THE EVOLUTION OF LIFE-HISTORY TIMING IN A LEAFMINING MOTH, *PHYLLONORYCTER MESPILELLA* (HÜBNER)

by

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The evolution of life-history timing in a leafmining moth,

Phyllonorycter mespilella (Hübner)

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ABSTRACT: In this study, I investigated the evolution of seasonal variation in the life-history timing of a multivoltine leafmining moth, *Phyllonorycter mespilella* (Hübner). During development, *P. mespilella* pass through two larval feeding stages known as the sap-feeding (SF) and tissue-feeding (TF) stages. Phenotypic selection by parasitoids on the duration of the SF stage (SF duration) was measured in field populations, and in artificial patches of larvae with manipulated development times. Variation in the direction, form and causes of selection was apparently caused by frequency-dependent changes in the behaviour of the parasitoids. Fall photoperiod caused extensions of the SF and TF larval stages, increases in pupal weight, and induction of diapause dormancy. Genetic variation in SF duration was detected under a fall larval photoperiod, as was a genotype by environment (GxE) interaction for SF duration across photoperiodic environments. The decision to host feed or oviposit on hosts with different fitness payoffs by female parasitoids was examined in a dynamic optimization model. The model predicted that hosts of higher fitness value should be used for oviposition under most conditions, and that lower-value hosts should be used either for oviposition or host feeding, depending on age, egg load, metabolic reserves, host availability and the magnitude of difference in fitness payoffs. A genetic algorithm model predicts that variation in the timing of a two-stage life history will be maintained when predator attack on early and late life-history stages is frequency-dependent. Variation is eroded when the predator prefers to consume the second stage. Frequency-dependent parasitoid attack and a GxE interaction are potential mechanisms for the maintenance of variation in SF duration in *P. mespilella.*

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CHAPTER 1: INTRODUCTION

Phyllonorycter mespilella (Hübner) (Lepidoptera: Gracillaridae) is a leafmining moth that feeds on the foliage of apple trees in the Okanagan Valley of British Columbia (B.C.), Canada (Cossentine & Jensen, 1992). Leafminers of the genus *Phyllonorycter* develop through two larval stages with distinct feeding biologies (Pottinger & Leroux, 1971). The first three larval instars feed on plant sap and are called sap-feeders (SF), and the last two larval instars feed on leaf tissue and are called tissue-feeders (TF). The life history of *Phyllonorycter* species thus proceeds through the following stages: egg, SF larva, TF larva, pupa, adult (Figure 1.1). Extended durations of the SF stage have been observed in fall generations in a number of *Phyllonorycter* species (Maier, 1984; Laing *et al.*, 1986; Barrett & Brunner, 1990a). This study investigated evolutionary influences on seasonal variation in the timing of larval development in *P. mespilella.*

Direct measurements were made of phenotypic selection by hymenopterous parasitoids on the timing of the transition between the SF and TF stages. Although parasitoids are assumed to be strong selective influences on traits in herbivorous insects (Price *et al.*, 1980; Jeffries & Lawton, 1984; Lawton, 1986; Bernays & Graham, 1988; Rausher, 1992), direct measurements of selection have rarely been attempted (Rausher, 1992; Weis *et al.*, 1992). Measurements of phenotypic selection on the date of transition to the TF stage (TF date) in field populations of *P. mespilella*

Egg → SF larva → TF larva → Pupa → Adult

FIGURE 1.1 Stages in the life history of *Phyllonorycter mespilella*.

are reported in Chapter 2. An experiment is presented in Chapter 4 in which selection on the duration of the SF stage (SF duration) is measured in artificial patches of larvae with manipulated development times. The pattern of parasitoid oviposition on SF and TF larvae was measured both in field populations and in artificial patches (Chapters 2 and 4). Considerable variation was found in the form, direction and causes of selection. Variation in the host selection behaviour of parasitoids appears to cause some of the variation in selection on SF duration.

Photoperiodic induction of seasonal changes in development time is also examined (Chapter 3). Seasonal extensions of the duration of SF development in *Phyllonorycter* species have previously been referred to as "summer diapause" (Laing *et al.*, 1986). The responses of *P. mespilella* larvae to photoperiod that are reported here indicate that seasonal extensions of larval development are part of a winter "diapause syndrome" (*sensu* Tauber *et al.*, 1986). Heritability and genotype by environment (GxE) interactions are measured for SF and TF development times across summer and fall photoperiods. Genetic variation in developmental traits in larvae is a prerequisite for a response to selection to occur. GxE interactions may constrain responses to selection across environments and thus reduce the potential for the evolution of adaptive phenotypic plasticity.

Frequency-dependent changes in the host-selection behaviour of parasitoids are responsible, in part, for variation in phenotypic selection on SF duration (Chapter 4).

A dynamic optimization model of the decision to host feed or oviposit on a host by a female parasitoids is presented in Chapter 5. This model compares optimal host decisions for two host types with different fitness payoffs. Results of simulation runs of the model provide a functional explanation for the seasonal changes in the rate of oviposition on SF larvae that were observed in this study (Chapters 2 and 4).

This study presents evidence from two different measurements that genetic variation for SF duration exists (Chapter 3 and 4). Frequency-dependent attack by parasitoids on SF and TF larvae may play a role in maintaining variation in SF duration (Chapter 4). Frequency-dependent selection by predators has been widely discussed as a mechanism for maintaining variation in traits that are involved in prey detection and acceptance (Clarke, 1964; Clarke, 1969; Ayala & Campbell, 1974; Hubbard *et al.*, 1982; Greenwood, 1984; Gendron, 1987; Allen, 1988; Endler, 1988). A genetic algorithm model of frequency-dependent selection on the timing of a two-stage life history is presented in Chapter 6. Optimal development strategies are compared for conditions where predation on the two life stages is frequency-dependent, and where predation is preferentially directed toward the second life stage.

CHAPTER 2: PHENOTYPIC SELECTION BY PARASITOIDS ON THE TIMING OF LIFE HISTORY IN A LEAFMINING MOTH

Natural selection by parasitoids is assumed to affect the evolution of a diversity of traits in herbivorous insects (Price *et al.*, 1980; Jeffries & Lawton, 1984; Lawton, 1986; Bernays & Graham, 1988; Rausher, 1992). Although a variety of methods exist for measuring and analyzing phenotypic selection in natural populations (Lande & Arnold, 1983; Endler, 1986; Mitchell-Olds & Shaw, 1987; Schluter, 1988; Crespi, 1990), attempts to measure selection by parasitoids on traits of herbivorous insects have been rare (Rausher, 1992; Weis *et al.*, 1992). This paper describes measurements of phenotypic selection on the timing of the life history of a leafmining moth, with particular reference to the selective influence of parasitoids.

Many animals undergo sequential changes in feeding mode, or habitat, during juvenile development. Differences in growth rates and mortality risks between habitats, and the time constraints imposed by seasonality or reproductive schedules, are predicted to select for optimal timing of niche changes during the life history (Rowe & Ludwig, 1990). For example, fledging times in Alcid seabirds are influenced by differences in growth rates and predation risks between nest and ocean habitats (Ydenberg, 1989). Differences in parasitoid attack rates between larval stages of herbivorous insects are ubiquitous (Vinson, 1976; Vinson & Iwantsch, 1980; van Alphen & Vet, 1986; Strand, 1986; Godfray, 1994), and should have a similar

selective influence on the timing of life histories.

The importance of particular ecological factors as selective influences is dependent on spatial and temporal variation in overall phenotypic selection. The direction, form and causes of selection have been found to vary between years, locations and life-history stages for a number of organisms (Kalisz, 1986; Schluter *et al.*, 1991; Weis *et al.*, 1992). The contribution of parasitoids to the outcome of selection will depend on variation in their relative influence compared to other mortality factors. Assessment of the importance of parasitoids as selective influences thus requires analysis of selective episodes within and between populations, years and life history stages.

Phyllonorycter mespilella (Hübner) is a multivoltine, leafmining moth with two stages of larval development that differ in feeding mode, and risk of parasitoid attack. This paper tests the hypothesis that different rates of parasitoid oviposition between larval stages can select on variation in the timing of the life history. I measured phenotypic selection in one population of *P. mespilella* during the fall generation of 1991, and in another population during summer and fall generations in 1993, and assessed the importance of parasitoids as selective influences in relation to other mortality factors.

Natural History

Leafmining moths of the genus *Phyllonorycter* (Lepidoptera: Gracillaridae) feed on the foliage of deciduous trees (Pottinger & Leroux, 1971; Miller, 1973; Askew & Shaw, 1979a; Maier, 1982; Maier, 1984; Laing *et al.*, 1986; Barrett & Brunner, 1990a; Cossentine & Jensen, 1992). Two stages occur during larval development: the sap-feeding (SF) and tissue-feeding (TF) stages (Pottinger & Leroux, 1971). SF larvae feed on plant sap by shearing cells, and excavate a blotch-shaped mine. TF larvae feed on plant tissue in a chamber formed by spinning silk threads across the bottom surface of the mine. Larvae complete development and pupate inside this feeding chamber. Summer generation adults emerge from mines on trees. Individuals of the diapausing generation overwinter as pupae in mines within leaves on the ground.

Insect parasitoids can cause mortality in their hosts either by oviposition or by host feeding (Godfray, 1994). Host feeding occurs when an adult female parasitoid feeds on the hemolymph of a host (Jervis & Kidd, 1986). The causes of parasitoid-induced mortality differ between SF and TF stages in many *Phyllonorycter* species. Parasitoids of the genera *Pnigalio* and *Sympiesis* (Hymenoptera: Eulophidae) oviposit predominantly on TF larvae, and host feed predominantly on SF larvae (Askew & Shaw, 1979b; Maier, 1982; van Driesche & Taub, 1983; Laing *et al.*, 1986; Casas, 1989; Barrett & Brunner, 1990b; Varela & Welter 1992). If the phenology of parasitoid oviposition is concurrent with the first appearance of TF larvae, and if parasitoids prefer TF larvae for oviposition, then individuals that enter the TF stage

earlier will have a higher risk of mortality from parasitoid oviposition. Oviposition attack by parasitoids may thus select for later times of transition to the TF stage (TF date) (Laing *et al.*, 1986).

In this study, I quantified phenotypic selection by parasitoids on TF date in *Phyllonorycter mespilella* (Hübner), particularly in the diapausing generation. The duration of the SF stage in diapausing generations of *Phyllonorycter* spp. has been frequently observed to be longer than in summer generations (Maier, 1984; Laing *et al.*, 1986; Barrett & Brunner, 1990a). Currently, there is no functional explanation for this change in life-history timing. Parasitoid oviposition during the SF stage may also select on variation in TF date. Oviposition attack on SF larvae is rare in summer generations, but has been observed to increase in frequency during fall generations of *Phyllonorycter* species (Barrett & Brunner, 1990b; Varela & Welter, 1992). Measurement of phenotypic selection caused by mortality during the SF stage is impossible because the phenotype (TF date) of individuals that die during the SF stage is never expressed. Here, I present measurements of phenotypic selection on the variation in TF date remaining after mortality in the SF stage has occurred.

MATERIALS AND METHODS

Leafminer and Parasitoid Species

Phyllonorycter mespilella (Hübner) feeds on the foliage of apple trees in the Okanagan and Similkameen Valleys of British Columbia (B.C.), Canada (Cossentine &

Jensen, 1992). Three generations per year are completed in the Okanagan Valley of B.C. where the study was conducted (Cossentine & Jensen, 1992). No overlap between the three generations occurred at the Summerland study site in 1991 (R. McGregor, unpublished data). *Pnigalio flavipes* (Ashmead) and a *Sympiesis* species (Hymenoptera: Eulophidae) are the most common parasitoids attacking *P. mespilella* in the Okanagan Valley of B.C. (Cossentine & Jensen, 1992). *P. flavipes* and *Sympiesis marylandensis* Girault accounted for 94% of all parasitoids reared from leafmines collected at Summerland in 1991 (R. McGregor, unpublished data).

Measurement of phenotypic selection

The study was conducted at research orchards of Agriculture Canada at Summerland, B.C. between August and November of 1991, and at Kelowna, B.C. between June of 1993 and May of 1994. The Summerland and Kelowna sites are separated by 39 kilometers, and are considered to harbor distinct populations of *P. mespilella.* In 1991, during the oviposition period of the second generation of *P. mespilella*, a total of 718 third-generation leafmines were individually marked as they appeared on foliage. In 1993, 748 second-generation leafmines were marked during the oviposition period of the first generation, and 1073 third-generation leafmines were marked during the oviposition period of the second generation. In both years, all mines were visited daily until the date of transition to the TF stage. The Julian date on which the first wrinkle appeared on the lower surface of a mine was recorded as

the date of transition to the TF stage (TF date).[1]

In 1991, the marked mines were collected when the leaves began to drop from the trees in November. The marked mines were taken to the laboratory and dissected. Each individual was assigned to one of three categories: survived, parasitized, or other. Individuals that survived were TF larvae, or pupae, that were alive at the time of dissection. At the time of dissection, no SF larvae were found alive. Parasitized individuals had an associated parasitoid egg, larva or pupa. Individuals in the "other" mortality category had died of unknown causes; no parasitoids were found in these mines. The "other" mortality category includes individuals that were fed upon by parasitoids. Host-feeding mortality could not be distinguished in dissections.

In 1993, the second-generation mines were collected after the end of the flight of second-generation adults, returned to the laboratory and dissected. The third-generation mines were collected when leaves began to drop from the trees in November, placed in screen bags, and staked to the orchard floor. These leafmines were collected in May, returned to the laboratory, and dissected. Individuals from both generations were assigned to the same three categories as in 1991. However, in 1993 individuals that were assigned to the "survived" category were those that had successfully emerged from pupae.

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[1] Longitudinal wrinkles appear on the lower surface of the blotch-shaped mines as silk strands spun by fourth instar larvae begin to contract. The appearance of these wrinkles is the first external evidence that the larva has molted to the TF stage.

Data analysis

Standard univariate selection statistics were calculated for TF date for those individuals that survived the SF stage. Directional selection intensity was calculated as:

$$i = \frac{\overline{X_a} - \overline{X_b}}{s}$$

where $\bar{X}_b$ is the mean TF date before selection, $\bar{X}_a$ is the mean TF date after selection, and s is the standard deviation of TF dates before selection (Endler, 1986). Selection on the variance of the phenotypic distribution was quantified by calculating the statistic j from the following formula:

$$j = \frac{v_a - v_b}{v_b}$$

where v_b is the phenotypic variance of TF date before selection, and v_a is the variance after selection (Endler, 1986). A two-tailed t-test was used to test the significance of values of i, and a two-tailed F-test was used to test the significance of values of j (Endler, 1986).

Values of i and j were calculated for data from each of the three selective episodes and tested for significance: (1) the third generation of 1991 at Summerland, (2) the second generation of 1993 at Kelowna, and (3) the third generation of 1993 at Kelowna. Individuals that died of parasitism were removed from these three data sets to create reduced data sets that included only "other" mortality. A third data set was created, for each selective episode, from which individuals that died of "other" causes were removed, thus including only parasitism mortality. Values of i and j were thus calculated and tested for significance for: (1) all of the data, (2) data with only "other" mortality included, and (3) data with only parasitism mortality included. The reduced data sets were analysed to examine the influence of particular mortality sources on phenotypic selection in each selective episode.

Survival functions were generated using a program that fits cubic spline regressions to survival data (Schluter, 1988). The data were searched within the range -5 to 10 for the value of the smoothing parameter that minimized the Generalized Cross Validation score (Schluter, 1988). Standard errors around survival functions were estimated from the predicted values of cubic spline regressions fit to 200 bootstrap samples of the data (Effron, 1981; Schluter, 1988). Survival functions were generated from the same data sets used to calculate selection statistics above.

RESULTS

Third Generation 1991 - Summerland, B.C.

The relationship between TF date and survival was examined for the 273 larvae that survived the SF stage (Table 2.1). Directional selection intensity was positive and significantly different from zero when calculated for (1) all of the data, (2) the data with only parasitism mortality included, or (3) the data with only other mortality included (Table 2.2). Values of the variance selection statistic, j, were not significantly different from zero (Table 2.2). The probability of survival rises monotonically from early TF dates to later TF dates (Figure2.1). Parasitism mortality has a stronger effect on survival at early TF dates, but both parasitism and other mortality sources contribute to the increasing nature of the survival function (Figure 2.2). The proportion of oviposition attack was higher on TF larvae than on SF larvae (Oviposition occurred on 14.8% of SF larvae *vs.* 35.5% of TF larvae, χ^2=52.4, df=1, $p<0.001$, Table 2.1). This heterogeneity in parasitoid attack between SF and TF stages is required if phenotypic selection by parasitoids on TF date is to occur.

Phenotypic selection on TF date clearly occurred for individuals that survived the SF stage in this population in 1991. Positive directional selection on TF date was detected, and individuals with later TF dates had much higher survival probabilities. Oviposition by parasitoids is the primary cause of this phenotypic selection, but mortality sources other than parasitism also contributed to the observed selection. Host feeding by parasitoids on TF larvae may cause this additional component of

TABLE 2.1. Mortality data for marked mines of *Phyllonorycter mespilella*. All marked mines were dissected in the laboratory and assigned to larval stage (Sap-feeder (SF) or tissue-feeder (TF)) and fate category (Survived, Parasitized or Other). The number of individuals in each combination of larval stage and fate category is given. Numbers in parentheses give the percentage of individuals in each larval-fate category, and are expressed as a percentage of all marked larvae for the SF stage, and as a percentage of those larvae that survived the SF stage for the TF stage.

Location and year	Generation	Larval stage	Parasitized	Other	Survived
Summerland 1991	Third	SF	106	339	273
(Total n=718)			(14.8)	(47.2)	(38.0)
		TF	97	109	67
			(35.5)	(39.9)	(24.5)
Kelowna 1993	Second	SF	13	192	543
(Total n=748)			(1.7)	(25.7)	(72.6)
		TF	161	64	318
			(29.7)	(11.8)	(58.6)
Kelowna 1993	Third	SF	38	114	921
(Total n=1073)			(3.5)	(10.6)	(85.8)
		TF	46	702	173
			(5.0)	(76.2)	(18.8)

TABLE 2.2. Selection statistics for date of transiton to the tissue-feeding stage (TF date) in *Phyllonorycter mespilella*. Directional selection intensity, i, and variance selection intensity, j, were calculated for the entire data set, and for the data with only parasitism mortality, or other mortality, included.

Location and year	Generation	Data grouping	Selection statistics[2]	
			i	j
Summerland 1991	Third	All	0.53 ***	-0.014 ns
		Parasitism	0.57 ***	-0.010 ns
		Other mortality	0.31 *	-0.064 ns
Kelowna 1993	Second	All	-0.08 ns	-0.182 ns
		Parasitism	-0.05 ns	-0.142 ns
		Other mortality	-0.029 ns	-0.016 ns
Kelowna 1993	Third	All	-0.27 ***	-0.200 ns
		Parasitism	0.04 ns	-0.104 ns
		Other mortality	-0.29 ***	-0.179 ns

[2] Statistics were tested for significance using a two-tailed t-test for i, and a two-tailed F-test for j [ns=p>0.05, **=p<0.05, ***=p<0.001].

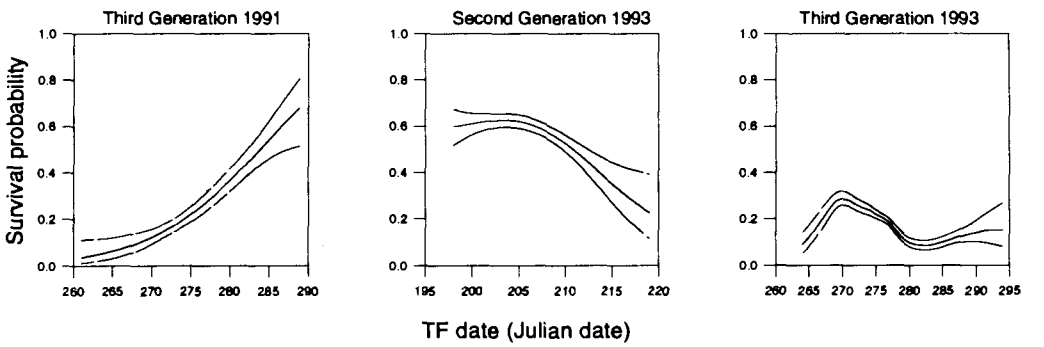

FIGURE 2.1. Probability of survival to the pupal stage as a function of date of transition to the tissue-feeding stage (TF date) for data from all marked *P. mespilella* larvae. Survival functions are cubic spline regressions of survival on TF date. Solid curves are the fitted functions and dashed lines represent prediction intervals (±1 SE) around predicted values for each function from 200 bootstrap subsamples of each dataset. Functions are shown for the Summerland site in the third generation of 1991, and the Kelowna site in the second and third generations of 1993.

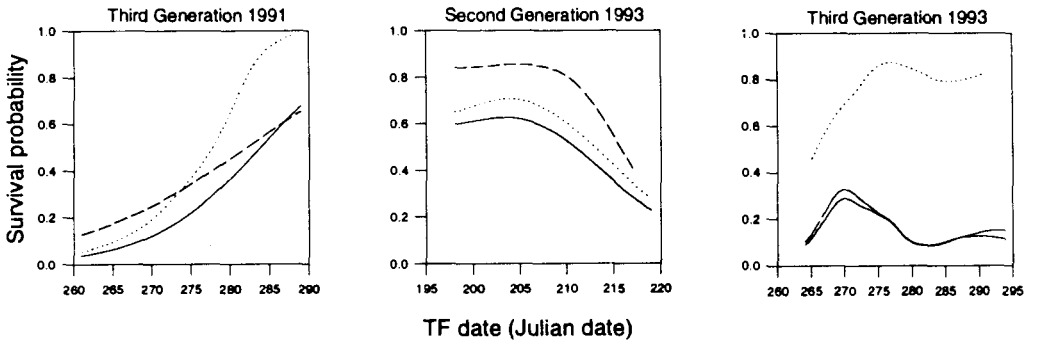

FIGURE 2.2. Probability of survival to the pupal stage as a function of date of transition to the tissue-feeding stage (TF date) for full and partial data from marked *P. mespilella* larvae. Survival functions are cubic spline regressions of survival on TF date. Solid curves are the fitted functions from the entire data sets from Summerland in the third generation of 1991, and Kelowna in the second and third generations of 1993. The dotted curves show functions generated from subsets of data with only parasitism mortality included. The dashed curves show functions generated from subsets of data with only other mortality included.

selection. Host feeding is coincident with parasitoid oviposition activity, and could also cause higher mortality on individuals with earlier TF dates.

Second Generation 1993 - Kelowna, B.C.

Directional selection intensities calculated for the second generation of 1993 at Kelowna were not significantly different from zero for (1) the entire data set, (2) for data with only parasitism mortality included, or (3) for data with only other mortality included (Table 2). Values of the variance selection statistic, j, were not significantly different from zero (Table 2). Although directional selection could not be detected, the cubic spline survival function shows a decreasing trend with increasing TF date (Figure 1). The shape of the survival function is similar when only parasitism mortality, or only other mortality, are included in the data (Figure 2). The proportion of oviposition attacks were again higher on TF larvae than on SF larvae (29.7% on TF larvae *vs.* 1.7% on SF larvae, χ^2=210.2, p<0.001, Table 1).

Third Generation 1993 - Kelowna, B.C.

Directional selection intensity was negative and significantly different from zero for (1) all of the third generation data from Kelowna in 1993, or (2) the data with only other mortality included (Table 2). When only parasitism mortality was included, directional selection intensity was positive, but not significantly different from zero (Table 2). Calculated values of the variance selection statistic, j, were not significantly

different from zero (Table 2). The survival function for the Kelowna population in the third generation of 1993 has an intermediate peak in survival probability near Julian date 270, and very low survival at the highest TF dates (Figure 1). The function generated from data with only other mortality included has a very similar shape (Figure 2). When only parasitism mortality is included, the function shows the lowest survival at early TF dates and higher survival at later TF dates. The proportion of oviposition attacks were similar between SF and TF larvae (3.5% on SF larvae *vs.* 5.0% on TF larvae, $\chi^2=2.6$, $p>0.05$, Table 1).

Negative directional selection on TF date occurred in the third generation of 1993 at Kelowna. By far, the largest proportion of mortality at this site in the third generation of 1993 was from sources other than parasitism (76.2% of TF larvae, Table 1). Marked mines from the third generation were left in the orchard over the winter of 1993-1994 and much of this mortality was presumably caused by death during the winter. If that assumption is correct, then overwinter mortality caused the negative directional selection on TF date measured in this population in 1993.

Seasonal changes in parasitoid oviposition attack

The proportion of parasitoid oviposition on SF versus TF larvae varied between generations at both the Summerland and Kelowna sites. Parasitoid females at Summerland oviposited more often into SF mines in the fall generation of 1991 (Proportion of ovipositions on SF larvae: Third generation 52%, Table 1), than in the

two summer generations of 1991 (Proportion of ovipositions on SF larvae: First generation 0%, Second generation 5%, R. McGregor, unpublished data). A similar seasonal increase in oviposition rate into SF mines was observed at the Kelowna site in 1993 (Proportion of ovipositions on SF larvae: Second generation 7%, Third generation 45%, Table 1).

DISCUSSION

Variation in phenotypic selection on TF date

Parasitoids caused positive directional selection on TF date in the Summerland population in the third generation of 1991. In the Kelowna population in the third generation of 1993, mortality sources other than parasitism caused negative directional selection. Oviposition by parasitoids caused non-uniform mortality across the range of TF date at Kelowna in the third generation of 1993, but this did not affect the outcome of selection. Overwintering mortality was clearly the most important cause of selection in this population in 1993. Individuals with later TF dates may pupate at smaller sizes and with fewer fat reserves, and thus have lower overwintering survival. Opposing selection by parasitoids and overwintering mortality on TF date may occur in third-generation populations of *P. mespilella.* Conflicting selection pressures at different life history stages occur in many taxa, and may be a phenotypic mechanism underlying life-history trade-offs (Schluter *et al.*, 1991).

Selection also varied between the two generations in 1993. Negative

directional selection occurred on TF date in the third generation at Kelowna in 1993, but no directional or variance selection on TF date was detected in the second generation. Parasitoids caused lower survival at late TF dates in the second generation, and lower survival at early TF dates in the third generation (Figure 2). The effects of parasitism mortality on the shape of the survival functions were thus opposite in direction.

The relative importance of parasitoids, and other mortality factors, as selective influences on TF date varied between generations, sites and years. Studies that have assessed the extent of variation in phenotypic selection in other taxa have also found considerable spatial and temporal variation (Kalisz, 1986; Schluter & Smith, 1986; Weis *et al.*, 1992). The importance of phenotypic selection by parasitoids on long-term evolution of TF date in *P. mespilella* will depend on variation in the timing and intensity of parasitoid attack, and in the strength of other selective influences.

Seasonal changes in parasitoid oviposition attack

Seasonal increases in the rate of oviposition attack on SF larvae occurred at both the Summerland and Kelowna sites. Similar increases in oviposition on SF larvae have also been observed for other *Phyllonorycter* species that are attacked by *Pnigalio flavipes* and *Sympiesis marylandensis* (Barrett & Brunner, 1990b; Varela & Welter, 1992). The increase in attack rate on SF larvae corresponds with seasonal changes in the timing of the leafminer life history. In fall generations, TF larvae do

not occur until a later date. SF larvae are presumably encountered more frequently by parasitoids searching for hosts during the fall generation. The number of oviposition attacks per unit time on SF larvae may increase in the fall because of an adaptive shift in host preference that occurs in response to changes in encounter rates with the different host types (Mangel & Roitberg, 1989; Roitberg *et al.*, 1992; Li *et al.*, 1993; Roitberg *et al.*, 1993). Alternatively, parasitoids may show no preferences for particular larval stages, and may attack SF larvae more often in the fall simply because they are the most often-encountered type of host. Regardless of the mechanism, the pattern of parasitoid oviposition appears to change in response to the relative frequencies of SF and TF larvae.

If parasitoid attack rates depend on local frequencies of SF and TF larvae, then the form of phenotypic selection on TF date may also be frequency dependent. The survival advantage of delaying the transition to the TF stage would be much diminished if the local frequency of SF larvae were high, and parasitoids oviposited more frequently into SF mines. Frequency-dependent prey selection has been proposed as a mechanism for the maintenance of colour polymorphisms in prey species (Greenwood, 1984; Gendron, 1987; Allen, 1988; Endler, 1988). Additive genetic variation for the duration of the SF stage (SF duration) has been detected in both the Summerland and Kelowna populations (R. McGregor, unpublished data). Frequency-dependent changes in the oviposition pattern of parasitoids could play a role in maintaining genetic variation in the timing of the transition between the SF and TF

stages in *P. mespilella.*

Conclusions

This study has shown that oviposition by parasitoids can select on the timing of life-history events in a herbivorous insect. However, the relative importance of parasitoids and other mortality sources as selective influences varied between populations, generations and years. Phenotypic selection by parasitoids on TF date depends on oviposition attack that is specific to a particular developmental stage of the host. Because such stage-specific oviposition is common in parasitoids (Vinson, 1976; Vinson & Iwantsch, 1980; van Alphen & Vet, 1986; Strand, 1986; Godfray, 1994), selection by parasitoids on the life-history timing of herbivorous insects may occur in many taxa.

CHAPTER 3: THE INFLUENCE OF PHOTOPERIOD ON THE PLASTICITY OF LARVAL DEVELOPMENT

In multivoltine temperate-zone insects, seasonal changes in the timing of life history events are common (Tauber *et al.*, 1986; Danks, 1987). In particular, the duration of larval development is often longer in diapausing generations than in non-diapausing generations (Tauber *et al.*, 1986; Danks, 1987). Induction of diapause and associated changes in life-history timing occur in response to seasonal changes in photoperiod in many insect taxa (Beck, 1980; Tauber *et al.*, 1986; Danks, 1987). This paper examines the influence of photoperiod on the timing of larval development in a leafmining moth.

Phenotypic plasticity is the variation in the phenotypes of quantitative traits caused by responses to environmental conditions (Stearns, 1989). The changes in phenotype may be caused by non-adaptive responses to environmental variation. For example, body size may vary with variation in the conditions for growth. However, if particular changes in phenotype are favoured by natural selection, phenotypic plasticity can be adaptive (Stearns, 1989). The range of phenotypes expressed by particular genotypes across environments are known as norms of reaction (Via & Lande, 1985; Stearns & Koella, 1986; Via, 1987; Stearns, 1989). The development times of diapausing and non-diapausing insect larvae define a norm of reaction across fall and summer environments.

Because selective influences on larval development time may be different in summer and fall, the optimal development time may differ between diapausing and non-diapausing generations. Response to selection is determined by both the level of genetic variation that exists for the trait within environments, and the level of genetic variation in phenotypic plasticity that exists across environments (Via & Lande, 1985; Via, 1987; Carrière & Roitberg, 1995). Heritable variation in phenotypic plasticity can be detected by measuring genotype by environment (GxE) interactions (Via, 1984; Via & Lande, 1985; Via, 1987; Falconer, 1989). GxE interactions may constrain the response to selection across environments, and thus maintain genetic variation in quantitative traits (Via & Lande, 1987; Gillespie & Turelli, 1989).

This study investigates seasonal changes in the timing of larval development of *Phyllonoryter mespilella* (Hübner), a species that feeds on the foliage of apple trees in British Columbia (B.C.), Canada (Cossentine & Jensen, 1992). Multivoltine leafmining moths of the genus *Phyllonorycter* (Lepidoptera: Gracillaridae) have two distinct stages of larval development, the sap-feeding (SF) and tissue-feeding (TF) stages (Pottinger & Leroux, 1971). The first three larval instars (SF) enlarge a blotch-shaped mine by shearing cells and feeding on plant sap. Larvae of the last two instars (TF) feed on plant tissue and complete their development in a chamber formed by spinning silk threads across the bottom of the mine. Extensions of larval development time have been observed in several *Phyllonorycter* species in the final generation of the year (Pottinger & Leroux, 1971; Maier, 1984; Laing *et al.*, 1986; Barrett &

Brunner, 1990a; Varela & Welter, 1992).

Body size, fat bodies and other metabolic storage organs are often larger in diapausing individuals of many insect taxa (Tauber *et al.*, 1986; Danks, 1987). For example, overwintering pupae of *Phyllonorycter blancardella* are larger than pupae from summer generations, and have larger fat bodies (Pottinger & Leroux, 1971). Increased size and accumulation of fat presumably function to increase overwinter survival of dormant individuals. This study also examines seasonal variation in pupal weight in *P. mespilella.*

Three main questions are addressed here: (1) Do seasonal changes in photoperiod affect the timing of larval development, the resulting size of pupae, and the induction of diapause in *P. mespilella*? (2) Does genetic variation exist for the durations of the SF and TF larval stages, and for pupal weight, in summer and fall photoperiod environments? Response to selection in different photoperiod environments requires genetic variation in larval development time and pupal weight. (3) Do GxE interactions exist for SF duration, TF duration, and pupal weight across photoperiod environments? GxE interactions may constrain the evolution of optimal plasticity of larval development and pupal size in *P. mespilella.*

MATERIALS AND METHODS

Experimental design and data collection

Pupae of *Phyllonorycter mespilella* were collected at an orchard in Summerland, B.C. in August of 1992, during the second generation. Three generations of *P. mespilella* are completed annually in the vicinity of Summerland, B.C. (Cossentine & Jensen, 1992). The field-collected pupae were dissected from leafmines, weighed, and sexed in the laboratory. Each pupa was placed in a gelatin capsule, and randomly assigned to a growth chamber with either a 16-hour, or a 13-hour, photoperiod. The photoperiod experienced by the parental generation of leafminers during emergence, mating and oviposition will be referred to as the maternal photoperiod. A 13-hour photoperiod occurs at Summerland during early September. *P. mespilella* larvae reared at a 13-hour photoperiod have longer durations of the SF stage than larvae reared at a 16-hour photoperiod (R. McGregor, unpublished data).

Growth chambers were set at 22°C, and the daily accumulation of degree days was monitored using Omnidata Biophenometers equipped with thermistor temperature probes (Omnidata International, Logan, Utah). The developmental threshold for a closely related species, *Phyllonorycter elmaella* Doganlar & Mutuura, in adjacent Washington State, U.S.A., is 4.7°C (Barrett, 1988). As no value was available for the developmental threshold of *P. mespilella*, degree days above a threshold of 5°C were recorded for this experiment.

Upon emergence, adult males and females were placed in pairs in gelatin capsules for 24 hours to induce mating. Mated female leafminers were transferred individually into oviposition cages that contained two apple seedlings (30-40 cm in height) for 24 hours. If they did not oviposit during the first 24 hours, surviving females were released for a second 24 hour period onto the same seedlings. If oviposition occurred during the first 24 hours, surviving females were released for a second 24 hours onto fresh seedlings.

The resulting full-sib families of leafminers were split by placing one seedling at a 16-hour photoperiod, and the other at a 13-hour photoperiod. The photoperiod experienced by families of larvae during development will be referred to as the larval photoperiod. This procedure produced four treatment combinations of maternal and larval photoperiod: 16/16, 16/13, 13/16, and 13/13 (maternal photoperiod (hours)/larval photoperiod (hours)). The split-family two-environment design (Groeters & Dingle, 1987) allowed assessment of the relative influences of maternal and larval photoperiod on larval development, the measurement of genetic variation for larval development traits, and the detection of GxE interactions.

All mines were labelled individually on appearance, and the larvae were allowed to develop to the pupal stage. Larvae were observed daily for transitions between the SF and TF stages, and between the TF and pupal stages. The number of degree days between the oviposition date and the date of transition to the TF stage

was recorded as the SF duration. The date on which the first wrinkle appeared on the lower surface of the SF mine was recorded as the date of transition to the TF stage (see Chapter 2). The number of degree days between the date of transition to the TF stage and the date of pupation was recorded as the TF duration. The date of pupation was determined by shining a bright light through each leafmine, and checking for pupal morphology in the silhouette. Pupae were dissected from the mines, and weighed, on the day of pupation. After weighing, pupae were placed individually in gelatin capsules, placed in a growth chamber at 22°C under a 16-hour photoperiod, and monitored for adult emergence.

Maternal effects have been observed to influence the expression of a number of life-history traits in insects (Mousseau & Dingle, 1991). Direct maternal control of various aspects of life history occurs in many species, and is examined in this experiment by measuring the influence of maternal photoperiod on larval development of offspring. The influence of maternal photoperiod on development and pupal size is determined by comparing mean SF duration (S), TF duration (T), and pupal weight (PW) between treatments with the same larval photoperiod, and different maternal photoperiod. Means are thus compared between the two 16-hour larval photoperiod treatments (16/16 and 13/16) and the two 13-hour larval photoperiod treatments (16/13 and 13/13).

Data analysis

The oviposition protocol resulted in nineteen full-sib families that had at least one larva developing at each larval photoperiod. The influence of photoperiod treatment on variation in S, T, and PW was analyzed for individuals from these families by least-squares analysis of variance (ANOVA) (All statistical analyses were conducted using the MGLH module of Systat (Wilkinson *et al.*, 1992)). Means of S, T, and PW were distinguished among treatment groups using Tukey tests. Nine larvae reared at a 16 hour larval photoperiod had extremely long SF duration (S>800 degree days), and entered diapause dormancy. Data for these nine individuals were excluded from the analysis of treatment effects. The means of S, T, and PW were compared between this group, the remainder of the larvae reared at 16-hour photoperiod, and the larvae reared at 13-hour photoperiod using ANOVA and Tukey tests.

A two-factor ANOVA was used to examine the influence of larval photoperiod (PHOTO), and FAMILY (full-sib family) on S, T, and PW. A significant interaction between full-sib family and photoperiodic environment (PHOTO*FAMILY) would provide statistical evidence of the existence of GxE interaction, and thus of genetic variation in phenotypic plasticity (Groeters & Dingle, 1987; Via, 1987). Heritability of S, T, and PW in each larval photoperiod environment was estimated as:

$$h^2 = \frac{2(\sigma^2_s)}{\sigma^2_t}$$

where σ^2_s is the among-sibship variance component, and σ^2_t is the total phenotypic variance, which is calculated as the sum of the among and within-sibship variance components (Falconer, 1989). ANOVA was conducted on S, T, and PW for each larval photoperiod with FAMILY as a fixed effect. Variance components were calculated from the among and within-sibship mean squares from these analyses (Falconer, 1989). Standard errors of heritability estimates were calculated as in Falconer (1989).

RESULTS

Larvae reared at 13-hour photoperiods (16/13 and 13/13) had significantly higher mean S, T, and PW than those reared at 16-hour photoperiods (16/16 and 13/16) (Table 3.1). Maternal photoperiod did not affect mean T or PW at either larval photoperiod, or mean S for larvae reared at 16-hour photoperiod (Table 3.1). However, mean S, for larvae reared at 13 hours, was significantly higher following a maternal photoperiod of 13 hours (13/13) *vs.* 16 hours (16/13) (Table 3.1). This indicates a maternal influence on the duration of SF development for larvae reared at 13-hour photoperiods.

Pupal morphology varied between larval photoperiod treatments. Pupae reared at a 13-hour photoperiod had a distended shape, and a yellow to light brown colour. None of these pupae emerged when held in a growth chamber at 21°C under a 16-hour photoperiod, and they are assumed to have entered diapause dormancy. Nine

TABLE 3.1. Mean (±1SE) values for life-history characters of *Phyllonorycter mespilella* compared between photoperiod treatments (n=220). Treatments are combinations of maternal and larval photoperiod in hours (maternal/larval).[1]

Photoperiod Treatment (hours/hours)	Life-history characters[2]		
	S (degree-days)	T (degree-days)	PW (mg)
16/16	416 ± 10 a	154 ± 3 a	1.22 ± .02 a
16/13	523 ± 11 b	333 ± 9 b	1.75 ± .05 b
13/16	416 ± 8 a	151 ± 2 a	1.15 ± .02 a
13/13	582 ± 15 c	331 ± 9 b	1.81 ± .04 b

[1] Means within a column followed by the same letter are not significantly different at α=0.05.

[2] S=sap-feeding duration, T=tissue-feeding duration, PW=pupal weight

pupae reared at a 16-hour photoperiod had a similar morphological appearance to the pupae reared at 13-hour photoperiod. These 9 individuals had longer SF durations than either the larvae reared at a 13-hour photoperiod, or the remaining larvae reared at 16-hour photoperiod. The TF durations and pupal weights of these individuals were not significantly different from larvae reared at 13-hour photoperiods (Table 3.2). These 9 individuals did not emerge as adults, and are assumed to have entered diapause dormancy. The remaining pupae reared at 16-hour photoperiod were slender in shape, and had a dark brown colour. The majority of these pupae (91%) emerged as adults when held in a growth chamber at 21° under a 16-hour photoperiod. The remainder died of what appeared to be desiccation, but none entered diapause dormancy.

Family means of S, T, and PW for the two larval photoperiod environments are plotted in Figures 3.1, 3.2, and 3.3. Crossing reaction norms, usually characteristic of GxE interactions (Via, 1987), are evident for all three characters. However, a significant interaction between larval photoperiod and full-sibship (PHOTO*FAMILY) was detected only for S (Table 3.3). This significant PHOTO*FAMILY interaction is statistical evidence of a GxE interaction for SF duration. Heritability estimates for S, T, and PW were low relative to their standard errors (Table 3.4). Using an arbitrary criterion that estimates of parameters must be at least twice their standard errors to be significantly different from zero at an α level of 0.05, only the estimate of h^2 for S at 13 hour larval photoperiod could be distinguished from zero ($h^2=0.45 \pm 0.20$, Table 4).

TABLE 3.2. Mean (±1SE) for life history characters of diapausing and non-diapausing *Phyllonorycter mespilella* larvae reared at summer and fall larval photoperiods (n=229).[3]

Larval Photoperiod (hours)	Diapausing or non-diapausing	Life history characters[4]		
		S (degree-days)	T (degree-days)	PW (mg)
16	non-diapausing	416 ± 6 a	152 ± 2 a	1.18 ± 0.01a
16	diapausing	1123 ± 50b	294 ± 26b	1.59 ± 0.13b
13	diapausing	554 ± 10c	332 ± 7 b	1.78 ± 0.03b

[3] Means within a column followed by the same number are not significantly different at α=0.05.

[4] S=sap-feeding duration, T=tissue-feeding duration, PW=pupal weight

TABLE 3.3. Two-factor analysis of variance in life-history characters of *Phyllonorycter mespilella* where larval photoperiod (PHOTO) and full-sibship (FAMILY) are the factors (n=19 full-sib families).

Life-history character[5]	Source of variation	F-value	df	p
S	PHOTO	82.5	1	<0.001
	FAMILY	1.5	18	0.082
	PHOTO*FAMILY	1.7	18	0.041
T	PHOTO	469.7	1	<0.001
	FAMILY	1.5	18	0.111
	PHOTO*FAMILY	1.2	18	0.302
PW	PHOTO	240.1	1	<0.001
	FAMILY	1.8	18	0.029
	PHOTO*FAMILY	1.4	18	0.149

[5] S=sap-feeding duration, T=tissue-feeding duration, PW=pupal weight

TABLE 3.4. Heritability estimates (h^2±1SE) for life-history characters of *Phyllonorycter mespilella* and analysis of variance among full-sibships for these traits at summer and fall larval photoperiods (n=19 full-sib families and df=18 for all F-values).

Life-history character[6]	Larval Photoperiod (hours)	h^2	F-value	p
S	16	0.20 ± 0.17	1.6	0.075
	13	0.45 ± 0.20	2.6	0.001
T	16	0.01 ± 0.12	1.0	0.510
	13	0.18 ± 0.16	1.6	0.074
PW	16	0.19 ± 0.17	1.6	0.078
	13	0.19 ± 0.16	1.6	0.066

[6]S=sap-feeding duration, T=tissue-feeding duration, PW=pupal weight

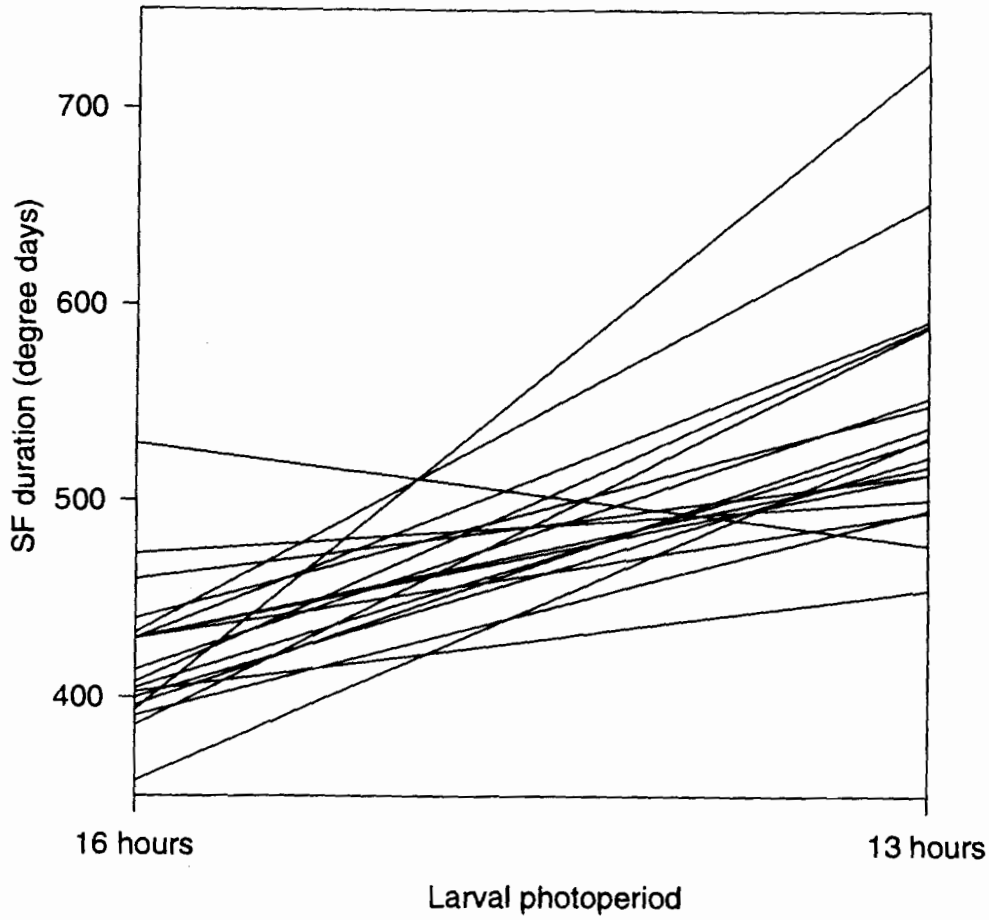

FIGURE 3.1. Mean sap-feeding duration (S) for nineteen full-sib families of *Phyllonorycter mespilella* reared under summer (16 hours) and fall (13 hours) larval photoperiod. The lines in the figure connect family means for summer and fall larval photoperiods (reaction norms).

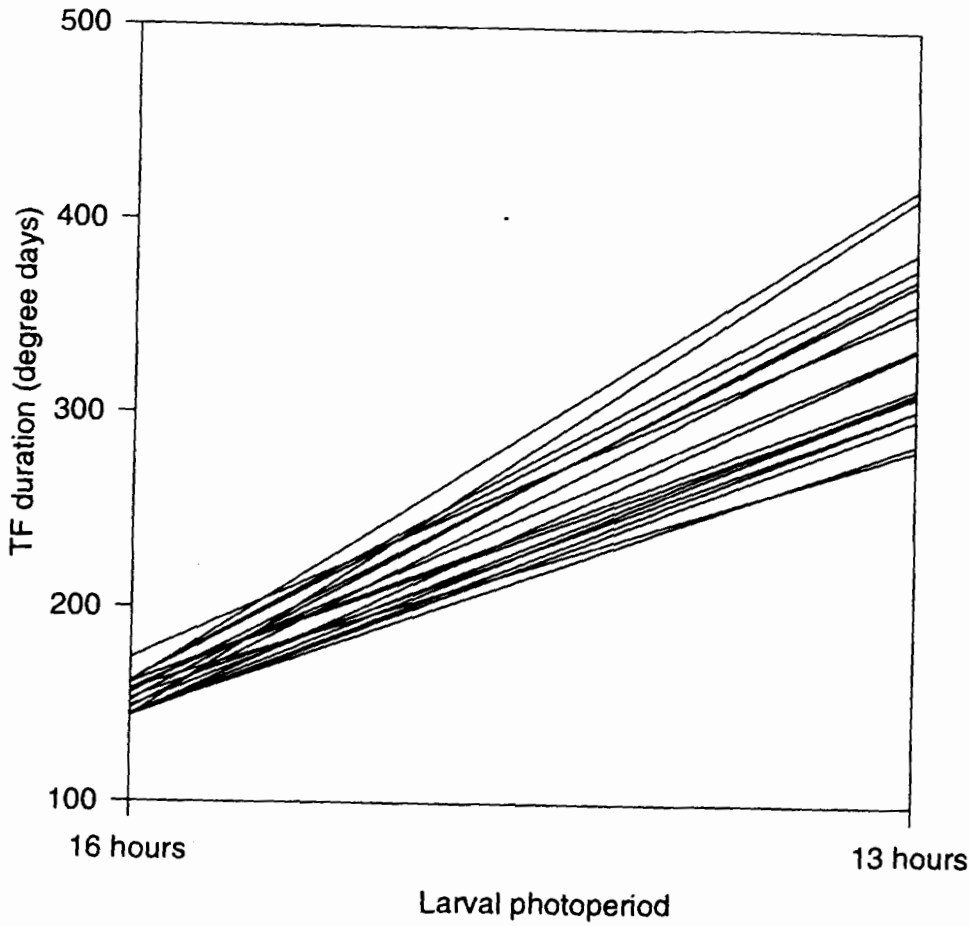

FIGURE 3.2. Mean tissue-feeding duration (T) for nineteen full-sib families of *Phyllonorycter mespilella* reared under summer (16 hours) and fall (13 hours) larval photoperiod. The lines in the figure connect family means for summer and fall larval photoperiods (reaction norms).

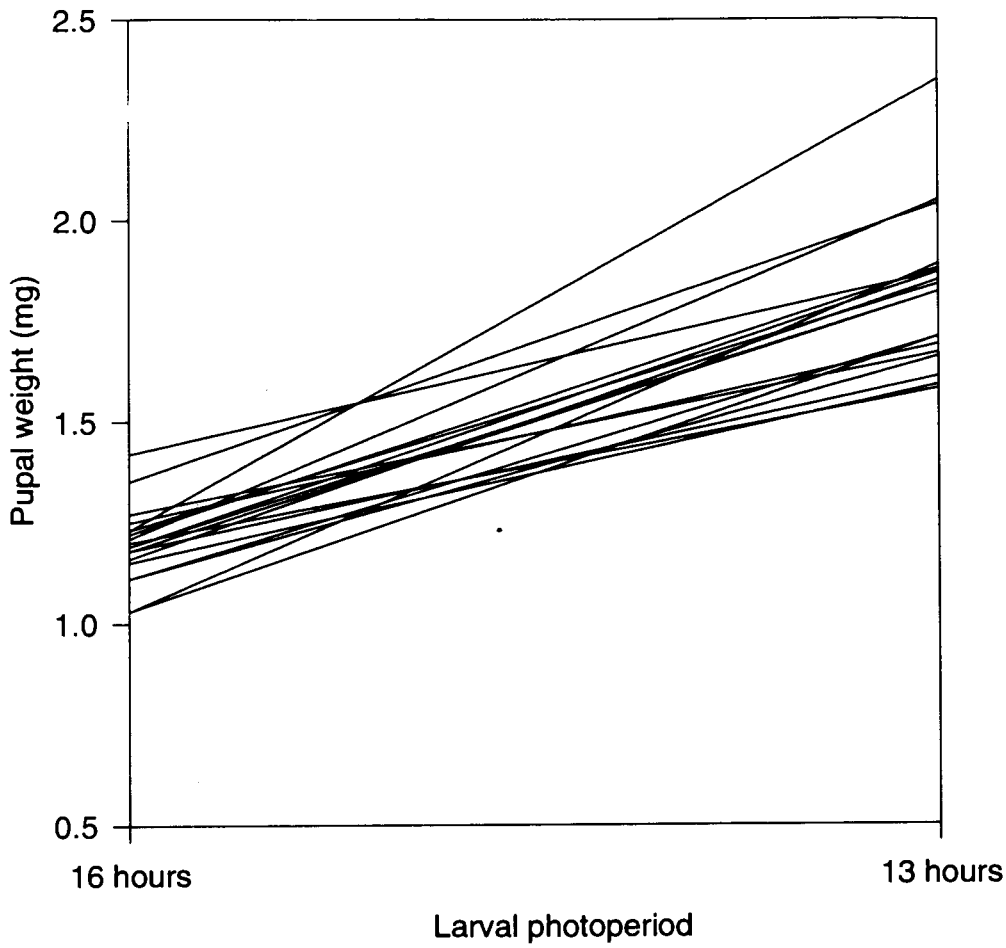

FIGURE 3.3. Mean pupal weight (PW) for nineteen full-sib families of *Phyllonorycter mespilella* reared under summer (16 hours) and fall (13 hours) larval photoperiod. The lines in the figure connect family means for summer and fall larval photoperiods (reaction norms).

Because the estimates of heritability are derived from full-sib families, they include, and may be inflated by, the contributions of dominance genetic variance and common environmental variance (Falconer 1989).

DISCUSSION

Fall photoperiod causes profound changes in the pattern of larval development in *P. mespilella.* The durations of both the SF and TF stages are prolonged, individuals pupate at a larger size, and pupae enter diapause dormancy. In another *Phyllonorycter* species, pupae were larger as were the larger fat bodies in diapausing individuals (Pottinger & Leroux, 1971). Longer feeding periods in larvae of *P. mespilella* destined for diapause may be required to accumulate fat reserves that are needed to survive the winter. The response of larvae to fall photoperiod explains the prolonged development times that have been frequently observed in diapausing generations of *Phyllonorycter* species (Pottinger & Leroux, 1971; Maier, 1984; Laing *et al.*, 1986; Barrett & Brunner, 1990a; Varela & Welter, 1992). The extensions of development time culminate in dormancy, and are thus components of winter diapause.

Maternal control of traits related to development time and diapause are common in insects (Mousseau & Dingle, 1991). The most common environmental cue that induces these maternal effects is photoperiod (Mousseau & Dingle, 1991). Fall maternal photoperiod caused increases in SF duration for *P. mespilella* larvae that were reared at fall photoperiod. This is evidence of a maternal influence on larval

development time in *P. mespilella*. However, the photoperiod experienced by females during emergence, mating and oviposition did not affect the TF duration or pupal weight of offspring in *P. mespilella*, or the SF duration of larvae reared at summer photoperiods. Larval photoperiod is clearly more important than maternal photoperiod in inducing diapause-related changes in larval development in this species.

Diapause was induced in nine larvae that were reared at summer photoperiod. These individuals always occurred in broods that contained other non-diapausing individuals, and were usually offspring of mothers from summer photoperiods (7 of the 9 cases). Female *P. mespilella* may "hedge their bets" (Seger & Brockman, 1987) by ovipositing mixtures of diapausing and non-diapausing eggs in summer generations, in order to ensure the survival of some offspring in the event of an early winter. Such "bet-hedging" oviposition strategies have been observed in a number of insect taxa (Tauber *et al.*, 1986; Mousseau & Dingle, 1991).

Heritability estimates could not be distinguished from zero except for the estimate for SF duration (S) measured at fall larval photoperiod. Because the families of leafminers in this experiment were reared on different host plants, estimates of h^2 may have been inflated by common environment effects caused by differences in host plant quality. However, if we assume that variation between seedlings was minimal (which is likely because of their identical rearing conditions), the heritability estimate for S suggests that genetic variation exists for this trait at fall photoperiods. No

evidence of genetic variation for TF duration (T) or pupal weight (PW) was found. Detection of a GxE interaction for SF duration indicates that genetic variation also exists for phenotypic plasticity in this trait.

Genetic variation apparently exists for SF duration, but not for TF duration, or pupal weight. GxE interaction across photoperiods may play a role in maintaining genetic variation for SF duration. Response to selection in different photoperiod environments may be constrained by the GxE interaction, resulting in the maintenance of genetic variation in SF duration. Genetic variation in quantitative traits can be maintained by GxE interactions because of disruptive selection acting on traits between environments (Via & Lande, 1987; Gillespie & Turelli, 1989). Heritable variation in phenotypic plasticity is detected by measuring GxE interactions, but is better quantified by estimating genetic correlations between "character states" of the trait measured in different environments (Via, 1984; Via & Lande, 1985; Via 1987; Falconer, 1989). Further evaluation of the hypothesis that a GxE interaction maintains variation in SF duration would benefit from more precise measurements of heritability, and from estimates of across-environment genetic correlations.

CHAPTER 4: PHENOTYPIC SELECTION ON LIFE HISTORY TIMING: THE INFLUENCE OF FREQUENCY-DEPENDENT ATTACK BY PARASITOIDS

Insect parasitoids are assumed to act as selective influences on a variety of traits in herbivorous insects (Jeffries & Lawton, 1984; Lawton, 1986; Bernays & Graham, 1988; Price *et al.*, 1980). The within-generation consequences of natural selection on quantitative traits can be determined by making direct measurements of phenotypic selection in natural populations (Lande & Arnold, 1983; Endler, 1986; Mitchell-Olds & Shaw, 1987; Schluter, 1988; Crespi, 1990; Wade & Kalisz, 1990). However, attempts to measure selection by parasitoids on traits in herbivorous insects have been rare (Rausher, 1992; Weis *et al.*, 1992). In this chapter, I describe measurements of phenotypic selection by parasitoids on the timing of life history events in a leafmining moth. In particular, I report on variation in phenotypic selection that is caused by frequency-dependent plasticity in the host-selection behaviour of parasitoids.

The fitness function, derived from measurements of phenotypic selection, formalizes the relationship between phenotype and a measure of fitness (Schluter, 1988). Phenotypic manipulations can be used to expand the range of phenotype over which the function is estimated in a natural population, or to increase the precision of the estimate in the tails of the phenotypic distribution where fewer data are available (Schluter, 1988; Anholt, 1991). The fitness consequences of phenotypic manipulations

have been studied for traits such as clutch size, male tail length, and testosterone levels in birds (Dijkstra *et al.* 1990; Andersson, 1982; Møller, 1994; Ketterson & Nolan, 1992), egg size in lizards (Sinėrvo *et al.*, 1992), and body size in damselflies (Anholt, 1991). Phenotypic selection, and fitness functions, can also be measured and compared between groups of individuals with manipulated phenotypic distributions. Moreover, detection of differences in phenotypic selection between treatment groups with differing phenotypic distributions can indicate the existence of frequency dependent selection (Schluter, 1994; Wade & Kalisz, 1990).

When predation or parasitism are the causes of selection, variation in phenotypic selection can result from variation in the behaviour of the predators or parasites. Many animals change their rates of acceptance for different prey types based on the local distribution of prey (Krebs & McCleery, 1984; Shettleworth, 1984; Stephens & Krebs, 1986). Such variation in prey-selection behaviour may, in turn, result in variation in natural selection on characters that determine the distribution of prey phenotypes. In animals with sequential life-history stages that vary in their risk of predation, natural selection should act on the timing of transitions between stages to minimize mortality from predation (Rowe & Ludwig, 1990). If the predator's attack rate on different life-history stages varies with the frequency distribution of those stages, then the form of selection on life-history timing will be frequency dependent.

Insect parasitoids often display oviposition preferences for particular

developmental stages of their hosts (Vinson, 1976; Vinson & Iwantsch, 1980; van Alphen & Vet, 1986; Strand, 1986; Godfray, 1994). This stage-specific oviposition can act as a selective influence on the timing of life history transitions (Chapter 2). However, oviposition behaviour in parasitic insects is often plastic, and can vary in response to the local distribution of host types (Mangel & Roitberg, 1989; Roitberg *et al.*, 1992; Li *et al.*, 1993; Roitberg *et al.*, 1993). When oviposition preferences vary with the distribution of hosts available, the form of selection on the timing of life history may change. The influence of variation in parasitoid behaviour on phenotypic selection can then be determined by comparing fitness functions between treatment groups of host insects with manipulated life history timing. Here, I present results of such a manipulation for *Phyllonorycter mespilella* (Hübner) a leafmining moth that feeds on the foliage of apple trees in the Okanagan Valley of British Columbia (B.C.), Canada (Cossentine & Jensen, 1992).

Phyllonorycter species complete two sequential stages during larval development: the earlier sap-feeding (SF), and later tissue-feeding (TF) stages (Pottinger & Leroux, 1971). *Pnigalio flavipes* (Ashmead) and a *Sympiesis* species (Hymenoptera: Eulophidae) are the most common parasitoids attacking *P. mespilella* in the Okanagan Valley of B.C. (Cossentine & Jensen, 1992). Parasitoids of these genera oviposit predominantly on TF larvae, and host feed predominantly on SF larvae (Askew & Shaw, 1979a; Maier, 1982; van Driesche & Taub, 1983; Laing *et al.*, 1986; Casas, 1989; Barrett & Brunner, 1990b; Varela & Welter 1992). Stage-specific

oviposition attack by Eulophid parasitoids on TF larvae can cause directional selection on the date of transition to the TF stage (TF date) in the diapausing generation of *P. mespilella* (Chapter 2). Individuals of the diapausing generation that have later TF dates have a higher probability of survival from parasitism than those that enter the TF stage earlier. This apparently occurs because the individuals with later TF dates complete the TF stage of larval development after the most intense period of parasitoid oviposition. However, overwintering survival is higher for individuals with earlier TF dates (Chapter 2). A trade-off may thus exist between avoiding parasitism and ensuring overwintering survival.

Parasitoid oviposition on SF larvae is more frequent in the fall generation than in summer generations in *P. mespilella* (Chapter 2). Seasonal increases in oviposition on SF larvae by Eulophid parasitoids have been observed in other *Phyllonorycter* species (Barrett & Brunner, 1990b; Varela & Welter 1992). Host-acceptance decisions by parasitoids attacking larvae of *Phyllonorycter* species may depend on the local frequencies of SF and TF larvae. SF larvae may be accepted more often for oviposition when TF larvae are rare, as is the case early in the fall generation. The survival advantage of delaying TF development to avoid parasitoid attack will be reduced if the probability of oviposition attack in the SF stage increases. Thus, phenotypic selection on TF date, and the form of the fitness function, may depend on the frequencies of SF and TF larvae.

In this study, phenotypic selection by parasitoids on SF duration in *P. mespilella* was measured in artificial patches of larvae with manipulated phenotypic distributions. Patches were created containing individuals with varying SF durations, resulting in different distributions of SF and TF larvae during the period of parasitoid attack. The main objective was to compare parasitoid oviposition, and resulting phenotypic selection on SF duration, between these patch types. The fitness function for SF duration was also estimated for individuals caged to exclude parasitoids, and heritability of SF duration was estimated for full-sib families of larvae. A secondary objective was to characterize a potential trade-off between survival from parasitism and overwintering survival by quantifying the relationship between SF duration, pupal weight and overwintering survival.

MATERIALS AND METHODS

Experimental design and data collection

Pupae of *Phyllonorycter mespilella* were collected during the second generation in August of 1993 in a research orchard located at Kelowna, B.C. At least three generations of *P. mespilella* are completed each year in the Okanagan Valley (Cossentine & Jensen, 1992). Leaves containing pupae were collected and returned to the laboratory. Pupae were dissected from leafmines, sexed, and placed individually in gelatin capsules. Each pupa was randomly assigned to a growth chamber (set at a temperature of 25°C) with either a 16-hour or a 13-hour, photoperiod.

Upon emergence, pairs of adult male and female *P. mespilella* were placed in pairs in gelatin capsules for 24 hours to induce mating. Mated female leafminers were transferred individually into oviposition cages that contained single apple seedlings 30-40 cm in height. After 24 hours the females were removed from cages, and the seedlings, with resulting full-sib families of leafminers, were held in the same growth chamber for six days. Oviposition occurred over a seven day period (August 17th to 23rd). At the end of the six-day pre-treatment, seedlings were transferred to a greenhouse, and held at ambient photoperiod until all other oviposition and photoperiod pre-treatments were complete.

On August 29th, all seedlings were returned to the orchard in Kelowna, B.C. Photoperiod pre-treatments were chosen to simulate different points in the growing season. Seasonal changes in photoperiod cause changes in the duration of development and mediate the induction of diapause in *P. mepsilella* (Chapter 3). The ambient photoperiod at Kelowna in early September is approximately 13 hours. Thus, individuals pre-treated with a 13-hour photoperiod experienced little change in photoperiod when they were placed in the orchard. In contrast, individuals pre-treated with a 16-hour photoperiod experienced a decrease in photoperiod when they were placed in the orchard. It was predicted that larvae treated with a 13-hour photoperiod would respond as if less growing time was available before winter, resulting in shorter SF durations. The larvae treated with 16-hour photoperiods were predicted to respond as if more growing time was available before winter, resulting in longer SF durations.

Twelve patches, each consisting of 10 seedlings on 1-meter high platforms, were placed at the base of separate orchard trees. On their platforms, the leaves of individual seedlings were at the same height as the lowest foliage in the orchard trees. Three types of patch were created: patches consisting of 10 seedlings pre-treated with 16-hour photoperiod, patches consisting of 5 seedlings from each of the two photoperiod pre-treatments, and patches consisting of 10 seedlings pre-treated with 13-hour photoperiod. These will be referred to as 16h, mixed, and 13h patch types, respectively. Each type of patch was replicated four times and all twelve were arranged in a rectangular grid with 15 meters between each position. Patches were randomly assigned to each position. In addition, two patches were created that contained 10 seedlings from each photoperiod pre-treatment. These patches were caged to exclude parasitoids, and located 15 meters from the edge of the treatment grid. All leafmines on the seedlings were individually marked and numbered.

Leafmines were checked daily for the transition between the SF and TF stages of development. The date on which the first longitudinal wrinkle appeared on the bottom surface of the leafmine was recorded as the date of transition to the TF stage (TF date). The duration of the SF stage was calculated and recorded for each larva as the number of days between the date of oviposition and the TF date.

After the TF dates were recorded, the seedlings were left in the orchard until October 27th. At this time, they were returned to the laboratory, and each marked

leafmine was dissected. Each individual was assigned to one of three categories: survived, parasitized, or other. Individuals that had survived were TF larvae or pupae that were alive at the time of dissection. Parasitized individuals had an associated parasitoid egg, larva, or pupa. Individuals in the "other" category had died of unknown causes.

Surviving pupae from the caged patches were weighed and placed individually in small plastic cups containing a moistened piece of cotton wick. These pupae were placed in an outdoor screenhouse for the duration of the winter. In May of 1994, these pupae were observed for adult emergence. Pupae that emerged successfully were recorded as having survived the winter. Those pupae that did not emerge were considered to have died during the winter.

Analysis of phenotypic manipulations

In order to determine if pre-treatment photoperiod and patch type affected variation in SF duration, least-squares analysis of variance (ANOVA) was conducted on SF duration for data from the twelve exposed patches (All statistical analyses were conducted using Systat, Version 5 (Wilkinson *et al.*, 1992)). Mean SF durations were compared between pre-treatment photoperiods and among patch types using Tukey tests. The number and proportion of SF and TF larvae present in each patch type was calculated for each Julian date during the experiment. The cumulative proportion of TF larvae appearing in the different patch types was compared by pairwise

Kolmogorov-Smirnov two-sample tests.

Analysis of survival from parasitism

Standard univariate selection statistics were calculated for SF duration for those individuals that survived the SF stage. Directional selection intensity was calculated as:

$$i = \frac{\overline{X}_a - \overline{X}_b}{s}$$

where $\bar{X}_b$ is the mean SF duration before selection, $\bar{X}_a$ is the mean SF duration after selection, and s is the standard deviation of SF duration before selection (Endler, 1986). Selection on the variance of the phenotypic distribution was quantified by calculating the statistic j from the formula:

$$j = \frac{v_a - v_b}{v_b}$$

where v_b is the phenotypic variance of SF duration before selection, and v_a is the variance after selection (Endler, 1986).

Values of selection statistics were calculated for each replicate patch in the experiment, and ANOVA was conducted on these values of i and j with patch-type as a factor. This analysis was done to determine if there were significant differences in the values of selection statistics among the three patch types. Data was then combined from the four replicates of each patch type to create pooled data sets for 16h, mixed and 13h patches. Values of i and j were calculated for these pooled data, and for subsets of these data from which individuals of one of the two mortality categories had been removed. The calculation of selection statistics from these reduced data sets allowed assessment of the contribution of individual mortality sources to phenotypic selection. Values of selection statistics that were calculated from pooled data were tested for significant differences from zero using standard tests.[1]

Survival functions were generated for each patch type using a program that fits cubic spline regressions to survival data (Schluter, 1988). The data were searched within the range -5 to 10 for the value of the smoothing parameter that minimized the Generalized Cross Validation score (Schluter, 1988). Standard errors around survival functions were estimated from the predicted values of cubic spline regressions fit to 200 bootstrap subsamples of the data (Effron, 1981; Schluter, 1988).

All measurements of selection, in this experiment, were made on individuals

[1] A two-tailed t-test was used to test the significance of values of i, and a two-tailed F-test was used to test the significance of values of j, as described in Endler (1986).

that survived the SF stage. Because the phenotype (SF duration) of individuals that die during the SF stage is never expressed, no information can be derived regarding selection during that stage. Phenotypic selection on SF duration may be caused by mortality sources that act during the SF stage, but it cannot be directly quantified.

Analysis of overwintering survival

Survival functions and selection statistics for SF duration and pupal weight were calculated as above for overwintering survival of individuals that survived to the pupal stage.

Heritability of SF duration

Heritability of SF duration for caged individuals from each photoperiod pretreatment was estimated as:

$$h^2 = \frac{2(\sigma^2_s)}{\sigma^2_t}$$

where σ^2_s is the among-sibship variance component, and σ^2_t is the total phenotypic variance, which is calculated as the sum of the among and within-sibship variance components (Falconer, 1989). Analysis of variance by full-sib family was conducted on SF duration separately for larvae from 13- and 16-hour photoperiod pre-treatments. Variance components were calculated from the among and within-sibship mean

squares from these analyses (Falconer, 1989). Standard errors of heritability estimates were calculated as in Falconer (1989).

RESULTS

Phenotypic manipulations

Mean SF duration was longer for individuals pretreated with a 16-hour photoperiod (45.4±0.4 days) than for those pretreated with a 13-hour photoperiod (41.7±0.4 days, $F=44.3$, $df=1$, $p<0.001$). Mean SF duration was shorter for 13h patches (40.9±0.4 days) than for 16h patches (45.1±0.4 days), or mixed patches (44.8±0.5 days, $F=27.6$, $df=2$, $p<0.001$). However, there was no significant difference in mean SF duration between 16h patches and mixed patches.

A larger proportion of larvae reach the TF stage at earlier dates in 13h patches than in mixed (Kolmogorov-Smirnov two-sample test, $p<0.05$) or 16h patches (Kolmogorov-Smirnov two-sample test, $p<0.01$) (Figure 4.1). Although there is no difference in mean SF duration between 16h and mixed patches, the time course of appearance of TF larvae varies between these patch types (Kolmogorov-Smirnov two-sample test, $p<0.01$). More larvae reach the TF stage at earlier dates in mixed patches than in 16h patches.

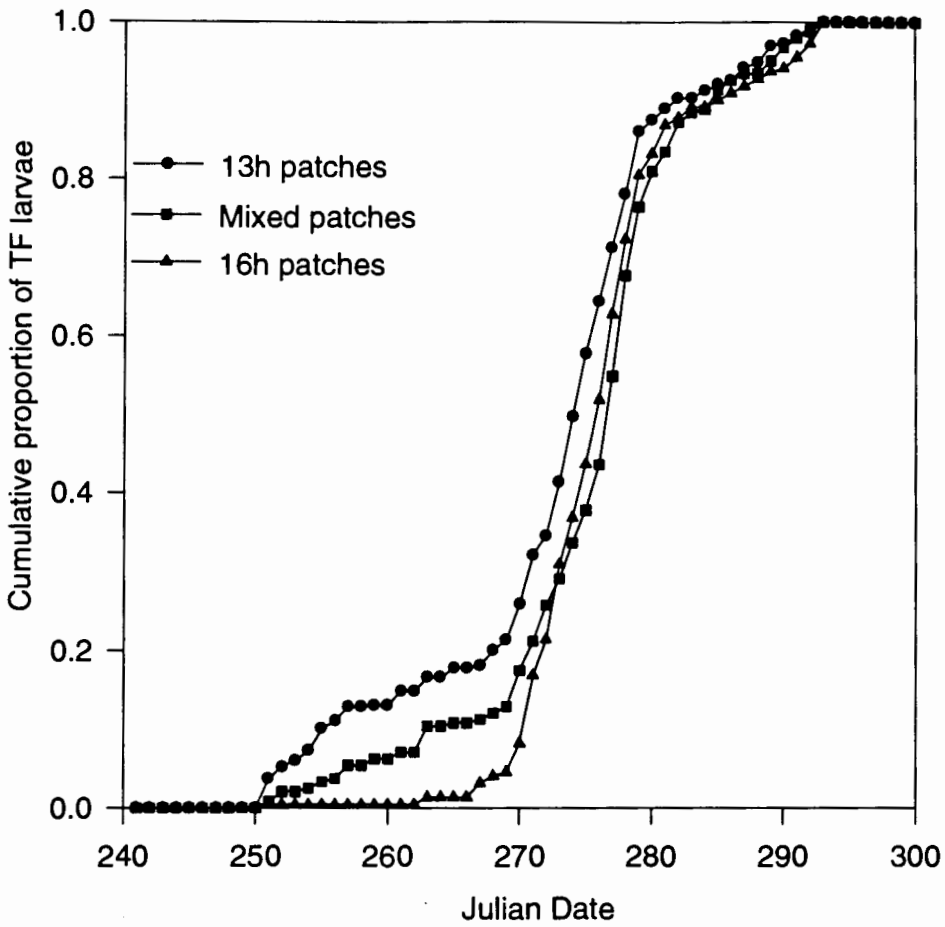

FIGURE 4.1: Comparison of cumulative proportions of tissue-feeding (TF) larvae appearing in artificial patches of larvae with manipulated development times. The figure shows the cumulative proportion of individuals within 13h, mixed and 16h patches that had completed the sap-feeding (SF) stage at each Julian date in 1993.

The pattern of parasitoid oviposition

Approximately equal numbers of parasitoid eggs were oviposited in SF and TF mines in mixed and 16h patches (Figure 4.2). More eggs were oviposited in TF mines, than in SF mines, in 13h patches (Figure 4.2). A larger proportion of parasitoid eggs were oviposited in TF mines in 13h patches than in either 16h patches (χ^2=14.7, df=1, p<0.001), or mixed patches (χ^2=15.4, df=1, p<0.001). The proportion of eggs oviposited in SF and TF mines did not differ between mixed patches and 16h patches (χ^2=0.001, df=1, p=0.98).

Phenotypic selection on SF duration

No significant differences in directional selection intensity (i) could be detected among the patch types when values were compared among replicate patches (F=0.78, df=2, p=0.49). Similarily, no significant influence of patch type was detected on values of the variance selection statistic (j) compared among replicate patches (F=3.18, df=2, p=0.09). However, because of low sample size (i.e. only four replicates of each patch type), the power of these tests was low, and the chance of Type II error was high (β>0.70 for i, and β=0.67 for j (Zar, 1984)). Further analysis will be presented for selection statistics calculated from pooled data for each patch type.

Positive directional selection on SF duration was detected for both 13h and mixed patches (Table 4.1). Values of directional selection intensity were positive and

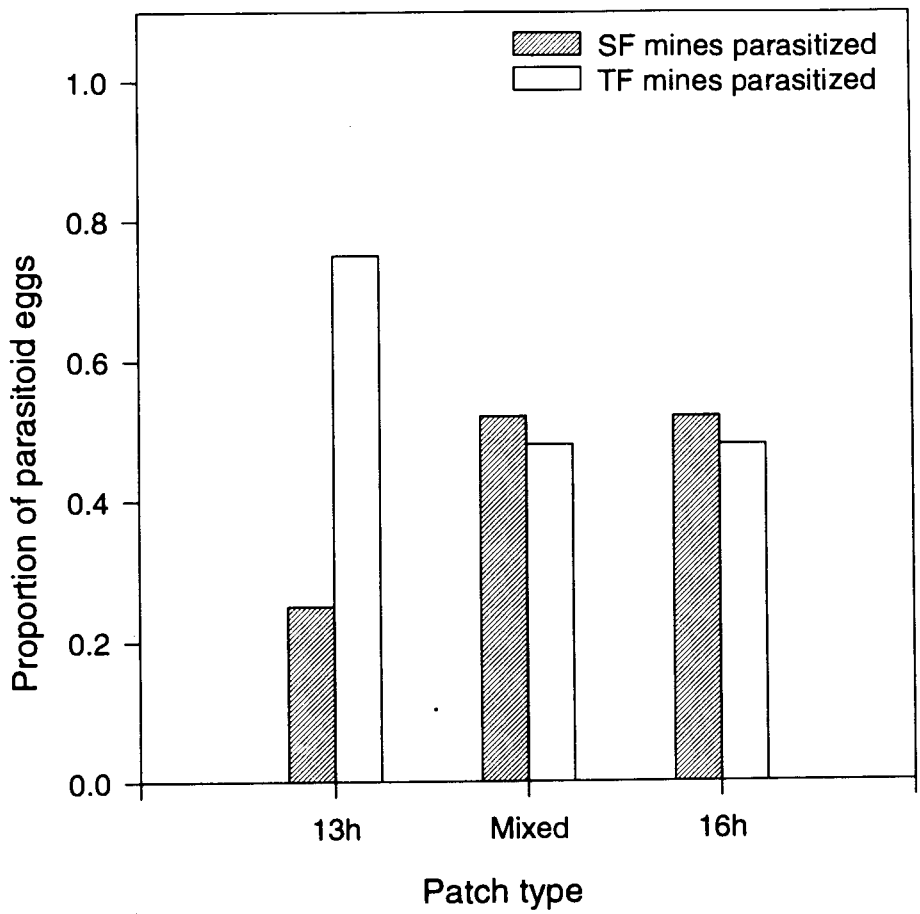

FIGURE 4.2: Proportion of parasitoid eggs oviposited in sap-feeding (SF) and tissue-feeding (TF) mines in 13h, mixed and 16h patches.

TABLE 4.1. Selection statistics for within-generation survival on sap-feeding (SF) duration for exposed patches (13h, mixed and 16h), and patches caged to exclude parasitoids. Directional selection intensity, i, and variance selection intensity, j, were calculated for all data for each patch type, and for data with only parasitism mortality, or other mortality, included.

Patch type	Data grouping	Selection statistics[2] i	j
13h	All	0.28 **	-0.21 ns
	Parasitism	0.18 ns	0.003 ns
	Other mortality	0.22 *	-0.27 *
Mixed	All	0.33 **	-0.35 *
	Parasitism	0.34 **	-0.37 *
	Other mortality	0.16 ns	-0.19 ns
16h	All	0.20 ns	0.14 ns
	Parasitism	0.22 ns	0.07 ns
	Other mortality	0.06 ns	0.10 ns
Caged	All	0.02 ns	-0.01 ns

[2] Statistics were tested for significant differences from zero using a two-tailed t-test for i, and a two-tailed F-test for j [ns=p>0.05, *=p<0.05, **=p<0.01].

significantly different from zero when calculated for pooled data for both of these patch types. When i was calculated for data with only parasitism mortality included, the value was significantly greater than zero for mixed patches, but not for 13h patches. When only other mortality was included, the value of i was significantly greater than zero for 13h patches, but not for mixed patches. Values of directional selection intensity calculated for 16h patches, and patches caged to exclude parasitioids, were not significantly different from zero.

Fitness functions for SF duration in 13h, mixed and 16h patches show an increase in survival probability with increasing values of SF duration (Figure 4.3). The increase in survival with higher SF duration is consistent with the detection of positive directional selection on SF duration in 13h and mixed patches. Although significant directional selection on SF duration was not detected in 16h patches, survival probability was still higher for individuals with longer SF durations. For larvae caged to exclude parasitoids, survival probability was high across the range of SF duration, with the exception of a dip in the function at SF duration equal to 35 days (Figure 4.4). Survival was virtually complete for individuals with SF durations less than 33 days. In exposed patches, survival probability was very low for individuals with SF durations between 20 and 30 days. Parasitism mortality, and mortality from other unidentified sources that are excluded by cages, are responsible for the differences between fitness functions for exposed and caged patches. These other sources of mortality may include host feeding by parasitoids on TF larvae.

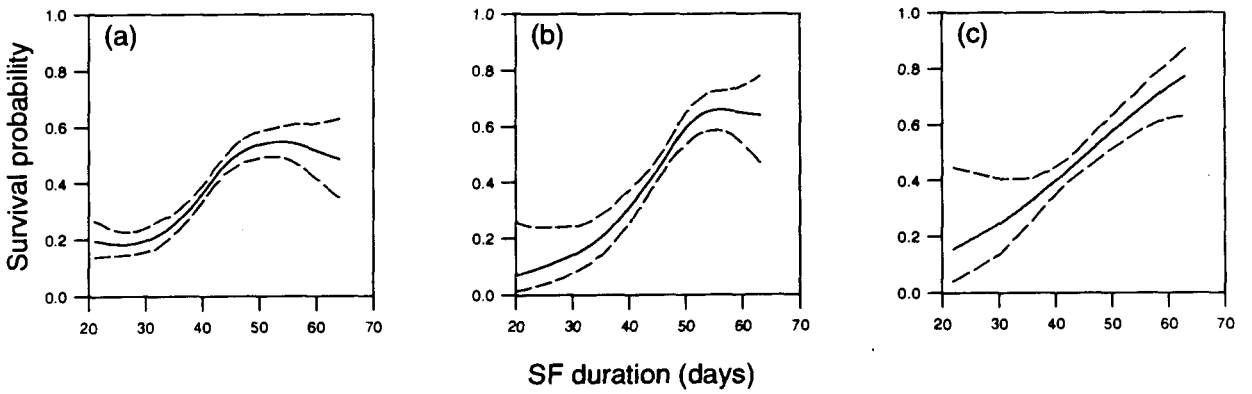

FIGURE 4.3: Fitness functions for within-generation survival *vs.* sap-feeding (SF) duration in (a) 13h patches, (b) mixed patches, and (c) 16h patches. Solid curves are the fitted cubic spline functions and dashed lines represent prediction intervals (±1 SE) for predicted values of the functions based on 200 bootstrap subsamples of the data sets.

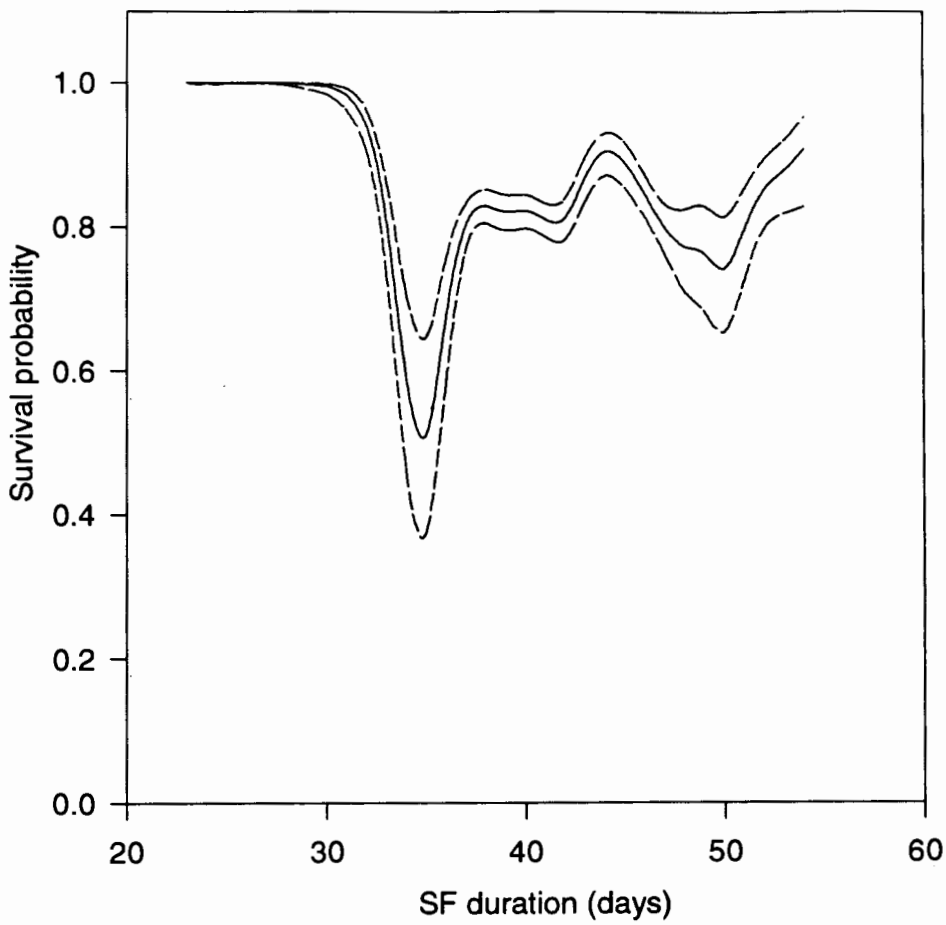

FIGURE 4.4: Fitness function for within-generation survival *vs.* sap-feeding (SF) duration in patches caged to exclude parasitoids. Solid curve is the fitted cubic spline function and dashed lines represent prediction intervals (±1 SE) for predicted values of the function based on 200 bootstrap subsamples of the data set.

Negative selection on the variance of SF duration was detected in mixed patches. Values of the variance selection statistic, j, were negative and significantly different from zero when calculated for all of the data, or for data with only parasitism mortality included (Table 4.1). Significant negative values of j can indicate the existence of stabilizing selection (Lande & Arnold, 1983; Endler, 1986). However, because negative values of j can also be caused by the reduction in variance associated with directional selection (Endler, 1986), and because no internal survivorship peak occurs in the fitness function for mixed patches, it is unlikely that stabilizing selection on SF duration occurred. In 13h patches, the value of j was only significantly different from zero when calculated from data with only other mortality included. Because a significant value of j was obtained only when calculated from a reduced data set this result will not be considered as evidence of selection on phenotypic variance in 13h patches. No values of j were significantly different from zero for 16h patches, or for patches caged to exclude parasitoids.

Overwintering survival, SF duration and pupal weight

Overwintering survival probability was higher in individuals with larger pupae (Figure 4.5). However, directional and variance selection statistics for pupal weight were not significantly different from zero (Table 4.2). Similarly, overwinter survival probability increases slightly with increasing SF duration (Figure 4.6), but no significant directional or variance selection on SF duration was detected (Table 4.2).

TABLE 4.2. Selection statistics for overwintering survival as a function of pupal weight and sap-feeding (SF) duration.

Life-history Character	Selection statistics[3] i	j
Pupal weight	0.04 ns	-0.23 ns
SF duration	0.07 ns	0.05 ns

[3] Statistics were tested for significant differences from zero using a two-tailed t-test for i, and a two-tailed F-test for j [ns=p>0.05].

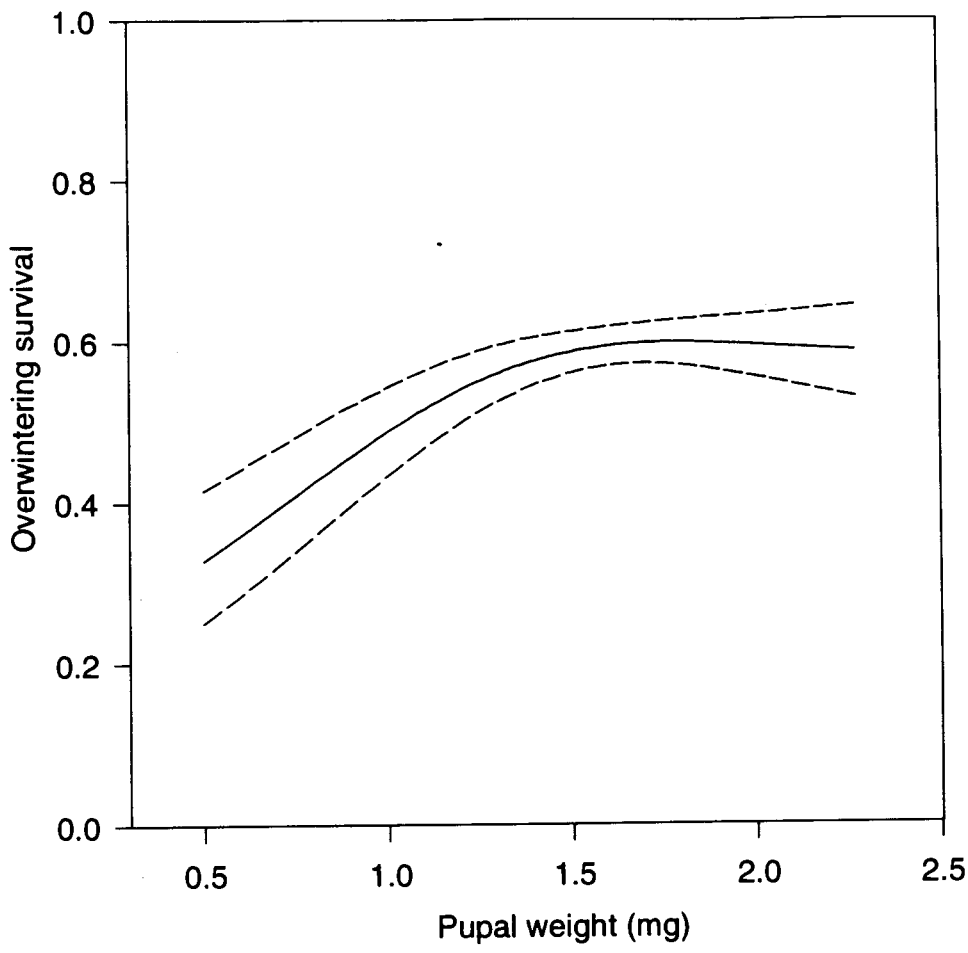

FIGURE 4.5: Fitness function for overwintering survival *vs.* pupal weight (PW). Solid curve is the fitted cubic spline function and dashed lines represent prediction intervals (±1 SE) for predicted values of the function based on 200 bootstrap subsamples of the data set.

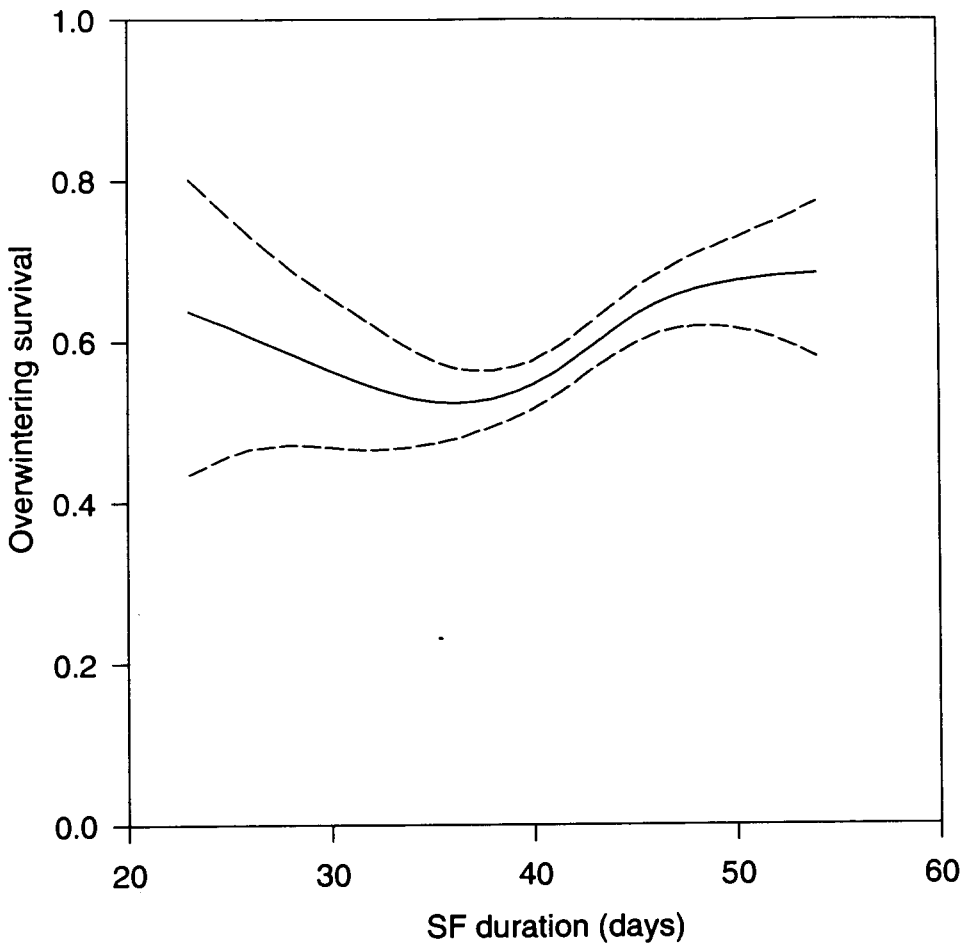

FIGURE 4.6: Fitness function for overwintering survival *vs.* sap-feeding (SF) duration. Solid curve is the fitted cubic spline function and dashed lines represent prediction intervals (±1 SE) for predicted values of the function based on 200 bootstrap subsamples of the data set.

Heritability of SF duration

Heritability (h^2) of SF duration was estimated separately for caged individuals from each of the two photoperiod pretreatments. For individuals pretreated with 13h photoperiod, h^2 was 0.75±0.19 (n=16 full-sib families), and, for individuals pretreated with 16h photoperiod, h^2 was 0.60±0.16 (n=21 full-sib families). Both estimates are large compared to their standard errors, and indicate the existence of additive genetic variation for SF duration. However, the estimates are derived from full-sib families and are may be inflated by the effects of common environments shared within sibships (Falconer, 1989). Because each family of leafminers, in this experiment, developed on the same host plant, differences in host plant quality may have caused variation between families to be overestimated.

DISCUSSION

Positive directional selection was detected on the duration of sap-feeding development in *P. mespilella* in this experiment. No variation in the magnitude of directional selection could be detected among patches with different relative frequencies of SF and TF larvae. However, significant positive directional selection was measured only in two of the three patch types (13 and mixed patches), and the causes of selection varied between these two patch types. Changes in the pattern of parasitoid oviposition may contribute to these differences in selection among patch types. Discussion below focuses on changes in parasitoid oviposition, differences in phenotypic selection among patch types, and the trade-off between survival from

parasitism and overwintering survival.

The pattern of parasitoid oviposition

Parasitoid oviposition on SF and TF larvae was frequency-dependent. The patch types varied in the time course of appearance of TF larvae, and thus in the relative frequencies of SF and TF larvae that occurred at particular times. Parasitoids searching concurrently in the three patch types presumably had different encounter rates with SF and TF larvae. Oviposition occurred on TF larvae more frequently when more TF larvae appeared earlier in the season (as in 13h patches). When fewer TF larvae were present early in the season (as in mixed or 16h patches), a larger proportion of parasitoid oviposition occurred on SF larvae.

Increases in the rate of oviposition on SF larvae by *Pnigalio* and *Sympiesis* species have previously been observed during the diapausing generation of *Phyllonorycter* species (Barrett & Brunner, 1990b; Varela & Welter 1992; Chapter 2). Because TF larvae appear later during the fall generation (Maier, 1984; Laing et al, 1986; Barrett & Brunner, 1990a; Varela & Welter, 1992), a seasonal increase in acceptance of SF larvae for oviposition may occur in response to a lower encounter rate with TF larvae. Seasonal changes in acceptance of SF larvae for oviposition may reflect an adaptive shift in larval-stage preference that is mediated by encounter rates with SF and TF larvae. Many parasitic insects alter oviposition preferences based on the availability of different host types (Mangel & Roitberg, 1989; Roitberg et al, 1992;

Li et al, 1993; Roitberg et al, 1993). Alternatively, female parasitoids may accept SF larvae more often in the fall simply because they are the most abundant host type during the period of search.

Much of the discussion concerning the mechanisms of frequency-dependent prey selection has focused on differential detection by predators of prey phenotypes having similar nutritional value (Gendron, 1987; Allen, 1988; Endler, 1988). Detection of rare phenotypes is lower because predators employ a search image for abundant prey types, because they reject novel prey types, or because experience with abundant prey types increases the rate of their detection (Murdoch *et al.*, 1975; Gendron, 1987). Frequency-dependent selection is also expected when prey phenotypes are of different value and equally detectable (Hubbard *et al.*, 1982; Greenwood, 1984). Above some critical frequency, the most valuable prey is taken exclusively. Below this threshold, less valuable prey types are incorporated into the diet dependent on the availability of preferred prey. This scenario is analogous to that of parasitoids searching for *P. mespilella* larvae. TF larvae are larger in size, and should be a more valuable host type for oviposition. TF larvae should always be used for oviposition when encountered, and SF larvae should be increasingly used for oviposition as the relative frequency of TF larvae decreases below some critical threshold.

Phenotypic selection on SF duration

No differences in the magnitude of selection statistics were detected when comparisons were made among the twelve replicate patches by patch type. However, significantly positive directional selection on SF duration was detected in both 13h and mixed patches, but not in 16h patches. In addition, the causes of directional selection varied between 13h and mixed patch types. Selection in 13h patches was caused by sources of mortality other than parasitism, and selection in mixed patches was caused by parasitism mortality. Explanations for variation in the causes and form of selection on SF duration are discussed below.

Although differences in the causes and outcomes of selection were observed between patches, the fitness functions are remarkably similar. All three fitness functions increase continuously with increasing SF duration. In the absence of parasitoids, survival probability was high across the range of SF duration, and no directional selection was detected. The differences in fitness functions, and in phenotypic selection, detected between caged and exposed patches must have been caused by mortality sources that were excluded by caging larvae. Parasitoid oviposition was identifiable as the cause of directional selection in mixed patches. Other types of parasitoid-induced mortality, like host feeding, may have caused selection in 13h patches.

Eulophid parasitoids of *Phyllonorycter* species host feed predominantly on SF

larvae (Askew & Shaw, 1979a; Maier, 1982; van Driesche & Taub, 1983; Laing *et al.*, 1986; Casas, 1989; Barrett & Brunner, 1990b; Varela & Welter 1992). However, in patches where TF larvae appear earlier, parasitoids may host feed more frequently on TF larvae. Unfortunately, it was not possible to identify host feeding as a mortality source when leafmines were dissected. The primary function of host feeding in parasitoids is to gather nutrients required for egg maturation (Jervis & Kidd, 1986). Newly-emerged female parasitoids may search for larvae primarily for host feeding, and not oviposition. In that case, individuals that enter the TF stage at the earliest date (short SF duration; 13h patches) may experience an increased risk of host feeding. Host feeding on the TF larvae that appear first would cause mortality of individuals with the shortest SF durations, resulting in positive directional selection.

The observed differences in phenotypic selection occurred between patches with different frequency distributions of SF duration. Parasitoids that encountered different relative frequencies of SF and TF larvae showed differences in the pattern of oviposition (and perhaps host feeding) with respect to these larval stages. The frequencies of individuals within a patch expressing various SF durations determined the eventual encounter rates of parasitoids with SF and TF larvae. This, in turn, determined the pattern of parasitoid attack, and the outcome of selection. Phenotypic selection on SF duration was thus frequency-dependent. The outcome of selection depended on the phenotypic distribution of SF duration.

Heritable variation in SF duration was measured in this experiment and it is possible that frequency-dependent selection by parasitoids plays a role in its maintenance. Frequency-dependent selection is predicted to maintain polymorphisms of characters related to predator detection in prey species (Greenwood, 1984; Gendron, 1987; Allen, 1988; Endler, 1988; Mani *et al.*, 1990). However, the different prey types considered here are sequentially occurring life history stages, and all individuals must express both prey "phenotypes". The frequencies of SF and TF larvae result from the phenotypic distribution of SF duration. The target of selection is thus SF duration, and not a character directly determining the prey phenotype to which the parasitoid responds. If frequency-dependent selection maintains genetic variation in SF duration, this must occur by a more complex mechanism than that previously considered for maintenance of prey polymorphisms.

The form of selection on SF duration should vary as the season progresses. The frequencies of SF and TF larvae encountered by searching parasitoids will change continuously in time as more individuals complete the SF stage. Parasitoids may change their pattern of attack on larvae in response to these temporal changes in the proportions of larval stages. Similarly, the risk of mortality in the SF or TF stage, and the form of the fitness function, will vary in time. Substantial variation in selection could result from variation in either the phenology of parasitoid attack or the phenotypic distribution of SF duration within a population. Direct measurement of phenotypic selection on SF duration that occurs during the SF stage is impossible

because the phenotype of individuals that die during the SF stage cannot be determined. Because of this empirical difficulty, and because of the apparently complex form of selection, examination of this problem in a theoretical context would be valuable.

Overwintering survival, SF duration and pupal weight

In all three patch types, individuals with longer SF durations had the highest probability of survival before winter. Because most growth in *Phyllonorycter* species takes place in the TF stage (Pottinger & Leroux, 1971), and because only a finite growth period exists in the fall generation (before the onset of winter), individuals that extend their SF durations may sacrifice productive growth time to escape parasitism. Growth in diapausing insects is often directed toward storage organs like the fat body (Tauber *et al.*, 1986; Danks, 1987). Nutrients stored in the fat body presumably function to increase overwinter survival. Diapausing pupae of *P. blancardella* have larger body sizes and larger fat bodies than non-diapausing individuals (Pottinger & Leroux, 1971). If individuals with larger body size, and more stored fat, have a higher probability of surviving the winter, then a trade-off may exist between survival from parasitism and overwinter survival.

P. mespilella individuals with the longest SF durations had a slightly higher probability of overwintering survival. Similarily, individuals with larger pupal weights had a higher probability of overwintering survival. However, overwintering survival

did not cause significant directional or stabilizing selection on either SF duration, or pupal weight. Growth costs associated with longer SF durations do not apparently reduce the probability of overwintering survival. The data does not support the hypothesis that a trade-off exists between survival from parasitism and overwintering survival.

Conclusions

In this study, variation in phenotypic selection on the timing of life history transitions in *P. mespilella* resulted from variation in the phenotypic distribution of larval-stage durations (SF duration). Changes in selection resulted from frequency-dependent changes in the pattern of attack by parasitoids. Variation in parasitoid attack, and the resulting variable selection on life history timing, may depend on the plasticity of oviposition preferences in response to the distribution of hosts. Plasticity of parasitoid behaviour may thus be an important cause of variation in phenotypic selection on traits in herbivorous insects.

CHAPTER 5: DYNAMIC HOST-UTILIZATION BEHAVIOUR IN PARASITOIDS: HOST FEEDING AND OVIPOSITION ON HOSTS WITH DIFFERENT FITNESS PAYOFFS

Much has been learned about the functional basis of behaviour in insect parasitoids by applying theoretical tools derived from evolutionary ecology. Because of the close relationship between oviposition and lifetime reproductive success, it is assumed that natural selection has a strong influence on oviposition behaviour in parasitoids. Methods from optimal foraging theory like dynamic optimization models (Mangel & Clark, 1988) have been used to study adaptive variation in oviposition behaviour. In these models, variation in optimal host selection and clutch-size is predicted as the age and egg load of the foraging parasitoid vary (Mangel, 1987; 1989; Mangel & Clark, 1988). Information external to the forager, like variation in the availability of hosts, can also influence optimal oviposition behaviour (Mangel & Roitberg, 1989; Roitberg *et al.*, 1992). Foraging females are assumed to display plasticity of behaviour, influenced by age, egg load, availability of hosts and other aspects of state, to maximize fitness by balancing current and future reproductive effort.

Many adult parasitoids feed on the hemolymph of their hosts (Jervis & Kidd, 1986). Host-feeding behaviour is most common in parasitoid taxa that mature eggs throughout their adult lives (synovigenic parasitoids) (Jervis & Kidd, 1986). The

nutrients derived from host-feeding in synovigenic parasitoids are primarily used for the maturation of eggs, and the most common type of host-feeding is "non-concurrent and destructive" (Jervis & Kidd, 1986). That is, different host individuals are used for oviposition and feeding, and host-feeding renders hosts unsuitable for later oviposition. Foraging female parasitoids are faced with the decision of whether to oviposit, or to host feed, when a host is encountered. This decision defines a behavioral trade-off between oviposition, to increase current reproductive output, and host-feeding, as an investment in future reproduction (Rosenheim & Rosen, 1992; Collier *et al.*, 1994; Heimpel & Rosenheim, 1995).

In many species, host-feeding and oviposition behaviour are size- or stage-specific (Barrett & Brunner, 1990b; Kidd & Jervis, 1991; Murdoch *et al.*, 1992; Rosenheim & Rosen, 1992; Godfray, 1994; Heimpel & Rosenheim, 1995). Oviposition is often confined to, or preferentially directed toward, larger or later stage hosts, while host-feeding occurs primarily on smaller or earlier stage hosts. In solitary parasitoids, the size of offspring resulting from oviposition on larger hosts is often higher than that from smaller hosts. Because body size and measures of fitness like lifetime fecundity in females and longevity in males are often positively correlated (Charnov *et al.*, 1981; King, 1987; Visser, 1994), there are clear fitness benefits to oviposition on larger hosts. Host-feeding may preferentially occur on smaller hosts because they are inferior as oviposition sites. This evolutionary explanation for size-selective host-feeding has been widely discussed (Kidd & Jervis, 1991; Murdoch *et al.*,

1992; Rosenheim & Rosen, 1992; Collier *et al.*, 1994; Godfray, 1994; Heimpel & Rosenheim, 1995), but, to date, it has not been examined in a formal theoretical context. Other adaptive explanations for size-selective host-feeding, like differences in handling times, or mortality costs, between small and large hosts, have been evaluated theoretically using simulation models (Kidd & Jervis, 1991).

Dynamic optimization models have been used to study both the physiological function of host-feeding (Chan & Godfray, 1993), and the influence of age and egg load on the decision to host-feed or oviposit (Collier *et al.*, 1994). Models presented by Collier *et al.* (1994) predict that parasitoids should host-feed only when completely devoid of eggs, unless there is a delay between host-feeding and maturation of eggs. Such delays occur because of the time required for conversion of nutrients into mature eggs. When even a short delay exists between host-feeding and the maturation of eggs, an egg-load threshold exists above which oviposition should occur, and below which host-feeding should occur. Parasitoids in these models encounter a single host type of uniform fitness value.

In this chapter, a similar model is presented in which two host types of different fitness value are encountered by searching parasitoids. The optimal pattern of host utilization is determined for these two host types. The primary objective of the study is to determine whether differential fitness contributions between host types can, of itself, provide a functional explanation for size-selective host-feeding and

oviposition. Because the distribution of hosts in the field is likely to vary, the model is also run under different rates of encounter for the two host types. A secondary objective is to determine if the optimal behaviours, predicted by the model, vary with the relative availability of high and low fitness hosts.

THE MODEL

Optimal behaviours are calculated for host encounters, under all combinations of egg load and age, by the technique of reverse iteration (Mangel & Clark, 1988). This model is directly analogous to the model of Collier *et al.* (1994) that has a one-time-step delay between host-feeding and egg maturation. In each time step, a female parasitoid either encounters or does not encounter a single host. On encounter with a host, a female can feed on the host, or oviposit an egg on the host. Females encounter two host types (1 & 2) with probabilities λ_1 and λ_2 (λ_{tot} is defined as the sum of λ_1 and λ_2). Attacks by parasitoids are assumed to have no effect on the availability of hosts in the next time unit. The model is run over a finite number of time steps which will be referred to as the time horizon (T), and which is equivalent to the length of a female's life. Eggs remaining in a female's abdomen at the time horizon are wasted and do not contribute to lifetime reproductive success. Females survive from one time step to the next with a constant finite probability, β.

The state variable x is defined as the number of eggs that a female has in her abdomen (egg load). It is assumed that there is a maximum capacity for eggs in a

female's abdomen (x_{max}). The state variable y represents a metabolic bank that is accumulated by host-feeding. Each host-feeding event adds one unit to y whether feeding occurs on host type 1 or host type 2. A maximum value of y is assumed beyond which host-feeding is not possible, because crop volume has reached capacity (y_{max}). Each unit of y produces an additional mature egg in each time step, but with a delay of one time unit after host-feeding has occurred. The fitness payoffs for oviposition onto type 1 and type 2 hosts are defined as FIT_1 and FIT_2. FIT_1 is assumed to be larger than FIT_2.

The dynamic programming equation is as follows:

$$F(x,y,t,T) = \beta*(1 - \lambda_{tot})*F(x+1, y-1, t+1, T)$$
$$+ \beta*\lambda_1*\max\{FIT_1 + F(x, y-1, t+1, T); F(x+1, y, t+1, T)\}$$
$$+ \beta*\lambda_2*\max\{FIT_2 + F(x, y-1, t+1, T); F(x+1, y, t+1, T)\}$$

where F(x,y,t,T) is the parasitoid's expected remaining reproductive success at time t for an egg load of x, and a value of the host-feeding bank of y. The first term of the equation describes time steps in which no host is encountered, and a single egg is matured from the host-feeding bank (x becomes x+1, and y becomes y-1, in the next time step). The second term describes encounters with hosts of type 1. The model calculates whether oviposition or host-feeding maximizes expected reproductive success. Regardless of the decision, an egg is matured from the host-feeding bank. Oviposition adds the fitness payoff FIT_1 to the equation, and host-feeding adds one unit to the host-feeding bank (Note that the value of y remains constant when host

feeding occurs because one unit of y is added by host feeding, and one unit is removed to mature an egg). The third term of the equation describes encounters with type 2 hosts. Again, the optimal decision is determined by calculating which behaviour returns the highest expected reproductive success. A fitness payoff FIT_2 is added for oviposition on type 2 hosts, but the fitness consequences of host-feeding are identical to those when type 1 hosts are encountered. Optimal behaviours for encounters with both host types are recorded for all values of x, y, and t. Egg maturation occurs at each time step regardless of host encounter except when the value of y=0, and no nutrients are stored for maturation of eggs.

There are no time costs for handling hosts incorporated in this model. Host feeding and oviposition are assumed to occur within the time unit in which a host is encountered. Under these conditions, rejection of a host will never maximize fitness. If a host is rejected for oviposition, it will always be accepted for host feeding because there is no handling cost. Parasitoids choose between feeding on a host, or ovipositing, at each encounter. Host rejection is thus not included in the terms for encounter with type 1 and 2 hosts in the equation above. Finite handling times for host feeding and oviposition occur in nature, and may vary between host types of different fitness payoff. However, they are not considered here. The model also does not consider competition between adult parasitoids. This may occur either by direct interactions between adults, or by competition for oviposition sites. No interactions between adults occur in the model, and no hosts are encountered that have been

previously parasitized or fed upon. The above simplifying assumptions disregard the effects of handling time and intraspecific competition in order to focus attention on the influence of fitness payoffs on the decision to host feed or oviposit.

RESULTS

Assuming no genetic constraints, the model shows that parasitoids will accrue higher fitness when they use hosts of higher fitness for oviposition, and not for host-feeding. Type 1 hosts are always accepted for oviposition except when either no eggs are available (x=0), or when the host-feeding bank is empty and only one egg remains in the abdomen (x=1 and y=0). This prediction for optimal utilization of type 1 hosts was consistent when the model was run for different levels of host availability (λ_{tot}), metabolic reserves (y), and relative fitness of host types (FIT_1 vs. FIT_2).

Optimal utilization of type 2 hosts depends strongly on the relative fitness payoffs for the two host types. Until late in the parasitoid's life, there is a constant asymptotic value of egg load above which oviposition is optimal, and below which host feeding is optimal. This value of egg load will be referred to as the oviposition threshold. As the fitness payoff for type 2 hosts increases, relative to that for type 1 hosts, the oviposition threshold decreases (Figure 5.1). When the fitness value of type 2 hosts is low, they are used for host-feeding at low egg loads, and for oviposition at high egg loads. At somewhat higher fitness values, type 2 hosts are used for oviposition except at very low egg loads. When the fitness value of the two hosts is

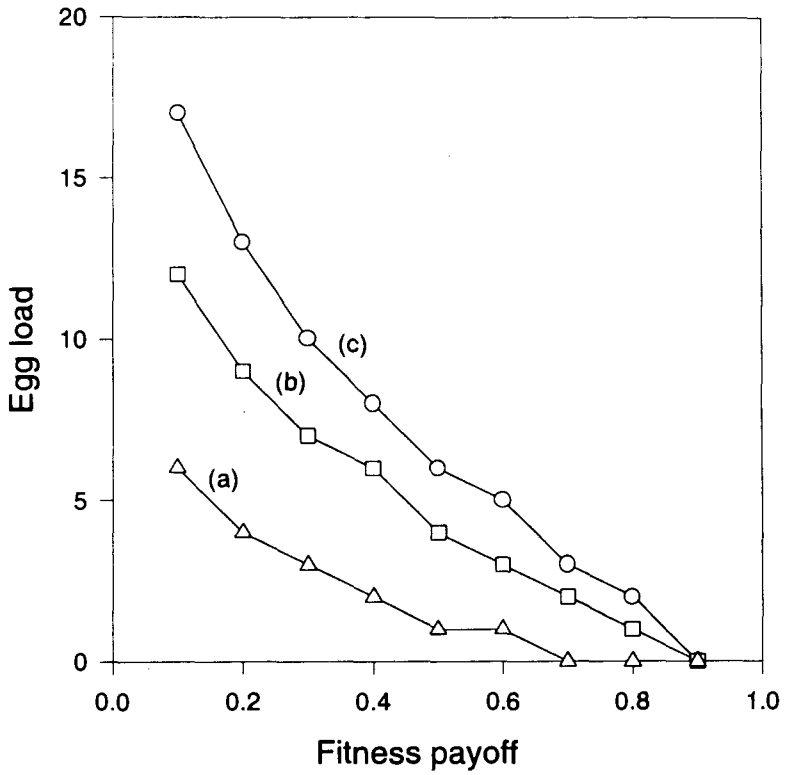

FIGURE 5.1: Egg-load thresholds for oviposition on type 2 hosts as a function of fitness payoff and host availability (λ_{tot}). Curves shown are for: (a) λ_{tot}=0.2, (b) λ_{tot}=0.6, and (c) λ_{tot}=1.0. Equal encounter rates with the two host types ($\lambda_1=\lambda_2$), and an intermediate value of the host-feeding bank (y=3) are assumed. Other parameter values are set at: FIT_1=1.0, β=0.99, x_{max}=20, y_{max}=5 and T=1000. At egg loads above the lines parasitoids accept type 2 hosts for oviposition, and at egg loads below the lines type 2 hosts are used for feeding.

similar there are no differences in the pattern of optimal host utilization for type 1 and type 2 hosts (eg. at FIT_2= 0.9 in Figure 5.1c). Under these conditions, host feeding only occurs on type 2 hosts at egg loads of zero, as is the case for type 1 hosts.

The predicted relationship between oviposition threshold and type 2 fitness payoff has the same decreasing trend for different values of overall host availability (λ_{tot}) (Figure 5.1). However, the oviposition thresholds are lower when host availability is lower. When hosts are relatively rare (Figure 5.1a), type 2 hosts are accepted for oviposition at lower egg loads than when hosts are more plentiful (Figure 5.1c). In addition, when hosts are rare, a larger fitness difference between host types is required before differences in host utilization behaviour are predicted (Figure 5.1a). When λ_{tot}= 0.2, the oviposition threshold is zero for type 2 fitness payoffs between 0.7 and 0.9. Under these circumstances, no difference in optimal behaviour is predicted for the two host types even with a 30% difference in fitness payoff.

Near the end of the parasitoid's life the oviposition threshold falls to zero (Figure 5.2). Females accept type 2 hosts for oviposition at lower egg loads as the time horizon approaches. This time dependence of the oviposition threshold occurs because eggs remaining in a female's abdomen at the time horizon do not contribute to fitness. It is optimal to accept lower quality hosts for oviposition, late in life, in order not to waste remaining eggs. The oviposition threshold for type 2 hosts is also lower when the contents of the host-feeding bank (y) are higher (Figure 5.2).

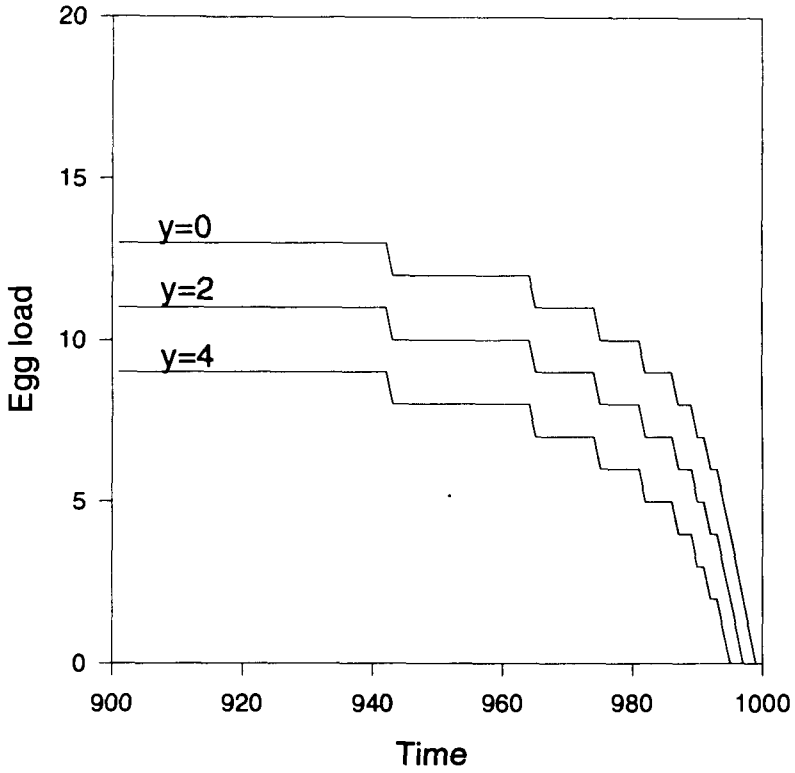

FIGURE 5.2: Egg-load thresholds for oviposition on type 2 hosts as a function of time. Simulations are shown for three values of the host-feeding bank (y). Other parameter values are set at: $\lambda_1 = \lambda_2 = 0.4$, $FIT_1 = 1.0$, $FIT_2 = 0.3$, $\beta = 0.99$, $x_{max} = 20$, $y_{max} = 5$ and $T = 1000$. At egg loads above the lines parasitoids accept type 2 hosts for oviposition, and at egg loads below the lines type 2 hosts are used for feeding.

Oviposition on type 2 hosts is optimal at lower egg-loads when more nutrients are stored that can be used to mature additional eggs.

The relative encounter rate with type 1 and type 2 hosts also affects the optimal behaviour for encounters with type 2 hosts. Host ratio is defined as the ratio of the encounter rate for type 1 hosts to that for type 2 hosts (host ratio = λ_1 / λ_2). The oviposition threshold for type 2 hosts decreases with host ratio (Figure 5.3). When type 1 hosts are rare compared to type 2 hosts (host ratios below 1), the threshold for oviposition on type 2 hosts is lower. This dependence of the oviposition threshold on host ratio is strongest when the fitness payoff for type 2 hosts is very low (Figure 5.3d). However, the same decrease in oviposition threshold with host ratio occurs when type 2 hosts have higher fitness payoffs (Figure 5.3a).

DISCUSSION

The model presented above provides a simple functional explanation for size-dependent host-feeding and oviposition in parasitoids. When there are differences in fitness value between hosts, it is predicted that higher-fitness hosts should be used for oviposition unless egg load approaches zero. Female parasitoids maximize lifetime reproductive success by ovipositing on larger hosts, which presumably have higher fitness value. Smaller, lower-fitness hosts are used for host feeding unless egg load is high, or the parasitoid is close to the end of its life, or high-fitness hosts are rarely encountered. When the lower-fitness host has a very low payoff relative to the

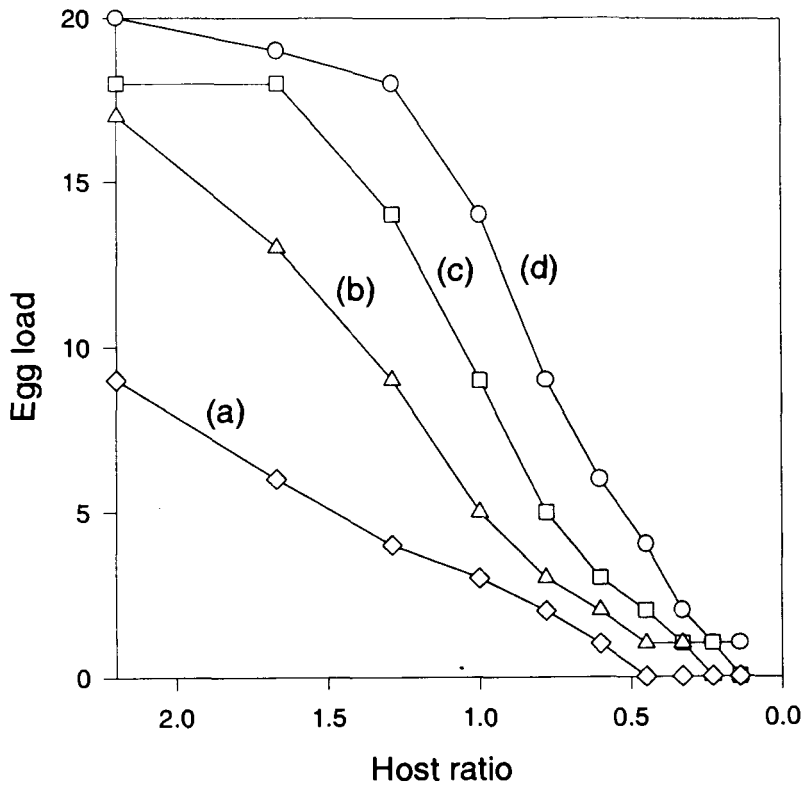

FIGURE 5.3: Egg-load thresholds for oviposition on type 2 hosts as a function of host ratio where host ratio is the ratio of the encounter rates of type 1 (λ_1) and type 2 hosts (λ_2). The overall availability of hosts is constant for the simulations shown (λ_{tot}=0.8). Results are shown for four values of the fitness payoff for type 2 hosts: (a) FIT_2=0.7, (b) FIT_2=0.5, (c) FIT_2=0.3, and (d) FIT_2=0.1. Other parameter values are set at: FIT_1=1.0, y=3, β=0.99, x_{max}=20, y_{max}=5 and T=1000. At egg loads above the lines parasitoids accept type 2 hosts for oviposition, and at egg loads below the lines type 2 hosts are used for feeding.

higher-fitness host, and overall host availability is relatively high, it is used almost exclusively for host-feeding. Under these circumstances, host-feeding and oviposition are specific to particular host types.

The theory presented here is directly analogous to that for optimal sex ratio in solitary hymenopterous parasitoids. Female parasitoids in the Hymenoptera can control the sex of their offspring, and often oviposit male eggs in small hosts, and female eggs in larger hosts (King, 1987). It is assumed that male fitness in parasitoids is not as closely related to size as female fitness. Thus, it is optimal for male offspring to be oviposited on, or in, smaller hosts. A size threshold is predicted below which only male eggs should be laid (Charnov *et al.*, 1981). A similar size-dependence of host feeding is predicted by the model presented here. Females host-feed on smaller, low-value hosts and oviposit on larger hosts. No sex-related differences in fitness for male and female eggs were incorporated into this model. It would be interesting to determine the optimal allocation of sex between host types in a model that included host feeding, and allowed females to determine sex when ovipositing.

The only previous theoretical study on size or stage-selective host feeding and oviposition in parasitoids focused on differences in handling time, and in mortality factors, between early and late-stage hosts (Kidd & Jervis, 1991). Simulation models predicted a selective advantage to host feeding on early life-stages if the handling time

for feeding on early-stage hosts was lower than that for late-stage hosts. It was also predicted that mortality of offspring would be higher for individuals that oviposit on early-stage hosts than for those that oviposit on late-stage hosts, resulting in selection for late-stage oviposition. Finally, if host feeding was confined to early-stage hosts, there was a reduced probability of offspring mortality from host feeding by the parent. These factors are likely to influence the evolution of stage-specific host feeding and oviposition. However, because of the direct impact on offspring fitness, it is probable that size-related differences in payoff between hosts also have a strong selective influence on host utilization behaviour. Empirical estimates of mortality factors, handling times and fitness payoffs for different host types are required to assess the relative importance of these influences.

Oviposition on low-fitness hosts is predicted to occur at lower egg-load thresholds when high-fitness hosts are rare. Lifetime reproductive success is maximized by accepting inferior hosts for oviposition when the probability of encountering high-quality hosts is low. A number of parasitic insects adjust their oviposition preferences according to the relative availability of different host types (Mangel & Roitberg, 1989; Roitberg *et al.* 1992; Li *et al.*, 1993; Roitberg *et al.*, 1993). Such changes in oviposition preference can cause differences in the intensity of attack on different sizes or life-stages of the host insect. For example, oviposition by parasitoids on early-larval stages of *Phyllonorycter* leafminers (Lepidoptera: Gracillaridae) is more frequent in the fall generation than in summer generations

(Barrett & Brunner, 1990b; Varela & Welter, 1992; R. McGregor, unpublished data; Chapter 2). This occurs because the late-stage hosts are encountered at a lower rate in the fall due to diapause-related changes in development time. Plasticity in parasitoid behaviour in response to the availability of host stages causes frequency-dependent oviposition on early and late larval stages that, in turn, leads to frequency-dependent selection on life-history timing in the host (Chapter 4).

Size-selective host feeding and oviposition can also have consequences at the population level. Density-dependent recruitment to the parasitoid population occurs when adult parasitoids destructively remove young immature individuals by host feeding (Murdoch *et al.*, 1992). As the density of adult parasitoids and the resulting rate of host-feeding on young immatures increases, parasitoid recruitment decreases because of the removal of potential oviposition hosts by host feeding. This "pseudo-density-dependent" effect can have either stabilizing or destabilizing effects on parasitoid-host population dynamics depending on parameter values (Murdoch *et al.*, 1992). The model of Murdoch *et al.* (1992) assumes that size-selective parasitoid behaviour is fixed. Plasticity of size-dependent behaviour in response to the host distribution may also have a substantial influence on population dynamics (Murdoch, 1994). Traditional theoretical explanations for the persistence of parasitoid and host populations have, for the most part, not been substantiated in empirical studies (Murdoch, 1994). Approaches that incorporate state-dependent plasticity of parasitoid behaviour may be vital in identifying new mechanisms for population stability (Mangel

& Roitberg, 1992; Murdoch, 1994).

This chapter presents a simple evolutionary theory for size-selective host utilization behaviour in parasitoids. Differences in fitness payoff between hosts of different sizes (or stages) cause host feeding to occur on smaller hosts, and oviposition to occur on larger hosts. Host-utilization decisions for lower-fitness hosts are dependent on age, egg load, and the availability of hosts. The model presented above makes the critical assumption that size and fitness are positively correlated in parasitoids.Laboratory measurements of lifetime fecundity in females, and longevity in males, indicate that this assumption is not unreasonable (Charnov *et al.*, 1981; King, 1987; Visser, 1994). However, measurements of the relationship between size and other components of fitness like searching efficiency, and measurements of parasitoid fitness in the field have only recently been attempted (Visser, 1994).

The decision to host feed or oviposit on a host by a searching parasitoid presents the opportunity to directly examine, through observations of individual behaviour, the trade-off between current and future reproduction (Rosenheim & Rosen, 1992; Collier *et al.*, 1994; Heimpel & Rosenheim, 1995). In this context, it has recently been shown that *Aphytis melinus* DeBach and *Aphytis lingnanensis* Compere (Hymenoptera: Aphelinidae) females host feed on smaller hosts and oviposit on larger hosts (Rosenheim & Rosen, 1992; Heimpel & Rosenheim, 1995). Clearly, there is a need for further empirical examination of the ideas presented here. It would

be particularily interesting to test the influence of host availability on the plasticity of these behaviours because of the potential consequences at the population level.

CHAPTER 6: THE INFLUENCE OF FREQUENCY-DEPENDENT PREDATION ON THE TIMING OF LIFE-HISTORY TRANSITIONS

Frequency-dependent natural selection occurs whenever the fitness of a phenotype expressed in a population varies with the relative frequencies of other phenotypes that are present (Endler, 1986). When prey selection by a predator varies with the frequencies of prey phenotypes that occur in a population, the form of natural selection on the prey character will be frequency-dependent. For example, positive frequency-dependent (or apostatic) prey selection occurs when the most abundant prey type is represented disproportionately in the diet of a predator (Clarke, 1969; Hubbard et al, 1982; Gendron, 1987). Less-abundant prey types have a higher rate of survival from predation until they become more abundant and the predator "switches" its foraging effort to the formerly rare prey (Murdoch et al, 1975; Gendron, 1987). This process can cause the maintenance of polymorphisms in a variety of prey characters by several mechanisms related to the foraging behaviour of predators (Clarke, 1964; Clarke, 1969; Ayala & Campbell, 1974; Hubbard *et al.*, 1982; Greenwood, 1984; Gendron, 1987; Allen, 1988; Endler, 1988; Mani *et al.*, 1990; Getty, 1993). This chapter presents a model in which frequency-dependent predation on different life-history stages indirectly maintains variation in the timing of life history in a prey species.

Any prey character whose phenotypic distribution in a population affects

predator detection or acceptance may be subject to frequency-dependent selection. Predators often feed at different rates on different life-history stages of their prey. The timing of transitions between life-history stages will affect the relative frequencies of individuals in different stages that are present in the population at any particular time. If the predation rates on different life-history stages vary with their relative frequencies, then frequency-dependent selection may occur on the timing of the life history. Consider a hypothetical life history in which a prey species develops through two life history stages that are both fed upon by a predator. We will assume that the predator can distinguish between prey individuals of different stages. The "phenotypes" that are subject to prey detection and acceptance are the sequential developmental stages that each prey individual must pass through. However, the target of selection, in this case, is a character determining the timing of the transition between stages, and not a character directly determining predator detection and acceptance.

Frequency-dependent selection of the type described above must occur by a more complex mechanism than that when prey phenotypes are specific to particular individuals in a population. Here, each individual allocates development time to two "phenotypes" which are subject to predation. The relative frequencies of early and late life stages will change continuously in time as more individuals make the transition to the second stage. If predation rates on the life-history stages vary with their relative frequencies, then the risk of predation in a particular stage will also vary continuously

in time. There may also be size-related differences in the fitness value of prey types for the predator. Predators may prefer prey from later life-history stages because they are larger and have a higher nutritional content. The discussion of mechanisms for frequency-dependent prey selection has mainly considered differential detection of prey types of similar fitness value (Gendron, 1987; Allen, 1988; Endler, 1988). However, frequency-dependent selection is also predicted to occur when prey are of different value and equally detectable (Hubbard *et al.*, 1982; Greenwood, 1987). Higher-value prey are preferred under most conditions, and lower-value prey are consumed below a critical frequency of the high-value prey (Hubbard *et al.*, 1982).

The evolution of timing in a two-stage life history is examined below using a genetic algorithm (GA) model. GA's are optimization models that employ a procedure analogous to biological evolution to solve design problems (Goldberg, 1989; Sumida *et al.*, 1990). Recently, GA's have been used to solve optimization problems in evolutionary biology similar to those previously addressed using dynamic programming (Sumida *et al.*, 1990). The GA procedure tests a population of individuals with different strategies against a fitness function over successive generations. Because strategies are optimized in a population of individuals in GA models, it requires little modifiction to make the fitness of strategies dependent on the frequencies of other strategies present in the population. The GA technique is thus ideal for testing ideas about frequency-dependent selection.

In the model presented here, predation occurs on individuals in early and late life-history stages. It is assumed that no differences in detection exist for the two stages, and that more growth occurs during the second stage. The model determines the stage durations that maximize survival and growth under these conditions. The main question being addressed is whether frequency-dependent predation on these two life-history stages can maintain variation in life-history timing. A secondary question is whether preference by the predator for the second life stage will affect optimal life history timing. Optimal allocation of time between life stages is compared between situations where predators adjust their acceptance of prey types exactly to the frequencies that are present, and situations where predators have different degrees of preference for the second life stage.

GENETIC ALGORITHMS

Genetic algorithm models test alternative designs, or strategies, against an objective function that tests the viability of designs. The strategy (or design) of interest is coded on a "chromosome". Each locus of the chromosome represents some aspect of the strategy and has a number of possible values. The loci are typically arranged in a linear array. The following operations occur in the course of running a GA:

1. A population of chromosomes is randomly generated usually including at least one copy of all possible strategies.
2. Fitness values are assigned to the chromosomes based on some objective (or

fitness) function.

3. Parent chromosomes are selected for the next generation by a weighted random procedure. Individuals with larger fitness are more likely to be selected for the next generation.

4. Crossing-over and mutation operators are applied to the selected chromosomes with some probability in order to introduce more variation into the population.

5. Fitness values are assigned to this new population using the objective function.

6. The procedure is iterated for a number of generations until an optimal strategy, or stable variation, emerges.

A number of biological terms are used in the description of these models. When using GA's to solve problems in evolutionary biology, it is important to emphasize that the chromosomes, mutations and crossing-over events are only analogous to the actual underlying biological processes.

THE MODEL

In this model, chromosomes encode the amount of time individuals will spend developing in two sequential life-history stages. The chromosome has two "loci" that encode integer values for days of development in life stages 1 and 2 (t_1 and t_2). A population of individuals is randomly generated (population size= n_{pop}), and the fitness of the chromosomes is evaluated by allowing the population to develop through a

simulation of one growing season. The main components of the simulation are growth functions for the two life stages, and an attack function for predation. For individuals that survive predation, fitness is evaluated as body size, which is the amount of growth that has occurred during the season. Body size is determined by the growth curves, and the development times encoded on the chromosome.

Growth functions for both life stages rise asymptotically to a maximum after which no further growth is possible (Figures 6.1). Body size as a function of time in the first stage of development ($s_1(t)$) is given by the equation:

$$s_1(t)=\max_1(1-e^{-0.1t})$$

where max_1 is the asymptotic size limit for stage 1. Body size as a function of time in the second stage ($s_2(t)$) is given by the equation:

$$s_2(t)=c*\max_2(1-e^{-0.1t})$$

where max_2 is the asymptotic size limit for stage 2, and c is a growth constraint factor. Growth in stage 2 is assumed to be constrained by the size of the individual at the end of the first stage of growth. The value of c is given by:

$$c=\frac{s_1(f)}{\max_1}$$

where $s_1(f)$ is the value of s_1 at the time of transition to stage 2. For all runs considered here individuals are assumed to have twice the growth opportunity in the second stage as they do in the first stage (max_1=30 and max_2=60). It is also assumed

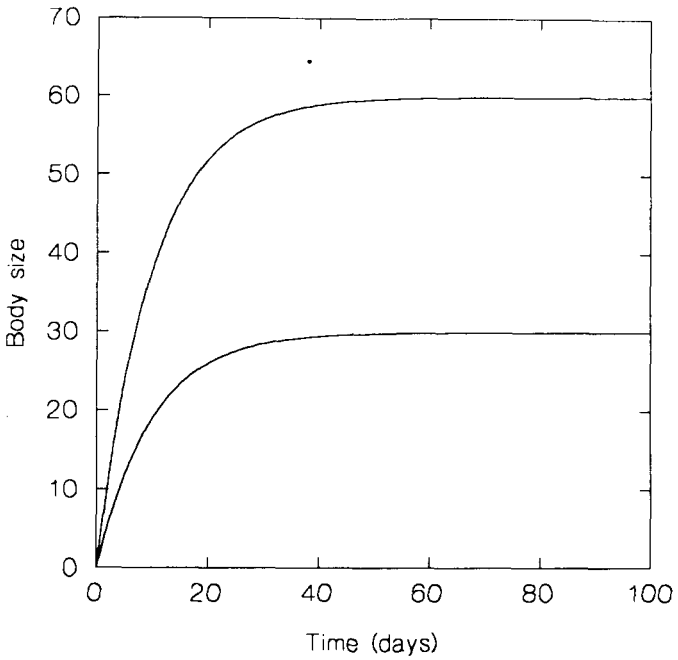

FIGURE 6.1. Growth functions for development of stage 1 and stage 2 individuals. The lower curve shows growth in stage 1, and the upper curve shows growth in stage 2. See text for equations for growth.

that food availability is high and that development is never impeded by a shortage of food.

The probability of predation (a) changes during the season as would be expected if predator feeding followed a seasonal phenology. The time-course of predation probabilty (a) is normally distributed across the period of development of the prey species, and is given by the equation:

$$a = \frac{1}{\sigma\sqrt{2\pi}} e^{-((t-\mu)^2/2\sigma^2)}$$

where μ is the value of time (t) at the peak of the function and σ is the standard deviation of a (σ=16 for all runs considered here). The peak of the predation function (μ) can be moved to different points of the season to simulate different phenologies of predation (Figure 6.2). The proportion of predation risk that occurs on stage 1 and 2 individuals is determined at each time step by the proportions of the two stages present in the population. That is, the total probability of predation is divided between the stages based on their current proportions. Under these conditions, predation risk changes through the season based on the predation function and the frequencies of the two life-history stages.

The proportion of predation that occurs on stage 1 individuals is varied using a preference factor (pref) (Figure 6.3). Increasing values of pref correspond to increasing levels of preference by the predator for attack on the larger stage 2

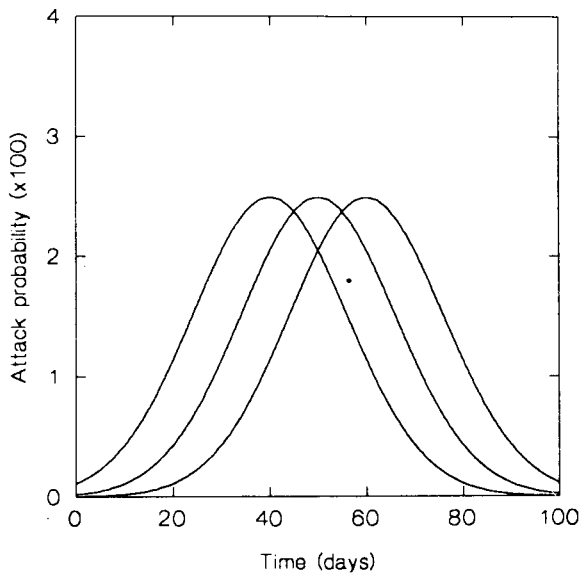

FIGURE 6.2. Probability of predator attack as a function of time during the growing season. Curves are shown for three values of peak predation risk (μ=40, 50, and 60). See text for predation equation.

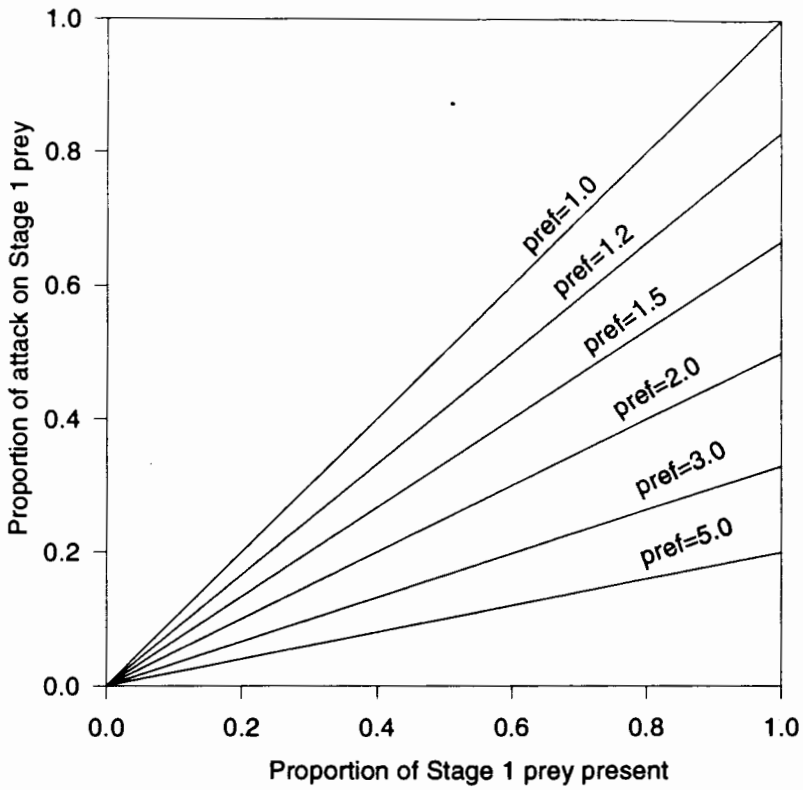

FIGURE 6.3. Proportion of predator attack that is directed toward individuals in life-stage 1 as a function of the proportion of individuals currently occupying life-stage 1 in the population. Curves are shown for six different values of the preference factor (pref).

individuals. When pref=1, predation risk is adjusted perfectly to the proportions of stage 1 individuals that are present in the population. This is considered to be analagous to a neutral form of frequency-dependent prey selection. When pref has higher values, the proportion of stage 1 individuals that are attacked by the predator decreases relative to the proportion present in the population. Predation occurs by a stochastic process for each individual in the population at each time step. The probability of predation for each life stage is calculated as described above, and these probabilities are used to determine survival from predation for each individual by a Monte Carlo process. Any individual that is attacked by a predator is assigned a fitness of zero. Surviving individuals complete growth, and are assigned fitness values, according to the growth functions and the program encoded on their chromosomes.

The growing season is a finite, deterministic period (T) after which no further growth can occur. Individuals are assumed to enter a non-feeding life stage after completing stage 2 development. The period immediately after the growing season may represent a period where reproduction occurs, or where individuals enter a dormant state in order to survive seasonally adverse conditions. Individuals with growth programs that exceed the length of the growing season are assigned a fitness of zero. It is assumed that they have missed a discrete opportunity for reproduction that occurs immediately after the growing season, or that they have died because they did not enter dormancy before the onset of adverse conditions. Individuals that complete

growth before the end of the season incur a fitness cost for metabolic maintenance (f_{loss}). This cost is subtracted daily from the accumulated body size for the period of time between the end of development and the end of the growing season (time=T).

Once values of fitness are assigned for all individuals in the population, parents are selected for the next generation. A weighted random procedure is used to select parents such that individuals with higher fitness are more likely to be chosen. Individuals are selected until a new population of size n_{pop} is filled. Crossing-over and mutation occur with probabilities p_c and p_m. When crossing-over occurs, alleles are exchanged between pairs of chromosomes in the selected population. This procedure increases the efficiency of search for optimal strategies by creating new combinations of alleles (Goldberg, 1989; Sumida *et al.*, 1990). Mutation causes random changes in the values of t_1 and t_2 on the selected chromosomes. The mutation procedure replaces alleles lost through fixation or deletion. Mutation rates are usually low in GA models because a high level of mutation can impede progress toward the optimal solution (Sumida *et al.*, 1990). No direct relationship is implied between crossing-over and mutation in this model, and the actual biological processes they refer to. The fitness for individuals of the new population is calculated as above. The entire procedure is iterated for a number of generations (n_{gen}), and the resulting strategies are recorded.

Common parameter values used for runs of the model are listed in Table 6.1. In the results of runs reported here, most parameters are held constant. Results are

presented for single runs of the model where populations of 500 individuals have evolved for 500 generations. The effects of variation in predator preference (pref) and phenology of predator attack (μ) on optimal development strategies are presented below.

TABLE 6.1. Common parameter values for the genetic algorithm model. Values of parameters for all runs of the model are listed below. Values of predator preference (pref) and peak of predation risk (μ) vary between runs and are discussed in the text.

Parameter	Symbol	Value
Population size	n_{pop}	500
Number of generations	n_{gen}	500
Maximum growth (stage 1)	max_1	30
Maximum growth (stage 2)	max_2	60
Length of season	T	100
Metabolic maintenance cost	f_{loss}	0.5
Cross-over probability	p_c	0.1
Mutation probability	p_m	0.0001

RESULTS

The position of the peak of predation risk (μ) influences the optimal development patterns that persist after 500 generations of selection. When predation occurs early in the season and is frequency-dependent (pref=1), the mean development times (t_1 and t_2) for both stages are relatively long (Figure 6.4). In contrast, when predation occurs late in the season, mean t_1 remains high, and mean t_2 occurs at a much lower level. With late-season predation, individuals are under selection to complete their development before the peak period of predator attack. This is reflected in a reduced allocation of time to stage 2 development. Substantially more variation in t_1 and t_2 remains after 500 generations for intermediate values of μ (Figure 6.4). Variation in t_1 is maintained over a range of values of μ (μ=30-60 days), and variation in t_2 is also maintained when μ=40 days. When predation occurs very early or very late in the season, little variation in t_1 and t_2 remains after selection (Figure 6.4).

When predators have a strong preference for stage 2 individuals (pref=3), little variation in t_1 or t_2 remains after selection across the entire range of μ (Figure 6.5). When pref=3, mean values of t_1 are higher for most values of μ than when pref=1. When μ is between 40 and 70 days, and pref=3, mean values of t_1 are extremely high. Because the risk of predation in stage 1 is low when pref=3, individuals remain in this stage during the peak period of predator attack in order to reduce predation during stage 2. When predation risk is concentrated near the end of the season

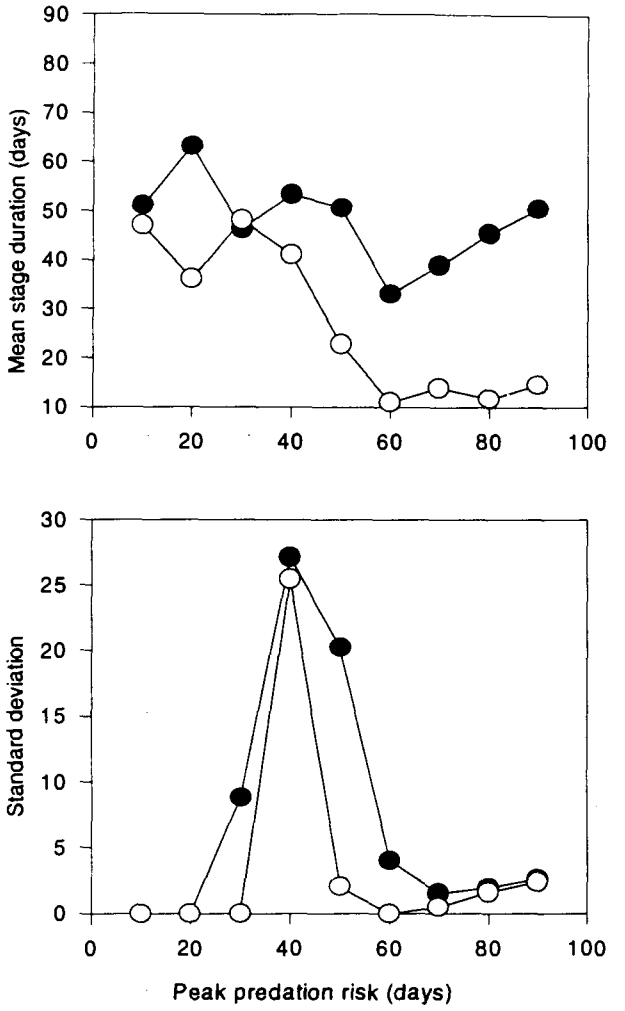

FIGURE 6.4. Means and standard deviations of stage durations (t_1 and t_2) as a function of the day during the season on which predation risk is the highest (peak predation risk (μ)) with predator preference (pref) set to 1. Black circles are for mean and standard deviation of t_1 and white circles are for mean and standard deviation values of t_2.

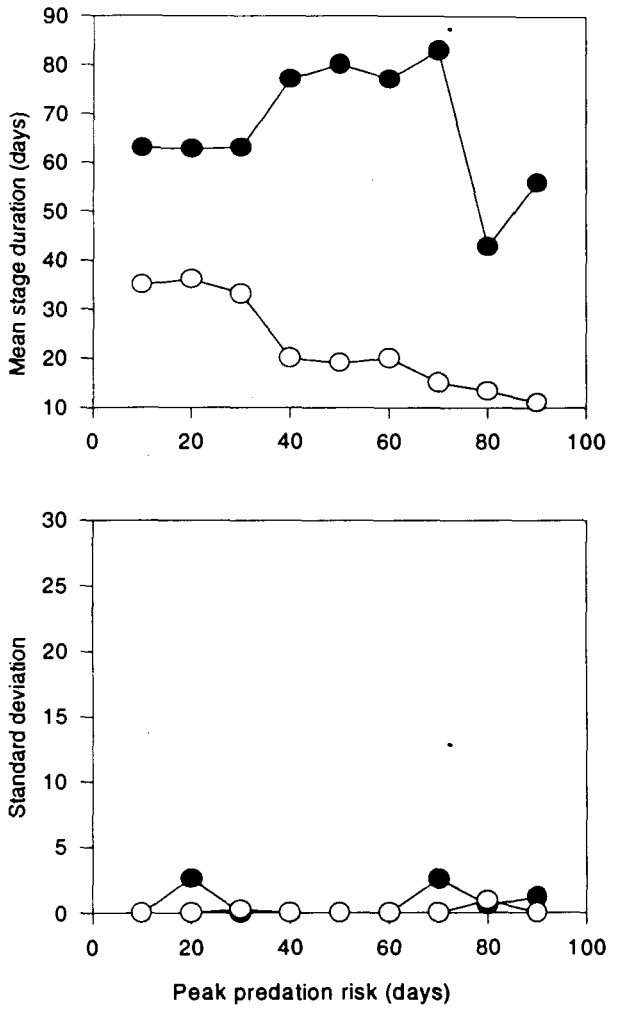

FIGURE 6.5. Means and standard deviations of stage durations (t_1 and t_2) as a function of the day during the season on which predation risk is the highest (peak predation risk (μ)) with predator prefererence (pref) set to 3. Black circles are for mean and standard deviation of t_1 and white circles are for mean and standard deviation of t_2.

(μ=80-90 days) the mean values of both t_1 and t_2 are relatively short because of selection to complete development before peak predation risk occurs.

Under frequency-dependent predation (pref=1), substantial variation in both t_1 and t_2 are maintained when predation risk reaches a maximum at μ=40 days. Considerable variation in t_1 is also maintained when pref=1 and μ=50 days. This study is primarily concerned with the maintenance of variation in life-history timing under frequency-dependent predation. Further results will be presented for runs of the model where predation risk occurs midway through the season (μ=40 and 50 days) and the highest level of variation in life-history timing is maintained.

After 500 generations of selection, several strategies persist for development in stage 1 and 2 when predation risk peaks at 40 days and pref=1 (Figure 6.6a). When a strong preference for predation on stage 2 (pref=3) exists, only one developmental strategy remains after 500 generations (Figure 6.6b). The value of t_1 is very long, and the value of t_2 is very short. With pref=3, time allocation to stage 2 is limited to the most productive period of growth as determined by the growth function (see Figure 6.1). Under the same conditions, the duration of stage 1 development by far exceeds the period of productive growth (Figure 6.1, Figure 6.6b). Stage 2 development is delayed until after the peak of predation risk as a strategy to avoid predation. When predation risk peaks at 50 days and pref=1, a wide range of strategies persist for stage 1 development after 500 generations of selection, while only short durations for stage

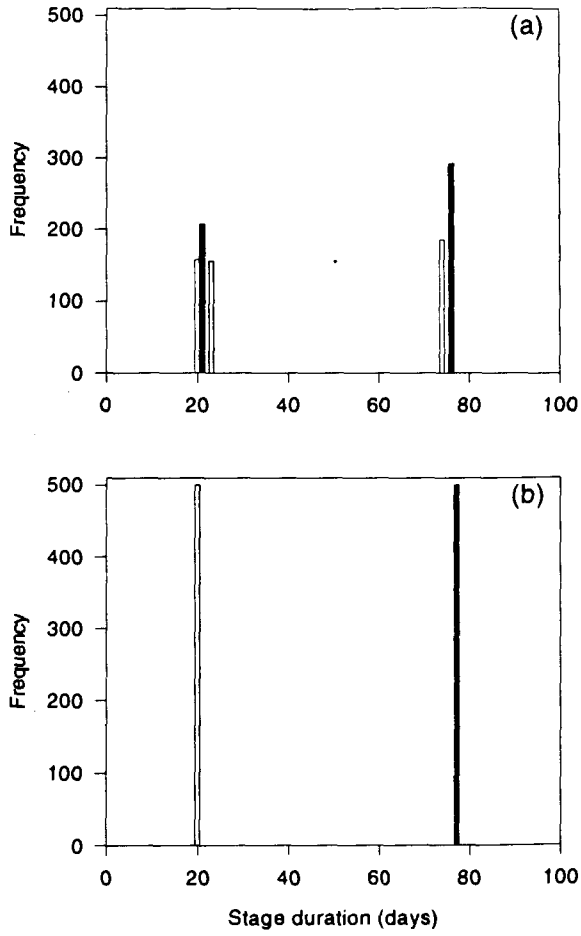

FIGURE 6.6. Frequency distribution of stage durations (t_1 and t_2) for two levels of predator preference with the day of peak predation risk (μ) set at 40: (a) pref=1 and (b) pref=3. Black bars are for surviving values of t_1. White bars are for surviving values of t_2.

2 remain (Figure 6.7a). When pref=3 and μ=50 days, only one developmental strategy remains after selection. Only individuals with a short duration for stage 2 and a long duration for stage 1 survive (Figure 6.7b).

Increasing values of predator preference cause a sharp decrease in the amount of variation that is maintained in t_1 and t_2 when μ=40 days (Figure 6.8). Mean t_1 increases and mean t_2 decreases as this variation is eroded, corresponding to the loss of short strategies for stage 1 development and long strategies for stage 2 development. A similar dependence of variation in t_1 on preference is observed when μ=50 days (Figure 6.9). However, this variation is maintained over a wider range of preference than when μ=40 days. When μ=50 days, little variation in t_2 is maintained even when predation is frequency-dependent.

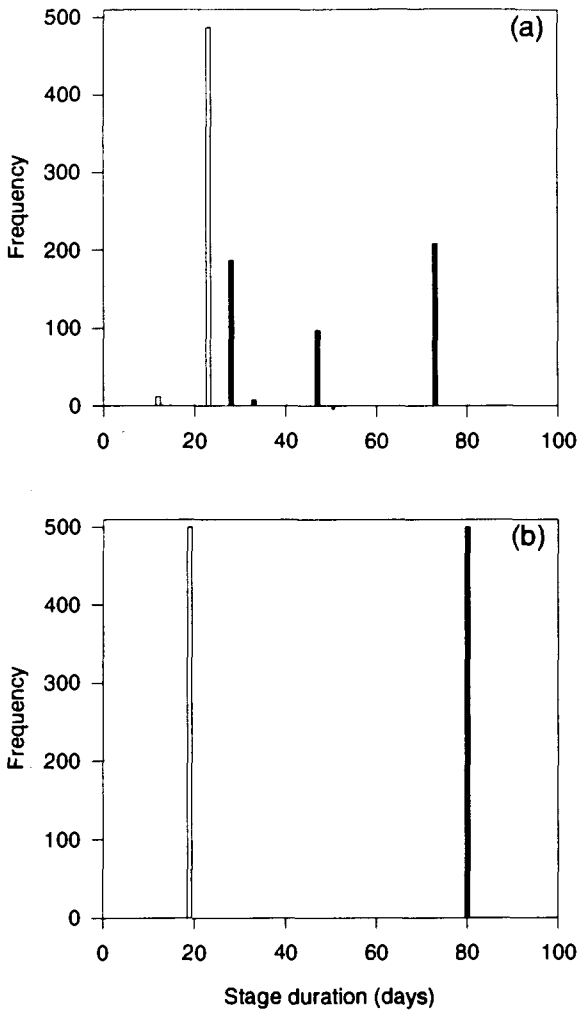

FIGURE 6.7. Frequency distribution of stage durations (t_1 and t_2) for two levels of predator preference and with the day of peak predation risk (μ)set at 50: (a) pref=1 and (b) pref=3. Black bars are for surviving values of t_1. White bars are for surviving values of t_2.

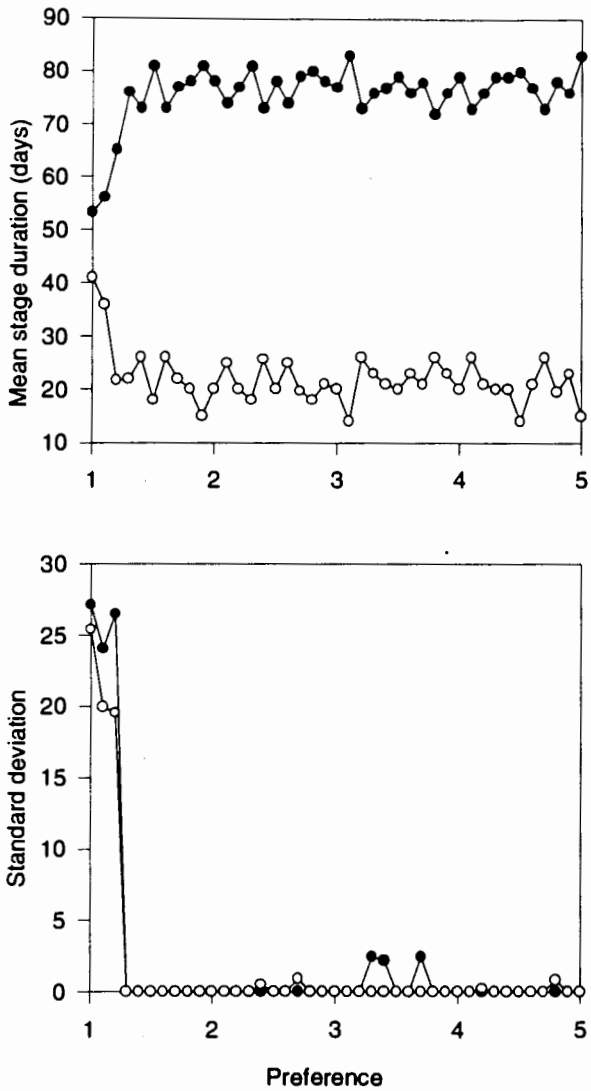

FIGURE 6.8. Means and standard deviations of stage durations (t_1 and t_2) as a function of predator preference with peak predation risk (μ) set at 40 days. Other parameters are as in Table 1. Black circles are for mean and standard deviation of t_1 and white circles are for mean and standard deviation of t_2.

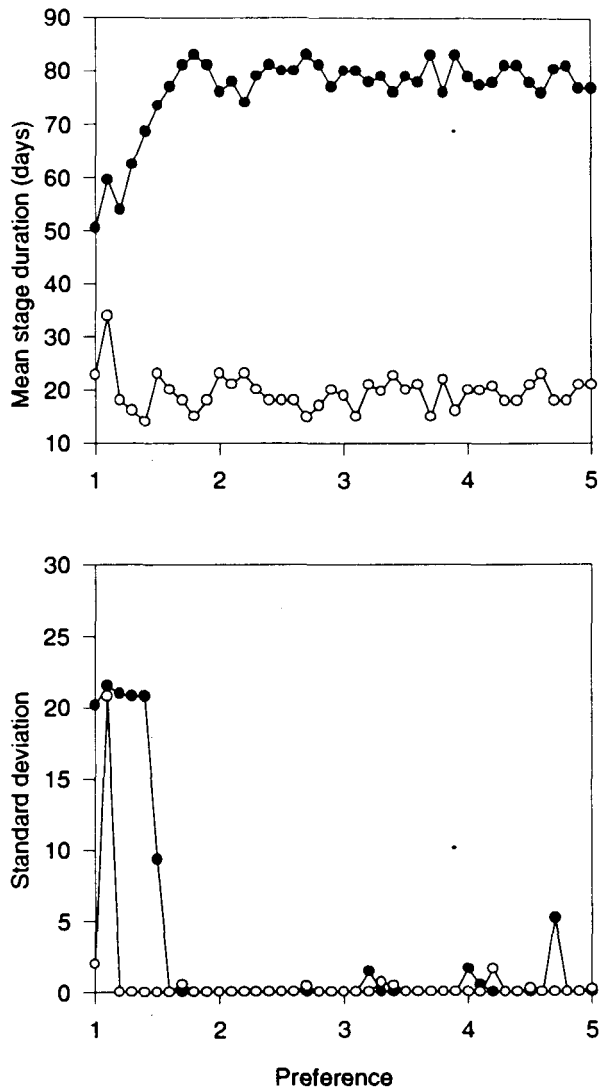

FIGURE 6.9. Means and standard deviations of stage durations (t_1 and t_2) as a function of predator preference with peak predation risk (μ) set at 50 days. Other parameters are as in Table 1. Black circles are for mean and standard deviation of t_1 and white circles are for mean and standard deviation of t_2.

DISCUSSION

The results of the model support the hypothesis that frequency-dependent predation on the two life-history stages maintains variation in the timing of the life history. When predator attack on the two life-stages occurs exactly according to the relative proportions of the two stages present in the population, a number of developmental strategies persist. As predator preference for the second stage increases, variation in stage-durations decreases rapidly. Variation is maintained by a neutral form of frequency-dependent selection. Frequency-dependent changes in the pattern of predation may occur because of adaptive adjustment of foraging effort by predators toward the relative abundance of prey types. Alternatively, predation may be frequency-dependent when predators show no preferences for prey types and simply accept prey in the proportions encountered. Whatever the mechanism, predation that occurs according to the proportions of the two life-history stages maintains variation in life-history timing.

The form of frequency-dependent predation that is presented here has not been previously discussed. Studies of frequency-dependent predation have focused on characters that are directly involved in prey detection and selection (Clarke, 1964; Clarke, 1969; Ayala & Campbell, 1974; Hubbard *et al.*, 1982; Greenwood, 1984; Gendron, 1987; Allen, 1988; Endler, 1988; Mani *et al.*, 1990; Getty, 1993). The character under selection in this study only indirectly determines the distribution of prey types that, in turn, causes changes in the pattern of predation. The phenotypic

distribution of early-stage development time (t_1) determines the frequencies of the two life stages that occur at any particular time in the season. The pattern of predator attack changes based on the current frequencies of the stages. This process causes maintenance of variation in the timing of the life history (i.e. in t_1 and under some conditions in t_2).

Adjustment of foraging effort based on the frequency distribution of prey types is quite common. Many predators adjust their prey preferences based on the local availability of different prey (Krebs & McCleery, 1984; Shettleworth, 1984; Stephens & Krebs, 1986). The distribution of host types also influences the acceptance of hosts for oviposition in parasitic insects (Mangel & Roitberg, 1989; Roitberg *et al.*, 1992; Li *et al.*, 1993; Roitberg *et al.*, 1993). When the prey types encountered by a predator, or parasite, are different life-history stages of the prey species, frequency-dependent selection of the type described above is predicted. However, it is likely that different sequential life-history stages will have different fitness value for predators because of size-related differences in nutritional value. If different life-history stages have different fitness payoffs, predators may prefer late life-history stages. Strong preferences for late stages will likely cause directional selection to extend the duration of the first stage, thus delaying the transition to the second stage until after the peak period of predation has passed.

The timing of life-history transitions is under selection to minimize mortality

risks and maximize growth benefits in different life stages (Rowe & Ludwig, 1991; Mangel & Ludwig, 1992). The evolutionary consequences of mortality by predation will be quite different depending on whether frequency-dependent selection or directional selection occur on stage durations. The form of selection will vary depending on a number of factors including the growth curves for different stages, fitness differences between prey types, and plasticity of predator behaviour. The phenology of predation also influences the nature of selection. The frequency-dependent effect discussed above is strongest when the peak of predation risk occurs midway through the life history. Empirical estimates of growth curves and fitness payoffs for prey, and the phenology and plasticity of predator attack, will aid in the assessment of the potential for selection in particular systems.

More to the point, the existence of frequency-dependent selection on life history timing is best demonstrated by directly measuring phenotypic selection in the field. Selection can be measured in groups of individuals with manipulated phenotypic distributions of life-history timing. Detection of differences in phenotypic selection between such treatment groups constitutes evidence for frequency-dependent selection (Wade & Kalisz, 1990; Schluter, 1994). Variation in phenotypic selection by parasitoids on life-stage durations in a leafmining moth was measured in artificial patches of larvae with manipulated life-history timing (Chapter 4). Frequency-dependent changes in host-selection behaviour of the parasitoids were apparently responsible for observed differences in selection. More measurements of this sort will

be required to determine how often this type of selection occurs in other taxa.

CHAPTER 7: GENERAL DISCUSSION AND CONCLUSIONS

The publication of a seminal paper by Price *et al.* (1980) heralded the pursuit of tri-trophic level approaches in the study of the evolution of interactions between plants and herbivorous insects. The intent of the paper by Price *et al.* (1980) was to focus attention on the probable role of parasitoids and predators as selective influences on traits in herbivorous insects. In the interim, the third trophic level has been incorporated increasingly into studies of plant-insect interactions (eg. Barbosa & Letourneau, 1988). However, quantitative studies of selection by third-trophic-level organisms on traits in their herbivorous hosts have remained rare (Rausher, 1992; Weis *et al.*, 1992).

In this thesis, measurements were made of phenotypic selection by parasitoids on the timing of life history in a leafmining moth. Variation in selection was detected between populations, seasons, and generations. Results from a manipulative experiment (Chapter 4) indicate that variation in selection was likely caused by variation in the host selection behaviour by the parasitoid. Considerable attention has focused recently on the dynamic nature of host-selection behaviour in parasitic insects (Mangel 1987, 1989; Mangel & Clark 1988; Mangel & Roitberg 1989; Roitberg *et al.*, 1992; Roitberg *et al.*, 1993). Evolutionary consequences of variation in parasitoid behaviour for the host species have not previously been addressed. Changes in the pattern of oviposition behaviour occurred in response to the distribution of hosts

encountered by searching parasitoids (Chapters 2 and 4). Frequency-dependent parasitoid attack on the SF and TF larval stages caused variation in phenotypic selection on the timing of the life history (Chapter 4).

Evidence from sib-analysis indicates that genetic variation for the duration of the SF stage exists (Chapters 3 and 4). Several potential mechanisms have been proposed for the maintenance of genetic variation in life history traits (Roff, 1992; Stearns, 1992). A GxE interaction for SF duration across photoperiodic environments may play a role in maintaining variation. However, the conditions under which GxE interactions can maintain variation in quantitative traits have not yet been resolved (Via & Lande, 1987; Gillespie & Turelli, 1989). Frequency-dependent selection is another mechanism by which variation can be maintained in quantitative traits (Clarke, 1964; Clarke, 1969; Ayala & Campbell, 1974; Hubbard *et al.*, 1982; Greenwood, 1984; Gendron, 1987; Allen, 1988; Endler, 1988). Frequency-dependent variation in oviposition behaviour by parasitoids may cause maintenance of genetic variation in SF duration in *P. mespilella* (Chapter 4). Frequency-dependent predation on sequential life-history stages can maintain variation in the timing of stage transitions under a variety of conditions (Chapter 6).

This study focused on phenotypic selection that occurred on the life-history timing of *P. mespilella* in the final generation of the year. This focus was motivated by the frequent observation of changes in life-history timing in fall generations of

Phyllonorycter species (Pottinger & Leroux, 1971; Maier, 1984; Laing *et al.*, 1986; Barrett & Brunner, 1990b; Varela & Welter, 1992). The timing of life-history events is more variable in diapausing larvae of *P. mespilella* than in non-diapausing larvae (Chapter 3). Selective influences may vary between different generations on traits in a multivoltine insect. Because individuals from summer generations must emerge and locate a mate in order to reproduce, there may be selection to synchronize the timing of juvenile development in the summer. Individuals from the fall generation have no requirement for emergence until the spring of the following year. As a result the timing of larval development may be less constrained in the diapausing generation. With the constraint of reproductive timing removed, selective factors like parasitoids may have a stronger influence on life-history timing in the fall generation. This may explain why selection by parasitoids was detected in fall, but not summer generations (Chapter 2).

Both dynamic host utilization behaviour by parasitoids and variation in stage durations of hosts are mechanisms that potentially stabilize parasitoid-host population dynamics (Smith & Mead, 1974; Murdoch, 1994). This study provides an evolutionary explanation for the persistence of both of these phenomena. Parasitoids should use hosts of different fitness value for host feeding or oviposition depending on the relative availability of different host types (Chapter 5). Theory predicts that optimal behaviours will change as the relative frequencies of low and high-value hosts vary. The resulting frequency-dependent host-selection behaviour, in turn, leads to

maintenance of variation in stage durations (Chapters 4 and 6). The methods of evolutionary ecology may be important in identifying new mechanisms for population regulation in parasitoid-host systems (Murdoch, 1994). Integration of the theoretical approaches of evolutionary ecology and population dynamics is currently at a very early stage (Mangel & Roitberg, 1992; Murdoch, 1994), but clearly deserves further attention.

Because insect parasitoids often display preferences for particular life-stages of their hosts for oviposition (Vinson, 1976; Vinson & Iwantsch, 1980; van Alphen & Vet, 1986; Strand, 1986; Godfray, 1994), parasitoids may often act as selective influences on life-history timing in herbivorous insects. Response to selection by parasitoids will depend on variation in the form, strength and direction of phenotypic selection, as well as on the genetic architecture of the traits. Direct measurements of selection in other taxa are required to determine the incidence of selection on life-history timing by parasitoids, and to further assess the general importance of parasitoids as selective influences on traits in herbivorous insects.

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