

AGE-DEPENDENT ASSOCIATIVE LEARNING

BY EXERISTES ROBORATOR (F.)

(HYMENOPTERA : ICHNEUMONIDAE)

by

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Age-dependent Associative Learning by Exeristes roborator (F.)

(Hymenoptera : Ichneumonidae).

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ABSTRACT

Females of the polyphagous ichneumonid parasitoid, Exeristes roborator (F.), that were conditioned for 10 days to hosts in a specific habitat demonstrated associative learning by responding with ovipositor probes to the habitat alone. Females conditioned immediately after eclosion showed a higher frequency, intensity, and retention (approximately 7 days) of learned behavior than those conditioned 5 or 10 days after eclosion, despite the fact that newly-eclosed females did not probe for hosts with their ovipositors until 4 or 5 days after eclosion. This is the first demonstration of age-dependent learning ability in an insect parasitoid.

The reduction in the learning ability of females within a short time after eclosion may impart a selective advantage to E. roborator by reducing the costs of learning, or may reflect developmental constraints on the process of learning. The results suggest that carefully controlled associative learning could be exploited to improve the efficiency of host location by parasitoids released in biological control programs.

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INTRODUCTION

Parasitism of a host by an insect parasitoid is the result of the successful completion of a complex series of events. Douth (1959) has divided this series into 4 components : 1) host habitat finding, 2) host finding, 3) host acceptance, and 4) host suitability for the development of immature stages. To this list Vinson (1975) has added a fifth component, host regulation, the process by which the ovipositing female and/or her progeny change the physiology, morphology, development, or behavior of hosts (Vinson and Iwantsch 1980).

Together, the first 3 components comprise the process of host selection (Vinson 1976). Many physical, chemical, physiological, and behavioral factors are involved in host selection by parasitoids (Douth 1959; Vinson 1976). Associative learning by the female parasitoid can be one of these factors. Arthur (1966,1967) showed that the polyphagous ichneumonid, Itopectis conquisitor (Say), learned to associate the color, size, and shape of a habitat with the presence of hosts. Taylor (1974) demonstrated that associative learning played a role in host selection by the oligophagous ichneumonid, Nemeritis canescens (Gravenhorst), a result supported by Arthur (1971), who showed that this parasitoid learned to associate habitat odor with its hosts. Bracon mellitor Say, a polyphagous braconid, learned to engage in ovipositional probing in response to the isolated chemical methyl parasept, an artificial dietary component retained in its hosts' hemolymph, after repeated

exposure to contaminated hosts (Vinson et al. 1977).

Learning also played a role in host acceptance by Trichogramma embryophagum Hartig, an egg parasitoid of moths, and Pseudeucoila bochei Weld, a larval parasitoid of Drosophila spp.. Both parasitoids learned to distinguish unparasitized hosts from those already parasitized by members of their own species (van Lenteren and Bakker 1975; Klomp et al. 1980).

The involvement of learning in host selection is not limited to parasitoids of the order Hymenoptera. Monteith (1963) demonstrated that the tachinid, Drino bohémica Mesnil, learned to associate habitat movement with the presence of hosts.

It is also possible that pre-imaginal conditioning of parasitoid larvae, as apparently occurs in N. canescens (Thorpe and Jones 1937), could influence host selection by adult females. However, Monteith (1958) and Arthur (1965) found that pre-imaginal conditioning did not occur in D. bohémica and Bessa harveyi Townsend, and I. conquisitor, respectively.

Evidence of learning is seen when changes in individual behavior occur as a result of experience. Associative learning includes all types of learning which result in the establishment of an association between a stimulus and a unit of behavior previously unlinked, due to experience. Conditioning falls into this category (Thorpe 1963).

The fact that learning plays a role in host selection by some parasitoids could have important implications for their use as biological control agents in pest management. The success of inundative release programs as well as many programs in which

insects are released for establishment depends upon the ability of parasitoids reared in an artificial environment to locate their target hosts in a totally different natural environment as soon as possible after release. When reared in an insectary, parasitoids may become conditioned to associate characteristics of this artificial environment with hosts and their habitats, or be conditioned to accept factitious hosts in place of natural ones. Thus they may be incapable of efficient host habitat location, host location, or host acceptance once released. While they might eventually become reconditioned to a new environment or host, heavy losses in the interim due to predation, weather, or dispersal away from the target infestation might reduce their numbers to ineffective levels.

Conversely, it might be possible to exploit this learning ability to 'train' parasitoids to become more efficient at locating target hosts in their natural habitats, and thus increase the success and impact of inundative release or establishment programs. Conditioning could be especially useful in overcoming the effects of rearing parasitoids on factitious hosts and in concentrating the attack of a polyphagous parasitoid on a target pest when other potential hosts are present.

Exeristes roborator (F.) is a polyphagous ichneumonid ectoparasitoid native to Europe. After locating a host habitat, females of this species search with their antennae for host larvae concealed in plant material. When hosts are found, possibly through chemical cues, females thrust their ovipositors

through the plant material into the host's body, paralyzing it by injecting venom or by causing mechanical damage. Thrusting of the ovipositor is not random, but occurs in response to the presence of a host, although females will sometimes probe an area that a host has recently vacated. After paralyzing a host larva, females lay one or more eggs on or near the larva. In the laboratory, females will oviposit on living, dead, paralyzed, and unparalyzed hosts (Baker and Jones 1934).

The natural hosts of E. roborator include such important pests as the European corn borer, Ostrinia nubilalis (Hübner), the European pine shoot moth, Rhyacionia buoliana (Schiff.), and the pink bollworm, Pectinophora gossypiella (Saunders) (Thompson 1957). E. roborator has been released in biological control programs directed against these 3 pests in various locations in the western hemisphere. In the 1920's and 1930's in eastern North America and in the 1920's in Guam it was released against O. nubilalis but it did not become established (Baker et al. 1949; MacLeod 1962; McGugan and Coppel 1962; Clausen 1978b). In the same areas of North America it was again released in the 1950's against R. buoliana, once more without success (MacLeod 1962; McGugan and Coppel 1962; Clausen 1978a). Its release against P. gossypiella in the southern United States, Mexico, and Puerto Rico in the 1930's and 1940's was also a failure (Oatman 1978). This parasitoid has also been employed unsuccessfully against the sugarcane borer, Diatraea saccharalis (F.), which is not a natural host, in the southern United States (Clausen and Oatman 1978). E. roborator

did not become permanently established at any release site (Clausen 1978b; Clausen and Oatman 1978; Oatman 1978; Krombein et al. 1979). A suggested reason for the failures in northern regions is a lack of seasonal synchronization between the parasitoid and its hosts (Baker and Jones 1934).

A preliminary study by L.M. Friskie¹ (unpublished) suggested that associative learning plays a role in host habitat location by E. roborator. My objectives were: 1) to verify the occurrence of associative learning in E. roborator and 2) to investigate the effect of age on the learning ability of E. roborator females.

MATERIALS AND METHODS

Rearing and Maintenance of Experimental Insects

In a modification of Baker and Jones' (1934) system, E. roborator were reared as larvae and pupae in individual cells in plexiglass trays kept under an 8 h light : 16 h dark regime at 24-26°C and 90% R.H. (Syed 1984). They were fed late-instar larvae of Galleria mellonella (L.) that had been coddled, i.e. killed by immersion in water at 60°C for 60 sec. Fresh food was placed in each cell every second day until the E. roborator larvae had pupated. On average, males and females, respectively,

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pupated after 9 and 11 days as larvae and eclosed 10 and 12 days later.

Verification of Learning and Investigation of Effect of Age on Learning Ability

Groups of females of 3 different ages were conditioned to a specific host habitat for 10 days. The conditioning period was followed by a 2-week testing period during which the responses of these conditioned insects and unconditioned control insects to this habitat were monitored. As in Arthur's (1966, 1967) studies on associative learning in I. conquisitor, probing of the habitat with the ovipositor was deemed to be a positive response to the habitat, indicating that females were searching for hosts there. Both the proportion of females responding to the habitat and the number of times the females probed with their ovipositors were recorded.

Every morning newly-eclosed females were collected from the rearing trays and placed in 30x30x45 cm cages, where they were either subjected to conditioning immediately or held for 5 or 10 days. Each day's females were marked on the thorax with a spot of model paint (Testor Corp., Weston, Ont.) of the same color to identify them. Those females subjected to a post-eclosion holding period were moved to identical cages for conditioning at the beginning of the photoperiod on the sixth or eleventh day after they had eclosed. While in holding and conditioning cages females had access to water, sugar, and honey, and were allowed to mate.

During the conditioning period (Table 1) females were given host larvae placed between the folds of a Kimwipe[®] (Kimberly-Clark Corp., Toronto, Ont.), held together by the lid and body of a 150 ml styrofoam cup (Stax Plastics, Ltd., Toronto, Ont.). This 'egg cup' apparatus (Fig. 1), the host habitat, was inverted and placed in the centre of the cage floor. Sections of the cup were cut away to give the females access to the Kimwipe surface, where they thrust their ovipositors through the Kimwipe into the larvae, fed upon the larval exudates to obtain protein needed for egg maturation (Baker and Jones 1934), and oviposited through the Kimwipe. On each morning of the conditioning period fresh egg cups were placed in the conditioning cages and left for the duration of the photophase.

Unconditioned control females were treated in exactly the same manner except that they were given host larvae exposed on the cage floor (Table 1) instead of in an egg cup.

At the end of the photoperiod on their tenth day of conditioning, females were removed from conditioning cages, assigned to test treatment groups (Table 1), and placed in identical cages where they remained for the testing period. Unconditioned females in Group I were controls monitored for any innate response to an empty egg cup. Females in Group II were conditioned controls monitored for their response to a full egg cup to determine what proportion of conditioned insects could normally be expected to respond when hosts were present, and what their probing frequencies would be. Females

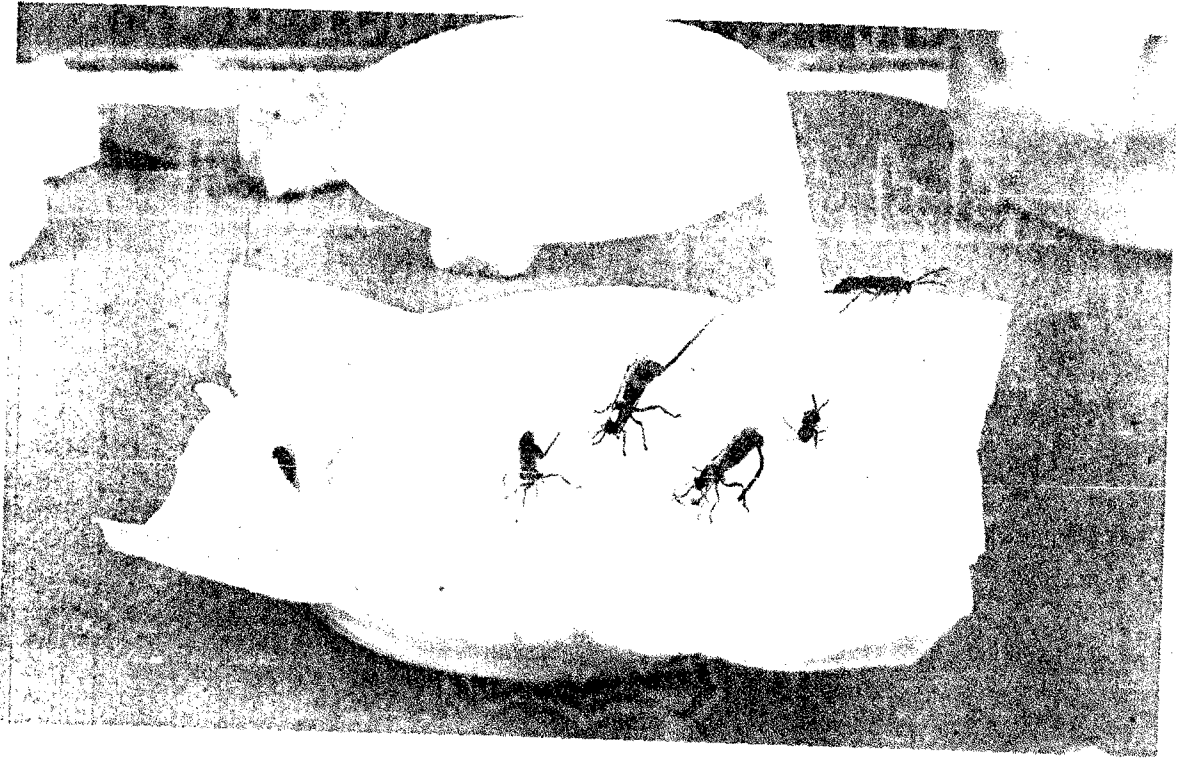
Table 1. Description of conditioning and post-conditioning test treatments for E. roborator females.

Test Group ^a	Conditioning Treatment ^b	Test Treatment ^b
I (Unconditioned Controls)	Given 5 host larvae exposed on cage floor for duration of photo-phase each day for 10 days.	Given empty egg cup for 1 h each day for 14 days.
II (Conditioned Controls)	Given 5 host larvae in egg cup for duration of photo-phase each day for 10 days.	Given 5 host larvae in egg cup for 1 h each day for 14 days.
III (Unfed Experimentals)	"	Given empty egg cup for 1 h each day for 14 days.
IV (Fed Experimentals)	"	Given empty egg cup for 1 h, then host larvae exposed on cage floor for 1 h each day for 14 days.

^aFor convenience, control groups are referred to throughout the manuscript as unconditioned and conditioned, despite the possibility that insects in Group I could have been conditioned to the cage floor as a host habitat.

^bHost larvae in all cases were G. mellonella coddled for 60 sec at 60°C (Syed 1984).

Figure 1. E. roborator females ovipositing through Kimwipe onto host larvae in an egg cup.



in Group III were monitored for their response to an empty egg cup to determine if they demonstrated associative learning, and associated the empty egg cup with hosts. Females in Group IV were also monitored for their response to an empty egg cup, but they were then exposed to host larvae (on which they fed freely), to determine whether or not nutritional status affected ability to respond to an empty egg cup over the testing period.

Individuals within each group were remarked on the thorax with different colors of model paint. All had access to water, sugar, and honey in the testing cages, but no males were present.

On each day of the testing period all groups were monitored for their response to the appropriate egg cup apparatus (Table 1). A fresh egg cup was placed in the cage at the beginning of each monitoring period and removed immediately after. The number of times each female probed the apparatus during this time was recorded. A probe was counted if the ovipositor was unsheathed and inserted into any part of the egg cup. Group IV females were exposed to host larvae immediately after completion of the monitoring period. The order in which groups were tested was varied from day to day, and the procedure was repeated until 20 females had been tested in each group.

Development of Probing Activity by Females After Eclosion

Newly-eclosed, individually-marked females were placed in a conditioning cage with water, sugar, honey, and males. On each of the 13 days immediately following eclosion a fresh egg cup containing 5 coddled G. mellonella larvae was placed in the cage at the beginning of the photoperiod and left all day. Ovipositor probing of the egg cup by the females was monitored for 1 h at the same time each day. Twenty females were observed, and the proportion of females probing, as well as the number of times they probed, was recorded.

Statistical Analysis

The proportions of responding females in each group were converted using an arcsin transformation and compared using a Newman-Keuls test modified for testing proportions². The numbers of times females probed were compared using the nonparametric STP test for multiple comparisons (Sokal and Rohlf 1981). Standard errors were calculated for percentages using the method of Parker (1979) and for means using the method of Sokal and Rohlf (1981).

²Dr. R.D. Routledge, Department of Mathematics, Simon Fraser University, Burnaby, British Columbia. V5A 1S6. Unpublished procedure.

RESULTS AND DISCUSSION

E. roborator females conditioned for 10 days to egg cups containing host larvae, and thereafter given empty egg cups for 1 h/day, responded positively with ovipositor probes into the empty egg cups (Figs. 2,3; Tables 2,3). Therefore, they are capable of learning to associate a habitat with a host, as are other non-specific parasitoids (Arthur 1966, 1967, 1971; Monteith 1963; Taylor 1974).

Females subjected to the 10-day conditioning regime immediately after eclosion showed a higher frequency (Fig. 2, Table 2), intensity (Fig. 3, Table 3), and retention (Figs. 2,3; Tables 2,3) of the learned response than those that began their conditioning 5 or 10 days after eclosion. Thus young females are more capable of learning and retaining the learned response than older ones. This is the first record of age affecting the learning ability of an insect parasitoid.

The superior learning ability of newly-eclosed females, as evidenced by ovipositor probing into an empty egg cup, is somewhat surprising, because the majority of females tested did not begin to probe with their ovipositors and feed on larval exudates until 4 or 5 days after eclosion (Figs. 4,5). Evidently early exposure to the host in its habitat contributes to the learning process in E. roborator females even though they are unable to respond with ovipositor probing when exposed to hosts during this post-eclosion maturation period. Perhaps immediate learning is of adaptive advantage in promoting

Figure 2. Percent of test females in 4 groups conditioned at 3 different ages responding to an egg cup during a 1 h monitoring period on each day of a 14-day testing period.

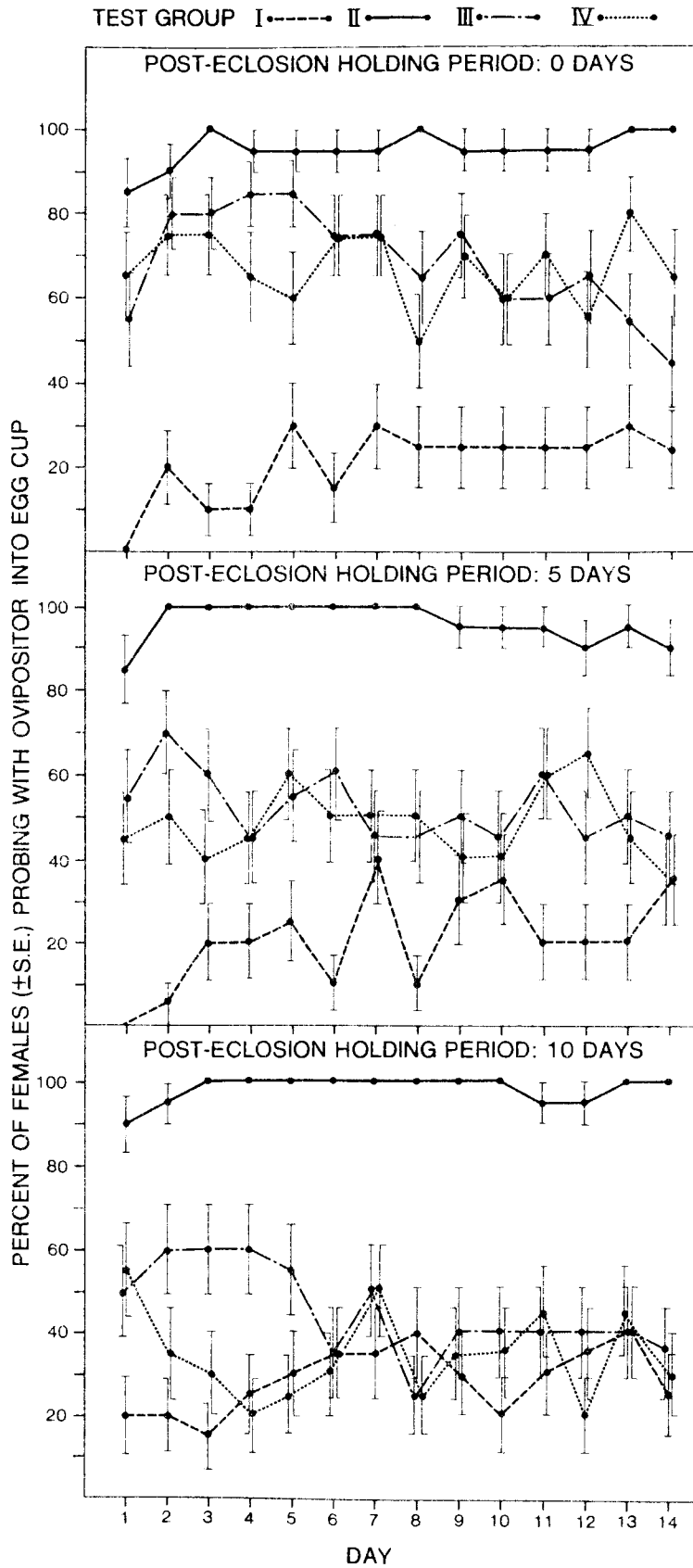


Figure 3. Mean number of ovipositor probes into an egg cup by test females in 4 groups conditioned at 3 different ages during a 1 h monitoring period on each day of a 14-day testing period.

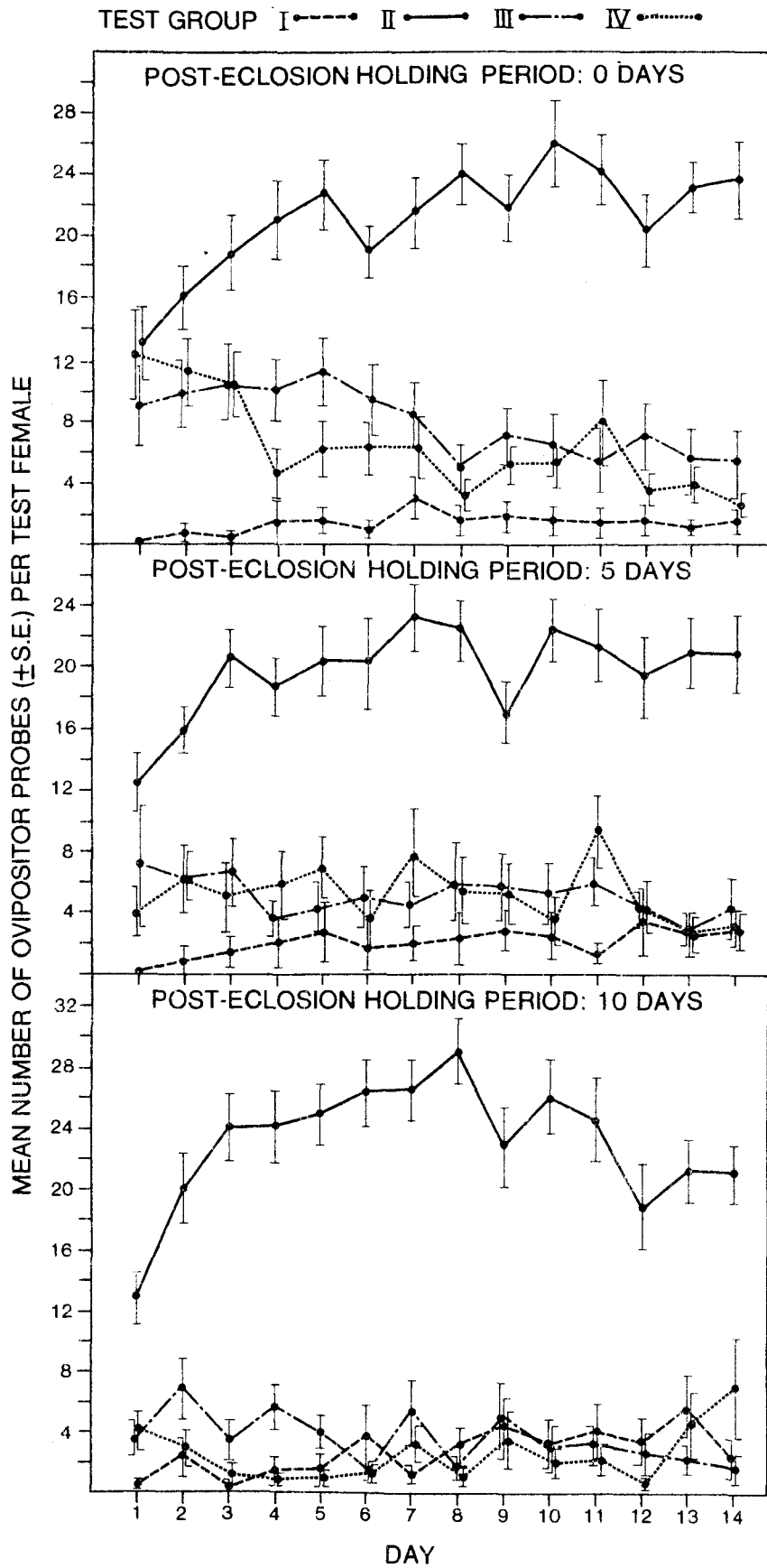


Table 2. Results of statistical comparisons between percent of 20 females in 4 test groups responding with ovipositor probes into egg cups, Newman-Keuls test modified for comparing proportions, $P < 0.05$. Different letters indicate significant differences between values shown for each day in Fig. 2.

Post- Eclosion Holding Period	Test Group ^a	Comparison of Differences by Test Day													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
0 days	II	a	a	a	a	a	a	a	a	a	a	a	a	a	a
	III	a	a	b	ab	ab	a	a	b	a	b	b	b	bc	bc
	IV	a	a	b	b	bc	a	a	b	a	b	b	b	b	b
	I	b	b	c	c	c	b	b	c	b	b	c	c	c	c
5 days	II	a	a	a	a	a	a	a	a	a	a	a	a	a	a
	III	b	b	b	b	b	b	b	b	b	b	b	bc	b	b
	IV	b	b	bc	b	b	b	b	b	b	b	b	b	b	b
	I	c	c	c	b	b	c	b	c	b	b	c	c	b	b
10 days	II	a	a	a	a	a	a	a	a	a	a	a	a	a	a
	III	b	b	b	b	b	b	b	b	b	b	b	b	b	b
	IV	bc	bc	bc	c	b	b	b	b	b	b	b	b	b	b
	I	c	c	c	c	b	b	b	b	b	b	b	b	b	b

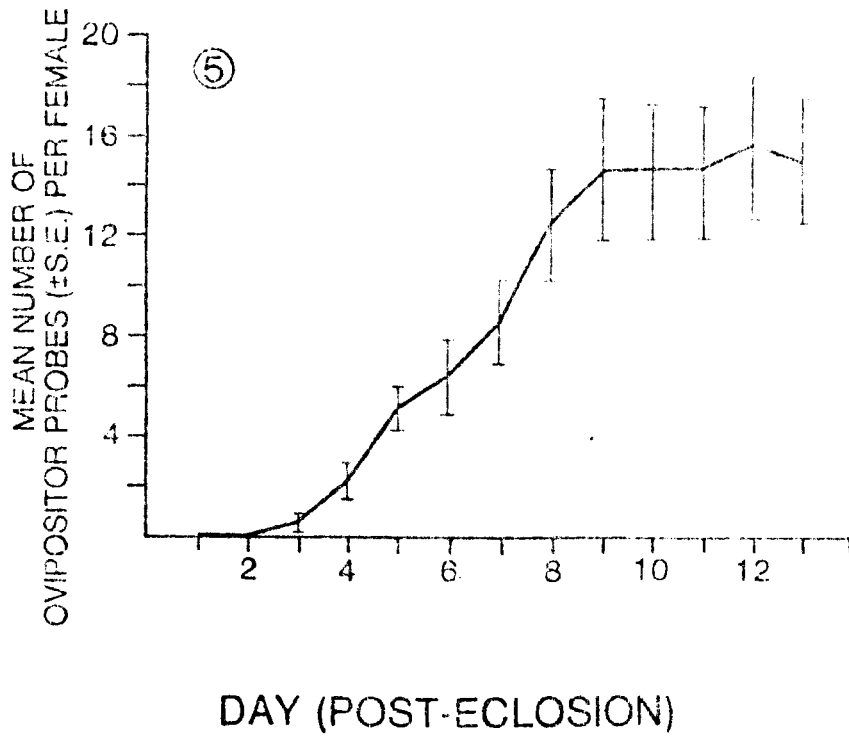
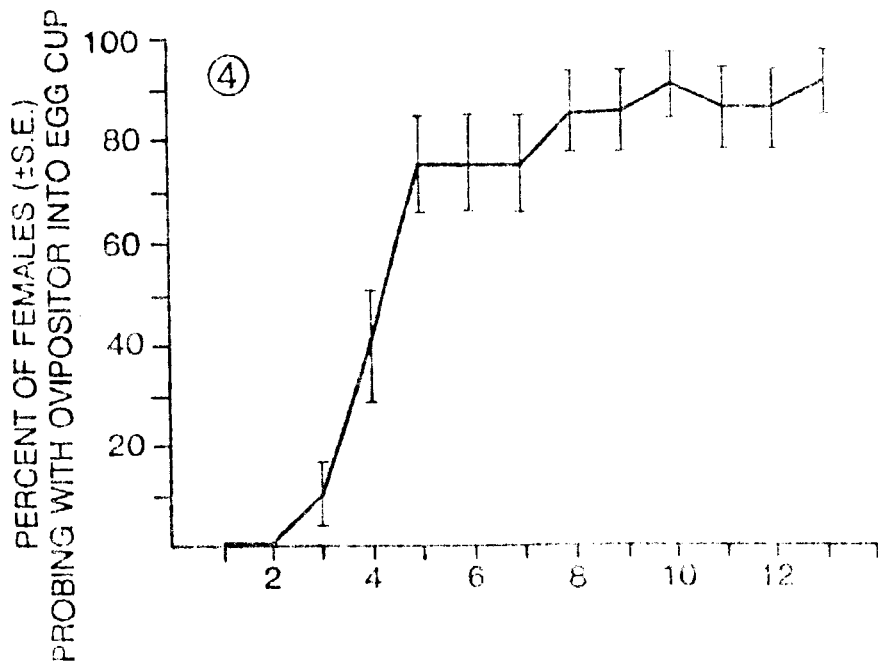
^aTest Groups (Table 1) ranked according to most consistent response level in Fig. 2.

Table 3. Results of statistical comparisons between ovipositor probes/female in 4 test groups, STP test, $P < 0.05$. Different letters indicate significant differences between distributions of values from which means shown in Fig. 3 were calculated.

Post- Eclosion Holding Period	Test Group ^a	Comparison of Differences by Test Day													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
0 days	II	a	a	a	a	a	a	a	a	a	a	a	a	a	a
	III	a	a	a	b	b	b	b	b	b	b	bc	b	bc	b
	IV	a	a	a	b	bc	b	bc	b	b	b	b	b	b	b
	I	b	b	b	c	c	c	c	b	c	b	c	b	c	b
5 days	II	a	a	a	a	a	a	a	a	a	a	a	a	a	a
	III	b	b	b	b	b	b	b	b	b	b	b	b	b	b
	IV	b	b	b	b	b	b	b	b	b	b	b	b	b	b
	I	c	c	b	b	b	c	b	b	b	b	c	b	b	b
10 days	II	a	a	a	a	a	a	a	a	a	a	a	a	a	a
	III	bc	b	b	b	b	b	b	b	b	b	b	b	b	b
	IV	b	b	bc	c	b	b	b	b	b	b	b	b	b	b
	I	c	b	c	bc	b	b	b	b	b	b	b	b	b	b

^aTest Groups (Table 1) ranked according to most consistent response level in Fig. 3.

Figures 4,5. Percent of females (Fig. 4) probing with ovipositors into an egg cup and mean number of probes/female (Fig. 5) during a 1 h observation period for females exposed to a full egg cup all day for 13 days immediately following eclosion.



efficient early location of hosts on which to feed, thus providing essential nutrients for vitellogenesis.

Responses by the unconditioned controls of all 3 ages in Group I (Table 1) were consistently low throughout the testing period, as measured by both participation rate (Fig. 2) and number of probes/female (Fig. 3). A supplemental control experiment was completed in which 10 females were conditioned for 10 days immediately after eclosion to an empty egg cup, and then tested for 1 h/day for 5 days for responses to an identical egg cup. The responses of these females were compared with those of females in 3 other groups equivalent to Groups I, II, and III (Table 1). There was no difference between the responses of females exposed to an empty egg cup for 10 days and those of females treated as unconditioned Group I insects (Appendix 1). Therefore, the possibility that conditioning to the cage floor as a host habitat deterred females in Group I from responding to the empty egg cup during the testing period can be discounted.

In contrast, responses by the conditioned controls in Group II remained high throughout the testing period (Figs. 2,3), and were significantly higher than responses by Group I females on all test days (Tables 2,3). Therefore, no effect associated with the egg cup or with aging could have caused the decline of learned responses over time by females in Groups III and IV (Figs. 2,3; Tables 2,3). As well, the lower responses of females in Groups III and IV conditioned 5 and 10 days after eclosion (Figs. 2,3; Tables 2,3) cannot be attributed to a decreasing ability to respond with age, because

Group II females of all 3 ages maintained similar high levels of response (Figs. 2,3).

The learned responses of unfed experimentals in Group III (Table 1) were generally similar to those of Group IV females which were given host larvae each day immediately after the 1 h exposure to the empty egg cup (Figs. 2,3). On the one occasion that participation rates (Table 2) and intensity of response (Table 3) were significantly different between the 2 groups (day 4 for females held 10 days post-eclosion), responses by the fed experimentals were lower than those by the unfed experimentals.

It is evident that neither an adverse effect associated with the egg cup or with aging, nor a lack of opportunity to feed on host larvae caused the decline in learned response by unfed females in Group III. Therefore, this decline can be attributed to a lack of retention of the learned behavior. It is difficult to determine how long a significant degree of learned behavior is retained by E. roborator females conditioned immediately after eclosion, but a strong learned response was seen for at least 7 days after conditioning ceased. After this time at least some females stopped displaying learned behavior. Arthur (1966, 1967) obtained similar results with I. conquisitor females, which showed a strong conditioned response for 8 days.

Non-specific parasitoids may derive distinct benefits from employing associative learning in host selection despite Cowie and Krebs' (1979) and Nachman's (1981) contention that the investment of time and energy required to gather the information

necessary to learn is relatively great for an insect parasitoid, which might be expected instead to depend upon innate behavior.

Arthur (1966, 1967) suggested that learning to associate a habitat with a host increases the host-finding efficiency of polyphagous parasitoids while maintaining their potential to find hosts in many diverse habitats, and reasoned that for a parasitoid with a limited range of hosts occupying similar habitats this learning ability would be less useful. In support of this hypothesis Arthur (1971) demonstrated that associative learning in an oligophagous parasitoid was much less persistent than in a polyphagous parasitoid. Vinson (1976) and Vinson et al. (1977) concluded that associative learning increases the success of polyphagous parasitoids by permitting efficient exploitation of different hosts, which vary in abundance with time and place, and from year to year. Thus associative learning would allow generalists such as E. roborator to concentrate their searching on the most productive habitats at any given time.

Associative learning can play a role in oviposition site selection by other generalistic insects. Jaenike (1982) concluded that associative learning in oviposition site selection by Drosophila melanogaster Meigen permitted increased use of available resources, closer correspondence between oviposition on and abundance of fluctuating resources, and spread of genes increasing fitness on particular hosts. He hypothesized that all these factors could contribute to the adaptive evolution of both phytophagous and parasitic insects.

Some potential selective advantages that could accrue to an herbivorous insect employing associative learning may be pertinent in oviposition site selection by adult females of the apple maggot, Rhagoletis pomonella (Walsh) (Prokopy et al. 1982). These include reduction of dispersal and associated mortality, and, again, concentration of searching by the insect on the most abundant host. Prokopy et al. (1982) also suggested that associative learning increases the foraging efficiency of R. pomonella by increasing its host acceptance rate. This advantage might also accrue to some parasitoids.

Learning to associate habitats with hosts only during a limited time in early adult life might impart a selective advantage to E. roborator females by reducing their overall investment of time and energy in learning while still giving them the opportunity to gain most of the benefits of learning. This compromise would only be possible if the individual female, after becoming conditioned to the habitat of one of her hosts, were able to continue locating this same species at the developmental stage necessary for successful parasitization for the rest of her reproductive life, and if continued experience locating this host in the same habitat reinforced the learned association, as in Group II females (Figs. 2,3). This may be true for E. roborator females (Baker and Jones 1934), and would make learning to identify new host habitats later in life less important. However, the utilization of different host species by individual females would have to be more thoroughly investigated before it could be concluded that they would not

need to form new associations between hosts and their habitats throughout adulthood. For females of I. conquisitor, which must attack different host species during their lives (Arthur 1967), it would not be of adaptive advantage for learning ability to be age-dependent. In contrast to females of E. roborator, I. conquisitor females learned successfully when first exposed to hosts 8 to 10 days after eclosion (Arthur 1967).

Alternatively, developmental constraints on the processes underlying learning in E. roborator females may limit to a critical post-eclosion period the time during which the ability to learn to associate habitats with hosts can be acquired. Thus females held for 5 or 10 days after eclosion without hosts may not have been able to develop the capacity to learn to any significant degree.

If females develop the ability to learn in early adulthood and retain it throughout their lives, they could be reconditioned to new host habitats, as Prokopy et al. (1982) demonstrated with R. pomonella.

Demonstration of reconditioning in E. roborator females would support the hypothesis that it is the capacity to learn that is developed during a critical period following eclosion. It would refute the hypothesis that the formation of associations between hosts and their habitats is restricted to this brief period. However, acceptance of the former hypothesis would demand that the basic processes of learning in E. roborator and I. conquisitor would be different, because I. conquisitor females do not need early exposure to hosts in order to learn to

associate a habitat with a host (Arthur 1967).

It could be argued that nutritional deficiencies caused by not allowing females to feed on host larvae during the post-eclosion holding period created a constraint on their learning ability. However, newly-eclosed females given immediate access to hosts did not begin to feed on them until 4 or 5 days after eclosion (Figs. 4,5), yet they displayed a learning ability much superior to that of females held 5 or 10 days before being exposed to hosts (Figs. 2,3; Tables 2,3). Clearly this early non-feeding period is critical to the development of associative learning in E. roborator females, and thus it can be concluded that nutritional deficiencies early in adult life were not responsible for the reduced learning ability of the older insects.

The potential for exploitation of associative learning to improve the efficiency of host location by parasitoids released in biological control programs has been pointed out (Arthur 1967; Vinson 1976; Vinson et al. 1977), but never tested. My results indicate that when employing a parasitoid in which learning is age-dependent the timing of conditioning would be critical. In order to condition such an insect effectively it would be necessary to identify accurately the boundaries of the period during which associative learning can occur, and to determine what factors the parasitoid could be conditioned to respond to in the field.

Moreover, in assessing the potential of a parasitoid as a biological control agent, it would be necessary to know how

long it would retain a significant degree of conditioning in the field, as well as whether or not the conditioned parasitoid would be capable of switching to alternative hosts in new habitats if populations of the target host were low.

APPENDIX 1

Statistical analysis of differences between test groups
in supplemental control experiment.

Table 4. Results of statistical comparisons between responses to egg cups by E. roborator females in 4 test groups. Different letters indicate significant differences for each test day.

Response Assessed	Test Group ^a	Comparison of Differences by Test Day ^b				
		1	2	3	4	5
Percent of 10 females responding	II	a	a	a	a	a
	III	a	a	a	a	b
	I	b	b	b	b	c
	IV	b	b	b	b	c
Ovipositor probes/female	II	a	a	a	a	a
	III	a	a	a	a	b
	I	b	b	b	b	c
	IV	b	b	b	b	c

^aTest Groups I-III identical to Groups I-III in Table 1. Test Group IV given an empty egg cup in conditioning period and an empty egg cup in testing period.

^bComparisons for percent of females responding by Newman-Keuls test modified for comparing proportions, $P < 0.05$, and for ovipositor probes/female by STP test, $P < 0.05$.

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