 2

b: Kashihara, Nara, May 4, 2022. n = 4

c: Ôiso, Kanagawa, Apr 23, 2022. n = 1

d: Minuma, Saitama, May 26, 2022. n = 1

e: Kashihara, Nara, Jul 21, 2022. n = 2

f: Tsurumi, Kanagawa; Jul 28, 2022. n = 2

g: Tsurumi, Kanagawa; Aug 17; 22; 29, 2022. n = 4

h: Kashihara, Nara; Aug 22, 2022. n = 2

i: Tsurumi, Kanagawa; Sep, 12, 2022. n = 1

j: Setagaya, Tokyo; Sep, 26, 2022. n = 5

a_2: Kashihara, Nara, Apr 12–13, 2022. n = 1

k: Saku, Nagano, May 29, 2020. n = 1

l: Hokuto, Yamanashi, Jun 5, 2022. n = 1

m: Tsurumi, Kanagawa, Oct 2, 2022, n = 1

n: Tsurumi, Kanagawa, Jun 26, 2022. n = 1

o: Tsurumi, Kanagawa, Jul 2, 2022. n = 1

p: Tsurumi, Kanagawa, Jul 10, 2022. n = 1

q: Kashihara, Nara; Jul 21–23, 2022; Aug 22, 2022. n = 8

The numerals in each cell indicate the percentage of individuals that responded (same as in Tables 5 and 6). Whether the response was valid or not was determined as follows. When each substance was released into a glass tube in which wasps were trapped, 1: the wasps bent their abdomen and waved their wings violently, valid; wasps did not display any special behavior, not valid

Identification of wasps was performed according to Iwata (1982)

A comparison between Method A and Method B for the same individual larva showed a more sensitive response to Method A than to Method B. This indicates that the wind generated by the wasp wing, not just the odor response, may be involved in the defensive behavior of the larvae.

A comparison of the results of *S. charonda* and *A. metis* revealed that the response of the larva becomes stronger as it ages. It has been pointed out that the final instar is the most vulnerable period for larvae from wasp attack, and it is understandable that the response becomes stronger

Table 5 Responses of *Sasakia charonda* larvae to chemical samples

Origin/Date	a	b		c	d		e	f	g	h	i		j	
Method Examined substances	A	A	B	A	A	B	A	B	B	B	A	B	A	B
2,3-butanedione	100	50	13	100	33	25	50	100	0	0	25	25	20	0
2-butanol	0	50	0	100	0	0	75	100	0	0	38	13	60	0
hexane	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2-methyl-3-buten-2-ol	0	0	0	100	50	0	75	0	0	0	75	13	40	0
1-penten-3-ol	0	38	0	100	33	0	50	0	0	0	88	0	40	20
3-pentanol	100	25	0	60	67	0	50	0	0	0	50	0	60	0
3-pentanone	0	13	0	60	50	0	25	100	60	0	75	13	40	0
3-hydroxy-2-butanone[1)]	0	13	0	60	33	0	0	0	0	0	13	0	0	0
3-methyl-3-buten-1-ol	100	13	0	100	0	0	50	0	20	0	50	0	80	0
2-methyl-1-butanol	100	50	0	80	17	0	25	100	20	14	75	0	0	0
2,3-butandiol [2)]	0	0	0	100	17	0	0	0	0	14	13	0	0	0
3-methyl-2-butenal	100	25	0	100	0	0	50	100	20	14	25	0	0	0
cyclohexanol		25	0	0	17	0	75		0	29	13	0	40	0
3-methyl-2-buten-1-ol		25	0	0	0	0	25		0	14	0	13	0	0
hexanal		0	0	0	0	0	50		0	43	0	25	0	0
benzaldehyde		25	0	100	50	0	50		0	0	63	13	0	0
6-methyl-5-hepten-2-one		25	13	100	0	0	75		0	0	75	0	0	0
nonanal		0	0	100	0	0	25		40	14	13	13	40	0
geranylacetone		0	0	0	0	0	50		0	0	0	0	0	0

a: Hokuto, Yamanashi, Jun 1124, 2020, last (6th) instar. $n = 5$

b: Hokuto, Yamanashi, May 28, 2022, 5th instar. $n = 8$

c: Hokuto, Yamanashi, May 28, 2022, last instar. $n = 5$

d: Saku, Nagano, May 29, 2022, 5th instar. $n = 6$

e: Saku, Nagano, Jun 10, 2022, last instar. $n = 4$. In this experiment, rechargeable air pump 1500 YH-760 (Hapyson) was used

f: Kashihara, Nara, Jul 26, 2020, third instar. $n = 10$

g: Kashihara, Nara, Apr 12, 2022, 3rd instar. $n = 5$

h: Kashihara, Nara, May 4, 2022, 4th instar. $n = 7$

i: Kashihara, Nara, May 26, 2022, 5th stage larva. $n = 8$

j: Kashihara, Nara, Nov 15, 2022, 3rd instar. $n = 5$. In this experiment, rechargeable air pump 1500 YH-760 (Hapyson) was used

at this time. Although the larvae of Papilionidae project the osmeterium even during the first instar stage, Apaturinae larvae appear to differ from Papilionidae larvae by having a reduced capacity for oral odorant production in their early instars. Considering the individual responses of the larvae, individuals that responded strongly to one substance tended to respond to many other substances too. As the genetic differences between the individuals of each institution used in the experiment do not seem to be large, these individual differences were considered to be linked to differences in their physiological condition. The results from Ōiso, Kanagawa, Aug 15, 2022, suggest that the sensitivity was considerably lower at the prepupal stage. The weak response of individuals after October may be the result of the drop in temperature, and it is also possible that various individuals began to prepare for overwintering and would have altered metabolic tempos as a result.

The defensive substances in the osmeterial emissions of Papilionidae species have been studied (Ômura et al. 2006), and their effects on two species of ants in Japan have been analyzed in detail (Honda 1983a). According to this previous study, these substances mainly consist of monoterpenoids, sesquiterpenoids, aliphatic acids, and their esters. Thus, the oral odorant components of the four Japanese Apaturinae larvae are very different from those of Papilionidae. As shown in Table 2, the defensive odors of the larvae of *S. charonda* and the three related species are mainly derived from C_4–C_5 alcohols. The synthesis pathways are unknown, and the mechanisms involved merit further investigation. Further, Kandori et al. (2022) reported that the long horn-like

Table 6 Responses of other three Apaturinae larvae to chemical samples

Species	*H. assimilis*							*H. persimilis*								*A. metis*				
Origin/date	a	b	c	d		e		f	g	h		i		j		k	l	m		n
Method Examined substances	B	B	B	A	B	A	B	A	A	A	B	A	B	A	B	A	B	A	B	A
2,3-Butanedione	67	100	100	0	0	80	0	0	20	22	0	0	0	100	100	100	25	20	20	67
2-Butanol	0	0	0	0	0	80	0	0	0	78	11	100	0	100	0	0	0	20	20	67
hexane	67	0	0	0	0	0	0	0	0	0	0	33	0	0	0	0	0	20	0	0
2-Methyl-3-buten-2-ol	0	0	0	0	0	0	0	0	0	33	0	67	0	100	0	0	0	20	0	0
1-Penten-3-ol	67	0	0	0	0	20	0	0	20	44	0	33	0	0	0	50	0	20	0	33
3-Pentanol	100	0	0	0	0	100	0	0	20	22	0	67	0	0	100	0	0	40	0	0
3-Pentanone	67	100	100	0	0	60	0	0	0	22	0	0	0	100	0	50	0	40	0	33
3-Hydroxy-2-butanone[1)]	0	0	0	0	0	0	0	100	0	67	0	67	0	0	0	50	0	0	0	67
3-Methyl-3-buten-1-ol	67	0	0	100	0	80	0	0	40	56	0	67	0	100	0	0	0	0	0	33
2-Methyl-1-butanol	100	0	0	0	0	0	0	0	40	33	0	100	0	0	0	50	0	0	0	0
2,3-Butandiol[2)]	0	0	100	0	0	0	0	0	0	0	0	33	0	0	0	0	0	0	0	0
3-Methyl-2-butenal	67	0	100	0	0	80	0	0	0	56	0	33	0	100	0	50	0	0	0	67
Cyclohexanol		0	0	0		60	20		0	0	0	33	0	0	0		0	0	0	0
3-Methyl-2-buten-1-ol		0	0	0		0	0		0	0	0	0	0	0	0		0	0	0	67
Hexanal		100	0	0		100	0		60	11	0	0	0	0	0		0	0	0	67
Benzaldehyde		0	0	0		0	0		0	0	0	0	0	0	0		25	20	0	33
6-Methyl-5-hepten-2-one		0	0	0		20	0		0	0	11	0	0	0	0		25	0	0	33
Nonanal		100	100	0		0	0		0	0	0	0	0	0	0		25	0	0	0
Geranylacetone		0	0	0		0	0		0	0	0	0	0	0	0		0	0	0	0

a: Tsurumi, Kanagawa, Jul 23, 2020, last instar. $n=3$

b: Minuma, Saitama, Apr 22, 2022, 5th (Final) instar. $n=1$

c: Ôiso, Kanagawa, Apr 22, 2022, 5th (Final) instar. $n=1$

d: Ôiso, Kanagawa, Aug 15, 2022, Prepupae. $n=1$. In this experiment method A, a rechargeable air pump 1500 YH-760 (Hapyson) was used. In this experiment, prepupae moult into pupae, thus, we could not get complete results from this individual

e: Tsurumi, Kanagawa, Aug 17–27, 2022, last instar. $n=5$. In this experiment method A, a rechargeable air pump 1500 YH-760 (Hapyson) was used

f: Setagaya, Tokyo, Aug 1, 2020, 5th (Final) instar. $n=1$

g: Kashihara, Nara, Apr 12, 2022, 3rd instar. $n=5$

h: Nara, Nara, Oct 20, 2022, 2nd–3rd instar, $n=9$. In this experiment, a rechargeable air pump 1500 YH-760 (Hapyson) was used

i: Ôtsu, Shiga, Oct 25, 2022, 3rd instar, $n=3$. In this experiment, a rechargeable air pump 1500 YH-760 (Hapyson) was used

j: Kashihara, Nara, Nov 15, 2022, 3rd instar. $n=1$

k: Minuma, Saitama, Oct 20, 2020 Last instar, $n=2$

l: Kashihara, Nara, Apr 12, 2022, 3rd–4th instar. $n=4$

m: Saku, Nagano, May 29, 2022, 2nd instar. $n=5$

n: Saku, Nagano, Jun 10, 2022, two 3rd instar and one final instar. In this experiment, a rechargeable air pump 1500 YH-760 (Hapyson) was used

projections on the heads of *Hestina japonica* (Felder, 1862) butterfly larvae protect them from their natural enemies, but they did not make any mention of oral odorants produced by these larvae. As above, Hayashi and Holikawa were able to perceive the smell, we wonder as to why Kandori et al. (2022) could not notice these odorants.

The odor-detecting systems of some ants have been researched morphologically and electrophysiologically (Hashimoto 1992; Ozaki et al. 2005; Ruchty et al. 2009; Sharma et al. 2015), but this research was limited to the substances that are involved in cuticular wax. The olfactory system of ants should still be researched to assess their responses to additional substances. Our own results for ants responses were inconsistent. This might be related to the fact that ants will assess individuals as probable nest mates using very minute features of their cuticular hydrocarbon pheromones (Ruchty et al. 2009). However, among the chemicals in larval oral odorants, 2-butanol induced a

Fig. 4 Responsive behavior of ants (*Pristomyrmex punctatus*) to the volatiles. Setagaya, Tokyo, Aug 01, 2020

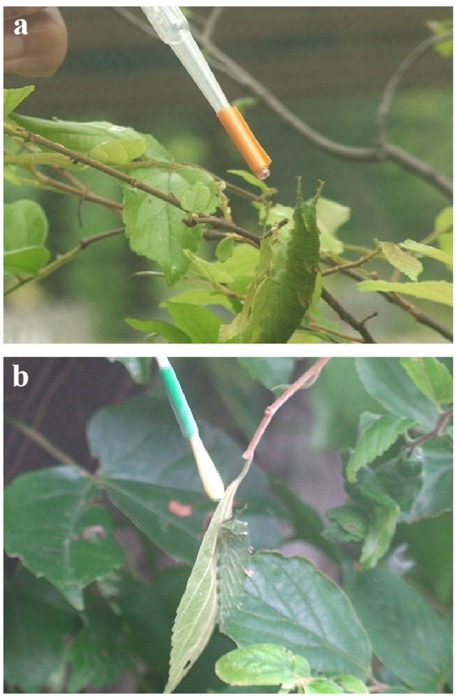

Fig. 5 Non-responsive behavior to the substances. **A** For *Sasakia charonda* larvae. **B** For larvae of *Hestina assimilis* and the other two species

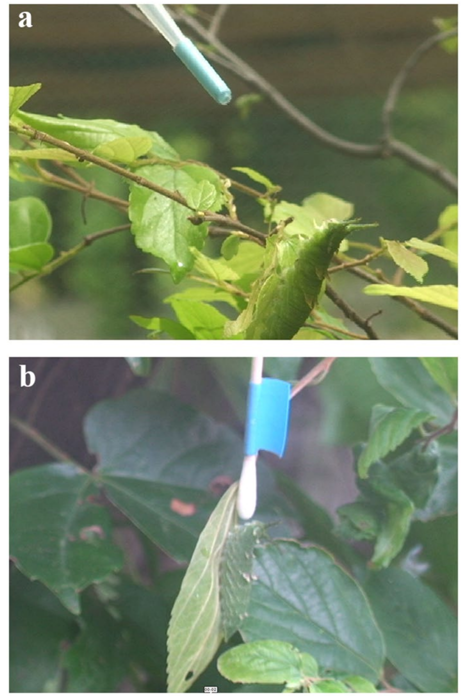

Fig. 6 Hostile response of *Sasakia charonda* larvae to the examined volatiles

Fig. 7 .

repellent behavior in almost all of the ants examined. As 2-butanol was the only substance detected in all four Japanese Apaturinae larval oral odorants, this chemical must be the key repellent. Notably, these substances may also affect Apaturinae larvae by serving as alarm cues. Research on the olfactory responses of Lepidoptera larvae has only been performed with substances emitted from their host plants (e.g., Dethier 1973; Van Loon 1990; Malmgren 2011; Rharrabe et al. 2014; Asaoka and Shibuya 1995). Our results indicate that the larvae are also sensitive to substances that they themselves produce and emit. Thus, our results may enrich the study of Lepidoptera olfaction. Many wasps responded to 3-methyl-2-buten-1-ol, benzualdehyde, 6-methyl-5-hepten-2-one, Nonanal, and (*E*)-geranylacetone, but considering that these substances are found in the volatiles produced by flowers, these responses seem to have a distinct significance toward 2,3-butanedione, etc. In fact, all five *Polistes jokahamae* and one *P. nipponensis* used on Sep 26, 2022, and Oct 2, 2022, respectively, which were collected on *Cayratia japonica* (Vitaceae: Vitales) flowers, showed notable responses to the substances found from flower scents

(Knudsen et al. 2006). Sasagawa et al. (1989) showed that JH is implicated in the physiological regulation responsible for the age-linked division of labor or polyethism in *Apis mellifera* Linneaus, 1758 (Apidae: Hymenoptera); such a phenomenon may also exist in wasps. Although some of the tested individuals were observed to respond to hexane, we also considered that 2,3-butanedione and 2-butanol influenced them, as those trials were performed just prior to hexane exposure. In this experiment, we examined the reaction to each substance in the order of elution of the substance by GC. Actually, in the experiment performed on Nov 15, 2022 (Column j of Table 5 and Column j of Table 6), we first examined the reaction to hexane. Further experiments with electro-antennograms, as well as with ants, would be necessary to validate this.

The larvae of Apaturinae, Charaxinae, Boblinae, and Satyrinae species have a pair of horns on their heads, which they use defensively when attacked by a predator (Teshirogi 2016). Nymphalinae, Heliconinae, and Limenitinae larvae have many spines on their body, and these can be used for physical defense against their predators. Moreover, *Heliconius* larvae have a chemical defense system that is also found in Danainae and Ithomiinae larvae. In contrast, an effective chemical defense system is not known in Charaxinae, Boblinae, and Satyrinae larvae. In the present study, we showed that the four Japanese Apaturinae larvae have a chemical defense system, raising the question of whether Apaturinae, Charaxinae, Boblinae, and Satyrinae larvae found outside Japan, which are morphologically similar to Japanese Apaturinae larvae, also bear such a chemical defense system. This could be a direction for future research.

In our study, we could not obtain sufficient numbers of *H. assimilis*, *H. persimilis*, or *A. metis* larvae, because a method of mass-breeding of these three species, such as that used for *S. charonda*, has not yet been established successfully. We hope that other researchers will tackle this vital issue. In conclusion, we showed that *S. charonda* and three other Apaturinae larvae use oral odorants to repel their predatory ants and wasps.

Acknowledgements We thank Mr. Shôji Akiyama (died 2016) and Ms. Nobuko Hibi (died 2018), who began breeding *S. charonda* in Kashihara City, Nara, in 1980 and provided instructions to Taro Hayashi on how to breed it. We sincerely thank Dr. Fumiaki Kimura, general manager of Kashihara City Museum of Insects, and his staff who assisted in rearing *S. charonda* for this study. Mr. Yoshinori Kunimoto, Mr. Katsumi Inoue, and Mr. Kenichi Murakami also substantially assisted in our research. The Museum of Sasakia charonda in Hokuto, Yamanashi and Park Parada, Saku, Nagano allowed us to perform research in their institutions. Mr. Naoto Hirosawa, Mr. Takeshi Takezawa, and Mr. Masahiro Andoh, of Showa Denko Materials Techno Service Co., Ltd., performed GC–MS analysis and responded to our questions. Prof. Fumio Yokohari from Fukuoka University provided us with information about current research articles on oral odorant reception by ants. Dr. Mamoru Terayama kindly identified the ants examined in this study. Mr. Akira Takasaki and Mr. Mikio Sasaki provided us with information regarding the habitats of *A. metis* and *Salix* plants in our neighborhood. Dr. Kei-ichi Honda advised us on information in the literature. We also wish to express our gratitude to Mr. Katsuhisa Toyama, Ms. Haruko Inoue, Mr. Takao Kanasugi, and Mr. Takayoshi Matsumura who also provided general information in the field. We would like to thank the two anonymous reviewers and editor for their constructive inputs that helped improve the manuscript. Finally, we thank Editage staff who provided suggestions to improve the English and content of this article. This work was partially supported by JSPS KAKENHI (18K06393 to FT).

Funding Funding was provided by JSPS KAKENHI (Grant no. 18K06393 to FT).

Declarations

Conflict of interest Not applicable.

References

Asaoka K, Shibuya T (1995) Morphological and electrophysiological characteristics of the epipharyngeal sensilla of the silkworm, *Bombyx mori*. Entomol Exp Appl 77:167–176. https://doi.org/10.1111/j.1570-7458.1995.tb01997.x

Dethier VG (1973) Electrophysiological studies of gustation in lepidopterous larvae II. Taste spectra in relation to food-plant discrimination. J Comp Physiol 82:103–134. https://doi.org/10.1007/BF00696148

Füssel U, Dötterl S, Jürgens A, Aas G (2007) Inter- and intraspecific variation in floral scent in the genus *Salix* and its implication for pollination. J Chem Ecol 33:749–765. https://doi.org/10.1007/s10886-007-9257-6

Hashimoto Y (1992) Unique sensillum structure of the formicid labial palpi (Hymenoptera). Hum Nat 1:57–62

Hirose Y (1981) Ecology of the citrus swallowtail, *Papilio xuthus* with special reference to its population dynamics. Yadoriga 105(106):1–10 (**in Japanese**)

Honda K (1983a) Defensive potential of components of the larval osmeterial secretion of papilionid butterflies against ants. Physiol Entomol 8:173–179. https://doi.org/10.1111/j.1365-3032.1983.tb00346.x

Honda K (1983b) Evidence for *de novo* biosynthesis of osmeterial secretions in young larvae of the swallowtail butterflies (*Papilio*): deuterium incorporation in vivo into sesquiterpene hydrocarbons as revealed by mass spectrometry. Int J Trop Insect Sci 4:255–261. https://doi.org/10.1017/S1742758400001247

Inoue TA (2020) Butterflies who I could meet in Japan in 2020–2. JESUTIO 244:15 (**in Japanese**)

Iwata K (1982) Encyclopedia of Japanese wasps and their ecology. Kodansha, Tokyo

Kandori I, Hiramatsu M, Soda M, Nakashima S, Funami S, Yokoi TK, Tsuchihara K, Papaj DR (2022) Long horns protect *Hestina japonica* butterfly larvae from their natural enemies. Sci Rep 12:2835. https://doi.org/10.1038/s41598-022-06770-y

Knudsen JT, Eriksson R, Gershenzon J, Ståhl B (2006) Diversity and distribution of flower scent. Bot Rev 72:1–120. https://doi.org/10.1663/0006-8101(2006)72[1:DADOFS]2.0.CO;2

Leme FM, Schönenberger J, Staedler YM, Teixeira SP (2020) Comparative floral development reveals novel aspects of structure and diversity of flowers in Cannabaceae. Bot J Linn Soc 193:64–83. https://doi.org/10.1093/botlinnean/boaa004

Malmgren L (2011) Response to olfactory stimuli in gregarious *Pieris brassicae* caterpillars. http://stud.epsilon.slu.se

Ohshima I, Tanikawa-Dodo Y, Saigusa T, Nishiyama T, Kitani M, Hasebe M, Mohri H (2010) Phylogeny, biogeography, and host–plant association in the subfamily Apaturinae (Insecta: Lepidoptera: Nymphalidae) inferred from eight nuclear and seven mitochondrial genes. Mol Phylogenet Evol 57:1026–1036. https://doi.org/10.1016/j.ympev.2010.09.018

Ozaki M, Wada-Katsumata A, Fujikawa K, Iwasaki M, Yokohari F, Satoji Y, Nisimura T, Yamaoka R (2005) Ant nestmate and non-nestmate discrimination by a chemosensory sensillum. Science 309:311–314. https://doi.org/10.1126/science.1105244

Ômura H, Honda K, Feeny P (2006) From terpenoids to aliphatic acids: further evidence for late-instar switch in osmeterial defense as a characteristic trait of Swallowtail butterflies in the Tribe Papilionini. J Chem Ecol 32:1999–2012. https://doi.org/10.1007/s10886-006-9124-x

Rharrabe K, Jacquin-Joly E, Marion-Poll F (2014) Electrophysiological and behavioral responses of *Spodoptera littoralis* caterpillars to attractive and repellent plant volatiles. Front Ecol Evol 2:5. https://doi.org/10.3389/fevo.2014.00005

Ruchty M, Romani R, Kuebler LS, Ruschioni S, Roces F, Isidoro N, Kleineidam CJ (2009) The thermo-sensitive sensilla coeloconica of leaf-cutting ants (*Atta vollenweideri*). Arthropod Struct Dev 38:195–205. https://doi.org/10.1016/j.asd.2008.11.001

Sasagawa H, Sasaki M, Okada I (1989) Hormonal control of the division of labor in adult honeybees (*Apis mellifera* L.): I. Effect of methoprene on corpora allata and hypopharyngeal gland, and its α-Glucosidase activity. Appl Entomol Zool 24:66–77. https://doi.org/10.1303/aez.24.66

Sharma KR, Enzmann BL, Schmidt Y, Moore D, Jones GR, Parker J, Berger SL, Reinberg D, Zwiebel LJ, Brei B, Liebig J, Ray A (2015) Cuticular hydrocarbon pheromones for social behavior and their coding in the ant antenna. Cell Rep 12:1261–1271. https://doi.org/10.1016/j.celrep.2015.07.031

Terayama M, Kubota S, Eguchi K (2014) Encyclopedia of Japanese ants. Asakura. Tokyo

Teshirogi M (2016) Nymphalid butterflies of the world. Hokkaido University Press **(in Japanese)**

Tollsten L, Knudsen JT (1992) Floral scent in dioecious *Salix* (Salicaceae)—a cue determining the pollination system? Plant Syst Evol 182:229–237. https://doi.org/10.1007/BF00939189

van Loon JJA (1990) Chemoreception of phenolic acids and flavonoids in larvae of two species of *Pieris*. J Comp Physiol A 166:889–899