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Chemical convergence in a guild of facultative myrmecophilous caterpillars and host plants

Chemical convergence in caterpillars

LUAN DIAS LIMA[1], JOSÉ ROBERTO TRIGO[2,†], and LUCAS AUGUSTO KAMINSKI[1,*]

[1] Programa de Pós-Graduação em Biologia Animal, Departamento de Zoologia, Universidade Federal do Rio Grande do Sul, CEP 91501-970, Porto Alegre, Rio Grande do Sul, Brazil. ORCiDs: LDL: 0000-0001-5414-3427; LAK: 0000-0002-6468-0960. +55 (51) 3308-7702 ; *Corresponding author: E-mail: lucaskaminski@yahoo.com.br

[2] Departamento de Biologia Animal, Instituto de Biologia, Universidade Estadual de Campinas, CEP 13083-970, Campinas, São Paulo, Brazil. ORCiD: 0000-0003-3028-5353. [†] Author deceased November 28th 2017

Abstract. 1. Ants are a strong selective pressure on herbivorous insects, however, some caterpillars live in symbiosis with them using chemical defensive strategies.

2. We aimed to investigate the adaptive resemblance between cuticular hydrocarbons (CHCs) in multitrophic systems involving a guild of facultative myrmecophilous caterpillar species (Lepidoptera: Lycaenidae), tending ants (Hymenoptera: Formicidae) and host plants from three families. Our study hypothesis was that the caterpillars' CHCs would resemble their host plants (chemical camouflage).

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3. We analyzed the CHCs using gas chromatography/mass spectrometry. The Morisita's similarity index (SI) of CHC profiles were compared between caterpillar species with different types of ant association (commensal or mutualistic), ants and host plants.

4. We found strong convergence between caterpillars' CHCs and plants, especially in commensal species that do not provide secretion rewards for ants. Moreover, we found unexpected chemical convergence between mutualistic caterpillar species that offer nectar reward secretions.

5. These results show that caterpillars acquire CHCs through diet that vary according to the host plant species and type of ant association (commensalism or mutualism). The 'chemical camouflage' in myrmecophilous caterpillars may have arisen as a defensive strategy allowing coexistence with ants on plants, whereas 'chemical conspicuity' may evolve in the context of honest signaling between true mutualistic partners.

6. We suggest the existence of both chemical Müllerian and Batesian mimicry rings between myrmecophilous caterpillars. In multimodal systems, the cuticular chemical blends can play a key adaptive role to decrease ant attacks and increase caterpillars' survival.

Key words. Ant-plant-herbivore interactions, chemical camouflage, chemical crypsis, chemical phytomimesis, chemical strategy, symbiosis.

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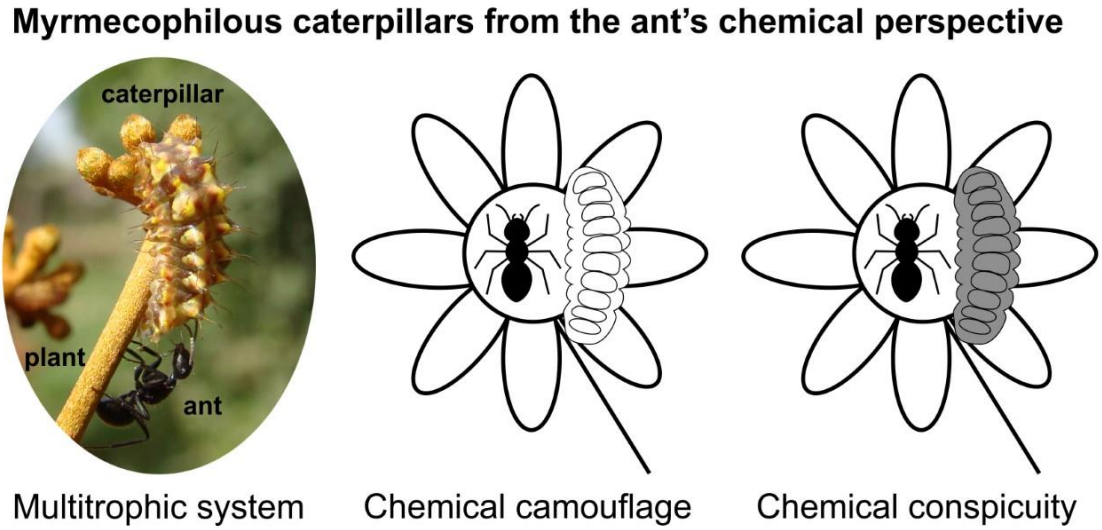
Graphical abstract

- Chemical camouflage can be a defensive strategy against ants in myrmecophilous caterpillars.
- The 'chemical conspicuity' is proposed as a new strategy mediated by cuticular hydrocarbons in myrmecophilous caterpillars.
- Chemical mimicry rings between myrmecophilous caterpillars may occur, specially between mutualistic species that produce nectar rewards for ants.



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Introduction

Herbivorous insects suffer a top-down effect by predators and parasitoids (Vidal & Murphy, 2018). Strategies that minimize the detection or the attack after detection as well as deceive these natural enemies will be positively selected and spread within herbivorous insects (see Ruxton *et al*., 2004). Among the top predators in terrestrial ecosystems, ants patrolling foliage are considered a strong selective pressure on these insects (Floren *et al*., 2002). However, some myrmecophilous herbivores such as hemipterans (e.g. aphids, scales, coccids, whiteflies, leafhoppers, and treehoppers), lepidopteran caterpillars (Lycaenidae and Riodinidae), and galls (induced by aphids and cynipid wasps) can release liquid rewards rich in sugar (honeydew) which attract tending ants (see Pierce *et al*., 2002; Pierce, 2019; Pringle, 2020). This relationship is named trophobiosis, a symbiotic relationship between ants and the trophobiont insects that they attend (Gibernau & Dejean, 2001). The attraction of tending ants can protect the trophobiont herbivores against predators and parasitoids as a conditional mutualism since the ants patrolling foliage behave aggressively against any foreign arthropod (see Rico-Gray & Oliveira, 2007 and references therein for examples with hemipterans).

The trophobiotic interaction is particularly interesting in lycaenid and riodinid caterpillars. They do not excrete the excess of the rich sugar phloem as honeydew-producing hemipterans do, they produce costly secretion rewards through specialized glands (Daniels *et al*., 2005; Kaminski & Rodrigues, 2011). This secretion in lycaenid caterpillars has been reported to manipulate behavior of tending ants (Hojo *et al*., 2015). Furthermore, myrmecophilous caterpillars possess behavioral traits and sets of specialized 'ant-organs' used for chemical and acoustic communication with tending ants (Malicky, 1970; DeVries, 1990; Fiedler *et al*., 1996; Casacci *et al*., 2019). However, a question remains: would be the nutritional reward sufficient to deter the ant aggressive

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behavior in trophobiont caterpillars, or did additional defensive mechanisms evolve to avoid predation? Several studies showed that tending ants may prey upon honeydew-producing aphids (e.g. Sakata *et al*., 1995; Fischer *et al*., 2001). Thus, one alternative strategy can be the 'chemical camouflage' (sensu von Beeren *et al*., 2012) which occurs when an operator does not detect an emitter as its chemical cues blend with the environment and no reaction is caused in the operator. This strategy is found in the myrmecophilous treehopper *Guayaquila xiphias* (Fabricius, 1803) (Hemiptera: Membracidae) due to the high similarity of cuticular hydrocarbons (CHCs) of its host plant, *Schefflera vinosa* (Cham. & Schltdl.) Frodin & Fiaschi (Araliaceae) and seems to solve the dilemma of attracting aggressive tending ants, since these predators did not recognize the hemipteran as prey (Silveira *et al*., 2010). Following this approach, we aimed to investigate the adaptive resemblance between CHC profiles in three multitrophic systems involving a guild of facultative myrmecophilous caterpillars, tending ants and host plant species (Fig. 1). These caterpillars are polyphagous and have polychromatism color pattern associated with visual camouflage (see Monteiro, 1991). Thus, our hypothesis was that the caterpillars would have CHC profiles that resemble their host plants, suggesting chemical camouflage as an additional defensive mechanism against aggressive tending ants.

Materials and methods

Study Site and Organisms

Collections were carried out in two areas of Cerrado savanna in São Paulo state, southeastern Brazil a small fragment belonging to the Laboratório Nacional de Luz Síncrotron in Campinas (22º48'S, 47°03'W), and a reserve of the Estação Experimental de Mogi-Guaçu, Mogi Guaçu (22°18'S, 47°10'W). In both sites, the vegetation consists

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of a dense scrubland of shrubs and trees, classified as cerrado sensu stricto (Oliveira-Filho & Ratter, 2002). The collection and transport of the specimens were authorized by the Biodiversity Authorization and Information System (SISBIO) by the license No. 62345-1.

Neotropical lycaenid butterflies are included in three subfamilies, with the tribe Eumaeini being the most diverse (Robbins, 2004). In general, Eumaeini caterpillars are polyphagous and feed on reproductive tissue (buds and flowers) of host plants (see Robbins & Aiello, 1982; Kaminski *et al*., 2012; Silva *et al*., 2014). Caterpillars of known Eumaeini are engaged in low degree of facultative interactions with tending ants and several species seem to have lost myrmecophily (Fiedler, 1991; LAK, unpublished data). We collected Eumaeini immatures (eggs and caterpillars) on three host plant species that are commonly used by these caterpillars in the study site (see Kaminski & Freitas, 2010; Kaminski *et al*., 2010b, 2012; Rodrigues *et al*., 2010): *Byrsonima intermedia* A. Juss. (Malpighiaceae), *Pyrostegia venusta* (Ker-Gawl.) Miers (Bignoniaceae) and *S. vinosa* (Araliaceae). On the inflorescences of these plants we collected six lycaenid species:

1) *Allosmaitia strophius* (Godart, [1824]) – oligophagous caterpillar specialized on inflorescences of Malpighiaceae (Kaminski & Freitas, 2010). Although the females prefer to oviposit on plants with ants (Bächtold *et al*., 2014), the caterpillars have a non-functional dorsal nectary organ (DNO). That is, they do not establish a typical food-for defense mutualistic interaction with them, being considered a commensal myrmecophilous (i.e. an organism that indirectly interacts with ants) (Kaminski & Freitas, 2010; Silva *et al*., 2014).

2) *Chalybs hassan* (Stoll, 1790) – polyphagous caterpillar on flower buds of Araliaceae, Fabaceae, Sapindaceae, and Sterculiaceae (LAK, unpublished data) with DNO, but without evidence on its functionality and role in the myrmecophily.

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3) *Michaelus thordesa* (Hewitson, 1867) – polyphagous caterpillar, but it occurs more frequently on tubular flower buds of Bignoniaceae, living inside of the flowers. It is a facultative myrmecophilous with functional DNO (Silva *et al*., 2014; Thiele & Kaminski, in prep.).

4) *Parrhasius polibetes* (Stoll, 1781) – polyphagous caterpillar with facultative myrmecophily and functional DNO since the third instar whereas the first and second are commensal myrmecophilous (Kaminski *et al*., 2010a, 2012; Rodrigues *et al*., 2010).

5) *Rekoa marius* (Lucas, 1857) – polyphagous caterpillar with facultative myrmecophily and functional DNOs on the third and last instars (Monteiro, 1991; Silva *et al*., 2014; Faynel *et al*., 2017).

6) *Rekoa stagira* (Hewitson, 1867) – polyphagous caterpillar with facultative myrmecophily and functional DNOs on the third and last instars (Faynel *et al*., 2017; LAK, unpublished data).

On the host plants, we collected samples of two ant species, *Camponotus blandus* (Smith, 1858) and *Camponotus crassus* Mayr, 1862. These species are very common in Cerrado and are involved in most of the interactions between ants and trophobionts on plants, including myrmecophilous caterpillars (e.g. Alves-Silva *et al*., 2013; Bächtold, 2014; Lange *et al*., 2019).

The hatched eggs and caterpillars were reared on buds or flowers of their host plants in an uncontrolled environment in the laboratory. For further chemical analysis, the desired developmental stage (different larval instar) of butterflies, as well the buds or flowers of host plants, and tending ants were killed by freezing and kept frozen at -20ºC until the extraction of CHCs. When it was available, we collected both the second instar, which does not produce a reward secretion and the fourth instar (last instar from now on), which may produce it. We collected the following samples for further analysis: *B.*

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intermedia (N = 2 buds, N = 1 flowers); *P. venusta* (N = 1 buds, N = 1 flowers); *S. vinosa* (N = 1 buds); *A. strophius* (N = 2 last instars on *B. intermedia*); *C. hassan* (N = 1 last instar on *S. vinosa*); *M. thordesa* (N = 1 last instar on *P. venusta*); *P. polibetes* (N = 1 second instar, N = 2 last instars on *B. intermedia*, N = 4 last instars on *P. venusta*, and N = 1 last instar on *S. vinosa*); *R. marius* (N = 1 last instar on *P. venusta* and N = 1 last instar on *S. vinosa*); *R. stagira* (N = 1 last instar on *B. intermedia*); *C. blandus* (N = 1 pool of 20 workers) and *C. crassus* (N = 1 pool of 20 workers).

Extraction and Identification of Cuticular Compounds

CHCs of the organisms were extracted following Portugal & Trigo (2005). The organisms were dipped in 5 ml of *n*-hexane (95%, Ultra-Resi-Analysed J.T.Baker) for 5 min and then removed with a forceps. The hexane was subsequently treated with anhydrous Na_2SO_4, filtered, and evaporated gently in a stream of N_2. The CHC extracts were analyzed using electron impact gas chromatography–mass spectrometry in a gas chromatograph (Hewlett Packard 6890) equipped with a column HP-5MS (5% phenyl methyl siloxane capillary 95%, 30 m x 250 µm x 0.25 µm; Hewlett Packard) directly coupled to a mass selective detector (Hewlett Packard 5973). All analyses were performed under the following conditions: 250°C temperature of injection; 60°-300°C to 2°C/min, 20 min at 300°C program temperature; helium 1 mL/min as carrier gas; ionization energy of 70 eV and a range of 40–600 amu; splitless injection mode, 1 µl injected. All samples were analyzed without and with co-injection with consecutive *n*-alkane standards for the determination of Kovats Retention Index (KI). The alkanes and alkenes were identified by their KI (Carlson *et al*., 1998) and by fragmentation patterns (Nelson *et al*., 1981; Pomonis, 1989; Carlson *et al*., 1999; Howard, 2001). The identification of alkenes was confirmed after derivatization with dimethyldisulfide for determination of double-bond

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position (Francis & Veland, 1981). The fragmentation pattern of *n*-alcohols was compared with the literature (Wang *et al*., 2007). The identification of *n*-alcohols was confirmed after derivatization with *N*-methyl-*N*-trimethylsilytrifluoruoacetamide (MSTFA; 100 mL MSTFA, 80°C, 1 h) according to Menéndez *et al*. (2005). We tentatively assigned some cuticular compounds (e.g. squalene-like) by using the NIST Mass Spectral Search Program (Agilent Technologies, Version 2.0 f. 2008) together with the mass fragmentation interpretation of Budzikiewicz *et al*. (1967). The other compounds remained as unknown.

Statistical Analyses

To test our hypothesis, we calculated the percentage of absolute abundance of the compounds found in the cuticular extracts by taking the compounds as 100%. From these data, we calculated the relative abundance, that is, the quantity of each separate compound expressed as a percentage of the total occurrence of the class of substance. We compared the percentage of similarity of the CHC profiles of the species studied by a hierarchical cluster analysis with a Morisita's similarity index (SI). This index varies from 0% (no similarity) to about 100% (complete similarity) (Krebs, 1999). We used an analysis of similarity (ANOSIM) for testing significant differences based on the percentage of similarity of the CHC profiles between species. To facilitate comparisons, we partitioned the data into two separate analyses. First, we divided species in three groups: caterpillars (group 1), ants (group 2), and plants (group 3). Second, we divided species in four groups: commensal caterpillars (group 1), mutualistic caterpillars (group 2), ants (group 3), and plants (group 4). In these analyses, it was possible to calculate R values from close or equal 0 (total similarity) to 1 (total difference) between the groups (see Clarke, 1993). As *C. hassan* does not have the kind of ant association (commensal or mutualistic) confirmed

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it was not analyzed in the second comparison. All the analyses were performed on PAST software (Version 4.03). We considered values above 80% similarity between caterpillars and host plant as possible cases of chemical camouflage strategy. This value was defined based on the bioassays carried out by Silveira *et al*. (2010) who showed this value to be sufficient to significantly reduce detection in myrmecophilous insects chemically camouflaged against *Camponotus* ants.

Results

The analyses by gas chromatography–mass spectrometry of the cuticular extracts revealed a high similarity between lycaenid caterpillars and their host plants corroborated by the ANOSIM (R_{ANOSIM} = 0.20; $P < 0.05$) (Figs. 2-3 and 5; Tables 1 and S1-S5). In general, the lycaenid caterpillars and the host plants showed *n*-alkanes (C27 and C29) as their main components (Figs. 2-3; Tables S1-S3). The highest SIs occurred between lycaenids that fed on *B. intermedia* varying from 35% to 98%, followed by those that fed on *P. venusta* varying from 55% to 90%, when on *S. vinosa* similarity was low, varying from 23% to 34% (Figs. 2-3 and 5; Table 1). We found evidence of chemical camouflage (similarity> 80%) in four lycaenid caterpillar species: *A. strophius* (98%), *P. polibetes* (second instar) (96%) and *R. stagira* (96%) on *B. intermedia*, and *R. marius* on *P. venusta* (90%) (Fig. 5). The highest values of convergence with the host plant were found in the commensal myrmecophilous that do not have functional DNO gland that produce nectar rewards ANOSIM (R_{ANOSIM} = -0.08; $P > 0.05$), i.e. *A. strophius* (98%), as well as the second instar of *P. polibetes* (96%) (Figs. 2-3 and 5; Tables 1 and S5).

Last instars of the myrmecophilous species *P. polibetes* and *R. marius* have conspicuous and less variable profiles, less affected by the host plant composition. Surprisingly, there were also high SIs between some caterpillar species regardless of the

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host plant used. For example, last instars of *M. thordesa* and *P. polibetes* (from 84% to 95%) and last instars of *C. hassan* and *M. thordesa* (76%) that were higher than those with their host plants (Figs. 2-3; Table 1). In general, *P. polibetes* SIs in the last instar were higher with other lycaenids (25% to 95%) than with the host plant used (27% to 66%) (Figs. 2-3; Table 1). Moreover, according to ANOSIM the mutualistic caterpillars did not have a high similarity with plants ($R_{ANOSIM} = 0.55$; $P < 0.05$; Table S5).

In contrast, the chemical similarity between lycaenid caterpillars and *Camponotus* ants was lower with a different pattern of cuticular compounds (SIs <23%, ANOSIM $R_{ANOSIM} = 1$; $P < 0.05$) (Figs. 4-5; Tables 1, S1, and S4-S5). *Camponotus blandus* and *C. crassus* workers showed mainly branched alkanes and unidentified compounds, and had a SI between them of 28% (Figs. 4-5).

Discussion

We found a high similarity of the CHC profiles (>80%) in some species of lycaenid caterpillars and host plants from two plant families analyzed in this study, thus confirming our initial hypothesis. The fact that the CHC profiles were congruent with the plants the caterpillars were feeding on suggest these caterpillars can acquire these profiles through their diet, as it has been shown that the diet can be essential on the chemical camouflage strategy (e.g. Akino *et al*., 2004; Lohman *et al*., 2006; Lima *et al*., in prep.). This kind of chemical camouflage, also called chemical crypsis or phytomimesis was suggested by Espelie *et al*. (1991) who found a similarity between the cuticular lipids of herbivorous insects and their host plants (see Akino *et al*., 2004; Akino, 2005, 2008; von Beeren *et al*., 2012; Lima & Kaminski, 2019). It was later reported for the first time in a non-trophobiont caterpillar of *Biston robustum* Butler 1879 (Geometridae) (Akino *et al*., 2004; Akino, 2005). Caterpillars of the butterfly *Mechanitis polymnia* (Linnaeus, 1758)

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(Ithomiinae) and the moth *Cydia pomonella* (Linnaeus, 1758) (Tortricidae) as well as larvae of the beetle *Chelymorpha reimoseri* Spaeth, 1928 (Chrysomelidae) and wasps also possess this defensive strategy against chemically oriented predators, including ants, for example (Portugal & Trigo, 2005; Piskorski *et al*., 2010; Massuda & Trigo, 2014; Ranganathan *et al*., 2015).

This strategy has been shown to avoid ants to recognize trophobiont treehoppers as prey (Silveira *et al*., 2010) and to reduce ant attacks even in the absence of honeydew rewards (Wang *et al*., 2018). However, to our knowledge, the chemical camouflage was only demonstrated in Lycaenidae with the entomophagous caterpillars of *Feniseca tarquinius* (Fabricius, 1793) which has a similar lipid composition to that of their aphid prey which is mutualistic with ants (Youngsteadt & DeVries 2005; Lohman *et al*., 2006). Silveira *et al*. (2010) suggested that chemical camouflage could also occur on myrmecophilous lepidopteran caterpillars and that this strategy could function as insect analogs of extrafloral nectaries for ants. Thus, this is the first evidence of chemical camouflage on trophobiont caterpillars. It is known that ants may prey on trophobionts species producing less honeydew (Sakata *et al*., 1995) and that lycaenid caterpillars can use their secretions to appease the aggressive behavior of ants (Hojo *et al*., 2015).

Nonetheless, our results suggest that lycaenid caterpillars can use an additional CHCs-mediated strategy through chemical background matching even when they are unable to secrete honeydew. This happens either because they were on the second instar when their DNOs that produce nutritious secretions for ants are not functional (see Fiedler, 1991; Kaminski *et al*., 2010a) or when they are in the pupal stage when DNOs are not retained (Mizuno *et al*., 2018). Indeed, some of the highest values of similarities between caterpillars and host plants that we found were obtained for *A. strophius*, a commensal myrmecophilous that does not produce nectar rewards (Kaminski & Freitas, 2010;

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Bächtold *et al*., 2014). The cuticular compounds of the facultative myrmecophilous lycaenid pupa *Lycaeides argyrognomon* (Berstrasser, 1779) contains not only CHCs but also several long-chained aliphatic aldehydes which suppress ant aggression even after certain myrmecophilous organs are non-functional (Mizuno *et al*., 2018).

Moreover, myrmecophilous lycaenid caterpillars have several adaptations such as chemical defense from exocrine glands, hairiness, the thickness of larval cuticle, and/or construction of silken shelters that avoid ant aggression (Malicky, 1970; Pierce *et al*., 2002). They also have ant-associated organs: pore cupola organs (PCOs) that supposedly secrete substances to pacify ants, tentacle organs (TOs) and DNO (Pierce *et al*., 2002). Lycaenid caterpillars with specialized parasitic lifestyle may use chemical or acoustic mimicry as well (e.g. Nash *et al*., 2008; Barbero *et al*., 2009). However, the low similarity of the CHC profile between caterpillars and ants studied here discards the hypothesis of this kind of chemical mimicry in facultative myrmecophilous interactions.

On the other hand, lycaenids are also benefited from ant protection and grooming (Hölldobler & Wilson, 1990; Fiedler *et al*., 1996; Hojo *et al*., 2014b) and ants may use the non-congruent CHCs of *Narathura japonica* (Murray, 1875) to learn how to recognize this mutualistic partner whereas they do not do the same with the non-ant-associated lycaenid, *Lycaena phlaeas* (Linnaeus, 1761) (Hojo *et al*., 2014b). According to Hojo *et al*. (2014a) multiple chemical signatures, not only CHCs, may be important for a myrmecophilous to exploit ants. Our study found high SIs among lycaenid species in unrelated genera. This convergence neither provides chemical camouflage on host plants nor chemical mimicry with tending ants. Thus, we propose the 'chemical conspicuity' as a strategy mediated by CHCs in myrmecophilous caterpillars. In this scenario, we can hypothesize the existence of Müllerian mimicry rings between myrmecophilous species with functional DNO, i.e. honest signal between true mutualistic partners (Rossato &

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Kaminski, 2019). Batesian mimicry could also occur when caterpillars that invest little energy in secretions mimic CHCs of conspicuous caterpillars that secrete better caloric rewards for ants. A similar scenario was proposed by Oliver & Stein (2011) that caterpillar species lacking DNO could chemically mimic rewarding ones to gain protection by tending ants. Extensive comparative studies can reveal whether these chemical strategies can occur at the community level.

This, together with other studies, reinforces the importance of chemical camouflage to be selected in herbivorous insects to live in host plants frequently visited by ants (Akino, 2008; Silveira *et al*., 2010). The chemical camouflage may have arisen as a defensive strategy of caterpillars allowing coexistence with ants on plants. In multimodal systems with signals and cues between caterpillars and ants (see Casacci *et al*., 2019) the CHC composition can play a key adaptive role to decrease ant attacks and increase their protection and consequently their survival. Myrmecophilous caterpillar assemblages can be quite rich in species that use different plant resources that vary in terms of nutritional quality and enemy-free space (Seufert & Fiedler, 1996; Rodrigues *et al*., 2010; Silva *et al*., 2014). Our study adds a new adaptive layer of variation regarding the degree of similarity and camouflage efficiency that different host plants can offer for myrmecophilous caterpillars. We hope that our study can serve as an incentive for further studies with the chemical interface of caterpillar-ant-plant interactions.

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interface in plant-ant-butterflies' interactions. Daniela Rodrigues and Adilson Moreira kindly assisted with the fieldwork. We are grateful to Sabrina C. Thiele for providing the *Michaelus* photo and to Fábio S. do Nascimento, Geraldo L. G. Soares and Viviane G. Ferro for critically reviewing the manuscript. This study was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001 as a PhD grant to LDL. JRT acknowledges grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (2011/17708-0) and Conselho Nacional de Pesquisa (CNPq) (2009/304473-0). LAK acknowledges grants from CNPq (140183/2006-0), FAPESP (10/51340-8), PNPD-CAPES, and National Geographic Society (#WW-224R-17).

Contribution of authors

All authors contributed to the study conception and design. LDL carried out the statistical analyses and wrote the manuscript. JRT carried out the chemical analyses and drafted the study. LAK collected the organisms, coordinated the study and critically revised the manuscript. All authors gave final approval for publication.

Conflicts of interest

The authors declare they have no conflicts of interest.

Data availability statement

The data that support the findings of this study are openly available at https://doi.org/10.6084/m9.figshare.12584765.

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Table 1. Morisita's similarity index (mean ± standard error) of the shared cuticular hydrocarbons of lycaenid caterpillars, host plants, and tending ants. Similarity values > 0.8 in bold

	Host plants			**Lycaenid caterpillars**							**Tending ants**	
Lycaenid caterpillars	*Byrsonima intermedia*	*Pyrostegia venusta*	*Schefflera vinosa*	*Allosmaitia strophius*	*Chalybs hassan*	*Michaelus thordesa*	*Parrhasius polibetes* (S)	*Parrhasius polibetes* (L)	*Rekoa marius*	*Rekoa stagira*	*Camponotus blandus*	*Camponotus crassus*
Allosmaitia strophius (L)*	**0.93 ± 0.02**	–	–	–	0.39 ± 0.00	0.25 ± 0.01	**0.85 ± 0.00**	0.34 ± 0.01	0.39 ± 0.05	**0.95 ± 0.00**	0.03 ± 0.00	0.08 ± 0.00
Chalybs hassan (L)**	–	–	0.34	0.39 ± 0.00	–	0.76	0.54	0.78 ± 0.02	0.62 ± 0.03	0.54	0.09	0.09
Michaelus thordesa (L)	–	0.55 ± 0.00	–	0.25 ± 0.01	0.76	–	0.36	**0.80 ± 0.06**	0.58 ± 0.01	0.41	0.05	0.04
Parrhasius polibetes (S)*	**0.90 ± 0.03**	–	–	**0.85 ± 0.00**	0.54	0.36	–	0.51 ± 0.03	0.54 ± 0.11	**0.92**	0.06	0.13
Parrhasius polibetes (L)	0.44 ± 0.04	0.60 ± 0.02	0.27	0.34 ± 0.01	0.78 ± 0.02	**0.80 ± 0.06**	0.51 ± 0.03	0.77 ± 0.05	0.66 ± 0.01	0.51 ± 0.03	0.14 ± 0.02	0.10 ± 0.01
Rekoa marius (L)	-	**0.82 ± 0.08**	0.23	0.39 ± 0.05	0.62 ± 0.03	0.58 ± 0.01	0.54 ± 0.11	0.66 ± 0.01	-	0.53 ± 0.09	0.06 ± 0.01	0.15 ± 0.04
Rekoa stagira (L)	**0.94 ± 0.02**	-	-	**0.95 ± 0.00**	0.54	0.41	**0.92**	0.51 ± 0.03	0.53 ± 0.09	–	0.03	0.10

*Commensal myrmecophily, dorsal nectary organ (DNO) non-functional; L = last instar; S = second instar. **Lack of data on DNO functionality and kind of ant association.

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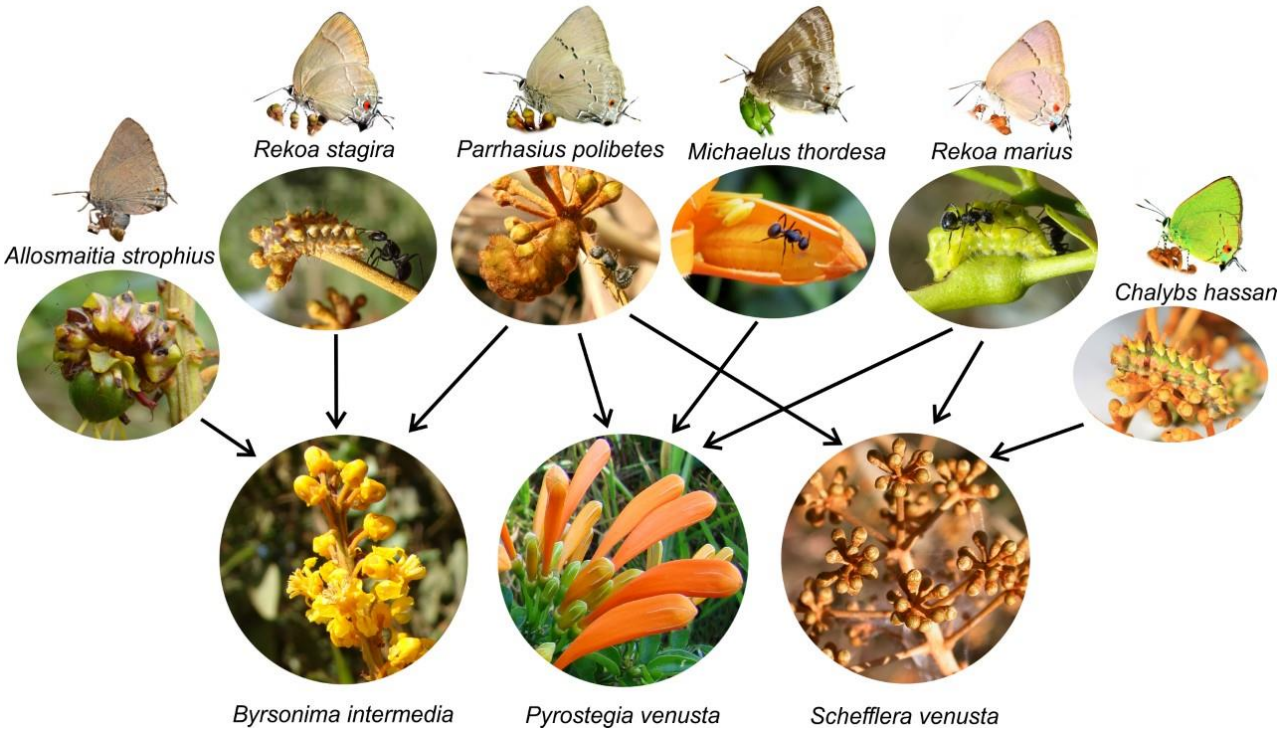

Fig. 1.

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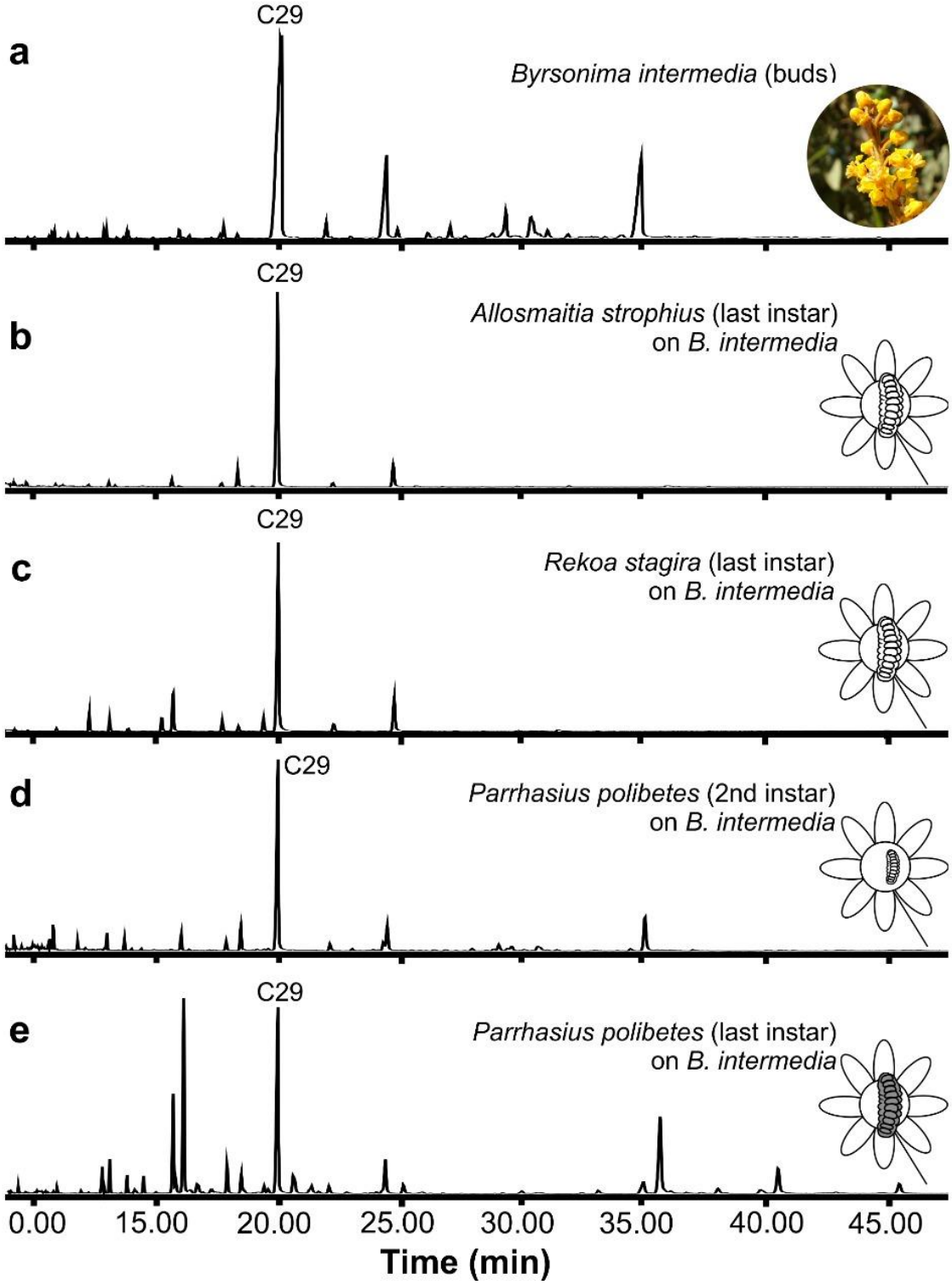

Fig. 2.

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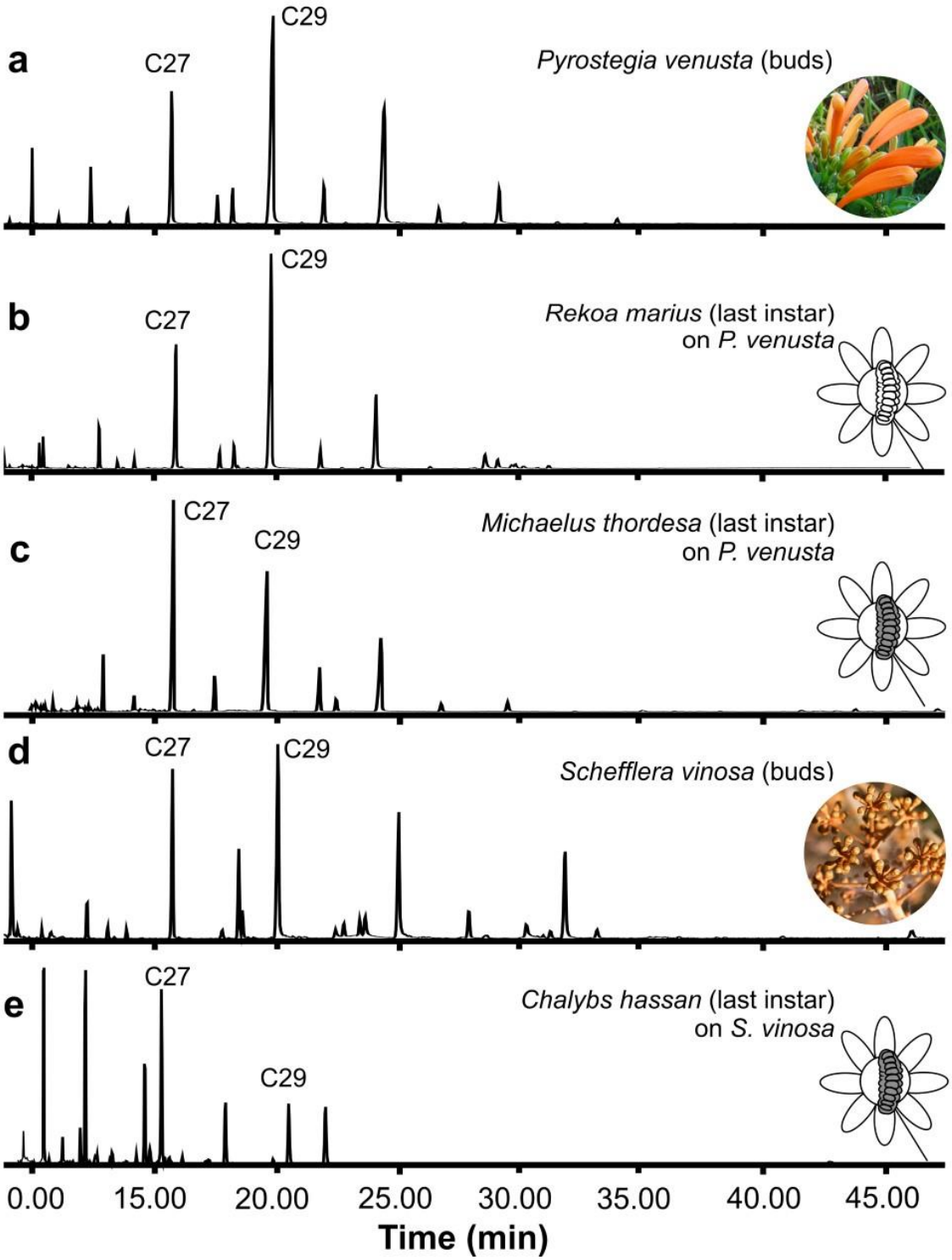

Fig. 3.

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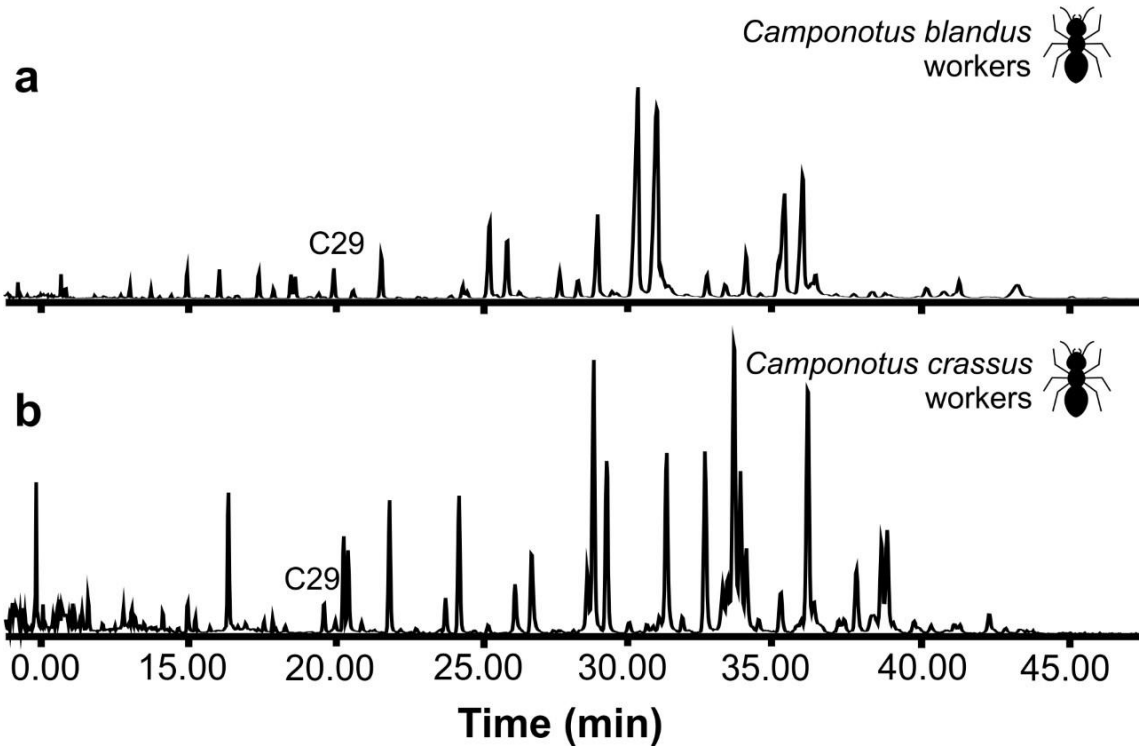

Fig. 4.

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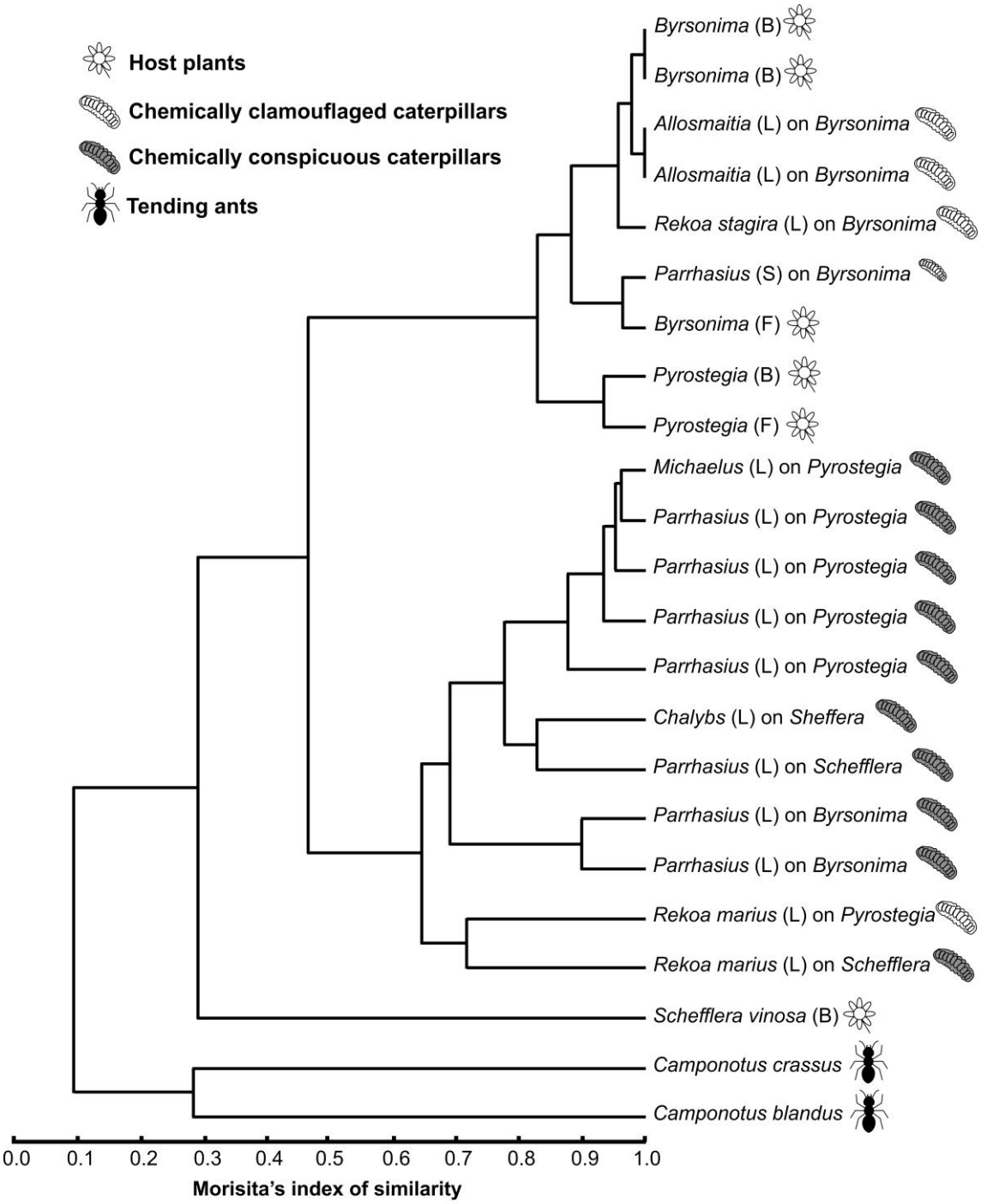

Fig. 5.

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Figure legends

Fig. 1. Multitrophic systems studied involving a guild of six facultative myrmecophilous caterpillars (Lepidoptera: Lycaenidae), tending ants (Hymenoptera: Formicidae) and host plant species in three families, Malpighiaceae, Bignoniaceae and Araliaceae, respectively. Photo of *Michaelus thordesa* caterpillar by Sabrina Thiele ©

Fig. 2. Chromatograms of the cuticular compounds of *Byrsonima intermedia* (Malpighiaceae) buds (a) and associated facultative myrmecophilous caterpillars (Lepidoptera: Lycaenidae) (b-e). b) last instar of *Allosmaitia strophius*, c) last instar of *Rekoa stagira*, d) second instar of *Parrhasius polibetes*, e) last instar of *P. polibetes*. Schematic white colored caterpillar represent cases of 'chemical camouflage' strategy (similarity> 80%) while gray colored caterpillars are 'chemical conspicuity' strategy

Fig. 3. Chromatograms of the cuticular compounds of host plants (a, d) and associated facultative myrmecophilous caterpillars (Lepidoptera: Lycaenidae) (b-c, e). a) *Pyrostegia venusta* (Bignoniaceae) buds (a), b) last instar of *Rekoa marius* on *P. venusta*, c) last instar of *Michaelus thordesa* on *P. venusta*, d) buds of *Schefflera vinosa* (Araliaceae), and e) last instar of *Chalybs hassan* on *S. vinosa*. Schematic white colored caterpillar represents cases of 'chemical camouflage' strategy (similarity> 80%) while gray colored caterpillars are cases of 'chemical conspicuity'

Fig. 4. Chromatograms of the cuticular compounds of tending ants (Hymenoptera: Formicidae) a) *Camponotus blandus* workers, b) *Camponotus crassus* workers

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Fig. 5. Hierarchical cluster analysis (Morisita's similarity index) of the shared cuticular hydrocarbons of facultative myrmecophilous caterpillars (Lepidoptera: Lycaenidae), host plants, and tending ants. B = bud; F = flower; L = last instar; S = second instar

Supporting Information

Table S1. Relative abundance (%) of cuticular compounds of the host plant *Byrsonima intermedia*, lycaenid caterpillars, and ants. Only compounds and values above 5% are shown

Compounds	Retention Index	*B. intermedia* (Bud)	*B. intermedia* (Bud)	*B. intermedia* (Flo)	*A. strophius* (last)	*A. strophius* (last)	*P. polibetes* (2nd)	*P. polibetes* (last)	*P. polibetes* (last)	*R. stagira* (last)	*C. blandus*	*C. crassus*
C_{25}	2500	-	-	-	-	-	-	-	11.84	-	-	-
x-$C_{27:1}$	2674	-	-	-	-	-	-	8.41	11.98	-	-	-
C_{27}	2700	-	-	-	-	-	-	17.04	13.47	10.15	-	-
Squalene	2828	-	-	-	5.73	7.19	5.22	-	-	-	-	-
C_{29}	2900	69.91	70.52	49.65	80.25	78.47	47.05	22.19	15.12	60.71	-	-
C_{31}	3100	21.41	22.48	13.76	10.64	11.94	6.81	-	-	11.90	-	-
x:$C_{32:1}$	3183	-	-	-	-	-	-	-	-	-	-	8.52
C_{32}	3200	-	-	-	-	-	-	-	-	-	-	5.49
x-$C_{33:1}$	3280	-	-	-	-	-	-	-	-	-	-	6.27
17-, 15-, 13-Me-C_{33}	3334	-	-	-	-	-	-	-	-	-	16.70	6.00
15,19-, 13,17-. 9,13-DiMe C_{33}	3356	-	-	-	-	-	-	-	-	-	18.60	-
x-$C_{34:2}$	3364	-	-	-	-	-	-	-	-	-	-	10.87
x-$C_{34:2}$	3373	-	-	-	-	-	-	-	-	-	-	5.10
x-$C_{33:2}$	3462	-	-	-	-	-	-	-	-	-	-	8.68
15-, 13-, 11-, 9-Me-C_{35}	3530	-	-	-	-	-	-	-	-	-	7.50	-
Unknown triterpen	3536	-	-	18.27	-	-	11.20	-	-	-	-	-
15,19-, 13,17-DiMe-C_{35}	3556	-	-	-	-	-	-	-	-	-	9.30	-
11,16-DiMe-C_{35}	3558	-	-	-	-	-	-	13.34	12.04	-	-	-
Unknown	?	-	-	-	-	-	-	5.10	6.62	-	6.67	5.21

Table S2. Relative abundance (%) of cuticular compounds of the host plant *Pyrostegia venusta* and lycaenid caterpillars. Only compounds and values above 5% are shown

Compounds	Retention Index	*P.venusta* (Bud)	*P. venusta* (Flo)	*M. thordesa* (last)	*P. polibetes* (last)	*P. polibetes* (last)	*P. polibetes* (last)	*P. polibetes* (last)	*R. marius* (last)
C_{23}	2300	-	-	-	12.84	-	5.56	9.58	12.00
C_{25}	2500	-	-	25.66	18.39	23.53	19.55	11.17	7.06
C_{26}	2600	-	-	6.07	-	-	-	-	-
C_{27}	2700	15.02	20.18	36.09	28.69	27.76	29.67	23.72	12.71
C_{28}	2800	-	-	6.52	-	-	-	-	-
Squalene	2828	-	-	-	-	7.72	-	12.50	-
C_{29}	2900	33.28	48.37	13.03	12.22	13.76	17.50	14.75	20.09
C_{31}	3100	23.37	16.81	-	-	-	-	-	20.66
C_{33}	3300	5.16	-	-	-	-	-	-	-
15,19-, 13,17-DiMe-C_{35}	3556	-	-	-	6.70	-	-	-	-

Table S3. Relative abundance (%) of cuticular compounds of the host plant *Schefflera vinosa* and lycaenid caterpillars. Only compounds and values above 5% are shown

Compounds	Retention Index	*S.vinosa* (Bud)	*C. hassan* (last)	*P. polibetes* (last)	*R. marius* (last)
Unknown	?	9.80	-	-	-
C_{23}	2300	-	-	-	6.67
x-$C_{25:1}$	2471	-	-	-	20.89
C_{25}	2500	-	-	-	10.17
C_{27}	2700	12.91	25.13	22.60	14.43
Squalene	2828	7.00	6.09	-	-
2-Me-C_{28}	2861	-	7.80	-	-
x-$C_{29:1}$	2873	-	-	8.55	-
Hexacosanol	2899	22.00	-	-	-
C_{29}	2900	-	18.00	22.76	12.39
C_{31}	3100	-	-	-	12.39
Otacosanol	3105	13.00	-	-	-
Branched with 35 C	3326	-	-	7.45	-
15,19-, 13,17-, 9,13-DiMe-C_{33}	3356	-	-	8.05	-
Unknown triterpen	3361	10.84	-	-	-

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Table S4. One-way ANOSIM comparison between the relative proportions of cuticular hydrocarbons of the profiles of lycaenid caterpillars, host plants and *Camponotus* workers

	Caterpillars		Ants		Plants	
	R	P	R	P	R	P
Caterpillars	-	-	1	0.0067	0.2094	0.0329
Ants	1	0.0067	-	-	0.6354	0.0768
Plants	0.2094	0.0329	0.6354	0.0768	-	-

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Table S5. One-way ANOSIM comparison between the relative proportions of cuticular hydrocarbons of the profiles of lycaenid caterpillars, host plants and *Camponotus* workers

	Commensal caterpillars		Mutualistic caterpillars		Ants		Plants	
	R	P	R	P	R	P	R	P
Commensal caterpillars	-	-	0.744	0.0048	1	0.0924	-0.08642	0.5548
Mutualistic caterpillars	0.744	0.0048	-	-	1	0.0101	0.5535	0.0007
Ants	1	0.0924	1	0.0101	-	-	0.6354	0.068
Plants	-0.08642	0.5548	0.5535	0.0007	0.6354	0.068	-	-