



# Multimodal defensive strategies in larvae of two *Hemichroa* sawfly species

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## Abstract

The two European sawfly species in the genus *Hemichroa* are a contrast in behaviour and appearance, since *H. crocea* is gregarious and brightly coloured, whereas *H. australis* is solitary and cryptic. Here, their defensive strategies are compared by integrating further components. In both species, ventral glands are minute, and no distinctive volatiles were detected by chemical analysis; hence, these exocrine glands are probably irrelevant in defence. Ethanol extracts of body parts were feeding deterrent to ant workers of *Myrmica rubra*, especially the integument of *H. australis* which was more deterrent than that of *H. crocea*. Single, living larvae of *H. crocea* were also attacked more frequently by ants. In contrast, single larvae of *H. crocea* are reluctantly taken by the bird *Parus major* that readily feeds on *H. australis*. The larvae of both species jerk their abdomen to physically defend themselves and/or to increase their (visual) warning signal (*H. crocea*). The larvae of *H. crocea* can scratch the host plant leaf with the tip of their abdomen to produce a sound assumed to convey information in intraspecific communication. However, this behaviour was also elicited from *H. australis*, when disturbed, which suggests that it may have another function. The defensive strategy is multimodal in both species. The principal differences are the reliance on gregariousness in *H. crocea*, as opposed to the use of integumental chemicals in *H. australis*.

## Keywords

Hymenoptera, Tenthredinidae, defence strategy, ants, behaviour, sounds, chemical ecology

## Introduction

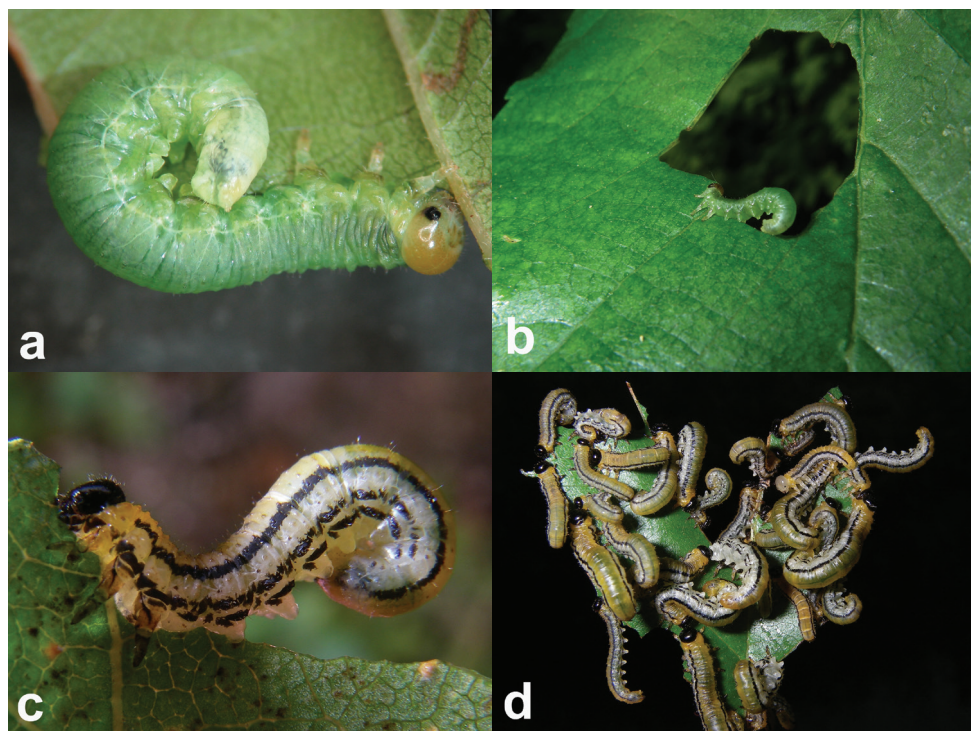
The sawfly genus *Hemichroa* (Hymenoptera, Tenthredinidae, Nematinae) constitutes a small group of 13 recognized species (Taeger et al. 2010). The larval stage is described only for the species *H. australis* (Serville, 1823), *H. crocea* (Geoffroy, 1785), and *H. militaris* (Cresson, 1880) (Lorenz and Kraus 1957, Smith 1975). The geographic distribution is Palearctic for *H. australis*, Palearctic, Nearctic and Oriental for *H. crocea*, and Nearctic for *H. militaris* (Taeger et al. 2010). Only the two former species occur in Europe, where they are quite common. Both species feed mainly on *Alnus* (Betulaceae) (Taeger et al. 1998). Other host-plant genera of *Hemichroa* are *Betula* (Betulaceae), *Carpinus*, *Corylus* (Corylaceae), *Amelanchier*, *Crataegus*, and *Prunus* (Rosaceae) (Smith 1975). The phylogenetic position of *Hemichroa* is closest to *Platycampus* (Nyman et al. 2006) that feeds on *Alnus* and has extremely cryptic larvae (Boevé and Angeli 2010). The species *H. australis* is cryptic and solitary, whereas *H. crocea* is brightly coloured, gregarious (Lorenz and Kraus 1957, Boevé and Pasteels 1985; Fig. 1) and sometimes a serious pest (Escherich 1940–1942, Kriegl 1964).

Nematinae larvae are characterized by the presence of ventro-abdominal exocrine glands which are turned inside out to emit volatiles used in defence (Boevé and Pasteels 1985). The glands vary in size across species, but they are clearly reduced in *Hemichroa* (with a glandular surface of 0.03 mm<sup>2</sup>; see Boevé and Pasteels 1985), and their chemical composition remains unknown. A unique facet of *H. crocea* larvae is their ability to scratch the leaf's surface with protuberances on their caudal segment, producing a stridulatory sound (Hopping 1937). These sounds are thought to maintain cohesion of the larval group, and to direct individuals to profitable, fresh leaves (Hograefe 1984). Similar communication by vibrational signals via a substrate is known for other sawflies such as the pergid *Perga affinis* (Carne 1962, Fletcher 2008).

It is likely that the defensive strategy of *Hemichroa* larvae is multimodal, combining behavioural, visual, chemical, and acoustic traits. This paper examines two aspects of their defence by using a comparative approach. The principal purpose was to determine whether or not *H. australis* and *H. crocea* – which display opposite appearance and gregariousness – also differ in other (behavioural and chemical) traits, and in the consequent effectiveness of their defensive strategies. Another aspect was to test whether and how acoustic cues are involved in defence.

## Methods

Larvae of *Hemichroa* were collected in Belgium and identified following Lorenz and Kraus (1957). Voucher specimens are kept in the Royal Belgian Institute of Natural Sciences. Throughout the text, the sawfly collection reference number is given between square brackets.



**Figure 1.** Pictures of larvae of the two studied *Hemichroa* species. **a, b** *H. australis*, solitary (body length ca. 18 mm) **c, d** *H. crocea*, gregarious (body length ca. 20 mm). Field host-plant [sawfly collection reference number]: **a** *Alnus glutinosa* [P2553] **b** *A. glutinosa* [P3999] **c** *Betula verrucosa* [P3225] **d** *A. glutinosa* [P3230]

Field observations were performed and documented with Pentax Optio W10 and Nikon Coolpix P300 cameras. An audio file was obtained in indoor conditions with a Zoom H4n digital recorder, its microphones being placed a few cm from a leaf harbouring a group of *H. crocea* larvae.

Ventral glands were dissected from larvae preserved in 70 % ethanol, then mounted between glass slides and plates. Glands were also dissected from larvae stored at  $-30^{\circ}\text{C}$  and thawed, to be analysed via solid sample injections by gas chromatography-ion-trap mass spectrometric detection (GC-ITD) as described in Boevé et al. (1992).

Hemolymph was collected with glass capillaries from live larvae. Afterwards, the larvae were frozen and the thawed specimens dissected to isolate integument and internal organs (mainly the digestive tract). The three samples from a batch of larvae were extracted in ethanol, then filtered, dried, and dissolved in sugar water. The laboratory, dual-choice bioassay consisted of comparing the number of ant workers of *Myrmica rubra* feeding on sugar water *versus* sugar water plus extract. Another bioassay consisted of placing a single live larva in the presence of 20 ants; the number of ants attacking the larva was counted, and the behavioural interactions were noted. All experimental procedures are detailed in Boevé (2010).

## Results

### Behaviour

The larvae of *H. crocea* and *H. australis* settle on the edge of a leaf, firmly gripping with their thoracic legs. Younger larvae make a hole in the leaf, thus feeding on the inner leaf edge, whereas older larvae feed on the outer edge, which is especially the case for *H. crocea* (Hopping 1937).

If disturbed, the larva places its body on the leaf side opposite to the source of disturbance, especially so in *H. australis*. Larvae can also perform defensive movements with the abdomen. These movements are either hearable scratch sequences (see Introduction; Suppl. material 1), or single quite violent jerks. Both abdominal movements were observed in *H. crocea* and *H. australis*. Larvae performed jerks when disturbed by approaching and attacking ant workers, or when I approached them, or when I directed my finger towards them. Since *H. crocea* is gregarious, jerking by one individual could be imitated by others, leading to ‘waves’ of jerks within a group (Suppl. material 2). This was elicited by an external disturbance as much as by an internal one in that the larvae were disturbing each other. When many larvae settled on one leaf and consumed a major part of it, scratching behaviour was virtually impossible because there was almost no leaf surface available. A few times in the field, my approach provoked scratching in a larva of *H. australis* (i.e. different larvae, locations, and dates; Suppl. material 3) and, if the environment was calm, I could hear it from nearly a meter away. After a while, however, the larva stopped scratching, probably do to habituation or fatigue.

### Morphology and chemistry of ventral glands

The minute and flattened ventral glands of *H. crocea* and *H. australis* are associated with only one pair of retractor muscles. The secretory layer is composed of only about 25 glandular cells on each side of the pouch.

Only small amounts of chemical compounds were detected by analysing a whole ventral gland. These were alkanes with an odd number of carbon atoms from 23 to 27 in *H. crocea*, and 21 to 27 in *H. australis*.

### Defensive efficiency

All extracts at a starting-test concentration of 8 mg DW extract / ml sugar water significantly deterred ants (Tab. 1). Internal organs proved to be the most deterrent body parts in both *Hemichroa* species. At this concentration, the total number of feeding ants was not significantly different between the two species, neither by comparing the hemolymph extract with the control solution ( $P = 0.742$ , Fisher exact probability test, two-tailed), nor by similarly considering the internal organs ( $P = 0.617$ ). However, it

**Table 1.** Feeding deterrence rates of extracts of *Hemichroa* larvae against *M. rubra* ants.

Species	Extract	8.0 mg DW/ml	2.6 mg DW/ml	0.8 mg DW/ml
<i>H. australis</i>	Hemolymph	61** (93)	7 (198)	8 (141)
	Integument	65** (126)	24* (124)	6 (128)
	Internal organs	87** (169)	39** (154)	9 (200)
<i>H. crocea</i>	Hemolymph	57** (139)	36** (115)	3 (218)
	Integument	31* (283)	-2 (182)	-
	Internal organs	91** (129)	45** (131)	6 (175)

The deterrence rate is the percentage of  $(C-T)/(C+T)$ , where C and T are the total numbers of ants feeding on the control and test solution, in a 12-replicated test. Values for C+T are given between parentheses. The starting extract solution was tested also in two logarithmic dilutions. For each test, the paired number of ants was compared with the Wilcoxon signed-rank test, two-tailed: (\*)  $P < 0.05$ , (\*\*)  $P < 0.01$ . (-) Not tested.

was significantly different for the integument ( $P < 0.001$ ) with *H. australis* being more deterrent than *H. crocea*. Testing dilutions of the starting concentration confirmed these results for the integument and internal organs, but indicated that the hemolymph may be more deterrent in *H. crocea* (Tab. 1).

A single living larva of *H. australis* was significantly less likely to be attacked (by a mean  $\pm$  SD of  $3.5 \pm 2.6$  ants) than one of *H. crocea* ( $6.5 \pm 1.8$ ) ( $P < 0.05$ , Mann-Whitney test, two-tailed). Both sawfly species made violent body movements while being attacked. Conversely, *H. australis* provoked clearer signs of distress in the ants than *H. crocea*.

## Discussion

Anti-predator defensive mechanisms often act in concert, but are dynamically modulated so as to produce specific responses to threats that vary in type, time, and intensity (Rowe and Harpin 2013). This is well illustrated in the two studied *Hemichroa* species in which visual, chemical, behavioural, and possibly acoustic components were revealed.

Ventral glands are greatly reduced compared to other Nematinae species. The detected alkanes, from heneicosane to heptacosane, were not unique to *Hemichroa* or the Nematinae, but correspond to those hydrocarbons generally occurring on the cuticular surface of insects (Blomquist and Bagnères 2010). As far as known, they are devoid of any particular interspecific repellent effect. Thus, the chemical defence in both species does not rely on a volatile glandular secretion, contrasting the situation in other Nematinae species (e.g. Boevé et al. 1992). However, another type of chemical defence exists because the extracts of all body parts proved to be deterrent to ants, notably the integument of *H. australis* (Tab. 1). This result was in accordance with the greater defensive efficiency of individual larvae of the latter species when confronted with 20 ants. Moreover, the mechanical resistance of the integument of *H. australis* is twice as

high as that of *H. crocea* (U. Schaffner and Boevé, unpublished results). The physical barrier is of particular importance in defending against invertebrate predators such as ants. But, the bird *Parus major* readily feeds on single larvae of *H. australis*, while only reluctantly accepting those of *H. crocea* (see Boevé and Pasteels 1985). Thus, a single *H. crocea* is better defended against birds than a single *H. australis*, the reverse being true against ants. In natural conditions, gregariousness of *H. crocea* probably enhances its defence against birds and may compensate for the relatively low defence efficiency of each individual against ants.

Both sawfly species exhibit similar abdominal movements. A larva can switch between jerking and scratching within a short period. The jerks are a common defensive behaviour among Nematinae larvae, and they can knock down a foraging ant or parasitoid, but are inefficient against birds as a physical defence (Boevé and Pasteels 1985). However, the intensity of the warning signal is increased by the gregarious behaviour of *H. crocea* larvae. The scratching behaviour is unusual among insects. In *Hemichroa*, there is good evidence that it plays a role in defence such as an acoustic and/or vibrational warning signal (Suppl. materials 1 and 3) that may function against birds as well as predatory invertebrates. It is reported here in *H. australis* for the first time. This solitary species obviously does not use scratching to communicate with conspecifics. It seems that this behaviour is used less frequently than jerking, and only when first encountering an antagonist, which may explain why Hograefe (1984) considered it as non-existent in this species. Interestingly, the larva of *H. militaris* does not possess caudal protuberances (Smith 1975), which raises the question whether it performs scratching or not. Furthermore, Dyar (1895: p. 305) says of the gregarious larvae of *Nematus ventralis* Say, 1824 (Nematinae): “The larvae scratch the leaf with their anal prongs and make a rasping sound”. In *H. crocea*, the use of scratching in intraspecific communication is plausible, although counterarguments to the conclusions of Hograefe (1984) would be that undamaged foliage not only supposes leaves with higher nutritional quality, but also a larger leaf surface on which the behaviour can be executed, independently of its function. Moreover, it remains unclear why the larvae on the heavily eaten leaf perform frequent scratch sequences at the beginning of the experiment; the experiment itself possibly disturbed the larvae that may have responded by a ‘defensive’ behaviour.

## Conclusion

Scratching is known to be a way of inter-individual communication in *H. crocea*. However, it is concluded here that the behaviour may be part of the defensive strategy in this gregarious species as well as in the solitary *H. australis*. There are gradual, behavioural responses to increasing levels of disturbance, with hiding (behind the leaf) followed by scratching, and finally jerking. The defensive arsenal is multimodal, involving behavioural traits as well as visual (gregariousness; brightly coloured *versus* cryptic integument), chemical (water-soluble chemical compounds), and acoustic (sounds by



scratching) traits. The divergence between the two defensive strategies is gregariousness in *H. crocea* and integumental chemicals in *H. australis*. The identity of these chemicals remains unknown. They may be plant-derived since the digestive tract (as main part of the internal organs) was overall the most active extract tested. The comparison of the defensive strategies between the two *Hemichroa* species reveals, 1) obvious contrasts in larval appearance and gregariousness, 2) points of similarity in jerking, scratching and in the absence of functional ventral glands, and 3) different defensive efficiencies against ants and birds, with single larvae of *H. crocea* being better defended against birds, whereas *H. australis* against ants.

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## Supplementary material I

### Audio of scratch sequences performed by a group of larvae of *H. crocea*

Author: Jean-Luc Boevé

Data type: audio

Explanation note: Audio (mp2; duration 3min25sec) of scratch sequences performed by a group of larvae of *H. crocea*. Larvae were collected in the field (Ave-et-Auffe, 08.X.2013) [P3799], but sounds recorded in indoor conditions (10.X.2013, between 9 and 10 PM). For clarity, the original audio was 20 dB amplified, followed by a 24dB background noise reduction. Note that the third scratch sequence is quite loud. Following Hograefe (1984), a scratch sequence comprises 3–5 behavioural units lasting  $224 \pm 6$  milliseconds each.

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## Supplementary material 2

### **Group of larvae of *H. crocea* performing rather synchronized jerks with their abdomen**

Author: Jean-Luc Boevé

Data type: video

Explanation note: Video (m4v; duration 19sec) in field conditions of a group of larvae of *H. crocea* performing rather synchronized jerks with their abdomen. The larvae are those shown in Fig. 1d.

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## Supplementary material 3

### **Larva of *H. australis* performing the leaf scratching behaviour**

Author: Jean-Luc Boevé

Data type: video

Explanation note: Video (m4v; duration 40sec) in field conditions of a larva of *H. australis* performing the leaf scratching behaviour. Larva is the one shown in Fig. 1b.

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