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HOST-INSTAR PREFERENCE OF *PRAON PEQUODORUM* VIERECK (HYMENOPTERA:

APHIDIIDAE) AND EFFECT OF PARASITISM ON THE REPRODUCTIVE

POTENTIAL OF THE HOST, *ACYRTHOSIPHON PISUM* (HARRIS) (HOMOPTERA:

APHIDIDAE)


by


Richard Sequeira

B. Sc. (Agric.), Mahatma Phule Agricultural University,

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Name:                    Richard Vincent Sequeira

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Examining Committee:

Chairman:

_____
Dr. B. D. Roitberg

_____
Dr. M. Mackauer, Senior Supervisor

_____
Dr. J. H. Borden, Professor

_____
Dr. H. R. MacCarthy, Adjunct Professor

_____
Dr. Bryan Frazer, Agriculture Canada Research
Station, Vancouver, External Examiner

Date approved 27 Nov 116

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Host-instar preference of Praon pequodorum Viereck (Hymenoptera:

Aphidiidae) and effect of parasitism on the reproductive potential

of the host, Acyrthosiphon pisum Harris (Homoptera: Aphididae)

Author: _____

(signature)

Richard Vincent Sequeira

(name)

_____

(date)

# ABSTRACT

Oviposition preference of the aphid parasite, *Praon pequodorum* Viereck (Hymenoptera: Aphidiidae), for apterous instars of the pea aphid, *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae), was investigated in the laboratory. In separate experiments, the parasite was offered equal numbers of the four host instars, in pairs and together, simultaneously. The preference pattern was evaluated by fitting log-linear models to the total number of eggs laid in each instar, and the total number of aphids of each instar attacked, as determined by dissection. The calculated probability ratios and their 95% confidence intervals indicated a preference for larger instars. The order of preference was Instar I < (II = IV) < III when instars were offered in pairs, and I < (II = III = IV) when all four instars were provided simultaneously.

The effect of parasitism of different instars on the expected population growth of the pea aphid was examined by the estimation of the intrinsic rate of natural increase, $r_m$. Instars I, II, and III did not reproduce after parasitization. Aphids parasitized in the fourth instar were able to reproduce for a short period prior to death; their $r_m$ was about 50% less than that of unparasitized controls. Parasitism of pre-reproductive and reproductive adults did not affect $r_m$ significantly.

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Host-stage preference is an important determinant of a parasite's effectiveness in controlling the host population. Host size, behaviour, and availability influence preference. Also, the experimental design and method of evaluation may bias conclusions about preference. Possibly due to other biological characteristics, the control potential of *P. pequodorum* appears to be lower than its host-instar preference pattern would indicate.

# ACKNOWLEDGMENTS

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# CHAPTER I

## GENERAL INTRODUCTION

The premise of classical biological control is that organisms are held at low, noninjurious levels by their natural enemies (Huffaker *et al*. 1976). Many introduced and some native pests of agricultural importance have been successfully controlled by the use of their parasites (Hagen *et al*. 1971; Rabb 1971). Much information on the biology and host relationships of insect parasites has come from laboratory investigations which provide data that are useful in characterizing host-parasite interactions and identifying what kind of information is important for the planning and evaluation of biological control (Doutt *et al*. 1976; Waage & Hassell 1982).

The effectiveness of a parasite in controlling its host appears to be dependant on several factors, including biological and ecological characteristics of the parasite and the host, as well as the environment (Mackauer & van den Bosch 1973; Doutt *et al*. 1976; Waage & Hassell 1982). The significance of any particular factor will depend on the situation and on the characteristics of the host-parasite association.

Many aphid species have overlapping generations (Dixon 1985). As a result, several developmental stages (instars) may be available at a given time for parasitization or predation by natural enemies. Whereas several studies have shown that parasitized aphids survive for some time and may even reproduce

for a certain period before death (Campbell & Mackauer 1975; Liu & Hughes 1984), the effect of this lag period on the parasite's control potential is not well known.

The life history and habits of the pea aphid were studied by R. E. Campbell (1926), Cooke (1963), A. Campbell (1974), among others. The aphid is a common pest of leguminous crops such as alfalfa, *Medicago sativa* L., and peas, *Pisum sativum* L. The aphid infests the leaves, stems, petioles, and flower buds, and feeds on the sap. It is easily reared on broad bean, *Vicia faba* L. Under laboratory conditions, reproduction by the pea aphid is parthenogenetic, viviparous and thelyotokous, and the progeny are predominantly apterous. There are four nymphal instars followed by a short pre-reproductive period in the adult stage. The distinguishing features of the four instars are given in A. Campbell (1974).

The life history and bionomics of the hymenopterous family Aphidiidae were reviewed in detail by Stary (1970). Females of most Aphidiidae are arrhenotokous. Unmated females lay haploid eggs, which develop into males, whereas mated females lay haploid and diploid eggs, which develop into males and females, respectively. Each successful oviposition results in a single egg being laid in the hemocoel of the host. Superparasitism is common under laboratory conditions. Irrespective of the number of eggs laid in an individual host, usually only one parasite larva completes its development within a single host.

The parasite larva feeds internally on the aphid until only the integument remains. The hardened integument, which develops unique characteristics depending on the parasite species, is called a mummy. The mature *Praon* larva spins its cocoon below the mummy.

I used the pea aphid, *Acyrthosiphon pisum* (Harris), and its parasite, *Praon pequodorum* Viereck, as a model system, to address the following questions: 1) How does a parasite distribute its eggs among different developmental stages of its host? and 2) What influence does the observed egg distribution pattern have on the growth and reproduction of the host?

# CHAPTER II

## HOST INSTAR PREFERENCE BY *PRAON PEQUODORUM* VIERECK (HYMENOPTERA: APHIDIIDAE)

## Introduction

The subject of host preference by insect parasites (parasitoids) has received considerable attention in recent years. Many examples can be found in the literature of parasites that attack several host species but prefer one (Drooz & Fedde 1972; Calvert 1973; van Alphen 1980). In addition, when several developmental stages of the host species are available for parasitization, many parasites prefer one or several of these stages over others (Lingren *et al*. 1970; Miller 1970; Richerson & Deloach 1972; Mackauer 1973, 1983; Duodu & Davis 1974; Hopper & King 1984).

Preference is indicated if the relative frequency of parasitized host classes differs from the relative frequency of available host classes (Mackauer 1983; Liu *et al*. 1984). This definition of preference can be applied to a variety of situations including cases in which the parasite has a choice between different host species as well as cases in which different developmental stages of a single host species are available. Furthermore, a definition based on relative preference permits, through experimentation, the identification of some of the factors that may influence parasite choice. Host

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characteristics such as sound (Quednau 1967), shape (Vinson 1968), size (Richerson & Borden 1972), and movement (Richerson & Deloach 1972), have been shown to influence a parasite's choice.

Preference for certain host instars by some parasites of aphids has been reported (Wiackowski 1962; Fox et al. 1967; Stary 1970 p. 168; Mackauer 1973, 1983; Liu et al. 1984). Instar-preference appears to be the outcome of a complex interaction between host and parasite. Once a searching parasite female has encountered a host, she must decide if the host is acceptable and, if acceptable, whether to lay an egg or continue searching. This decision is unlikely to be based on any single factor; more likely, several factors will be involved. Accordingly, an appropriate measure of preference would be one that reflects the complexity of the decision-making process. Such a measure, expressed as the probability that a host will be parasitized, would serve as a basis for analysing the host-acceptance behaviour of parasites and, further, could allow one to relate this behaviour to the dynamics of the host-parasite interaction in a meaningful way.

My objective was to examine host-instar preferences, if any, of *Praon pequodorum* Viereck, a parasite of the pea aphid, *Acyrthosiphon pisum* (Harris), in the laboratory.

## Materials and Methods

*Maintenance of insect colonies*

Stock colonies of pea aphids were maintained in the laboratory on potted broad bean plants, *Vicia faba* L. cv. 'Broad Windsor'. Bean seeds were planted in garden mix soil. Adult aphids were transferred to fresh bean plants each week to provide a succession of vigorous colonies.

A culture of *P. pequodorum* was maintained in the laboratory. It was established in 1982 from a sample obtained from Dr. D. J. Sullivan of Fordham University, Bronx, N.Y., U.S.A. Mated female parasites were allowed to parasitize third- and fourth-instar aphids from a stock colony, kept in wax-paper cups. After 8-10 h, the parasites were removed by aspiration and the aphids were transferred to potted bean plants in plexiglass rearing cages (33 x 34 x 44 cm), kept at room temperature. Mummified aphids were collected from the colony and held separately. Upon emergence, male and female parasites were fed a solution of honey and water. A new batch of parasites was thus produced every week. To enhance parasite longevity, the stock culture was maintained in a controlled environment chamber at 10±1 °C.

*Design of Experiments*

Two types of experiments were conducted in order to detect preferences, if any, in *P. pequodorum*, for four different age classes of the pea aphid, namely, 18±4, 48±4, 114±4, and 138±4

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h; these ages correspond to the four nymphal instars of apterous viviparous pea aphids. In the first set of experiments, all four aphid instars were compared in pairs; in the second set of experiments, all four instars were available to parasites at the same time.

For all experiments, synchronous colonies of aphids were obtained by the transfer of adult aphids to bean plants for 8 h. Any offspring produced during this time were reared on bean plants at $20\pm1$ °C and 50-55% R.H. Individuals from each age class were marked by antennal amputation (Mackauer 1972) to distinguish the different age classes.

Female parasites intended for use in experiments were maintained at $20\pm1$ °C after emergence. For all experiments, I used "naive" females, i.e., females that had not been exposed to aphids prior to the experiment. This was done to avoid the influence of experience on choice of host. All tests were done in 15.5 x 4 cm plastic cages (Mackauer and Bisdee 1965a) that contained a bean shoot placed in tap water.

Experiment A was designed to compare all of the six possible pairwise combinations of instars I, II, III, and IV, each combination called a host group. Each group included 24 mated female parasites which were placed together with their paired hosts, one parasite per cage, for 6 h. Each cage contained 30 aphids of each of the two host instars in that group. The aphids were allowed to settle on the bean shoots for 24 h prior to the

introduction of the parasites. After 2-3 days, samples of 15 of each instar in each host group were dissected, and the number of parasite eggs found per aphid was recorded. For a given group, the numbers of eggs laid in each of the two instars within each cage were summed to obtain two grand totals. Similarly, grand totals of the numbers of hosts attacked in each of the two instars were obtained for every group. The results of all six groups were analysed as a single experiment with a completely balanced incomplete block design.

In Experiment B, fifteen individuals of each of the four instars were exposed together to a single parasite. In this case, a sample of ten aphids from each of the four instars was dissected, and the numbers of eggs found per aphid recorded. The experiment was replicated nine times with nine different parasites. The numbers of eggs laid in each aphid and the numbers of aphids attacked in each instar were summed over all replicates to obtain two grand totals for each of the four instars.

*Statistical Analysis*

The data were analysed in two parts. In the first part, parasite oviposition was considered to follow a Poisson distribution which was analysed by the fitting of a log-linear model with the aid of a statistical package for Generalized Linear Models, GLIM (Baker & Nelder 1978). The data were cross-classified in accordance with two factors, instar and host

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group. All parasites were considered to be identical in that their egg-laying habits presumably did not differ significantly from each other. Hierarchial log-linear models were fitted to the grand totals of the numbers of eggs laid/aphid from experiments A and B.

The second part of the analysis was technically the same as the first but, in this case, the totals of interest were not the numbers of eggs laid/aphid, but rather the numbers of hosts attacked. That is, the presence of one, two, or more eggs in an aphid meant that the aphid had been parasitized.

*The Log-linear Model*

Useful reviews on the construction and use of log-linear models include those of Fienberg (1970) and Heisey (1985). The analysis of multi-dimensional tables and categorical data were discussed in detail by Fienberg (1977), Haberman (1978), and McCullagh & Nelder (1983).

The models used in the analysis were constructed as follows: Given an *IxJ* table with instar and group as row and column variables, respectively,

Let $\Theta_{ij} = \ln(\lambda_{ij})$,

where $\lambda_{ij}$ is the expected number of "occurrences" in instar $i$ when placed in the comparative situation of column $j$. Occurrence

means, for the first part of the analysis, that one egg was deposited while, for the second part, it means that an aphid was parasitized.

The model has the form

$$\theta_{ij} = \mu + \alpha_i + \beta_j,$$

where $\Sigma_i \alpha_i = 0$ and $\Sigma_j \beta_j = 0$.

Analogous to the ANOVA model, $\alpha_i$ and $\beta_j$ represent instar and host group effects, respectively, and $\mu$ is the grand mean of ln(ocurrence probability). Thus, instar $i$ and host group $j$ are assumed to contribute a given amount ($\pm$) to ln(occurrence probability).

One writes $\theta_{ij}$ for the value estimated, or fitted, by the data analysis, and $\theta_{i+}$ for the arithmetic mean of $\theta_{i1}$, $\theta_{i2}$, $\theta_{i3}$, $\theta_{i4}$, $\theta_{i5}$, and $\theta_{i6}$ (note that three of these are "phantom" cells). Just as $\theta_{i+}$ is a composite estimate of ln(expected number of occurrences) for instar $i$ in general, so also $\lambda_{i+}$, defined as $\exp(\theta_{i+})$, is a composite estimate of the expected number of occurrences.

Extending this idea, $\theta_{2+} - \theta_{1+}$ is a general comparison of instars II and I on the natural logarithmic scale and is an estimate of the true $\theta_{2+} - \theta_{1+}$, while $\lambda_{2+} / \lambda_{1+}$ is an estimate of $\lambda_{2+} / \lambda_{1+}$ and is a general occurrence rate ratio as well as

an occurrence probability ratio of instars II and I. Note that $\lambda_{i+}$ and $\lambda_{i+}$ are actually estimated and true geometrical means across groups for instar $i$. By obtaining a composite estimate of the expected number of occurrences for instar $i$, one assumes that instar $i$ contributes a fixed amount to ln(expected number of occurrences), irrespective of the group. Such an assumption is necessary to determine the structural relationship between instar and group. The purpose of pooling the six host groups into one analysis is that, under the entirely reasonable model assumption, additional information (and hence shorter confidence intervals) can be brought to bear upon, say, $\lambda_{3+}/\lambda_{4+}$, from indirect evidence in groups 2 and 5 and elsewhere, to enhance and sharpen the direct evidence in group 6. The standard errors used to calculate confidence intervals for the probability ratios were adjusted to account for overdispersion.

The goodness of fit of the models to the data was measured by the deviance and the changes in this deviance for the larger model.

## Results

### Instar preferences of P. pequodorum

The experimental results suggest that *P. pequodorum* exhibits a distinct preference pattern. When the parasite was offered different host instars in pairwise combinations (Experiment A, Table 2.3), the pattern can be summarized as the order of

preference for the four instars, as follows:

$$I < (II = IV) < III$$

However, when all four instars were presented simultaneously (Experiment B, Table 2.4), this preference pattern changed to:

$$I < (II = III = IV)$$

Although both patterns differ slightly from each other, as should be expected, both indicate that instar I was least preferred by *P. pequodorum* while instars II and IV were either equally acceptable as, or were slightly less acceptable than, instar III.

The estimator of preference is the ratio of choice probabilities or Poisson counts, $P_{i/j}$, on the logarithmic scale (Tables 2.3-2.6). This estimator is a direct function of the probability that, given a choice between any two instars, the parasite will prefer one over the other. A $P_{i/j}$ estimate of 1.00 indicates no preference. A $P_{i/j}$ significantly greater than 1.00 indicates distinct preference for the instar shown in the numerator. The significance of any particular estimate of $P_{i/j}$ is determined by its 95% confidence interval. If the interval does not include a value of 1.00, one can conclude that, under these experimental conditions, the parasite exhibits a distinct preference. By contrast, a confidence interval that includes 1.00, suggests a lack of preference. Any values close to 1.00

should be interpreted with caution. For example, $P_{II/IV} = 1.027$ for Instar II/Instar IV (Table 2.3) indicates that *P. pequodorum* did not distinguish between Instars II and IV. Estimated $P_{I/j}$ values for Experiment B (Table 2.4) are apparently conservative, probably due to an insufficient number of replicates.

In the paired comparison experiments, the total number of eggs laid in the different series varied. The number of eggs laid increased as the parasites were presented with older aphids (Table 2.1). Egg totals thus increased from group 1 to group 5 but decreased sharply in group 6, in which the parasites were offered instars III and IV. In Experiment B (Table 2.2), the egg totals again increased with the instar, but were smaller than any of the totals in Experiment A. Superparasitism was observed in both experimental series. The percentage of superparasitism was generally less than 10% of the total number of eggs laid.

*Goodness of fit*

The difference between the observed value (full model) and the fitted value (current model) of the variable is the deviance. It is an indicator of how well the model fits the data. In the process of fitting hierarchial Log-Linear models to a given set of data by iterative methods such as those used in the analysis presented here, the deviance is calculated for each cycle. Iterations are terminated when a suitable significance level is achieved. The significance of the deviance is then tested using tabled $x^2$ values.

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In the first part of the analysis (Experiment A), which was based on the numbers of eggs/aphid, the drop in deviance of 41.6 with 3 degrees of freedom was obtained by fitting a model consisting of the grand mean $\mu$ and instar effect $\alpha_i$ as compared to a model consisting only of the grand mean $\mu$. By further adding the group effect, $\beta_j$, an additional drop in deviance of 125.8 with 5 degrees of freedom was obtained. Comparing these two statistics with their respective degrees of freedom with tabled $\chi^2$ values, one finds that both are highly significant (P < 0.0001). The same procedure was followed for the second part of the analysis of Experiment A, which was based on the numbers of hosts attacked. The drop in deviance from fitting $\alpha_i$ after $\mu$ was 31.4 with 3 degrees of freedom. A further drop in deviance of 97.3 with 5 degrees of freedom was obtained after adding the group effect $\beta_j$. Both these statistics were highly significant (P < 0.0001).

The final model consisting of the grand mean, instar effect and group effect had a deviance of 9.2 with 3 degrees of freedom (P = 0.03). The small P-value suggests a highly significant effect of instar and group in improving the fit of the model. By introducing additional terms representing complex interactions between $\alpha$ and $\beta$, it might have been possible to reduce the deviance, but this change would have made the model more complex, a fact that could have contributed to difficulties of interpretation.

Experiment B, in which all host instars were exposed together, required special consideration because there were no eggs laid by any of the parasites in aphids of instar I. In order to get around this problem, Jeffry's Prior Distribution was used, in which, an amount of 0.5 was added to the totals of each instar before the data were analysed as a one-way ANOVA design of a Log-linear model.

The models fitted to the results of Experiment B were tested for goodness-of-fit as above. A model consisting only of the grand mean, when fitted to the results based on the number of eggs/aphid, resulted in a deviance of 59 with 3 degrees of freedom ($P < 0.0001$). When instar effect $\alpha$ was added to the model, the deviance dropped to 0, with 0 degrees of freedom. Since this model could not be tested, the model with only the grand mean was considered for the computation of preference probability ratios. Results based on the number of hosts attacked were fitted similarly to a model consisting only of the grand mean. This resulted in a deviance of 57 with 3 degrees of freedom ($P < 0.0001$). The conclusion, again, is that the model provided a good fit to the data.

Table 2.1 Summary of host-instar preference by *Praon pequodorum* (n = 24 females) for each of four instars offered in pairs. Totals are based on 15/30 aphids dissected in each instar in each replicate.

Host group and instar totals

| Variable | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | I | III | I | IV | II | III | II | IV | III | IV |
| No. hosts dissected | 360 | 360 | 360 | 360 | 360 | 360 | 360 | 360 | 360 | 360 | 360 | 360 |
| No. eggs laid | 77 | 132 | 102 | 176 | 110 | 182 | 123 | 177 | 169 | 175 | 103 | 50 |
| No. hosts attacked | 76 | 125 | 99 | 157 | 109 | 162 | 121 | 162 | 156 | 160 | 103 | 50 |
| Eggs laid/aphid attacked: mean | 806 | 1.03 | 936 | 1.11 | 1.01 | 1.10 | 976 | 1.08 | 1.07 | 1.05 | 1.00 | 542 |
| S.D. | 428 | 263 | 293 | 177 | 034 | 135 | 216 | 127 | 101 | 264 | 0.00 | 509 |
| % eggs wasted | 1.30 | 5.30 | 2.94 | 10.8 | 0.91 | 11.0 | 1.63 | 8.47 | 7.69 | 8.57 | 0.00 | 0.00 |
| Variance/mean ratio | 813 | 712 | 778 | 740 | 715 | 716 | 693 | 672 | 686 | 680 | 716 | 864 |

Table 2.2 Summary of host-instar preference by *Praon pequodorum* (n = 10 females) for each of four instars provided simultaneously. Totals are based on 10/15 aphids dissected in each instar in each replicate.

| Variable | Instar Totals | | | |
|---|---|---|---|---|
| | I | II | III | IV |
| No. hosts dissected | 90 | 90 | 90 | 90 |
| No. eggs laid | 0 | 26 | 37 | 41 |
| No. hosts attacked | 0 | 24 | 35 | 39 |
| No. eggs laid/aphid attacked: mean | 0 | 1.06 | 1.04 | 1.03 |
| S.D. | | .167 | .111 | .083 |
| % eggs wasted | 0 | 3.85 | 2.70 | 2.44 |

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Table 2.3 Estimated probability ratios, $P_{i/j}$, of instar preference and their 95% confidence intervals for experiment A (number of eggs laid/aphid)

| HOST GROUP | INSTAR MAKEUP | ESTIMATED | | |
|---|---|---|---|---|
| | | LOW | $P_{i/j}$ | HIGH |
| 1 | II/I | 1.1175 | 1.5256 | 2.0828 |
| 2 | III/I | 1.5512 | 2.1325 | 2.9318 |
| 3 | IV/I | 1.0950 | 1.4851 | 2.0141 |
| 4 | III/II | 1.0311 | 1.3978 | 1.8950 |
| 5 | II/IV | 0.7698 | 1.0273 | 1.3709 |
| 6 | III/IV | 1.0332 | 1.4359 | 1.9955 |

Table 2.4  Estimated probability ratios, $P_{i/j}$, of instar preference and their 95% confidence intervals for experiment B (number of eggs laid/aphid)

| HOST GROUP | INSTAR MAKEUP | LOW | ESTIMATED $P_{i/j}$ | HIGH |
|---|---|---|---|---|
| 1 | II/I | 2.2227 | 52.9845 | 1263.04 |
| 2 | III/I | 3.1757 | 74.9634 | 1769.55 |
| 3 | IV/I | 3.5236 | 83.0132 | 1955.73 |
| 4 | III/II | 0.8003 | 1.4148 | 2.5012 |
| 5 | IV/II | 0.8965 | 1.5667 | 2.7379 |
| 6 | IV/III | 0.6677 | 1.1074 | 1.8365 |

Table 2.5  Estimated probability ratios, $P_{i/j}$, of instar preference and their 95% confidence intervals for experiment A (number of hosts attacked)

| HOST GROUP | INSTAR MAKEUP | LOW | ESTIMATED $P_{i/j}$ | HIGH |
|---|---|---|---|---|
| 1 | II/I | 1.0168 | 1.3736 | 1.8555 |
| 2 | III/I | 1.3968 | 1.9031 | 2.5930 |
| 3 | IV/I | 0.9786 | 1.3178 | 1.7746 |
| 4 | III/II | 1.0302 | 1.3856 | 1.8634 |
| 5 | II/IV | 0.7844 | 1.0423 | 1.3849 |
| 6 | III/IV | 1.0477 | 1.4441 | 1.9906 |

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Table 2.6 Estimated probability ratios, $P_{i/j}$, of instar preference and their 95% confidence intervals for experiment B (number of hosts attacked)

| HOST GROUP | INSTAR MAKEUP | LOW | ESTIMATED $P_{i/j}$ | HIGH |
|:---:|:---:|:---:|:---:|:---:|
| 1 | II/I | 2.1356 | 51.0089 | 1218.33 |
| 2 | III/I | 3.0850 | 72.9665 | 1725.79 |
| 3 | IV/I | 3.4299 | 80.9635 | 1911.19 |
| 4 | III/II | 0.8013 | 1.4305 | 2.5538 |
| 5 | IV/II | 0.8998 | 1.5872 | 2.8000 |
| 6 | IV/III | 0.6646 | 1.1096 | 1.8525 |

## Discussion

In the experiments described in the previous section, preference was measured based on the numbers of each instar parasitized among those available during a fixed period. Given that all instars were equally available and had an equal probability of being parasitized, the numbers of each instar parasitized then represent the numbers of encounters between the parasite and individuals of that instar that led to successful oviposition. Expressed as a probability, this number reflects the preference of the parasite for that instar. Each host instar represents a particular combination of related factors or attributes. Thus, the probability of an instar being parasitized can be interpreted as the degree of attractiveness of that particular combination. On a relative scale, the ratio of any two probabilities enables different instars to be ranked in their order of attractiveness for oviposition. This ranking order is a measure of how the parasite apportions its eggs among different host instars.

Obviously, a study of instar preference would entail answering the question: "What is the effect of different host instars on parasite choice?" Until fairly recently, hypotheses based on such a question were analytically intractable, due to the fact that the data resulting from preference studies were in the form of counts (i.e. discrete) and, strictly speaking, could not be analysed by methods intended for continuous data. The

results of most preference studies to date have been presented in one of two ways; either as the percentage of each instar attacked or as an index of preference (Mackauer 1983). Some of the indexes of preference were reviewed by Cock (1978); however, except for Manly's (1972) index $\beta$, which was used by Hopper & King (1984), they have not been used in analysing instar preference.

One of the methods proposed for the analysis of categorical data is by log-Linear Models (Fienberg 1970). This approach has been shown widely applicable to and appropriate for many different situations (Haberman 1978; McCullagh & Nelder 1983). Heisey (1985) demonstrated the appropriateness of this method for the testing of resource selectivity hypotheses based on Manly's selectivity measure.

In studying preferences under laboratory conditions, the experimental design or method of evaluation becomes important. A design that exposes a parasite to all host instars at the same time more closely approximates a field situation than, for example, paired comparisons; in this regard, the latter can be considered a standard. It has been used in some of the recent studies on instar preferences (Liu *et al.* 1984; Hopper & King 1984). A single method of evaluation, however, may not provide all the information. Some alternative methods, such as the method of paired comparisons, are intuitively appealing. Paired comparisons allow for the decomposition of a complex experimental environment into smaller, less complex, study

units. They are useful also in the identification of subtle behavioural components that would otherwise go unnoticed. However, paired comparisons do not allow one to extrapolate the results to a universe containing other than the different choice categories within any one comparison. When applied to instar preference, this restriction would be unrealistic because, under field conditions, an aphid parasite will normally be exposed to all the instars of its host. A more realistic approach, and one that will yield more meaningful information, would be to 'pool' the various groups into a single analysis. The extent to which the results from both experimental designs agree would indicate not only the sensitivity of the alternative design (i.e., paired comparisons) but also the consistency of the attribute(s) being measured. The latter is of particular importance because it shows that, given a particular set of experimental conditions, the parasite will exhibit the same pattern of preference every time, within the bounds of experimental variation and intrinsic differences between parasite females.

In the paired comparisons of Experiment A, the addition of the group effect to the model with the grand mean and instar effect resulted in a highly significant drop in deviance, indicating that the addition of the group effect improved the fit of the model. This means that the probability of a paired instar being parasitized was strongly influenced by the other member of the pair. This can also be seen in the differences among groups in the total number of eggs (see Table 2.1). Thus,

the factors that influence every act of oviposition in, say, group 1 (Instar I *vs* II), may be subtly different from those influencing oviposition in, say, group 2 (Instar I *vs* III).

In moving from Experiment A to Experiment B, the environment in which the parasite makes a choice or exercises a preference has become more complex, with a wider variety of choices, each exerting it's own effect on the parasite's behaviour. The net effect is a change in oviposition behaviour that is reflected not so much in the preference pattern of Experiment A but in the discrepancy between corresponding $P_{i/j}$ estimates of the two experiments.

In comparing the results of Experiments A and B, the discrepancy in the magnitudes of the ratios of instars II, III and IV compared to I becomes conspicuous. For example, in Table 2.3, the $P_{i/j}$ estimate in row 1 (instar II/instar I) is 1.5256; the corresponding estimate in Table 2.4 is 52.9845. One might be tempted to speculate on the difference between these two estimates, although it is not possible to assign, definitively, any biological significance to it. In Experiment A, the presence of individuals of other instars in the same environment at the same time seems to affect the parasite's preference for a particular instar. In Experiment B, it appears that encountering hosts of different sizes serves to stimulate the parasite to search for preferred instars. It is probably this effect that manifests itself as the discrepancy between corresponding ratios.

It should also be noted that there is probably a confounding factor inherent in the design of Experiment B. If parasite choice is strongly biased in favour of older instars *viz*, II, III and IV, then the parasite had three times as many of the preferred instars to choose from. This condition would make it difficult to assess the relative preference rating of first instar individuals.

The results presented in this paper, suggest that *P. pequodorum* generally distributes most of its eggs among the higher instars of *Acyrthosiphon pisum*, but, under certain conditions, may exhibit a partial preference for any one of the higher instars. Reports on instar preferences among aphid parasites were reviewed by Stary (1970, p. 168). A critical examination of some of the reports on, for example, *Praon exsoletum* Nees (Schlinger & Hall 1960), *Trioxys complanatus* Quilis (Schlinger & Hall 1961), and *Praon volucre* Haliday (Beirne 1945) reveals that the results presented are unsuitable for drawing inferences about the instar preferences of these parasites. At best, the data allow one to conclude that most stages of the host(s) were suitable for parasite oviposition and larval development.

Wiackowski (1962) was the first to investigate the instar preference of *Aphidius smithi* Sharma & Subba Rao, a parasite of the pea aphid. He found that second and third nymphal instars were prefered for oviposition over first and fourth instars. Fox *et al.* (1967) noted a strong preference by *A. smithi* for the

early first instar when instars were offered separately, one at a time. However, Mackauer (1973, 1983) was unable to confirm these results. He first used the method of paired comparisons in studying the host selection and host suitability of *A. smithi*. The parasite was offered different pairwise combinations of host age groups. Each combination had a test age group consisting of aphids belonging to one of seven age groups (0-4 h old to 192 h old) and a standard age group consisting of 48h-old aphids. He found that the parasite did not distinguish between instars II, III and IV, but attacked instar I much less frequently than the others.

Recently, Liu *et al*. (1984) described the instar preference of *Aphidius sonchi* Marshall, a parasite of the sowthistle aphid, *Hyperomyzus lactucae* (L). They found that when all the instars were exposed simultaneously to the parasite, more eggs were laid in instars II and III than in IV or I. One of the hypotheses being tested was that egg laying within any instar followed a random distribution, which further implied that parasites searched for hosts at random. The authors found that, when each instar was offered separately to the parasite, the resulting egg distributions were, in most cases, indistinguishable from random. Random search by *A. sonchi* was thus shown to be a valid assumption. They used a weighted analysis of variance technique to analyse preference among the different instars. Their analyses, as the one presented in this paper, do not, however, provide any information on the overall egg distribution between

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the different instars. Such a composite egg distribution may be expected, in most cases, to differ from Poisson (van Alphen 1980). This is to be expected when a parasite prefers some host instars over others.

Liu *et al*. (1984) suggested that one component of the effect of host size may be reflected in random search by the parasite. The larger the host, the more likely it is to be encountered and parasitized. Countering this is the fact that larger hosts may be more difficult to parasitize due to the increased defensive response of the host, like struggling or a greater proclivity to fall off the host plant. This behaviour would result in more eggs being laid in possibly less preferred instars which represent a balanced blend of all the factors involved in the interaction.

Parasite experience is another factor that can influence preference. Samson-Boshuizen *et al*. (1974) showed that the degree of success of parasitization depends on prior exposure to the host. They exposed females of *Pseudeucoila bochei* Weld (Hymenoptera: Cynipidae), a parasite of *Drosophila melanogaster* Meig., to larvae of the host for varying periods of time. The parasite females were 'naive', that is, they had not been exposed to hosts prior to the experiment. The number of eggs laid, as determined by dissection, did not match the number of apparent ovipositions, as determined by observing the parasites. When the experiment was repeated with the same females following varying periods of isolation from hosts, they observed marked

improvement in the rates of success. Handling time also decreased with experience. Apparently, some parasite females required several trials, followed by a period of respite to be able to co-ordinate properly the various parts of the oviposition act, such as the flexing and positioning of the ovipositor, mobilization of male sperm, and the movement of the egg through the ovipositor.

Host instar preference has also been reported for other insect host-insect parasite systems. Hopper & King (1984) found that *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae) showed strong preference patterns for some instars of *Heliothis* spp. (Lepidoptera: Noctuidae). The wasps preferred third-instar larvae most, fourth- and second-instar larvae next, followed by first- and fifth-instar larvae. van Alphen (1980) showed that *Tetrastichus* spp. (Hymenoptera: Eulophidae), which are gregarious egg parasites of asparagus beetles, *Crioceris asparagi* (L), distinguished between young and old beetle eggs. The parasites preferred older eggs (>3days old) for oviposition, but fed generally on younger eggs (0-2 days old). Instar preference has also been shown in *Asobara tabida* Nees (Braconidae: Alysiinae) (van Alphen & Drijver 1982), *Anagyrus indicus* Shafee *et al.* (Hymenoptera: Encrytidae) (Nechols & Kikuchi 1985), *Campoletis sonorensis* (Cameron) (Hymenoptera: Ichneumonidae) (Isenhour 1985).

For all the parasites discussed so far, the fact that preference is not rigid and that eggs are laid in most of the

instars available for parasitization indicates that an assessment of the different types of hosts available may be one of the criteria that determine the eventual apportioning of eggs. The degree to which parasites control their host populations will depend , in large part, on the availability of host stages that are suitable for parasite larval development. Because not all stages of the host are equally suitable and because the relative abundance of the most suitable stages varies both spatially and temporally, parasitic insects have developed an array of behavioural, ecological, and physiological adaptations to utilize their hosts (Doutt 1964). Instar preference can be viewed as an adaptation to host utilization. A knowledge of host selection contributes to an understanding of the population dynamics of the host and parasite species, and is vitally important for the development and implementation of biological control and IPM programs, as well as in assessing ongoing programs (Nechols & Kikuchi 1985).

From the foregoing, I conclude that *P. pequodorum* is least likely to oviposit in first instars of the pea aphid when other instars are available. As a result the effects of parasitism on host population growth are expected to be much less than what they might be. This aspect will be examined in the next section of the thesis.

## CHAPTER III

## THE EFFECT OF PARASITISM BY *PRAON PEQUODORUM* VIERECK ON

## REPRODUCTION AND POPULATION GROWTH OF THE PEA APHID

### Introduction

Among the chief characteristics of a successful biological control agent is the ability to effect a signifcant depression in the host population (Beddington *et al.* 1978; Huffaker & Kennett 1969; van Lenteren 1980). In the interactions between insect hosts and their parasites (parasitoids), various factors are thought to be important in determining the effectiveness of parasites to control the host population. These include biological attributes of the parasite such as its reproductive potential, searching ability, host selection behavior, sex ratio, fecundity, time-to-adult and host specificity, as well as other factors such as effect of parasitism on host reproduction, synchronization, and adaptedness to the environment (Hassell & Waage 1984; Huffaker *et al.* 1971; Mackauer & van den Bosch 1973; van Lenteren 1980; Waage & Hassell 1982).

Different factors may vary in their relative importance, depending on the situation, and, in general, it is difficult to rate factors on their importance in determining the effectiveness of parasites (Mackauer & van den Bosch 1973). One such factor is the effect of parasitism on the host's reproductive potential.

In the study of aphids and their parasites, several authors have observed that if aphids are parasitized in the early instars, they become mummified before reproducing, but when parasitized in the later instars or in the adult stage, they may produce some offspring before becoming mummified (see Stary 1970, p. 187). In such systems, the stage of the host at which it is parasitized, or in other words, the pattern of host-instar preference exhibited by the parasite, becomes important in assessing the effectiveness of the parasite in controlling the host population (Liu & Hughes 1984; Campbell & Mackauer 1975).

Although 'instar' preference in aphid parasites is quite well documented (see previous chapter), few attempts have been made to assess the importance of such a behavioral adaptation with regard to the parasite's control potential. The attempts to date include studies by Campbell & Mackauer (1975), Liu & Hughes (1984), Mackauer & Kambhampati (1984), and Rabasse & Shalaby (1979).

In this chapter, I describe experiments conducted to examine the effect of parasitism by *Praon pequodorum* Viereck on the reproductive potential of its host, the pea aphid. I shall compare the intrinsic rate of natural increase, $r_m$, calculated from life tables of pea aphids parasitized at different developmental stages (instars), and show that host-instar preference is an important factor in determining the effectiveness of parasites.

## Materials and Methods

Cultures of *A. pisum* and *P. pequodorum* were maintained as described in the previous chapter. A synchronous colony of nymphs of the pea aphid was produced by allowing adult aphids to reproduce on potted broad-bean plants for 4 h. Any nymphs produced during that period were transferred to uninfested bean plants and maintained at 20 ± 1 °C at 50-55% RH with a photoperiod of 24 h. Aphids of known age were produced by allowing the nymphs to develop until they reached the desired age. Mated, 2- to 3-day-old female parasites from the stock colony were used in all the experiments. The females had been allowed to parasitize aphids prior to the experiments.

*Design of Experiments*

Experiments were designed to compare the effects of parasitism on different developmental stages of *A. pisum*. Twenty pea aphid nymphs were placed in gelatin capsules (size 00), one aphid per capsule. One female wasp was introduced into each capsule and allowed to parasitize the aphid. To prevent superparasitism, the wasps were allowed to attack each aphid once only. Aphids that had been attacked were transferred to small, numbered clip cages, each capable of holding a single adult aphid. The cages were fastened to the ventral surface of bean leaves, one cage per leaf, where the aphid was reared until mummification. The cages were maintained in a controlled environment chamber at 20 ± 1°C and 50-55% RH, under continuous

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light.

The cages were examined daily when dead aphids and any
progeny produced were counted and removed. The instar, the
number of progeny produced, and the age at which death and
mummification occurred were recorded for each aphid. This
procedure was repeated for different age groups of the pea aphid
(Table 3.1). Unparasitized aphids maintained under identical
conditions served as controls.

The effect of parasitism by *P. pequodorum* on the
reproductive potential of the pea aphid was assessed by
constructing life tables for ten groups of aphids differing in
age at the time of parasitism between 0.5 and 9.0 days. Average
daily fecundities (age-specific fecundity), $m_x$, and survival
rate, $l_x$, were calculated. From these data, the intrinsic rate
of increase, $r_m$, for each group, was calculated from the
Lotka-Euler equation (Andrewartha & Birch 1954)

$$\Sigma \exp\{-r_m x\}\, l_x\, m_x = 1$$

by iterative substitution of the value of $r_m$. Other biological
statistics such as doubling time (DT), generation time (T),
gross reproductive rate (GRR), and net reproductive rate (Ro)
were also calculated. The data were jackknifed according to the
procedure described by Sokal & Rohlf (1981, p. 795) in order to
estimate 95% confidence limits around the estimated values of
$r_m$.

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The relationship between the host age at parasitization, (AHP), and the length of reproductive period after parasitization, (LRP), was investigated by fitting a linear regression equation to the data. Non-linear regression models were fitted to the mean total fecundity (MTF), $r_m$, and DT with the aid of a statistical package (Bio-Medical Data Processing, BMDP).

## Results

*Host development*

Parasitized pea aphids lived, on an average, 7.58 ±0.4 days (mean ± SD) from the time of parasitization to death. A complete external cocoon was observed under the empty aphid skin generally 24 h following the death of the parasitized aphid. Aphids that were parasitized as early first instars, and late first and second instars, mummified in the third and fourth instar, respectively. Those that were parasitized as third and fourth instars mummified as adults. The oviposition success of the female wasps, expressed as the proportion of parasitized aphids that mummified among those in each group, was only 25% in groups 1 and 2, but was generally above 50% in groups 3-10 (Table 3.1). From my observations, it appeared that the wasps had considerable difficulty in parasitizing first-instar nymphs successfully. This was confirmed later when only 5/20 aphids parasitized in each of groups 1 and 2 mummified. However, the

wasps apparently had no difficulty parasitizing older aphids. The number of mummies in groups 3-10 ranged from 10/20 in group 10 to 20/20 in groups 5 and 6.

*Host reproduction*

Aphids parasitized at age ≥4 days produced some offspring before mummification (Table 3.1). In each group, reproduction began six days after birth. The $m_x$ values for groups 6-10 (host age 5-9 days at parasitization) were similar to the corresponding control values, but declined abruptly as the aphid neared death. $m_x$ values for group 5 (host age 4 days) were considerably lower than those of the corresponding control group (Figure 3.1). The length of the reproductive period (LRP) was correlated with the age of the aphid at parasitization (AHP). For $3.5 \leq AHP \leq 9.5$ days, LRP could be predicted from a linear regression equation of the form

$$Y = 1.21943X - 2.72295$$

where $Y$ is the predicted length of the reproductive period of a host parasitized at age X (regression $r^2 = 0.983$, $F = 226.91$, $P < 0.0001$). For aphids that reproduced after parasitization, as age at parasitization increased, the mean total fecundity (MTF) increased as an exponential function of age. MTF for aphids of age $4.0 \leq X < +\infty$ could be predicted from the equation

$$Y = 107.9018/[1 + \exp(3.9717 - 0.5627X)]$$

where $Y$ is the predicted MTF for host age X (regression MS = 6254.44, $F = 148.88$, $P < 0.005$).

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The maximum attainable rate of growth of a population of stable age-distribution is defined as the intrinsic rate of natural increase, $r_m$ (Andrewartha & Birch 1954). Under the experimental conditions of this study, only aphids parasitized at age 4 days or older contributed to population growth. For groups 5 and 6 (host ages 4 and 5 days, respectively), $r_m$ increased exponentially and approached asymptotically a value of 0.5. Values for the different groups are shown in Table 3.2. A curvilinear regression equation was fitted to $r_m$ values for host age $4.0 \leq X < +\infty$ (Figure 3.3). The equation is of the form

$$\hat{Y} = 1/[0.2857/(X - 3.8386) + 2.0079]$$

where $\hat{Y}$ is the predicted $r_m$ for the pea aphid parasitized at age X (regression MS = 0.03938, F = 135.32, P < 0.005).

Doubling time, DT, is the time in days that is required by a population to double in numbers; it is inversely proportional to $r_m$ (Andrewartha & Birch 1954). This relationship is seen in the non-linear regression equation fitted to the observed values of DT for host age $4.0 \leq X < +\infty$ (Figure 3.3). The equation is of the form

$$\hat{Y} = [0.2053/(X - 3.8298)] + 1.3938$$

where $\hat{Y}$ is the predicted value of the doubling time for host age at parasitization X (regression MS = 1.12 , F = 487, P < 0.005).

Table 3.1 Development and reproduction of 10 groups of 20 pea aphids parasitized at different ages by *Praon pequodorum* Viereck.

| Group no | Host age in days and instar at parasitization | No. mummified/20 | Instar at mummification | Parasitization to death in days, PPD $\bar{x}$ (SE) | Mean reproductive period in days, LRP $\bar{x}$ (SE) | Mean total fecundity, MTF $\bar{x}$ (SE) |
|---|---|---|---|---|---|---|
| 1 | 0.5 | 5 | III | 8.00 (0) | | |
| 2 | 1.0 (I) | 5 | IV | 7.40 (0.24) | | |
| 3 | 2.0 (II) | 13 | IV | 7.15 (0.10) | | |
| 4 | 3.0 (III) | 16 | A | 7.19 (0.10) | | |
| 5 | 4.0 (IV) | 20 | A | 7.25 (0.10) | 1.70 (0.15) | 7.5 (0.72) |
| 6 | 5.0 (A) | 20 | A | 7.15 (0.08) | 3.65 (0.13) | 27.7 (0.57) |
| 7 | 6.0 (A) | 15 | A | 7.67 (0.13) | 4.87 (0.13) | 40.9 (1.10) |
| 8 | 7.0 (A) | 16 | A | 7.94 (0.06) | 6.00 (0.13) | 57.0 (1.40) |
| 9 | 8.0 (A) | 12 | A | 8.00 (0) | 7.00 (0) | 70.5 (0.73) |
| 10 | 9.0 (A) | 10 | A | 8.10 (0.10) | 8.00 (0) | 73.5 (1.50) |
| control | | | | | 14.35 (0.50) | 109.8 (1.50) |

Table 3.2 Demographic characteristics of the pea aphid parasitized at different ages by *Praon pequodorum* Viereck.

| Age at parasit- ization in days AHP | Gross reprod- uctive rate GRR | Net reprod- uctive rate Ro | Generation time T | Doubling time in days DT | Finite rate of natural increase R | Intrinsic rate of natural increase $r_m$ (SE) |
|---|---|---|---|---|---|---|
| 4.0 | 7.5 | 7.5 | 7.51 | 2.60 | 1.307 | .265 (0.013) |
| 5.0 | 27.7 | 27.7 | 7.70 | 1.60 | 1.542 | .434 (0.004) |
| 6.0 | 40.9 | 40.9 | 7.90 | 1.48 | 1.598 | .467 (0.004) |
| 7.0 | 57.0 | 57.0 | 8.18 | 1.40 | 1.639 | .496 (0.004) |
| 8.0 | 70.5 | 70.5 | 8.50 | 1.41 | 1.650 | .499 (0.003) |
| 9.0 | 73.5 | 73.5 | 9.10 | 1.48 | 1.600 | .468 (0.005) |
| control | 109.8 | 109.8 | 9.57 | 1.43 | 1.630 | .485 (0.003) |

Figure 3.1 Age-specific survival $l_x$ (solid lines) and daily fecundity rates $m_x$ (broken lines) of pea aphids parasitized at different ages. Arrows indicate age of host at parasitization, AHP. A) AHP=4; B) AHP=5; C) AHP=6; D) AHP=7; E) AHP=8; F) AHP=9; G) unparasitized (control).

$l_x$      $m_x$

MEAN AGE IN DAYS

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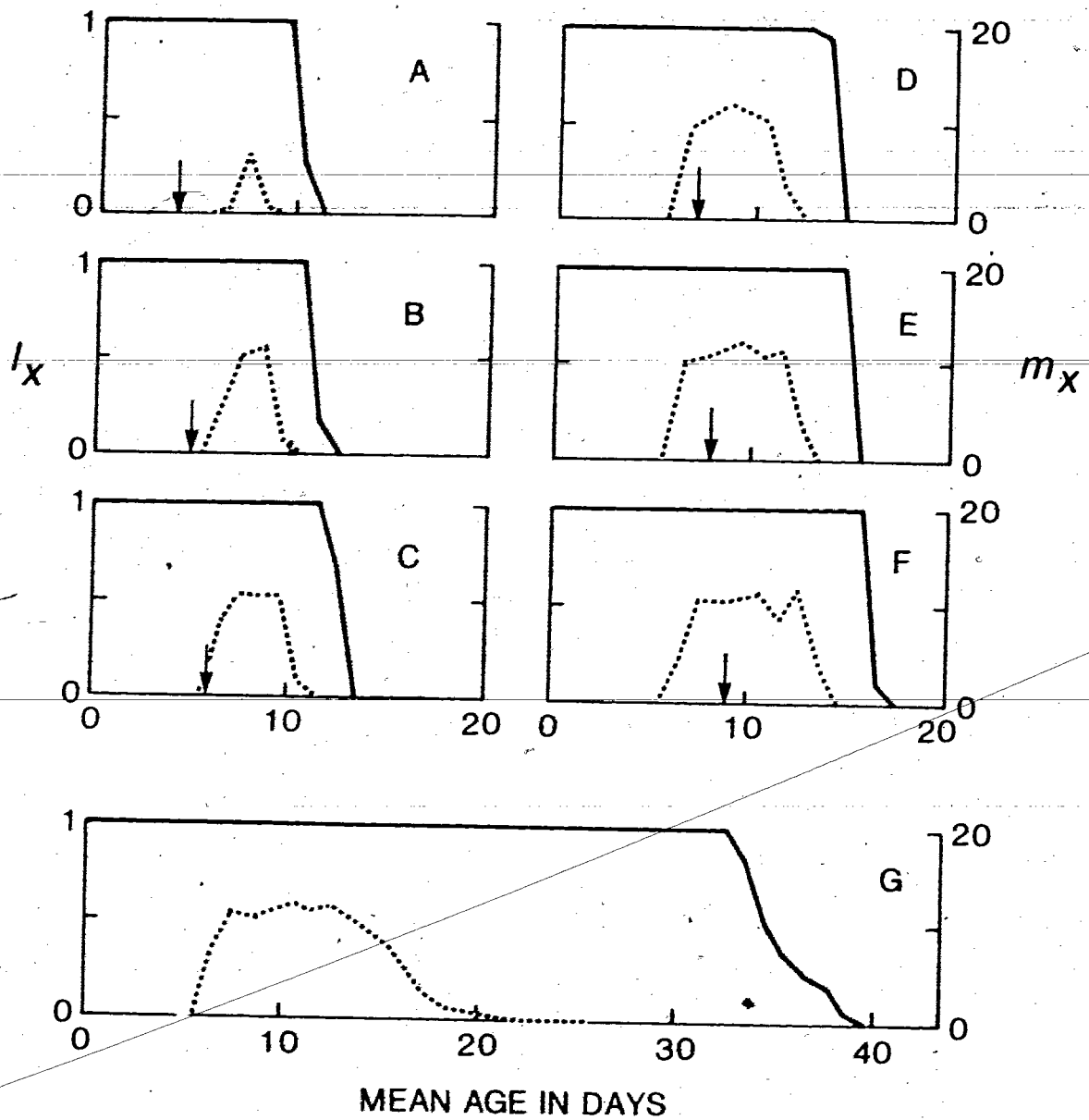
Figure 3.2 The relationship between the mean total fecundity, MTF, and the mean age at parasitization. Fecundity of unparasitized (•) aphids was plotted against the mean age at which reproduction ceased.

MEAN AGE AT PARASITIZATION (days)

MEAN TOTAL FECUNDITY
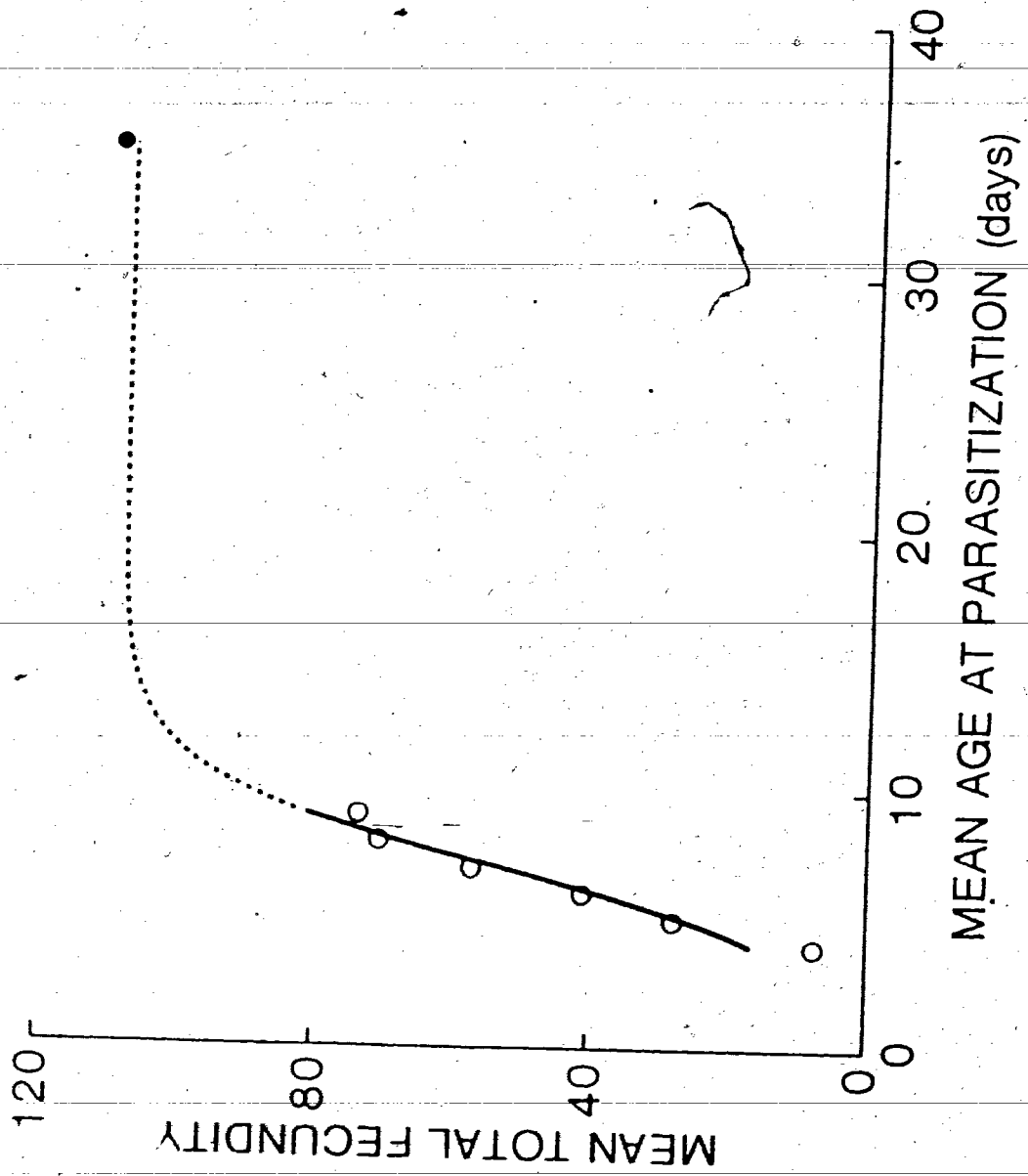
Figure 3.3 The relationship between the intrinsic rate of natural increase, $r_m$ (o), doubling time, DT (◻), and the mean age at parasitization. Values for the unparasitized control group (● and ◼) were plotted against the mean age at which reproduction ceased.

DT

2.8

2.4

2.0

1.6

1.2

$r_m$

DT

40

30

20

10

MEAN AGE AT PARASITIZATION (days)

0.50

0.45

0.40

0.35

0.30

0.25

$r_m$

## Discussion

The intrinsic rate of natural increase, $r_m$, is useful as a means for describing the growth potential of a population under a specified set of conditions (Southwood 1966). Changes in $r_m$ can reflect changes either in environmental conditions or in host physiology, e.g., under the influence of parasitism, or both. Thus, $r_m$ can be used as a b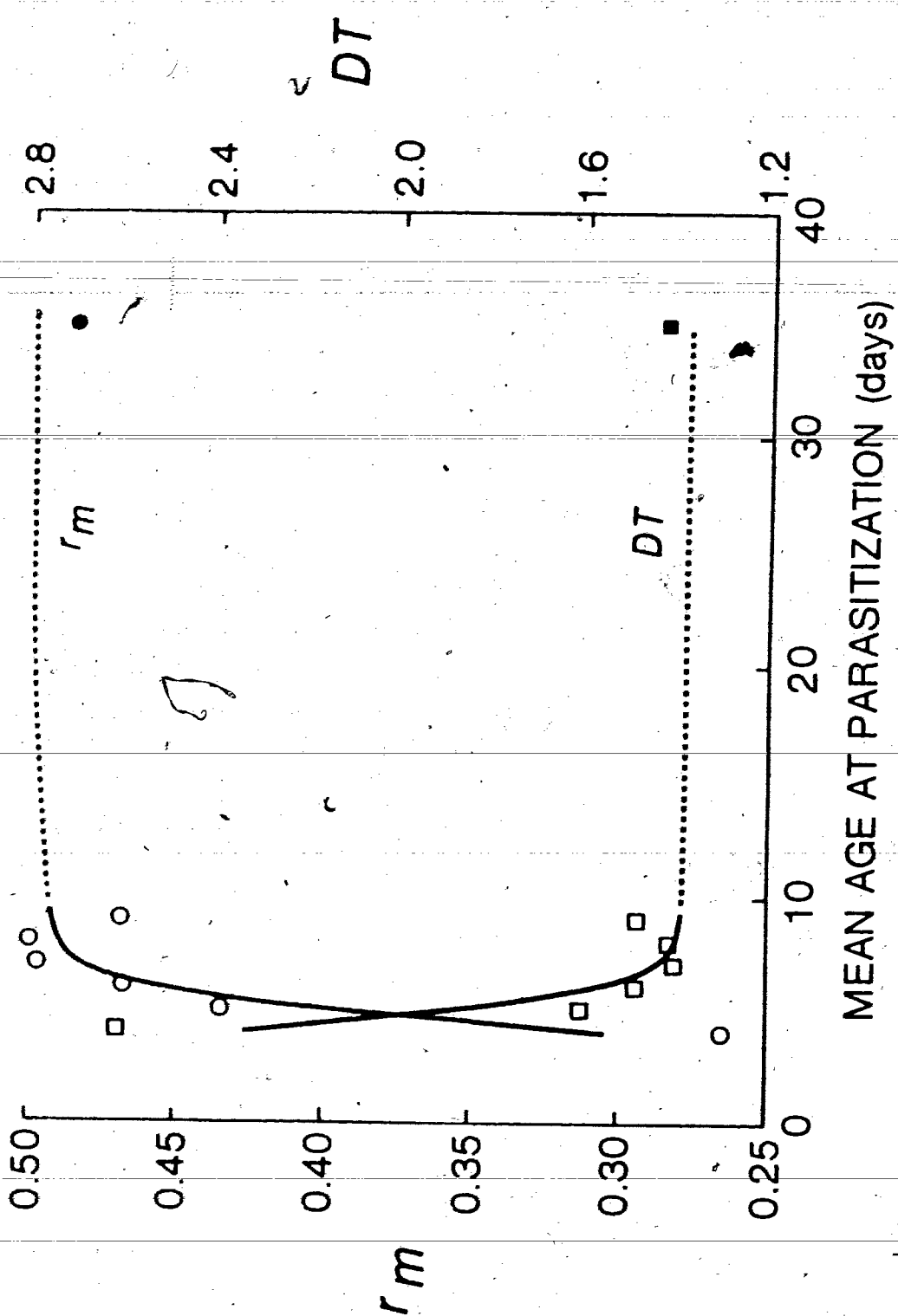io-climatic index in assessing the pest potentialities of insects (Messenger 1964). Based on these considerations, one could evaluate the pest potentiality of the pea aphid under a given set of conditions, such as parasitism by *P. pequodorum*.

Values of $r_m$ in Table 3.2 indicate that parasitism by *P. pequodorum* of host-instars I, II and III contributes significantly to aphid population reductions, as these instars do not normally survive to the adult stage, and hence do not reproduce. Aphids parasitized in the fourth instar have an $r_m$ value approximately one-half that of unparasitized aphids. This implies that parasitism of a large proportion of fourth-instar nymphs from the population would also be of significance in checking aphid population growth. Therefore, assuming that the parasite has a choice between all the host instars simultaneously, the influence of parasitism on host reproduction will depend, among other things, on the parasite's preference for particular instars.

Other studies have shown that, for Aphidiidae, the severity of parasitogenic effects on the host depends mainly on the age of the host at parasitization. *Aphidius sonchi* Marshall is a primary parasite of the sowthistle aphid *Hyperomyzus lactucae* (L). The host-parasite interactions between *A. sonchi* and *H. lactucae* have been studied in considerable detail (Liu & Hughes 1984; Liu *et al*. 1984; Liu 1985). The parasite lays most of its eggs in the second, third, and fourth instars of the host. Aphids that are late second instars (60-64h) or older at the time of parasitization are able to reproduce. The fecundity of parasitized aphids increased with host age at parasitization.

Mackauer (1973) examined, among other things, the preferences of *A. smithi* for different instars of the pea aphid. He found that second, third, and fourth instars were more frequently attacked than first instars. Campbell & Mackauer (1975) compared this ovipositional behaviour with the $r_m$ of parasitized instars. Apterous as well as alate pea aphid nymphs that were four days or older at the time of parasitization were able to contribute significantly to population growth.

Other aphid parasites that have been studied with regard to their impact on the host's $r_m$ include *Diaeretiella rapae* (M'Intosh), a parasite of the cabbage aphid, *Brevicoryne brassicae* (L) (Mackauer & Kambhampati 1984), and *Aphidius matricariae* Hal., a parasite of the green peach aphid, *Myzus persicae* Sulz. (Rabasse & Shalaby 1979).

In the previous chapter, I have described host-instar preference in *P. pequodorum*. I have shown that the parasite distributes most of its eggs among instars II, III, and IV. This behaviour, in itself, would appear to indicate that *P. pequodorum* is potentially useful for biological control. Information on the field biology and ecology of *P. pequodorum* is limited, but the reports to date, notably those of Mackauer & Bisdee (1965b), A. Campbell (1974), and Mackauer & Kambhampati (1986), indicate that the parasite is not very effective in the field.

A. Campbell (1974) conducted a detailed investigation into the seasonal dynamics of the pea aphid and its associated parasites in the southern interior of British Columbia. He found that the pea aphid is attacked by several parasites including *Aphidius smithi* Sharma & Subba Rao, *A. ervi* Haliday, *A. pisivorus* Smith (=*pulcher* Baker), and *Praon pequodorum* Viereck. Under controlled laboratory conditions, a comparison of some of the attributes of these parasites showed that *P. pequodorum* had a lower fecundity and a longer time-to-adult than the other three parasites (Table 3.3). *A. smithi*, *A. ervi*, and *A. pisivorus* were observed to have a time-to-adult, on the physiological time scale, of 178.6, 196.8, and 187.9 day-degrees (°C), respectively, whereas *P. pequodorum* required 199.6 day-degrees. Consequently, the *Aphidius* spp. were more abundant than *P. pequodorum* in early spring, when aphids were scarce. In addition, *A. smithi* prefered host instars II, III, and IV for

oviposition, thereby indicating a possible overlap of instar preferences with *P. pequodorum* in the field. Information on host-instar preferences of *A. ervi* and *A. pisivorus* is lacking.

The results of a long-term survey of the relative abundance of primary parasites of the pea aphid in British Columbia, during the period 1971 to 1984, were reported by Mackauer & Kambhampati (1986). Samples of mummified pea aphids were collected in 1971, 1972, 1983, and 1984 from two climatic zones, in the interior and coastal regions. In the interior regions the percent representation of *P. pequodorum* was 6.56, 10.23, 1.43, and 2.38 for the four years, respectively. In the coastal regions, *P. pequodorum* represented 9.54, 13.76, 0, and 0% of the samples of mummified pea aphids collected during the four years.

The observed differences among the various members of the parasite community and possible competition for preferred host instars may explain the apparent ineffectiveness of *P. pequodorum*. However, in multi-parasitized aphids, larvae of *P. pequodorum* usually survive when competing with larvae of *A. smithi* and possibly other *Aphidius* species (Chow & Mackauer 1984). This intrinsic superiority may explain why *P. pequodorum* has, so far, managed to retain a (relatively minor) position in the parasite community.

From the foregoing, the potential for effective control that *P. pequodorum* could have achieved from its preference patterns for host instars appears to be offset by its other

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Table 3.3 Some biological characteristics of four primary parasites of the pea aphid determined under controlled laboratory conditions. (Data on fecundity and adult longevity are from Dr. M. Mackauer, quoted in A. Campbell 1974; times-to-adult were taken from Table VII in A. Campbell 1974).

| Species | Fecundity[1] | Adult[2] longevity (days) | Time-to-adult (days) |
|---|---|---|---|
| *A. smithi* | 774 ±86.1a | 7.3 ±0.73a | 13.05 |
| *A. ervi* | 567 ±56.0b | 7.7 ±0.69a | 14.39 |
| *A. pisivorus* | 316 ±38.4c | 6.4 ±0.39a | 13.57 |
| *P. pequodorum* | 199 ±18.5d | 6.9 ±0.65a | 15.30 |

[1] Mean ±SE; means followed by the same letter are not significantly different ($p = 0.05$), while means followed by different letters differ significantly ($p = 0.05$) (t-test). Parasites were reared at $20.5 \pm 0.5\ ^{\circ}C$, 55% RH, and a photoperiod of 16L/8D h.
[2] Mean ± SE (accuracy of measurement: range ± 0.12 days). reared at $19.7 \pm 1\ ^{\circ}C$, 55% RH.

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characteristics such as low fecundity and prolonged time-to-adult. However, this does not in any way imply that *P. pequodorum* could not be managed so as to enhance pea aphid control.

In general, the effect of parasitism is the outcome of complex interactions between the parasite larva and the host (Vinson & Iwantsch 1980; Mackauer 1986). If a parasitized host is able to achieve reproductive age before the parasite larva developing within it reaches the stage of destructive feeding (Stary 1970), it may reproduce briefly before death. The impact of parasitism on the $r_m$ of the host is dependant on host age at the begining of parasitism. The growth and development of the parasite larva, in turn, will be affected by factors such as temperature (Liu *et al*. 1984) and host quality (Mackauer 1986). Preference patterns exhibited by aphid parasites for host instars are important in studying host-parasite interactions. Together with other biological characteristics of the parasite, host-instar preference should be useful in assessing or predicting parasite performance.

# CHAPTER IV

## GENERAL DISCUSSION AND CONCLUSIONS

Host-instar preference by a parasite results in individuals of particular instars being parasitized in greater numbers than expected from their frequency in the environment. Expressed as a probability pattern, the egg distribution of *P. pequodorum* suggests that pea-aphid instars II, III, and IV are more likely to be parasitized than instar I.

Reproduction and development of an organism in nature is affected by environmental as well as by intrinsic factors. A measure of the significance of any particular factor can be obtained by the evaluation of changes in the intrinsic rate of natural increase, $r_m$, of the population under a specified set of conditions. Instar preference results in some aphid instars suffering higher mortality than others. The manner in which this differential mortality affects the expected population growth of the pea aphid is well summarized by the $r_m$ statistic.

The importance of host-instar preference is reflected in the outcome of several biological control programs. Succession among, and distribution and phenology of three introduced parasites, *Aphelinus semiflavus* Howard (Eulophidae), *Praon exsoletum* Nees (=*palitans* Muesebeck) (Aphidiidae), and *Trioxys complanatus* Quilis (=*utilis* Muesebeck) (Aphidiidae), of the spotted alfalfa aphid, *Therioaphis trifolii* (Monell), were investigated by van den Bosch *et al.* (1964). The data were

collected in study plots in different climatic areas of southern California, *i.e.*, 1] low desert, 2] south coastal, including (a) extreme south coastal, and (b) inner south coastal; 3] western Mojave desert, and 4] southern San Joaquin Valley. Aggregate yearly catches of adult wasps, from 1957-1962, were analysed. In 1957, data gathered in areas 1, 3, and 4 showed that *Praon* predominated over *Trioxys* in areas 3 and 4. Data for *Aphelinus* were first available in 1959; they showed that this species predominated in area 2a. By 1962, *Trioxys* had replaced *Praon* and *Aphelinus* in all four study areas. The observed predominance of *Trioxys* was attributed by van den Bosch *et al*. (1964) to the parasite's oviposition preference for younger aphids, among other factors. This preference could have the double effect of negatively affecting the growth of *T. trifolii* to a relatively greater extent, because aphids attacked in the earlier stages do not reproduce, as well as giving *Trioxys* a competitive advantage over *Praon* and *Aphelinus*.

Biological control of fruit flies in Hawaii represents a classic example of succession within a community of imported parasites, attributed primarily tc differences in the host-stage attacked. In mid-1948, two braconid wasps, *Opius vandenboschi* Fullaway (=*persulcatus*) and *O. longicaudatus* (Ashmead), were imported and released in large numbers to control the Oriental fruit fly, *Dacus dorsalis* Hendel. By summer 1949, *O. longicaudatus* had increased rapidly, and up to 50% of fruit fly larvae in collected fruits were found parasitized. However, by

December 1949, *O. vandenboschi* had become the dominant parasite and alone accounted for the parasitism of 65-80% of fruit fly larvae (van den Bosch & Haramoto 1950). At about the same time, fruit fly larvae mummified by another *Opius* species, *O. oophilus* Fullaway, were found. This species was thought to have been accidentally introduced together with *O. longicaudatus* and *O. vandenboschi*. By fall 1950, *O. vandenboschi* was replaced by *O. oophilus* as the dominant parasite. The latter continued to maintain this position in the parasite complex (van den Bosch & Haramoto 1951). The primary reason for this clear succession of competing species is that *O. longicaudatus* attacks only large, older larvae, which are often less vulnerable to attack due to their cryptic habitat. *O. vandenboschi* attacks the more vulnerable, small larvae, whereas *O. oophilus* oviposits in fruit fly eggs; the latter is intrinsically superior to the other species (van den Bosch & Haramoto 1953, cited in Bennett *et al.* 1976).

The alfalfa weevil, *Hypera postica* (Gyll.) (Coleoptera: Curculionidae), is an important pest of alfalfa in the United States (Hagen *et al.* 1971). *Bathyplectes curculionis* (Thompson), an ichneumonid larval parasite, and *Tetrastichus incertus* Ratz., a eulophid larval parasite, were imported and released in different states between 1910 and 1925 (Chamberlin 1925, 1926, 1933). The parasites were, in general, quite effective in controlling this pest, although in competition with each other, parasitism by *T. incertus* decreased, while parasitism by *B.*

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*curculionis* increased, because the latter oviposited in earlier larval stages of the host.

Parasitism of a particular stage of the host is, in some cases, dependant on the accessibility of that stage to the parasite. For example, a study of the parasite fauna of pyralid and noctuid stem borers (Lepidoptera) in Malaysian Borneo indicated that egg parasites were more effective than larval or pupal parasites (Rothschild 1970). The cryptic habitat of the older larval stages often made them inaccessible to their parasites, whereas the eggs, laid externally on plant parts, were easily found by parasites.

The arguments presented in this paper suggest that a study of host-instar preference patterns exhibited by an insect parasite, or members of the parasite community that attack one particular host species, is a useful tool in analysing host-parasite interactions. Host size, behaviour, and availability influence preference. The experimental design and method of evaluation may bias conclusions about preference. For hosts that are susceptible to parasitism in most of their developmental stages, the host stage(s) attacked will be a primary determinant of the effectiveness of the parasite(s).

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