



Your chemical coat tells me you are my delicacy: a predatory stink bug uses cuticular hydrocarbons to identify prey

Huai-Jun Xue¹ · Jing Wei^{1,2} · Zheng-Zhong Huang^{1,2} · Wen-Zhu Li¹ · Xing-Ke Yang¹

Received: 22 August 2017 / Accepted: 19 March 2018 / Published online: 22 March 2018
© Springer International Publishing AG, part of Springer Nature 2018

Abstract

Extensive studies have shown that cuticular hydrocarbons (CHCs) are among the major cues that allow many insects to identify interspecific and intraspecific variation between individuals. CHCs often have mutually nonexclusive functions that can provide multiple types of signals, while their role in predator–prey interactions has received little attention. Here, we used a predatory stink bug, *Zicrona caerulea* (Hemiptera: Pentatomidae), and one of its favorite flea beetle prey, *Altica viridicyanea* (Coleoptera: Chrysomelidae), to test the hypothesis that CHCs of prey are important chemical cues for a predator. Two-choice bioassays using dead beetles and glass dummies clearly indicated that the CHC profile of *A. viridicyanea* is the pivotal cue in prey identification for *Z. caerulea*. The results also suggested that the role of acoustic and visual cues can be ignored in prey recognition at close range.

Keywords Cuticular hydrocarbon · Chemical cue · Predation · Prey preference · *Altica* · *Zicrona*

Introduction

Cuticular hydrocarbons (CHCs) of insects often are comprised of straight chain, unsaturated and methyl-branched complex mixtures with the chain lengths ranging from 21 to 50 and, in some rare cases, even 70 carbon atoms (Blomquist and Bagnères 2010; Ginzl and Blomquist 2016). In addition to acting as a desiccation barrier, the nonvolatile CHCs may have mutually nonexclusive functions and can provide multiple chemical communication signals in many insects (Ginzl and Blomquist 2016). While CHCs are involved in several important functions in insects such as species recognition (Peterson et al. 2007; Zhang et al. 2014), sex and breeding status discrimination (Dietemann et al. 2003; Monnin 2006; Scott et al. 2008), nestmate and kin recognition (Lahav et al. 1999; Thomas et al. 1999; Wagner et al. 2000; Lorenzi et al.

2004; Lihoreau and Rivault 2009; Rahman et al. 2016) and chemical mimicry (Akino et al. 1999; Endo and Itino 2013) their role in predator–prey interactions has received little attention outside of several hymenoptera species (Uma and Weiss 2010; Koedam et al. 2011; Endo and Itino 2013; Rutledge et al. 2014; Wang et al. 2014; Ranganathan et al. 2015; Binz et al. 2016; Ginzl and Blomquist 2016).

Our previous studies showed that CHCs were used by *Altica* Geoffroy (Insecta: Coleoptera: Chrysomelidae) species in inter- and intra-specific individual discrimination. In mating choice tests, the male beetles not only can recognize conspecific females from those of closely related species (Xue et al. 2016a), but also can distinguish males from females and distinguish sexually mature females from immature ones, partly using CHCs (Xue et al. 2016b).

Zicrona caerulea (Linnaeus) is a cosmopolitan species (Rider and Zheng 2002) with the common name “blue shieldbug” belonging to the Pentatomidae family (Hemiptera). The bug favours leaf beetles as prey, and *Altica* is its most common prey (Phillips 1977; Wang et al. 2005; Bantock and Botting 2013). Because of a deep blue-green metallic luster similar to that of *Altica* adults, the bug was suggested as a case of aggressive mimicry (Bantock and Botting 2013; also see Fig. 1). In addition to other areas, *Z. caerulea* was often found preying on larvae and adults of *Altica* species in Beijing, China, including *Altica cirsiicola*

Communicated by Thomas Schmitt.

✉ Huai-Jun Xue
xue@ioz.ac.cn

¹ Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China

² University of Chinese Academy of Sciences, Beijing 100049, China



Fig. 1 The blue shieldbug *Zicrona caerulea* preys on a flea beetle *Altica viridicyanea*

Ohno, *A. fragariae* Nakane and *A. viridicyanea* (Baly) (H.J. Xue, personal observation). Here, we tried to use the “*Zicrona-Altica*” system to test the hypothesis that the stink bug predator uses CHCs to identify prey.

Materials and methods

Insects

Adults of *Z. caerulea* (length 5.0–7.0 mm) and *A. viridicyanea* (length 3.0–4.0 mm) were collected from field populations in Liuchun (40.11°N, 116.01°E), Changping, Beijing and then maintained in growth chambers held at 16:8 LD and 25 °C. *A. viridicyanea* was fed their normal host plants *Geranium nepalense* (Sweet) and *Z. caerulea* was fed with larvae and adults of *A. viridicyanea*.

Prey bioassays

In a previous study, six (semi-) volatile and 19 nonvolatile CHC components (relative percentage > 0.5%) were identified from hexane extracts of *A. viridicyanea*. After solvent evaporation, most of the remaining chemicals in hexane extracts are nonvolatile CHC components (Xue et al. 2016b). In the present study, hexane was used as a solvent to strip the CHCs of beetles and to coat the dummies with CHCs.

In the prey preference bioassays, we constructed arenas using Petri dishes (9.0 × 1.2 cm) lined with moistened filter paper. Three bioassays were carried out in a temperature-controlled room held at 25–27 °C under natural light conditions.

In bioassay I, one killed beetle with intact CHCs and one killed beetle stripped of its CHCs were presented to a stink bug. Randomly selected individuals of *A. viridicyanea* were

killed by freezing at –30 °C for 20 min. To strip the CHCs, each beetle was dipped in 0.8 mL hexane (Ourchem, Sinopharm Chemical Regent Co., Ltd, Shanghai, China) for 15 min six times. Previous GC-FID analysis showed that nearly all of the CHCs were removed by this wash step (Xue et al. 2016b). The dead specimens were glued (Koni-shi glue, Japan) to a small piece of triangular filter paper (length = 1.0 cm), then to the wall of a Petri dish (the two specimens were about 2.5 cm apart) (c.f. Xue et al. 2016a, b). The solvent was evaporated in a chemical fume hood before tests.

In bioassay II, we utilized a similar setup to bioassay I, but one beetle with CHCs stripped and one beetle with CHCs stripped and then reapplied were presented to a stink bug. For the second group, CHCs were extracted by soaking each beetle in 40 μL hexane for about 15 min. Then CHC-free specimens were dipped in the above extracts to reapply CHCs, and the solvent was evaporated in a chemical fume hood.

In bioassay III, black glass dummies (diameter = 3 mm) were used instead of beetles. To obtain beetle cuticular extracts, each beetle was dipped in 40 μL hexane for 15 min. One glass dummy was submerged in 80 μL cuticular extracts (each dummy was treated with the hexane extracts of two beetles because its surface area is much larger than that of a beetle) and the other dummy just treated with hexane, then the solvent was evaporated in a chemical fume hood. These two dummies were glued on the bottom of Petri dishes about 2.5 cm apart from each other.

In all bioassays, stink bugs were starved for 24 h to increase their predatory desire before feeding tests. Forty-two replicates were conducted for each bioassay. One-hour bioassays were carried out because trial tests showed that predation percentage exceed 50% during that period. The number of successful predations and the prey preferences were recorded during the tests. In bioassays I and II, a predation was considered successful when the bug inserted its rostrum into the beetle for longer than 1 min; in bioassay III, a successful predation was recorded when the bug exhibited proboscis-protruding behavior (Yasuda 1997), i.e., tried to insert its rostrum into the glass dummy.

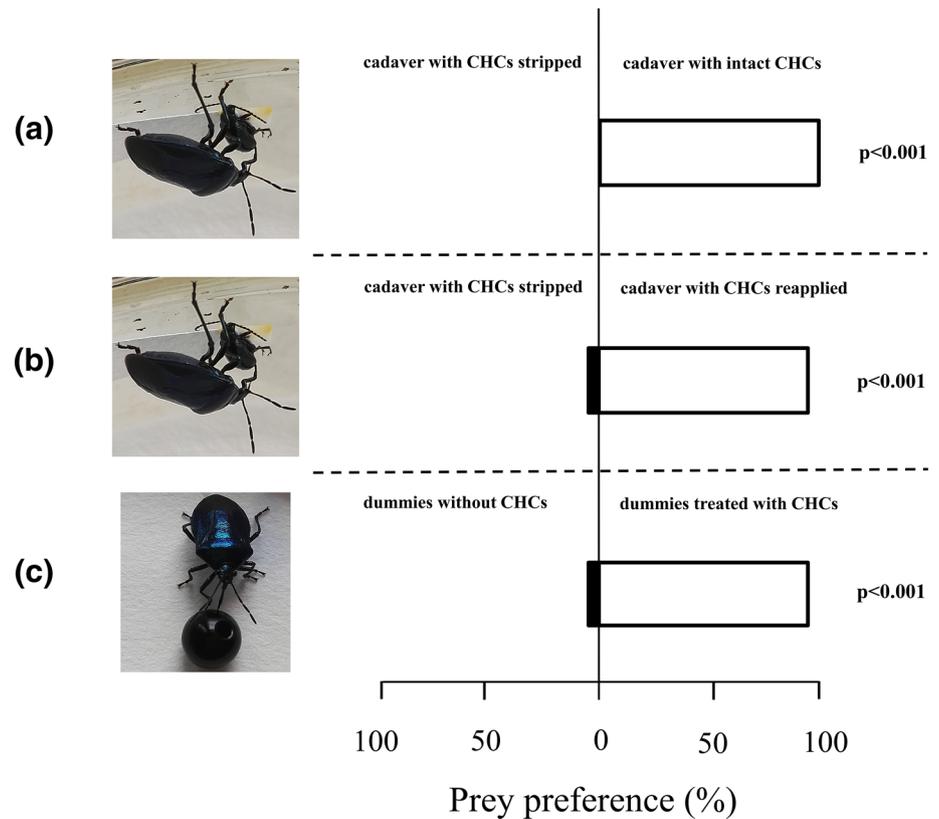
Data analysis

Prey preference was analyzed using Chi-square (χ^2) tests in SPSS 18.0 (IBM, Armonk, NY, USA).

Results and discussion

The results of bioassay I showed that all *Z. caerulea* individuals preferred *A. viridicyanea* with intact CHCs to those with CHCs stripped (Fig. 2a). In bioassay II, only one *Z.*

Fig. 2 *Zicrona caerulea* prey preference in two-choice tests. **a** Dead *Altica viridicyanea* with cuticular hydrocarbons (CHCs) stripped versus cadaver with intact CHCs; **b** dead *A. viridicyanea* with CHCs stripped versus cadaver with CHCs stripped and reapplied; **c** dummies without CHCs versus dummies treated with CHCs of *A. viridicyanea*. Forty-two replicates were conducted for each bioassay, 26, 26 and 24 predations were observed in bioassay I, II and III, respectively, during the tests



caerulea preyed on a beetle without CHCs in a total of 26 successful predation events ($\chi^2 = 22.154$, $p < 0.001$; Fig. 2b). Similarly, in bioassay III, only one *Z. caerulea* selected a dummy without CHCs in a total of 24 successful predation events ($\chi^2 = 20.167$, $p < 0.001$; Fig. 2c).

The results of bioassays I and II suggested that beetle CHCs are important cues for the stink bug predator. In bioassay I, one beetle was treated with hexane, so the effect of the solvent may affect predatory behavior. In bioassay II, both beetles were treated with hexane, so the effect of the solvent could be ruled out. Although gas chromatography analysis showed that nearly all of the CHCs were removed during the hexane wash step (Xue et al. 2016b), some queries may be raised because other chemicals (such as esters, free fatty acids, alcohols, ketones and sterols) on the beetle surface may bias the predatory behavior of *Z. caerulea*. In bioassay III, the only difference between the two dummies was whether they were coated with beetle CHCs or not (almost all of the chemicals left after solvent evaporation were non-volatile CHCs). Therefore, the results further verified that CHCs play a pivotal role in prey recognition for *Z. caerulea*.

Olfactory, visual and acoustic cues from prey are often used by insect predators to assess food information (Wang et al. 2014). Several wasps were reported as efficiently using olfactory cues to locate prey, for example, the digger wasp, *Liris niger* (Anton and Gnatzy 1998); European

beewolf, *Philanthus triangulum* (Herzner et al. 2005); the social paper wasp, *Mischocyttarus flavitarsis* (McPheron and Mills 2007); the spider-hunting wasp, *Sceliphron caementarium* (Uma and Weiss 2010); digger wasp *Trachypus boharti* (Koedam et al. 2011); a ground-nesting wasp *Cerceris fumipennis* (Rutledge et al. 2014) and the hornet *Vespa velutina* (Wang et al. 2014). In most cases, a combination of olfactory and visual cues facilitates predators in detecting, locating and identifying their prey. The different types of cues may play different roles in different stages of predation. In the paper wasp *Mischocyttarus flavitarsis*, olfactory cues play a significant role in both short-range and long-range location of prey, while visual cues are negligible in foraging behavior (McPheron and Mills 2007). Some other insect predators, for example, the stinkbug *Podisus maculiventris* use vibrations produced by prey as cues for prey location (Pfannenstiel et al. 1995). In the present study, the hypothesis that prey CHCs act as important chemical cues for a stink bug predator was supported by direct behavioral evidence. Our study using dead beetles and dummies rejected the likelihood of acoustic signals in prey identification. Likewise, the results of bioassay III using dummies instead of beetles also indicated visual signals do not play a decisive role in predation behaviour. The bioassays were carried out in a limited space in the present study (Petri dishes with diameter = 9 cm), no doubt nonvolatile CHCs play a pivotal role

at a close range while the visual function at a distance still cannot be rejected.

Previous studies showed that the CHC profiles of *Altica* are species-specific (Xue et al. 2016a, b), furthermore, although *Z. caerulea* prefers *Altica* species to other insects, it is not a strict specialist predator. For example, it also preys upon *Leptinotarsa decemlineata* (Chrysomelidae) and caterpillars (Lepidoptera) occasionally (Shu et al. 2012). This suggests that the chemical cues eliciting predatory behavior may be common chemicals existing in various insect prey (Yasuda and Wakamura 1996). Alternatively, the bug may respond to multiple chemical cues released by prey during prey-search behavior (Binz et al. 2016).

The chemical compounds triggering predatory behavior have been seldom identified. A long-chain alcohol, (Z)-11-eicosen-1-ol, was suggested as an essential component of the prey recognition cue for the European beewolf, *Philanthus triangulum* (Herzner et al. 2005). Behavioral bioassays showed that the stinkbug *Eocanthecona furcellata* mainly uses two volatile chemicals from extracts of *Spodoptera litura* to locate its prey: *n*-pentadecane attracts the bugs and Ephytol stimulates their proboscis-protruding behavior (Yasuda and Wakamura 1996; Yasuda 1997). For the “*Zicrona-Altica*” system, further studies are required to identify the behaviorally active compounds, which will give deeper insights into the mechanism of predatory behavior.

Acknowledgements We thank Rui-E Nie and Ming-Xia Guo for assistance in field collecting and bioassays, anonymous reviewers for helpful comments and Elizabeth McHone for helpful comments and English editing. This study was supported by the National Natural Science Foundation of China (Grant No. 31272302) awarded to H.J.X.

References

- Akino T, Knapp JJ, Thomas JA, Elmes GW (1999) Chemical mimicry and host specificity in the butterfly *Maculinea rebeli*, a social parasite of *Myrmica* ant colonies. *Proc R Soc B* 266:1419–1426
- Anton S, Gnatzy W (1998) Prey specificity and the importance of close-range chemical cues in prey recognition in the digger wasp, *Liris niger*. *J Insect Behav* 11:671–690
- Bantock T, Botting J (2013) British Bugs, an online identification guide to UK Hemiptera. <http://www.britishbugs.org.uk/index.html>
- Binz H, Kraft EF, Entling MH, Menzel F (2016) Behavioral response of a generalist predator to chemotactile cues of two taxonomically distinct prey species. *Chemoecology* 26:153–162
- Blomquist G, Bagnères A (2010) Insect hydrocarbons: biology, biochemistry and chemical ecology. Cambridge Press, Cambridge
- Dietemann V, Peeters C, Liebig J, Thivet V, Hölldobler B (2003) Cuticular hydrocarbons mediate discrimination of reproductives and nonreproductives in the ant *Myrmecia gulosa*. *Proc Natl Acad Sci USA* 100:10341–10346
- Endo S, Itino T (2013) Myrmecophilous aphids produce cuticular hydrocarbons that resemble those of their tending ant. *Popul Ecol* 5:27–34
- Ginzel MD, Blomquist GJ (2016) Insect hydrocarbons: biochemistry and chemical ecology. In: Cohen E, Moussian B (eds) Extracellular composite matrices in arthropods. Springer, New York, pp 221–252
- Herzner G, Schmitt T, Linsenmair KE, Strohm E (2005) Prey recognition by females of the European beewolf and its potential for a sensory trap. *Anim Behav* 70:1411–1418
- Koedam D, Morgan ED, Nunes TM, Patricio E, Imperatriz-Fonseca VL (2011) Selective preying of the sphecoid wasp *Trachypus boharti* on the meliponine bee *Scaptotrigona postica*: potential involvement of caste-specific cuticular hydrocarbons. *Physiol Entomol* 36:187–193
- Lahav S, Soroker V, Hefetz A, Vander Meer RK (1999) Direct behavioral evidence for hydrocarbons as ant recognition discriminators. *Naturwissenschaften* 86:246–249
- Lihoreau M, Rivault C (2009) Kin recognition via cuticular hydrocarbons shapes cockroach social life. *Behav Ecol* 20:46–53
- Lorenzi MC, Sledge MF, Laiolo P, Sturlini E, Turillazzi S (2004) Cuticular hydrocarbon dynamics in young adult *Polistes dominulus* (Hymenoptera: Vespidae) and the role of linear hydrocarbons in nestmate recognition systems. *J Insect Physiol* 50:935–941
- McPherson LJ, Mills NJ (2007) Influence of visual and olfactory cues on the foraging behavior of the paper wasp *Mischocyttarus flavitarsis* (Hymenoptera: Vespidae). *Entomol Gen* 30:105–118
- Monnin T (2006) Chemical recognition of reproductive status in social insects. *Ann Zool Fenn* 43:515–530
- Peterson MA, Dobler S, Larson EL, Juárez D, Schlarbaum T, Monsen KJ, Francke W (2007) Profiles of cuticular hydrocarbons mediate male mate choice and sexual isolation between hybridising *Chrysomachus* (Coleoptera: Chrysomelidae). *Chemoecology* 17:87–96
- Pfannenstiel RS, Hunt RE, Yeargan KV (1995) Orientation of a hemipteran predator to vibrations produced by feeding caterpillars. *J Insect Behav* 8:1–9
- Phillips WM (1977) Observations on the biology and ecology of the chrysomelid genus *Haltica* Geoff. in Britain. *Ecol Entomol* 2:205–216
- Rahman S, Hajong SR, Gévar J, Lenoir A, Darrouzet E (2016) Cuticular hydrocarbon compounds in worker castes and their role in nestmate recognition in *Apis cerana indica*. *J Chem Ecol* 42:444–451
- Ranganathan Y, Bessière J, Borges RM (2015) A coat of many scents: cuticular hydrocarbons in multitrophic interactions of fig wasps with ants. *Acta Oecol* 67:24–33
- Rider DA, Zheng LY (2002) Checklist and nomenclatural notes on the Chinese Pentatomidae (Heteroptera) I. Asopinae. *Entomotaxonomia* 24:107–115
- Rutledge CE, Silk PJ, Mayo P (2014) Use of contact chemical cues in prey discrimination by *Cerceris fumipennis*. *Entomol Exp Appl* 153:93–105
- Scott MP, Madjid K, Orians CM (2008) Breeding alters cuticular hydrocarbons and mediates partner recognition by burying beetles. *Anim Behav* 76:507–513
- Shu M, Eyoumu W, Luo QH, Liu WW, Feng LK, Mou LS, Ma N, Wang PL (2012) Predation potential of *Zicrona caerulea* (Linnaeus) to the *Leptinotarsa decemlineata* (Say) low instar larvae. *J Environ Entomol* 34:38–44
- Thomas ML, Parry LJ, Allan RA, Elgar MA (1999) Geographic affinity, cuticular hydrocarbons and colony recognition in the Australian meat ant *Iridomyrmex purpureus*. *Naturwissenschaften* 86:87–92
- Uma DB, Weiss MR (2010) Chemical mediation of prey recognition by spider-hunting wasps. *Ethology* 116:85–95
- Wagner D, Tissot M, Cuevas W, Gordon DM (2000) Harvester ants utilize cuticular hydrocarbons in nestmate recognition. *J Chem Ecol* 26:2245–2257
- Wang SY, Cui JZ, Li WZ, Zhang Y (2005) The feeding habits of the genus *Altica* and biological significance. *Chin Bull Entomol* 42:385–390

- Wang ZW, Chen G, Tan K (2014) Both olfactory and visual cues promote the hornet *Vespa velutina* to locate its honeybee prey *Apis cerana*. *Insects Soc* 61:67–70
- Xue HJ, Wei JN, Magalhães S, Zhang B, Song KQ, Liu J, Li WZ, Yang XK (2016a) Contact pheromones of 2 sympatric beetles are modified by the host plant and affect mate choice. *Behav Ecol* 27:895–902
- Xue HJ, Zhang B, Segrales KA, Wei JN, Nie RE, Song KQ, Liu J, Li WZ, Yang XK (2016b) Contact cuticular hydrocarbons act as a mating cue to discriminate intraspecific variation in *Altica* flea beetles. *Anim Behav* 111:217–224
- Yasuda T (1997) Chemical cues from *Spodoptera litura* larvae elicit prey-locating behavior by the predatory stink bug, *Eocanthecona furcellata*. *Entomol Exp Appl* 82:349–354
- Yasuda T, Wakamura S (1996) Behavioral responses in prey location of the predatory stink bug, *Eocanthecona furcellata*, to chemical cues in the larvae of *Spodoptera litura*. *Entomol Exp Appl* 81:91–96
- Zhang B, Xue HJ, Song KQ, Liu J, Li WZ, Nie RE, Yang XK (2014) Male mate recognition via cuticular hydrocarbons facilitates sexual isolation between sympatric leaf beetle sister species. *J Insect Physiol* 70:15–21