Journal of Insect Physiology 56 (2010) 14–20

Contents lists available at ScienceDirect

Journal of Insect Physiology

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

Regulation of wing formation and adult development in an aphid host, *Aphis fabae*, by the parasitoid *Aphidius colemani*

Amalia Kati, Jim Hardie *

Faculty of Natural Sciences, Department of Life Sciences, Imperial College London, Lees Building, Silwood Park Campus, Ascot, Berks, SL5 7PY, UK

ARTICLE INFO

Article history:
Received 23 February 2009
Received in revised form 7 August 2009
Accepted 17 August 2009

Keywords:
Adult development
Aphidius colemani
Aphis fabae
Host regulation
Venom
Wing development

ABSTRACT

Nymphs of presumptive winged gynoparae of *Aphis fabae* (Hemiptera: Aphididae), were exposed to female parasitoids, *Aphidius colemani* (Hymenoptera: Aphidiidae) and stung once with the ovipositor. Wing development was inhibited and, when aphids were parasitised during the early stages, they did not reach the adult stage but mummies with rudimentary or no wingbuds are observed in the host's fourth-stadium. These and previous studies have suggested that wing development may be inhibited by factor(s) from the maternal parasitoid injected into the host at the time of oviposition. In an attempt to identify such factor(s), saline extracts of whole female parasitoids, abdomens, ovaries and venom glands were prepared. When a saline extract of venom glands was injected into late-second-stadium aphids, many develop to fourth-stadium nymphs with rudimentary wingbuds, indicating an effect on wing formation but also showed developmental arrest and often died when attempting to moult to the adult stage. It appears that host death may be related to physiological/biochemical interactions of parasitoid and host rather than just late stage parasitoid larvae ingesting the host's vital organs. Injections with extracts into later host stadia gave similar results with regard to development to the adult, although aphids injected in the late-fourth-stadium develop normally to the adult stage with no effect on wing formation. The results indicate that the earlier the injection before the final moult the greater the effect of the injected extract on preventing adult development.

Extracts prepared from head + thorax do not affect aphid development and the results indicate that there is an active factor(s) – likely a protein – in the female parasitoid's venom that disrupts wing development and/or inhibits development to the adult stage. Surprisingly, injections of extracts from male parasitoids have similar effects but the location and function of such a factor(s) in males are unknown.

1. Introduction

Koinobionts (endoparasitoids that allow their hosts to feed and grow for some time after parasitism) like *Aphidius colemani* (Viereck) (Hymenoptera: Aphidiidae) spend part of the life cycle as larvae living in intimate contact with their host. To their own nutritional advantage, they do not damage vital organs so that the host remains alive and active. However, they may manipulate the host's behaviour, biochemistry and physiology for their own benefit (Godfray, 1994). As a result, host tissues that are not essential for survival are used for the parasitoid's nutrition and welfare. Typically, parasitised insects undergo developmental arrest and eventually die as soon as the parasitoid becomes independent of the host. Parasitoid development interferes with the host's immune system, hormone titres and causes changes in behaviour (Beckage and Gelman, 2004). These parasitoids are known as regulators because they disrupt host development, morphology or behaviour primarily by interfering with the host's endocrine system (Lawrence, 1986).

Parasitism may induce morphological changes in the host insect. The cause of these changes appears to be oviposition-induced stress, venom injected into the host with the egg or factors produced by the developing parasitoid larvae (Vinson and Iwantsch, 1980). Non-vital, metabolically active tissues are shut down and parasitoid larvae gain a nutritionally enhanced environment. Evidence presented by Digilio et al. (2000) indicates that *Aphidius ervi* causes complete castration in its host (*Acyrthosiphon pisum*) and interferes with adult development. It is suggested that the parasitoid venom acts as a host regulation factor during parasitism, directly degenerating the female aphid gonads. Falabella et al. (2007) have described a dimeric venom protein from *A. ervi* which induces apoptosis in the host's ovarioles by causing oxidative stress.

One of the impressive effects that aphid parasitoids have on their hosts is the inhibition of wing development (apterisation).

* Corresponding author. Tel.: +44 0207 594 2259.
E-mail addresses: j.hardie@imperial.ac.uk, katiamalia@yahoo.gr (J. Hardie).

0022-1910/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jinsphys.2009.08.010

After parasitism, aphid nymphs that should develop into winged adults, develop into wingless or partially winged adults/mummies (Johnson, 1959; Christiansen-Weniger and Hardie, 1998). In addition to this apterisation, after parasitisation during the first or second host stadium, aphids do not reach the adult stage but are killed and mummified during the fourth-stadium. Consideration of progression of host and parasitoid development makes it possible that some host regulation factors introduced at oviposition, and possibly contained in the venom, are responsible for inhibition of wing development (Hardie and Christiansen-Weniger, 2001). There is no clear explanation on how this is achieved but those authors conclude that a direct effect on the wingbud tissues, without interference with the endocrine system, is most likely.

The aim of the present study was to investigate wing formation after parasitism in an aphid and the mechanism of how any changes might be effected. The black bean aphid, *Aphis fabae* Scopoli (Hemiptera: Aphididae), and the parasitoid, *A. colemani*, were chosen because short-day rearing of *A. fabae* allows the induction of the gynopara morph (a winged female that, in the field, flies from the summer to the winter host plant and produces the wingless sexual females) such that nearly 100% of neonatal individuals are guaranteed develop into winged adults (Hardie, 1980). Thus, *A. colemani* were allowed to parasitise presumptive winged gynoparae of different ages and the effects on wing formation and development through the stadia were monitored. This was important as the age when the aphids are parasitised is known to be crucial for the effected changes in the parasitoid/aphid systems. A microinjection method was then used for a series of experiments where non-parasitised black bean aphids were injected with extracts from female and male (initially as a control) parasitoid tissues. The injections allow for parasitoid material to be inserted into the body of the aphid host and thus the hypothesis that host regulating factors injected during oviposition are responsible for the effects could be tested without the confounding effects of parasitoid egg/larva development and/or activity. The aim of investigating wing formation was to an extent frustrated when many extract-injected insects died before the final moult and so effects on adult development are also studied. Heat and protease treatments provided an initial attempt to define the nature of the active factor(s).

2. Materials and methods

2.1. Insects

A. fabae gynoparae were reared on tick bean seedlings (*Vicia faba* L.) at 15 ± 1 °C, in short-day conditions (LD 12:12 h) (Hardie, 1980). *A. colemani* were reared at 19 ± 1 °C, LD 16:8 h, on the green peach-potato aphid, *Myzus persicae* (Sulzer), maintained on Brussels sprout plants (*Brassica oleracea* var. *gemnifera* L., cultivar Bedford-Winter Harvest).

Gynoparae of known ages were obtained by placing short-day exposed adults on bean plants for 24 h to reproduce. The mothers were removed and the progeny reared to the required age and stadium for experiments.

Only after moulting to the third stadium are wingbuds visible and the adult winged form is predictable. Third- and fourth-stadium presumptive winged gynoparae were inspected for the presence of wingbuds and those with no visible wingbuds were excluded from the experiments as they would develop into wingless adults.

2.2. Parasitism

Groups of gynoparae, of the same age, were placed in a Petri dish (50-mm diameter) with a female parasitoid from the stock culture and observed. Aphids that were stung by the parasitoid were removed immediately to avoid multiple oviposition and any insects that may have received a second sting were discarded. The aphids were then returned to bean plants and placed in the short-day cabinet to develop. For the control, the same procedure was followed without the female parasitoid placed in the Petri dish. After parasitisation, aphids were inspected daily for the presence of mummies or adults and the presence of normal wingbuds (fourth-stadium nymphs or mummies) or fully developed wings (adults), using a dissecting microscope.

2.3. Extract injection

2.3.1. Preparation of parasitoid extracts

Parasitoids were collected from the rearing cages and anaesthetised with CO_2. For the preparation of whole female or male parasitoid extract, separate sexes were put into an Eppendorf tube with the required volume of Pringle's saline (Pringle, 1938) and ruptured with a pellet pestle. After centrifugation at $13{,}000 \times g$ for 5 min, the supernatant was recovered and adjusted to the required concentration of parasitoid equivalents/μl (0.1, 0.01 and 0.1 eq/μl for female and male whole-insect extracts, respectively). For the preparation of female or male abdomen or head + thorax extracts, parasitoids were bisected at the petiole using a razor blade fragment and strictly speaking the head + -thorax segments include the first abdominal segment, the propodeum. Afterwards, both parts were kept separately in Eppendorf tubes and the two extracts were prepared as described above (0.1 eq/μl for both female and male abdomen or head + -thorax extracts).

For the venom extract, the abdomens of intact female parasitoids were placed in a drop of Pringle's saline on a microscope slide and the ovipositor was pulled out gently with fine forceps. Both ovaries and venom glands remain attached to the ovipositor and were placed in saline for the ovary + venom extract (1.5 eq/μl), whereas only the venom glands or only the ovaries were kept in saline for the venom (1.5, 0.15, 0.015 eq/μl) or ovary (1.5 eq/μl) extract, respectively. The tissues were ruptured in an Eppendorf tube with a pellet pestle and after centrifugation at $13{,}000 \times g$ for 5 min, the supernatant recovered. Venom extract was also either treated at 100 °C (Eppendorf tubes containing the extracts were placed in a beaker with boiling water for 5 min, then centrifuged for 5 min at $13{,}000 \times g$ and the supernatant recovered) or digested with protease (Protease K-Acrylic beads, P0803, Sigma–Aldrich, St Louis, MO, USA) (extract incubated with protease at 37 °C for 3 h, centrifuged at $13{,}000 \times g$ for 1 min, the supernatant was placed in a clean tube and centrifuged again at $13{,}000 \times g$ for 1 min and finally the supernatant was recovered). For the protease treatment the control comprised venom extract held at 37 °C for 3 h, without protease. All extracts were used immediately or stored at −80 °C for future use. The procedures were similar to those described by Digilio et al. (1998, 2000).

2.3.2. Injection procedures

Aphids of known ages and stadia were obtained as above. Late-second-stadium gynoparae were 6 days old, early-third-stadium gynoparae were 7 days old, late-third-stadium gynoparae were 10 days, early-fourth-stadium gynoparae were 11 days and late-fourth-stadium gynoparae were 16 days old.

A. fabae gynoparae were injected with extracts prepared from the whole body, the abdomen, and the head + thorax of female or male parasitoids as well as with extracts of venom, ovaries and venom + ovaries from female parasitoids. Saline injections were used as a control, together with sham injections (where the glass pipette was just inserted into the body and carefully removed, without any injection into the aphid). Furthermore, 100 °C-treated or protease-treated venom extracts were also tested for activity.