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HOST MARKING: SOURCE OF A SUBSTANCE THAT RESULTS IN HOST DISCRIMINATION IN INSECT PARASITOIDS

BY

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Two species of female parasitoids, *Cardiochiles nigriceps* and *Micropletis croceipes* (*Hymenoptera: Braconidae*) were found to discriminate between superparasitized and nonparasitized hosts. The source of the host marking pheromone was found to be the alkaline (Dufour's) gland in both species.

The ability of insect parasitoids to distinguish between parasitized and nonparasitized hosts (SALT, 1934; ULLYETT, 1945) and pre-searched and unsearched areas (PRICE, 1970) has been described. Such discriminatory ability allows for optimum survival of progeny and utilization of hosts and is considered to be a common behavioral element among parasitoids (DOUTT, 1959).

SALT (1937) reported that female *Trichogramma evanescens*, an egg parasitoid, could detect odors left by another female on the host egg two days earlier. The odor could be removed by water from glass beads over which the females had previously walked and the water solution (when painted on eggs) would protect the egg from further parasitism. The source of the odor was undetermined. Similar results have been reported for *Telenomus sphingis*, another egg parasitoid (RABB & BRADLEY, 1970). In several larval parasitoids both physical (ULLYETT, 1936) and chemical (FISHER & GANESALINGAM, 1970) changes in the host have been suggested as rendering a host less susceptible to further parasitism.

The purpose of this investigation was to determine the possibility of host discrimination by two species of braconid parasitoids, *Cardiochiles nigriceps* VIERECK and *Micropletis croceipes* (CRESSON). Also the possible source of the host marking pheromone was investigated.

Materials and Methods

The two braconid parasitoids used in this investigation were reared in the laboratory from their host the tobacco budworm, *Heliothis virescens* (F.). The adult parasitoids were maintained in the laboratory on a solution of honey and water. The hosts, *H. virescens* were reared in the laboratory on artificial medium (VANDERZANT *et al.*, 1962).

The ability of both species of female parasitoids to discriminate between previously parasitized and nonparasitized larvae was tested by randomly placing ten control (nonparasitized) and ten parasitized larvae on a piece of filter paper in a 10 cm. Petri dish. Control larvae were marked with a small drop of a water soluable dye for easy identification. Two female parasitoids of one species were introduced into the Petri dish and their choice for oviposition recorded. The attacked individuals were removed immediately after stinging (oviposition) and replaced with a similarly treated individual so that a 1:1 ratio was maintained at all times. This process was repeated until 36 observations (stinging ovipositional attacks) were made. A statistical analysis using the sign test (DIXON & MASSEY, 1968) was applied. A value of 12 or less is significant at the 95% level of probability. Replicate tests with each species of parasitoid were carried out.

In the second experiment superparasitized larvae (larvae which were stung two or more times by the same parasitoid species) were used in place of the larvae parasitized once. Larvae were superparasitized by placing 20 second instar larvae in a Petri dish with several female parasitoids of the same species. The parasitoids were replaced every 30 minutes for a period of two hours and were observed to ensure that superparasitism occurred. Following superparasitism ten superparasitized larvae were marked with a small drop of water soluable dye on the head and were randomly placed on a piece of filter paper in a 19 cm. Petri dish. Ten control larvae were marked with dye on the posterior abdomen and were randomly placed in the same Petri dish. Two female parasitoids of the same species were introduced and their choice for oviposition recorded as described above.

A third set of experiments were performed to determine the source of the marking pheromone for both species. The reproductive system was removed from adult female parasitoids by gently grasping and pulling the last two abdominal segments and ovipositor from the rest of the abdomen. The alkaline gland (Dufour's), poison gland and calyx region of the ovary were isolated. Concentrated solutions of the poison gland or calyx region of the ovary were prepared by macerating ten to fifteen glands in an equal volume of physiological saline (PRINGLE, 1938). Due to the oily nature of the alkaline gland, these glands

were macerated in a 15 % acetone water solution which appeared to disperse the oil. Second instar *H. virescens* larvae were treated by dipping each larvae in a solution of the contents of each organ to be tested. Ten control (dipped in saline or 15% acetone water solution) and ten larvae treated with the gland solution were marked with dye as described and placed randomly in a Petri dish. Two female parasitoids of the same species as the source of the gland extract were introduced into the Petri dish and their choice for oviposition recorded as described above.

Results and Discussion

The results (table 1) show no evidence of discrimination by either species of parasitoid between nonparasitized and larvae parasitized once by the same species of parasitoid. However, following superparasitism a significant degree of discrimination was found by both parasitoids against hosts superparasitized by their own species (table 1). The marking pheromone appeared to act as a repellent since adult female parasitoids moved away from treated larvae.

TABLE 1

The acceptance of nonparasitized, parasitized and superparasitized hosts by two species of braconid parasitoids.

Parasitoid	No. larvae attacked (a)	
	nonparasitized	parasitized
Cardiochiles nigriceps	20	16
	18	18
Micropletis croceipes	19	17
	21	15
	nonparasitized	superparasitized
Cardiochiles nigriceps	28	8*
	29	7*
Micropletis croceipes	27	9*
	26	10*

(a) Total of 36 observations for each treatment.

* Significant at the 95 % level of probability or greater using the sign test.

No changes in the behavior of the host was observed following parasitism. Pheromone specificity was indicated since hosts superparasitized by one species of parasitoid were not discriminated against by the second species of parasitoid.

In an effort to determine the source of the host marking pheromone, larvae were treated with solutions of the contents of various glands associated with the female reproductive system. The alkaline gland solution was the only solution to show a significant degree of activity of the glands investigated (table 2). Both species of parasitoids attacked the control hosts in preference to hosts treated with the alkaline gland solution of their respective species. There was no significant discrimination against larvae treated with either the poison gland or calyx fluid from the ovary of either species of parasitoid by the respective female parasitoid. Interspecific activity of the glands was not investigated since no interspecific discrimination was noted using superparasitized larvae.

TABLE 2

The acceptance of host larvae topically treated with solutions of the accessory reproductive glands of two species of parasitoids.

Treatment	*No. larvae attacked* (a) Control	Treated
Cardiochiles nigriceps		
Poison gland	21	15
	18	18
Calyx fluid	19	17
	20	16
Alkaline gland	30	6*
	35	3*
Micropletis croceipes		
Poison gland	17	19
	19	17
Calyx fluid	21	15
	20	16
Alkaline gland	26	10*
	26	10*

(a) Total of 36 observations for each treatment.

* Significant at the 95 % level of probability or greater using the sign test.

The selective advantage of a host marking pheromone appears obvious. In the case of egg parasitoids (SALT, 1937; RABB & BRADLEY, 1970) one ovipositional attack resulted in discrimination by other female parasitoids. However, in this study discrimination occurred only after superparasitism. Such a situation may be of particular advantage to larval parasitoids such as *C. nigriceps* and *M. croceipes*.

Many of the parasitoids potential larval hosts are not accessible to them because many of the hosts feed within the fruiting structures of the plant and are out of reach of the parasitoid. Those hosts which are accessible may thwart the females stinging attack due to the host's aggressive response (HAYS & VINSON, 1972). Thus an allowance for more than one stinging attack ensures that available hosts will be parasitized. As the number of stinging attacks increases the probability that one will have been successful in oviposition approaches certainly and the advantage of further attacks approaches zero. A decrease in attractiveness of these hosts as further ovipositional sites due to a build-up of marking pheromone would become more important.

RÉSUMÉ

Marquage des hôtes : la source d'une substance qui permet la discrimination des hôtes par les insectes parasites.

Deux espèces de parasites femelles, *Cardiochiles nigriceps* et *Micropletis croceipes* (*Hymenoptera : Braconidae*), sont capables de distinguer les hôtes superparasités et les nonparasités. On a trouvé que la source de la pheromone qui marque les hôtes est la glande alkaline de Dufour dans les deux cas.

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