

Drosophila parasitoid–host interactions: vibrotaxis and ovipositor searching from the host's perspective

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Two strains of *Drosophila* differing in host movement were simultaneously offered to a female parasitoid of either *Leptopilina heterotoma* or *Asobara tabida*. The number of encounters with the moving and nonmoving host strains was independent of larval movement for *L. heterotoma* whereas a highly significant effect of movement was found for *A. tabida*. This increased encounter rate of *A. tabida* with moving larvae resulted from the interaction of this parasitoid's searching strategy (vibrotaxis) and the polymorphic behaviour of the hosts. We conclude that differences in searching mode of two parasitoids of *Drosophila* larvae, *A. tabida* and *L. heterotoma*, can influence individual host susceptibility.

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Deux lignées de *Drosophila* distinctes par leur façon de se mouvoir ont été offertes simultanément à une femelle parasitoïde de *Leptopilina heterotoma* ou d'*Asobara tabida*. Le nombre de rencontres avec la lignée hôte mobile ou la lignée non mobile est indépendant des mouvements de la larve chez *L. heterotoma*, alors que les mouvements ont un effet très significatif sur *A. tabida*. L'augmentation de la fréquence des rencontres entre *A. tabida* et les larves en mouvement résulte de l'interaction entre la stratégie de recherche du parasitoïde (vibrotactisme) et le comportement polymorphe des hôtes. Il faut conclure que les différences dans le mode de recherche des deux parasitoïdes des larves de *Drosophila*, *A. tabida* et *L. heterotoma*, peuvent influencer la sensibilité d'un hôte au parasitisme.

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Introduction

The process of parasitizing a host is a sequential one. First, a parasitoid must find an appropriate host habitat. Having found a habitat, the host must be located within it. Finally, the suitability of the host must be evaluated before host acceptance (Doutt 1964). In the case of wasps parasitic on fruit flies, the parasitoid will oviposit an egg inside a larval host if the host is suitable. When such parasitization has been successful an adult wasp ecloses from the pupal case instead of a fly.

Vet and van Alphen (1984) have provided indirect evidence that the parasitoids *Asobara tabida* and *Leptopilina heterotoma* use different searching modes to detect host larvae within a food patch. *Asobara tabida* is thought to use vibrotaxis, vibration caused by host movement, as the host detection stimulus. This parasitoid shows a walk–stop locomotory pattern while searching within a food patch. After the host has been located through vibrotaxis, the ovipositor is used to probe it (van Alphen and Drijver 1982; Vet 1984). In contrast, *L. heterotoma* continuously walks over the substrate while rhythmically probing the surface with its ovipositor. This random probing of the ovipositor into the food patch is known as ovipositor searching (van Lenteren 1976). Can the behaviour of a host affect its susceptibility to parasitoids? The purpose of the present study was to determine whether differences in host locomotory behaviour had a significant effect on encounter rate with two different parasitoid species.

In the field, *Drosophila melanogaster* larvae feed on fermenting fruit where they are susceptible to different hymenopteran parasitoids. In temperate climates, such parasitoids are predominantly *Asobara tabida* Nees (Braconidae: Alysiinae) and

Leptopilina heterotoma Thomson (Eucoilidae). We decided to determine whether larval movement influenced host susceptibility since field-collected *D. melanogaster* larvae show a polymorphism in larval locomotory behaviour (Sokolowski 1980; Sokolowski *et al.* 1986); larvae of the "rover" phenotype show high locomotion while feeding in a food patch, whereas "sitter" larvae have low locomotory behaviour.

Differences in parasitoid searching modes suggest that when *A. tabida* is presented with a food patch containing a mixture of moving and motionless larvae it should be more successful at locating the moving (rover) host whereas *L. heterotoma* should show no difference in its ability to locate either host morph. To test this hypothesis unambiguously, we used two strains of *D. melanogaster* at two temperatures as the rover and motionless host strains: (i) the *WW* strain, which behaves as a rover at 20°C and 29°C and (ii) the *sh^{ts}* strain, a paralytic mutant strain which forages as a rover at 20°C but cannot move at 29°C (Grigliatti *et al.* 1973). At 29°C the predicted differences in host encounter rates should be shown; the host encounter rates at 20°C, where the two strains behave similarly, serve as a control for interstrain differences.

Materials and methods

Parasitoids

Asobara tabida (strain Sospel '83) was obtained from Chris Mollema, Department of Population Biology, University of Leiden, The Netherlands. The strain was initiated with 40–50 inseminated females collected in fruit-baited traps in the fall of 1983 in Sospel, France. This strain of *A. tabida* was chosen for the present study because it readily parasitizes larvae of *Drosophila melanogaster* (C. Mollema, unpublished data). *Leptopilina heterotoma* (strain Rosenburgh '81) origi-

TABLE 1. A comparison of location of *WW* and *sh^{ts}* strains of *D. melanogaster* by two parasitoid species, *Leptopilina heterotoma* and *Asobara tabida*

	No. of wasps tested (df)	Total no. of encounters with larvae		χ^2	<i>p</i>
		<i>WW</i>	<i>sh^{ts}</i>		
<i>Leptopilina heterotoma</i>					
Control, 20°C	8	185	182	4.1	ns
Treatment, 29°C	8	212	191	1.5	ns
<i>Asobara tabida</i>					
Control, 20°C	9	189	186	2.6	ns
Treatment, 29°C	9	224	33	74.2	<0.0001

nated from fruit-baited traps in a coppice wood in Voorschoten, The Netherlands. Van Strien-Liempt and van Alphen (1981) outline rearing methods for both parasitoids.

Hosts

Two strains of larvae of *Drosophila melanogaster* were used as hosts. The *WW* strain had been reared in the laboratory in Leiden for 20 years, having been initiated by crossing four wild-type strains (Bakker 1961). The shibire (*sh^{ts}*) is a temperature-sensitive paralytic mutant strain (Grigliatti *et al.* 1973). Larvae of this strain perform wild-type feeding and locomotory behaviour at temperatures less than 29°C (M. B. Sokolowski, unpublished data). At temperatures of 29°C and higher, *sh^{ts}* larvae are paralyzed and perform neither feeding nor locomotory movements. For the purpose of the present study it is important to note that at 20°C both *WW* and *sh^{ts}* larvae perform the rover pattern of foraging whereas at 29°C *WW* larvae are rovers and *sh^{ts}* show a motionless phenotype.

Host habitat

Hosts foraged on *Saccharomyces cerevisiae* (Hansen) (baker's yeast, Engedura, Gist Brocades, Delft, The Netherlands). The habitat consisted of a glass-covered petri dish (4.5 cm in diameter and 0.9 cm high) containing a smooth layer of 4% agar (4.5 cm in diameter and 0.3 cm deep) on top of which a yeast suspension (25 g living dry Engedura yeast per 100 mL H₂O) was thinly painted in a circular patch. Fifteen first-instar larvae 24 h post-hatching (at 20°C) of each of the *WW* and *sh^{ts}* strains were randomly placed on the yeast patch. One of the two strains of larvae had been feeding on yeast with carmine, which coloured its digestive tract red. In this way, the two strains of larvae could be readily distinguished under the microscope. Each strain was coloured in alternate experiments. Colouring with carmine does not influence larval encounter rate by *A. tabida* (van Alphen and Nell 1982) or *L. heterotoma* (Bakker *et al.* 1967).

Bioassay

Each set of experiments was performed at two temperatures: 29.5 ± 0.5°C (treatment) and 20 ± 0.5°C (control). Observations were made with the aid of a low-powered binocular microscope and a cool light source. Twenty-four hours before the experiment, all wasps were given oviposition experience by allowing them to search for and parasitize 24 h post-hatching larvae of both strains (van Lenteren 1976). The test procedure was as follows. One female of *A. tabida* or *L. heterotoma* was introduced into the test dish 5 min after the larvae were placed on the yeast patch. At 29°C this was a sufficient time for larvae of the *sh^{ts}* strain to become paralyzed. Behavioural observations were made so that the number of probes with the ovipositor directed at each strain (*WW* or *sh^{ts}*) was recorded. A probe resulted in either rejection or parasitization of the host. The number of rejections was defined as the number of times the wasp inserted her ovipositor into the larva without ensuing oviposition. Rejections occurred when a wasp probed a previously parasitized host. *Leptopilina heterotoma* and *A. tabida* are solitary parasitoids which lay only one egg per host larva; both species normally reject previously parasitized hosts. From earlier observations we know that, when the ovipositor remains inside the larva for 20 s in *L.*

heterotoma or 10 s in *A. tabida* we may assume that oviposition takes place. The total number of encounters was calculated to be equal to the number of rejections plus the number of parasitizations performed by one female wasp in an experiment. Larvae were neither removed nor replaced once they were parasitized. Either of the following two criteria were used to end the experiment: (i) the wasp left the yeast patch and did not return to it for at least 5 min or (ii) the wasp remained on the patch for more than 1 h. Eight trials for each of the two treatments (29°C and control (20°C) temperatures were performed with *L. heterotoma* and nine for each temperature were performed with *A. tabida*.

Results

The data in Table 1 strongly support our hypothesis. We found no significant difference in the total number of encounters of *L. heterotoma* with *WW* as compared with *sh^{ts}* larvae at both the control and treatment temperatures. The efficiency of ovipositor searching was not affected by differences in host locomotory behaviour at 29°C.

The pattern for *A. tabida* was strikingly different. When host locomotory behaviour was not polymorphic in the patch, *A. tabida* encountered both strains of host equally. At 29°C, however, when host behaviour was polymorphic, the difference in the total number of encounters was highly significant (*p* < 0.0001). The patch contained a mixture of "moving" and "non-moving" hosts. The number of encounters with the moving host (*WW*) was approximately seven times higher than with the nonmoving host (*sh^{ts}*).

Occasionally a *sh^{ts}* larva was mechanically moved by a *WW* larva that had crawled under it. At these times, *A. tabida* was seen to probe a *sh^{ts}* larva. Most of the 33 *sh^{ts}* encounters in the nine experiments with *A. tabida* at 29°C resulted from this type of puppetry.

In these experiments temperature was used as a tool to modify the behaviour of hosts in a patch from monomorphic (all rovers) to polymorphic (a mixture of rovers and motionless larvae). The effect of temperature *per se* on the total number of encounters with a parasitoid of either species was not significant.

The number of rejections, the number of times the wasp inserted her ovipositor into the larva without ensuing oviposition, showed the same pattern as was found for the total number of encounters (Table 1). The large number of rejections was due to the gradual increase in the number of parasitized hosts, which were rejected at subsequent encounters. At 20°C, *L. heterotoma* rejected 94 *WW* and 90 *sh^{ts}* hosts and at 29°C it rejected 116 *WW* and 116 *sh^{ts}* hosts. At 20°C, *A. tabida* rejected 95 *WW* and 82 *sh^{ts}* hosts. At 29°C the number of rejections was much higher for *WW* hosts than for *sh^{ts}* (130 compared with 13, $\chi^2 = 49.1$, *p* < 0.0001). Again, the only significant difference was in the

TABLE 2. Number of parasitizations by *Asobara tabida* and *Leptopilina heterotoma* of *WW* and *sh^{ts}* strains of *D. melanogaster*

	No. of wasps tested (df)	Total no. of parasitizations of larvae		χ^2	p
		<i>WW</i>	<i>sh^{ts}</i>		
<i>Leptopilina heterotoma</i>					
Control, 20°C	8	132	92	1.4	ns
Treatment, 29°C	8	96	75	1.2	ns
<i>Asobara tabida</i>					
Control, 20°C	9	94	104	1.0	ns
Treatment, 29°C	9	94	20	26.6	<0.005

greater number of parasitizations by *A. tabida* of *WW* hosts compared with *sh^{ts}* hosts at 20°C (Table 2).

Discussion

In the present study we show that differences in host behaviour have a profound effect on the total number of encounters and parasitizations by the larval endoparasitoids *A. tabida* but not on those by *L. heterotoma*. We believe that this difference reflects the two distinct searching strategies, vibrotaxis and ovipositor searching, used by these parasitoids. We must conclude in answer to our earlier question that indeed the behaviour of a host can affect its susceptibility to a parasitoid. Specifically it is the interaction between *Drosophila* larval locomotory behaviour and *A. tabida*'s search mode of vibrotaxis that influences host susceptibility. To further develop these ideas we will discuss the relevant literature on vibrotaxis, address whether our findings can be extended to the rover-sitter polymorphism, and finally, briefly discuss the evolutionary implications of these findings.

Glas and Vet (1983) studied host location by *Diachasma alloeum*, a parasitoid of *Rhagoletis pomonella*, the apple maggot fly (which mostly lays one egg in small fruit such as hawthorn). Nonrandom ovipositor probing occurred on a fruit containing a moving larva; random probing occurred on a fruit containing a larva previously paralyzed by a wasp. They concluded that *D. alloeum* primarily detects individual larvae by vibrotaxis. Van Alphen and Janssen (1982) studied host-species preference of *A. tabida*. They observed two potential host "defence" strategies: (i) after detection of a parasitoid some species of larvae stayed motionless and (ii) others performed vigorous wriggling, turning about their axis when probed with an ovipositor. Both of these behaviours could potentially minimize parasitization by *A. tabida*. A. Janssen (personal communication) suggested that the vigorous wriggling behaviour may cause the wasp to cease searching for hosts and begin elaborate preening movements.

In the present study we chose *WW* and *sh^{ts}* strains of *D. melanogaster* as hosts to maximize the differences in host movement so that any subtle differences in host detection by the two parasitoid species would be measurable. Can we expect our results to parallel those we would find if *sh^{ts}* was replaced by a sitter strain? The qualitative differences between the rover, sitter, and *sh^{ts}* phenotypes are as follows: (i) rover larvae perform high levels of locomotory and feeding behaviour, (ii) sitter larvae display low levels of locomotory behaviour and high levels of feeding, and (iii) paralyzed *sh^{ts}* larvae do not perform locomotory or feeding movements. The major difference be-

tween *sh^{ts}* and sitter strains is in the amount of feeding movement. We also know that locomotory and feeding behaviour are under independent genetic control (Sokolowski 1980). We do not know, however, how much both feeding and locomotory movements contribute to *A. tabida*'s ability to detect hosts through vibrotaxis. If locomotory movements are significantly more important than feeding ones then *sh^{ts}* may be a good simulation for a sitter strain. If the reverse is true, then the results of the present study using rovers and *sh^{ts}* will not predict the results that would be obtained with rovers and sitters. The second reason for caution about extending our results to rovers and sitters is that differences between rovers and sitters in locomotory behaviour are stronger in the second and third instars than in the first instar, which is the stage of larva used for parasitization in the present study. Over evolutionary time, however, we expect any small differences in the host detection of rovers as compared with sitters to be reflected in changes in frequency of the two morphs.

Recently Carton and David (1985) have shown greater parasitization rates of *Leptopilina boulardi* on strains of nondigger compared with digger *D. melanogaster* larvae. Their digger strain shows the rover phenotype whereas their nondigger strain shows the sitter phenotype. T. C. J. Turlings (unpublished data) found that *A. tabida* parasitizes hosts that are found deeper in the medium more frequently than *L. heterotoma*. Vet and Bakker (1985) have demonstrated that *L. boulardi* has a host detection strategy intermediate to those of *A. tabida* and *L. heterotoma*; it uses both vibrotaxis and ovipositor searching. These studies and the present one demonstrate that the host population should not be assumed to comprise homogeneous behavioural clones.

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