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Impact of host behavioral defenses on parasitization efficacy of a larval and adult parasitoid

**Annabelle Firlej · Éric Lucas · Daniel Coderre ·
Guy Boivin**

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Abstract Koinobiont parasitoid females when attacking host species are faced with barriers at various levels, host behavioral defenses represent one of these barriers. We present data of the effects of host behavioral defenses on host preference of larval and adult parasitoid. We quantified the effects of defensive behaviors of the exotic host, *Harmonia axyridis* (Pallas), and the indigenous host, *Coleomegilla maculata* lengi Timberlake (Coleoptera: Coccinellidae), on the handling time and attack preference of the indigenous parasitoid, *Dinocampus coccinellae* Schrank (Hymenoptera: Braconidae). Successful egg development was also recorded as a consequence of host behavioral defense and unsuitability. Female parasitoids were offered in an interspecific choice test, adult or larvae of both *H. axyridis* and *C. maculata*, and in an intraspecific choice test, larval and adult stage of *H. axyridis*. Adult *H. axyridis* exhibited a greater number of defensive behaviors compared to adult *C. maculata* or larval *H. axyridis* resulting in significantly longer handling time by the parasitoid. Our results suggest that host acceptance cues used by the generalist parasitoid *D. coccinellae* are inadequate to evaluate adult *H. axyridis*. These results provide support to the hypothesis that *H. axyridis* represents an evolutionary trap for *D. coccinellae*.

Keywords Host-parasitoid relationship · Behavioral defenses · *Harmonia axyridis* · *Coleomegilla maculata* · *Dinocampus coccinellae*

Introduction

Koinobiont parasitoid females must overcome various levels of defenses in order to successfully parasitize their hosts. Host defensive behaviors influence the parasitoid parasitism success because they directly impact the parasitoid's individual fitness by increasing host handling time, thus decreasing the instantaneous rate of fitness gain (de Farias and Hopper 1999; Gerling et al. 1990; Gross 1993; Völkl and Stadler 1996; Walker and Hoy 2003). In extreme cases, behavioral defenses can harm or even kill parasitoid

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A. Firlej (✉)
Institut de Recherche en Biologie Végétale,
Université de Montréal, 4101 Sherbrooke est,
Montréal H1X 2B2, QC, Canada
e-mail: afirlej@yahoo.com

É. Lucas
Université du Québec à Montréal, Case postale 8888,
succursale centre-ville, Montréal H3C 3P8, QC, Canada

D. Coderre
Université du Québec, 475 rue du Parvis,
Québec G1K 9H7, QC, Canada

G. Boivin
Centre de Recherche et de Développement en
Horticulture, Agriculture et Agroalimentaire Canada,
Saint-Jean-sur-Richelieu J3B 3E6, QC, Canada

females (Potting et al. 1999). Host behavioral defenses thus affect host acceptance, decreasing the rate of parasitism and eventually shaping parasitoid host-preference (Gross 1993).

Active host defensive behaviors are usually divided into two categories (Godfray 1994; Gross 1993). Firstly, behaviors that allow hosts to escape from a parasitoid female, such as aphids that evade parasitoids by walking away or dropping off the plant (Braendle and Weisser 2001; Chau and Mackauer 1997; Dill et al. 1990; Losey and Denno 1998), are categorized as evasive behaviors. Secondly, behaviors that drive away, disable or kill parasitoid females are categorized as aggressive behaviors. For example, caterpillars defend themselves aggressively by jerking their head, regurgitating, spitting and biting (Gentry and Dyer 2002; Lederhouse 1990; Potting et al. 1999; Singer and Stireman 2003).

The relationship between host defensive behaviors and female parasitoid host preference has mostly been studied by comparing female host choice between different host stages according to their behavioral defenses rather than comparing interspecific hosts (Allen 1990; Barrette et al. 2008; Chau and Mackauer 1997, 2000; Cornell et al. 1987; Gerling et al. 1990; Walker and Hoy 2003). Moreover, many studies have used a single host-choice experimental design which is more adapted to the evaluation of host suitability and thus more prone to erroneous conclusions regarding host preferences (Mansfield and Mills 2004).

We studied the effect of behavioral host defenses on parasitism using a model composed of an exotic host, *Harmonia axyridis* Pallas, an indigenous host, *Coleomegilla maculata lengi* Timberlake and a generalist indigenous parasitoid, *Dinocampus coccinellae* Schrank. The Asiatic multicolored lady beetle, *H. axyridis*, was introduced in the early 20th century to the southern United States as a biological control agent (Tedders and Schaefer 1994) and has since invaded most of North America (Chapin and Brou 1991; Colunga-Garcia and Gage 1998). The twelve-spotted ladybeetle, *C. maculata*, is an indigenous coccinellid species to North America (Gordon 1985) that co-occurs in some habitats with *H. axyridis* (Hoogendoorn and Heimpel 2004; Musser and Shelton 2003). The parasitoid *D. coccinellae* is a generalist Braconidae with a known host range of nearly 30 coccinellid species (Hodek and Honěk 1996). *Dinocampus coccinellae* produce only females by thelitokous parthenogenesis (Balduf 1926).

In Quebec, parasitism by *D. coccinellae* reaches levels of 32.1% on *C. maculata* but only 4.6% on *H. axyridis* (Firlej et al. 2005). This supported the general assumption that, in North America, *H. axyridis* shows a low susceptibility to pathogens, nematodes, parasitoids and predators (see review in Koch 2003). In addition, *H. axyridis* shows aggressive behaviors against heterospecific coccinellids in intraguild interactions (Michaud 2002; Sato et al. 2005; Snyder et al. 2004; Yasuda et al. 2001, 2004) but whether it displays such defensive behaviors against parasitoids has not been documented. Previous studies that looked at choice tests between different hosts by *D. coccinellae* females never considered defensive behaviors as a factor influencing parasitism success (Koyama and Majerus 2008; Obrycki 1989; Orr et al. 1992).

We suggest that host behavioral defenses, along with the host immune system, could be a major factor explaining the low susceptibility of the North Americain strain of *H. axyridis* to *D. coccinellae*. *Dinocampus coccinellae* females readily attack North American *H. axyridis* in laboratory (Hoogendoorn and Heimpel 2002) but Firlej et al. (2007) and Hoogendoorn and Heimpel (2002) also demonstrated that successful development of *D. coccinellae* eggs and immatures are very low within North American *H. axyridis*. Firlej et al. (2007) hypothesized that immune response of *H. axyridis* may play a role in parasitoid resistance by encapsulating *D. coccinellae* eggs. These postulations were confirmed by observations in electronic microscopy (Firlej et al. unpublished data).

Here, we focused on the effect of host behavioral defenses on host handling time and female *D. coccinellae* attack preference using paired-choice tests. Usually, parasitoid host preference is determined by recording oviposition in the offered host which directly relates to parasitoid fitness. *Dinocampus coccinellae* oviposition in *H. axyridis* remains difficult to evaluate due to the presence of an immunological resistance to *D. coccinellae* (Firlej et al. unpublished data). We therefore measured successful egg development as a fitness parameter taking into account that it reflected a combined effect of host behavioral defense and host unsuitability. Because *D. coccinellae* females can encounter larvae and adults of both host species together in fields

(Musser and Shelton 2003), we tested the effect of host behavioral defenses on parasitism in situation of interspecific (*D. coccinellae* with a choice among *C. maculata* and *H. axyridis* as either larvae or adults) and intraspecific host choice (*D. coccinellae* with a choice among larvae and adults of *H. axyridis*).

Materials and methods

Rearing colonies

Coleomegilla maculata, H. axyridis (Coleoptera: Coccinellidae) and *D. coccinellae* (Hymenoptera: Braconidae) were collected from corn fields from southern Quebec, Canada (45°21′N; 73°09′W) and maintained in laboratory for approximately two years with regular introductions of wild individuals to maintain genetic variability. Coccinellids were reared on a liver-based artificial diet (Firlej et al. 2006), grounded pollen, Mediterranean flour moth eggs (*Ephestia kuehniella* Keller) and aphids (*Acyrtosiphon pisum* Harris). The coccinellids had ad libitum access to all food sources that were renewed every two days. Parasitoids were reared on *C. maculata* adults by placing 30 *C. maculata* adults with four *D. coccinellae* adult females every two days, for 24 h. Parasitized *C. maculata* were incubated for a three week period to allow larval parasitoid development. Emerged adult *D. coccinellae* were kept in Petri dishes (5 cm diameter) with water and honey. Insects were reared under 20 ± 2°C, 60 ± 10% RH and a LD 16:8 h photoperiod.

Test procedure

Both parasitoid and host behaviors were observed i) in interspecific paired-choice tests where a single two days old, experienced *D. coccinellae* female was placed in a Petri dish (10.5 cm diameter) with either three *C. maculata* and three *H. axyridis* adult females or three *C. maculata* and three *H. axyridis* fourth instar larvae, and ii) in intraspecific paired-choice tests where a single two days old, experienced *D. coccinellae* female was placed in a Petri dish (10.5 cm diameter) with three *H. axyridis* adult females and three *H. axyridis* fourth instar larvae. Adult, one week old *C. maculata* and female *H. axyridis* with uniform body size (mean pronotum width ±SE: 3.20 ± 0.25 mm for *H. axyridis* and 2.39 ± 0.09 mm for *C. maculata*) were used for experimentation. To avoid biased host preference, only female adult coccinellids were offered as *D. coccinellae* have been shown to prefer females over males (Davis et al. 2006). Fourth instar larvae with uniform body size (mean body weight ±SE: 18.06 ± 2.9 mg for *H. axyridis* and 11.63 ± 2.3 mg for *C. maculata*) were also used for experimentation. Each choice test represented a treatment with 15 replicates. New parasitoid female and hosts were used for each replicate. Preliminary experiments showed that experienced females laid 4.4 ± 2.8 eggs in the following choice tests regardless of the combination supplied. In order to experience the females, they were individually placed in Petri dishes (10.5 cm diameter) for 1 h with six hosts of the same type (species or stages) offered in the subsequent choice test. After given an experience with hosts, females were then isolated with water and honey for 1 h prior to the choice test. Prior to each test, all hosts were marked with a small drop of white acrylic paint, on elytra for adult hosts or on spines for larval hosts in order to facilitate their visual identification. Only hosts stung by wasps were kept in order to monitor parasitoid egg development. Preliminary observations showed that acrylic paint did not influence the host choice behavior of the female parasitoid and previous studies (Richerson and DeLoach 1972) showed that colors did not influence the female's host preference.

Behaviors recorded

The number and duration of behaviors realized by parasitoid females (Table 1) were recorded with "The Observer" software (version 4.0; Noldus 1991). Occurrence of defensive behaviors expressed by hosts against parasitoids (Table 1) were noted. When first encountering a potential host, *D. coccinellae* female examine it with its antennae, the wasp then bends its abdomen with the ovipositor directed toward the host. In a rapid movement, the female inserts its ovipositor inside the host's abdomen and either accepts the host and lays an egg or rejects it and removes its ovipositor without depositing an egg (Richerson and DeLoach 1972; Orr et al. 1992). Behavioral recordings were initiated when the parasitoid female was released in the Petri dish (10.5 cm diameter) and lasted for a period of 30 min. In all experiments, each parasitoid female interacted with at least one host type offered.

Table 1 Description of parasitoid and host behaviors recorded

Parasitoid behaviors	
Antennal examination	Contacting the host with antennae followed by examination from the antennae
Ovipositor bending	Abdomen placed between legs with the ovipositor directed toward the host
Ovipositor attack	Quick stinging of the host with the ovipositor, ovipositor returns thereafter to horizontal position
Cleaning	Cleaning of the body with legs
Walk	Walking in the petri dish
Stop	Without any movements
Host defensive behaviors	
Attack	Attack of the parasitoid female with mandibles
Escape	Running away
Leg movements	Scratching movement from the metathoracic legs to kick the parasitoid ovipositor

Hosts stung were kept in rearing at 20 ± 2°C and 50–60% RH for five days (i.e. time needed to observe developing eggs inside host abdomen) and their abdomens were dissected thereafter in phosphate buffered saline (PBS) under binocular to check for egg presence. We therefore distinguished between (1) a host attacked by the female parasitoid, indicating host was accepted following visual and antennal examination, and (2) a successfully infected host shown by the successful egg deposition and egg development after a five-days incubation period.

To evaluate the proportion of ovipositor insertions that were not followed by egg deposition (host rejection), one inexperienced two days old *D. coccinellae* female was placed in the presence of one week old *C. maculata* or *H. axyridis* adult female and observed during 30 min. Each test was replicated ten times with new parasitoid females and hosts each time. The number of stings performed by the parasitoid female in each host was recorded and hosts were dissected immediately in PBS buffer to recover eggs laid inside each host with a stereomicroscope.

Statistical analysis

The sequence of host encounters and behaviors expressed can influence the female parasitoid's subsequent behaviors. The Cox's proportional hazard model (Cox 1972) has been used to identify behavioral mechanisms determining patch residence time of animals across several taxa, including for several parasitoid species (Wajnberg 2006; Wajnberg et al. 2006; Martel et al. 2008). We used the Cox's proportional hazard model to estimate the influence of previous host encounter by *D. coccinellae* on the latency period prior to attacking a host. Here, we used "antennal examination" by *D. coccinellae* on one host type as covariate and measured its influence on the latency of occurrence of "ovipositor attack" on *H. axyridis* in interspecific comparison and on adult *H. axyridis* in intraspecific comparison. The equation model is:

$$h(t) = h_0(t)\exp\left\{\sum_{i=1}^{p} \beta_i z_i\right\} \tag{1}$$

Where $h(t)$ is the hazard rate, $h_0(t)$ is the baseline hazard, t is the time elapsed since the last ovipositor attack on *H. axyridis* and β_i the regression coefficients that give the relative contributions of p covariates z_i. The effect of covariates is given by the term $\exp\{\beta_i z_i\}$, the hazard ratio. When the hazard ratio is greater than one, the covariate (here antennal examination) reduces the latency between two ovipositor attacks on *H. axyridis*. The significance of the model was assessed with a likelihood ratio test (Collett 1994).

Both mean number of host encounters and mean number of host behavioral defenses of each host type (species or stages) were compared within a choice test between each host types with a paired t-test (Sokal and Rohlf 1981). The proportion of each defensive behavior type were compared using a Wilcoxon signed rank-test (Sokal and Rohlf 1981). The total time spent on each host type (species or stages) was compared within each choice test by a paired t-test (Sokal and Rohlf 1981). We calculated the mean handling time by summing the time spent by the parasitoid manipulating one host type (antennal examination + ovipositor bending + ovipositor insertion) and dividing it by the sum of encounters with this host type. The parasitoid attack preference was calculated by dividing the number of encounters followed by an ovipositor insertion in a host type by the number of encounters with this host type. The number of eggs that successfully developed represented the number of developed eggs observed inside each host divided by the number of ovipositor insertions observed in those hosts. Because of the lack of independence between parasitoid interactions in paired-choice tests (Mansfield and Mills 2004), we paired data for the analysis. The mean handling time was compared within a treatment between host types (species or stages) with a paired t-test (Sokal and Rohlf 1981) whereas the attack preference and the number of eggs that successfully developed were compared with a Wilcoxon signed rank-test (Sokal and Rohlf 1981). Finally, the proportion of ovipositor rejection was compared between *H. axyridis* and *C. maculata* adults with a χ^2 analysis (Sokal and Rohlf 1981). All statistical tests were performed with the JMPin software (SAS Institute Inc.) (Sall and Lehman 1996).

Results

Interspecific host choice

Adults

The parasitoid encountered *H. axyridis* adults significantly more often than *C. maculata* adults ($t_{14} = -3.20$, $P = 0.0064$) (Fig. 1). Adults of *H. axyridis* expressed more behavioral defenses in the presence of the parasitoid than did *C. maculata* ($t_{14} = 2.14$, $P = 0.0500$) (Table 2) and *H. axyridis* adults expressed more leg movements than did *C. maculata* against the parasitoid ($z = 2.35$, $P = 0.0186$) (Fig. 2). The mean handling time was longer ($t_{14} = 4.02$, $P = 0.0013$) and the number of eggs that successfully developed was lower ($z = 10.50$, $P = 0.0310$) for *H. axyridis* than *C. maculata* adults (Table 2). When assessed five days after oviposition, no eggs were found in *H. axyridis* adults. The attack preference did not differ between host species ($z = 28.00$, $P = 0.117$) (Table 2). *Dinocampus coccinellae* spent significantly more time interacting with *H. axyridis* (75.4%) than with *C. maculata* (24.6%) ($t_{14} = 4.28$, $P = 0.0008$).

Larvae

The mean number of encounters with larvae of the two host species did not differ significantly ($t_{14} = 0.24$, $P = 0.8146$) (Fig. 1). When a female *D. coccinellae*

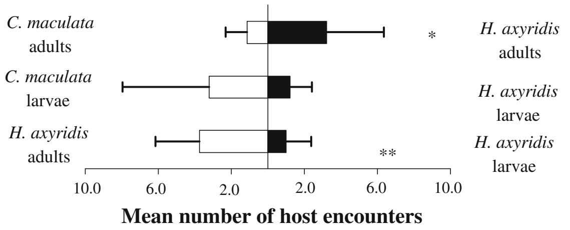

Fig. 1 Mean number (+SE) of host encounters by *D. coccinellae* in a paired-choice tests (*$P < 0.05$ and **$P < 0.01$ with a paired t-test)

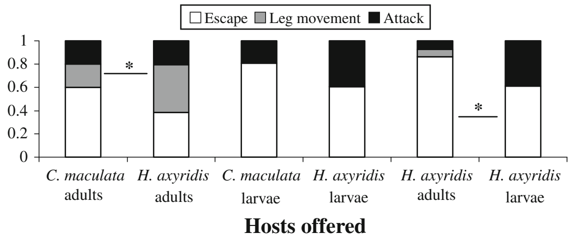

Fig. 2 Proportions of behavioral defenses expressed against the parasitoid in paired-choice tests (*$P < 0.05$ with a Wilcoxon signed-rank test for a same behavioral defense compared between hosts offered)

Table 2 Hosts and parasitoid parameters (mean ± SE) in paired-choice tests

	Interspecific host choice				Intraspecific host choice	
	H. axyridis adults	*C. maculata* adults	*H. axyridis* larvae	*C. maculata* larvae	*H. axyridis* adults	*H. axyridis* larvae
Number of host behavioral defenses*	3.20 ± 3.14 a	1.13 ± 1.18 b	1.20 ± 1.20 a	3.20 ± 4.75 a	3.73 ± 2.43 a	1.00 ± 1.36 b
Handling time (s)*	29.44 ± 14.43 a	16.40 ± 7.40 b	11.88 ± 3.35 a	11.27 ± 2.89 a	21.72 ± 9.54 a	10.53 ± 4.88 b
Attack preference**	0.25 ± 0.09 a	0.16 ± 0.20 a	0.41 ± 0.12 a	0.33 ± 0.19 a	0.35 ± 0.11 a	0.22 ± 0.19 b
Number of eggs that successfully developed**	0.00 ± 0.00 b	0.33 ± 0.47 a	0.52 ± 0.30 a	0.45 ± 0.37 a	0.004 ± 0.018 b	0.35 ± 0.35 a

Within a treatment, different letters in the same row indicate significant differences with: * a paired t-test ($P < 0.05$); and ** a Wilcoxon signed rank-test ($P < 0.05$)

was given the choice between larvae of *H. axyridis* and *C. maculata*, the mean number of host defenses, the mean handling time, the attack preference and the number of eggs that successfully developed did not differ (Table 2). Also, *H. axyridis* and *C. maculata* larvae did not exhibited different behavioral defenses against the parasitoid (Fig. 2). *D. coccinellae* spent 45.4% of their time with *H. axyridis* and 54.5% with *C. maculata* ($t_{14} = -0.67$, $P = 0.5119$).

Intraspecific host choice

We observed a higher mean number of encounters with adult compared to larvae of *H. axyridis* ($t_{14} = -3.96$, $P = 0.0014$) (Fig. 1). *Harmonia axyridis* adults displayed higher mean number of behavioral defenses in the presence of the parasitoid compared to the larvae ($t_{14} = -2.73$, $P = 0.0016$) (Table 2). Furthermore, the proportion of the different behavioral defenses used by *H. axyridis* against the parasitoid differed between adults and larvae ($z = -2.21$, $P = 0.0269$) (Fig. 2): adults used more escape to defend themselves against *D. coccinellae* than did larvae. The mean handling time ($t_{14} = 4.64$, $P = 0.0005$) and attack preference ($z = 35.50$, $P = 0.025$) were higher for *H. axyridis* adults. However, the number of eggs that successfully developed was lower ($z = 22.50$, $P = 0.004$) in *H. axyridis* adults than in larvae (Table 2). *Dinocampus coccinellae* spent significantly more time with adult *H. axyridis* (79.7%) than with larvae (20.3%) ($t_{14} = 6.74$, $P < 0.0001$).

Host rejection

No significant differences were observed in the proportion of host rejection following ovipositor insertion between adult *H. axyridis* and *C. maculata* ($\chi^2_1 = 0.15$, $P = 0.6983$). In *C. maculata* and *H. axyridis* adults, respectively 28 and 24% of the ovipositor insertion were followed by egg deposition.

Cox proportional hazards model

In the interspecific choice tests, the mean latency between two ovipositor attacks on *H. axyridis* ranked from 246.4 ± 122.2 s when parasitoid females were confronted with adults of both coccinellid species to 200.2 ± 85.4 s when confronted to larvae of both species. When confronted to adults and larvae of

Table 3 Frequency of covariates (mean occurrence per sequence of attack), estimate regression coefficient (β), standard error of β, exponential (β) and likelihood ratio test results showing covariates with a significant effect ($P < 0.05$)

Treatments	Dependent variable	Covariates	Frequency	β	SE (β)	Exp (β)	χ^2	*df*	*P* value
C. maculata adults-*H. axyridis* adults	Ovipositor attack of *H. axyridis*	Antennal examination *C. maculata*	1.316	−0.033	0.043	0.968	0.39	1	0.528
		Antennal examination *H. axyridis*	2.495	0.057	0.043	1.059	1.94	1	0.163
C. maculata larvae-*H. axyridis* larvae	Ovipositor attack of *H. axyridis*	Antennal examination *C. maculata*	2.015	−0.024	0.023	0.977	0.71	1	0.400
		Antennal examination *H. axyridis*	2.097	0.162	0.059	1.176	14.17	1	<0.001
H. axyridis larvae-*H. axyridis* adults	Ovipositor attack of adults	Antennal examination larvae	1.144	0.098	0.052	1.104	3.00	1	0.083
		Antennal examination adults	2.205	0.270	0.054	1.310	33.13	1	<0.001

H. axyridis, latency between two ovipositor attacks on adults decreased to 179.5 ± 83.3 s. Table 3 describes the influence of antennal examination on the aforementioned latency in interspecific and intraspecific choice tests. Antennal examination of adult or larval *C. maculata* had no significant influence on the latency period prior to an ovipositor attack on adult *H. axyridis* by the female parasitoid. Antennal examination of adult *H. axyridis* had no influence on the latency period prior to an ovipositor attack on adult *H. axyridis* when the parasitoid was confronted to adults of both host species. That being said, antennal examinations significantly decreased by 1.176 the latency period when in contact with larvae of both host species. Antennal examination of adult *H. axyridis* resulted in a decrease of 1.310 of the latency period before an ovipositor attack of adult *H. axyridis* by the female parasitoid when confronted to both larvae and adults of *H. axyridis*.

Discussion

As expected, the exotic host *H. axyridis* expressed more behavioral defenses against females of the indigenous parasitoid *D. coccinellae* compared to the indigenous host *C. maculata*. These defenses significantly increased the parasitoid handling time from 135% to 217% depending of the host considered.

Host defenses occurrence and effect

Adult *H. axyridis* appeared to exhibit more defensive behaviors than adult *C. maculata* or larval *H. axyridis* and frequently disrupted the ovipositional sequence of *D. coccinellae*, significantly increasing parasitoid handling time. This supported previous findings demonstrating that *H. axyridis* is the most aggressive and better defended species among coccinellid guild (Michaud 2002; Sato et al. 2005; Snyder et al. 2004; Yasuda et al. 2001, 2004) and that young stages are less defensive than adults against parasitoids (Chau and Mackauer 2000; Gerling et al. 1990; Mackauer et al. 1996; Walker and Hoy 2003). We demonstrated that *H. axyridis* could express behavioral defenses not only against predators of the same guild, but also against parasitoids.

The parasitoid took longer to handle adult *H. axyridis* compared to the other interspecific adults or intraspecific larvae. Defensive behaviors were shown to protect hosts by increasing parasitoid handling time (Gerling et al. 1990; Gross 1993; Potting et al. 1999; Walker and Hoy 2003). Usually, better-defended hosts receive fewer eggs than hosts with weaker defenses (Gross 1993). However, *D. coccinellae* are able to reject hosts on the basis of internal examination following ovipositor insertion (Davis et al. 2006; Sloggett et al. 2004). Based on the number of eggs recovered immediately after oviposition, the proportion of host rejections did not differ between adult exotic and indigenous hosts, 76% for *C. maculata* and 72% for *H. axyridis*, respectively. Furthermore, following host acceptance and egg deposition, the egg can still be destroyed by the host's immune system (Strand and Pech 1995). The fact that no parasitoid egg was recovered from adult *H. axyridis* five days after oviposition suggests that these eggs have been destroyed by the host's immune system. While the capacity of the cellular immune response of adult *H. axyridis* to encapsulate *D. coccinellae* eggs has been shown (Firlej, personal observation) the immune response of *C. maculata* against *D. coccinellae* eggs has never been studied.

Host preference

Similar studies have been conducted in Britain where *D. coccinellae* females were shown to have the same attack rate on *H. axyridis* and on the seven-spotted lady beetle, *Coccinella septempuncata*, although *H. axyridis* greater mobility resulted in a higher encounter rate with the parasitoid females (Koyama and Majerus 2008). Similarly, we demonstrated that *D. coccinellae* females encountered more often adults of *H. axyridis* over other hosts (*C. maculata* adults or *H. axyridis* larvae). Contrary to the findings reported in Koyama and Majerus (2008), we showed that attack rate was higher on adult *H. axyridis* (Table 2). This suggests that *D. coccinellae* females prefer adults rather than larvae *H. axyridis*. Criteria of host preference were well studied in the multi-host species system associated to *D. coccinellae*. Females *D. coccinellae* prefer large hosts, female over male and adults over larvae for parasitization (Davis et al. 2006; Geoghegan et al. 1998; Obrycki 1989; Orr et al. 1992; Richerson and DeLoach 1972). In theory, this preference is adaptive as parasitism is more successful and development time is shorter in adult hosts (Firlej et al. 2007; Geoghegan et al. 1998; Obrycki et al. 1985). However, adult *H. axyridis* used in our study did not support *D. coccinellae* progeny development as previously demonstrated (Firlej et al. 2007). This would indicate that female *D. coccinellae* inaccurately assessed the internal quality of adult *H. axyridis*. The criteria used by *D. coccinellae* to assess host quality, such as its size or stage, do not appear to be associated with parasitism success when interacting with adults of the exotic host *H. axyridis*.

Our results indicate that attacking the larval stage of *H. axyridis* is a better choice. Contrary to what has been observed with other coccinellid host species (Geoghegan et al. 1998; Obrycki et al. 1985), larvae of *H. axyridis* were more suitable for the development *D. coccinellae* than adults (Firlej et al. 2007). This suggests that adult *H. axyridis* found parasitized by *D. coccinellae* in field samples (Firlej et al. 2005; Hoogendoorn and Heimpel 2002) probably originated from *D. coccinellae* attacks on *H. axyridis* larvae.

These findings could also be interpreted as an indication that the parasitoid was better adapted to overcome the behavioral defenses of the indigenous host *C. maculata* rather than those of the exotic host *H. axyridis*. Supposing that the exotic host species was to encounter indigenous natural enemies in its new environment, its behavioral defenses could favor its escape from biological control by natural enemies without any coevolutionary history (Keane and Crawley 2002; Prenter et al. 2004). However, these results are based on a single replicate of host interactions (one exotic species *vs* one indigenous species), and further tests using other sets of exotic/indigenous species would be needed to confirm this interpretation.

The fact that the *D. coccinellae* population used in our experiment was reared on *C. maculata* could have brought a bias to our results. Parasitoid laboratory rearing on *H. axyridis* is impossible considering the low success of parasitism (Firlej et al. 2007). The *D. coccinellae* females used here were reared on *C. maculata* and have never experienced contact with *H. axyridis*. However, to reduce this bias, the female parasitoids were giving an experience with *H. axyridis* before testing on both hosts. Because the level of successful parasitism in natural populations of *H. axyridis* is very low (Hoogendoorn and Heimpel 2002; Firlej et al. 2005), one can assume that most of the *D. coccinellae* emerge from *C. maculata* populations. Our experimental conditions therefore mimic the natural situation where an indigenous parasitoid emerge from an indigenous host and is faced with both indigenous and exotic hosts.

Our results provide additional support to the egg sink hypothesis of Hoogendoorn and Heimpel (2002) and in general to the "evolutionary trap" hypothesis applied to biological invasions (Schlaepfer et al. 2005). The latter hypothesis predicts that indigenous species could be trapped by their evolutionary responses when confronted to exotic species: the set of cues used to recognize a host does not provide an honest evaluation of the quality of the exotic host. *Dinocampus coccinellae* has shown a preference for large coccinellids (Richerson and DeLoach 1972) and we showed that females spent more time interacting with adult *H. axyridis* compared to *C. maculata* with an associated fitness cost as the exotic host is unsuitable for development because of an immunological response which reduces egg hatching success (Firlej et al., unpublished data). Because female *D. coccinellae* incorrectly assess the suitability of adult *H. axyridis* as hosts, the parasitoid could experience a decrease in population density when the population of *H. axyridis* is high compared to other coccinellid host species. However, the defensive behaviors of *H. axyridis* could decrease the effect of the evolutionary trap by increasing the parasitoid handling time therefore decreasing its oviposition rate and the likelihood of loosing eggs in an unsuitable exotic host.

Populations of *H. axyridis* and *D. coccinellae* coexist in Japan where *D. coccinellae* can develop successfully on *H. axyridis* although with a lower success than on other indigenous Japanese coccinellids (Maeta 1969; Koyama and Majerus 2008). This suggests that Canadian *D. coccinellae* did not yet have enough time to adapt and overcome behavioral and physiological defenses from the newly introduced *H. axyridis* populations. In the long term, if a sufficient selective pressure is exerted on Canadian *D. coccinellae* populations, we could observe the selection of isofemale lines able to develop on Canadian *H. axyridis* populations.

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