CHAPTER 5. PREDATION OF <u>HELICONIUS</u> EGGS AND LARVAE

In Chapter 4, larval growth ability on the different types of host plant is discussed. This is a principal component of host plant quality as experienced by the immature stages of <u>Heliconius</u>. In this chapter a second major component will be discussed: predation and parasitism of <u>Heliconius</u> eggs and larvae as influenced by the host plant, or "host plant related" predation. Both aspects of host plant quality are intimately related, as will be discussed in Chapter 6.4b.

Several instances of host-plant-related predation and parasitism in <u>Heliconius</u> are discussed in Benson, et al. (1976) and Gilbert (1975). These authors regard this type of predation on <u>Heliconius</u> as a biotic antiherbivore defense on the part of the plant, pointing out that <u>Passiflora</u> vines actively secrete nectar which attracts many kinds of predaceous insects. Some of these insects are known to consume the eggs and larvae of <u>Heliconius</u> as well as the nectar, thus benefitting the plant beyond the cost of producing the nectar. It is believed that predaceous ants and hymenopteran parasitoids are the chief participants in this mutualistic interaction with <u>Passiflora</u> (Benson et al. 1976; Gilbert 1975; Bentley 1977). It is also conjectured that <u>Heliconius</u> have counter-adapted to these defenses by avoiding host plants with ants on them and by placing their eggs such that they are relatively safe from predation (Benson et al. 1976). However, beyond these general observations, little is known about the details

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of these interactions.

By comparing the types of predation characteristic of the different Passiflora species it may be possible to add to this knowledge somewhat. For instance, does the type of predation (ant or parasitoid) correlate with the structure of the extrafloral nectaries? These nectaries are extremely variable in their size, shape, and placement on the different Passiflora species, and it is plausible that these differences are functional in attracting different types of predaceous insects. Similarly, it should be possible to correlate the types of predation with egg placement behavior in the female Heliconius, assuming that egg placement is a response to predation pressure as suggested above.

Carnivorous and parasitic insects are known to be a major factor controlling the populations of herbivorous insects in nature. Southwood (1974) has proposed that the type of agent regulating herbivore populations (1974) is in part a function of herbivore abundance, which may then result in more than one stable equilibrium between insect and host plant. For example, an insect pest species may have a stable low-density equilibrium which is maintained for long periods and then switch to a high-density equilibrium which will also persist unless interfered with. At each equilibrium the insect is primarily controlled by a different factor. Southwood proposes that predation and intraspecific competition for food are two such factors, but suggests that there may be more than two factors operating

on any given insect population. While there is no evidence in this
study for direct intraspecific competition (see Chapter 3.4 and 6.3)
the possibility remains there will be different types of predation in
the low-density forest habitat as opposed to the high-density second-
growth habitat. While it will not be possible to prove that the
observed differences are strictly a function of Heliconius or
Passiflora abundance as opposed to habitat quality, the results will
be suggestive as to how natural populations of insect herbivores are
regulated.

Host-plant-related predation may also help to explain why
H. melpomene is host-specific on P. oerstedii. It is not sufficient
to argue that because Passiflora is abundant in H. melpomene's
habitat, H. melpomene is able to select the host plant that yields
the highest growth rate. While it may well be the case that P.
oerstedi is the most favorable plant in terms of larval growth, there
are still several other species of Passiflora that yield high growth
rates but are not used by H. melpomene (see Chapter 4.4a). This type
of host specialization has been termed "ecological monophagy"
(Gilbert 1976). If it were found that due to predation, the eggs
and larvae of H. melpomene have much higher rate of survivorship on
P. oerstedii than the other available Passiflora, the basis of the
ecological monophagy would be explained. In addition, such a finding
would suggest that insects become host-specific not to increase their
digestive efficiency or growth rate per se, but to more effectively

avoid predation (see also Brower 1958). By specializing on a single host plant, an insect would be able to specialize its predator avoidance tactics to the predators associated with that plant.

The host-plant-related predators and parasitoids (and the resulting predation) were investigated at La Selva in three different ways, as will be described in turn. These are: studies of (1) larval survivorship at La Selva, (2) predators of Heliconius found on Passiflora plants, and (3) egg placement by ovipositing Heliconius. The implications of the results with regard to the structuring of the Heliconius-Passiflora community will be discussed in Chapter 6.2 and 6.3.

5.2 Larval Survivorship at La Selva

There are two principal reasons for observing the larvae of Heliconius under natural conditions at the field site. One is to monitor developmental rates, as described in Chapter 4.3c. The other is to directly measure mortality rates in the larvae under observation. Ideally, larval survivorship on the different host plants under natural conditions should be a good approximation to the "quality" of those host plants, combining the effects of host plant palatability and host-plant-related predation. However, individual variation in survivorship is of course very great, and a great many larvae would have to be observed for significant differences to be obtained.

Fifty-five larvae were observed at La Selva during fall, 1976. Thirteen of these were moved from the garden area to plants in the field; the other 42 were found on <u>Passiflora</u> in the field and were not touched. None of the transferred individuals survived for more than three days. In several instances it was found that the act of examining the larvae caused the entire host plant to be damaged by some unknown animal; thereafter care was taken to minimize contact with the host plant. The larvae were periodically re-visited every two days and their stage of development recorded. The probability of surviving two days or more was calculated for each instar, as was the average number of days survived. Taking the data for the last four instars together it is possible to compare survivorships for first instar and later-instar individuals. As shown in Figure 5.1, later-instar individuals have a significantly higher survivorship ($p < .001$). Aside from this, no statistically significant trends were found in the data. Mortality in <u>Heliconius</u> larvae is high, on the order of 95%, if the data in Figure 5.1 is extrapolated throughout larval life. As in many organisms the younger stages appear to be more vulnerable to mortality than the larger individuals.

5.3 Predators and Parasitoids of Heliconius Found on Host Plants

As described in Chapter 2, <u>Passiflora</u> (including "<u>Tetrastylis</u>") host plants are found in all parts of the field site in various habitats. By systematically visiting these plants it was possible to collect

Heliconius eggs (Chapter 3.2), observe larvae, and sample other insects found on the host plants. These include herbivorous "flea beetles" (Alticini, Chrysomelidae; see Appendix 6.4) and ants of various types. Egg parasitoids were reared from the collected eggs for identification and to determine the parasitism rate.

5.3a Methods

In 1975, 110 _Passiflora_ vines in six habitats were selected for study (see Appendix 6.1). Accessibility along trails and diversity of species and habitat were the major considerations in selecting vines. Each vine was checked at three day intervals and the following data recorded: Date, time of day, plant I.D. #, number of new shoots, number of stems with mature leaves lacking new growth, ants present, eggs present, whether or not eggs are parasitized, larvae present, and flea beetles present. Ants, eggs, larvae, and flea beetles were collected for identification.

Ants were identified to the genus level when possible by Donald H. Feener and Sally Levings of the University of Texas; most of the smaller ants could not be identified with certainty. The microhymenopteran egg wasps were identified as being in two families, the Encyrtidae and the Scelionidae, by E. E. Grissell of the Florida Department of Agriculture.

5.3b Ants Found on Passiflora

The results of the ant identifications are given in Appendix 6.2. There is no striking association between any ant species and

any Passiflora species, and most of the ants may be found on any of the Passiflora. Neither is there an association between ant species and plant size, as checked near ground level. The only differentiation among ant species in this data is between habitats. Here it is clear that (1) Ectatomma tuberculatum is most commonly found in edge habitats and (2) the majority of ants found on forest Passiflora are small, undetermined species. The sample from the garden "habitat" is also distinctive, consisting almost exclusively of Ectatomma sp., Camponotus, and Pseudomyrmex. The effects of these differences in ant composition on Heliconius survivorship are not known.

In Figure 5.2, the presence or absence of ants on Passiflora is analyzed, ignoring species differences. The proportion of times that ants were present out of the total number of plants examined is here termed ant frequency. There is no striking difference among habitats in ant frequency, except that the edge habitat has a much greater proportion of ants present (p < .001) than any of the other habitats. These consist almost exclusively of Ectatomma tuberculatum. With respect to plant size, the larger Passiflora have a greater ant frequency than the smaller vines (p < .001), as might be expected if ant recruitment varies with plant size (Bentley 1977). If Heliconius larval mortality is correlated to the presence of ants on a given Passiflora, it is expected that, other things being equal, smaller vines will be preferred by ovipositing Heliconius. This may explain why species such as H. cydno, H. melpomene, and H. erato seek out scattered

small plants for oviposition.

The ant frequency on _Passiflora_ also varies with _Passiflora_ species (Figure 5.3). Three _Passiflora_ have ants present well over 50% of the time while some other species, notably _P. oerstedii_ and _P. costaricensis_, have ants less than 15% of the time. Three other species have intermediate ant frequencies. These differences correlate with the structure of the extra-floral nectaries. The highly ant-attractive plants, _P. auriculata_, _P. vitifolia_, and _P. ambigua_, all have large extrafloral nectaries. The nectaries of the first two species are cup-shaped appendages on the petiole, while _P. ambigua_ has large swellings on the petiole which secrete nectar through an opening. The least ant-attractive plant is _P. costaricensis_, which lacks extrafloral nectaries entirely. _P. oerstedii_, another plant with few ants present, has small nectaries on long stalks. The _Passiflora_ intermediate in attractiveness have either short, stalked nectaries (_P. "Tetrastylis" lobata_ and _P. coriacea_), or small nectaries on the leaf blade (_P. biflora_). The data from _P. quadrangularis_ was obtained from one vine in poor condition and is probably not representative of that species' ant-attractiveness. The quantity and quality of nectar produced by these nectaries is not known (except _P. biflora_: see Durkee 1977), but it is plausible that ants are more attracted to nectaries with greater flow rates or nutritional content (see Bentley 1977). In other _Passiflora_ species there is evidence that nectar secretion rate is correlated to nectary size (Durkee 1977).

5.3c Egg Parasitism

The proportion of eggs collected that were found to be para-
sitized (egg parasitism rate) was determined for the different habit-
ats and for the different species of Passiflora at the field site.
However, the rates thus calculated are not necessarily a true estimate
of the probability of an egg being parasitized in nature. This is
because collecting an egg eliminates the possibility of parasitism
after the time of collection, which biases the results toward an
underestimate of the true parasitixm rate. The opposite bias, resul-
ting from the fact that the time spent on the host plant (subject
to collection) by a parasitized egg is longer than the time spent by
a normal egg (10 as opposed to 4 days), is not a factor here since
all the eggs on a plant were collected at three day intervals. These
considerations should not affect the results of comparing egg parasit-
ism rates because all eggs collected should be affected in approx-
imately the same way.

In Figure 5.4 egg parasitism rates are given for five habitats
at the field site. The majority of the data are from the September
1975 study described above, but it has been supplemented by parasitism
data taken in 1976. The results indicate that the frequency of egg
parasitism changes from high values (near 50%) in the early succession-
al areas to zero in the forest habitat (Kolmogorov-Smirnov Goodness-of-
Fit test, $p < .001$ that frequency does not change with habitat; Sokal
and Rohlf 1969). Out of 22 eggs collected in the forest areas, none

were found to be parasitized.

The egg parasitism rates were also calculated for each species of Passiflora, as shown in Figure 5.3. Here it is evident that P. oerstedii has a much higher rate of egg parasitism than any other Passiflora species, suggesting that this species is in some way more attractive to the egg parasitoids. Figure 5.5 tests this hypothesis by controlling habitat type. In two habitats, the garden and the early succesisional areas, P. oerstedii was found to have signifi-cantly more egg parasitism than the other Passiflora species combined (Fisher's exact probability; p = .009 and p = .034, respectively; Sokal and Rohlf 1969). Thus, egg parasitism varies strongly with difference in habitat, and, like ant frequency, it varies with the species of host plant.

5.3d Strategies of Biotic Plant Defense

The data given above suggest that the different Passiflora species have evolved differences in attractiveness to beneficial predaceous insects. Some species, such as P. vitifolia, have evolved large extrafloral nectaries which are very attractive to ants and perhaps other predaceous insects. Other species such as P. oerstedii have small nectaries which are less attractive to ants, and which may (hypothetically) be more attractive to parasitoid wasps. It is quite possible that ants act antagonistically toward the tiny parasitoids when the two encounter, and that the plant may encourage the presence of parasitoids by providing small, stalked nectaries that are not

attractive to ants. Since the parasitoids were found primarily in second growth habitat it is predicted that the P. oerstedii-type of extrafloral nectary will be found principally in second-growth-inhabiting Passiflora

Among the La Selva Passiflora three of the six forest-inhabiting species have highly ant-attractive properties and the other three species are undetermined in this respect. In the early successional areas two species are ant-attractive, two are ant-"repellent" (including the parasitoid-attractive P. oerstedi), and two are intermediate in attractiveness to ants. This pattern suggests that forest-inhabiting Passiflora will often be found to have large, ant-attractive nectaries which is probably the most effective form of biotic defense in that habitat. In second growth both ants and parasitoids are present, and the Passiflora there will have nectaries designed to attract either ants or parasitoids, which suggests that both of these predators are effective means of biotic defense in that habitat. Thus, Passiflora species may be committed to certain strategies of biotic (extrafloral nectary) defense which are appropriate only in certain habitats. However, much more data is necessary to evaluate this hypothesis.

5.3.e Correlations with Host Density

The data in Figure 5.4 suggest that the frequency of egg parasitism may be density-dependent with respect to the abundance of Heliconius eggs, which are much rarer (per hectare of habitat) in

forest understory than in second growth. This implies that either the parasitoids are somewhat host-specific to Heliconius or that the abundance of other host eggs varies in the same way as Heliconius. In contrast, ant frequency does not markedly vary between habitats, suggesting that ant predation is not density-dependent at this level of comparison. The overall result is that the intensity of predation on Heliconius eggs and larvae should increase as Heliconius density increases, with an increasing component of mortality due to egg parasitism. This increasing predation pressure may help to explain why the ratio of Heliconius abundance to Passiflora abundance is reduced in the early second growth areas, as discussed in Chapters 2.5 and 6.3.

5.4 Egg Placement in Heliconius

As shown above, the type of predation affecting the eggs and larvae of Heliconius varies according to habitat and species of host plant. By observing oviposition behavior in Heliconius it should be possible to test the hypotheses that (1) plants with predaceous insects are avoided by ovipositing females, and (2) that egg placement on the host plant is optimal for avoiding predators. The first hypothesis has received anecdotal support based on field observations (Benson et al. 1976). The second hypothesis has received experimental support, showing that small insects are safer from ant predation when attached to tendrils than to other parts of the plant (Benson

et al. 1976). This may explain why the young larvae of most _Heliconius_ feed on tendrils for the first few instars of life; they are safer there from predation. What is not explained is why some _Heliconius_ place their eggs on tendrils while others do not.

The hypothesis that ovipositing female _Heliconius_ avoid host plants with ants present was tested indirectly using the data described above (Chapter 5.2). If the hypothesis were true then there should be a negative correlation between ants and eggs found on the _Passiflora_; i.e. host plants should tend to have either ants present or eggs present but nto both. The data in Figure 5.6 indicate that there is no such negative correlation. In fact, a _positive_ correlation was found. This was due to approximately 50 cases where plants were unsuitable to either ants or ovipositing _Heliconius_. By considering only plants with new growth shoots, the correlation between ants and eggs falls to zero. Thus, there is no evidence here that _Heliconius_ avoid ovipositing on plants with ants on them. This is not to say that _Heliconius_ females never avoid ant-infested host plants, but in this sample the predicted results of such behavior are not evident.

Egg placement behavior was observed directly during the single-plant presentation experiments described in Chapter 3.3b. While it was not possible to observe the reactions of the butterflies with respect to the presence of predators, it was feasible to record which part of the host plant was selected for oviposition. Plant parts

selected include shoot-tip leaf clusters, young, uncoiled tendrils, leaf tips, leaf blades, stipules, stems and petioles, coiled tendrils, non-photosynthetic tissues such as bark and dead leaves, and finally, "off the plant entirely." The data are given in complete form in Appendix 4.3.

Figure 5.7 summarizes the tendency of each species to lay their eggs on young tendrils and on shoot-tip leaf clusters ("meristems"), the two most prevalent egg placement sites on the host plants. Two species are tendril-layers almost exclusively; H. cydno and H. hecale. The other three species tested seldom oviposit on tendrils but prefer the shoot tips instead; these are H. melpomene, H. erato, and H. charitonia. The latter species (along with H. sara) chooses shoot tips almost exclusively.

Because egg parasitism occurs chiefly in second-growth habitats it would be predicted that Heliconius in these habitats would place their eggs so as to minimize such parasitism. In contrast, the forest-inhabiting species are predicted to place their eggs so as to avoid ant predation, because egg wasp predation is not a factor there. The pattern of egg placement shown in Figure 5.7 is suggestive as to the validity of these predictions. The forest-inhabiting H. cydno oviposits on tendrils, where the eggs and hatching larvae are relatively safe from ant predation. The second-growth inhabiting H. erato, H. melpomene, and H. charitonia do not oviposit on tendrils, where, in spite of being relatively safe from ants, they may well be highly

vulnerable to parasitoids. By ovipositing in the shoot-tip leaf
cluster these species are placing their eggs in a complicated,
folded mass of leaves which may well decrease their chances of being
detected by searching parasitoids (J. Waage, personal communication).
Because the habitat preferences are not clear, the two other species,
H. hecale and H. sara, may or may not fit the predicted pattern.

5.5 Summary

Larval mortality of Heliconius under natural conditions was
found to be high in all habitats, on the order of 95%, with first
instar individuals suffering the heaviest losses. Mortality due to
egg parasitism was high in early-successional habitats but zero in the
forest habitat. Other types of egg mortality were not investigated.
The fact that egg parasitism only occurs in the high-density Heliconius
habitat implies that the low-density forest dwelling species are not
regulated by egg parasitism. In contrast, egg parasitism is probably
an important regulating factor in the high-density second growth
populations (see also Ehrlich and Gilbert 1973).

The ant community found on Passiflora was investigated and
it was found that larger host plants tend to have ants on them more
often than smaller plants. Most of the habitats have approximately
equal probabilities of ant presence on the Passiflora, but certain
Passiflora species differ strongly in their attractiveness to ants.
These differences appear to be due to the structure and function of

the extrafloral nectaries on the <u>Passiflora</u>. The rates of egg parasitism correlate in a different way to <u>Passiflora</u> nectary structure, and it is suggested that because ants and egg parasitoids are incompatible on the same host plant, the different <u>Passiflora</u> species are adapted to attract one or the other type of <u>Heliconius</u>-feeding predator.

Egg placement on host plants was found to be correlated to habitat preference in the <u>Heliconius</u> tested. Apparently, the tendril-ovipositing behavior of some <u>Heliconius</u> is a defense against ants whereas the shoot-tip ovipositing behavior is a defense against egg parasitism.
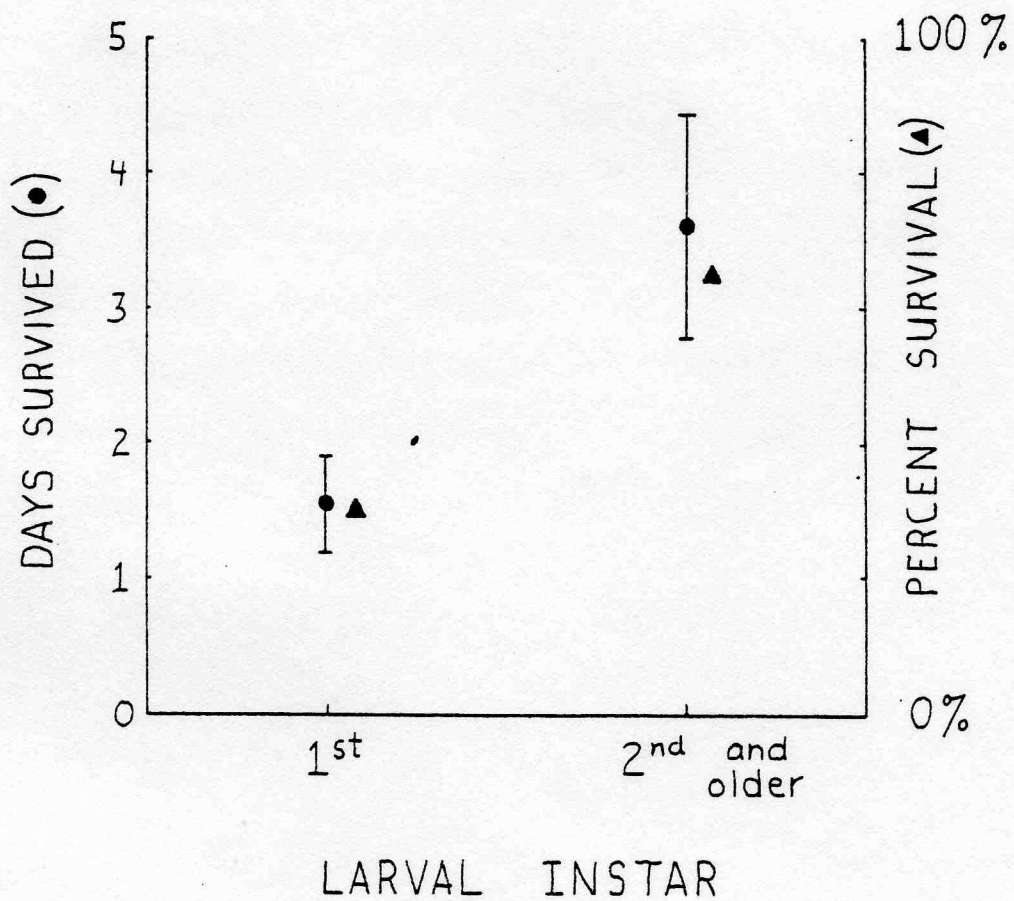
Figure 5.1 Larval survivorship in La Selva <u>Heliconius</u>.
Data are mostly from <u>H.cydno</u> and <u>H.hecale</u>
larvae. Percent survival was calculated
in two-day periods.

Habitat:	Ants present	No ants present	
Forest	49	46	52%
Cacao	34	47	42%
Old second growth	33	28	54%
Edge	54	2	96%
Garden	72	91	44%
Early second growth	37	61	38%

Plant size:	Ants present	No ants present	
Small	87	106	45%
Medium	127	157	45%
Large	67	34	66%

	+ Ants −				+ Ants −	
Edge	54	2		Large	67	34
Habitat:				Plant size:		
Other	225	273		Med. or Sm.	214	263

(G=64.98, df=1, p .001)   (G=15.58, df=1, p .001)

Figure 5.2   Frequency of ant visitation to Passiflora vines
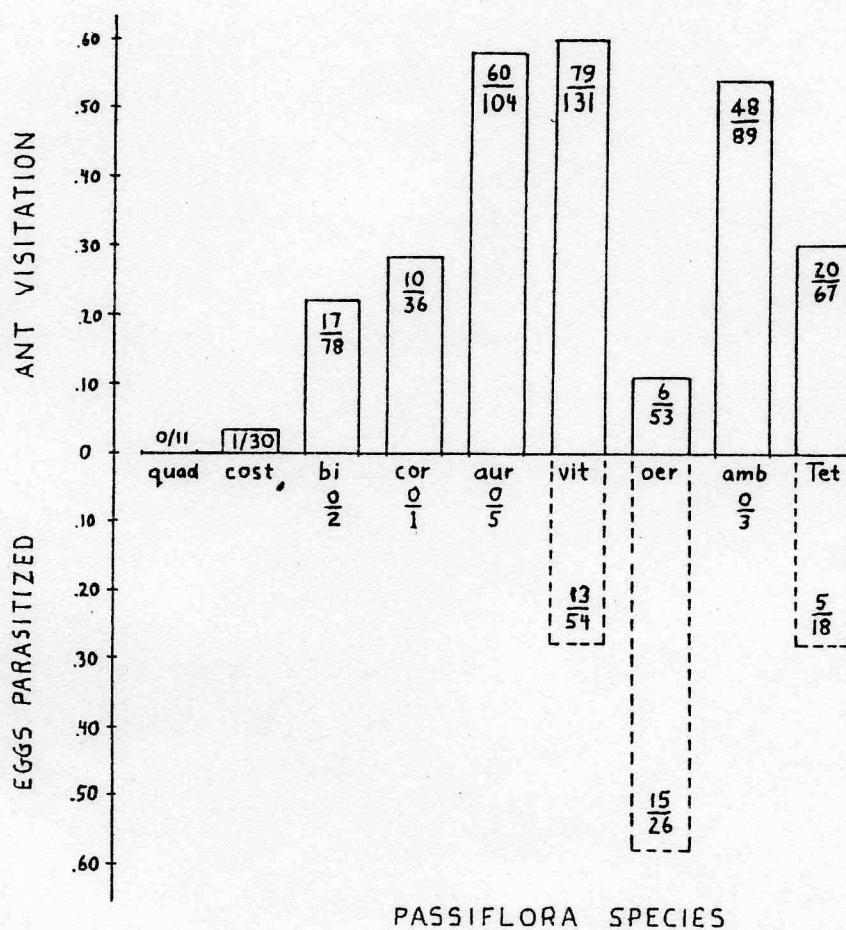in different habitats and of different sizes.

Figure 5.3 Predators of Heliconius eggs and larvae
found on Passiflora species. See text
for further explanation. quad=quadran-
gularis, cost=costaricensis, bi=biflora,
cor=coriacea, aur=auriculata, vit=viti-
folia, oer=oerstedii, amb=ambigua, Tet=
"Tetrastylis" lobata.

		Parasitized	Not Parasitized	
Forest	'75	0	12	0%
	'76	0	9	0%
	Total	0	22	0%
Succession Plots	'75	7	3	70%
	'76	2	5	28%
	Total	9	8	53%
Edge	'75	2	14	14%
Garden	'75	16	35	32%
	'76	7	15	32%
	Total	23	50	32%
Cacao	'76	1	8	11%
Total		35	102	26%

Figure 5.4 Frequency of egg parasitism at La Selva. Contingengency table G-statistic: G= 65, df=7, p<.005.

Early second growth: # not
(successional plots)   #parasitized parasitized

	#parasitized	# not parasitized
P. oerstedii	9	4
non-oerstedii	1	7

Host plant: (label at left, between P. oerstedii and non-oerstedii rows)

(Fisher's exact test: p=.034)

Garden area: # not
                                #parasitized parasitized

	#parasitized	# not parasitized
P.oerstedii	6	3
non-oerstedii	11	43

Host plant: (label at left, between P.oerstedii and non-oerstedii rows)

(Fisher's exact test: p=.009)

Figure 5.5   Egg parasitism in two habitats at La Selva.

All vines

Eggs

	+	−	
Ants +	19 (15.6)	85 (88.4)	= Positive correlation
Ants −	26 (29.0)	167 (164.0)	

Vines with new growth

	+	−	
Ants +	19 (18.7)	85 (85.3)	= No correlation
Ants −	26 (25.7)	117 (117.3)	

Figure 5.6   Frequency of Passiflora vines with and without ants and eggs of Heliconius. Expected frequency in parentheses.
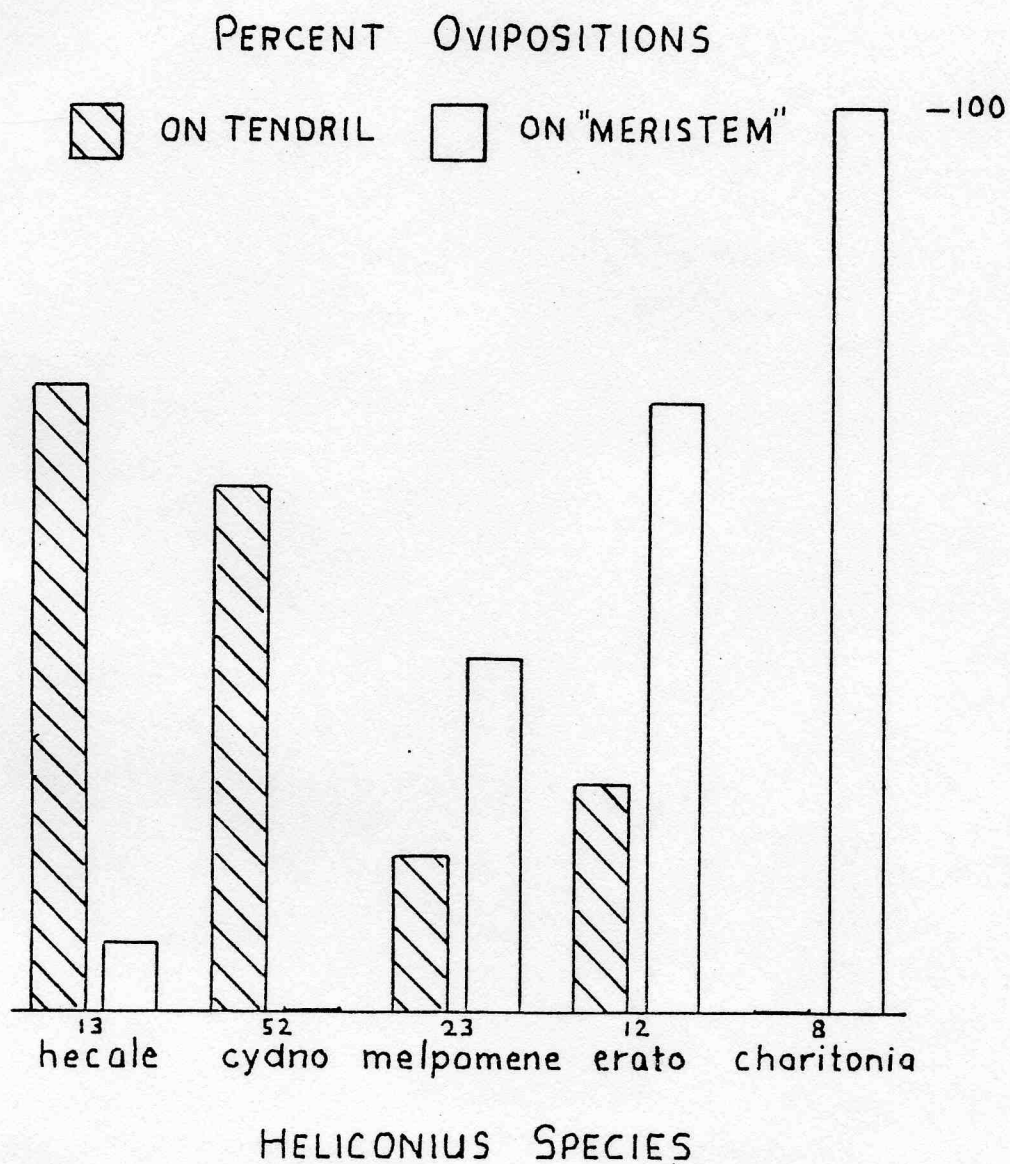
Figure 5.7 Egg placement in five <u>Heliconius</u> species.
Eggs may be layed on green, uncoiled tendrils,
on shoot-tip leaf clusters ("meristems"), or
on other plant parts (not represented).