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Deleterious Effects of Low Temperature Exposure on Learning Expression in a Parasitoid

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In this paper, we review the learning capacities of insect parasitoids. We present data on the learning capacity of the parasitoid wasp, *Anaphes victus* (Hymenoptera: Mymaridae), in the host (egg) discrimination process. In addition, we examine the effect of low temperature exposure on the wasp's learning. Our results showed that *A. victus* females learned rapidly to recognize their own chemical cues that they left on the host eggs, and retained this learning from patch to patch. Conspecific chemical cues left on the eggs took more time to be learned, but two learning trials induced a prolonged memory for the cues. Our results also showed that the use of learned, conspecific chemical cues was more affected by cold exposure than was the use of learned personal cues.

Learning in Parasitoids

Insect parasitoids develop on or in a single host and kill it (Eggleton & Gaston, 1990). More than 100,000 species of insect parasitoids are known and while 75% of these species are Hymenoptera, the parasitoid lifestyle has evolved also in Diptera (flies), Coleoptera (beetles), Neuroptera (lacewings), Lepidoptera (butterflies) and Trichoptera (caddisflies). Learning in parasitoids was demonstrated as early as 1937 by Thorpe and Jones, but research on the topic did not flourish until the end of the 1980s. Most studies focused on learning by Hymenoptera, but Diptera parasitoids were also shown to be able to learn visual cues in the host location process (Montheith, 1963, Stireman, 2002). Nowadays, learning has been shown to occur throughout the life of adult parasitoids: in the search for a mate, the localization of hosts and host habitats, the choice of the host species and instar, in the host discrimination process (defined as the ability to recognize an already parasitized host), and in competition (Papaj & Lewis, 1993; Turlings, Wäckers, Vet, Lewis, & Tumlinson, 1993; van Baaren & Boivin, 1998a; van Lenteren & Bakker 1975; Vet, Lewis & Carde, 1995). More recently, Baeder and King (2004) showed that males of the wasp *Nasonia vitripennis* (Hymenoptera: Pteromalidae) learned to associate a color with the reward of a virgin female.

Most learning studies have focused on the female learning capacities during parasitization. After emergence, female parasitoids must find a host in their environment. Since cues originating from the host itself are of low intensity, female parasitoids generally use cues from the host habitat for host localization. After parasitoids gain some experience with their host's habitat, they use that knowledge and employ directional searching for habitat and micro-habitat location (e.g., Geervliet, Vreugdenhil, Dicke, & Vet, 1998), and for host-stage and age selection (Dutton, Mattiacci & Dorn, 2000). For example, the host plant preference of the parasitoid wasp *Aphidius colemani* (Hymenoptera: Aphidiidae) is induced by chemical cues encountered on the aphid "mummy" at the time of emergence (the mummy is the parasitized aphid, containing only the nymph of the parasitoid inside the skin of the dead aphid). However, these initial preferences can be changed by subsequent foraging experiences (Storeck, Poppy, van Emden, & Powell, 2000). Also, females of the wasp *Trichogramma evanescens* (Hymenoptera: Trichogrammatidae) have been shown to associate the sex pheromones of their host (pyralid moths) with successful parasitization (Schöller & Prozell, 2002).

Generally, it is considered that learning enables the parasitoid to specialize on the most valuable resources through the selective increase in responsiveness to, and the creation of preferences for certain key stimuli, and that learning can change initial random search patterns to directed search behaviour resulting in increased host encounter rate (Vet et al., 1995). Learning to seek a specific resource and bypass others may significantly increase egg laying rate and reproductive success (e.g. Turlings et al., 1993). Learning associated with behaviours related to oviposition is predicted to be advantageous when increases in foraging efficiency on the more abundant host species outweigh the disadvantages of ignoring higher quality, but less abundant host species (Cunningham & West, 2001). Further, associative learning can be used to seek stimuli with positive fitness effects and to avoid stimuli with negative fitness effects (Dukas & Duan, 2000). For instance, after finding a host, a female parasitoid must oviposit in (endoparasitoids) or on (ectoparasitoids) the host. The importance of experience in shaping oviposition in egg parasitoids (wasps that parasitize eggs rather than larvae or adults) is that learning allows for a more efficient exploitation of the host (Al-Wahaibi & Walker, 2000).

The parasitoid wasp *Microplitis croceipes* (Hymenoptera: Braconidae) has been shown to be capable of complex learning, or multiple task learning. This species can exhibit multiple conditioned behaviours in feeding and reproduction (Wäckers, Bonifay & Lewis, 2002). Non-social species like parasitoids frequently have to alternate between discrete tasks, in particular between searching for hosts and foraging for food sources. Lewis and Takasu (1990) demonstrated that host- and food-associated learning are separate processes whose expression is dependent on the hunger state of the parasitoid. This behavioural complexity makes parasitoids particularly suited for the study of multiple task learning (Wäckers et al., 2002).

The capacity to distinguish between two closely related stimuli has received relatively little attention in parasitoids. However, a few studies have shown that this capacity is well developed in parasitoids. For instance, females of the wasp *Microplitis croceipes* (Hymenoptera: Braconidae) discriminate between different chain-length alcohols, and between alcohols with different positions of func-

tional groups (Meiners, Wäckers & Lewis, 2003). In addition, wasps can adjust their degree of discrimination between similar odours according to the profitability of the information in terms of host encounter rate (Meiners et al., 2003).

Many insects interact with competitors that may limit their ability to use a resource. In non-fatal encounters, the forager could benefit from learning the extent of the danger of such interactions. For example, aphid parasitoids often interact directly with ants when foraging and ovipositing in aphids attended by honey-dew-collecting ants which often protect the aphids (the wasp's carbohydrate source) against natural enemies. However, after some non-aggressive ant encounters, experienced female parasitoids change their behaviour by reducing their searching speed, approaching ants from the side and even from the front, retreating less often in response to an approaching ant and reducing the "safe distance" (the distance to an ant that elicits escape behaviour). These experienced females have a significantly higher rate of oviposition than do naive females (Völkl, 2001).

Numerous studies have also shown that parasitoid behaviour is affected by prior, similar behaviour. For example, the oviposition behaviour of a female on a patch containing hosts can be affected by the behaviours that she performed in a previous patch (Babendreier & Hoffmeister, 2002; Boivin, Fauvergue & Wajnberg, 2004; Keasar, Ney-Nifle, Mangel, & Swezey, 2001; Outreman, Le Ralec, Wajnberg, & Pierre, 2005; van Baaren, Outreman & Boivin, 2005).

Learning During the Host Selection Process in Parasitoids

In contrast to predators that consume their prey, hosts parasitized by female parasitoids remain in the environment. Therefore, they may be encountered by other foraging female parasitoids, particularly when intense intra- and inter-specific competition occurs for a limited number of hosts. For solitary parasitoids, one host allows for the development of a single larva. Laying an egg in a host already parasitized by another female (i.e., superparasitism) results in larval competition and the first larva to hatch generally has the advantage. Consequently, for most parasitoid species, discriminating a host already parasitized represents a strong selective advantage, preventing wasted eggs and time (Godfray, 1994). Understandably, such host discrimination has been found in almost all species studied (Godfray 1994; van Alphen & Visser, 1990).

It was first thought that learning would not be involved in host discrimination. The belief was that conspecific cues used to recognize a parasitized host should be genetically determined (Papaj & Lewis, 1993; van Alphen, van Dijken & Waage, 1987; van Lenteren & Bakker, 1975). However, van Baaren and Boivin (1998a) showed that females of the wasp *Anaphes victus* (Hymenoptera: Mymaridae) did indeed use learning in host discrimination. When they encounter a series of parasitized hosts, the females begin by rejecting these hosts following ovipositor insertion (sting rejection), and in the process learn to associate the presence of an external odour cue with the presence of an immature in the host. Gradually, after more than ten such rejections, they switch to antennal rejection, which occurs twenty times faster than sting rejection. Consequently, antennal rejection allows females to save considerable time during patch exploitation. Although the learning that underpins antennal rejection is forgotten within four hours, females can de-

posit most of their eggs in a single day and never survive more than four days in the laboratory, so the learning need not be retained for very long periods. It is interesting that these wasps learn their own external chemical cues faster than they learn those of conspecifics, which may allow them to avoid parasitizing the same host twice (van Baaren & Boivin, 1998a).

As in most learning experiments, the benefits of learning involved in host selection have been measured in time savings. Only a few studies have directly measured fitness gains due to learning in this context (Dukas & Bernays, 2000; Dukas & Duan, 2000; Mery & Kawecki, 2002).

Learning and Cold Exposure

It is common to store the larvae of biological pest control agents such as parasitic wasps under cold conditions (Hofsvang & Hagvar, 1977). Storage of these natural enemies of certain pest species assures their availability in sufficient numbers when they are released into the environment. Therefore, the development of storage techniques for biocontrol agents is considered of utmost importance in order to provide flexibility and efficiency in their mass production, to synchronize the desired developmental stage for peak release, and to make available standardized stocks for use in research (Leopold, 1998; Ravensberg, 1992). However, storage techniques must ensure the availability of quality natural enemies (Bigler, 1994, Tezze & Botto, 2004).

Numerous studies have shown that parasitoids can be stored at cold temperature for a few weeks without significant damage to their quality, evaluated by measuring adult emergence time (developmental time from pupa to adult emergence, i.e., the time between the end of cold storage and adult emergence); number of emerged adults per number of parasitized eggs; fecundity of the stored adults and of their first generation, proportion of deformed adults and proportion of females (Archer & Eikenbary, 1973, Hofsvang & Hagvar, 1977, Tezze & Botto, 2004, and references therein). However, negative effects do appear after a long cold exposure, for example, more than 50 days in a *Trichogramma* species, (Tezze & Botto, 2004), including mortality, and decreases in fecundity and mobility.

However, few studies have evaluated the effects of cold storage on wasp behaviour. Only one study has shown that females of the wasp *Microplitis demolitor* Wilkinson (Braconidae) emerging from chilled pupae are unable to respond to an attractive odour (volatile semiochemicals), even after just four days of chilling (Hérard, Keller, Lewis, & Tumlinson, 1988). Besides this study, sub-lethal effects of cold exposure on parasitoids have received little attention and only a little information is available on the effect of cold on foraging behaviour. However, we do know that cold storage can affect the behaviour of the wasp *A. victus* (van Baaren et al., 2005) because it reduces the number of ovipositions made on good quality patches (i.e., patches containing healthy hosts) and increases ovipositions on patches containing hosts already parasitized by conspecifics, and it also affects time allocation between patches. However, cold storage for three weeks did not affect the total number of eggs deposited by the wasps in a day or the wasps' host discrimination abilities. However, longer cold storage (6 to 12 weeks) was more

deleterious; it affected patch time allocation, the number of deposited eggs, and the superparasitism rate.

In the following experiments, we studied in detail the learning capabilities of the wasp *A. victus* (Hymenoptera: Mymaridae) in discriminating between an unparasitized and a parasitized host. Our aim was to determine how learning was used when the wasp visited patches that differed in host quality, and if the learning allowed the wasp to save time while foraging. Then we tested whether or not cold exposure during development affected the learning capabilities of the adult females.

Method

Subjects

The host beetle *Listronotus oregonensis* LeConte (Coleoptera: Curculionidae) and its solitary wasp parasitoid *Anaphes victus* were laboratory reared (Boivin, 1988) from individuals originally captured at Sainte-Clotilde, Quebec (45°09'N, 73°41W). *Anaphes victus* is a micro-Hymenoptera of the family Mymaridae. These wasps are characterized by their small size (less than 1 mm in length), dark color and their wings that bear long setae. For all experiments, the host (beetle) eggs were 2-4 days old, the preferred stage for female wasps (Picard, Auclair & Boivin, 1991). Experimental female wasps were isolated at emergence, mated and used the following day. Just before an experiment, they were allowed to oviposit once in one unparasitized *L. oregonensis* egg.

Procedure

Experiment 1: Effect of Patch Quality on Learning. Each female wasp was presented with three successive patches containing 16 host eggs, placed regularly in 4 lines of 4 eggs on a wet filter paper on a 1.5 x 1.5 cm grid. The time interval between contacts with two successive patches was 90 to 150 min during which time the female was isolated in a plastic capsule. Four different environments were used.

In the first environment (the "Good environment"), the three successive patches contained only unparasitized host eggs. In the second environment (the "Poor environment"), the three successive patches contained only parasitized host eggs. In the third environment (the "Optimist environment"), the first patch contained only unparasitized eggs, the second patch contained 50% unparasitized and 50% parasitized eggs, and the third contained only parasitized eggs. In the fourth environment (the "Pessimist environment"), the first patch contained only parasitized eggs, the second contained 50% unparasitized and 50% parasitized eggs, and the third contained only unparasitized hosts. In the latter three environments, eggs had been parasitized by a conspecific female one hour before the test. In the mixed patches, parasitized and unparasitized eggs were placed alternatively in the grid in order to obtain the same encounter rate for each category of host. Ten different females were used for each environment, and the test conditions were randomized.

Experiment 2: Effect of Cold Exposure on Learning. *Anaphes victus* overwinters as quiescent first and second instar larva and so cold storage, even at 2°C, does not induce diapause (Boivin, 1994). This suggests that cold storage could be used prior to releasing *A. victus* in biological control programs (Picard et al., 1991). In these experiments, parasitized eggs containing second instar *A. victus* larva were stored at 4°C, the usual temperature of parasitoid storage (Tezze & Botto, 2004), for 3, 6 or 12 weeks. After the low temperature exposure, the parasitized eggs were placed at 24°C until the adults emerged. Females were isolated at emergence, mated and used when they were 24h old. Just before the experiment, they were allowed to oviposit once in an unparasitized *L. oregonensis* egg. These females were tested in the optimist environment and their behaviour was compared to females not exposed to cold. Ten different females were used for each cold exposure duration.

For each condition tested (good environment, bad environment, optimist environment, pessimist environment, and cold-stored females tested in the optimist environment), behavior was video-recorded between the moment the female began to search inside the patch until she left the patch for

more than 60 s. In each test, when a host was encountered, the duration of two behaviors were recorded: antennal rejection and sting rejection. Sting rejection could be differentiated from oviposition by the absence of stereotypical movements accompanying egg passage (van Baaren, Boivin & Nénon, 1995).

Statistical Analyses

    Learning curves. In the present study, the learning capacity of the parasitic wasps was evaluated by the increase in the proportion of antennal rejections with the rank of host encounter (i.e., first, second, third, etc…). In each experiment, the proportionate changes of antennal rejection with encounter order were described by a sigmoid curve using the Verhulst equation (1838):

$$y = \frac{a}{[1 + b.\exp(-c.x)]}$$

    This equation is defined by three parameters, where a represents the maximum of the y-axis (i.e., maximum proportion of antennal rejection observed in a given treatment), and both b and c are free parameters that adjust the shape of the curve. The value of these three parameters and their confidence intervals were calculated by using the "nls" function of S-plus® statistical software (MathSoft, Cambridge, MA; Venables & Ripley, 1997).

    To show the phenomenon more clearly, the types of rejection were pooled for the ten females tested and for each consecutive encounter. In order to compare the shape of the curve according to the treatment, the coordinates of the point of inflection of the corresponding curve were calculated (Verhulst, 1838), using:

$$\begin{cases} x_i = \dfrac{\ln(b)}{c} \\[2mm] y_i = \dfrac{a}{2} \end{cases}$$

    The lower the x_i-value, the faster is the learning process (change of behavior from sting rejection to antennal rejection), and a y_i-value close to 0.5 suggests that all females used antennal contact for host rejection after a given number of encounters.

Duration of the host rejection behaviors

    In both experiments, the duration of both antennal and sting rejections (the durations were calculated using the digital event-recorder "The Observer" (Noldus, 1991) were compared. In Experiment 2, the effect of cold exposure on the duration of both antennal and sting rejections was also tested. These analyses were done by means of generalized models assuming a Gamma error as a link function (McCullagh & Nelder, 1989) using S-plus® statistical software (MathSoft, Cambridge, MA; Venables & Ripley, 1997).

Results

Effect of Environmental Quality on Learning Use

    In the following text, "self learning" refers to a female learning to recognize her own external chemical cues left on a host egg, whereas conspecific learning refers to learning to recognize the chemical cues left by other females. We found that whatever the quality of the environment, the wasps used their learning ability in the host discrimination process. For each treatment, all females learned to use information acquired by antennal contact for host rejection (for each treatment,

the y_i-value of the inflexion point was close to 0.5; Table 1). However, the change in proportion of antennal rejection with the rank of host encounter (i.e., first, second, third, etc…) depended on the habitat quality and the rank of the visited patch.

In the good environment, females oviposited in each healthy host encountered. In the first patch (Figure 1, A1), when females started to encounter self-parasitized hosts (due to patch depletion), they rejected the first self-parasitized hosts after a sting without oviposition. Then, the host rejection behaviour changed quickly and, after six encounters with self-parasitized eggs, all rejections were based on antennal contact. In both the second (Figure 1, A2) and third patches (Figure 1, A3), most females immediately rejected self-parasitized eggs via antennal contact indicating that they had not forgotten what they had learned.

In the poor environment, females encountered only conspecific-parasitized eggs and rarely oviposited in the first two (0 to 2 times) or the third patch (1 to 5). In the first patch, most rejections in the first seven encounters were sting rejections (Figure 1, B1) after which their behaviour gradually changed to antennal rejection (i.e., conspecific-learning). After 14 encounters, antennal rejection reached 100%. In the second patch (Figure 1, B2), females began by rejecting parasitized eggs using sting rejection, suggesting that they had forgotten what they had learned in the prior patch. However, the x_i-value of the graph's inflexion point was reduced by two (Table 1). In the third patch (Figure 1, B3), 100% of the eggs were rejected by antennal contact after the first encounter.

In the optimist environment, the learning curve obtained for the first visited patch was similar to the one obtained in the first patch of the good environment (Table 1; Figure 1, C1, versus Figure 1, A1). In the second patch, females first encountered conspecific-parasitized eggs, and after some ovipositions in healthy eggs, encountered a mixture of self- and conspecific parasitized eggs (Figure 1, C1, and Figure 1, C3, respectively). Both self- and conspecific parasitized eggs were never accepted for oviposition. Self-parasitized eggs (Figure 1, C2) were rejected immediately through antennal contact. However, for conspecific parasitized eggs, the inflexion point, (where 50% of the rejections were antennal) occurred at about four encounters (Figure 1, C3). Note that conspecific-learning occurred more quickly after self-learning (Figure 1, C3) than without that prior experience (Figure 1, B1). In the third patch (Figure 1, C4), the curve suggests that some forgetting has occurred.

In the pessimist environment, the conspecific-learning curves obtained in the first two patches were similar to those obtained in the first two patches of the poor environment (Table 1 and Figure 1, D1, and Figure 1, D2, respectively). In addition, even if parasitic wasps had never previously encountered eggs that they had already parasitized, they immediately rejected such eggs after an antennal contact in the second patch (Figure 1, D3). So, in the pessimist environment, self-learning was faster after a conspecific-learning (Figure 1, D3) than without it (Figure 1, A1, and Figure 1, C1). This ability to immediately reject self-parasitized eggs by antennal rejection was retained when wasps visited the third patch (Figure 1, D4).

Table 1

Adjusted Sigmoid Curves Showing the Change of Rejection Type by Females of Anaphes victus in Four Different Environments. The Curves were Fitted to the Sum of Behaviours of 10 Different Females for Each Consecutive Encounter. (SD: standard deviation, CI: confidence interval).

Treatment	Patch Quality	Hosts encountered	Model parameters									Inflexion Point	
			A	S.D.	95% CI	b	S.D.	95% CI	c	S.D.	95% C.I.	x_i	y_i
Good environment	FP (GQ)	Self-parasitized	0.996	0.006	[0.983 ; 1.008]	7.851	2.143	[3.461 ; 12.24]	1.452	0.162	[1.119 ; 1.785]	1.419	0.498
	SP (GQ)	Self-parasitized*	-	-	-	-	-	-	-	-	-	-	-
	TP (GQ)	Self-parasitized	1.031	0.088	[0.839 ; 1.223]	0.187	0.081	[0.010 ; 0.364]	0.158	0.222	[-0.324 ; 0.642]	-10.538	0.515
Poor environment	FP (PQ)	Conspecific-parasitized	0.948	0.032	[0.880 ; 0.948]	21.397	12.86	[-5.622 ; 48.41]	0.556	0.109	[0.327 ; 0.785]	5.505	0.474
	SP (PQ)	Conspecific-parasitized	0.968	0.019	[0.927 ; 1.008]	7.98	3.026	[1.565 ; 14.39]	0.771	0.126	[0.503 ; 1.039]	2.692	0.484
	TP (PQ)	Conspecific-parasitized	0.996	0.007	[0.973 ; 1.011]	31.387	11.15	[-8.242 ;71.01]	4.851	0.656	[3.454 ; 6.248]	0.71	0.498
Optimist environment	FP (GQ)	Self-parasitized	1.005	0.012	[0.978 ; 1.031]	4.45	1.605	[1.129 ; 7.770]	0.969	0.176	[0.605 ; 1.334]	1.539	0.502
	SP (MQ)	Self-parasitized*	-	-	-	-	-	-	-	-	-	-	-
		Conspecific-parasitized	0.990	0.010	[0.966 ; 1.011]	19389.000	26538.000	[-37175 ; 75954]	2.660	0.360	[1.898 ; 3.431]	3.711	0.495
	TP (PQ)	Conspecific-parasitized	0.993	0.020	[0.948 ; 1.038]	3.032	1.099	[0.689 ; 5.376]	0.709	0.155	[0.377 ; 1.041]	1.563	0.496
Pessimist environment	FP (PQ)	Conspecific-parasitized	0.915	0.049	[0.811 ; 1.019]	13.115	9.405	[-6.728 ; 32.96]	0.488	0.136	[0.200 ; 0.775]	5.272	0.457
	SP (MQ)	Conspecific-parasitized	0.986	0.014	[0.956 ; 1.016]	19.818	9.41	[0.190 ; 39.47]	1.063	0.160	[0.729 ; 1.397]	2.808	0.493
		Self-parasitized*	-	-	-	-	-	-	-	-	-	-	-
	TP (GQ)	Self-parasitized*	-	-	-	-	-	-	-	-	-	-	-

Note.*no model was fitted as no sting rejection were observed; FP = First Patch; SP = Second Patch, TP = Third Patch; GQ = Good Quality; MQ = Mixed Quality; PQ = Poor Quality; SD: Standard deviation; CI: Confidence Interval
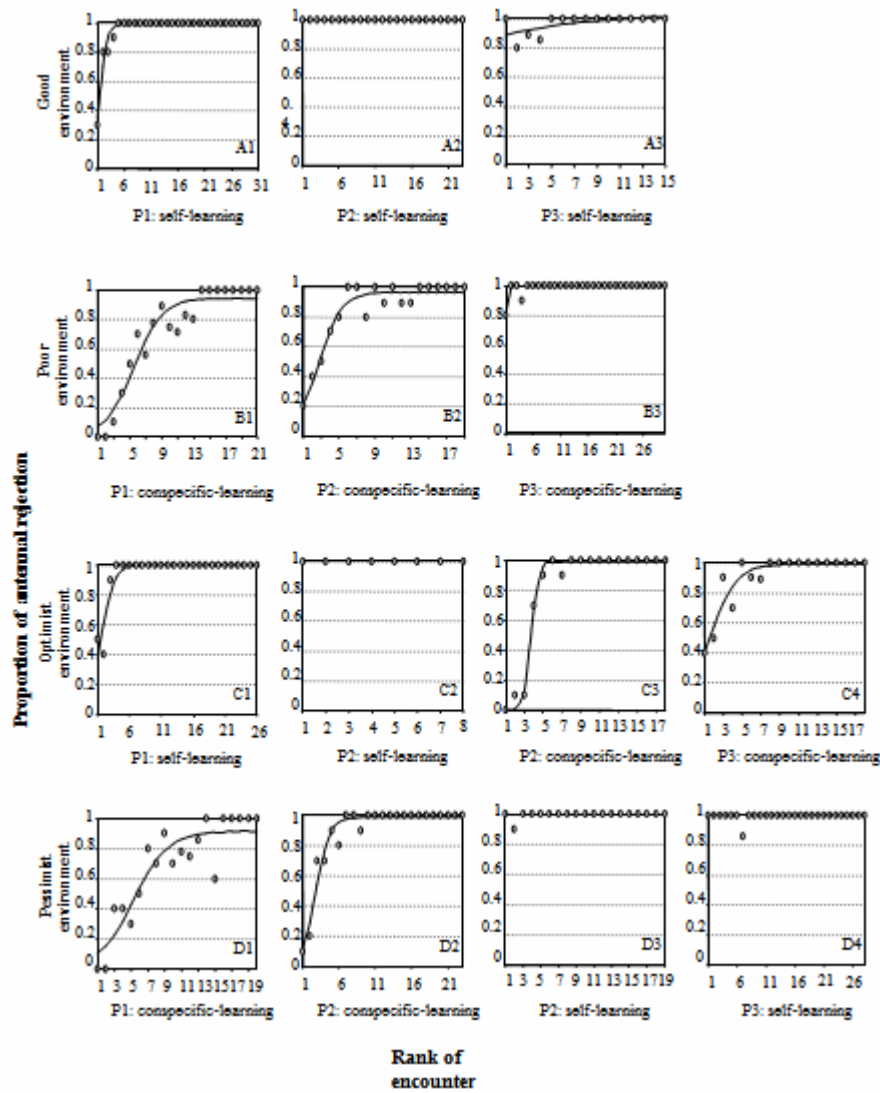
Figure 1. Graphs A1 to D4: Learning curves in each of three patches in four different environments (good, poor, optimist and pessimist), by 10 *A. victus* females. For patches containing two types of eggs (self- and conspecific parasitized), two curves were proposed. See table 1 for mathematical parameters of the curves. First, second and third patches refer to the three successive patches that were offered to the females. For each graph, the x axis represents the rank of encounters with eggs (first, second, third, etc…) and the y axis represents the % of antennal (versus sting) rejection. Antennal and sting rejection represent 100% of the rejections. "Self learning" refers to a female learning to recognize her own external cues on a host egg, whereas "conspecific learning" refers to learning to recognize the cues left by other females.

Effect of Cold Exposure on Learning

Cold exposed females were offered the same three patches used in the optimist environment. Table 2 summarizes the fitted curves and their parameters. For

some treatments, the Verhulst logistic model did not fit the data well. For these treatments, we used either linear or polynomial regressions depending upon which model explained the highest amount of variation (Figure 2).

Overall, increased durations of cold exposure reduced the learning capabilities of the wasps. Further, for all cold exposure durations, rejection of parasitized eggs after antennal contact was never used by all females (i.e., the y_i-value of the inflexion point was always below to 0.5) (Table 2, Figure 2, B1-D3).

In the first patch, cold exposed females presented more sting rejections than control wasps not exposed to cold (Figure 2); After three and six weeks of cold exposure, the proportion of antennal rejection reached only 60% and 70% respectively (Figure 2, B1, Figure 2, C1). After twelve weeks of cold exposure, females never used their own external cues to reject parasitized eggs (Figure 2, D1).

In the second patch, females initially encountered only conspecific parasitized eggs followed by a mixture of self- and conspecific parasitized eggs as a result of patch depletion. Here, self-learning was affected proportionate to the duration of cold exposure. While the control females immediately recognised their own external cues (Figure 2, A2), wasps exposed to cold for three weeks did not (Figure 2, B2). The self-learning was progressively poorer after longer cold exposures (Figure 2, C2, D2). Conspecific learning in the second patch was similarly affected by cold exposure. After three and six weeks of cold exposure, the switch to antennal rejection was slow and never complete (Figure 2, B3, Figure 2, C3). Females exposed to cold for twelve weeks never learned the conspecifics cues (Figure 2, D3).

Again, in the third patch, the time needed to learn to recognise conspecific cues increased after three or six weeks of cold exposure compared to controls (Figure 2, B4, C4 and A4, respectively). No females exposed to cold for twelve weeks rejected conspecific-parasitized eggs via antennal rejection (Figure 2, graph D4 is not presented because no antennal rejection occurred).

Durations of the Different Types of Rejection

In the first experiment, the duration of an antennal rejection was significantly shorter than the duration of a sting rejection (5.45 ± 0.34 ($n = 1510$) and 53.54 ± 2.50 ($n = 254$) respectively, $F = 356.94$, $df = 1$, $p < 0.0001$, GLM test). The same result was found in the second experiment (6.49 ± 0.54 ($n = 577$) *versus* 75.23 ± 2.06 ($n = 597$) respectively, $F = 827.10$, $df = 1$, $p < 0.0001$, GLM test). While cold exposure did not affect the duration of antennal rejection ($F = 0.72$, $df = 3$, $p = 0.53$, GLM test), the duration of sting rejection increased with the duration of cold exposure (no exposure: 51.52 ± 3.80 ($n = 62$); three weeks: 67.56 ± 2.52 ($n = 176$); six weeks: 78.68 ± 3.52 ($n = 198$); twelve weeks: 90.02 ± 5.40 –n =161); $F = 14.78$, $df = 3$, $p < 0.001$, GLM test).

Table 2

l Curves Showing the Change of Rejection Type by Females of Anaphes victus in Four Different Treatments (0, 3, 6 and 12 Weeks of Cold Exposure). The Curves were Fitted to the Sum of Behaviours of 10 Different Females by Treatment. (SD: strandard deviation, CI: confidence interval).

Treatment	Patch Rank	Parasitized hosts encountered	Model fitting data	a	S.D.	95% CI	b	S.D.	95% CI	c	S.D.	95% C.I.	Inflexion point x_i	y_i
No cold exposure	A	SP	LGC	1.005	0.012	[0.979 ; 1.032]	4.45	1.605	[1.129 ; 7.770]	0.969	0.176	[0.605 ; 1.134]	1.539	0.502
	B	SP	No model fitting*	-	-	-	-	-	-	-	-	-	-	-
		CP	LGC	0.999	0.010	[0.966 ; 1.011]	19389	26538	[-37175 ; 75954]	2.666	0.360	[1.898 ; 3.431]	3.711	0.495
	C	CP	LGC	0.993	0.020	[0.948 ; 1.039]	3.032	1.099	[0.689 ; 5.376]	0.709	0.155	[0.377 ; 1.041]	1.563	0.496
Three weeks of cold exposure	A	SP	LGC	0.583	0.092	[0.387 ; 0.778]	13.985	58.612	[-105.4 ; 133.4]	0.957	1.375	[-1.946 ; 3.858]	2.755	0.292
	B	SP	LGC	0.890	0.084	[0.623 ; 1.158]	11.473	26.583	[-73.13 ; 96.08]	2.245	2.035	[-4.232 ; 8.723]	1.085	0.445
		CP	LR	0.041	0.021	[-0.006 ; 0.088]	0.005	0.157	[-0.347 ; 0.356]	-	-	-	-	-
	C	CP	LGC	0.897	0.075	[0.734 ; 1.062]	19.437	54.436	[66.99 ; 165.8]	0.586	0.177	[0.200 ; 0.972]	6.655	0.448
Six weeks of cold exposure	A	SP	LGC	0.729	0.186	[0.301 ; 1.157]	11.374	23.3	[-42.37 ; 65.11]	0.628	0.584	[-0.721 ; 1.972]	3.880	0.364
	B	SP	PR	- 0.118	0.113	[-0.607 ; 0.370]	0.663	0.693	[-2.321 ; 3.647]	- 0.171	0.910	[-4.087 ; 3.745]	-	-
		CP	LGC	0.491	0.231	[0.194 ; 0.788]	60.48	160.02	[-301.5 ; 422.4]	0.647	0.483	[-0.447 ; 1.741]	6.338	0.245
	C	CP	LR	0.065	0.017	[0.024 ; 0.104]	-0.098	0.139	[-0.390 ; 0.194]	-	-	-	-	-
Twelve weeks of cold exposure	A	SP	PR	- 0.018	0.005	[-0.031 ; -0.005]	0.1979	0.063	[0.047 ; 0.347]	- 0.245	0.152	[-0.606 ; 0.115]	-	-
	B	SP	LR	- 0.050	0.028	[-0.174 ; 0.074]	0.1667	0.078	[-0.174 ; 0.507]	-	-	-	-	-
		CP	LR	- 0.007	0.003	[-0.015 ; 0.000]	0.064	0.023	[0.011 ; 0.118]	-	-	-	-	-
	C	CP	No model fitting**	-	-	-	-	-	-	-	-	-	-	-

Note. *No model was fitted as no sting rejection were observed; **No model was fitted as no antennal rejection were observed; SP = Self-parasitized; CP = Conspecific parasitized; LR: Linear regression; LGC: Logistic growth curve; PR: Polynomial regression
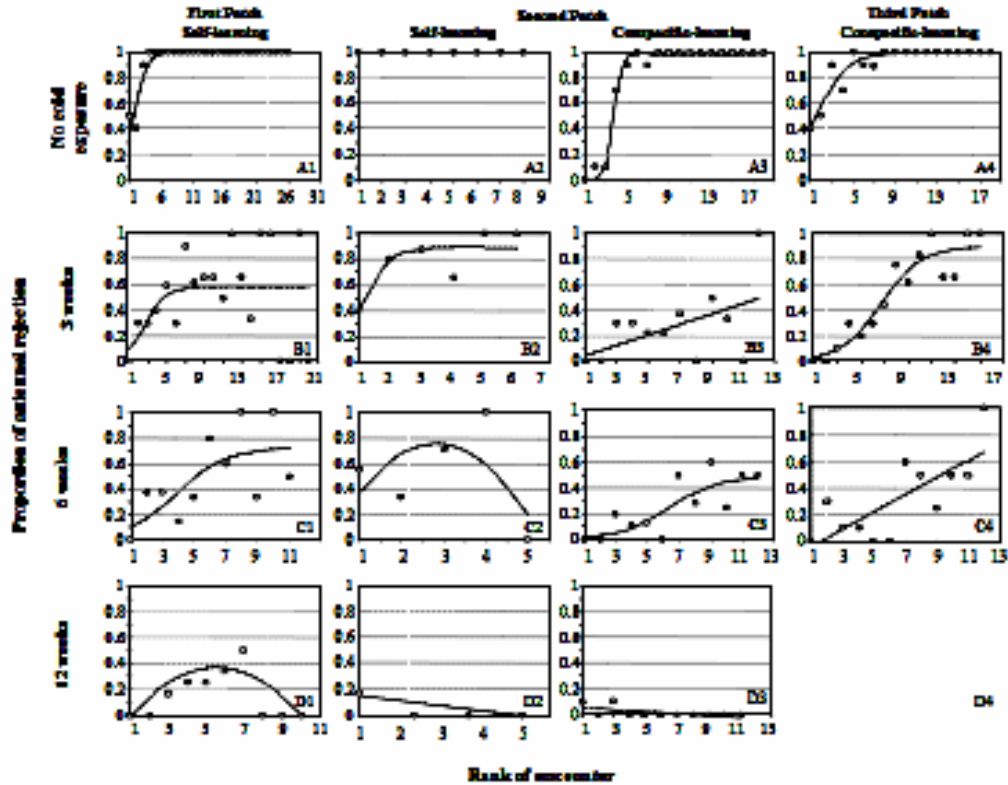
Figure 2. Graphs A1 to D3: Learning curves by 10 females of *A. victus* exposed to 4°C for 0, 3, 6 and 12 weeks, in the three patches of the optimist environment. For patches containing two types of eggs (self- and conspecific parasitized), two curves were proposed. See table 2 for mathematical parameters of the curves. First, second and third patches refer to the three successive patches that were offered to the females. For each graph, the x axis represents the rank of encounters with eggs (first, second, third, etc…) and the y axis represents the % of antennal (versus sting) rejection. Antennal and sting rejection represent 100% of the rejections. "Self learning" refers to a female learning to recognize her own external cues on a host egg, whereas "conspecific learning" refers to learning to recognize the cues left by other females.

Discussion

The data presented here support previous findings demonstrating that female *A. victus* can learn to associate information about a host egg acquired via the act of ovipositioning with that acquired at or around the same time via antennal contact with the egg. This allows the wasp to replace longer duration sting rejections with much faster antennal rejections (van Baaren & Boivin, 1998a). In addition, learning the cues associated with an egg previously parasitized by the wasp herself occurs more quickly than learning the cues associated with a host egg parasitized by another wasp of the same species. Further, the former can last from two to four hours before it is totally forgotten (van Baaren & Boivin, 1998a). Our study also showed that *A. victus* females learn as well in a poor (host sparse) environment than in a good (host rich) environment suggesting that the quality of envi-

ronment does not interfere with the nature or the rapidity of learning in this species.

One major new finding is that foraging experiences affected the wasps' performance: Females used the information that they were acquiring more quickly when foraging in a third patch than when foraging in the second patch, even if they were isolated for the same amount of time between patches. Papaj and Prokopy (1989) suggested that to be considered learning, a change of behaviour has to disappear if the stimulus that drove that change is no longer present. Generally, olfactory memory persists only 3 to 7 days in insects (e.g., Du, Poppy, Powell, & Wadhams, 1997) although persistence of up to 14 days has been shown in the parasitoid wasp *Hyssopus pallidus* (Hymenoptera: Eulophidae) (Gandolfi, Mattiacci & Dorn, 2003). In bees, an odour learned and forgotten is relearned more rapidly than a new odour, which indicates that a memory trace can persist without behavioural output (Menzel, 1983; Menzel, Erber & Masuhr, 1974). A similar process could explain the behavioral changes observed in *A. victus*. In the poor treatment, females learned to reject eggs parasitized by conspecific females following antennal contact. When placed in the second patch, these females forgot partially what they have learned in the first patch. However, when confronted with parasitized eggs in the third patch, the females immediately rejected them following antennal contact. In the parasitoid wasp *Leptopilina boulardi*, two different types of memory were shown: a short-term memory, which persisted 1 to 2 hours and a long-term memory, which persisted more than 24h, but no more than 3 days (Kaiser, Perez-Maluf, Sandoz, & Pham-Delegue, 2003).

We also found that female *A. victus* learned conspecific cues more rapidly after learning her own cues and vice-versa. The effect of previous learning of different cues could be compared to transfer, where learning a behavioural pattern in one task influences the learning of a behavioural pattern in another task (Smith, 1991). If the performance of subjects on the second task learned is better than on the first, the transfer is said to be positive. Positive transfer effects have been observed in bee motor learning on natural flowers: Bees do better at handling a second flower species than they do when they begin as entirely naive foragers (Chittka, Gumbert & Kunze, 1997). In humans, as in bees, the degree of transfer depends strongly on the similarity between tasks (Chittka et al., 1997). In *A. victus*, a chemical similarity between self and conspecific cues (van Baaren & Boivin, 1998b) could explain the positive transfer occurring between these tasks. When learning different but similar stimuli, an organism may use a categorization process that accelerates learning of all stimuli within a category (e.g., Zhang, this volume). This performance is not a simple form of learning (habituation, sensitization or associative learning), but somewhat more complex, and was shown in honeybees (Giurfa, 2003). Again, the faster learning of conspecific cues by females having learned their own cues may be due to categorization of these chemical cues.

In *A. victus*, the adaptive value of learning parasitized host-related cues seems to be a gain of time, in that antennal rejection is approximately twenty times faster than sting rejection. Antennal rejection may also reduce risk of predation because females are more vulnerable during lengthy sting rejections than during the shorter antennal rejection process. This gain of time also allows females to increase their fitness; a female that rejects parasitized hosts quickly gains time to

oviposit in the patch's remaining unparasitized hosts. This type of time savings has also been shown in the parasitoid wasp *Pholetesor bicolour* (Hymenoptera: Braconidae) when it discriminates between two larval stages of its host, one a sap-feeder and the other a tissue feeder. The average time required to parasitizing the tissue feeder is 1015 s, whereas the average time required for parasitizing a sap-feeder host is only 297 s. Females of this species were able to learn to choose the latter, more profitable resource (Dutton et al., 2000).

Deleterious Effect of Cold Storage on Learning

In parasitoids, few data are available on the effects of cold temperature exposure on behaviour. Tezze and Botto (2004) showed a decrease in locomotion capabilities in the wasp *Trichogramma* (Hymenoptera: Trichogrammatidae), particularly after 50 days of cold storage. In the wasp *Microplitis demolitor* (Hymenoptera: Braconidae), holding the pupae at 13°C for four days made most of the emerging females unresponsive to volatile semiochemicals (Hérard et al., 1988). Females were exposed to host frass before the experiment and while control females learned this odour and showed sustained flight towards it, females exposed to low temperature did not, possibly as a result of a loss of learning capacities. Previously, we have shown that in *A. victus*, patch exploitation strategies are affected by cold exposure (van Baaren et al., 2005). Here, we have shown that cold exposure also has a deleterious effect on the capacity of *A. victus* females to learn, or at least on their capacity to use what they have learned about parasitized host egg cues, and that the degree of the effect is correlated with the duration of cold exposure. Although the ability to learn self- and conspecific generated cues associated with the parasitized host egg were affected, the latter suffered most. Previously, van Baaren and Boivin (1998a) showed that learning self-generated cues associated with parasitized host eggs was easier and faster than learning cues from conspecifics or from females of a different species. Results presented here suggest that difficult tasks can be made more difficult by cold exposure, though the precise mechanism by which this occurs is not known.

We do know that the behaviours of adult honey bees are influenced by the temperature experienced during pupal development (Tautz, Maier, Groh, Rossler, & Brockmann, 2003). Individuals reared during the pupal instar at the lowest temperatures within the natural range of 33-36°C perform more poorly in dance communication and olfactory learning than do bees that developed at higher temperatures. Groh, Tautz and Rossler (2004) proposed that the cellular activities and processes associated with neurometamorphosis during larval development may be affected by temperature. In honey bees, different sensory pathways converge on different central nervous system centres, including the mushroom bodies, where multimodal sensory integration can occur. Such centres allow the association of stimuli during learning and recall and they could be the substrate for complex forms of learning. Transfer of information between sensory modalities and processes underpinning the categorization of novel stimuli could also occur in these centres (Giurfa, 2003). In the lip of the mushroom body calyx, changes in microglomeruli numbers can occur at temperature differences of less than 1°C. Microglomeruli, therefore, represent a potential neuronal substrate for temperature-mediated effects on adult be-

haviour. In the mushroom body collar, microglomeruli numbers responded less sensitively, indicating that temperature has a differential influence on different brain neuropils. It was shown that the mushroom bodies have an important role in mediating behavioural plasticity, which may be especially true for complex tasks such as navigation, multimodal communication, learning, and memory (Zars, 2000).

In conclusion, the studies on learning in insects have focused mostly on the learning processes within some model species such as the honeybee and *Drosophila* whereas studies in parasitoids have focused more on the adaptive value of learning, and on the circumstances where learning is used. Our results on the effect of cold exposure on learning underline the need for studies looking at the mechanisms involved in these responses. In agriculture and forestry, the production of cold stored biological agents, with a good survival rate, a good longevity and fecundity, but a bad capacity to learn is undesirable. Learning of odours is used in all steps of the parasitoid life, and particularly for the steps of host habitat and microhabitat location. The sublethal effects on behaviour we observed could interfere with the reproduction capacity of parasitoids and have an impact on the population biology of insects.

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