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Responses of a Generalist and a Specialist Parasitoid (Hymenoptera: Eucoilidae) to Drosophilid Larval Kairomones

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Foraging parasitoids are thought to need more specific information than generalists on the presence, identity, availability, and suitability of their insect host species. In the present paper, we compare responses to host kairomones by two phylogenetically related parasitoid species that attack Drosophilidae and that differ in the width of their host range. As predicted, the behavioral response of the parasitoids to host kairomones reflected their difference in host range. The response of the specialist parasitoid Leptopilina boulardi *was restricted to contact kairomones from its natural hosts and one closely related species. In contrast, the generalist parasitoid* Leptopilina heterotoma *responded to contact kairomones of a variety of Drosophilidae species.*

KEY WORDS: Hymenoptera; *Leptopilina*; *Drosophila*; semiochemicals; kairomones; parasitoid; generalist; specialist; foraging behavior.

INTRODUCTION

One way to determine whether a trait is adaptive is to use the comparative approach. In this approach, phylogenetically related species are used to search for correlations between species characteristics and ecological factors (Harvey and Pagel, 1991; Brooks and McLennan, 1991). Ecological entomologists have successfully used the comparative approach to study the function of certain

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behavioral and life history traits in entomophagous arthropods (Sabelis, 1985; Vet and van Alphen, 1985; van Alphen and Vet, 1986).

In the present paper we compare a behavioral trait in two phylogenetically related parasitoid species that differ in host and habitat range but that otherwise have highly comparable, even overlapping, niches. This increases the likelihood of finding differences between the species that can be interpreted as adaptive. Both are parasitoids of larval Drosophilidae. The behavioral trait of interest is the parasitoid's response to larval extracts (potential kairomones) and we question whether differences in host range of the parasitoid is reflected in this behavioral response. Several authors have generated hypotheses on how dietary specialization (i.e., host range) affects parasitoid foraging (Sheehan, 1986; Vet *et al.*, 1990; Poolman Simons *et al.*, 1992; Vet and Dicke, 1992). Vet and Dicke (1992) addressed how the use of infochemicals by foraging parasitoids correlates with their dietary specialization at different trophic levels (i.e., host and host food range). They argue that the degree of dietary specialization sets the degree of specificity of the information needed for successful foraging for host larvae by the parasitoid. Specifically, specialists should need more specific information on the identity, presence, suitability and availability of their host species than should generalists. To test the hypotheses they suggest a species-comparison approach.

Here we study a specialist and a generalist *Leptopilina* species (Hymenoptera; Eucoilidae) that attack drosophilid larvae. *Leptopilina boulardi* (Barbotin *et al.*) is found mainly in tropical and subtropical areas and is a specialist parasitoid that attacks only hosts in fermenting fruits. Its main host is *Drosophila melanogaster* Meigen and it may parasitize some other species of the melanogaster subgroup such as *D. simulans* Sturtevant but with less success (Nordlander, 1980; Carton and Kitano, 1981; Carton *et al.*, 1986; Hertlein, 1986). *Leptopilina heterotoma* (Thomson) has an holarctic distribution and attacks a wide variety of Drosophilidae in several different microhabitats such as phloem sap of bleeding trees, fermenting fruits, decaying plant materials, and mushrooms (Carton *et al.*, 1986; Janssen *et al.*, 1988). Parasitoids are attracted to microhabitat (food substrate) odors (e.g., Vet, 1985b). After they arrive at the substrate they start searching for individual hosts by probing the substrate with their ovipositor. As is common in insect parasitoids, the next cue they respond to is a host-derived contact kairomone (Weseloh, 1981). For several *Drosophila* parasitoids, it is known that searching females respond to a water-soluble kairomone of host larvae. They increase the time spent searching on a patch when host kairomone is present (e.g., Dicke *et al.*, 1985; Vet, 1985a; Vet and van der Hoeven, 1984). The generalist *L. heterotoma* is reported to respond to kairomone of *D. melanogaster* (van Alphen *et al.*, 1984; Dicke *et al.*, 1985; Vet and van der Hoeven, 1984) and kairomone of *Scaptomyza pallida* (Zetterstedt) (Vet, 1985a). However, data on kairomone response by the specialist

L. boulardi are lacking. The present experiments compare the responses of these two parasitoids to larval extracts (potential kairomones) from a wide range of Drosophilid species.

MATERIALS AND METHODS

Parasitoids

L. heterotoma (strain S 1985) females were obtained from a laboratory culture (on D. *subobscura* Collin; for rearing medium see below), established from wasps reared from sap fluxes from bleeding trees in a deciduous forest in the Netherlands in 1985. Parasitoids and hosts were reared at 21°C on a 16L:8D photocycle. After emergence the wasps were stored at 12.5°C in clean agar vials with honey as food.

L. boulardi (strain G.317.1) females were obtained from a laboratory culture (on *D. melanogaster*; for rearing medium see below), established from wasps reared from prickley pear fruits (*Opuntia* sp.) in Tunisia in 1986. Parasitoids were reared on live baker's yeast (*Saccharomyces cerevisiae* Hansen) only, at 25°C on a 16L:8D photocycle. After emergence the wasps were stored at 12.5°C in clean agar vials with honey as food.

Drosophilid Species

Parasitoid response was tested to larval extracts from the drosophilidae species given in Table I, which also lists the taxonomic position of the drosophilid species as well as information on their substrate use. Parasitoid response was tested using larval extract from the following Drosophilidae species.

D. melanogaster and *D. simulans* laboratory cultures were established from individuals reared from fruit baits in orchards in the Netherlands. They were reared on a medium of agar, dead yeast, and sugar at a temperature of 25 and 21°C, respectively.

D. subobscura laboratory culture was established from individuals reared from sap fluxes of bleeding trees. It was reared on an apple medium (ground apple, water, sugar, agar, yeast) at a temperature of 21°C.

D. hydei Sturtevant (strain Leiden) laboratory culture was obtained from the Department of Genetics, University of Leiden. This strain was most likely established from adults caught from decaying vegetables. It was reared in the same way as *D. subobscura*.

D. phalerata Meigen laboratory culture originated from *Phallus impudicus* mushrooms and was reared on fresh commercially grown mushrooms (*Agaricus hortensis*) at a temperature of 21°C.

Scaptomyza pallida laboratory culture originated from decaying *Beta vul-*

Table I. Taxonomic and Ecological Information on Host Species

Species[a]	Subgenus	Species group	Habitat	References[b]
D. melanogaster	Sophophora	*melanogaster*	Fermenting fruit	Carton, 1977; Carton & Kitano, 1981; Parsons & Stanley, 1981
D. simulans	Sophophora	*melanogaster*	Fermenting fruit	Parsons & Stanley, 1981
D. subobscura	Sophophora	*obscura*	Mainly fermenting fruit, but also sap fluxes, decaying fungi, & plants	Lakovaara & Saura, 1982; Atkinson & Shorrocks, 1977; Janssen *et al.*, 1988
D. hydei	Drosophila	*repleta*	Decaying "plants"[c]	Atkinson & Shorrocks, 1977; Parsons & Stanley, 1981; Wasserman, 1982
D. phalerata	Drosophila	*quinaria*	Fungi	Shorrocks, 1982; Janssen *et al.*, 1988
Scaptomyza pallida	Parascaptomyza		Decaying plants	van Alphen *et al.*, 1991

[a] *D. melanogaster* and, to a lesser extent, *D. simulans* are natural hosts for *L. boulardi*. All species except for *hydei* are reported as natural hosts for *L. heterotoma*.
[b] For a taxonomic overview we refer to Wheeler (1981); other references concern distribution and biology (e.g., habitat use).
[c] Closely associated with man; found in decaying vegetables, generally in low numbers.

garis (sugar beet) leaves and was reared on decaying beet leaves (Vet *et al.*, 1984)

Larval Kairomone Extractions

Extracts were prepared from *D. melanogaster*, *D. simulans*, *D. subobscura*, and *D. hydei* as follows. 1 ml of a condensed yeast suspension containing 0.25 g of yeast (*Saccharomyces cerevisiae* Hansen) was evaporated in a Perspex ring (diameter, 22 mm) placed on agar in a petri dish. Two hundred late first- to early second-instar larvae were added to this yeast spot and incubated at 25°C for 20 h. After incubation, the yeast, larvae, and 1.5 ml of water were mixed and filtered over moist filter paper (54-mm diameter). The filtrate, containing potential kairomone of about 5 larval equivalents per drop of 0.035 ml, was stored in 0.3-ml aliquots at −20°C until use. Dicke *et al.* (1985) showed that freezing does not damage the activity of *D. melanogaster* larval kairomone.

Extracts from *D. phalerata* was prepared by putting 200 first- to early second-instar larvae in 3 g of ground decayed *Agaricus hortensis* mushroom on agar in a small petri dish and incubating this at 25°C for 20 h. After incubation, the mushroom, larvae, and 1.75 ml of water were mixed and filtered. The yield (potential kairomone of about 5 larval equivalents per 0.035 ml) and further treatment were as for *D. melanogaster*.

Extracts from *S. pallida* was prepared by putting 200 first- to early second-instar larvae in 2 g decayed beet (*Beta vulgaris*) leaf pulp on agar in a small petri dish and incubating this at 25°C for 20 h. After incubation, the beet leaf pulp, larvae, and 1.75 ml of water were mixed and filtered. Again, this yielded potential kairomone of about 5 larval equivalents per 0.035 ml. Further treatment was as for *D. melanogaster*.

Control Solutions A control solution was prepared for each larval extract in exactly the same manner as above but without larvae.

Experimental Procedure

Testing Patch. Five-hundredths milliliter of yeast paste (yeast: water, 1:4) was applied with a micropipette to the center of a small (diameter, 5.5-cm) glass petri dish which was three-fourths-filled with agar. Then one drop (=0.035 ml) of defrosted larval extract (or control extract) was added to the yeast spot using a Pasteur pipette. With a sterile glass rod the spot was mixed and enlarged to 2 cm in diameter. Excess moisture was allowed to evaporate, leaving a thin, moist, but not wet film on the agar. The petri dish lid was replaced until the patch was used for the test (within 5 h).

Test Animals. All wasps (mated females) were between 8 and 11 days old at the time of testing. One day prior to the test, they were given a 10 to 25-min oviposition experience on a yeast patch with a surplus of late first- to early second-instar *D. melanogaster* larvae (per vial, up to five female wasps simultaneously).

Test Procedure. Experienced female wasps were allowed to acclimatize from 12.5 to 20°C for 1 to 2 h. Each female was placed into a single small glass vial (3.5 ml) for at least 10 min prior to the behavioral test. The wasp was allowed to walk out of the vial onto the agar surface. If the wasp did not initiate searching on the patch within 10 min, it was discarded from the experiment, but this happened in a negligible number of cases. Patch times were recorded starting at the point when the female walked onto the patch and stopping when she left the patch for more than 30 s. Both a control and a larval extract test were performed simultaneously with the same species of wasps within a sheltered enclosure on a laboratory bench. Each female was tested only once and 15 females were tested per treatment.

RESULTS

Figure 1 shows the responses of both parasitoid species to the filtrates of larva infested substrates and to the control filtrates of their uninfested substrates. Both parasitoid species can respond to kairomone. However, as predicted there were differences between their responses to different larval extracts. The gen-

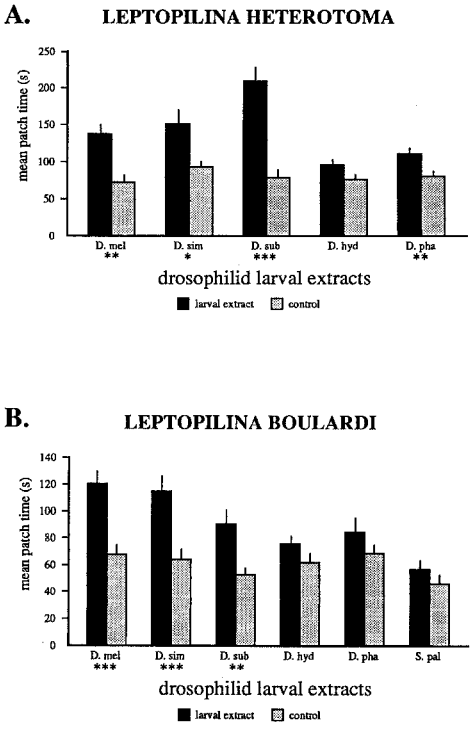

Fig. 1. Responses of a generalist and a specialist *Leptopilina* parasitoid species to extracts of host-infested (= larval extract) and uninfested (= control) substrates, measured as the mean time (with SE) females search on a patch with the extract added. A significant response to a larval extract is indicated by asterisks (Mann–Whitney U test between larval extract and its control): (*) $P < 0.05$; (**) $P < 0.01$; (***) $P < 0.001$. $n = 15$ for all treatments. (A) The generalist *L. heterotoma*. (B) The specialist *L. boulardi*.

eralist species *Leptopilina heterotoma* showed a significant response to the larval extract of all drosophilid species but *D. hydei* (Fig. 1a). Indeed, significantly more time was spent searching on patches that contained the larval extract compared to its control patch (Mann–Whitney U-test, $P < 0.05$). This confirms a similar bioassay (Vet, 1985a) where *L. heterotoma* significantly responded to the kairomone of *S. pallida* in decaying plant material.

Since the time spent on the control patches did not differ between the treatments (Kruskal–Wallis ANOVA, $P >> 0.05$), we compared the relative response to the different larval extracts (*D. hydei* excluded). There was no

significant difference in relative responses of *L. heterotoma* to the different host kairomones (Kruskal–Wallis rank sums, $P > 0.05$).

The results for *L. boulardi* show a more restricted response range (Fig. 1b). There was no response to extracts of *D. hydei*-, *D. phalerata*-, and *S. pallida*-infested substrates. Significant responses were limited to extracts of the fruit inhabiting species *D. melanogaster*, *D. similans*, and *D. subobscura*. There was no significant difference in relative response between these three treatments when tested with a Kruskal–Wallis ANOVA ($P = 0.07$). However, the response to *D. melanogaster* kairomone was significantly higher than that to *D. subobscura* kairomone when tested with a Mann–Whitney U test ($P = 0.026$).

DISCUSSION

The generalist parasitoid *L. heterotoma* and the specialist *L. boulardi* differ in the specificity of their response to drosophilid larval extracts. The generalist responds to extracts of five of six drosophilid species tested, while the response of the specialist was restricted to extracts of only three species, two of which are natural hosts found in fermenting fruit. Apparently *L. boulardi* does not respond to a general cue that is produced by any drosophilid larva that feeds in a substrate. These differences support the hypothesis that specialists need more specific information on the presence and suitability of their hosts than generalists, for which it may suffice to respond to more general cues while foraging (Vet and Dicke, 1992). As expected, *L. heterotoma* responds to kairomone of a wide range of drosophilid hosts from different taxonomic groups [also *S. pallida* (Vet, 1985a)] and living in different substrates (Table I). All host species *L. heterotoma* responds to are parasitized by this species in nature. Furthermore, there was no significant response ($P = 0.12$) to an extract of substrate infested with *D. hydei*; *L. heterotoma* has not been reported to use *D. hydei* larvae in nature. Although *D. hydei* has a cosmopolitan distribution (Wasserman, 1982), it does not seem to occur in the natural habitats of *L. heterotoma* (e.g., Shorrocks, 1982; Janssen *et al.*, 1988). Its occurrence is reported to be "associated with man" and decaying vegetable material is thought to be its major microhabitat (Table I). Laboratory experiments indicated that *L. heterotoma* does not readily accept *D. hydei* larvae for parasitization and survival in this host species is relatively low (van Lenteren, unpublished data).

The response of *L. boulardi* was restricted to *D. melanogaster* and *D. simulans*, both natural hosts, and to *D. subobscura*, an ecologically and taxonomically relatively closely related species. Laakovara and Saura (1982), who discuss the evolution of the *D. obscura* group (to which *D. subobscura* belongs), mention that, with respect to morphologic and allozyme patterns, the *D. obscura* group species is similar to the *D. melanogastar* group species (to

which *D. melanogaster* and *D. simulans* belong). Ecologically *D. subobscura* is related to *D. melanogaster* and *D. simulans* in that its microhabitat is mainly fermenting fruits, although it is less restricted to this type of substrate than the other two species (Shorrocks, 1977; Atkinson and Shorrocks, 1977; Shorrocks, 1982; Janssen *et al.*, 1988).

Chemical identification of the compounds in the host kairomone is needed to elucidate whether the specificity of the responses is reflected by the chemical composition of the cues. Dicke *et al.* (1985) isolated and purified kairomone of *D. melanogaster* but the chemical structure is still unidentified. Nemoto *et al.* (1987), who purified and compared kairomone components of larval feces of four phycitid moth species, hosts of the parasitoid *Venturia canescens*, reported that most compounds were common among species but that there were species specific quantitative differences in each of the components. If this is also true for the *Drosophila* parasitoid system, it would enable generalist natural enemies to use a general compound produced by many Drosophilidae and specialists to use quantitative differences in compounds to distinguish between species specific kairomones.

L. boulardi is a specialist at two trophic levels: it attacks just a few host species that occur in just one type of food substrate. This specialization is indeed reflected in the species' behavioral responses; females exhibit a clear preference for odors of their own substrate when tested in a choice situation (Vet 1985b), and as shown by the present results, their response to contact kairomones is limited to a few host species. It remains to be investigated whether the parasitoids can distinguish between the kairomone of the different species to which they responded. If so, kairomones may help them in assessing host identity and host presence, i.e., patch profitability.

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