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Host discrimination in *Dinocampus coccinellae* (Hymenoptera: Braconidae), a solitary parasitoid of coccinellid beetles

Takashi Okuda* and Piotr Ceryngier[1]

Department of Insect Physiology and Behavior, National Institute of Sericultural and Entomological Science, Tsukuba, Ibaraki 305-8634, Japan
[1] Institute of Ecology, Polish Academy of Sciences, Dziekanow Lesny near Warsaw, 05–092 Lomianki, Poland

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Abstract

The ability of a solitary parasitoid, *Dinocampus coccinellae* (Schrank) to discriminate between a parasitized and unparasitized host, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) was tested in the laboratory. A *D. coccinellae* female wasp was placed together with a host beetle and allowed to parasitize 3 successive times, and the time interval between these parasitizations was measured. The wasp successfully parasitized the host within 25 s on average and avoided superparasitism for 155 s after the 1st parasitization. The period of rejection between the 2nd and 3rd parasitization further extended to about 4 min. Furthermore, the behavioral pattern of a parasitoid wasp and 3 host beetles monitored by a video camera confirmed the host discrimination of *D. coccinellae*. The wasp was unable to discriminate a host recently parasitized by her genetically identical sister. A possible reason for weak host discrimination of *D. coccinellae* is discussed.

Key words: Superparasitism, host discrimination, solitary parasitoid, *Dinocampus coccinellae*, *Coccinella septempunctata*

INTRODUCTION

Parasitoids are often able to discriminate between parasitized and unparasitized hosts by using internal or external markers left by the previous wasp (Hofsvang, 1990). In solitary parasitoids, only one individual can successfully develop in a given host. However, superparasitism, i.e., the parasitization of already parasitized hosts has commonly been recorded (van Alphen and Visser, 1990; Speirs et al., 1991). *D. coccinellae* is one such solitary parasitoid frequently reported to superparasitize its adult hosts (Ogloblin, 1924; Balduf, 1926; Sluss, 1968; Maeta, 1969; Wright and Laing, 1978). Little is known, however, about host discrimination in this species (Ceryngier and Hodek, 1996). In the present paper, experimental investigations on host discrimination and host acceptance behavior are reported for the *D. coccinellae–C. septempunctata* system.

MATERIALS AND METHODS

Experiments were conducted at two sites, Ceske Budejovice in the Czech Republic and Tsukuba in Japan, in 1996 and 1997, respectively.

Insects. *D. coccinellae* were obtained from naturally parasitized adults of *C. septempunctata* which were collected from the field and used to maintain a laboratory culture. *D. coccinellae* is a parthenogenetic thelytokous species and females emerge from pupae having mature ovaries. Wasps obtained from the laboratory culture were used for parasitization immediately after emergence. They were kept at 20°C, 12 h light–12 h dark cycles and fed with 10% sucrose. *C. septempunctata* adult beetles used for parasitoid culture as well as those used for experiments were fed with aphids, *Acyrthosiphon pisum* (Harris).

Parasitoid wasps were conditioned before use in the experiments. Freshly emerged virgin wasps were allowed to parasitize 10 to 20 host beetles. The next day these wasps were again allowed to parasitize 10 host beetles just prior to experiments.

The wasp pursues the host beetle in its typical posture, i.e. with the abdomen flexed to the front between the legs and the ovipositor extending forward of the head. Egg deposition is easily recog-

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* To whom correspondence should be addressed at: E-mail: oku@nises.affrc.go.jp

nized by a powerful forward thrust of the ovipositor into the host (Hodek, 1973).

Sequential parasitization of a single host

(1) Host discrimination by self. A parasitoid wasp was placed together with a host beetle in a Petri dish (5 cm dia) and allowed to sequentially parasitize the host 3 times. The time intervals from release to first parasitization, from first parasitization to second and from second to third were recorded, respectively. The study consisted of 30 replicates. As a control, instead of sequential parasitization with the same host beetle, a parasitoid wasp parasitized 3 unparasitized host individuals and was allowed a single parasitization on each host. The time intervals between release to parasitization on the first host beetle, the second and the third one were recorded respectively. In this control experiment, 30 wasps and 90 host beetles were used. The parasitoid wasps typically respond to only mobile host beetles, thus some extreme data obtained with immobile host beetles were omitted.

(2) Host discrimination by sister. Sequential parasitizations of the same coccinellid by two parasitoid individuals were also tested to check whether chemical marking in hosts plays a role in host discrimination of *D. coccinellae*. Time from release to parasitization by the first wasp was measured. The parasitized beetle was immediately transferred to the second wasp to determine the same time parameter. The average time taken for the coccinellid beetles to be parasitized by the first wasp and then by the second one were calculated from 30 replicates with 60 wasps on 30 coccinellids. The two wasps used in an individual test were genetically identical, i.e., originated from the same parthenogenetically reproducing mother. Insects used in the experiments originated from Tsukuba, Japan. Statistical analyses were done using Mann-Whitney *U* test.

Parasitoid behavior placed with different 3 hosts. Analysis of parasitoid behavior placed together with 3 host beetles in a Petri dish (5 cm dia) was based on direct observations and recordings with a video camera. Overall, in the series of 7 replicates, 2 conditioned wasps and 21 coccinellids were used. The coccinellids were individually marked on the elytra. We measured the time from the moment the parasitoid detected any of the 3 hosts. Individual tests lasted 9 to 20 min. Encounters of parasitoids with hosts during the tests were categorized either resulting in host acceptance (attempts of the parasitoid to oviposit into an encountered beetle) or in host rejection (no attempts to oviposit after examination of the beetle, searching for another host continued). If successive encounters of the wasp with a certain host resulted in the same type of parasitoid response, i.e., in acceptance or rejection behavior, time intervals between the encounters were regarded to be host acceptance and host rejection periods, respectively. The time a parasitoid spent pursuing the beetle and trying to parasitize it was included within the period of host acceptance. Time intervals (1) from the beginning of the test to the first encounter with a given host, (2) between two successive encounters resulting in opposite parasitoid responses and (3) from the last encounter to the end of the test were considered to be the period of unknown relationship between the parasitoid and the host. Time distribution of ovipositions, host acceptance periods and host rejection periods were determined in relation to each beetle tested. Insects used in these experiments were obtained from C. Budejovice in Czech Republic.

RESULTS AND DISCUSSION

Conditioned wasps started pursuing as soon as they recognized the host beetles and successfully parasitized within 24.7 s (Fig. 1). After the first parasitization the wasps clearly showed host discrimination, so that the recently parasitized hosts were rejected by the wasps. The pre-parasitization period was significantly prolonged ($p<0.001$), with the second parasitization occurring 155.1 s after the first parasitization. The pre-parasitization period between the second and third parasitization was prolonged further ($p<0.005$) and the third parasitization took place 232.4 s after the second parasitization. In the control experiment a wasp was allowed to parasitize 3 unparasitized hosts sequentially; there was no significant difference among the pre-parasitization periods.

The behavioral pattern of a parasitoid wasp and 3 host beetles (Fig. 2) monitored by a video camera clearly confirmed that the *D. coccinellae* wasp exhibited host discrimination, e.g. recently parasitized hosts were rejected for a certain time and no successive oviposition on the same host was recorded. Wright and Laing (1978) noticed that *D. coccinellae* can oviposit repeatedly into the same

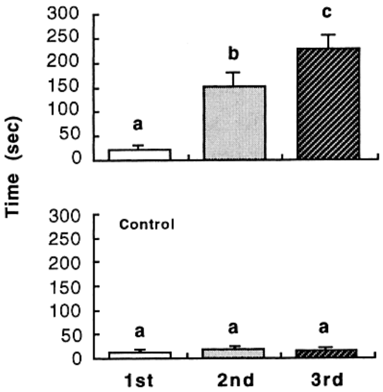

Fig. 1. Time taken for the first oviposition and between ovipositions of *Dinocampus coccinellae* spent in the test with sequential parasitism of the same *Coccinella septempunctata* beetle. Time intervals from the beginning of the test to the first parasitization (1st), between the first and second parasitization (2nd) and between the second and third (3rd) were recorded, with a stop-watch. In this experiment 30 wasps and 30 coccinellids were used. Control: Three unparasitized host beetles were sequentially parasitized by a wasp and the time intervals between release to parasitization of the first host beetle (1st), the second (2nd) and the third (3rd) one were recorded. In this experiment 30 wasps and 90 host beetles were used. Numbers within a column followed by the same letter are not significantly different ($p>0.05$) by Mann-Whitney *U*-test. Vertical lines indicate SD.

coccinellid with little or no restraint, which appears to be contradictory to our results. Our preliminary experiments indicated that wasps that are isolated from the hosts for more than one day tend to be aggressive and oviposit repeatedly (unpublished observation). Therefore, we pre-conditioned wasps before using them for experiments (see Materials and Methods).

The sequential parasitism of *C. septempunctata* beetles by pairs of sister wasps showed that *D. coccinellae* is unable to recognize a host recently parasitized by genetically identical sisters (Fig. 3), i.e. the average time of 20 s that the first wasp of the pair took to oviposit did not differ significantly from the 24 s measured for the second wasp (Mann-Whitney *U* test, $p=0.2242$). These data indicate that external markers (pheromones) which can provide easily detected information, can therefore probably be excluded. Even if we assume that the suspected markers differ among genotypes, a parasitoid female should be able to discriminate a host parasitized by its clonal sister.

It is still possible, although not very probable, that *D. coccinellae* recognizes parasitized coccinellids by internal probing with the ovipositor. Our

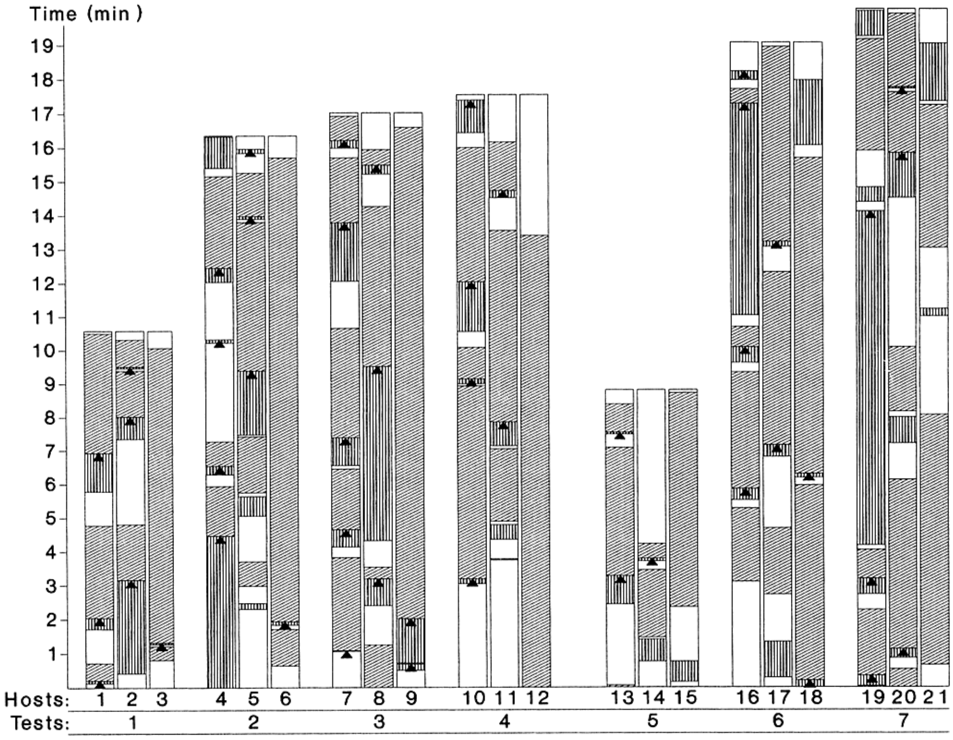

Fig. 2. Host acceptance behavior of one *Dinocampus coccinellae* placed with 3 adults of *Coccinella septempunctata*. ▥: host accepted, ▨: host rejected, □: unknown relation to the host, ▲: oviposition. Tests 1–4 were performed with one wasp, and tests 5–7 with another wasp.

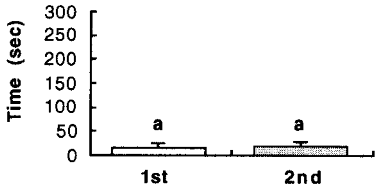

Fig. 3. Time taken for the first and second *Dinocampus coccinellae* individual to oviposit in the tests with sequential parasitism of the same *Coccinella septempunctata* beetle by a pair of sister wasps. Time from the release to its parasitization by the first wasp was measured. The parasitized beetle was transferred to a second wasp to determine the same parameter. Average times spent to parasitize the coccinellid by the first wasp (1st) and to superparasitize it by the second one (2nd) were calculated from 30 replicates (60 wasps and 30 coccinellids were used in this experiment). The two wasps used in an individual test were genetically identical, i.e., originated from the same parthenogenetically reproducing mother. Numbers within a column followed by the same letter are not significantly different ($p>0.05$) by Mann-Whitney *U*-test. Vertical lines indicate SD.

tests could not answer this question because internal markers, due to their nature (e.g., biochemical changes in host caused by the parasitization), can usually be detected only when parasitoid development is more or less advanced. We suppose, however, that the recognition of internally marked hosts may be difficult for *D. coccinellae* and perhaps for other euphorine parasitoids because the host stage attacked is the adult. Adult insects are usually better protected against natural enemies due to their hard exoskeleton and greater mobility compared to juvenile stages. Successful oviposition of *D. coccinellae* into such a well protected coccinellid beetle is, however, facilitated by host movement, when the vulnerable areas of its body (soft integument on the dorsal side of the abdomen, inter segmental membranes) are more easily accessible (Richerson and DeLoach, 1972; Orr et al., 1992). Consequently, oviposition into a walking coccinellid has to be very rapid (a fraction of a second) and there is probably not enough time for the ovipositor chemoreceptors to determine whether or not the host is marked internally.

Since probably neither external nor internal markers are used by *D. coccinellae* to recognize parasitized hosts, it seems that the only mechanism employed in host discrimination of the wasp is based on its ability to remember examined hosts for a certain time. Presumably, olfactory and/or visual cues, specific for individual beetles, play a part in this mechanism. The mechanism of self-host discrimination in the ichneumonid wasp *Pimpla nipponica* was clarified (Ueno and Tanaka, 1996), and failed to show that females of this species deposit chemical markers on a host during oviposition. They assumed that the host could smell different enough for a female wasp to discriminate between self- and conspecifically parasitized hosts. A solitary parasitoid, *Epidinocarsis lopezi* superparasitizes fewer hosts previously parasitized by itself than hosts parasitized by conspecifics, supporting an involvement of individual-specific markers in the host discrimination (van Dijken et al., 1992). Such a self-host discrimination mechanism is also reported in the ichneumonid wasp *Itoplectis naranyae* (Ueno, 1994)

Efficiency of host discrimination in parasitoid wasps might perhaps be related to distribution of the host insects in natural conditions. In an aphid parasitoid, *Ephedrus cerasicola*, 62% of the females still avoided superparasitism 24 h after the 1st parasitization (Hofsvang, 1988) and it was reported that host discrimination persists even longer (up to 120 h) (Solhaug, 1985, in Hofsvang, 1988). This sustained and efficient host discrimination, employing both external and internal markings, must be reasonably significant, because the host aphids (often genetically identical) are usually aggregated on plants. In contrast, such efficient host discrimination may not be necessary for optimal foraging in *D. coccinellae*. Taking into account the mobility of both coccinellids and their parasitoid, it is possible that the short periods of host rejection are, in natural conditions, long enough to significantly reduce the risk of superparasitism. On the other hand, the avoidance of superparasitism may not be the best strategy for parasitoids of high potential fecundity, since it may not be the number of eggs in the ovaries but the time available for searching and ovipositing which limits real fecundity (Liu and Morton, 1986). It appears that *D. coccinellae* belongs to that category of parasitoids. Balduf (1926) found about 100 mature eggs per ovary in newly emerged *D. coccinellae* females and estimated their potential fecundity to range between 200 and 400 eggs. Wright and Laing (1978) recorded that this fecundity is never realized, even when the hosts are permanently available throughout the parasitoid lifespan. The field data collected in autumn 1991 and 1992 from the lowland hibernaculum in Central Poland (vicinity of Warsaw) support this assumption, i.e. in the host populations studied, 22–64% of parasitized beetles contained more than one parasitoid egg and/or larva (Ceryngier, 2000).

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