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DEVELOPMENT OF OVIPOSITION BEHAVIOR OF *BRACHYMERIA INTERMEDIA*, A PARASITOID OF THE GYPSY MOTH, *LYMANTRIA DISPAR*.

A Dissertation Presented

by

YVONNE C. DROST

Submitted to the Graduate School of the
University of Massachusetts in partial fulfillment
of the requirements for the degree of

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September 1991

Department of Entomology

DEVELOPMENT OF OVIPOSITION BEHAVIOR OF *Brachymeria intermedia*, A PARASITOID OF THE GYPSY MOTH, *Lymantria dispar*.

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To Olivier,

and my parents

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Chapter 2 of this dissertation is included with permission of *Journal of Insect Behavior*.

ABSTRACT

DEVELOPMENT OF OVIPOSITION BEHAVIOR OF *BRACHYMERIA INTERMEDIA*, A PARASITOID OF THE GYPSY MOTH, LYMANTRIA DISPAR.

SEPTEMBER, 1991

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Brachymeria intermedia is an introduced endoparasitoid of the gypsy moth, *Lymantria dispar*. To gain understanding of the basic mechanisms by which *B. intermedia* influence population densities of the gypsy moth, the oviposition behavior of this parasitoid was studied. Both the physiological state and the informational state of the animal at a certain point in time may influence the development of the behavior. Factors of the physiological state here investigated are egg load and age of the female parasitoid. Factors of the informational state here investigated are the number of hosts previously encountered, the host species encountered and characteristics of the sites where pupae previously were encountered.

Chapter 1 contains the introduction and outline of the study. Chapter 2 shows that experience is an important factor influencing the rate of acceptance of hosts by the parasitoid. The sequence of behaviors before ovipositor insertion does not change with experience. Chapter 3 shows that parasitoids, deprived of hosts from emergence, accumulate eggs up to a certain level, but have a low rate of acceptance. Early exposure to pupae increases the rate of acceptance. *B. intermedia* adjust their egg production to host availability. Chapter 4 shows that the rate of acceptance for an alternate host increased with age in parasitoids, deprived of hosts from emergence. Rearing the parasitoids on an alternate host and/or oviposition experience on an alternate host did not influence the rate of acceptance of the alternate host. After oviposition experience on the alternate host contaminated with kairomone, uncontaminated hosts were accepted at the same rate as gypsy moth. Chapter 4 shows that *B. intermedia* is able to use learned visual cues, thereby restricting their searching area to a microhabitat previously found to be profitable. Under laboratory conditions and under semi-natural conditions, parasitoids could be trained to search for pupae either on the ground or on a tree model. Subsequent training to the other microhabitat reversed this effect.

The studies show that both the physiological state and the informational state of the parasitoid influence oviposition behavior. Questions are raised and discussed as to the extent of polyphagy of *B. intermedia* in North America.

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CHAPTER I

INTRODUCTION AND OUTLINE OF THE STUDY

A. Introduction

Brachymeria intermedia (Nees) is an introduced parasitoid and now a predominant mortality factor of the gypsy moth, *Lymantria dispar* L. in North America (Ticehurst et al., 1981; Elkinton et al., 1988). The gypsy moth was accidentally introduced in North America more than a century ago and is probably the most serious pest of forest and shade trees (Fuester and Ramaseshiah, 1988). Efforts to control gypsy moth populations by releasing natural enemies have only been partly successful. Population explosions have not been prevented and the gyspy moth continues to expand its range, especially into southern forests. Effective biological control of gypsy moths (and insect pests in general) requires understanding of the foraging behavior of their natural enemies. During the past fifteen years increasingly attention has been given to the behavioral interactions between parasitoids and their hosts. This interaction may be rather complex, because it involves three trophic levels, the food of the host, the host itself and the parasitoid. The process by which parasitoids search and select suitable hosts for oviposition is referred to as host selection. The main objective of the dissertation is to determine which factors influence the development of host-selection behavior in *B. intermedia*.

B. The host: *Lymantria dispar*

The gypsy moth is very polyphagous, and in Europe and North America is a defoliator primarily of hardwoods, especially oaks (*Quercus)*, but after the larvae are half grown they also attack conifers (Fuester and Ramaseshiah, 1988). It was brought in the United States from France to cross it with the silkworm to get more vigorous silkworm colonies. In 1869, gypsy moths escaped from the colony in Medford, Massachusetts and the species readily established itself. Without their natural enemies, within 20 years gypsy moth populations increased dramatically and since then there has been a continuous spread at approximately 10-15 km per year (Wallner, 1988). During the last decades of the 19th century, research was mainly descriptive and aimed at the biology and behavior of the gypsy moth, and the influence of native parasitoids and predators. When populations exploded, attempts were made to import foreign parasitoids and predators, of which *B. intermedia* was one.

C. The parasitoid: *Brachymeria intermedia*

B. intermedia is a parasitoid of the family Chalcididae. Several releases, the first in 1905 in Massachusetts, of *B. intermedia* from

France and Italy were considered unsuccessful because *B. intermedia* was never recovered from the field (Howard and Fiske 1911; Burgess and Crossman 1929; Dowden 1935). One *B. intermedia* was recovered in 1942 from a leafroller pupa but in 1965 *B. intermedia* was recovered in larger numbers from gypsy moth in several Connecticut localities (Leonard 1966). *B. intermedia* is presumed to have remained at low densities following the first introductions, because it was not well adapted to the environmental conditions in New England.

The life cycle of *B. intermedia* starts inside a host pupa, as an endoparasitoid. The egg hatches in about 48 hours, and the young larvae start feeding immediately. Young larvae combat, and only one, rarely two, larva(e) develop in a host pupa. Development time varies greatly with temperature, but the average time in each stage during the normal season of development is 2 days in the egg stage, 2 days in each of four larval instars, 3 days in the last larval instar, 2 days as a prepupa and 13 days as a pupa, making a total of 28 days (Dowden, 1935). When held at 20^0 - 25^0 C on a diet of honey and without hosts, some female *B. intermedia* introduced from Europe lived up to 150 days (Dowden, 1935). Adult females have been found to overwinter in loose bark or wood borer holes in dead trees and in leaf litter (Dowden 1935; Waldvogel and Brown 1978; Ticehurst 1978). The food source of adult *B. intermedia* in the field is unknown, but they are reared successfully on water and honey.

D. Concepts about host selection

A model used by many researchers who want to understand the behavioral mechanisms underlying the host-selection process is the division into four consecutive steps: host-habitat selection; host selection; host acceptance and host suitability (Salt 1935; Doutt 1964; Vinson 1975, 1981; Lewis et al. 1976; Arthur 1981; Weseloh 1981). Each step in this model narrows the area of search by the parasitoid. Host-habitat selection brings the parasitoid in an area containing several hosts or clusters of hosts. Cues such as the plant on which the host is feeding may be involved in this step. Within the habitat, the parasitoid has to find a suitable host for oviposition. During this host-selection step it is most likely that cues directly related to the host are involved (e.g. host-specific odors). Once a potential host has been found it will be accepted or not, based on again different cues perceived through contact with the host surface. Once it is accepted and the ovipositor is injected, the host can still be rejected based on cues from inside the host. In this study only the first three of the above mentioned categories are considered, and host-suitability was not considered.

There is some ambiguity in the literature about the definitions of host-habitat selection and host selection. Some researchers use host-habitat selection only for responses over long distances and divide host selection into two components, one at short distance within the habitat of the host and one at the contact level. Others divide habitat selection into macro-habitat selection and micro-habitat selection. Again others consider all airborne responses as habitat selection, irrespective of the distance over which the response takes place. It depends on the life style of the host species that is studied which definition is most appropriate.

The research here presented is concerned with the host-selection behavior of a pupal parasitoid of the gypsy moth. At high density, pupae of the gypsy moth occur on tree trunks and in forest litter, whereas at low density most pupae are found in the litter (Elkinton and Gould, 1988). Some trees in the forest contain host pupae, others do not. Thus the parasitoid has to find a tree or a typical place on the forest ground that contains pupae. This will be referred to as host-habitat selection (Chapter V). Subsequently, they have to find a pupa on the tree or in the leaflitter, that seems suitable for oviposition. This, we will refer to as host selection (Chapter II, III, IV). The process between contacting the pupa and insertion of the ovipositor will be referred to as the host-acceptance phase (Chapter II, III, IV).

E. State variables approach

A behavioral ecologist's approach to gain insight into the decision making process of the parasitoid is to assume that all decisions are made in order to maximize total fitness accumulated through ovipositions (Mangel 1989). So called state variables (McNamara and Houston 1986; Houston et al. 1988; Mangel and Clark 1986, 1988; Mangel 1987a, b) influencing oviposition decisions can be included into models of maximum lifetime fitness to estimate their ultimate importance. Physiological state variables may be egg load, or age. Informational state variables may be the estimated (by the parasitoid) fraction of unparasitized hosts available, where the estimation is made based on the previous encounter rate (Mangel 1988, 1989). In this study the informational state variable of host species will also be considered.

F. Learning and development of behavior

Animals in search of resources for food or oviposition have to deal with an ever changing environment. In order to respond to these changes, a certain degree of behavioral flexibility is required. Learning is an evolutionary adaptation that allows animals to make adjustments to changing environments. Most definitions used for learning are either too broad or too restricted. Papaj and Prokopy (1989) recommend the use of three criteria to specify learning. In short, these are: 1. behavioral changes as a result of experience should be repeatable; 2. behavior changes gradually with experience; 3. waning of the changed behavior in absence of continued experience (forgetting). Learning is ultimately characterized by the exclusion of phenomena that are not learned (see references in Papaj and Prokopy, 1989).

Learning and its counterpart "instinct" are somewhat loaded terms, and they have been the subject of fierce debate between zoologists and psychologists. The dichotomy between learning and instinct has been used to distinguish inherited behaviors from behaviors that were environmentally determined. However, the question is what one should call inherited or not. During the development from fertilized egg to adult, an organism is perpetually in contact with its environment and all processes are influenced by its environment. The genetic make up of an animal may broadly define the direction of differentiation, but environmental cues may then govern a substantial part of the differentation (Tierney 1986). Potential individual differentiation in development does not necessarily result in behavioral variability between individuals. When the environment during development is always the same for all immatures, learning of the same cues will be inevitable. If the potential behavioral variability between individuals is not taken in account, one might then conclude that the behavior was inherited while in fact it was not. Thus, it may be almost impossible to determine whether a behavior is inherited or learned, it is more a question of relatively innate and relatively environmentally determined (Jolly, 1972). If all members of a species respond in exactly the same manner under different environmental conditions, one can say the response is relatively innate. If, however, there is great variability among members of the same species in different environments one may say the response is relatively environmentally determined (Marler and Hamilton 1966; Jolly 1972).

Two common procedures to determine whether a behavior is relatively innate or relatively learned are deprivation experiments and teaching experiments. In deprivation experiments, all stimuli that are thought to be potential factors to be learned are removed from the environment of test individuals (Chapter II, III). If these individuals that never experienced the relevant cues then perform in the same way as individuals that did experience the cues, one can say the behavior is relatively innate. A prerequisite for deprivation experiments is knowledge about the stimuli influencing the behavioral repertoire of the studied animal, so that a considered choice can be made of stimuli to be deprived from the animal. If the animal does not respond to the relevant cues, no conclusion can be made, because the researcher may have used the wrong testing technique or the animals may have experienced different stimuli unknown to the researcher.

In teaching experiments, the animal is first deprived of the stimulus and then the stimulus is given under controlled conditions (Chapter IV, V). Again, if there is no response after training, for the reasons mentioned above no conclusions can be drawn .

G. Outline of the study

Development of oviposition behavior in *B. intermedia* was studied by observing and recording oviposition behavior of individual parasitoids, such that a continuous record of the behavior was obtained. Chapter II describes the behavioral components of oviposition behavior in *B. intermedia*. The sequential organization of the behaviors as well as the durations of the behaviors have been recorded for female parasitoids that were inexperienced at time of the test and of female parasitoids that had previously been exposed to hosts. To determine whether increased acceptance rates of experienced parasitoids were due to the physiological state of the females or to a change in the informational state, parasitoids were deprived of hosts for different periods of time, after which the acceptance rates and the egg load per female was determined (Chapter III). Chapter IV presents a study on the rate of acceptance of alternate hosts as a function of experience. If experience increases the rate of acceptance this could be a mechanism by which host switching occurs. *B. intermedia* were reared and/or given adult experience on an alternate host and on gypsy moth. Subsequently, the acceptance rates for both host species were tested. To assess the role of gypsy moth kairomone during host acceptance, parasitoids were tested for their response to alternate hosts that were contaminated with gypsy moth kairomone (Chapter IV). From observations of flight behavior of *B. intermedia* females in the rearing cages, in which parasitoids were kept by age class, the hypothesis was put forward that older females had developed a characteristic hovering flight above the petri dishes containing pupae. Chapter V presents experimental data on the ability of *B. intermedia* to use visual cues of structures that previously contained pupae. Learning of visual cues has been verified under laboratory conditions and under semi-natural conditions.

CHAPTER II

INFLUENCE OF EXPERIENCE

A. Introduction

The influence of adult experience on the foraging behavior of parasitoids has been shown in many recent papers (Vet 1983; Vet and van Opzeeland 1984; Wardle and Borden 1985; Drost et al. 1986, 1989; Hérard et al. 1988; Lewis and Tumlinson 1988; Zanen et al. 1989; Kaiser et al. 1989; Cardé and Lee 1989; Turlings et al. 1990). However, as noted by Vet and Schoonman (1988), most of the data available deal with host selection and host-habitat selection by means of olfactory cues. Less is known about the influence of experience on foraging decisions made by parasitoids once the host has been found, i.e. close-range host selection and host-acceptance behavior. Although parasitoids of moving hosts (larvae) generally have a very short host-acceptance phase, parasitoids of sessile hosts (eggs, pupae) often go through an elaborate examination of the host before oviposition (Vinson 1984). Behavioral plasticity at this level of the foraging process may therefore be more likely in parasitoids of sessile hosts.

The endoparasitic chalcidid *Brachymeria intermedia* was introduced into the United States at the beginning of this century as a biological control agent for gypsy moth, *Lymantria dispar* (Burgess and Crossman 1929, Leonard 1966). *B. intermedia* has been recovered from many species of Lepidoptera in Europe (Dowden 1935) and has been reared from lepidopterous and dipterous pupae in the laboratory (Dowden 1935; Minot and Leonard 1976), but its natural polyphagy in the United States is largely unknown.

The host-selection behavior of *B. intermedia* is guided partly by volatile kairomones (Cardé and Lee 1989) and partly by contact kairomones (Leonard et al. 1975; Minot and Leonard 1976). Adult experience in *B. intermedia* affects the time needed to find the kairomone odor source in an olfactometer as well as the propensity to accept a host (Cardé and Lee 1989). In the present paper we investigate the behavioral sequence leading to host acceptance, the behavioral changes that occur as a result of prior oviposition experience, and whether exposure of the wasps to odor of gypsy moth pupae affects host acceptance.

B. Materials and Methods

1. Insects

Gypsy moth egg masses were supplied by the USDA Methods Development Laboratory at the Otis ANG Base, MA. The larvae were reared on a wheat germ diet at 28 ± 1^0C in LD 16:8 h, 1400 lux (Bell et al. 1981). Pupae were collected from this laboratory colony or obtained directly from the USDA.

B. intermedia was reared on female gypsy moth pupae according to the method of Minot and Leonard (1976) and maintained at 28 ± 1^0C and 60 ± 15% RH. Parasitoids were collected randomly on the day of emergence, distributed equally over two experimental groups that were held separately in cages (20 x 20 x 20 cm) with water and honey. Female/male ratio was 2:1 in all cages. Females generally mate once within two days after emergence (Dowden 1935) but males continue to court mated females (Leonard and Ringo 1978). To avoid possible influences of courting males on the physiological state of the female, males were removed at the third day after emergence. One experimental group was withheld from hosts until the test (the inexperienced group). The other experimental group was provided with fresh gypsy moth pupae daily in a one pupa per female ratio, starting three days after emergence, until the day before the test (the experienced group). In a separate experiment, females were kept in separate growth chambers, one with pupal odor (a petri dish with 10 live pupae, covered with wire gauze, so that the parasitoids could not contact the pupae) and one without pupal odor; again starting three days after emergence until the day before the test.

2. Data Collection and Behavioral Repertoire

Tests were conducted between the 8th and 12th hours of the photophase (Minot and Leonard 1976) when females were 5-12 days old (Barbosa et al. 1986), at 28^0C and 11,000 lux. Behavioral assays were carried out in 10 cm diam. plastic petri dishes with a Whatman No. 1 filter paper on the bottom. One female *L. dispar* pupa, 3-5 days old, was placed on the center of the filter paper. The pupa could move freely; however, it generally did not move until the wasp inserted her ovipositor and this occasionally resulted in the parasitoid being thrown off the host. One female parasitoid was transferred to the lip inside of the dish cover and the dish was closed. The observation time was 10 min., unless a female left the pupa after drumming or was thrown off earlier. The occurrence and duration of the following behaviors of *B. intermedia* were recorded continuously with a TRS-80 model 100 computer:

Not on host: walking or standing still while the parasitoid is not in contact with the host.

Preening not on host: all preening activities, while not in contact with the host.

Preening: all preening activities, while standing on the host.

Antennal encounter: standing beside the pupa while drumming it with the tips of the antennae.

Walking: walking on the pupa, while the antennae are mostly parallel to the host surface and the tips of the antennae are touching the host surface intermittently.

Drumming: walking or standing still on the pupa, while the flagella are perpendicular to the host surface and the tips of the antennae drum the host surface intermittently.

Standing still: standing still on the pupa while none of the other described behaviors occur.

Grasping: grasping of the pupa by spreading the hind legs.

Inserting ovipositor: insertion of the ovipositor into the host.

Throwoff: loss of contact with the host as a result of the host's defensive behaviors [spinning and arching as described by Rotheray and Barbosa (1984)].

Some of the above behaviors have been previously described for *B. intermedia* ovipositing in *Galleria mellonella*, the wax moth (Tucker and Leonard 1977). Lashcomb et al. (1983) reported that feeding on host fluids commonly occurs in the laboratory; however, we and Tucker and

Leonard (1977) rarely observed it and in the present experiments it was never seen.

3. Sequence Analysis

To determine significant sequences of behavior and the influence of experience, a 10 x 10 x 2 contingency table was constructed where variable A was the preceding behavior (10 possible behaviors), variable B the following behavior (10 possible behaviors) and variable C the history of the parasitoid (two possible values: inexperienced and experienced) and analysed by log-linear models (Bishop et al. 1975; Fienberg 1980). Because there were so-called structural zero's in the table (impossible transitions as a result of the observer's definitions), the expected frequencies could not be calculated directly from the marginal totals, requiring iterative procedures (Bishop et al. 1975). We used the Iterative Proportional Fitting (IPF) function of PROC IML of SAS (Sas Institute 1988).

The expected frequency f_{exp} of one cell of the table can be expressed as:

$$\log(f_{exp}) = \mu + I_A + I_B + I_C + I_{AB} + I_{AC} + I_{BC} + I_{ABC}$$

μ is the geometric mean of the cell probability; I_A, I_B, I_C are the effects of variables A, B and C; I_{AB}, I_{AC}, I_{BC} are the effects of assocation between pairs of variables; and I_{ABC} is the parameter representing association between all variables. The log-linear models used in the analysis all contained the parameters μ, I_A, I_B, I_C, and I_{AC}. μ sets the overall effect, I_A, I_B, and I_C adjust for internal variation, because the margins were not fixed for any variable. I_{AC} needed to be included because the frequency of the preceding behavior (variable A) was not equal for inexperienced and experienced (variable C) females. Including the parameter I_{AC} ensured that the transition probabilities sum to 1.00 over each row.

The log-linear model assuming behavioral independence excludes parameter I_{ABC} and I_{AB}, i.e. $I_{ABC} = I_{AB} = 0$. Rejection of this model rejects the hypothesis of random association between acts or a 0^{th} order Markov chain (Colgan 1978) in favor of a 1^{st} order Markov chain. The model assuming a 1^{st} order Markov chain, but no influence of the history of the parasitoid on the transition probabilities includes I_{AB} and sets $I_{ABC} = I_{BC} = 0$. The model assuming influence of parasitoid history in addition to a 1^{st} order Markov chain only sets $I_{ABC} = 0$.

The fit of the log-linear models to the observed data was determined with the G - test. The degrees of freedom for the G - test were adjusted to the number of structural zero's and the number of estimated parameters according to Bishop et al. (1975):

$$\text{d.f.} = (T_e - z_e) - (T_p - z_p)$$

where T_e = # cells in the table (200); z_e = # structural zero's (60); T_p = # parameters fitted by the model; and z_p = # cells obtaining $f_{exp} = 0$ under the fitted model.

Freeman-Tukey deviates (Sokal and Rohlf 1981),

$$\sqrt{f_{obs}} + \sqrt{(f_{obs} + 1)} - \sqrt{(4f_{exp} + 1)}$$

where f_{obs} is the observed cell frequency and f_{exp} the expected cell frequency, show which transitions account for the poor fit of the fitted log-linear model, i.e. which transitions are affected by the excluded parameter(s). As a criterion for the deviates being large, the deviates were compared with

$$\sqrt{(\text{d.f.} * 3.841) / \text{Number of estimated cells}}$$

(Sokal and Rohlf 1981), where 3.841 is the Chi-square value for Á = 0.05, d.f. = 1.

4. Result Categories

At the end of each observation we categorized the outcome of the behavioral sequence as follows:

ACCEPT - ovipositor insertion occurred;
REJECT - Drumming the pupa, but never ovipositor insertion;
NODRUM - mounting the pupa, but never drumming;
NOMOUNT - no mounting;
THROWOF - observation stopped because the parasitoid was thrown off by the pupa.

5. Time Analysis

For each female the cumulative time engaging in a behavior was calculated. The non-parametric Wilcoxon Two Sample Test (Sokal and Rohlf 1981) was used to compare durations among groups.

C. Results

1. Sequence Analysis

A kinematic graph of all behavioral transitions occurring is given in Figure 2.1. To determine which of these behavioral transitions were significant and whether experience influenced the behavior, log-linear models were fitted to the data with G-tests.

The first model assumed random association between acts and no influence of experience. When this model was fitted to the data, the G-test gave a $P<0.001$ (Fig. 2.2A), which means that this model is not satisfactory to explain all the variability in the data. The significant Freeman-Tukey deviates indicate non-random transitions and are graphically represented in Figure 2.3. For this model we did not consider negative deviates, because we were not interested in behavioral transitions that do not or rarely occur. As is clear from Figure 2.3, the sequential relationship between behaviors is remarkably similar in inexperienced and experienced females. For many transitions, however, the Freeman-Tukey deviates were greater in experienced females, meaning they were more likely to occur in experienced females (Fig. 2.2A).

A second model assumed a first order Markov-chain and no influence of experience. Fitting this model to the data with the G-test gave a $P =0.0412$ (Fig. 2.2B), which means that a large part of the variation in our data is explained by behavioral interactions. The unexplained variation must have been due to the history of the female, i.e. experience. Figure 2.4 shows how inexperienced and experienced females

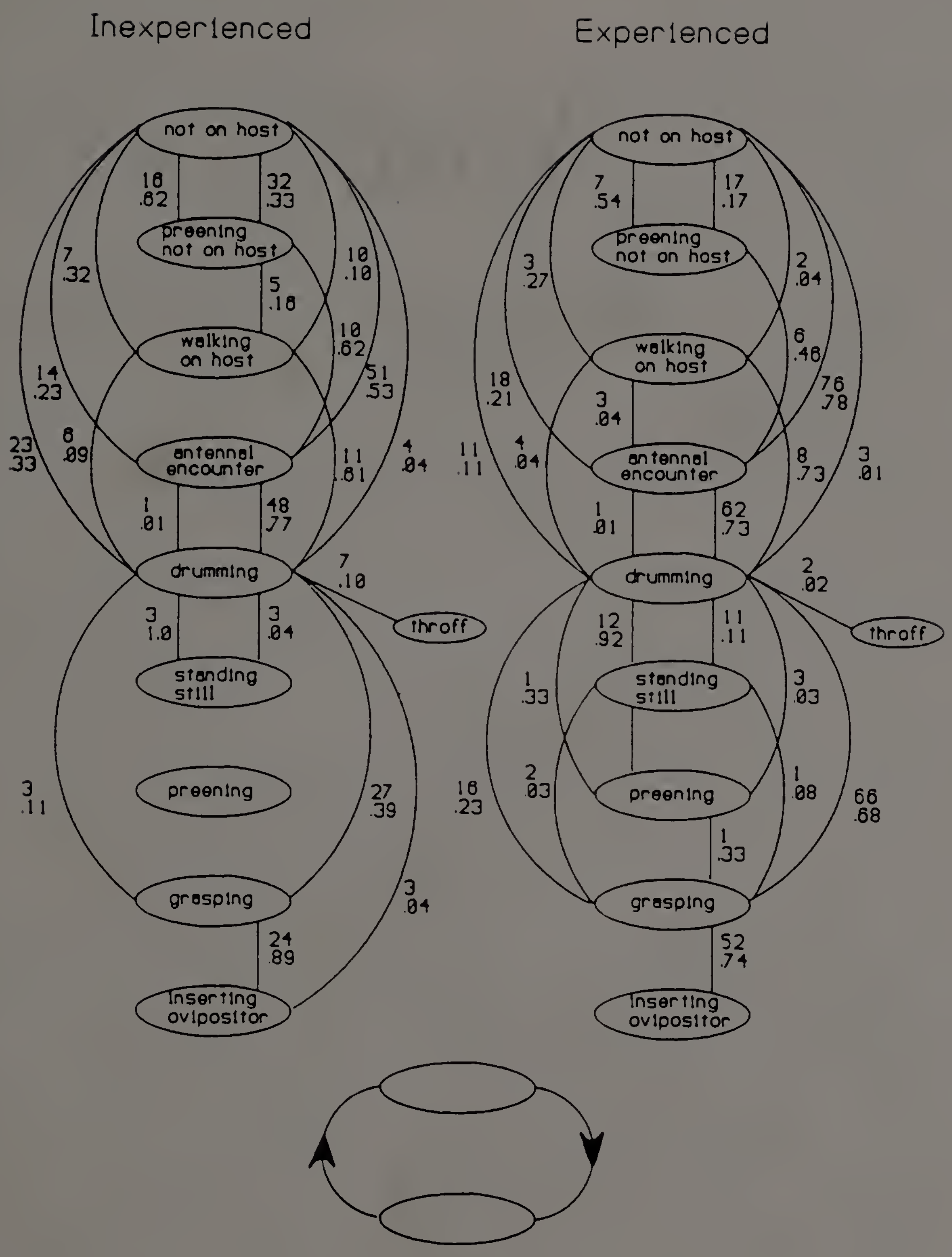

Fig. 2.1 Numbers of occurrence (first entry) and transition probabilities (second entry) of host acceptance behavior in *B. intermedia*.

Fig. 2.2 Expected frequencies (first entry) and Freeman-Tukey deviates (second entry) when different log-linear models are fitted to the transition frequencies. (n) **not on host**; (p) **preening not on host**; (W) **walking**; (E) **antennal encounter**; (D) **drumming**; (T) **throwoff**; (S) **standing still**; (P) **preening** on host; (G) **grasping**; (I) **inserting ovipostior**. (T) and (I) do not occur in rows because they are not followed by any behavior according to the definition. **(A)** Model I assuming random association between acts: G = 1189.87, d.f. = 108, P=0.0000, T_p = 38, z_p = 6, # estimated cells = 124, critical value for the Freeman-Tukey deviates (c.v.) = 1.83; **(B)** Model II assuming interaction between behaviors, but no influence of experience: G = 62.74, d.f. = 45, P = 0.0412, T_p = 110, z_p = 15, # estimated cells = 56, c.v. = 1.76; **(C)** Model III assuming behavioral interactions as well as influence of experience: G = 11.18, d.f. = 37, P = 0.9999, T_p = 119, z_p = 16, # estimated cells = 56, c.v. = 1.59.

(A)

INEXPERIENCED

	n	p	W	E	D	T	S	P	G	I
n	-	12.01 4.40	7.63 0.90	26.52 4.01	30.73 -6.90	-	1.03 0.16	0	9.96 -3.96	9.21 -3.74
p	6.30 2.26	-	2.01 1.66	7.06 1.06	6.21 -3.40	-	0.28 0.96	0	2.66 -1.00	2.46 -0.68
W	4.46 1.13	1.73 -0.40	-	3.61 -1.61	4.41 2.46	0.69 0.47	0.16 1.16	0	1.43 -0.16	1.32 -0.09
E	16.40 -1.02	7.12 -3.01	4.46 -1.93	-	16.20 6.34	2.66 -1.11	0.61 0.56	0	6.90 -2.66	6.46 -2.38
D	21.29 0.41	6.23 -3.41	6.16 0.44	16.17 -6.17	-	3.29 1.71	0.71 1.76	0	6.63 6.17	6.31 -1.39
S	0.71 0.46	0.27 0.97	0.17 1.12	0.60 0.67	0.70 1.76	0.11 1.22	-	0	0.23 1.03	0.21 1.06
P	0	0	0	0	0	0	0	-	0	0
G	6.62 -2.91	2.64 -0.99	1.66 -0.36	6.62 -2.62	6.76 -1.66	1.06 0.13	0.23 1.03	0	-	2.02 6.66

EXPERIENCED

	n	p	W	E	D	T	S	P	G	I
n	-	3.96 4.25	2.67 0.88	24.43 7.56	31.67 -8.92	-	3.26 -1.34	0.69 0.46	19.34 -6.92	11.66 -4.54
p	1.42 2.89	-	0.32 0.91	3.01 1.48	3.93 -1.87	-	0.40 0.60	0.06 1.26	2.36 -0.63	1.46 -0.20
W	1.16 1.34	0.41 0.79	-	2.60 -0.90	3.26 2.08	0.0 6 1.3 0	0.34 0.66	0.07 1.26	1.96 -0.67	1.21 0.00
E	11.41 1.77	3.95 -1.69	2.64 0.39	-	31.61 4.52	0.6 1 0.6 6	3.25 -1.33	0.66 0.46	19.19 -6.67	11.76 -4.61
D	14.41 -0.66	4.99 -2.16	3.21 0.61	30.69 -6.69	-	0.7 7 1.1 3	4.11 2.61	0.66 1.62	24.23 6.41	14.63 -6.36
S	1.40 -0.16	0.46 0.70	0.31 0.91	2.97 -1.16	3.66 3.01	0.0 7 1.2 7	-	0.06 1.26	2.36 -0.61	1.44 -0.19
P	0.32 0.91	0.11 1.22	0.07 1.26	0.67 0.60	0.67 0.29	0.0 2 1.3 6	0.09 1.26	-	0.63 0.65	0.32 0.90
G	8.67 -3.63	3.07 -1.23	1.98 -0.57	16.84 -8.32	24.57 -1.64	0.4 7 0.7 1	2.53 -0.19	0.63 0.65	-	9.13 6.36

(B)

	n	p	W	E	D	T	S	P	G	I
n	-	24.37 1.46	6.96 1.11	63.17 -1.66	2.49 0.93	-	0	0	0	0
p	16.20 0.01	-	3.62 0.60	11.27 -0.31	0	-	0	0	0	0
W	6.21 1.13	0	-	0	11.79 -0.16	0	0	0	0	0
E	13.62 0.17	0	1.26 -0.06	-	46.62 0.21	0.43 0.77	0	0	0.66 0.32	0
D	14.17 2.10	0	4.17 0.69	0.63 0.33	-	3.76 1.47	6.63 -1.20	1.26 0.33	36.76 -2.00	1.26 1.26
S	0	0	0	0	2.61 0.23	0	-	0	0.19 1.09	0
P	0	0	0	0	0	0	0	-	0	0
G	0	0	0	0	6.29 -0.97	0	0.66 0.62	0	-	21.16 0.65

	n	p	W	E	D	T	S	P	G	I
n	-	24.63 -1.61	7.04 -1.16	63.63 1.46	2.61 -0.91	-	0	0	0	0
p	6.60 0.17	-	1.48 -0.21	4.73 0.63	0	-	0	0	0	0
W	3.79 -0.29	0	-	0	7.21 0.37	0	0	0	0	0
E	16.36 -0.03	0	1.72 0.92	-	63.16 -0.12	0.67 0.60	0	0	1.16 0.76	0
D	19.63 -2.16	0	6.83 -0.70	1.17 0.03	-	6.25 -1.64	6.17 0.96	1.76 0.90	64.25 1.64	1.76 -0.41
S	0	0	0	0	12.19 0.02	0	-	0	0.81 0.35	0
P	0	0	0	0	1.00 0.16	0	1.00 0.18	-	1.00 0.18	0
G	0	0	0	0	13.71 0.65	0	1.44 0.54	0	-	54.85 -0.35

Fig. 2.2. Continued next page.

(C)

	n	p	W	E	D	T	S	P	G	I
n	-	17.00 0.06	4.67 -0.29	74.96 0.15	1.67 -0.35	-	0	0	0	0
p	5.07 0.66	-	1.09 0.10	6.88 -0.25	0	-	0	0	0	0
W	3.91 -0.35	0	-	0	7.07 0.42	0	0	0	0	0
E	18.72 -0.11	0	1.75 0.91	-	62.22 0.00	0.38 0.82	0	0	1.78 0.31	0
D	11.30 -0.02	0	3.30 0.47	1.14 0.05	-	1.62 0.41	11.03 0.06	3.00 0.13	66.26 0.00	0.47 0.72
S	0	0	0	0	12.01 0.07	0	-	0	0.96 0.22	0
P	0	0	0	0	0	0	0	-	0	0
G	0	0	0	0	16.04 0.05	0	1.97 0.17	0	-	51.53 0.10

	n	p	W	E	D	T	S	P	G	I
n	-	32.00 0.04	9.14 0.35	52.04 -0.11	3.35 0.44	-	0	0	0	0
p	17.93 -0.40	-	3.90 0.81	9.10 0.38	0	-	0	0	0	0
W	6.09 0.44	0	-	0	11.94 -0.20	0	0	0	0	0
E	13.27 0.26	0	1.25 -0.04	-	47.75 0.07	0.62 0.55	0	0	0.25 1.00	0
D	22.72. 0.11	0	6.70. -0.16	0.86. 0.31	-	7.38. -0.05	2.95 0.15	0	26.71. 0.10	2.58 0.37
S	0	0	0	0	2.97 0.15	0	-	0	0.04 1.33	0
P	0	0	0	0	0.41 0.79	0	0.18 1.11	-	0.72 0.44	0
G	0	0	0	0	2.99 0.13	0	0.05 1.33	0	-	24.42 -0.03

Fig. 2.2. Continued.

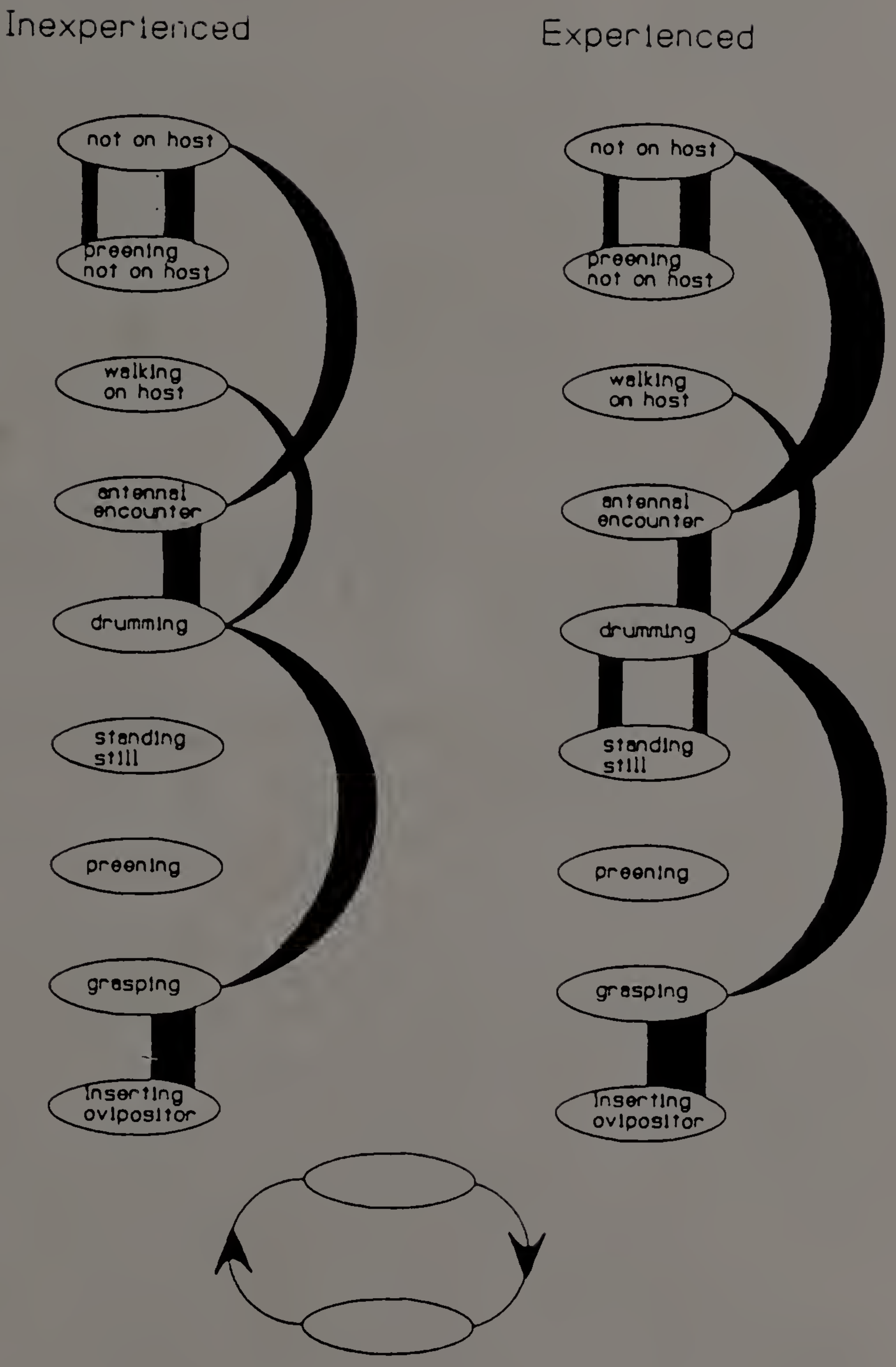

Fig. 2.3 Sequential organization of host acceptance behavior in inexperienced and experienced *B. intermedia*. The arrows indicate transitions where the occurrence of the succeeding behavior depends significantly on the occurrence of the immediately preceding behavior (first order Markov chain). The width of the arrows corresponds to the magnitude of the Freeman-Tukey deviates, fitting the log-linear model and assuming independence of behavioral acts (Model I).

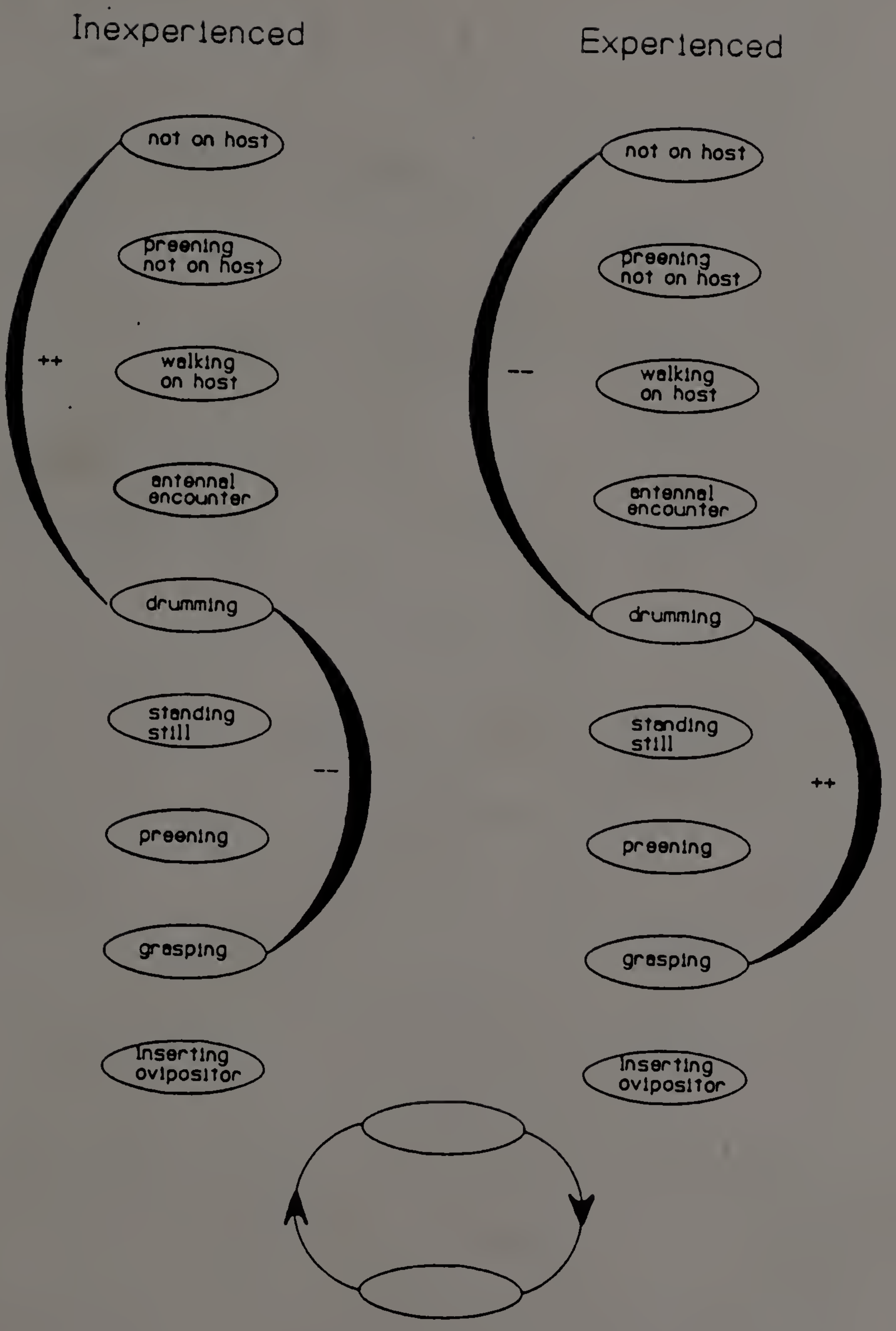

Fig. 2.4 Behavioral transitions that are significantly different between inexperienced and experienced females. The width of the arrows corresponds to the magnitude of the Freeman-Tukey deviates, fitting the log-linear model and assuming no influence of experience on the interaction between behavioral acts (Model II). "++" indicates stimulation, while "--" indicates inhibition.

differ in their behavior: the transitions influenced by female history are **drumming** to **grasping** and **drumming** to **not on host**. The transition probability of **drumming** to **grasping** increases after experience, while **drumming** to **not on host** decreases. Thus, after experience the association between **drumming** and **grasping** becomes stronger, because females do not leave the host and instead go over to **grasping**.

The third model assumes that, in addition to behavioral interaction, there are changes in transition probabilites after experience. Fitting this model to the data with the G-test gave a P = 0.99, which means that this model is satisfactory because it explains nearly all the variability in the data.

Thus, the main change in behavior with experience is that the transition probability from **drumming** to **grasping**, which preceeds ovipositor insertion, increases.

2. Result Categories

The number of females accepting the host was almost twice as high in experienced females as in inexperienced females (69.3 %, n=75; 36.0 %, n=75; P=0.05, G-test). For all other result categories there was no significant difference between inexperienced and experienced females. Some inexperienced females walked over the pupa without drumming it (NODRUM). This never happened with experienced females.

3. Time Analysis

Time spent **not on host** is significantly shorter in experienced females than in inexperienced females (Table 2.1). Time expenditures for all other behaviors were highly variable and are not significantly different between the experimental groups.

To determine whether experienced females were faster in finding and handling the host and whether there were differences in host-selection and host-handling times between accepting and rejecting females, we analyzed behavioral durations separately for accepting and rejecting females within each experimental group. Because observations ended when a parasitoid that drummed the host departed the host, host-finding time can be calculated as the sum of all behaviors off the host (**not on host + preening not on host**). The host-handling time is the sum of durations of all behaviors on the host. Host-finding time appears to decrease with experience, and females that eventually reject the host tend to have alonger host-finding time (Table 2.2). Host-handling time did not change with experience and was longer in accepting females irrespective of experience.

4. Exposure to Odor

When referring to experienced females, we have to consider whether it were the cues encountered, the performance of behaviors, or both that were vital to the change from being inexperienced, i.e., does a female have to find a pupa, contact the kairomone and perhaps oviposit, or is exposure to the odor of the pupae sufficient to improve host acceptance

Table 2.1

Time spent (in seconds) per behavioral component performed by inexperienced and experienced *B. intermedia*.

Behavioral component	Female History								
	Inexperienced				Experienced				
	n	mean	min	max	n	mean	min	max	P*
n not on host	73	76	2	181	73	47	2	180	<0.001
p preening	26	50	9	137	15	48	9	92	NS
W walking	13	7	0	24	9	3	1	10	NS
E antennal encounter	49	12	1	62	67	11	0	72	NS
D drumming	54	57	2	269	62	71	3	235	NS
S standing still	3	10	8	12	10	52	2	272	NS
P preening on host	-	-	-	-	2	14	11	17	NA
G grasping	25	4	0	14	53	9	0	77	NS
I inserting ovipositor	27	82	2	229	52	76	6	390	NS

* Wilcoxon Two Sample Test, $\alpha = 0.05$.

Table 2.2

Comparison of time spent host-finding and host-handling by inexperienced and experienced female *B. intermedia*, based on whether they accepted or rejected the host.

Result Category	Female History	N	Host-finding time (mean ± SE)*		Host-handling time(mean ± SE)*	
Accept	Inexperienced	27	53.9 ± 9.7	ab	153.9 ± 13.1	ba
	Experienced	52	33.7 ± 4.8	a a	165.1 ± 14.7	a b
Reject	Inexperienced	19	78.5 ± 11.6	a a	57.5 ± 10.3	a a
	Experienced	8	58.3 ± 14.4	ab	91.6 ± 17.0	aa

* Values followed by the same letters connected by lines are not significantly different (Wilcoxon Two Sample Test, $\alpha < 0.05$).

behavior?. To determine whether exposure to odor alone is sufficient, we tested females that were exposed to odor of gypsy moth pupae and females that were witheld from odor (by keeping them in another environmental chamber) in the same way as we did for the other experiments. Females had no oviposition experience when tested. We found that the percentage of tested females accepting the host was not different between these groups (inexperienced 33.3%, n=24; odor exposed 37.5%, n=24; P=0.549, G-test) and similar to inexperienced females of the first experiment (36%).

D. Discussion

There are two points in the oviposition behavioral sequence of *B. intermedia* females affected by oviposition experience. First, females with foraging experience approached the host significantly faster and were more apt to investigate it with their antennae than inexperienced females. Second, the transition from **drumming** to **grasping** was more likely to occur in experienced females, which resulted in a higher frequency of host-acceptance, whereas the transition from **drumming** to **not on the host**, leading to host-rejection, was inhibited in experienced females.

Interestingly, only experienced females that accepted the host were faster in host-finding, wheras the individuals that rejected the host were not significantly different from inexperienced females with regard to host-finding time. It is surprising that a considerable number of females in both experimental groups rejected the host, while the host quality was kept as uniform as possible throughout the experiment. Perhaps the parasitoids detect subtle differences in pupae that appear of equal quality to our standards. Alternatively, there may be individual variation in the parasitoids for the ability to determine host quality. Both inexperienced and rejecting females had long host-finding times. Perhaps this should be interpreted as low searching activity. The fact that they mounted and investigated the pupa thus would be an artifact of the small test arena. Most of the females that eventually made an antennal encounter with the host investigated it, and this did not change with experience, i.e. the transition **antennal encounter** to **drumming** did not change with experience, nor did the time spent **drumming**.

Papaj and Prokopy (1989) reviewed definitions of learning and proposed to use several criteria to determine whether a change in behavior is due to learning. Aside from repeatability (1) of the behavioral change, the criteria are: (2) that the behavior changes gradually with experience; and (3) that the change in behavior wanes when continued experience of the same type is absent. Using criteria 2 and 3 we can not yet verify if the change in behavior with experience in *B. intermedia* is due to learning. It is possible that physiological differences between inexperienced females and experienced females are responsible for the changes in behavior. The physiological state of the parasitoids was influenced by whether they were given pupae, but individual variation in physiological state, perhaps due to the number of eggs produced, may also be a factor influencing the behavioral response. For example, in *Venturia canescens* there is a positive correlation between the number of eggs in the oviducts and the time spent foraging (Trudeau and Gordon 1989). Thus, we have shown that in

B. intermedia the foraging behavior for gypsy moth pupae changes with experience at two points in the behavioral sequence. Further research is needed to reveal whether this is due to learning or a change in the physiological state of the female (apart from learning) and how plastic the foraging behavior is when different host species are encountered.

E. Acknowledgments

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CHAPTER III

EFFECT OF PHYSIOLOGICAL STATE

A. Introduction

A parasitoid encountering a potential host has to decide whether or not to parasitize. Under given levels of host availability this decision may be influenced by factors such as the number of mature eggs in the individual's ovaries and the age of the individual (Trudeau and Gordon 1989). Species in which oogenesis continues after oviposition has commenced (synovigenic *sensu* Flanders 1950) face different trade-offs (Mangel 1989) than species in which oogenesis has ceased when oviposition starts (proovigenic sensu Flanders 1950). If a proovigenic species is deprived of hosts, the acceptance rate for unsuitable or less suitable hosts may increase, because the female has the choice of 'dumping' her eggs or not using them at all. A synovigenic species deprived of hosts may stop producing eggs and wait until there are suitable hosts before resuming egg production. Thus, synovigenic species should be more capable of adjusting their reproductive physiology to host availability than proovigenic species.

Brachymeria intermedia is a synovigenic species (Barbosa and Frongillo 1979) that was introduced from Europe into the United States as a biological control agent for the gypsy moth, *Lymantria dispar*. *B. intermedia* females have six ovarioles (Dowden 1935) and resorb their eggs in response to short daylength (Barbosa and Frongillo 1979).

In the present paper we describe the effect of host deprivation and its timing on the egg load and the oviposition behavior of *B. intermedia*.

B. Materials and Methods

1. Insects

L. dispar larvae were reared on artificial medium (Bell *et al.* 1981). *B. intermedia* were reared on *L. dispar* pupae for more than 100 generations (Drost and Cardé 1990a). Adult parasitoids used for tests were held in a growth chamber at 28 ± 1° C, 40-70 %RH, LD, 16:8 h. DC-mains fluorescent lights yielded a light intensity of 1400 lux. Tests were carried out under the same conditions.

2. Experience

Parasitoids were collected on the day of emergence (Age=0) and transferred to screened cages (1 dm^3) where they had access to water and honey. Each cage held one female and one male. The males were removed on the third day following emergence. Because *B. intermedia* does not produce progeny during the first two days after emergence (Barbosa *et al.* 1986), the treatments were started on the third day. Parasitoids were randomly assigned to 5 groups that received pupae at different time intervals:

Group I - no pupae

Group II - 5 pupae on day 3 and 5 pupae on day 4

Group III - 5 pupae on day 11 and 5 pupae on day 12

Group IV - 1 pupa on days 3 to 12

Group V - 5 pupae on days 3 to 12

The *L. dispar* pupae used during the treatment period were 3-5 days old. The pupae were placed in the individual cages of the parasitoids from the 5-8th h of photophase, outside the growth chamber (same environmental conditions). This ensured that parasitoids left behind in the growth chamber were isolated from the odour of pupae. On the 12th day after emergence during the 10-12th h of photophase females were tested individually to determine whether they accepted a gypsy moth pupa for oviposition.

3. Test Procedure

A female was introduced into a 10 cm dia. Petri dish lined with Whatman No. 1 filter paper and containing one female *L. dispar* pupa in the center. When a female drilled the pupa, this was recorded as an acceptance and the female was allowed to complete the oviposition. After the test, the female parasitoid was stored in 70% alcohol for subsequent determination of the number of eggs left in her ovaries. Only vitellogenic eggs (opaque) approximately 0.3 mm long were counted as mature.

4. Dissections

Pupae were dissected 4 to 6 days after exposure to parasitoids (at which time moth emergence occurred from unsuccessfully attacked pupae) to determine the presence of *B. intermedia* larvae. We checked for larvae rather than eggs, because larvae were recovered more reliably. Thus the number of eggs laid may be underestimated slightly.

5. Statistical Procedures

Categorical data analysis was performed with the log-likelihood ratio test (G test). Multiple comparisons were made by comparing pairs of treatments of interest. The level of Á was adjusted to the number of comparisons (k) made: $Á' = 1-(1-Á)^{1/k}$.

Continuous variables were analysed with the Wilcoxon Two Sample Test in case of two treatments and the Kruskal Wallis Test in case of more than two treatments. Again Á was adjusted to the number of comparisons made.

C. Results

To get an impression of the daily progeny production of *B. intermedia* that have access to an ample supply of pupae, we graphed the mean number of daily progeny of females of Group V against the age of the females. Figure 3.1 shows that the mean progeny production increases during days 3,4 and 5, then stabilizes and tends to decrease again at day 12.

The relatively low acceptance rates (Table 3.1) of Groups III and V were expected, because females in these groups had parasitized 5 pupae on the morning of the test day and thus were assumed to be egg depleted. The acceptance rate by females deprived of hosts from emergence (Group I) was lower than that of Group II and Group IV. One explanation for this would be that egg production in *B. intermedia* is not initiated when

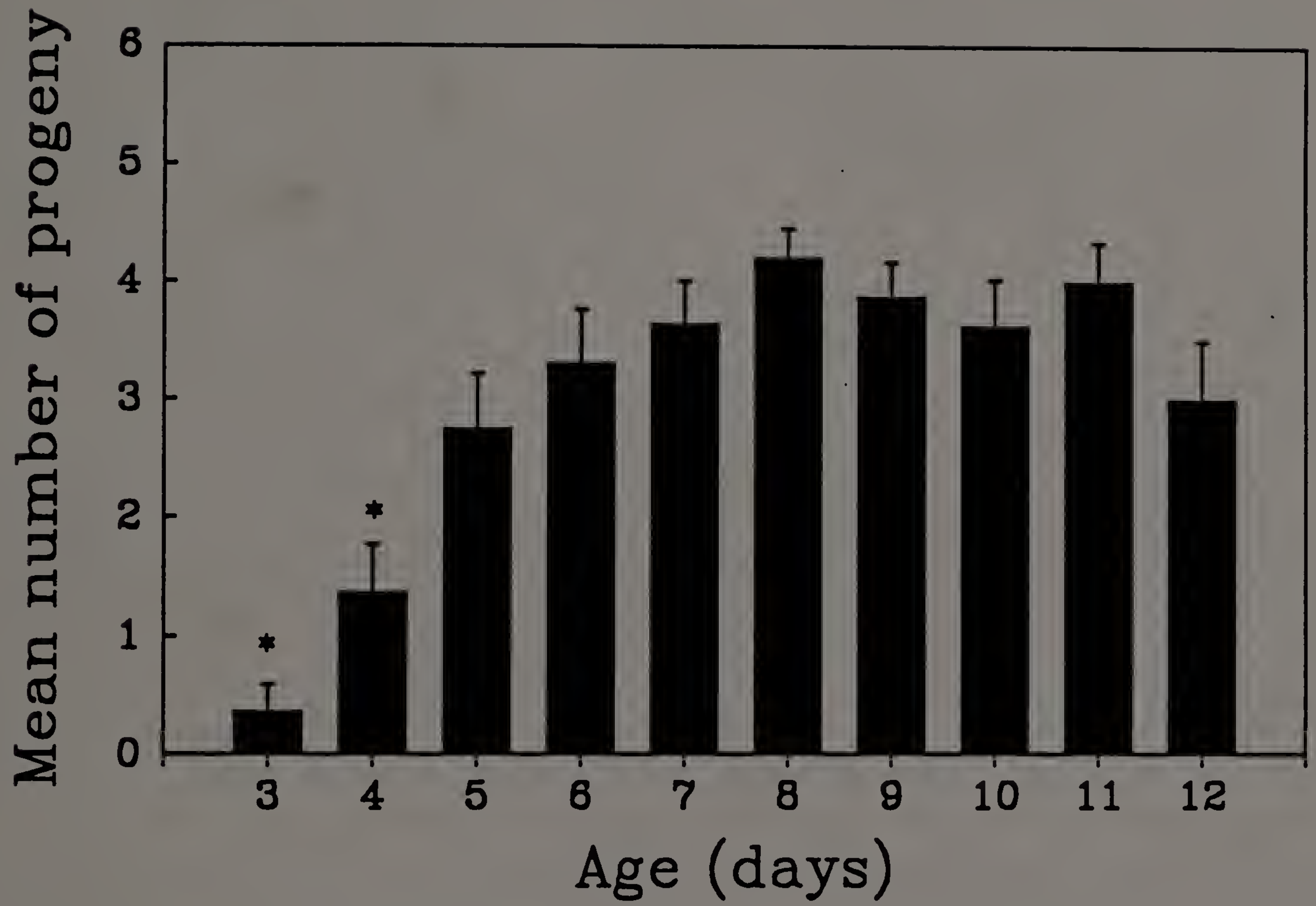

Fig. 3.1. Mean number of *Brachymeria intermedia* progeny recovered from *L. dispar* pupae exposed to individual females during the treatment period of Group V. Error bars represent the standard error of the mean. The number of parental females was 16 for all groups. Multiple comparisons using the Kruskal Wallis Test: All groups: $P < 0.02$; minus Age3: $P < 0.02$; minus Age 3 and Age 4: $P > 0.02$

Table 3.1

Acceptance rates and egg production in *B. intermedia* in response to different host-exposure regimes.

	TREATMENT GROUP				
TREATMENT VARIABLES	I	II	III	IV	V
Age (days) during host exposure	--	3-4	11-12	3-12	3-12
Age (days) during test	12	12	12	12	12
Duration (days) of host-deprivation prior to test	12	8	0	0	0
Host-density during exposure period	--	5	5	1	5
Total number of hosts to which exposed	0	10	10	10	50
RESULT VARIABLES					
N	17	16	16	17	17
% Acceptance*	18.0	50.0	44.0	56.0	29.0
Number (mean ± SE) of** eggs left in ovaries after test	8.82 (0.58)	11.14 (0.82)	5.58 (0.69)	5.33 (0.70)	8.00 (1.14)
Total number (mean ± SE)*** of eggs produced	8.82 (0.58)	13.36 (1.51)	10.83 (0.51)	13.42 (0.95)	40.10 (3.52)

* Multiple comparisons with G test, I vs II+III+IV: < 0.02; V vs II+III+IV: ns

** Multiple comparisons with Mann-Whitney U test, I vs II: < 0.02 ; II vs III: < 0.02; IV vs V: ns

*** Multiple comparisons with Mann-Whitney U test, I vs II: < 0.02 ; II vs III: ns; IV vs V: < 0.02

no hosts are encountered. However, host-deprived females do accumulate eggs (Table 3.1) and host acceptance rates do increase over time (Drost and Cardé 1991). A more likely explanation probably, is that egg production and host acceptance do not increase indefinitely, but up to a certain point and subsequently egg resorption takes place and acceptance rates decrease. This theory would fit the results that the egg load on day 12 was higher for females of Group II than for females of Group I, because Group II females were deprived for a shorter time and egg resorption has not yet occurred.

The total egg production (eggs laid + eggs left in the ovaries) varied with the total number of pupae encountered. Females of Groups II, III, and IV were exposed to a total of 10 pupae and had a similar total egg production (Table 3.1), whereas Group V females, exposed to 50 pupae total, had a much higher total egg production. Thus the egg production is adjusted to the number of hosts encountered.

If host deprivation had an effect on oviposition behavior, one would expect the latter to be correlated to egg load. However, correlations between percentage host acceptance and mean egg load (data from Table 3.1, Kendall's rank correlation coefficient Ó = 0.4) and between percentage host acceptance and mean total number of eggs produced (Kendall's rank correlation coefficient Ó = 0.2) were not significant. Group I females accepted far fewer pupae than those in Group II. Yet they had nearly as many eggs in their ovaries as Group II females. As suggested above, lower acceptance rates could have been caused by the longer host deprivation period in group I (12 days instead of 8 days) rather than by the early exposure to hosts in group II.

To determine the effect of duration of host deprivation we conducted an additional experiment in which females were deprived of hosts during either 8 or 12 days following exposure to pupae on days 3 and 4, with control groups for each age (Table 3.2). The percentage females accepting a host after 12 d deprivation (Group C) was not significantly different from Groups B and D with 8 d deprivation. Thus the duration of host deprivation does not affect the rate of host acceptance, but exposure to hosts does (Group A, Table 3.2). The number of eggs in the ovaries at time of the test again was higher if females had early exposure to hosts than if they had no previous exposure to hosts.

B. intermedia has only six ovarioles and it has been reported that this parasitoid carries a maximum of six mature eggs (Dowden 1935; Barbosa and Frongillo 1979). We found up to 15 eggs in some females. To determine whether these eggs were indeed all viable, we gave 14 females the early host exposure regime (as in group II of the first experiment) and on day 12 each female was allowed to oviposit in as many pupae as possible in as short a time as possible. Each oviposition was observed and immediately after the female left the host she was brought in contact with the surface of another unparasitized pupa. When the wasp did not start drumming immediately upon encountering the next unparasitized pupa, the observation was stopped. The production of up to 11 viable offspring (Table 3.3, female 1) indicated that *B. intermedia* accumulates eggs and can have more than 6 eggs ready to oviposit. Although, for this experiment, we did not determine gypsy moth emergence without parasitism we generally find 100% of gypsy moth emergence in the laboratory and therefore it is most likely that the

Table 3.2

Acceptance rates and egg production in *B. intermedia* in response to different host-deprivation durations.

TREATMENT VARIABLES	TREATMENT GROUP A	 B	 C	 D
Age (days) during host-exposure	--	3-4	3-4	7-8
Age (days) during test	12	12	16	16
Duration (days) of host-deprivation prior to test	12	8	12	8
Host-density during exposure period	--	5	5	5
Total number of hosts to which exposed	0	10	10	10
RESULT VARIABLES				
% Acceptance*	36.36	91.67	91.67	76.92
Mean (± SE) number of** eggs left in ovaries after test	7.40 (0.85)	11.83 (0.74)	9.58 (0.84)	12.69 (1.22)

* Multiple comparisons with G test, A vs B+C+D: < 0.02; D vs A+B+C: ns

** Multiple comparisons with Mann-Whitney U test, A vs B: < 0.02 ; B vs D: ns;
C vs D: ns

Table 3.3

Results of one continuous bout of oviposition behavior by 14 different females of *B. intermedia* (age 12 d) that parasitized hosts during days 3 and 4 but were subsequently deprived of hosts until day 12.

Individual Number	Fate of the *L. dispar* pupae.* (in order of exposure)	Total viable offspring produced
1	B B B B N B G B B N B B B B N	11
2	N N B B B N B G B B N G	6
3	N	0
4	G G	0
5	B B B B N B	5
6	B B N B B G G G	4
7	B N B B B B N B N N	6
8	B B B B B N B N N N	6
9	B B B B B N B	6
10	B B B B B G B B N G N B N G	8
11	B B B N B N B B	6
12	B B B B B B B B	8
13	N B B B B N B N N G B B	7
14	B B N N B B B	5

*G= *L. dispar* adult emerged; B= *B. intermedia* larva present; N= No moth emergence, no parasitoid larva detected.

deaths of pupae in the experiment were parasitoid-induced, as shown previously by Minot and Leonard (1976).

D. Discussion

B. intermedia females that were deprived of hosts for 12 days from emergence (Group I) showed a significantly lower host-acceptance rate than females that were partly deprived of hosts (Group II). This may be caused by a lack of oviposition experience in Group I females (Cardé and Lee 1989; Drost and Cardé 1990a), i.e. they did not recognize the pupae as suitable oviposition sites. Alternatively, the physical condition of Group I females may not have been optimal because they carried eggs that either were not fully developed or eggs that were developed but partly resorped. Either of these alternatives or both may have caused the low acceptance rates in Group I females. The experiments showed that females with oviposition experience on days 3 and 4 and subsequent host-deprivation for 12 days retained a high host-acceptance rate and accumulated many viable eggs. Thus, in this situation oviposition experience proved more important than temporary host-deprivation. It does not seem likely that egg production is induced by encountering hosts, since females accumulated eggs even when host-deprived from emergence. A decrease in host-acceptance in host-deprived parasitoids also occurs in *Brachymeria euploeae* (Schneider 1941) and was termed *Ovarialkrise*. Flanders (1950) described it as "ecological castration," or "a strong decrease in the readiness to oviposit in response to environmental conditions adverse for oviposition, which may be accompanied by complete egg absorption." In *B. intermedia*, adult females are the overwintering stage (Dowden 1935; Waldvogel and Brown

1978) and either short photoperiods or low temperatures induce oosorption in this species (Barbosa and Frongillo 1979). Schneider (1941) reported oosorption in *B. euploeae* in response to a critical number of eggs in the ovaries. Perhaps, this also occurs in *B. intermedia*, in addition to oosorption in response to photoperiod. The eggs in the ovaries of *B. intermedia* showed no visible indication of oosorption, such as shrivelled surfaces of the eggs, but the fact that host acceptance rates in completely deprived females were lower than in non- or partly-deprived females may indicate a behavioral anticipation of oosorption.

B. intermedia females exposed to pupae between days 3 and 16 retained an acceptance rate of over 50% and can accumulate up to 16 eggs. Females deposited these eggs in rapid succession and most of them hatched. This means that *B. intermedia* is not a monootene species (Barbosa and Frongillo 1979) but a polyootene species, i.e. with more than one mature egg in one ovariole.

As Mangel (1989b) has pointed out, frequently there are descriptions in the literature of insect behavior such as 'motivation to oviposit' or 'ovipositional drive', thus attempting to account for all variables that determine 'motivation to oviposit'. To understand how an insect becomes motivated to oviposit, it is essential to know which variables contribute to the behavioral outcome. Recently, dynamic optimal-foraging models to predict oviposition behavior of parasitoids have been developed. These models may play an important role in predicting the outcome of biological control efforts, provided that they are based on realistic assumptions. Whereas some models assume factors such as egg-production rate and host-encounter rate to be constant, dynamic-state-variable models include egg production rate and host-encounter rate as stochastic elements (Mangel 1989a, 1989b). In the latter models, the oviposition behavior is shaped by physiological state variables of the animal (such as age and egg load) and informational state variables (such as host-encounter). The stochasticity of these variables allows assumptions such as variable egg production in response to the environment. Egg load is often assumed to be directly correlated to oviposition behavior. We did not find such a direct correlation. Rather, oviposition behavior in B. intermedia is shaped by the interactions among the physiological state variables of age and egg load and the informational state variable host availability and experience.

E. Acknowledgments

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CHAPTER IV

HOST-SWITCHING POTENTIAL

A. Introduction

Generalist foragers are thought to have a stabilizing effect on the population density of their hosts if they tend to concentrate on the most abundant resource when searching for food, prey or oviposition sites. When this resource declines in density partly due to the forager, the animal switches to the resource that is currently the most abundant (Murdoch 1969). When strongly-preferred resources are involved, the tendency to switch is typically lower than when weakly-preferred resources are involved (Murdoch 1969, Cornell and Pimentel 1978).

Preference for a particular resource can be influenced by learning. For example, bumblebees (Heinrich 1979 1984; Laverty and Plowright 1988) and butterflies (Lewis 1986) can acquire specialized flower-handling techniques through instrumental conditioning, thereby increasing their foraging efficiency for a particular kind of flower. In apple maggot flies (Prokopy et al. 1982, 1986, Papaj and Prokopy 1986) and butterflies (Papaj 1986, Traynier 1986) acceptance of an alternate host plant for oviposition is influenced by prior experience with that host plant (for a review of learning in phytophagous insects, see Papaj and Prokopy 1989).

In parasitoids, which are a higher trophic level, both cues from the host organism and cues from the host habitat may be involved during host selection. Host specialists need to track their host on different food sources and thus may benefit from learning habitat cues related to the food of the host. This has been demonstrated in a number of parasitoids (Drost et al. 1988; Lewis and Tumlinson 1988; Zanen and Cardé 1991). Host generalists may benefit from learning cues of the most preferable host species. Different host species typically have different niches. They may live in the same macro-habitat, but different micro-habitats. Thus learning in host generalists may involve cues of the habitat as well as cues directly emanating from the host. Depending on the life style of the host it may be difficult to separate the food-cues (micro-habitat) from the host cues. When the host species are obligate to their micro-habitat, one could extend the study of host switching in the parasitoid to micro-habitat switching (Vet 1983; Vet and van Opzeeland 1984, 1985; Wardle and Borden 1985, 1986, 1989). On the other hand, Cornell and Pimentel (1978) demonstrated host switching in *Nasonia vitripennis* based on cues from the host by rearing the different host species on the same food medium.

Our study explored the capacity of *Brachymeria intermedia* (Nees), a pupal endoparasitoid of *Lymantria dispar* L., the gypsy moth, to switch hosts. *B. intermedia* was introduced in North America from the south of France and Italy where it appears to have a wide range of hosts. In North America it has been reared in the laboratory on many different host species (Dowden 1935, Minot and Leonard 1976). The few field recoveries of *B. intermedia* in North America from hosts other than the gypsy moth were all from totricids (Prokopy 1968, Leonard 1975).

It is not known what conditions lead to host switching in *B. intermedia*. Possibly only one or a few oviposition experiences on an alternate host increases the acceptance level for that host, as has been found for several other generalist parasitoids (Vet and van Opzeeland 1984, Kaiser et al. 1989, Turlings et al. 1989). Prior oviposition experience with *L. dispar* elevates by about two-fold acceptance of this species as a host (Cardé and Lee 1989, Drost and Cardé 1990). We investigated the capacity of *B. intermedia* to accept an alternate host and the influence of experience and host deprivation on the acceptance behavior. *B. intermedia* does not overlap in distribution with *H. lamae*, a bog-inhabiting species. However, *B. intermedia* develops successfully in this species in the laboratory.

B. Materials and Methods

1. Insects

L. dispar larvae were reared on artificial medium (Bell et al. 1981). A laboratory culture of *H. lamae* was established from females collected at Big Heath Bog, Acadia National Park, Maine on August 12, 1984 and reared on pinto-bean diet (see Schal et al. 1987). To establish a *B. intermedia* colony on *H. lamae*, host-deprived parasitoid females, 12-15 d old, were used and the females were left with the pupae for 5 days. The base colony of *B. intermedia* has been reared in our laboratory since 1986 (see Drost and Cardé 1990).

Parasitoids used in tests were from the third generation on *H. lamae* or from the base colony on *L. dispar*. Wasps were held in a growth chamber at 28 ± 1^{o}C, 40-80 %RH and 1400 lux. When a treatment involved experience with pupae, females with *L. dispar* pupae and females with *H.*

lamae pupae were held in separate growth chambers to prevent mixing of odors of the host species. All pupae used in tests were 3-5 d old. Tests were conducted during the 8th-12th h of photophase.

2. Infuence of Rearing and Adult Experience

Females reared on *L. dispar* or on *H. lamae* were tested on either host species for host acceptance before and after experience. The test arena consisted of a 10 cm petri dish, lined with Whatman No. 1 filter paper and with pupae placed in the center. *H. lamae* pupae are ca.1/5 the size of *L. dispar* pupae. To obtain comparable surface areas in both experimental situations, we used one female *L. dispar* pupa, or a cluster of five *H. lamae*.

One parasitoid was removed from the maintenance cage in a 2.5 cm petri dish and transferred to the lip inside the test-arena cover. The test arena was closed. Durations of the following behaviors were recorded (see also Drost and Cardé 1990):

NOT ON HOST: walking or standing still while the parasitoid is not in contact with the host;

PREENING NOT ON HOST: all preening activities while not in contact with the host;

DRUMMING: walking or standing still on the pupa, while the flagella are perpendicular to the host surface and the tips of the antennae drum the host surface intermittently;

ACCEPTING: drilling the host with the ovipositor.

Females were observed for a maximum of 3 min. An observation was stopped either because the female inserted her ovipositor into the host (i.e. accepted the host) or because 3 min. had past since the onset of the observation. The first host acceptance test was conducted when the females were 7-9 days old. Subsequently, to become "experienced" on a given species, each female was given 10 pupae overnight. Pupae were changed during the 5th hr of photophase. On the third day, parasitoids had no access to pupae from the 5th hr of photophase until tests were conducted starting at the 8th hr of photophase. Number of replicates of each treatment varied per day, but not within days. Hence, day-to-day variation was assumed to be equally distributed over all treatments.

3. Influence of Host Deprivation

Parasitoids were obtained from the base colony on gypsy moth and held in 15 cm petri-dish cages (with a 6 cm height wire screen strip between bottom and lid), at rate of five females and two males per cage. The males were removed when females were 3 d old. The host acceptance tests were the same as described for the previous experiment. For each replicate, wasps of different age groups, 1-3, 4-6, 7-9, 10-12 and 13-15 d (which had emerged on different days), were host deprived from emergence and were all tested for host acceptance on the same day.

4. Influence of Kairomone

Pilot experiments by Cardé and Lee (unpublished) showed that topical application of gypsy moth kairomone to *H. lamae* improved their acceptability for oviposition by *B. intermedia*. Apparently, the *H.*

lamae pupae adsorb or absorb the gypsy moth kairomone. This effect was used to investigate whether *B. intermedia* makes an association between a kairomone that it has experienced previously and an unfamiliar host. To contaminate *H. lamae* pupae with *L. dispar* kairomone, 50 *H. lamae* pupae were held with 50 *L. dispar* pupae in a 500 ml paper cup for 2 h where they were in contact with the *L. dispar* pupae. The *L. dispar* pupae were not reused. The host acceptance tests were the same as described for the previous experiments. Female parasitoids were allowed to complete one oviposition on a contaminated *H. lamae* pupa, and were then transferred to a clean *H. lamae* pupa or vice versa. A maximum observation time of 3 min was maintained.

5. Statistical Procedures

For analysis of response proportions, linear models were fitted on logit-transformed proportions. Hypotheses were tested using G=-2(log-likelihood ratio), which has approximately a Chi-square distribution (Sokal and Rohlf 1981).

Durations of behaviors were not normally distributed. Therefore, although we give means with standard errors in the tables, the durations were analyzed nonparametrically. Analysis of variance was performed using the Kruskal-Wallis test and pairwise comparisons with the Mann-Whitney U test, with Á adjusted according to the number of comparisons (k) made: $Á' = 1 - (1-Á)^{1/k}$ (Sokal and Rohlf 1981).

C. Results

1. Influence of Rearing and Adult Experience

B. intermedia females showed a higher initial host acceptance rate for *L. dispar* than for *H. lamae*, irrespective of the host on which they were reared (Table 4.1, 4.2). Host acceptance for *H. lamae* remained extremely low, even after experience. The effect of experience is contained in the residual which is not significant at the level of Á = 0.05, however the probability that the residual variation is due to random events instead of experience is 0.07. At a higher sample size this probably will come out significant.

The duration of time spent NOT ON HOST was always shorter with *L. dispar* as the host than with *H. lamae*. Experience on *L. dispar* further decreased the time spent NOT ON HOST even further. Experience on *H. lamae* had no significant effect on the time spent NOT ON HOST (Table 4.3). The duration of PREENING NOT ON HOST was significantly shorter when females were reared on *L. dispar* and tested on *L. dispar* as compared to the other situations of the experiment (Table 4.3). Preening of the antennae and other body parts were not distinguished, but preening of the antennae comprised about 90% of this behavioral category. The duration of DRUMMING was longer on *L. dispar*, irrespective of the rearing host and adult experience, except for the *H. lamae*-reared, *H. lamae*-experienced females.

Table 4.1

Percentage of *B. intermedia* females (n = 30 in all situations) reared on either *L. dispar* or *H. lamae* accepting either *L. dispar* or *H. lamae* when inexperienced (age = 7-9 d) and after adult experience (age - 10-12 d) with the same host as they were reared on.

Rearing Host	Test Host	First test INEXPERIENCED	Second test EXPERIENCED
L. dispar	*L. dispar*	66.7	80.0
	H. lamae	10.0	16.7
H. lamae	*L. dispar*	53.3	83.3
	H. lamae	6.7	10.0

Table 4.2

Summary of analysis of Table 4.1.

Source of Variation	df	G	P
Rearing Host	1	0.62	0.43
Test Host	1	97.77	0.00
Rearing Host x Test Host	1	0.15	0.69
Residual	4	8.61	0.07
Total	7	107.16	

Table 4.3

Mean durations of behaviors performed by *B. intermedia* reared on either *L. dispar* or *H. lamae* and exposed to either *L. dispar* or *H. lamae* when inexperienced (age = 7-9 d) and after adult experience (age 10-12 d) (same experiment as Table 4.1).

Nr	Rearing Host	Test Host	Phys. state	Mean sec ± SE (n)	Mann-Whitney U test: * = P < 0.017
		Behavior: NOT ON HOST			
1.	*L. dispar*	*L. dispar*	inexp	62.7 ± 9.5 (30)	vs.2: *,vs.3: *,vs.5: ns
2.			exp	30.8 ± 6.0 (29)	vs.1: *,vs.4: *,vs.6: ns
3.		*H. lamae*	inexp	120.2 ± 9.7 (30)	vs.1: *,vs.4:ns,vs.7: ns
4.			exp	131.4 ± 10.8 (30)	vs.2: *,vs.3:ns,vs.8: ns
5.	*H. lamae*	*L. dispar*	inexp	70.0 ± 9.4 (30)	vs.1:ns,vs.6: *,vs.7: *
6.			exp	25.8 ± 5.4 (30)	vs.2:ns,vs.5:ns,vs.8: *
7.		*H. lamae*	inexp	146.8 ± 8.6 (30)	vs.3:ns,vs.5: *,vs.8: ns
8.			exp	131.8 ± 11.7 (30)	vs.4:ns,vs.6: *,vs.7: ns
		Behavior: PREENING NOT ON HOST			
1.	*L. dispar*	*L. dispar*	inexp	18.0 ± 2.7 (13)	vs.2:ns,vs.3: *,vs.5: *
2.			exp	26.3 ± 5.7 (9)	vs.1:ns,vs.4:ns,vs.6: ns
3.		*H. lamae*	inexp	48.4 ± 8.5 (22)	vs.1: *,vs.4:ns,vs.7: ns
4.			exp	36.3 ± 8.6 (20)	vs.2:ns,vs.3:ns,vs.8: ns
5.	*H. lamae*	*L. dispar*	inexp	34.8 ± 5.3 (11)	vs.1: *,vs.6:ns,vs.7: ns
6.			exp	19.0 ± 4.6 (8)	vs.2:ns,vs.5:ns,vs.8: ns
7.		*H. lamae*	inexp	28.1 ± 9.8 (14)	vs.3:ns,vs.5:ns,vs.8: ns
8.			exp	44.0 ± 12.8 (13)	vs.4:ns,vs.6:ns,vs.7: ns
		Behavior: DRUMMING			
1.	*L. dispar*	*L. dispar*	inexp	50.6 ± 6.6 (26)	vs.2:ns,vs.3: *,vs.5: ns
2.			exp	42.4 ± 7.5 (30)	vs.1:ns,vs.4:ns,vs.6: ns
3.		*H. lamae*	inexp	19.3 ± 5.4 (12)	vs.1: *,vs.4:ns,vs.7: ns
4.			exp	15.6 ± 6.7 (14)	vs.2:ns,vs.3:ns,vs.8: ns
5.	*H. lamae*	*L. dispar*	inexp	49.8 ± 4.8 (28)	vs.1:ns,vs.6:ns,vs.7: *
6.			exp	34.6 ± 5.8 (29)	vs.2:ns,vs.5:ns,vs.8: *
7.		*H. lamae*	inexp	19.2 ± 6.0 (9)	vs.3:ns,vs.5: *,vs.8: ns
8.			exp	31.7 ± 11.3 (9)	vs.4:ns,vs.6: *,vs.7: ns

Table 4.4

Summary of analysis of Figure 4.1.

Source of Variation	df	G	P
Testhost	1	25.73	0.00
Age	4	28.01	0.00
Residual	4	2.73	0.61
Total	9	56.46	

2. Influence of Host Deprivation

The percentage of females accepting the host increased with the duration of host deprivation (Fig. 4.1). Both the test host and age were significant factors influencing the response levels, but there was no interaction between these factors (Table 4.4, non-significant residual). Again consequently more females accepted *L. dispar* than *H. lamae*. Nonparametric analysis of variance of behavioral durations with the Kruskal-Wallis test showed no effect of host deprivation on the time spent not on host (*L. dispar* as host, P = 0.09; *H. lamae* as host, P = 0.23), the time spent preening not on host (*L. dispar* as host, *P* = 0.42; *H. lamae* as host, *P* = 0.21) and the time spent drumming (*L. dispar* as host, P = 0.27; *H. lamae* as host, P = 0.89).

3. Influence of Kairomone

In no choice tests, the DRUMMING and ACCEPTING levels for *H. lamae* pupae that were contaminated with the kairomone of *L. dispar* were much higher than for clean *H. lamae* (Table 4.5). The test host was the only significant factor influencing the response levels (Table 4.6). Variation due to a different effect of the test host on % DRUMMING and % ACCEPTING is contained in the residual. This residual variation was not significant (Table 4.6).

When females were exposed to both types of hosts in succession, e.g. clean *H. lamae* and contaminated *H. lamae*, the response depended on the order in which both species were offered (Table 4.7). Clean *H. lamae* were accepted at a low rate as initial host, but if females had

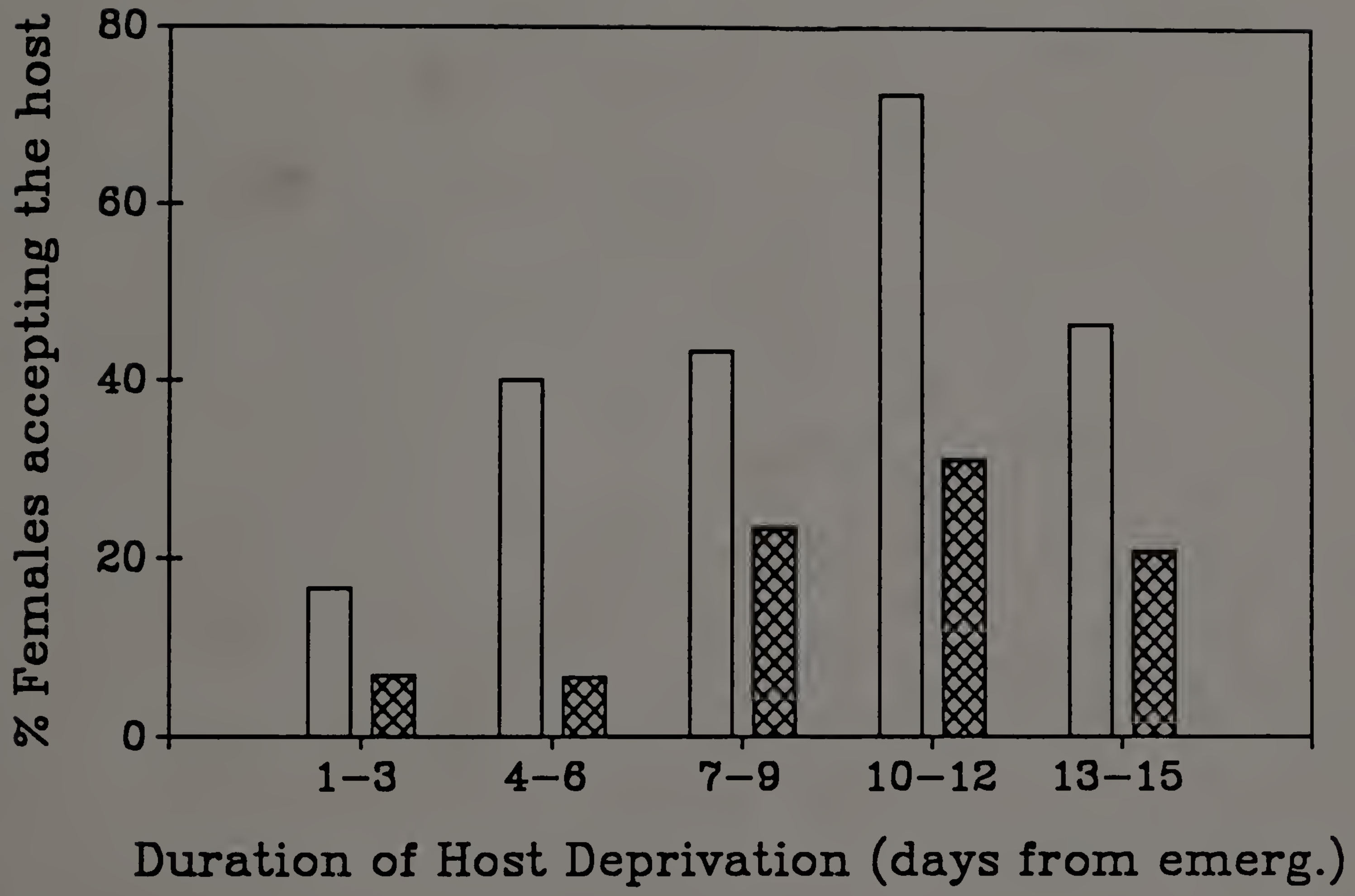

Fig. 4.1 Influence of host-deprivation on host-acceptance behavior of *B. intermedia*.

Table 4.5

Percentage of *B. intermedia* females reared on *L. dispar* drumming and accepting *H. lamae* with *L. dispar* kairomone, clean *H. lamae* and *L. dispar* (n = 25 in each situation).

Host	% DRUMMING	% ACCEPTING
H. lamae contaminated	52.0	44.0
H. lamae clean	12.0	8.0
L. dispar	78.0	48.0

Table 4.6.

Summary of analysis of Table 4.5.

Source of Variation	df	G	P
Testhost	2	33.75	0.00
Residual	3	4.78	0.19
Total	5	38.53	

Table 4.7.

Number of female *B. intermedia* reared on *L. dispar* accepting, drumming or not mounting either clean *H. lamae* or *H. lamae* contaminated with *L. dispar* kairomone. One group of females was exposed first to clean *H. lamae* and subsequently, irrespective of the outcome, to contaminated *H. lamae*. Another group of females was exposed to the reverse situation. N=20 for both groups.[*]

		SECOND HOST					
FIRST HOST		clean			contaminated		
	response	AC	DRUM	NOM	AC	DRUM	NOM
clean	ACCEPTING		--		1	0	0
	DRUMMING		--		3	0	0
	NOT MOUNTING		--		7	1	8
conta-minated	ACCEPTING	11	6	2		--	
	DRUMMING	0	0	1		--	
	NOT MOUNTING	0	0	0		--	

[*] The distribution of responses for both situations were significantly different (G-test, $P < 0.05$).

first accepted a contaminated *H. lamae*, many of them subsequently accepted clean pupae. Experience with clean *H. lamae* suppressed host acceptance levels for *H. lamae* contaminated with *L. dispar* kairomone.

D. Discussion

Because the gypsy moth is a univoltine species, *B. intermedia* is generally referred to as a species *requiring* alternate hosts (e.g. Fuester and Ramaseshiah 1989), but whether *B. intermedia* is a uni-, bi- or tri-voltine species and whether it really requires alternate hosts remains unresolved (Hoy 1976). The 1908-1927 releases in Massachusetts of *B. intermedia* from France and Italy were considered unsuccessful because *B. intermedia* was never recovered from the field (Howard and Fiske 1911, Burgess and Crossman 1929, Dowden 1935). One *B. intermedia* was recovered in 1942 from a leafroller (Tortricidae) pupa, but in 1965 *B. intermedia* was recovered in numbers from gypsy moth in several Connecticut localities (Leonard 1966).

It is unlikely that this established population was derived from a 1963 release of *B. intermedia* in Connecticut (Leonard 1966, Hoy 1976). Instead, *B. intermedia* is presumed to have remained at low densities following the first introductions, because it was not well adapted to the environmental conditions in New England. The strain that is now established in the northeastern United States may have quite different seasonal adaptation characteristics than the progenitor strains from southern Europe. Cold winters may have selected for a long dormancy period and uni- or bi-voltinism, reducing or possibly eliminating the requirement of alternate hosts. Several reports indicate that bi- or even univoltinism in *B. intermedia* is physiologically possible. When held at 21° - 25° C on a diet of honey and without hosts, *B. intermedia* introduced from Europe lived up to 150 days. One female held at 10° C even survived 22 months (Dowden 1935). Adult females have been found to overwinter in loose bark or wood borer holes in dead trees and in leaf litter (Dowden 1935; Waldvogel and Brown 1978; Ticehurst 1978). Thus, the host range of nearctic *B. intermedia* could be more restricted to gypsy moth than is generally assumed.

Host acceptance by *B. intermedia* females, reared on either *L. dispar* or *H. lamae*, was consistently higher for *L. dispar* than for *H. lamae*. Acceptance of *L. dispar* was elevated by adult experience on this host. *B. intermedia* females reared on *H. lamae* for three generations and females reared on *L. dispar* had very similar host acceptance rates for *L. dispar* (high) and for *H. lamae* (low).

When parasitoids were deprived of hosts from emergence there was an increasing tendency with age to accept either *L. dispar* pupae or *H. lamae* pupae. Nonetheless, the maximum host acceptance rate for *H. lamae* remained far below that for *L. dispar*. The decrease in host acceptance rates in females older than 10 d and thus longer deprived of hosts, confirms earlier findings. Drost and Cardé (1992) found that prolonged host deprivation (12 d) causes a reduction in egg load and low host acceptance rates probably due to oosorption.

Rearing *B. intermedia* on *L. dispar* for approximately 65 generations in our laboratory possibly could have diminished genetically-controlled traits for accepting alternate host species. On the other hand, *B. intermedia* from a culture that has been reared on *Galleria mellonella* L. (Pyralidae) preferred *L. dispar* over *G.*

mellonella (Minot and Leonard 1976, Tucker and Leonard 1977). In agreement with our present results, rearing the parasitoid on the alternate host had no effect on oviposition behavior of the female adult wasp (Minot and Leonard 1976, Tucker and Leonard 1977).

B. intermedia is increasingly apt to accept alternate hosts after they are deprived of *L. dispar* for 7-9 days and after 10-12 days this effect became significant at the level of Á = 0.05. Perhaps instrumental learning of alternate hosts only occurs when the parasitoids are host deprived. The duration of host deprivation necessary to increase acceptance rates for alternate hosts (and thus the chance to learn) may depend on the affinity of *B. intermedia* for the alternate host.

B. intermedia accepted *H. lamae* contaminated with *L. dispar* kairomone at the same rate as *L. dispar* pupae. After one oviposition on a contaminated *H. lamae* pupa, females also increased their acceptance levels of clean *H. lamae*. From Table 4.1 we know that experience with a clean *H. lamae* pupa does not increase the response to another clean *H. lamae*, so the increased acceptance reates must have been due to the fact that the *H. lamae* pupae were contaminated with *L. dispar* kairomone. Apparently, *B. intermedia* associated certain characteristics of the *H. lamae* pupa with the *L. dispar* kairomone. The nature of these characteristics (chemical, size) is unknown.

The kairomone of *L. dispar* appears to be more effective in eliciting a response in *B. intermedia* than the kairomone of some other Lepidoptera (Tucker and Leonard (1977)): washing the pupae of *Choristoneura fumiferana* (Clemens) (Tortricidae), and *G. melonella* with hexane lowered their acceptability as hosts, but *L. dispar* pupae were accepted by *B. intermedia* after three 1-hr hexane washings. The tendency of *B. intermedia* females to switch to an alternate host may be higher if the kairomone of the alternate host resembles that of the gypsy moth, for example, as might be the case when the alternate host is closely related to gypsy moth. On the other hand, *B. intermedia* is able to reproduce in many families of Lepidoptera and even in sawflies and Diptera (e.g. Howard and Fiske 1911, Dowden 1935). Most of these data are from laboratory experiments where the parasitoids had no choice but to oviposit in the host offered. Females confronted with only these hosts and no opportunity to disperse may eventually oviposit in these species, but in the field they may not accept such hosts and instead resorb their eggs. The reports of field recovery of *B. intermedia* are from a small range of Lepidoptera, and the identity of the *Brachymeria* spp. recovered can not be readily verified. The recovery of *B. intermedia* from North American tortricids may reflect the coincidental high population densities of the gypsy moth and the alternate hosts species (Prokopy 1968, Leonard 1975) rather than a routine strategy of host switching requisite to reproduction.

E. Acknowledgments

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CHAPTER V

USE OF VISUAL CUES DURING HOST SELECTION

A. Introduction

Arthur (1966, 1971) was the first to demonstrate that parasitoids *(Itoplectis conquisitor* and *Nemeritis canescens*) learn to associate novel odors with the presence of hosts. Although learning of odors as well as visual cues has been demonstrated in many phytophagous insects (see Papaj and Prokopy 1989 for a review), in parasitoids few studies have involved the learning of visual cues. Taylor (1974) showed that *Nemeritis canescens* can learn to hunt in a novel environment. After oviposition experience with the host larvae (*Anagasta kuehniella*) hidden under cloths, the parasitoids increased ovipositor probing of cloths. Wardle and Borden (1986) demonstrated that *Exeristes roborator* learn to hunt for host larvae hidden in an artificial egg cup. This parasitoid also learned the color, but not the brightness of light reflected by artificial host microhabitats in the laboratory (Wardle 1990). Both studies were at what we would call the patch level. In this paper we demonstrate learning at the macro-habitat level, defined here as a collection of patches that might or might not contain hosts.

We studied *Brachymeria intermedia*, an endoparasitoid of pupae of *Lymantria dispar*, the gypsy moth. Gypsy moth pupae are found in leaf litter on the forest floor and on bark, often in crevices on tree trunks and branches. Their spatial distribution varies with population density: at high density pupae are found in the leaf litter and on tree trunks, whereas at low density most pupae are in the leaf litter (Elkinton et al. 1989). The pupae are brown and therefore, even when they are attached to a tree trunk, they often may be difficult to discern visually. Kairomone odors emanating from gypsy moth pupae play an important role during micro-habitat location by *B. intermedia* (Tucker and Leonard 1977; Cardé and Lee 1989; Drost and Cardé 1990). Whether *B. intermedia* also uses odors of tree species that the gypsy moth feeds on is unknown, but this is unlikely, considering that gypsy moth feeds on numerous tree species and pupation of gypsy moth often occurs on non-host material. Thus, orientation to odors of the gypsy moth's host plants would be of dubious value. A likely strategy of the macro habitat for *B. intermedia* would be to use visual cues. In this paper, we hypothesized that visual cues play a role in the parasitoid's decision of where to concentrate hunting for hosts: either on trees or in the leaf litter or both.

B. Materials and Methods

B. intermedia were reared on *L. dispar* pupae (see Drost and Cardé 1990). Females were collected from rearing cages at age 5-8 d. These parasitoids had access to gypsy moth pupae since age 3 d, and only females that were observed parasitizing were collected for experiments.

1. Laboratory Experiments

Two cages of 30x30x50 cm were used as training and test cages. Models of a ground habitat and a tree habitat were made using 6 cm

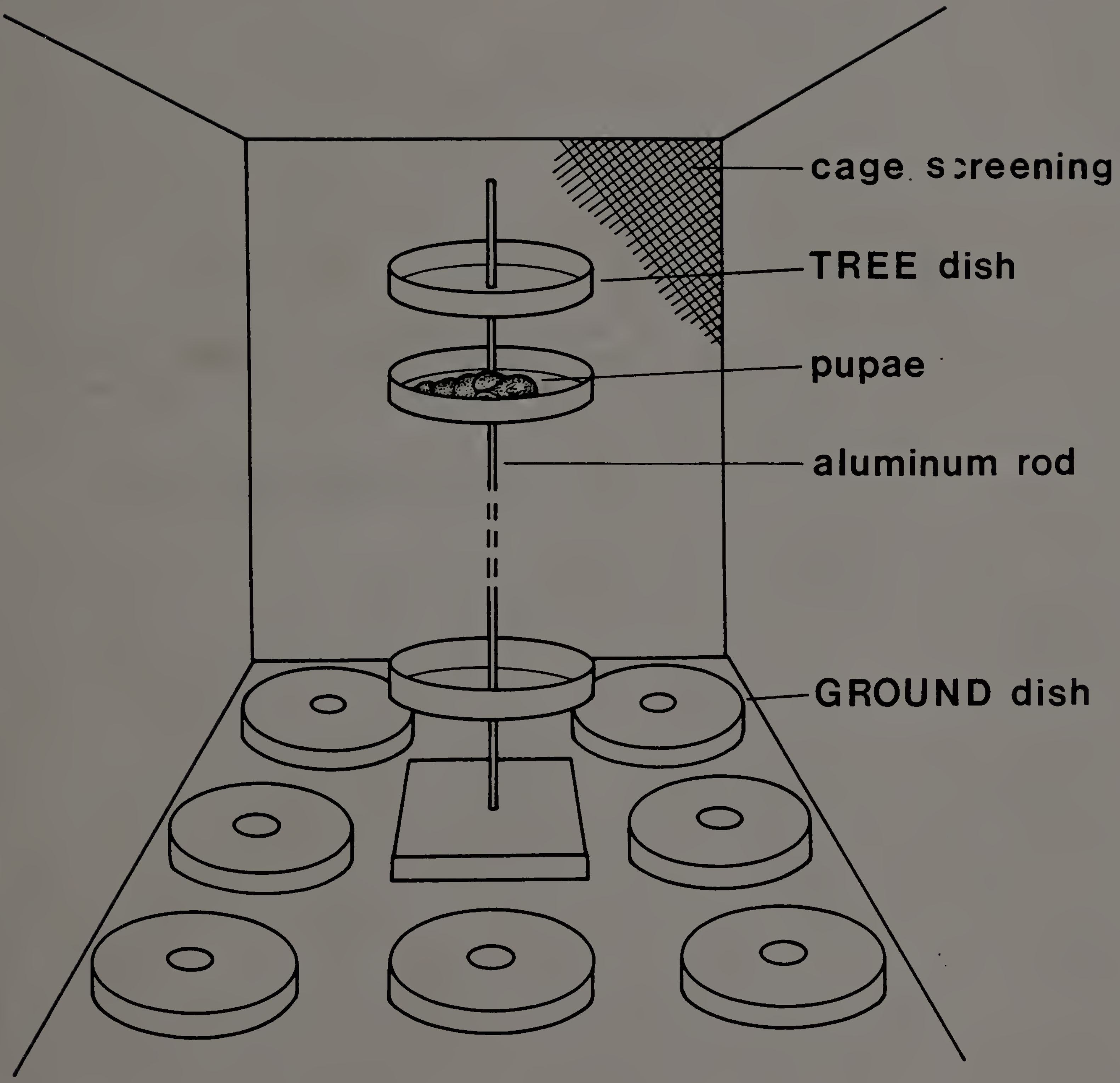

Fig. 5.1 Experimental set-up of the laboratory experiments.

diameter petri dishes (the micro-habitats) that were painted flat black on the outside. The tree-model (TREE) (Fig 5.1) consisted of a black 40 cm, 3 mm diameter aluminum rod, secured on a black wooden foot. Fifteen petri dish bottoms with a hole in the center were stacked on this rod, separated by 5 mm dia. rods of 3 cm length. The ground-model (GROUND) consisted of 15 petri dishes that were distributed evenly on the ground of the cage. The GROUND petri dishes had lids with a 2 cm diameter hole in the center. This shielded the pupae from the view of the flying parasitoids. Each cage contained both a GROUND and a TREE, a flask with a cotton wick in water, and a paper strip hanging from the ceiling and with drops of honey. The cages were set on a table with non-screened sides adjoining and screened sides on the front and the back (minimizing direct odor mixing between cages). White cardboard was put in between and on the sides of the cages to prevent interaction between insects in both cages. The light intensity in the cages was 1400 lux from DC-mains fluorescent lights and kept at a 16/8 h LD cycle, at 28 ± 3°C.

a) Experiment 1. In one cage two petri dishes of the GROUND type each held 10 gypsy moth pupae; in the other cage two petri dishes in the TREE type each held 10 gypsy moth pupae. On the first day of the experiment, 10 female parasitoids were introduced in the cage on the pupae in one of the dishes. Subsequently, they were left in these cages for four days, while the pupae were replaced daily. Every day two randomly chosen dishes either on the GROUND in one cage or the TREE in the other cage contained the pupae so that during the training period,

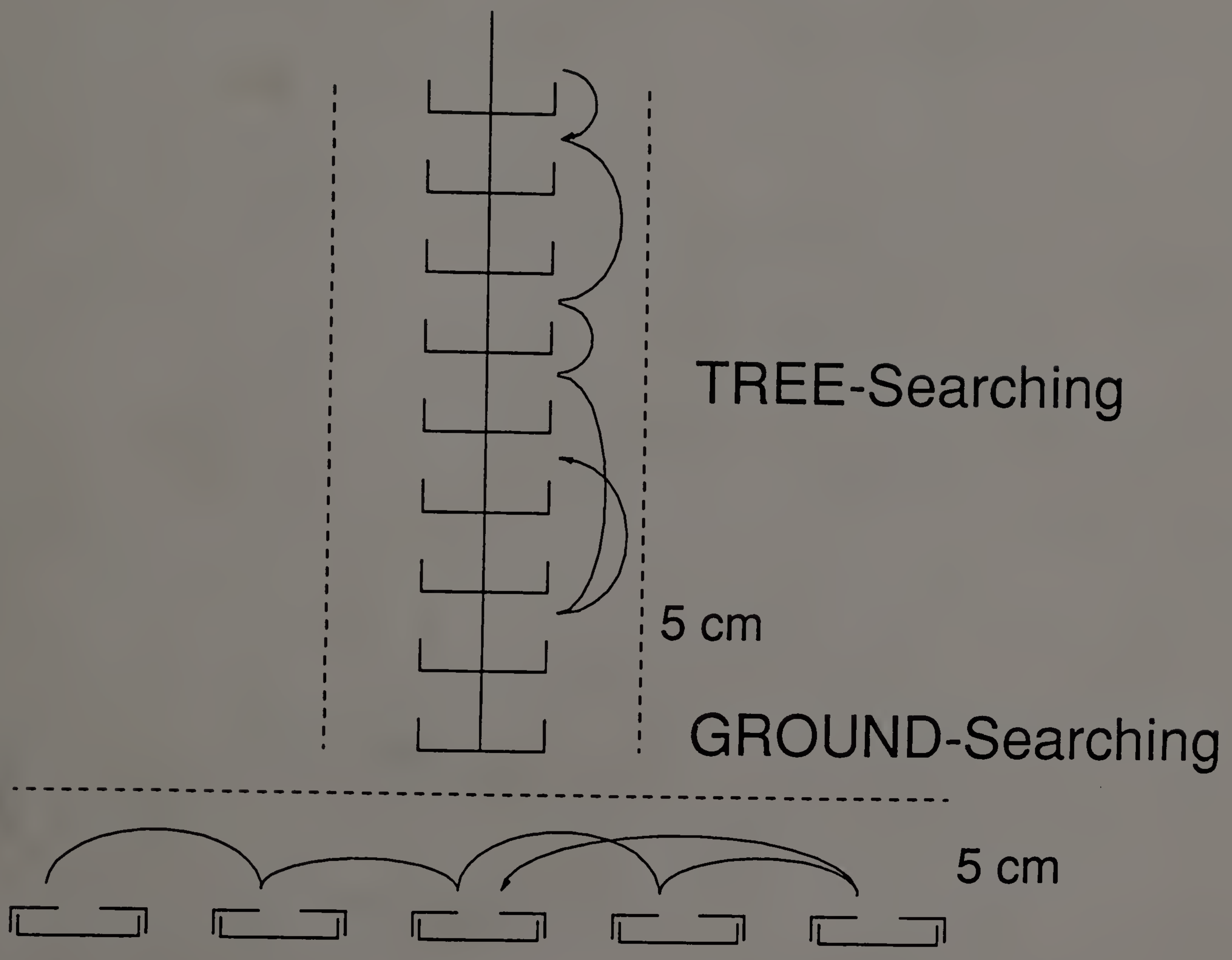

Fig. 5.2 Graphical respresentation of the definitions of TREE-searching and GROUND-searching.

parasitoid females would find pupae only in one model. On the fourth day, during the 13th h of photophase, the pupae were taken out, and the parasitoids were transferred to a 20 x 20 x 20 cm screened cage provisioned with water and honey. In this way the parasitoids were host deprived for 19-20 h before testing. We did not leave them in the training cages without pupae, because this might cause negative reinforcement, decreasing the response to the models. The training cages were subsequently used as test cages. All petri dishes were washed with water and soap, rinsed with water, 70 % ethanol, and hexane, and air dryed before they were used in the test. In addition, the test cage was wiped with water and ethanol to remove any traces of chemicals that parasitoids might have left behind during the training period.

The test situations were the same as the training situations, i.e. either 10 pupae in each of two dishes on the GROUND or 10 pupae in each of two dishes in the TREE. The following test procedure was maintained. One individual female was released on the paper strip containing honey. When she left this strip, the observation started. With a TRS-80 model 100 laptop computer, we recorded whether the parasitoid was in flight or and its position in the cage, which was near the tree, near the ground or elsewhere in the cage which we called non-oriented flight (Fig. 5.2). The petri dishes were numbered and we kept track of which dishes were approached within 5 cm distance before the parasitoid contacted a pupa. As soon as it found a pupa the observation was stopped and the female was removed from the cage. If no pupae were found, the observation stopped after 10 min. The experiment was replicated three times, with three different groups of parasitoids.

b) Experiment 2. To determine the reversibility of the learned responses found in experiment 1, we marked *B. intermedia* females individually on the thorax with liquid paper colors without aneasthesia. They were then trained to find pupae in either the GROUND or the TREE for three days, tested on the fifth day, trained on the other model for three days, and tested again. In the cages we used in experiment 1, training did not have a significant effect on the ability to find pupae in the TREE model, whereas it did for pupae in the GROUND model. Therefore, in experiment 2 we tested females only for their ability to find pupae in the GROUND model, following training on either the GROUND or the TREE model. The procedures of handling pupae and wasps were the same as in experiment 1.

2. Field experiments. Field tests were conducted on Orchard Hill, Amherst, MA from July 2 - July 11 1989. Wasps were trained in 1 x 1 x 2 (height) m screened wooden cages. TREE models were constructed from the upper part of the trunk of a young red maple (*Acer rubra*) tree. The trunks varied in diameter from 8-14 cm and were 1.5 m high. Trunks were supported on the bottom by a wooden cross. Twenty five wire gauze pockets (10.2 ± 1.39 cm) big enough to contain one female gypsy moth pupa were stapled at equal distances on the upper half of the TREE. GROUND models were constructed with leaf litter collected from a forest that also contained red maple. The pupae were placed on the wooden cross on the ground. Leaf litter (6 cm deep) was deposited on the ground, leaving the pupae exposed, so they could easily be recovered. A CONTROL cage that did not contain a tree trunk or leaf litter, contained pupae that were hung in wire gauze pockets on the screened walls, the

screened ceiling and the wooden floor of the cage. All cages contained two 30 ml plastic cups with cotton wicks and 5 % honey-water solutions. One cup was hung at 1 m above the ground, on the tree (at the same height in the corner of the cage for the CONTROL), and one cup was placed at the base of the tree on the ground (in the center of the cage on the ground for the CONTROL). Female parasitoids were trained for three days to forage for pupae in the TREE, the GROUND or the CONTROL situation. To avoid disturbing the parasitoids we did not replace pupae daily. At the end of the third day the parasitoids were collected and transferred to the smaller cages used in the lab experiments. We observed that *B. intermedia* spent the night at the ceiling of the test cages. Therefore, we kept the small cages overnight 1-1.5 m above the ground. For the test we used large 3 m diameter screened walk-in cages. At the center of this cage, we placed a 1 m diameter tray containing leaf litter and in at center of this tray a TREE model. A female parasitoid was placed on a gypsy moth pupa and as soon as she started to grasp the pupa, she was transferred to the base of the tree and the observation started. The same behaviors were recorded as described for Experiment 1. Because females that hovered above the ground also often searched the base of the tree, we separated the time spent searching the base of the tree from the time spent searching the upper part of the tree. When parasitoids searched the upper part of the tree they would fly upwards rather quickly to a level of 30 cm above ground, whereas the base of the tree was searched 5-10 cm above ground level. The transition from searching the base of the tree to searching the upper part of the tree was defined as crossing the level of 20 cm above ground.

3. Statistical Analysis

Nonparametric statistics were used to determine statistical significance of differences. We used the Kruskal-Wallis test for comparison of more than two treatments and for two treatments the Mann-Whitney U test or the Wilcoxon-matched-pairs test. For comparing percentages, the G-test was used on the original numbers.

C. Results

1. Laboratory Experiments

a) Experiment 1. Parasitoids displayed a characteristic searching behavior oriented to the TREE or the GROUND. While hovering and facing the TREE the parasitoids kept a distance from the TREE of <= 5 cm (see Fig. 5.2), thereby traveling from dish to dish and landing on the dish containing the pupae. Likewise, parasitoids searching the GROUND hovered within 5 cm above the GROUND-dishes. When TREE-trained females were tested with test pupae in the TREE, they never showed any GROUND searching (Table 5.1). When the test pupae were in the GROUND models some TREE-trained females would search the GROUND, but only for a few seconds. When the test pupae were in the TREE, the time spent searching the TREE was not significantly different between TREE-trained and GROUND-trained females. When flight was not oriented to the TREE or the

Table 5.1.

Influence of training on the time allocation in seconds (mean ± SE) by *B. intermedia* to the TREE model and the GROUND model.

Location of pupae during test	Training	N	Behavior		
			Hovering alongside TREE	Hovering above GROUND	Non-oriented flight
TREE	TREE	21	10.33 ± 2.26	0	26.24 ± 6.03
	GROUND	19	6.79 ± 2.09	6.09 ± 3.61	28.58 ± 10.25
P*			0.14	0.03	0.70
GROUND	TREE	23	34.57 ± 9.46	3.96 ± 1.83	65.26 ± 16.91
	GROUND	22	10.77 ± 2.94	17.05 ± 3.92	40.95 ± 10.50
P*			0.11	0.002	0.72

* Wilcoxon two-sample test, normal approximation (with continuity correction of 0.5)

Table 5.2

Influence of training on the number of petri dishes visited by *B. intermedia* in the TREE-MODEL and GROUND-MODEL.

Location of pupae during test	Training	N#	% females finding the pupae	Mean ± SE number of dishes visited in TREE-model	in GROUND-model
TREE	TREE	19	85.0	3.21 ± 0.62	0.05 ± 0.05
	GROUND	17	79.0	2.82 ± 0.65	0.89 ± 0.41
P*				0.47	0.05
P**			0.93		
GROUND	TREE	15	13.6	16.93 ± 1.20	1.07 ± 0.36
	GROUND	19	50.0	3.37 ± 0.98	4.42 ± 1.08
P*				0.005	0.003
P**			0.03		

* Wilcoxon two-sample test, normal approximation (with continuity correction of 0.5)

** G-test
# only females that hovered near at least one dish

GROUND it was generally not hovering, but a faster flight. The time spent in this flight type was not different between the test groups.

The fact that TREE pupae were more readily found than GROUND pupae is also demonstrated in Table 5.2. When test pupae were in the GROUND model, females trained on the TREE would continue to search the TREE repeatedly as is expressed in the large number of TREE dishes visited. The only group that visited a significant number of GROUND dishes was the GROUND-trained, GROUND-tested females.

b) Experiment 2. The above results show that the test with pupae in the TREE was much less discriminatory for the effect of the different training procedures than the test with pupae in the GROUND model. Thus in the second laboratory experiment we only tested *B. intermedia* with pupae in the GROUND model. The most recent training clearly modified the searching behavior of the parasitoids to a significant degree, demonstrating the flexibility of the response (Table 5.3).

2. Field Experiments

The time spent searching the base and upper part of the tree is shown in Table 5.4. The Kruskal-Wallis test only reveals that the three treatments are different, but not which treatments differ from each other. However, in all situations it is obvious which treatment contributes most to the significance of the Kruskal-Wallis test. Parasitoids trained to hunt for pupae on tree trunks spent more time hovering alongside the upper part of the tree than parasitoids that were trained to hunt on the ground, or on the screen (control). Likewise, parasitoids trained to hunt on the ground spent more time searching the leaf litter than parasitoids of the other two groups. Hovering alongside the base of the tree was not significantly different among the three treatment groups, but because the P-value generated by the Kruskal-Wallis test is 0.06 (close to 0.05), it is interesting to note that the time spent hovering alongside the base of the tree was lowest in the CONTROL group, which we would expect because these parasitoids were not trained to the tree or the ground. Also, the non-oriented flight-durations were largest in the CONTROL group but because the variation also was higher, there were no significant differences. In contrast to the parasitoids from the training groups, the CONTROL females quickly flew away from the test area to the screening of the cage and some females spent more than 2 min (maximum 127 s) hovering alongside the screening, whereas the maxima for the tree-trained parasitoids and the ground-trained parasitoids were 55 s and 68 s respectively.

D. Discussion

Both the laboratory and field experiments confirmed that *B. intermedia* is capable of learning new environments in which to hunt for host pupae. The parasitoids were able to find pupae hidden in petri dishes on the floor only after they had been trained to do this for four days. Thus learning can affect the reproductive success of the parasitoids by increasing the probability that a host is found.

In past research on learning in parasitoids, there has been considerable emphasis on the role olfactory cues (Vet 1983; Vet and van Opzeeland 1984; Wardle and Borden 1985; Drost et al. 1986, 1988; Hérard

Table 5.3

Influence of alternate training on time allocation in seconds (mean ± SE) to TREE-model and GROUND-model. The same group of females (individually marked) was trained twice and tested for their response when pupae were in the GROUND-model.

Training	N	Behavior		
		Hovering alongside TREE	Hovering above GROUND	Non-oriented flight
1st: TREE	16	39.69 ± 11.30	4.38 ± 2.00	63.78 ± 16.34
2nd: GROUND		6.06 ± 2.86	9.88 ± 3.43	38.50 ± 8.64
P*		0.02	0.04	0.17
1st: GROUND	14	14.50 ± 7.25	28.25 ± 8.01	30.50 ± 7.58
2nd: TREE		42.14 ± 11.41	11.00 ± 4.44	63.29 ± 11.85
P*		0.03	0.03	0.06

* Wilcoxon matched-pairs test, normal approximation (with continuity correction of 0.5)

Table 5.4

Influence of training on the time allocation (mean ± S.E.) by *B. intermedia* to the TREE and the GROUND in the field experiment. Females were released at the base of the tree. During the test, there was one *L. dispar* pupa on the ground and one on the top of the tree trunk.

Training	N	Behavior			
		Hovering alongside TREE	Hovering above GROUND	Non-oriented flight	Hovering alongside TREE base
TREE	21	45.48 ± 8.03	9.62 ± 3.13	13.57 ± 3.74	28.10 ± 6.10
GROUND	21	16.24 ± 2.02	34.90 ± 10.25	14.43 ± 3.96	32.29 ± 4.63
CONTROL	18	24.00 ± 6.06	13.00 ± 4.79	31.61 ± 9.53	18.39 ± 6.10
P*		0.0204	0.0635	0.4032	0.0604

*Probability that the three training groups are not different with respect to the behavioral durations, Kruskal-Wallis test, chi-square approximation.

et al. 1988; Lewis and Tumlinson 1988; Zanen et al. 1989; Kaiser et al. 1989; Cardé and Lee 1989; Turlings et al. 1990). For many parasitoids, olfactory cues are important during habitat finding as well as during host finding. Under laboratory conditions, many parasitoids are able to find hosts by using only kairomones, but in order to deal with the unpredictability of the field habitat, it may be more profitable for a parasitoid to use more than one sensory modality to find its host. Taylor (1974) developed stochastic models of the process of parasitism incorporating learning. When these models were tested on parasitism by *Nemerites canescens*, a model postulating learning of two cues fitted the experimental data better than a one cue model.

Initially, we intended to assess the effect of learning without pupae in the test cage, i.e. with only visual cues, but when there was no pupal odor, very few females initiated search during the observation time. On the other hand, the fact that parasitoids trained to hunt for pupae on the TREE never searched the GROUND, when the GROUND contained pupae, demonstrates that the searching is not exclusively guided by olfactory cues. We suspect that a sensory threshold for olfactory cues controls the initiation of searching behavior in *B. intermedia* and that the searching strategy, using visual cues the nature of which remains to be determined, is mainly determined by previous experience with visual cues associated with the presence of hosts. A similar process has been demonstrated in *Coccigomimus turionellae*. Sandlan (1980) showed that this pupal parasitoid, when exposed to host odor just prior to a test, responded much more strongly to contrast and three-dimensional effects than when they had not been exposed to odor.

We have demonstrated that *B. intermedia* is capable of learning visual cues during habitat finding, but its significance in natural situations remains to be verified. *B. intermedia* is typically recovered from high density gypsy moth populations (Ticehurst et al. 1978; Elkinton and Gould 1988), where pupae are in the leaf litter and on trees. Microclimatic differences may affect the phenology of pupation, whereas differences in predation and presence of other parasitoids (Smith 1988) may affect the comparative numbers of pupae in litter and on trees. Thus the location of pupae suitable for parasitization is rather unpredictable. Learning cues associated with the most profitable places to find pupae could decrease the time spent searching in unprofitable places, thus contributing to the optimal foraging strategy of the parasitoid.

The acquisition of new foraging methods through learning theoretically can result in a sigmoid (type III) functional response (Holling 1959,1965), which would have a stabilizing effect on the density of the host. Whether this is the case for *B. intermedia* is unknown. Because *B. intermedia* is only recovered from high density gypsy moth populations, Elkinton et al. (1989) conclude that *B. intermedia* is unlikely to play an important role in maintaining the apparent stability of low density gypsy moth populations. However, it appears to be an important factor in the collapse of high density populations (Ticehurst et al. 1978). The numerical response of *B. intermedia* is probably the main reason for this evident density dependence in parasitization of gyspy moth (Elkinton, personal communication). However, our findings show that there is also a functional response in *B. intermedia*. In the field, such a functional response may only be expressed in gypsy moth populations of relatively high density.

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CHAPTER VI

FINAL REMARKS

The main objective of this study was to determine which factors influence the development of host-selection behavior in *B. intermedia*. It was demonstrated that previous experience of the adult parasitoids with hosts increases host-acceptance rates. This is most likely due to an increased response to the gypsy moth kairomone in experienced females as shown by Cardé and Lee (1989) through the association of the kairomone with a suitable host. The process through which pre-existing weak responses increase through associative learning has been called 'alpha-conditioning' and may be a very common and functional kind of learning in parasitoids (Vet and Groenewold 1990; Carew et al. 1984; Gould and Marler 1984). The kairomone subsequently can be associated to a new oviposition substrate as is demonstrated in the experiments with *H. lamae* as the alternate host (Chapter IV): experience with gypsy moth kairomone applied to *H. lamae* elicits a response in *B. intermedia* to *H. lamae* pupae without kairomone (Chapter IV). Thus a link was established between an unconditioned stimulus (the kairomone) and a conditioned stimulus (the alternate host) that previously elicited virtually no response.

When laboratory reared parasitoids are released in the field, these effects should be taken in account. Wardle and Borden (1986) warn for adverse effects of learning in the laboratory, i.e. by conditioning on the wrong host. It seems that for *B. intermedia* there is no particular danger in this sense, since *B. intermedia* always exhibited a much stronger response to gypsy moth than to other host species (Chapter IV, and see also Tucker and Leonard 1977). By learning visual cues related to the environment in which pupae can be found *B. intermedia* increases its searching efficiency. Less time is spent at searching non-profitable places and pupae are found at a faster rate (Chapter V).

The searching efficiency of *B. intermedia* may also be influenced by the physiological state of the parasitoid. Host-deprivation causes a decrease in discrimination behavior in the sense that it increases response levels no matter what host is offered (Chapter IV). Prolonged host-deprivation (12 days), however, causes a decrease in response levels, probably due to egg resorption (Chapter III).

Parasitoids of larvae that move around face the reliability-detectability problem (Vet et al. 1991). Larvae-derived cues are the most reliable in indicating presence of the larvae, but generally are hard to detect, whereas food-derived cues may be less reliable but easier to detect. It thus makes sense for larval parasitoids to learn the easy detectable food-derived cues that are associated with the presence of hosts (Vinson et al. 1977; Vet 1983; Vet and van Opzeeland 1984; Drost et al. 1986; Lewis and Tumlinson 1988). Parasitoids of pupae not necessarily face the reliability-detectability problem, because pupae do not move around. The host may have evolved to pupate in hidden areas, but once the pupa has emerged it remains stationary. Learning of direct environmental cues associated to the location of the host may therefore be more profitable for pupal parasitoids than for larval parasitoids.

BIBLIOGRAPHY

Arthur, A.P. (1966). Associative learning in *Itoplectis conquisitor* (Say) (Hymenoptera: Ichneumonidae). *Canadian Entomologist* 98:213-223.

Arthur, A.P. (1971). Associative learning by *Nemeritis canescens* (Hymenoptera: Ichneumonidae). *Canadian Entomologist* 105:145-156.

Barbosa, P. and Frongillo, E.A. Jr. (1979). Photoperiod and temperature influences on egg number in *Brachymeria intermedia* (Hymenoptera: Chalcididae), a pupal parasitoid of *Lymantria dispar* (Lepidoptera: Lymantriidae). *Journal of the New York Entomological Society*, 87, 175-180.

Barbosa, P., Martinat, P. and Bennett, R. (1986). Consequences of maternal age and development of *Brachymeria intermedia* (Nees) and the mortality of its gypsy moth (*Lymantria dispar*) host. *Journal of Applied Entomology*, 101, 215-223.

Bell, R.A., Owens, C.D., Shapiro, M., and Tardif, J.R. (1981). Development of mass rearing technology. In Doane, C.C. and McManus, M.L. (eds.), *The gypsy moth: reseach toward integrated pest management*. USDA Technical Bulletin 1584: 599-655.

Bishop, Y.M.M., Fienberg, S.F., and Holland, P.W. (1975). *Discrete multivariate analysis: Theory and practice*. M.I.T. Press Cambridge, Mass.

Burgess, A.F., Crossman, S.S. (1929). Imported insect enemies of the gypsy moth and the brown-tail moth. *USDA Technical Bulletin* 86: 116-118.

Cardé, R.T., and Lee H.-P. (1989). Effect of experience on the responses of the parasitoid *Brachymeria intermedia* (Hymenoptera: Chalcididae) to its host, *Lymantria dispar* (Lepidoptera: Lymantriidae), and to kairomone. *Annals of the Entomological Society of America* 82: 653-657.

Carew, T.J., Abrams, T.W., Hawkins, R.D., and Kandel, E.R. (1984). The use of simple invertebrate systems to explore psychological issues related to associative learning. In Alkon, D.L., and Farley, J. (eds.), *Primary Neural Substrates of Learning and Behavioral Change*, Cambridge University Press, Cambridge, pp. 169-184.

Colgan, P.W. (1978). *Quantitative Ethology*. John Willey and Sons, Inc., New York.

Cornell, H., and Pimentel, D. (1978). Switching in the parasitoid *Nasonia vitripennis* and its effect on host competition. *Ecology* 59: 297-308.

Dowden, P.B. (1935) *Brachymeria intermedia* (Nees), a primary parasite and *B. compsilurae* (Cwfd.), a secondary parasite, of the gypsy moth. *Journal of Agricultural Research,* 50, 495-523.

Drost, Y.C., Lewis, W.J., Zanen, P.O., and Keller, M.A. (1986). Beneficial arthropod behavior mediated by airborne semiochemicals I. Flight behavior and influence of preflight handling of *Microplitis croceipes* (Cresson). *Journal of Chemical Ecology* 12: 1247-1262.

Drost, Y.C., W.J. Lewis, and J.H. Tumlinson. (1988). Beneficial arthropod behavior mediated by airborne semiochemicals. V. Influence of rearing method, host plant, and adult experience on host-searching behavior of *Microplitis croceipes* (Cresson), a larval parasitoid of *Heliothis. Journal of Chemical Ecology* 14: 1607-1616.

Drost, Y.C. and Cardé, R.T. (1990). The influence of experience on the sequential and temporal organization of host-acceptance behavior in *Brachymeria intermedia* (Chalcididae), an endoparasitoid of gypsy moth. *Journal of Insect Behavior,* 3, 647-661.

Drost, Y.C., and R.T. Cardé. (1992). Influence of host deprivation on egg load and oviposition behavior of *Brachymeria intermedia. Physiological Entomology* (in press).

Drost, Y.C. and Cardé, R.T. (1992). Host-switching in *Brachymeria intermedia* (Chalcididae), a pupal parasitoid of *Lymantria dispar. Environmental Entomology* (submitted).

Elkinton, J.S., and Gould, J.R., Liebhold, A.M., Smith, H.R. and Wallner, W.E. (1989). Are gypsy moth populations in North America regulated at low density? In *Lymantriidae: A comparison of features of new and old world tussock moths.* Proceedings USDA Forest Service, Northeastern Forest Experiment Station, Technical Report NE-123: 233-249.

Fienberg, S.E. (1980). *The analysis of cross-classified categorical data.* MIT Press Cambridge, Mass.

Flanders, S.E. (1950). Regulation of ovulation and egg disposal in the parasitic Hymenoptera. *Canadian Entomologist,* 82: 134-140.

Fuester, R., and G. Ramaseshiah. (1989). A comparison of the parasite complexes attacking two closely related lymantriids. In *Lymantriidae: A comparison of features of new and old world tussock moths.* Proceedings USDA Forest Service, Northeastern Experiment Station, General Technical Report NE-123.

Gould, J.L., and Marler, P. (1984). Ethology and the natural history of learning. In Marler, P., and Terrace, H.S. (eds.), *The Biology of Learning,* Springer-Verlag, New York, pp. 47-74.

Hérard, F., Keller, M.A., Lewis, W.J., and Tumlinson, J.H. (1988). Beneficial arthropod behavior mediated by airborne semiochemicals. IV. Influence of host diet on host-oriented flight chamber responses of *Microplitis demolitor* Wilkinson. *Journal of Chemical Ecology* 14: 1597-1606.

Heinrich, B. (1979). Majoring and minoring by foraging bumblebees; *Bombus vagans*: an experimental study. *Ecology,* 60: 245-255.

Heinrich, B. (1984). Learning in invertebrates. pp. 135-147 in *The Biology of Learning* Marler, P, and Terrace H.S. (eds.). Springer-Verlag, Berlin.

Houston, A., Clark, C.W., McNamara, J., and Mangel, M. (1988). Dynamic models in behavioral and evolutionary ecology. *Nature* 332:29-34.

Howard, L.O., and Fiske, W.F. (1911). The importation into the United States of the parasites of the gypsy moth and the brown-tail moth. *USDA Bureau of Entomology Bulletin* 91.

Hoy, M.A. (1976). Establishment of gypsy moth parasitoids in North America : an evaluation of possible reasons for establishment or non-establishment. In Anderson, J.F., and Kaya, H.K. (eds.), *Perspectives in Forest Entomology.* Academic Press, N.Y.

Jolly, A. (1972). *The evolution of primate behavior.* Macmillan Publishing Co., Inc., New York.

Kaiser, L., Pham-Delegue, M.H., and Masson, C. (1989). Behavioral study of plasticity in host preferences of *Trichogramma maidis* (Hym.: Trichogrammatidae). *Physiological Entomology* 14: 53-60.

Lashomb, J.H., N, Y.-S., and Metterhouse, W. (1983). Gypsy moth (Lepidoptera: Lymantriidae) pupal age as a determinant of suitability by *Brachymeria intermedia* (Hymenoptera: Chalcididae). *Environmental Entomology* 12: 855-857.

Laverty, T.M., and Plowright, R.C. (1988). Flower handling by bumblebees: a comparison of specialists and generalists. *Animal Behavior* 36: 733-740.

Leonard, D.E. (1966). *Brachymeria intermedia* (Nees) (Hymenoptera: Chalcididae) established in North America. *Entomological News* 77: 25-27.

Leonard, D.E. (1975). Parasitization of the spruce budworm,*Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae) by *Brachymeria intermedia* (Nees) (Hymenoptera: Chalcididae). *N.Y. Entomological Society* 87: 269-270.

Leonard, D.E., Bierl, B.A., and Beroza, M. (1975). Gypsy moth kairomones influencing behavior of the parasitoids *Brachymeria intermedia* and *Apanteles melanoscelus*. *Environmental Entomology* 4: 929-930.

Leonard, S.H., and Ringo, J.M. (1978). Analysis of male courtship patterns and mating behavior of *Brachymeria intermedia*. *Annals of the Entomological Society of America* 71: 817-826.

Lewis, A.C. (1986). Memory constraints and flower choice in *Pieris rapae*. *Science* 232:863-865.

Lewis, W.J. and Tumlinson, J.H. (1988). Host detection by chemically mediated associative learning in a parasitic wasp. *Nature* 331: 257-259.

Mangel, M. (1989a). Evolution of host selection in parasitoids: does the state of the parasitoid matter?. *American Naturalist* 133, 688-705.

Mangel, M. (1989b). An evolutionary interpretation of the "motivation to oviposit." *Journal of Evolutionary Biology,* 2, 157-172.

Mangel, M., and Clark, C.W. (1986). Toward a unified foraging theory. *Ecology* 67:1127-1138.

Mangel, M., and Clark, C.W. (1988). *Dynamic Modeling in Behavioral Ecology*. Princeton University Press, Princeton, NJ.

Marler, P., and Hamilton, W.J. III (1966). *Mechanisms of animal behavior*. Wiley, New York.

McNamara, J.M., and Houston, A.I. (1986). The common currency for behavioral decisions. *American Naturalist* 127:358-378.

Minot, C.M. and Leonard, D.E. (1976). Host preference and development of the parasitoid *Brachymeria intermedia* in *Lymantria dispar, Galleria mellonella*, and *Choristoneura fumiferana*. *Environmental Entomology*, 5, 527-532.

Murdoch, W.W. (1969). Switching in general predators: experiments on predator specificity and stability of prey populations. *Ecological Monograph* 39: 335-354.

Papaj, D. (1986). Conditioning of leaf-shape discrimination by chemical cues in the butterfly *Battus philenor*. *Animal Behavior* 34:1281-1288.

Papaj, D. and Prokopy, R.J. (1986). Phytochemical basis of learning in *Rhagoletis pomonella* and other herbivorous insects. *Journal of Chemical Ecology* 12:1125-1143.

Papaj, D.R., and Prokopy, R.J. (1989). Ecological and evolutionary aspects of learning in phytophagous insects. *Annual Review of Entomology* 34: 315-350.

Prokopy, R.J. (1968). Parasites of the leaf rollers *Archips argyrospilus* and *A. griseus* in Connecticut. *Journal of Economic Entomology* 61:348-352.

Prokopy, R.J., Averill, A.L., Cooley, S.S. and Roitberg, C.A. (1982). Associative learning in egglaying site selection by apple maggot flies. *Science* 218:76-77.

Prokopy, R.J., Papaj, D.R., Cooley, S.S., and Kallet, C. (1986). On the nature of learning in oviposition site acceptance by apple maggot flies. *Animal Behavior* 34:98-107.

Rotheray, G.E., and Barbosa, P. (1984). Host related factors affecting oviposition behavior in *Brachymeria intermedia*. *Entomologia experimentalis et applicata* 35: 141-145.

Sandlan, K. (1980). Host location by *Coccygomimus turionellae* (Hymenoptera: Ichneumonidae). *Entomologia experimentalis and applicata* 27: 233-245

SAS Institute Inc. (1988). *SAS/STAT™ User's Guide, Release 6.03 Edition*. Cary, NC: SAS Institute Inc. 1028 pp.

Schal, C., Charlton, R.E., and Cardé, R.T. (1987). Temporal patterns of sex pheromone titers and release rates in *Holomelina lamae* (Lepidoptera: Arctiidae). *Journal of Chemical Ecology* 13: 1115-1129.

Schneider, F. (1941). Eientwicklung und Eiresorption in den ovarien des puppenparasiten *Brachymeria euploeae*. *Zeitschrift für angewandte Entomologie*, 28, 211-228.

Smith, H.R. (1988). Predation: its influences on population dynamics and adaptive changes in morphology and behavior of the Lymantriidae. *USDA Forest Service, Northeastern Forest Experiment Station, Technical Report* NE-123:469-488

Sokal, R.R., and Rohlf, F.J. (1981). *Biometry*. Second Edition. W.H. Freeman and Company, NY.

Ticehurst, M., Fusco, R.A., Kling, R.P., and Unger, J. (1978). Observations on parasites of gypsy moth in first cycle infestations in Pennsylvania from 1974-1977. *Environmental Entomology* 7: 355-358

Tierney, A.J. (1986). The evolution of learned and innate behavior: Contributions from genetics and neurobiology to a theory of behavioral evolution. *Animal Learning and Behavior* 14:339-348.

Traynier, R.M.M. (1986). Visual learning in assays of sinigrin solution as an oviposition releaser for cabbage butterfly, *Pieris rapae*. *Entomologia experimentalis et applicata* 40: 25-33.

Trudeau, D. and Gordon, D.M. (1989). Factors determining the functional response of the parasitoid *Venturia canescens*. *Entomologia Experimentalis et Applicata*, 50, 3-6.

Tucker, J.E., and Leonard, D.E. (1977). The role of kairomone in host recognition and host acceptance behavior of the parasite *Brachymeria intermedia*. *Environmental Entomology* 6: 527-531.

Turlings, T.C.J., Scheepmaker, J.W.A., Vet, L.E.M., Tumlinson, J.H., and Lewis, W.J. (1990). How contact foraging experiences affect the preferences for host-related odors in the larval parasitoid *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae). *Journal of Chemical Ecology* 16: 1577-1589.

Vet, L.E.M. (1983). Host-habitat location through olfactory cues by *Leptopilina claviceps* (Hartig) (Hym.: Eucolidae), a parasitoid of fungivorous *Drosophila*: The influence of conditioning. *Netherlands Journal of Zoology* 33: 225-248.

Vet, L.E.M., Wäckers, F.L., and Dicke, M. (1991). The reliability-detectability problem for foraging parasitoids: usability of 1st and 2nd trophic level stimuli. *REDIA*, LXXIV(2): 1-5

Vet, L.E.M., and Groenewold, A.W. (1990). Semiochemicals and learning in parasitoids. *Journal of Chemical Ecology* 16: 3119-3135

Vet, L.E.M. and van Opzeeland, K. (1984). The influence of conditioning on olfactory microhabitat and host location in *Asobara tabida* (Nees) and *A. rufescens* (Foerster) (Braconidae: Alysiinae), larval parasitoids of Drosophilidae. *Oecologia* 63: 171-177.

Vet, L.E.M. and van Opzeeland, K.. (1985). Olfactory microhabitat selection in *Leptopilina heterotoma* (Thomson) (Hym.: Eucoilidae), a parasitoid of Drosophilidae. *Netherlands Journal of Zoology* 35: 497-504.

Vet, L.E.M., and Schoonman, G. (1988). The influence of previous foraging experience on microhabitat acceptance in *Leptopilina heterotoma*. *Journal of Insect Behavior* 1: 387-392.

Vinson, S.B., Barfield, R.D., and Henson, R.D. (1977). Ovipositional behavior of *Bracon mellitor* Say, a parasitoid of the boll weevil (*Anthonomus grandis* Boh.). II. Associative learning. *Physiological Entomology* 2: 157-164.

Vinson, S.B., (1984). Parasitoid-host relationship. In: Bell, W.J. and Cardé, R.T. (eds.) *Chemical Ecology of Insects*. Chapman and Hall, London, pp. 205-224.

Waldvogel, M.G. and Brown, M.W. (1978). An overwintering site of the gypsy moth parasite, *Brachymeria intermedia*. *Environmental Entomology,* 7, 782-783.

Wardle, A.R. (1990). Learning of host microhabitat colour by *Exeristes roborator* (F.) (Hymenoptera: Ichneumonidae). *Animal Behaviour* 39: 914-923.

Wardle, A.R. , and Borden, J.H. (1985). Age-dependent associative learning by *Exeristes roborator* (F.) (Hymenoptera: Ichneumonidae). *Canadian Entomologist* 117: 605-616.

Wardle, A.R., and Borden, J.H. (1986). Detrimental effect of prior conditioning on host habitat location by *Exeristes roborator.* *Naturwissenschaften* 73: 559-560.

Wardle, A.R. and Borden, J.H.. (1989). Learning of an olfactory stimulus associated with a host microhabitat by *Exeristes roborator.* *Entomologia experimentalis et applicata* 52: 271-279.

Zanen, P.O., and Cardé, R.T. (1990). Learning and the role of host-specific volatiles during in-flight host finding in the specialist parasitoid *Microplitis croceipes.* *Physiological Entomology* (in press).

Zanen, P.O., Lewis, W.J., Cardé, R.T., and Mullinix, B.G.(1989). Beneficial arthropod behavior mediated by airborne semiochemicals. VI. Flight responses of female *Microplitis croceipes* (Cresson), a braconid endoparasitoid of *Heliothis* spp. to varying olfactory stimulus conditions created with a circular turbulent jet. *Journal Chemical Ecology* 15: 141-167.