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HOST DISCRIMINATION IN A GREGARIOUS PARASITOID *NASONIA VITRIPENNIS* (WALKER) (HYMENOPTERA: PTEROMALIDAE)

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INTRODUCTION

Nasonia vitripennis (Walker) is a parasitoid on the pupa but within the puparium of a number of muscoid diptera. Wylie (1958) showed that the female had no power of discrimination between the parasitized and the unparasitized in her oviposition on pupae of *Musca domestica* when parasitoid eggs were present, but avoided oviposition on dead hosts or those containing well-developed parasitoid larvae, pupae or adults. Discrimination was only possible after the ovipositor had drilled through the puparium and made contact with the pupa within. Sense organs have been described on the ovipositor by Fulton (1933), Walker (1937), Clausen (1940), Varley (1941), Edwards (1954), Carthy (1958), Doutt (1959), Fisher (1959), Patton (1963), Quendhau & Hubsch (1964), and Weseloh (1969) and Dethier (1947), using an ichneumon *Nemeritis canescens*, suggested that these sense organs were chemoreceptors. Ganesalingham (1969) using the electron microscope showed a nerve supply to small plate-like structures in the same species. Since no detailed examination of the process of host discrimination has been made in a chalcid, the present study was undertaken. Since *Nasonia vitripennis* is a gregarious parasitoid and more than one clutch of eggs will develop in a single host, coupled with the fact that the eggs are laid on the host pupa and not inside it, it was considered worth re-investigating Wylie's (1958) results as a preliminary and then examining some of the factors involved in this discrimination.

MATERIALS AND METHODS

Nasonia vitripennis was maintained in culture on puparia of *Lucilia* sp. and *Calliphora* sp. as described by Whiting (1955). For electron microscopy, the ovipositor was vivisected from the female's abdomen and fixed, either in 5% cacodylate-buffered glutaraldehyde followed by 2% osmium tetroxide, buffered to pH 7·4 with veronal acetate (Sabatini, Bensch & Barrnett, 1963), or in 1% osmium tetroxide alone (Palade, 1952). The material was dehydrated in graded cold ethanols and embedded on Epon 812 or Araldite (Luft, 1961). Sections were cut on a Huxley ultratome and those with grey or silver interference colours mounted on copper grids. The sections were double-stained with 2% aqueous solution of potassium permanganate (7 min) followed by lead citrate (7 min) (Reynolds, 1963) and examined in an Akashi Tronscope 50.

For examination in the stereoscan microscope, dissected ovipositors were mounted on studs with silver paint as adhesive and then coated with gold palladium. In some

specimens the stylets were teased away from the sheath so that their inner surfaces, normally lining the egg canal, could be examined.

For an electrophoretic examination of the haemolymph of parasitized and unparasitized hosts, 3-day-old puparia were used. Half of these were untouched as controls and the other half were placed in 3 in × 1 in specimen tubes with female *Nasonia vitripennis* which were 3 days old, during which time they were given host puparia and then deprived of hosts overnight prior to the experiment. The ratio of puparia to parasitoids was 1:1 and the association lasted for 3 h. Experimental and control puparia were kept together for 3 days at 20 °C, after which they were dissected and the larvae of *N. vitripennis* were removed from the parasitized puparia. Samples of five pupae taken either from the parasitized or unparisitized puparia were homogenized in 1 ml distilled water, centrifuged and stored at 0 °C.

For disk electrophoresis a 7% running gel solution at pH 9 was polymerized in 7·5 × 0·5 cm glass tubes for 30 min. A spacer gel solution at pH 6·8 was then placed on top and left for 90 min in cool fluorescent light for polymerization to occur. Samples were placed directly on top of the spacer gel in the form of a crescent. Electrophoretic runs were continued for 90 min in a refrigerator using trisglycine buffer at pH 8·3 diluted × 10 before use. The current used was 2·5 mA per tube. The gel columns were stained for 30 min in 1% amido black in 7% acetic acid, followed by destaining at room temperature in 7% acetic acid for 36 h.

Methylene blue, Lowit's method and Viallane's method were used to stain nerves *in situ*.

OBSERVATIONS

It is now generally accepted that there are two sources of stimulus left on a host by one parasitoid, which can be detected by other parasitoids of the same species: first an external stimulus, the 'taste smell' of Salt (1937) or 'spoor effect' of Flanders (1951); and secondly, by the internal stimulus. The latter is only detectable after insertion of the ovipositor. Salt (1937) showed that the 'taste smell' factor operated in *Trichogramma evanescens* Westw. but Wylie (1958) showed that this did not operate in *Nasonia vitripennis*. He described an internal stimulus in this insect, but gave no detailed information concerning when it occurred or what it might be. The following experiment was therefore carried out in which the parasitoid was given the choice of acceptance or rejection, without alternative puparia as in Wylie's experiments.

(1) *Examination of discrimination*

Newly emerged females were provided with host puparia for 3 days and then deprived for 12 hr overnight. In each of a series of 3 in × 1 in specimen tubes were placed five healthy host puparia and five female *N. vitripennis*. After 3 h the parasitoids were removed thus leaving the host puparia with eggs laid in them. These 'parasitized' puparia were then presented for a period of 3 hr to fresh parasitoids which had been provided with host puparia previously for 3 days since their emergence from a host puparium, and had been starved overnight at periods of 12, 24, 36, 48, 60 hr, 3, 6, 9, 12 and 18 days after their initial parasitization. In each instance 3 in × 1 in specimen tubes were used and five host puparia were placed with five parasitoids. After the

Table 1. *Results of discrimination experiments*

Time after first oviposition	No. of pupae examined	No. of pupae superparasitized	Percentage superparasitized	Drilling observed
12 h	146	125	85·61	+
24 h	144	120	83·33	+
36 h	147	120	81·63	+
48 h	148	115	77·70	+
60 h	150	99	66·00	+
3 days	204	4	1·97	+
6 days	85	None	Zero	+
9 days	90	None	Zero	+
12 days	85	None	Zero	+
15 days	90	None	Zero	+
18 days	90	None	Zero	+

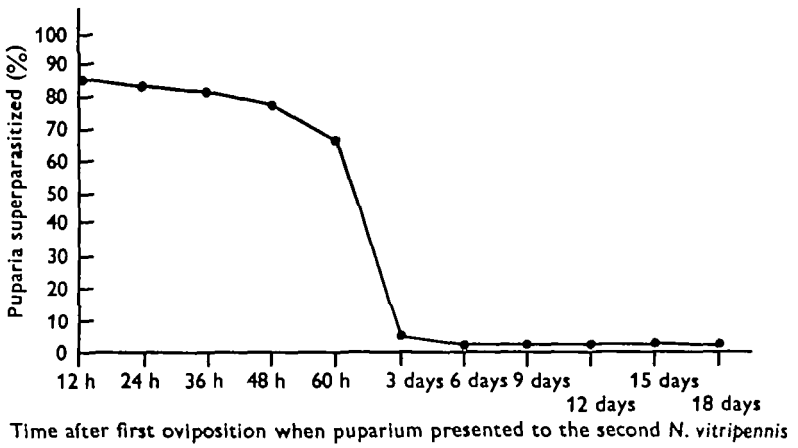

Fig. 1. Graph showing the percentage of offered parasitized puparia which were superparasitized at different times after the initial parasitization.

parasitoids had been removed, the puparia were opened and those containing a fresh clutch of eggs were noted. These puparia had thus been superparasitized. In each experimental period fresh parasitoids were used, so that factors of age and nutrition could be kept constant.

The results of this experiment are shown in Table 1 and Fig. 1. They showed that initially there is virtually no discrimination between previously parasitized and unparasitized host puparia by the females of *N. vitripennis*. At 60 hr there is a slight discrimination and then at 3 days almost 100 % discrimination. Examination of parasitized puparia at this stage showed that the melanization, resulting from stinging (Ratcliffe & King, 1967), had spread over the body surface and the heart had stopped beating. Thus, marked changes had occurred in these puparia, compared with unparasitized puparia of the same age. In each instance the decision to lay or not, that is to discriminate, came only after drilling had occurred. Thus it seems logical to suppose that discrimination depends upon an examination by the ovipositor tip of the inside of the host puparium and possibly the tissues of the pupa.

(2) *The ovipositor tip*

The structure and mode of action of the ovipositor has been described by Snodgrass (1933) in *Megarhyssa lunator* F. and *Tremex columba* (L), by Abbott (1934, 1935) in *Thalessa leucographa* (Grav), by Baumann (1924) in *Apis mellifera*, by Zander (1911) and Snodgrass (1931) in *Monodontomerus dentipes* (Dalman), by Bucher (1948) in *Euchalcidia caryobori* (Hanna), by Hanna (1934), in *Dahlbominus fuscipennis* (Zett), by Wilkes (1965) in *Eupelmus urozonus* (Dalman), by Delanoue & Arambourg (1965) in *Habrocytus cerealellae* (Ashmead), by Fulton (1933) in Mymaridae, by King & Copland

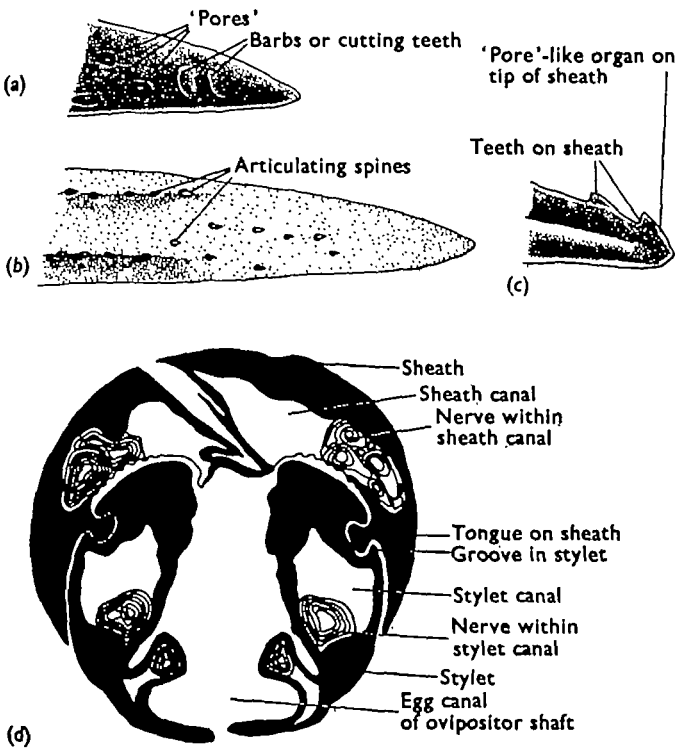

Fig. 2. Diagrams derived from stereoscan pictures:
(a) Outer surface of stylet tip showing teeth or barbs and the 'pores'.
(b) Inner surface of the stylet, which faces the egg canal. Articulating spines present.
(c) Tip of the sheath showing prominent teeth and a single pair of terminal sense organs.
Diagram derived from electronmicrographs:
(d) transverse section of the ovipositor shaft showing sheath and stylet canals with nerves within them. Section approximately 0·12 mm from tip of ovipositor shaft.

(1969) and in *Nasonia vitripennis* by King (1959). The gross structure of chalcid ovipositors has been described by Domenichini (1953) and Nikolskaya (1963). Varley (1941) described a number of sense organs on the ovipositor tip of *Eurytoma curta* (Walk). He said that at the distal end of each ventral valvula there were three slender tubules from the lumen of the valvula, a series of about thirty sensilla on the ventral valvula and several other structures. In addition to discrimination the ovipositor must drill into the host, inject venom, form a feeding tube and deposit the eggs within the

host puparium (King & Ratcliffe, 1969). The origin of the ovipositor has been discussed by Snodgrass (1933) and Smith (1970).

The shaft of the ovipositor consists of a sheath, formed by the two inner valvulae, and a pair of stylets formed from the ventral valvulae. Distally the sheath valvulae meet in the mid-line, the right one then overlaps the left and eventually fuses with it. Thus the ovipositor shaft has three components distally and four proximally. A ridge along each component of the sheath engages a groove on one of the stylets. Thus the stylets can slide freely along the main axis of the shaft. Normally one or both stylets protrudes beyond the tip of the sheath. The distal end of the sheath has several prominent teeth and at the tip a pair of small plate-like structures which are perhaps sensory. The stylets each have two small teeth at the end in the outer surface, and behind each of these an articulating spine. Similar spines occur at intervals along the outer surface.

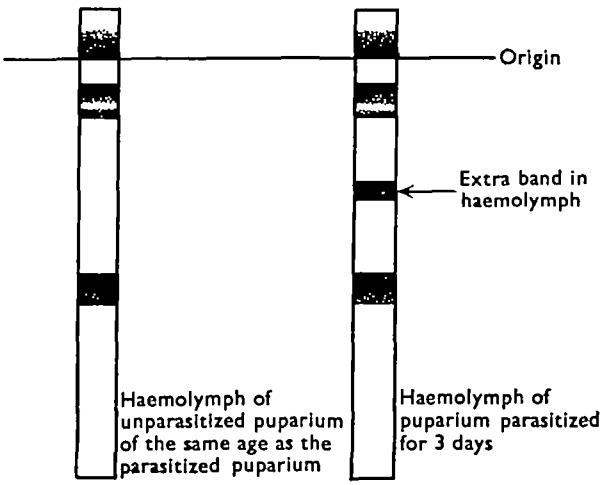

Fig. 3. Diagram of the gel columns resulting from disk electrophoresis experiments. The major bands present in the haemolymph of unparasitized host pupae are compared with those from the haemolymph of parasitized pupae. The latter has an extra conspicuous band.

Proximal to the spines there are three depressions in the cuticle. Each has a thin membrane and a central protuberance (Fig. 2). These were described as 'pores' by Fulton (1933), Varley (1941) and Edwards (1954). Stains, including methylene blue, Lowit's and Viallane's which are supposedly specific to varying degrees for nerves, give a positive reaction in the small ducts from the central canal of the stylet leading to these structures. The central duct also contains some positive strands. Electron microscope profiles indicate that nerves pass down the stylet canals and branch near the tip, presumably going to these 'pores'.

The position of the 'pores' and articulating spines behind the stylet teeth, and of the other spines on the inner surface of the stylet, means that they are protected from possible damage during the drilling process; and, during that process, the insect will always know whether the stylets are in touch with material, in a space, or how deeply the shaft has penetrated. The 'plate-shaped' structures and the 'pores' are similar to those described on the ovipositor of *Nemeritus canescens* by Ganesalingham (1969).

(3) *Composition of host haemolymph*

In the electrophoresis experiments there was no change in the haemolymph of a newly parasitized host puparium but, after 3 days, several extra bands showed up on the gel (Fig. 3). Thus chemical changes occur in the blood of the host following oviposition by *Nasonia vitripennis*; whether this results from larvae feeding, or a substance injected during oviposition, has not been determined. The present study necessitated only the demonstration of a difference in the chemical composition of the haemolymph of parasitized and unparasitized hosts, which was very pronounced (Fig. 3).

DISCUSSION

Varying degrees of discrimination between parasitized and unparasitized hosts have been recorded in a number of entomophagous endoparasitoids. It has been observed in: *Limerium validum* (Cres.) (Timberlake, 1912), *Spalangia muscidarum* Richardson (Pinkus, 1913), *Dacnusa areolaris* (Nees) (Haviland, 1922), *Platygaster hiemalis* (Hill, 1926), *Cardiochiles nigriceps* Vier. (Chamberlin & Tenhet, 1926), *Melittobia acasta* Walk. (Thompson & Parker, 1927), *Collyria calcitratar* (Grav.) (Salt, 1932; Walker, 1937) *Ascogaster quadridentata* Wesm. (Rosenberg, 1934), *Trichogramma evanescens* Westw. (Salt, 1934, 1936; Laing, 1938), *Telenomus ulyetti* Nixon (Jones, 1937), *Telenomus gifuensis* Ash (Hidaka, 1958), *T. basalis* Woll. (Wilson, 1961), *Microplectron fuscipennis* (Zett), *Oencyrtus kuvanae* (How.) (Lloyd, 1938) and *Caraphractus cinctus* (Jackson, 1966).

Some parasitoids can detect the presence of an egg or eggs laid by another member of their species within the host. This has been observed in *Platygaster hiemalis* (Hill, 1926), *Cardiochiles nigriceps* (Chamberlin & Tenhet, 1926), *Ascogaster quadridentata* (Rosenberg, 1934), *Spathius critolous* (Ayyar, 1941), *Mastrus carpocapsae* (Lloyd, 1956), *Horogenes chrysostictos* (Fisher, 1961 *a*), *Pseudeucoila bochei* (Bakker, Bagchee, van Zwet & Meelis, 1967). Many species however are unable to detect the presence of the egg stage within the host but later stages are detectable. This has been observed in *Microplectron fuscipennis* (Zett) (Ullyett, 1936), *Ooencyrtus kuvanae* (How) (Lloyd, 1938), *Angitia cerophaga* (Grav) (Lloyd, 1940), *Diadromus subtilicornis* (Grav), *D. collaris* (Frav) (Lloyd, 1942), *Nemeritis canescens* (Grav) (Fisher, 1961 *a*, *b*), *Spathius critolous* (Ayyar, 1941). *Nasonia vitripennis* falls into this category and can discriminate when hosts contain developing parasitoids older than 2½ days. Though Andrewartha & Birch (1954) assumed that ectoparasites would not recognize parasitized hosts from unparasitized hosts, a discriminative ability in some ectoparasites was observed by a few workers *Spathius critolous* Nixon (Ayyar, 1941); *Mastrus carpocapsae* Cush (Lloyd, 1956). The eggs of *Nasonia vitripennis* hatch in 24 hr and there are second instar larvae present when the discrimination occurs. When an ovipositor enters an already parasitized puparium the chances are against it piercing a parasitoid larva because of its relatively small size. The results of the electrophoresis experiments showed that, after parasitization, changes occur in the composition of the host haemolymph. This is similar to the situation described in *Nemeritis canescens* by Ganesalingham (1969). At the time when discrimination occurs the heart of the host has usually ceased beating

and the dark spot around the point of ovipositor entry has spread over the surface of the pupa. Thus the physical and chemical condition of a parasitized host differs from that of an unparasitized. Although the female *Nasonia vitripennis* makes a thorough examination of the host puparium with its antennae before drilling (Jacobi, 1939; Edwards, 1954), unless there is an exit hole through which parasitoids have emerged previously, the female is unable to discriminate until the ovipositor has entered the host (Wylie, 1958). A similar phenomenon has been described in *Aphelinus semiflavus* How. (Hartley, 1922), *Stenobracon deesae* (Narayanan & Chaudhuri, 1954), *Aphelinus diaspidis* How. (Quale, 1911), *Cheiloneurus noxius* Com. (Weseloh, 1969). Ganesalingham (1969) described sense organs in the ovipositor shaft, particularly the sheath of *Nemeritis canescens* and, using the electron microscope, showed a nerve supply in the sheath. This of course is a requisite of a sense organ. Numerous articulating spines are present on the inner surface of the stylets, at intervals on their outer surface and behind the teeth, which would make ideal touch receptors. The sheath has two small plate-like organs at the end, similar to those in *N. canescens*; though in that insect they are present in greater numbers.

The three 'pores' on the end of each stylet (Fulton, 1933; Varley, 1941; Edwards, 1954) are not true pores but have a membrane over them slightly lower than the normal stylet surface. The centre of the 'pore' membrane is raised into a small knob. The channels leading to this 'pore' gave a positive reaction with several nerve stains, and transverse sections of the stylets showed that nerves are present. The evidence thus suggests that these 'pores' are sense organs. Dethier (1947) working with *N. canescens* showed that the ovipositor reacted when placed in different solutions. Wiley (1958) stated that in *Nasonia vitripennis* the female's mouthparts always move vigorously for several seconds after the ovipositor enters a puparium with a living host inside which had not pupated, or after it pierces a pupa on which she later lays (and is thus suitable) but not after it enters a puparium whose contents are neither imbibed nor laid on. A similar behaviour pattern involving proboscis extension has been observed in several dipterous species when sugar solutions are applied to chemosensory hairs on their legs (Dethier, 1955). Since there are both physical and chemical changes in a parasitized host puparium after $2\frac{1}{2}$ days either chemoreceptors or sensitive touch receptors at the tip of the ovipositor could detect the change. Some sense organs are clearly articulating tactile setae and perhaps the larger pores on the stylets; and small ones on the sheath are chemoreceptors, though these, having a thin membrane, could equally well be tough receptors. Experimental evidence, however, is in favour of some chemosensitive structures.

The value of this discrimination to the parasitoid is perhaps to prevent the second clutch being eaten or being unable to feed because the host has been rendered unsuitable in some way by the first clutch. Since discrimination does not occur during the first 60 h it is unlikely that an avoidance of overcrowding is the purpose since extensive superparasitism can occur in the first 60 hr.

SUMMARY

1. Females of *Nasonia vitripennis* cannot distinguish newly parasitized hosts from unparasitized ones but after 60 hr parasitization the parasitized puparia are rejected as oviposition sites.

2. The sheath and stylets of the ovipositor have nerves passing down their canals leading to several types of receptor at the tip of the ovipositor shaft.

3. Differences are detectable in the chemical composition of host haemolymph after 3 days parasitization as compared with the unparasitized condition. These may be the factors bringing about discrimination.

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