COLOR POLYMORPHISM IN SPHINGID CATERPILLARS (LEPIDOPTERA: SPHINGIDAE)

By

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I did not grow up with a fascination for hawk moths. The caterpillar drawings of The Reverend A. Miles Moss lured me, and this dissertation is the result.

In writing, I have come to realize that the range of questions I am asking are most closely akin to those asked long ago by E.B. Poulton. Poulton has not received the general recognition that he deserves for his curiosity and insight about animal coloration and evolution. His experimental work falls short by modern standards, but he was experimental, and comparative, and he asked the right questions.

I dedicate this thesis to the memory of

Arthur Miles Moss, M.A., F.Z.S., F.E.S.
1873-1948

and

Edward Bagnall Poulton, D.Sc., M.A., F.R.S.
Hope Professor of Zoology, University of Oxford
1856-1943

I wish I had known them both.

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Sphingid caterpillars eat a lot and produce too much frass. In

nature, they are found near alligators and chiggers. For helping to rear caterpillars in my dungeon environmental chamber and to census them in the wild, I thank Carolina Murcia, Cathy Langtimm, Cynthia Ozburn, Alfonso Alonso, Doug Levey, Carlos Martinez del Rio, Alan Masters, Karen Hoffmann Masters, and John Watts. Vickie McDonald and Lincoln Brower are the finest gardeners I know, and taught me enough to keep most of my plants alive.

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Many polymorphic grasshoppers, butterfly pupae, and caterpillars are phenotypically labile, with color expression under environmental as well as genetic control. Sphingid caterpillars (Lepidoptera: Sphingidae) are polymorphic during one to four instars, with up to four color morphs per instar. I conducted experiments to understand the proximate factors causing sphingid caterpillars to switch colors, and addressed potential tradeoffs of being a particular morph in various environmental contexts. I focussed on Amphion floridensis, which is green or pink in the fourth instar, and conducted comparative experiments on three additional species.

In 35 Amphion broods, the incidence of pink ranged from 2% to 86%. Genetic crosses indicated that color determination is a threshold trait with quantitative genetic inheritance (heritability = 0.24 +/- 0.25). Temperature, food species, and leaf color significantly affected the incidence of pink, but rearing density and photoperiod did not. Pupae

of pink individuals were heavier, but pink females had lower fecundity than their green sisters. Foodplant species also affected morph frequencies in Eumorpha fasciata. Combined with published data, my experiments suggest that plant traits trigger color changes in many sphingids, and lead to nonrandom spatial distributions of morphs.

Behavioral differences also result in nonrandom morph distributions. Green Amphion larvae rested under leaves more often than on stems, whereas pink larvae did the reverse. Under natural conditions pink individuals gained less weight during the day than green ones, suggesting that resting away from leaves entails a cost. Similar leaf and nonleaf resting choices occurred among color morphs of Xylophanes tersa and Eumorpha fasciata, but not in Enyo lugubris or an earlier instar of E. fasciata.

Plasticity of color expression in sphingid caterpillars allows individuals to respond quickly to the phenotypic heterogeneity of their foodplants. Further experiments must clarify the adaptiveness of using foodplant cues to trigger color changes, and identify the major factors selecting for both phenotypic and behavioral variability.

CHAPTER 1
INTRODUCTION AND BACKGROUND

Introduction

> "It is noteworthy that both colours of dimorphic larvae are invariably of protective value: they are, in fact, nearly always the two chief tints of nature--green and brown." (Poulton 1890, p. 46)

Color polymorphism is common among free-living palatable invertebrates that face heavy visual predation. Comparative studies have elucidated both the mechanisms and functions of color polymorphisms in land snails, orthopteroid insects, and the pupae and adults of Lepidoptera (reviews in Rowell 1971; Ford 1975; Jones et al. 1977; Smith 1978; Hazel 1980; Fuzeau-Braesch 1985; Howlett and Majerus 1987). Although there is still debate over the relative importance of various selective factors in regulating morph frequencies in both snails and melanic moths (discussed in Jones et al. 1977; Howlett and Majerus 1987), it is clear that such polymorphisms are adaptations maintained in wild populations partly or entirely by natural selection (Endler 1986). In butterfly pupae, grasshoppers, and mantids, for example, there is considerable evidence that green/brown polymorphisms under environmental control give an individual the ability to become more cryptic in a "green and brown" spatially or seasonally heterogenous environment, and that increased background-matching can

reduce predation (reviewed in Rowell 1971; Hazel 1980; Fuzeau-Braesch 1985).

Caterpillar color polymorphism is widespread, but its occurrence, function, and control are not well-understood. One reason for the neglect of caterpillar color polymorphism may be a false confidence that the adaptiveness and mechanisms will be similar to those of butterfly pupae and grasshoppers. As Poulton (1890) noted, many polymorphisms in the Geometridae, Sphingidae, and Noctuidae involve green and brown forms. These exposed caterpillars live in heterogeneous environments and face heavy predation; facultative color polymorphism should allow an individual to respond to its particular surroundings and increase its crypsis. Although this model is probably true for many of the palatable caterpillars, it is not yet supported with experimental data, and it oversimplifies the tremendous diversity of the polymorphisms that are found in caterpillars. To borrow Poulton's words out of context, the green-brown paradigm for caterpillars may be "one of those deceptively feasible suggestions which are not tested because of their apparent probability" (1890, p. 118).

This Study

Abundant natural history records make it clear that color polymorphism in the Sphingidae is common, but do not reveal how morphs are distributed spatially, temporally, or geographically. Except for Grayson (1986), no one has sampled sphingid populations and tabulated morph frequencies. One of the most crucial pieces of information that

is lacking, therefore, is the distribution and abundance of color morphs in the wild. How much polymorphism actually exists in wild populations, under natural rather than laboratory conditions? Are there correlations with climate, habitat, or hostplants?

Why are sphingids polymorphic? Although naturalists assume that sphingid polymorphism is a defense against visually hunting predators, no experiment has adequately addressed the selective pressures faced by sphingid caterpillars, and how polymorphism affects their fitness.

What proportion of the difference between color morphs is due to genetic differences, and what proportion to nongenetic factors? What is the adaptiveness of sensitivity to particular environmental factors? Do species vary in the environmental influences on morph determination, and is this variation adaptive?

This dissertation is the beginning of a comparative study of the control and adaptiveness of color polymorphism in sphingid caterpillars. I have focussed on the proximate control of polymorphism, and on correlations between color and other traits. Direct tests of the function of sphingid color variation will be a major goal of future research. I will (1) quantify morph frequencies in natural populations of two sphingid species, Amphion floridensis and Eumorpha fasciata, and look for correlations between morph frequencies and environmental factors (Chapters 2 and 3); (2) examine the genetic and environmental control of the polymorphism in A. floridensis (Chapters 2 and 3); and (3) determine whether phenotypes differ in features other than color, by looking for behavioral (Chapter 4) and developmental (Chapter 5) variation among morphs in several species.

Finally, I will present general conclusions, and ideas for future research (Chapter 6).

Terminology: Polymorphism, Polyphenism, and Ontogenetic Color Changes

Polymorphism versus Polyphenism

Discontinuous phenotypic variation under simple Mendelian control ("genetic polymorphism", Ford 1940, 1965) is frequently contrasted with variation under environmental control ("polyphenism", Mayr 1963). In the former, the morph of each individual is determined directly by its genotype, whereas in the latter, genotypically identical individuals are phenotypically plastic. Sphingid color variation fits Mayr's definition of a polyphenism, but I will continue to use the term polymorphism, as do many authors discussing environmentally controlled discontinuous variation (e.g., Richards 1961; Nijhout and Wheeler 1982; Hardie and Lees 1985; Roff 1986). I have been criticized for this (H. Townes, E. Curio, G. Pasteur, pers. comms., each objected for a different reason), and therefore will give the justifications for my decision. (1) It serves no purpose to limit the use of "polymorphism" to Mendelian traits. The term polymorphism pre-dates the field of genetics (see Sang 1961, Hardie and Lees 1985), and is an unambiguous and useful term for discontinuous variation when the control mechanism is not known. Ford's equating of polymorphism with genetic polymorphism (1961, 1965, 1975) was based on the false assumption that environmental switch mechanisms controlling discontinuous variation were rare and difficult to evolve. (2) When Mayr (1963) defined polyphenism, it may have seemed like a straightforward alternative to

polymorphism. By now, however, both terms suffer from an abundance of not-quite-synonymous definitions. Thus some authors limit polyphenism to discontinuous variation (Hardie and Lees 1985), although Mayr's original definition included continuous variation. Polymorphism and polyphenism were originally envisioned as mutually exclusive (Mayr 1963), but polyphenism has been treated as a subset of polymorphism (Nijhout and Wheeler 1982; Walker 1985; Douglas 1986), and less frequently polymorphism as a subset of polyphenism (see Hardie and Lees 1985). (3) As in the nature/nurture debate in ethology, the polymorphism/polyphenism terminology artificially dichotomizes a continuum (Shapiro 1976). All traits are influenced both by the genotype of the individual and its environment. Although some traits have high heritability, with little or no environmental influence (e.g., many industrial melanics), many traits have significant genetic and environmental components to their variation. The distinction is not between genetic and environmental control mechanisms, therefore, but single-locus or two-locus systems and polygenic systems with measurable environmental effects. (4) Townes (pers. comm.) argued that the Greek root of polymorphism means "form", and therefore should be used only for morphological features. Although the Greek "η μορφη" is translated as "form", other definitions are "appearance" and "kind" (Liddell and Scott, 1977). In addition, using an alternative such as "polychromism" for discontinous color variation implies that color alone varies, which is often not true.

In summary, "polymorphism" is a useful general term for many types of discontinuous variation. Where the distinction is important, I will

call polymorphism under Mendelian genetic control, "genetic polymorphism", and polymorphism influenced by environmental conditions, "polyphenism" or "facultative polymorphism." "Polymorphism" alone will imply nothing about the underlying mechanism. Continuous color variation will not be called "polymorphism".

Ontogenetic Color Change versus Polymorphism

In discussing polymorphism, it is necessary to distinguish between color changes accompanying development, known as "ontogenetic color change" (Bückmann 1974), and polymorphism within a given developmental stage. In many swallowtail butterfly larvae, all early-instar larvae are brown-and-white "bird dropping mimics" while all mature larvae are green. Between instars the color change is striking, but during a particular instar all individuals are either fecal mimics or green. In this ontogenetic color change, all individuals go through the same changes, and there is no variability between individuals within an instar; the population is not polymorphic. In contrast, if individuals in a dimorphic species never switched from one phenotype to the other, the species would be polymorphic, but individuals would not undergo an ontogenetic color change. Many species show both polymorphism and ontogenetic color changes.

Sphingids undergo a color change after completion of feeding in the final instar, before wandering off of the plant to pupate. Because all individuals undergo the same change at the same point in development, this is an ontogenetic color change. In this dissertation

I am concerned with multiple forms occurring within an instar, and therefore the prepupal color changes will not be examined.

Sphingid Classification and Larval Biology: Background Information

Rothschild and Jordan (1903) produced a comprehensive revision of the Sphingidae that is still the major taxonomic reference on the family. The North American and African sphingids have been revised (Hodges 1971, Carcasson 1976), and D'Abrera (1986) recently published an "illustrated systematic list" of the entire family. Genera and species have multiple synonyms; for uniformity, I will use the synonyms found in D'Abrera (1986), which are the most recent and complete, even though this work is not a true taxonomic revision. I will follow Hodges' nomenclature for suprageneric groups.

The Sphingidae are a well-defined, uniform taxonomic group of slightly more than 1000 species. They have a worldwide distribution, with the highest diversity in the tropics (Schreiber 1978). The family is divided into two subfamilies, the Sphinginae and Macroglossinae, and five tribes (Table 1-1), based on characters of the male and female genitalia. The subfamilies, and the tribes of the Sphinginae, are generally considered robust; however, the definition of tribes in the Macroglossinae, and the relationships of genera within tribes, are not clear (Carcasson 1976; D'Abrera 1986; Hodges, pers. comm.).

Table 1-2 summarizes characteristics of the larvae in the two subfamilies and the five tribes. The diagnostic larval feature of the sphingids is the caudal "horn" or tail. All larvae have a tail in the

first instar that is as long as or longer than their body; the relative size of the tail decreases with each molt, and it disappears entirely in many mature larvae of the Philampelini and Macroglossini. The Smerinthini and Sphingini are cylindrical in shape; in contrast, in the Macroglossine tribes the larvae often have one or two enlarged thoracic segments into which they are able to retract their head and anterior thoracic segments. Larvae of the Smerinthini are distinct from the other four tribes, with a triangular head and, usually, a granulose cuticle.

The Literature on Sphingid Caterpillars

With the exception of Manduca sexta, the tobacco hornworm, few sphingid caterpillars have been subjects of experimental research. M. sexta was originally studied because of its economic importance, but it has become a major laboratory model for insect physiologists, because of its large size and tractability. The only extensive field experiments are those of Curio (1970a,b) on Erinnyis ello, and Casey (1976) on the thermal biology of Hyles lineata and Manduca sexta larvae. Other recent papers on sphingid behavior have been descriptive (Heinrich 1971; Dillon et al. 1983; Janzen 1980; Bernays and Janzen 1988). Comparative studies of caterpillar behavior and ecology that include sphingids are de Ruiter (1955), Herrebout et al. (1963), Heinrich (1979), Heinrich and Collins (1983), and Janzen (1984).

For the majority of sphingid caterpillars, therefore, information is only available in the natural history literature. Because sphingids are large, impressive moths, they were well-collected and described,

worldwide, in the nineteenth and early twentieth centuries. Most attention was devoted to adults, but descriptions of immature stages are fairly abundant. The best caterpillar descriptions are in Weismann (1882), Eliot and Soule (1902), Moss (1912, 1920), Mell (1922), Bell and Scott (1937), Sevastopulo (1938-1971), and Newman (1965).

All larval descriptions must be viewed with caution, because authors consistently omit sample sizes and rearing conditions and do not state if their information is based on direct observation or on previously-published accounts, or on live or preserved specimens. Most color descriptions are divorced from any information on ecology or behavior. Nevertheless, this literature can be used to generate hypotheses and predictions, and can corroborate, or raise doubts about, the generality of conclusions drawn from empirical studies.

I have tried to examine the literature critically and to separate the valuable from the unreliable. I have not weighed individual observations as heavily as those based on large samples, and I have depended primarily on major sources (Eliot and Soule 1902; Moss 1912, 1920; Bell & Scott 1937; Sevastopulo 1938-1971), whose careful observations cannot be faulted.

Sphingid Caterpillar Defenses

As external plant feeders, sphingid larvae are faced with numerous vertebrate and invertebrate predators. The majority of sphingids are known or assumed to be palatable, and many species are heavily attacked by birds, mammals, lizards, and predatory wasps (Eliot and Soule 1902; Rabb and Lawson 1957; Curio 1970a,b; Janzen 1984; pers. obs.).

Gregarious, aposematic, and presumably unpalatable sphingid species do occur (e.g., the neotropical genera Pseudosphinx and Isognathus, and the Old World Hyles euphorbiae), but they are in the minority.

With a few exceptions, sphingid caterpillars do not have any spines, dense hairs, or large tubercles. The caterpillars of many species regurgitate when disturbed, but most lack defensive glands. [Moss (1920, p. 378) described yellow droplets exuded "from any part of the skin" by aposematic Isognathus larvae, and Haber and Frankie (1983) reported that Callionima falcifera larvae secrete foam from glands on the prothorax.] They do not construct any protective retreats such as leaf rolls or silk tents. The caudal horn has been hypothesized to be sensory or to deflect predator attacks away from the head (Carcasson 1968, cited in Schreiber 1978), or to deter ichneumonid parasitoids (Schreiber 1978), but its function has not been demonstrated in any instar for any species. Because the tail's relative size and mobility decrease with each molt, it is probably functional primarily in the earliest instars. Without physical or chemical defense, therefore, the majority of sphingid caterpillars must rely on crypsis to escape predation.

Larval Size

The range of body sizes encompassed by the term "sphingid caterpillar" is considerable. Many sphingid moths are large, with forewing lengths of 5-9 cm and stout bodies up to seven centimeters long (D'Abrera 1986). Obviously the mature larvae of such moths are impressive: many reach body lengths of more than 10 cm (Bell and Scott

1937), and the mature larvae of Eumorpha typhon weigh 20 g (J.O. Schmidt, pers. comm.). These lepidopteran behemoths, however, are not the sphingid norm. Many species have forewing lengths of 2-3 cm. Among the smallest, Sphingonaepiopsis nanum has a forewing length of just 13 mm (D'Abrera 1986), and the mature caterpillar of Sphingonaepiopsis pumilio is 40 mm long (Bell and Scott 1937).

Size affects many aspects of a caterpillar's biology, for example its thermal ecology, predation risk, and potential resting sites. A newly-hatched sphingid larva is 4-6 mm in length. Throughout this dissertation, therefore, the reader should keep in mind the range of sizes that an individual passes through during its development, and the range of sizes of different species at the same developmental stage.

Hostplant Affinities

The sphingids use a wide array of woody and herbaceous families as foodplants. Janzen (1984, Janzen and Waterman 1984) surveyed the hosts of the sphingid and saturniid caterpillars in Santa Rosa National Park (Costa Rica), and concluded that sphingids fed on plants that were low in phenolics but rich in alkaloids and other small toxic molecules. Most species use one to several plant families as food, but a few are polyphagous (Acherontia styx eats plants in at least nine families, and Theretra oldenlandiae at least eight [Bell and Scott 1937]). The most common foodplants for the Macroglossinae are the Rubiaceae (used by at least 82 species in 20 genera), Vitaceae (65 species), Apocynaceae (25 species), Araceae (25 species), and Onagraceae (22 species) (Forbes 1958; Harris 1972). The foodplants of the subfamily Sphinginae have

not been tabulated, but many Sphingini use the Oleaceae, Solanaceae, Bignoniaceae, and Verbenaceae; and Smerinthini use the Leguminosae, Juglandaceae, Boraginaceae, and Salicaceae (Bell and Scott 1937; Forbes 1958; Hodges 1971; Harris 1972).

Larval Markings

Larvae are immaculate (without markings) when they hatch, but most acquire patterns in later instars. The most common pattern, especially in the Sphinginae, is a series of five to eight (usually seven) oblique lateral lines. In the Macroglossinae, obliques may be replaced by, or supplemented with, dorsolateral lines running from the head or thorax to the horn, and a mid-dorsal line. Other markings include longitudinal stripes in conifer-feeding species (Herrebout et al. 1963), irregular lateral blotches (e.g. in Enyo [Moss 1920]), and complete or interrupted bands around each segment.

Many Macroglossine larvae have markings that resemble vertebrate eyes. The ocellated Dilophonotini and Eumorpha labruscae have a single pair of eyespots on the thorax, and the Macroglossini have less detailed ocelli, which may or may not have a "pupil", located dorsolaterally on each thoracic and abdominal segment. The combination of thoracic eyespots and expanded thoracic segments makes several species into apparent snake mimics, most elegantly in E. labruscae, Hemeroplanes triptolemus, and H. ornatus (Moss 1920; Brower 1971; Pough 1988). Eyespots are not found in any larval Sphinginae.

Weismann (1882, recently summarized by Gould 1977) studied the ontogeny and phylogeny of sphingid caterpillar markings, and provided

hypotheses for the the selective value of several pattern types (oblique lines, longitudinal stripes, and ocellations). The development of the markings would merit a new examination in the context of the recent work on pattern formation in butterfly wings (Nijhout 1985), but is beyond the scope of this dissertation.

The Occurrence of Color Polymorphism in Larval Sphingids

Color polymorphism occurs in all tribes. In the most primitive group, in the tribe Dilophonotini (Carcasson 1976), Erinnyis spp. are cryptic and polymorphic, Pseudosphinx tetrio is aposematic, gregarious, and monomorphic, and Isognathus includes both cryptic and aposematic monomorphic species (Moss 1912, 1920; Curio 1965; Schneider 1973). Many genera contain both monomorphic and polymorphic species.

Polymorphism in four tribes involves a marked change in background color, from green to black, brown, red, or yellow. In the Smerinthini, however, polymorphism most often involves changes in the shade of the background color (e.g., from "whitish-green" to "blue-green" or "yellow-green") or the presence/absence of lateral spots or patches of color. This generalization has exceptions: polymorphism in some Sphingini (e.g., Sphinx ligustri) involves shades of green (Meldola 1882), and in some Smerinthini (Leucophlebia lineata) involves red or yellow morphs (but not brown or black [Bell and Scott 1937]). In a minority of species, for example Eumorpha fasciata (Philampelini) and Enyo lugubris (Dilophonotini) morphs differ in markings (maculation) as well as in background color.

Color Polymorphism in the Grape-Feeding Sphingids

Table 1-3 summarizes the available information on the colors of sphingid caterpillars feeding on the grape family (Vitaceae). I examined the Vitaceae because they are the foodplants of Amphion floridensis, the primary species I studied. This table demonstrates several important features of sphingid polymorphisms. [It also illustrates the spottiness of published descriptions.]

First, Poulton's generalization that alternative forms of caterpillars are "nearly always" green and brown obviously has many exceptions. Grape-feeding sphingids are also yellow, orange, purple, pink, and black. All species have green forms in at least one instar, but the alternative colors vary even among congeners feeding on the same foodplant. Clearly, if color polymorphisms are adaptations for crypsis in a variable environment, then the worlds of sphingid caterpillars are not simply "green and brown."

Second, the timing and extent of polymorphism are highly variable. All species are probably monomorphic in the first instar, but polymorphism can arise in any other instar. In general, the diversity of morphs seems to increase as larvae develop, but Amphion floridensis is polymorphic at intermediate instars and monomorphic in the final instar. In addition, species are not just dimorphic; they may be trimorphic, or have continuous color variation.

Third, this variability among species is apparent within large genera (Eumorpha, Theretra) as well as among grape-feeders as a group. Two species of Eumorpha feed on the Onagraceae rather than the Vitaceae. E. fasciata is similar to E. typhon in being polymorphic in

four instars (Moss 1912). Moss (l.c., plate XI) shows five forms of Eumorpha fasciata in the fourth instar (yellow, two shades of green, green-and-pink, and black), and three in the fifth (yellow, green, and brown). Yet E. eacus, which feeds on the same foodplant genus at the same time, is "as constant as fasciatus is variable, and is always green" (Moss 1920, p. 405).

Prolegomenon

Sphingid caterpillars provide exquisite examples of biological variation. Individuals undergo dramatic color changes during development, and there is variation in the sequence of changes among individuals; populations contain caterpillars of many colors simultaneously, and there will certainly be variation in the frequencies of color morphs among populations; species vary in the extent and timing of their color polymorphisms, and, as demonstrated by the grape-feeding sphingids, a habitat or community will contain species with diverse developmental sequences. Each level of variation poses its own questions, but in addition, affects and is affected by every other level. This dissertation begins the challenge of elucidating how and why such complex variation is maintained.

Table 1-1. Synonymy of the suprageneric classification used in the three major revisions of the Sphingidae.

Hodges 1971	Rothschild and Jordan 1903	Carcasson 1976
Subfamily Sphinginae	Division Asemanophorae	Subfamily Asemanophorinae
Tribe Sphingini	Subfamily Acherontiini	Tribe Acherontiini
Tribe Smerinthini	Subfamily Ambulicinae	Tribe Ambulicinae
Subfamily Macroglossinae	Division Semanophorae	Subfamily Semanophorinae
Tribe Dilophonotini	Subfamily Sesiinae	Tribe Dilophonotini
Tribe Philampelini	Subfamily Philampelinae (part)	Tribe Philampelini (part)
Tribe Macroglossini	Subfamily Philampelinae (part)	Tribe Philampelini (part)
	plus Subf. Choerocampinae	plus Tr. Choerocampini

Table 1-2. Summary of larval characteristics of the five tribes of sphingids.

SUBFAMILY:	SPHINGINAE		MACROGLOSSINAE		
TRIBE*:	Sphingini	Smerinthini	Diloph.	Philamp.	Macrog.
# Genera	40	65	25	2	60
# Species	150	200	130	20	400
Genera**	Manduca	Smerinthus Laothoe Mimas	Erinnyis Pseudosphinx Enyo	Eumorpha	Xylophanes Amphion Darapsa Sphecodina
Greatest diversity	Neotropical Nearctic	Indo-Australian Ethiopian	Neotropical	Neotropical	Indo-Australian Ethiopian Neotropical Nearctic
Shape	cylindrical anterior	slight taper to head not retractile triangular head granulated	tapers from segment 4 or 5 to head head & thorax retract into segs 4 & 5		
Horn	present in all instars		often disappears in later instars		
Common markings	obliques	obliques patches dorsolateral line	obliques longitudinal lines	obliques snake mimics	obliques lateral ocelli snake mimics
Common foodplants	Oleaceae Solanaceae Bignoniaceae conifers	trees & shrubs Anacardiaceae Leguminosae Salicaceae	Vitaceae Apocynaceae Rubiaceae	Vitaceae Onagraceae	Vitaceae Apocynaceae Rubiaceae Araceae

Compiled from Rothschild and Jordan (1903), Bell and Scott (1937), Hodges (1971), Harris (1972), Carcasson (1976), and Schreiber (1978).

* Diloph. = Dilophonotini; Philamp. = Philampelini; Macrog. = Macroglossini

** Genera that are discussed frequently in this dissertation.

Table 1-3. Color morphs of sphingid caterpillars that feed on the plant family Vitaceae.

Species	#fam[a]	Tl[b]	Developmental Pattern[c]	Reference[d]
Tribe Dilophonotini				
Enyo lugubris	V	6.5	g-g-G/Pi-G/Pi/G,Pu-G/Pu/G,Pu	1
		6.0		7
		6.0		4
E. gorgon	V		?-?-?-?-G/RBr	8
Tribe Philampelini				
Eumorpha anchemola	V	10.0	wh-pi-?-g-g	3
			?-g-g-?-G/Pi	4
			g-?-?-?-br	9
		10.5	g-g-g-?-br	10
E. pandorus	V	10.0	g-g-g-G/Br-G/GBr/Br.var	2
		7.5-10.0		7,11
			g-?-?-?-G/Br	12
		10.0	?-?-r-r-r	13[e]
E. satellitia	V		?-?-?-?-G/RBr	8
E. satellitia licaon			g-g-g-g-g	9
E. achemon	V	10.0	g-g-g-G/Br-G/GBr/Br.var	2
			g-g-g-g-br	14
			g-?-?-?-G/Br	12
		7.5-9.0		11,24
E. typhon	V	12.5	g-G/Pi-G/Pi-G/Pi/Y-G/Bu/Gr	15
E. vitis	V	7.0	g-g-g-G.var/Pi-G.var/Pi.var	3
		7.5		7
E. labruscae	V	9.5	?-br-br-br-br	3
			g-g-br-br-br	9
			g-g-gbr-br	10
Tribe Macroglossini				
Ampelophaga rubiginosa	V	9.5	g-g-g-g-G/O	5
A. khasiana khasiana	V+1	8.5	g-g-g-g-g	5
Acosmeryx naga	V+1	10.0	g-g-g-g-G/Pi	5
A. anceus subdentata	V		g-g-g-g-g	5
Panacra mydon mydon	V+1	9.5	g-g-g-g-G/G,Br	5
Hyles lineata livornica	V+5	8.5	?-?-?-?-G/Bl	5
			?-?-?-?-G.var/Bl	16
			g-gbl-var-var-G.var/Bl.var	17
			?-?-?-?-G/Br	18

Table 1-3 -- continued.

Species	#fam[a]	T1[b]	Developmental Pattern[c]	Referenced
Hippotion celerio	V+5	6.0	g-g-g-G/Br-G/Br	5
			g-g-G/Br-G/Br-G/Br	17
H. osiris			?-?-?-?-g	19[f]
			?-?-G/Br-G/Br-br	6
			?-?-?-?-br	20
	V+2		?-?-?-?-br	16
H. eson	V+3		?-?-?-?-G/Br.var	16
			?-?-?-?-G/Br	20
	V+5		?-?-G/Br-G/Br-br	6
Theretra clotho clotho	V+5	8.5	g-g-g-g-g.var-G/Br	5
T. gnoma	V+1		g-g-g-g-g.var-G/Br	5
T. latreillei lucasi	V+4	6.0	g-g-g-g-G/Br	5
T. alecto alecto	V+3	8.0	y-g-G/Dk-G/Dk-G/Br	5
			?-G/Pi-G/Pi-G/Pi-br	6
T. alecto cretica			?-?-?-?-g	21[e]
T. lycetus	V+1	8.5	g-gpi-rbr-rbr-G/RBr	5
T. old. oldenlandiae	V+7	8.0	g-g-bl-bl-bl	5
			g-bl-bl-bl-bl	6
			?-?-?-?-[Gr]/Pu	22
T. pallicosta	V+1	8.5	g-g-g-g-g-G/Br.var	5
T. capensis			?-?-?-?-G/R	19
			?-?-?-?-G.var/Br.var	16
Rhyncholaba acteus	V+3	7.0	g-g-g-G/Br-G/Br	5
			g-G/Pi-G/Pi-G/Pi-G/Br	6
Rhagastis aur. aurifera	V+1	8.5	g-g-g-g-g	5
			g-g-g-g-g-G/Br	6
R. confusa	V	9.0	?-g-g-g-g-g	5
R. olivacea	V+2	9.0	g-g-g-g-g-G/Br	5
	V+1	6.5	?-?-?-?-g	23
R. alb. albomarginatus	V+1	10.0	g-g-g-g-g-g	5
Cechenena mirabilis	V+2	10.0	g-g-g-g-g-G/Br	5
C. minor minor	V+2	8.5	g-g-g-g-g-br	5
C. lineosa	V+3	10.0	g-g-g-g-g-br	5
Sphecodina abbottii	V	6.0	g-g-g-g-g-G,Br/Br	2
		5.5-7.5		7,11,24
Deidamia inscripta	V	5.5	g-g-g-g-g-g	2
		5.0-5.8	g-g-g-g-g-g	7[f]
Amphion floridensis	V	6.5	g-g-G/Pi-G/Pi-br	1
		6.2-7.5		7,11
Darapsa myron	V+1	7.0	g-g-g-g-g-G/Br	1,2
		5.0-6.0		7,11,24

Table 1-3 -- continued.

[a] Number of plant families recorded as larval hosts.
V = Vitaceae; V + n = Vitaceae plus n other families
[b] Tl = total length of fully grown final instar larva in cm. Different authors measure length in different ways; these measures should be considered estimates.
[c] Developmental pattern: instars 1-2-3-4-5. Lower case letters indicate monomorphic instars; uppercase letters separated by a slash (/) indicate polymorphic instars. (However, where a single form is described in an instar, it may often be because the sample size was small.) Most sphingid species have five larval instars; instars are separated with a hyphen (-).

Color abbreviations

bl	Bl	Black
br	Br	Brown
be	Be	Blue
bu	Bu	Burgundy
g	G	Green
gr	Gr	Grey
o	O	Orange
pi	Pi	Pink
pu	Pu	Purple
r	R	Red
wh	Wh	white
y	Y	Yellow
dk	Dk	identified only as "dark" or "melanic"
ab	AB	intermediate (gy = green-yellow, rbr = red-brown)
a,b	A,b	color A with markings of color b
a.b.c	A.B.C	complex pattern of colors a,b,c
a.var	A.var	variable shades of color a
[a]		rare color morph (often N = 1)
?		color unknown

[d] References: 1: this study; 2: Eliot and Soule 1902; 3: Moss 1912; 4: Moss 1920; 5: Bell and Scott 1937; 6: Sevastopulo 1938-1947; 7: Beutenmuller 1902; 8: von Bonninghausen 1899; 9: Burmeister 1878; 10: Burmeister 1879; 11: Baynes-Reed 1882; 12: Harris 1839; 13: Lintner 1864; 14: Boisduval 1874; 15: J.O. Schmidt, pers. comm.; 16: Pinhey 1962; 17: Newman 1965; 18: Weismann 1882; 19: Fawcett 1901; 20: MacNulty 1970; 21: Butler 1880; 22: Forsayeth 1884; 23: Fellowes-Manson 1921; 24: Riley 1869.
[e] Known to be monomorphic from a large sample
[f] Sample size = 1 larva

CHAPTER 2
SOURCES OF VARIATION IN MORPH FREQUENCIES
IN AMPHION FLORIDENSIS

Introduction

In this chapter I will address the proximate factors influencing larval color variation in a common Florida sphingid, Amphion floridensis.

Phenotypic variance (V_P) is composed of genotypic differences among individuals (V_G), variation among individuals of a particular genotype due to environmental effects (V_E), and interactions between the genotype and the environment. That is, $V_P = V_G + V_E + V_{GXE}$ (Falconer 1981). With appropriate experiments, it is possible to partition the total phenotypic variance and determine the relative contribution of genetic and non-genetic factors. In addition, both V_G and V_E can be partitioned to examine the relative importance of the additive, dominance, and epistatic effects of genes, and such nongenetic sources as maternal effects, climate, and nutrition (Falconer 1981).

The genetic architecture of known color polymorphisms includes single-locus Mendelian traits (e.g., many moths with industrial melanism, Robinson 1971; Bupalus piniarius caterpillars, den Boer 1971), multilocus systems with varying degrees of linkage and epistasis (Cepaea snails, Jones et al. 1977; Chlosyne lacinia caterpillars,

Gorodenski 1969, Neck 1971); Phlogophora meticulosa caterpillars, Majerus 1983b; Eupteryx leafhoppers, Stewart 1986), and polygenic threshold traits with large environmental variance (swallowtail butterfly pupae, Hazel 1982, Sims 1983). Information on the genetics of larval polymorphisms in sphingids is almost nonexistent. Manduca sexta is the only species in which the genetic control of polymorphism has been explicitly determined, and offspring morph ratios from a small number of crosses are available for Deilephila elpenor (Federley 1916) and Laothoe populi (Grayson 1986).

Wild Manduca sexta caterpillars are monomorphic green in the wild (but see Chapter 3), although the closely-related M. quinquemaculata is often reported as dimorphic, or variably-shaded from green through black or brown (Lintner 1864, Beutenmuller 1895, Eliot and Soule 1902, Hudson 1966). In the 1970s, black larvae appeared in one brood of a laboratory stock of M. sexta, and a pure black strain was developed through intense artificial selection (Safranek and Riddiford 1975). The mutant larvae are green at early instars, becoming black in the fourth or fifth instar. The genetic model supported by Safranek and Riddiford (1975) is straightforward: color is controlled by one sex-linked gene. Modifier genes are apparently important as well, for crosses between black and wild-type individuals produced a range of intermediate phenotypes. E. Lampert and R.M. Roe recently found a new, pinkish-white mutant in their lab culture of M. sexta, that is a single-locus, recessive trait (E. Lampert, pers. comm.).

Based on segregation in an initial brood from a wild female, Federley (1916) hypothesized that the green/dark dimorphism in

Deilephila elpenor was controlled by a single gene, with the green allele recessive. Federley then reared only dark larvae, however, from single green x green, green x dark, dark x green, and dark x dark crosses. He concluded that the color forms did not differ genetically, but were controlled by an unidentified environmental factor. Robinson (1971) emphasized that these results were compatible with a polygenic, threshold model.

In Laothoe populi, larval color is influenced by environmental factors, indicating that it is not a Mendelian trait (Grayson 1986). In a small number of crosses, parental phenotypes were correlated with their offspring brood frequencies: most offspring of yellow-green crosses were yellow-green; most offspring of dull-green crosses were dull-green.

Thus, in the two mutant Manduca sexta strains polymorphism is controlled by a single gene, but in the naturally-occurring polymorphisms inheritance seems to be more complex. Combined with evidence of environmental effects on larval color, and variation in color within any one environment (Schneider 1973; Grayson 1986), the limited genetic data suggest, but do not demonstrate, that larval color is inherited as a polygenic threshold trait.

Threshold traits in natural populations have generally been ignored by quantitative geneticists and evolutionary biologists (Falconer 1981, Endler 1986). A threshold character varies discontinuously, but does not conform to Mendelian rules of inheritance (Falconer 1981). In a threshold trait, it is assumed that the discontinuous phenotypic classes are underlain by a continuous,

normally-distributed variable, called "liability" (Falconer 1965). Individuals with liabilities above a threshold value become one morph, and individuals below the threshold become another morph. Variance in liability in a population is governed by the same factors that govern any continuously-variable trait (genotype, environment, and interactions between the two), and can be partitioned by quantitative genetic techniques.

This Study

Amphion floridensis caterpillars are dimorphic green and pink in the third and fourth instar. By measuring the variation within and between broods in the proportion of fourth instar larvae that are pink, I will (1) document the level of phenotypic variation in a wild population; (2) determine the proportion of this variation that is due to additive genetic effects (the heritability of larval color); and (3) examine the effects of three nongenetic factors (temperature, photoperiod, and population density) on larval color. These data will also be examined for evidence of potential maternal effects, nutritional effects, and genotype-environment interactions.

Amphion floridensis

Amphion floridensis Clark (= A. nessus (Cramer)) is a small (wingspan = 3.7 - 5.5 cm) diurnal hawkmoth whose range extends from Florida to Kansas, and north into Canada (Hodges 1971). Adults are common in Gainesville, FL from late February through late September,

with peak abundances in mid-March through late June (pers. obs., based on bait trap collections). Larvae feed on the Vitaceae (grape family). I have found eggs in the wild and reared larvae successfully in the laboratory on Vitis rotundifolia (Muscadine grape), Ampelopsis arborea (peppervine) and Parthenocissus quinquefolia (Virginia creeper).

Amphion larvae are dimorphic green and pink in the third and fourth instars. In the first and second instar, all larvae are pale green, without markings. Fifth instar larvae are all dark brown, with faint black markings. Most fourth-instar larvae have indistinct white subdorsal lines, edged with darker green in the green larvae, and with darker pink in the pink larvae. A low percentage of the green fourth-instar larvae have pink subdorsal lines, and short pink oblique lines adjacent to their spiracles.

The pink color does not develop until several hours after ecdysis, and it disappears several hours before the next molt. The pink is most intense from late on the first day of each instar, through the second day. As the larva grows, the pink gradually fades, and the larva becomes pinkish-green, then completely green, on the last day of the instar. Development time from egg to pupa is approximately three weeks (at a temperature of 29 C on Ampelopsis arborea). The third instar lasts two to three days, and the fourth instar three to four days. The actual polymorphism, therefore, lasts one to two days during the third instar, and two to three days during the fourth instar.

Caterpillars of Amphion are difficult to find in the wild; in five years I have found 59 eggs and eight caterpillars, although I have collected countless bushels of foodplant. Nevertheless I have

collected up to 28 adults in a single bait trap in one day, suggesting that the species is common. Of the five fourth-instar larvae I have found, one was green, three were pink, and one was greenish-pink and parasitized by braconid wasps.

General Methods

All Amphion rearing experiments described in this dissertation generally followed the methods described below. Modifications for each experiment are described in the appropriate Methods sections.

Wild moth collections. Adults were captured in bait traps (Platt 1969) supplied with a mixture of cooked apples, molasses, brown sugar, yeast, and stale beer. Traps were tended every one to three days.

Egg collection. To obtain eggs, I placed females individually in black organza bags (25 x 30 cm) on freshly-picked branches of Vitis or Parthenocissus, which were kept in water. Females were put in a constant environment chamber at 29 C, on a 16L:8D light cycle. The eggs were collected daily and stored in glass vials or covered plastic dishes, with those from each female kept separate. Because sphingid eggs are firm and tough they could be picked off the leaves and bags by hand without damage. The plants were replaced every 2-3 days.

Each female was fed a solution of clover honey in water (25-35% honey by volume) once daily. For feeding, a piece of paper towel was soaked in a small quantity of the solution in a shallow dish. Each female in turn was placed on the towel, and her proboscis unrolled with a pin. Females were allowed to feed to satiation.

I determined the mating status of all wild-caught and some lab-paired females by post-mortem dissection. The spermatophore deposited by a male can be found easily in the female's bursa copulatrix.

Larval rearing. On the day before larvae hatched, a damp piece of filter paper and a small piece of Ampelopsis were added to the egg container. Larvae were allowed to feed for one day before being transferred to experimental cups (clear plastic, either 9.5 cm diameter x 5 cm high, or 11.5 cm diameter x 3.5 cm high) using a fine paintbrush.

For most experiments the initial group size was 30 larvae per container. At the late second or early third instar, the larvae were divided into three cups of 10; at the early fourth instar these were further divided into groups of two to four. Fresh food was provided daily, at which time each cup was wiped clean of frass and uneaten food.

Many groups of larvae were discarded after their fourth instar color was recorded. (Larvae were killed by freezing, released on foodplants outdoors, or given to captive birds.) In those groups which were being reared to emergence, however, the fourth instar larvae were separated by color. At the fifth instar all larvae from a particular mother were placed in larger plastic boxes (either 26x38x11cm or 17x30x9cm), one for pink and one for green larvae. The late fifth instar larvae were given damp paper towels or damp sphagnum moss in which to pupate, and sprayed with water daily until adult emergence. In broods used for outbred crosses, I separated pupae by sex.

Amphion larvae are not prone to disease; under these rearing conditions no pathogen outbreaks occurred in three years. Pupal mortality and poor adult emergence, however, were high in some broods, from undetermined causes. To control disease, the few sickly larvae were destroyed and their cup-mates placed in clean containers. All containers were soaked in a dilute bleach (sodium hypochlorite) solution between uses. Malformed pupae and adults were discarded.

The larvae were kept in a constant-temperature chamber at 29 C on a fixed light:dark cycle of 16 hours light:8 hours dark. Containers were kept on wire shelves, stacked 1-3 deep.

Food. Unless noted otherwise, the foodplant used in all experiments was the peppervine, Ampelopsis arborea. For some experiments Parthenocissus quinquefolia (Virginia creeper) and Vitis rotundifolia (Muscadine grape) were used.

I collected leaves daily in the early morning or early evening and kept them in plastic bags until used. On a few occasions when leaves were used one to two days after picking, they were kept refrigerated in a plastic bag with a damp paper towel. Ampelopsis leaves were collected primarily from large stands on the University of Florida campus, but leaves of all three species were also collected from numerous other sites in Gainesville.

Statistical analyses. All parametric analyses requiring a computer were done using SAS (version 6.0) on an IBM PC, or SAS (release 5.16, 1986), on a mainframe computer. Percentage data were arcsine transformed (Sokal and Rohlf 1981) and tested for normality before using parametric tests. Analyses of variance were run with PROC

ANOVA (if balanced) or PROC GLM, and Type III sums of squares are reported. Contingency tables were analyzed with G-tests, using Williams' correction (Sokal and Rohlf 1981).

Interbrood Variation under Controlled Rearing Conditions

I determined the frequency of color forms within and between broods by collecting wild females, having them oviposit in the lab, and rearing their offspring under controlled conditions.

Methods

I ran bait traps in two locations in Gainesville, FL. Collection sites were in mesic hammocks, 10 km apart on the edge of Paynes Prairie. One site was on the north rim, near the FL Dept. of Natural Resources, Division of Recreation and Parks District III office. The second site was on the west edge of the prairie, off of Williston Road (SR 121). I used wild females collected between March and July 1986.

The data for these experiments are the number of green and pink fourth instar larvae per family. Data are expressed as percent pink, and called the "brood frequency".

Results

The 35 families differed widely in the percent becoming pink in the fourth instar (Table 2-1, Figure 2-1). None of the broods was entirely green or entirely pink in the fourth instar; the percent pink ranged from 2 to 86 (Median = 30%).

For fifteen of the families I had enough eggs to rear more than one group under the same conditions, with at least 20 larvae per group. The replicates for each family were analyzed with G-tests, and no significant heterogeneity was found (all had $p > 0.1$, except the replicates for female 20, in which $p > 0.05$). Because replicates were homogeneous, I combined them, and Table 2-1 and Figure 2-1 give the overall percent pink for each family.

I compared the among-family and within-family variance for these 15 families with a one-way analysis of variance, and calculated the intraclass correlation coefficient (Sokal and Rohlf 1981). This indicates the proportion of the total variance that is due to differences among families. The correlation coefficient = 0.825, indicating that 82.5 percent of the total variance is due to differences among families, and only 17.5 percent is due to differences among the replicates within a family (Table 2-2).

Partitioning the interfamily variation

The major causes of the variance in the incidence of pink larvae will be factors that are consistent within broods, but vary from brood to brood. Because all larvae were reared under the same conditions, photoperiod and temperature are ruled out as causes of environmental variance (in this experiment). Because these broods came from wild-collected females, and were reared over a six-month period on leaves from wild plants, the three most probable causes of variance among families are (1) genetic differences, (2) maternal effects, and (3) nutritional effects. By examining these thirty-five broods in more

detail, I can look for evidence that any or all of these factors affect the brood frequency.

Season and site

Females were collected over a 20-week period from two sites on Paynes Prairie. Brood frequencies seem to be related both to the site at which the female was collected, and to the date on which she was collected (and, therefore, the dates on which her offspring were reared). Later broods tended to have a higher incidence of pink larvae (Figure 2-2).

I had assumed that I was sampling from a homogeneous population, because sphingid moths are strong fliers, and the habitat between the two collecting sites is fairly continuous. Examination of the data, however, indicates that this assumption is probably false. The broods from females collected on the west edge of the prairie were less pink than broods from females collected on the north edge (West: mean = 20% pink, N = 8 females; North: mean = 37% pink, N = 27 females).

Because females from the west site were collected earlier, on average, than the females at the north site (Median dates of collection: West = weeks 3-4; North = week 8), the two effects (Site and Season) were analyzed simultaneously with an analysis of covariance. The dependent variable in the analysis was the arcsine transformed percent pink of the broods, the independent class variable was collection site, and the covariate was week of collection. I first tested for heterogeneity of slopes in the regression of percent pink on week of collection, using an ANCOVA with the interaction term present

(Freund and Littell 1981). The interaction term was not significant (interaction $F(1,31) = 1.48$, $p = 0.23$), indicating that the two samples did not have different responses to season. I therefore repeated the ANCOVA without the interaction term.

Both the seasonal trend and the site differences approached but did not reach statistical significance (Table 2-3; Site, $p = 0.09$; Season, $p = 0.10$). The seasonal trend suggests that some of the interfemale variance may be due to changes in either (a) the mother's environment, or (b) the Ampelopsis used as larval food. The difference between the two sites may be due either to (a) genetic differences between the Amphion populations at the two sites, or (b) environmental differences at the two sites acting on the females.

Other evidence addressing the possibility of maternal effects

I looked for other evidence of maternal effects by comparing the brood frequencies among families that differed in the mating status or fecundity of the mother, and by looking at the relationship between female age and the brood frequency she produced.

Number of matings. The seven females that were mated more than once (with two or more spermatophores) had more pink offspring (mean = 45% pink) than the 24 singly-mated females (with a single spermatophore; mean = 33% pink), but the difference was not significant (Mann-Whitney test, $p = 0.30$; spermatophore count was not determined for four females, see Table 2-1).

Fecundity. The 35 wild females laid from 44 to 538 eggs before dying. There was no correlation between number of eggs laid and

percent pink (Pearson correlation on arcsine transformation of percent pink; $r = 0.0097$, $p = 0.956$).

Maternal age. Amphion females lay fertile eggs in captivity for up to 14 days, and the later eggs are significantly lighter than early eggs (Table 2-4). A decrease in egg size with female age is common in Lepidoptera, but there is little information on the consequences of this size change for the resulting larvae (Wiklund and Persson 1983, Chew and Robbins 1984). If larval color is correlated with egg or maternal quality in Amphion floridensis, then brood frequencies might change predictably as the female ages and her eggs become smaller. Amphion color is significantly affected by larval nutrition (Chapter 3); perhaps embryonic nutrition is also important.

To test whether the later eggs of females produce a different percent pink than early eggs, I compared the percent pink for the first and last days that 13 of the wild females produced eggs. These females laid two or more clutches of more than 20 eggs at intervals of at least 24 hours. No trend was found. The percent pink increased in four females, decreased in four females, and was unchanged in five females (Table 2-5). The mean percent pink was 29.5% on the first day, and 30.8% on the last day. Therefore egg size and female age do not affect larval color in Amphion floridensis.

Genetic Experiment

As a first step in elucidating the genetic architecture of the polymorphism in Amphion, I made controlled crosses among green and pink

individuals and looked at the changes in morph frequencies between parent and offspring generations.

This experiment addressed two questions:

1. Between-brood variation: Does the interbrood variation in the incidence of pink larvae have a genetic basis?

If some of the between-family variation in brood frequencies is genetic in origin, then green or pink individuals from primarily-pink broods should produce more pink offspring than green or pink individuals from primarily-green broods. If the between-brood variation is due largely to brood-specific environmental effects (maternal influences and foodplant quality), however, then the brood frequencies that individuals produce may not be related in any predictable manner to the incidence of pink among their siblings.

2. Within-brood variation: Do green and pink siblings differ from one another genetically, or is the difference in their phenotype due entirely to non-genetic factors?

If some of the within-brood variation in phenotype is genetic in origin, then pink individuals should produce more pink offspring than their green siblings. If the phenotypic difference, however, reflects only random environmental influences acting on the same genotype during development, the offspring brood frequencies of green and pink siblings should not differ.

Methods

Rearings followed the protocol described previously. This experiment was conducted from May to August 1986. The individuals used

as parents were the lab-reared offspring of the wild females discussed in the previous section (some of females 1 to 43, in Table 2-1). The mother in each cross is indicated by the cross number; roman numerals correspond to the arabic numeral of the mother.

Matings. Pairings were made between fresh adults following their emergence in plastic pupation boxes. Most moths were paired within a day of emergence, but some were transferred to screen cages until appropriate mates were available.

Amphion mate readily in the lab. A single male and female were fed and put together in a container for two days. They were not fed, but the cages were sprayed with water each day. A variety of containers were used successfully, including screen cylinders 14 cm high by 7 cm diameter, screen cages 30x30x30cm or larger, and plastic shoeboxes lined with a paper towel. The feature important for successful mating was a non-slippery vertical or overhead substrate (screen, paper, or cloth) for the male to grasp. After two days (or less if the pair was observed in copula) the female was fed and bagged for oviposition. Egg laying usually commenced two days after copulation.

A successful cross was defined as one in which mating took place, the female oviposited, and at least 15 larvae were reared to the fourth instar.

Crosses made. Initially a balanced design was intended, with green and pink crosses made among full siblings. By paired comparisons of green and pink siblings, this design would have answered the question of within-family variation. The regression of offspring

values on mid-parent values would have allowed me to address the sources of variation among broods, although there are complications in the interpretation of the parent-offspring regression under inbreeding (Falconer 1981).

The planned inbred crosses were not achieved, for three reasons: (1) Amphion is protandrous. Males emerge several days before their sisters, and do not survive long in captivity. Therefore, in some lines no males were available when their sisters finally emerged. (2) Mating success was about 50%. Of 66 pairings in 1986 whose mating status was determined by post-mortem dissection, only 34 females contained spermatophores. (3) In lines with a high incidence of one color morph, the number of individuals of the other morph available for pairings was limited. The probability of both sexes of the rarer morph emerging simultaneously was low.

From many families, therefore, I produced only green or only pink crosses, instead of one or more of each color. In other families, when many individuals emerged at once, more than one successful cross of a given color was made. Four inbred green x green crosses, for example, were made among siblings from wild female 10.

Because the early crosses gave unexpected results that were possibly due to inbreeding, I also made outbred crosses. Individuals of the same color, but from separate mothers, were mated. The parents were not selected systematically; I paired whichever individuals were available at the same time. Big families contributed more parents than small families, and the number of crosses using individuals from a particular family ranged from one to six. Crosses that have parents

from the same line can be determined by looking at the "Parent Line" column in Tables 2-6 and 2-7.

Eighteen green inbred, eight pink inbred, thirteen green outbred, and seven pink outbred crosses were successful. In the second generation one inbred green and three inbred pink crosses were successful. The unbalanced nature of the crosses, and the potential non-independence of crosses derived from the same family, precludes some rigorous statistical analyses. All analyses presented here must be considered exploratory, and all interpretations tentative.

Within-family data analysis. Parent and offspring brood frequencies were compared for each cross separately. In the inbred crosses, parent and offspring color frequencies were compared with a G-test of independence. This cannot be used in the outbred crosses, because the mother and father came from different broods. Outbred crosses, therefore, were analyzed with goodness-of-fit tests, with the mid-parent % pink [= (maternal % pink + paternal % pink)/2] used to calculate expected values for the offspring broods. For all crosses, values of G were adjusted with Williams' correction (Sokal and Rohlf 1981), and compared to X^2 with one degree of freedom.

Between-family data analysis: Calculating the heritability of a threshold trait. Parents with mean values of a quantitative trait that are higher than the population mean will tend to have offspring with mean values higher than the population mean (heavy parents would have heavy children, or short parents would have short children, for example). The heritability of the trait (ratio of additive genetic variance to total phenotypic variance) can be estimated from the

regression of offspring mean values on midparent values (Falconer 1981). If all of the variance in the population mean were due to additive genetic effects, then the heritability, and the regression of offspring on midparental means, would equal one. Because some of the variance will be due to environmental and/or non-additive genetic effects, however, the heritability, and the slope of the regression, will be between zero and one.

Individuals with liabilities below the threshold have one phenotype; individuals with liabilities above the threshold have a different phenotype. For Amphion, I will arbitrarily define liability with respect to pink: a pink larva has a liability above the threshold, and a green larva has a liability below the threshold. The percent of a brood that is pink (the incidence of pink), therefore, is the percent of the family with liabilities above the threshold.

The brood frequencies, expressed as percent pink, are an inappropriate form for a regression, and must be transformed. If it is assumed that the liability in each family is normally distributed, and that variances are equal, then the mean liability of each family can be calculated in standard deviations from the threshold. A brood with an incidence of 50% has a mean liability at the threshold, or zero standard deviations. A brood with a low incidence of pink has a mean liability farther below the threshold than a brood with a high incidence of pink. I converted the percent pink from Tables 2-6 and 2-7 to mean liabilities, using Appendix A of Falconer (1981).

I used an analysis of covariance to calculate the heritability of the family means. Offspring percent pink was the dependent variable,

mid-parent percent pink the covariate, and cross type (green or pink) the class variable. For each analysis, I first tested the homogeneity of the slopes of the green and pink classes, by demonstrating that the interaction of midparent value and color was not significant.

Results

Four major patterns emerge from this data set:

1. Offspring of pink crosses are more pink than their parents (Tables 2-6b and 2-7b). In seven of eight inbred crosses and six of seven outbred crosses, the difference between parent and offspring brood frequencies is significant. In the three second-generation crosses, however, the offspring percent pink is not significantly different from the parent percent pink.

2. Offspring of green crosses are not significantly more green than their parents. G-tests found the percent pink in the offspring significantly higher than in the parents in 11 of 18 inbred green crosses, and significantly lower in only 3 crosses (Table 2-6a). In the single second-generation green cross the offspring percent pink is also higher than the parent percent pink, although the difference is not significant. In contrast to inbred crosses, the offspring of outbred green crosses are neither more pink nor more green than the parental broods. Offspring are significantly more pink in five outbred crosses, significantly more green in two, and not significantly different in six (Table 2-7a).

3. Broods from pink crosses are more pink than broods from green crosses in both inbred and outbred crosses. The mean percent pink of

inbred broods was 38% for green versus 77% for pink; mean percent pink of outbred broods was 32% for green versus 72% for pink. This difference between green and pink crosses is shown by the distribution of points on Figures 2-3 and 2-4: most of the offspring brood frequencies for the pink crosses lie above those for the green crosses.

4. There is a positive correlation between the parental and offspring brood means for both inbred and outbred crosses. Parents from broods with many pink larvae produce broods with many pink larvae. Figures 2-3 and 2-4 present the data as incidences (percent pink); Figures 2-5 and 2-6 present the same data, transformed to mean liabilities.

Recall that this experiment is addressing two distinct questions: (1) do individuals of the same color from primarily pink and primarily green broods differ in their tendency to produce pink offspring; and (2) do green and pink individuals from the same brood differ in their tendency to produce pink offspring? The third result above demonstrates that green and pink larvae produce different brood frequencies and the fourth result demonstrates that parents from primarily green and primarily pink broods produce different brood frequencies. These results, however, are complicated by the fact that the pink crosses were made from broods with a higher incidence of pink than the green crosses (Table 2-8). The correlation between parent and offspring brood frequency is less obvious if the broods of each color are examined separately.

I therefore tested the interbrood and intrabrood effects simultaneously with an analysis of covariance. If brood frequencies

are heritable, then there will be a positive regression of offspring percent pink on mid-parent values, when adjusted for the difference between the means of green and pink crosses. If an individual's color affects its offspring, for a given mid-parent value, then the intercept for the pink crosses will be larger than the intercept for the green crosses.

For the outbred crosses, the regression coefficient for the analysis of covariance = 0.24, with a standard error of 0.25. Thus the estimate of the heritability of family means is 0.24 +/- 0.25. Obviously with such a large standard error this regression coefficient is not significantly different from zero (Table 2-9, $p = 0.36$). A larger sample size might support a non-zero heritability. Nevertheless the regression provides preliminary evidence that some of the variation among family means is due to additive genetic effects: a pink individual from a family that is 20% pink differs genetically from a pink individual from a family that is 80% pink.

The intercept for the pink outbred crosses was significantly higher than for the green outbred crosses (Table 2-9). This confirms that, for a given mid-parent value, pink larvae produced more pink offspring than green larvae: there is a genetic component to the intrafamily phenotypic variation. For example, green brood XVIg3 and pink brood XVIp4 both had mothers from brood 16 and fathers from brood 17. Despite their common mid-parent value (% pink = 23%), the pink cross produced 76% pink offspring, and the green cross only 58% pink.

A similar analysis of covariance was performed on the inbred crosses (Figure 2-5, Table 2-10). Again the brood frequencies of pink

and green crosses were significantly different, when they were adjusted for differences in mid-parent values ($p = 0.0006$). The regression coefficient, however, was much smaller than for the outbred cross ($0.088 +/- 0.267$), and far from being significant ($p = 0.75$). The regression coefficient of offspring on mid-parent values in inbred crosses is expected to be lower than in outbred crosses, because the heritability within inbred lines is lower than if the population were outbreeding (Falconer 1981). A larger number of inbred crosses would decrease the standard error of the estimate of heritability under inbreeding, but because these moths are unlikely to be inbred in the wild, the biological significance of such an estimate would be unclear.

In summary, the probability that a caterpillar will be pink is significantly correlated with the color of its parents. The regression coefficient provides suggestive evidence that an individual's phenotype is also correlated with the kind of brood its parents came from (primarily green or primarily pink), but this must be confirmed with larger samples.

Effect of Photoperiod and Temperature

Photoperiod and temperature are the two most important environmental cues in the control of many polyphenisms (Tauber et al. 1986). While no one has suggested that sphingid caterpillars are seasonal forms, this may be because no one has censused them over any period of time.

When Amphion larvae are reared at 29 C on a long photoperiod (16L:8D), there is high variation among families in the incidence of pink individuals. I reared siblings under four combinations of temperature and photoperiod, to test (1) whether the mean incidence of pink larvae was affected by photoperiod and/or temperature, and (2) whether the responses to photoperiod and temperature were consistent among families.

Methods

Each treatment was set up in a different environmental chamber. The temperature in each chamber was monitored continuously with a Tempscribe 7-day wind-up chart recorder. Morph frequencies were compared for larvae reared on two photoperiods (Long (L) = 16 h Light:8 h Dark; Short (S) = 12L:12D) at two temperatures (Warm (W) = 29 C; Cool (C) = 21 C).

Additional eggs from the 35 wild females used in the previous analysis were distributed among treatments. The eggs each female laid in a single day were divided into groups of 30; the remainders were also used in some cases. One batch of eggs from her first day of oviposition was always assigned to the warm/long treatment, and provided the data described in the previous section. The distribution of all other egg groups was haphazard; since I determined that the percent pink did not change as a female aged, this does not affect the results. For most broods a single replicate was reared in each chamber (other than warm/long); when additional groups of larvae were reared, the data were combined for analysis.

Two experiments were conducted:

Incomplete factorial: WL vs WS vs CL. Offspring from 22 wild females were reared in three incubators, but not all families were represented in all treatments. For analysis I used fourteen samples with at least 15 larvae in each of the three treatments.

Complete factorial: WL vs WS vs CL vs CS . Offspring from 17 lab crosses were reared in four incubators; for analysis I used the sixteen samples with at least 15 larvae in each of the four treatments. The parents were the first-generation offspring of wild females, and the data set includes offspring from both inbred and outbred crosses.

Results

Incomplete factorial design

More pink larvae were produced in the WL treatment (median = 30.5% pink) than in WS (median = 15.5%) or CL (median = 13%) (Table 2-11). For the 14 families with larvae reared under all three conditions, this variation among rearing environments was significant (Table 2-12).

Complete factorial design

When families were reared under all four combinations of temperature and photoperiod, warm/long conditions again produced the most pink larvae (Table 2-13). Data were analyzed with a three-way mixed-model ANOVA without replication (Table 2-14). Temperature and photoperiod were fixed variables, each with two levels, and family was a random variable. Brood IVg6 was excluded from the analysis because the sample size in CL was less than 15 larvae.

Significantly more pink larvae were produced at 29 C than at 21 C, but there was no significant difference between broods reared at long and short photoperiod. The interaction between temperature and photoperiod was significant: the temperature effect was much greater at the longer photoperiod. Neither the family x temperature nor the family x daylength interaction was significant. The families did not differ significantly in their responses to the two temperatures, or to the two daylengths.

Intrabrood correlations

The absence of family x environment interactions in the previous analysis indicates that the differences among families were consistent in the different environments. Overall the families with more pink larvae in one condition also had more pink larvae in another condition. For example, in the first experiment, broods from females 2 and 8 had few pink larvae in all three treatments, but those from females 13, 18, and 30 had many pink larvae. Figures 2-7 and 2-8 show the correlations between the percent pink in WL and WS, and WL and CL, in the first experiment.

Although the family x environment interactions were not significant, when the data are plotted differently they suggest that not all broods were responding to the change in environment in the same way. In Figure 2-9, the arcsine-transformed brood frequencies from the second experiment are plotted for WL on the left axis and for CL on the right axis. The slopes of the lines indicate how each family responded to the change in environment (Via 1986). Parallel lines indicate that

families responded similarly; convergent or crossing lines indicate that families responded dissimilarly. Figure 2-10 is a similar graph for WS and CS. Figure 2-9 demonstrates the homogeneity of the response to temperature. All but three of the broods had a considerably lower incidence of pink at the lower temperature. At the short photoperiod, although the majority of broods showed a lower percent pink at the lower temperature, the overall pattern was less consistent.

Because my sample sizes were small, and I did not have replicates for each brood in each environment, the confidence intervals of each brood frequency are very large. For example, for brood VIIIg4, which showed an apparent increase in percent pink at lower temperature, at both photoperiods, the confidence intervals are: WL, 4-31%; WS, 0-18%; CL, 6-42%; CS, 4-42%. The results shown in Figures 2-9 and 2-10, therefore, do not provide any real information on the magnitude of family x environment interactions. Without replicates within each environment, I cannot test for interactions. Each data point on Figures 2-9 and 2-10 should be a family mean, rather than a single estimate of the family's brood frequency.

Effect of Rearing Density

Many caterpillars, grasshoppers, and walking sticks show color changes correlated with population density, usually becoming darker at higher densities (Long 1953, Key 1957, Fuzeau-Braesch 1985). The function of this color change is obscure, and none of the proposed explanations is adequate. Despite our lack of understanding of the

phenomenon, its existence is well-established. Of the four sphingids that have been tested, two species (Cephonodes hylas, Sasakawa and Yamazaki 1967; Erinnyis ello, Schneider 1971) have density-related color changes, and two species (Mimas tiliae and Laothoe populi, Long 1953) do not. I reared caterpillars of Amphion floridensis at four densities and looked for correlated changes in morph frequencies.

Methods

Offspring of fourteen wild Amphion females caught in 1987 were used. Siblings were reared en masse through early- to mid-second instar (3-4 days after hatching) on Ampelopsis arborea under warm/long conditions. Forty larvae from each of 11 females were then reared through the fourth instar under the following conditions:

1 larva per cup, 10 cups per female = 10 larvae

2 larvae per cup, 5 cups per female = 10 larvae

5 larvae per cup, 2 cups per female = 10 larvae

10 larvae per cup, 1 cup per female = 10 larvae

The numbers of green larvae in each group were compared with a Friedman test. If density decreases the proportion of green larvae, then fewer green larvae should be found among those reared 5-10 per container than among those reared 1-2 per container.

Results

Amphion larvae reared at high density were significantly more likely to be green than larvae reared at low density (Table 2-15; Friedman test, $N=11$, $df = 3$, $X2r = 20.78$, $p < 0.001$).

This trend became evident early in the experiment, after rearing the first three broods. Inspection of the cups suggested that the differences were not due to density, but to food quality. I tended to give young larvae in small groups smaller quantities of leaves than young larvae at higher density. The leaves of the young, low-density larvae dried over 24 hours, unlike the leaves in higher density containers. To control for this factor, an additional experimental group was added, '1W' (1 Wet), consisting of singly-reared larvae with a damp piece of filter paper and a surplus of leaves added to the container.

Percent pink in groups 10 and 1W were compared for offspring of 11 females (partially overlapping with females in the previous experiment). If the difference between 1 and 10 in the previous experiment was due to larval density, then a similar difference would be found here. In fact, the percent pink did not differ in these two groups, demonstrating that the 'density' effect was not due to the number of larvae in the rearing containers (Table 2-16; Wilcoxon test, $p > 0.05$).

General Discussion

The control of color in Amphion floridensis is complex. The color polymorphism is not a genetic polymorphism, but a facultative polymorphism, or polyphenism. The high variability maintained within each temperature-photoperiod regime, and the absence of photoperiod

effects, indicate that this is an aseasonal rather than a seasonal polyphenism (Tauber et al. 1986).

The results are consistent with a model that color determination is a quantitatively-inherited threshold trait, with large non-genetic contributions to the phenotypic variation. My estimate of the heritability of family means was 0.24 +/- 0.25, for the population sampled, under long photoperiod at 29 C. The fact that some of the interbrood variation has an additive genetic basis demonstrates that the population is capable of responding genetically to natural selection.

Among the environmental factors I tested, temperature influenced morph frequencies, but photoperiod and rearing density did not. The data are consistent with the hypothesis that maternal effects and/or nutritional effects also influence morph frequencies. Chapter 3 will discuss nutritional effects on coloration.

Selection for color polyphenism

All of the wild females in these experiments produced both green and pink offspring. This implies that the fitness of a female is higher when she produces offspring of both colors than if she produced offspring all of one morph. Both frequency-dependent mortality (Ayala and Campbell 1974, Clarke 1979) and environmental heterogeneity (Hedrick et al. 1976, Tauber et al. 1986) are likely to be important factors in the evolution of sphingid color polymorphisms. Frequency-dependent predation on color variants has been demonstrated in numerous experiments with a variety of predators (summarized in Ayala and

Campbell 1974, Clarke 1979), and for Amphion, the probability that a caterpillar will be eaten will almost-certainly depend on the overall frequency of green and pink caterpillars in the population. In addition, plants are highly variable environments (Denno and McClure 1983; Willmer 1986), and selective pressures are likely to vary spatially and temporally.

Temperature effects on color

More individuals became pink at 29 C than at 21 C. What is the potential adaptiveness for an Amphion caterpillar of using temperature as a cue for color determination? What could be the relative advantage of being pink at higher temperatures?

Thermoregulation. Wing melanization significantly affects thermoregulation in many butterflies (e.g., Colias spp. [Watt 1968]), but results of other studies on the effect of body color on insect temperatures are contradictory (reviewed in Casey 1981). If the body temperature of green larvae were higher than the body temperature of pink larvae, under the same insolation levels, then green larvae would be at an advantage at low air temperatures and a disadvantage at high air temperatures. In fact, pink larvae became slightly but significantly warmer than green larvae when matched pairs were placed outdoors in full sunlight (Chapter 4). Therefore, the adaptiveness of the temperature-related color change does not lie in different thermal properties of green and pink larvae.

Interactions between temperature and behavior. The microclimate provided by a plant is highly variable (Willmer 1986), and many insects

take advantage of this variation to regulate their body temperature (Casey 1981). Casey (1976), for example, demonstrated that desert-dwelling sphingids, Hyles lineata and Manduca sexta, changed their resting behavior as the temperature rose. At low air temperatures, H. lineata caterpillars rested near the ground, which allowed them to raise their body temperature above ambient; as the temperature rose, they moved into the foliage, and decreased the difference between ambient and body temperatures. M. sexta larvae did not move to the ground, but at higher air temperatures they moved from the periphery to the shaded interior of their plants.

If overheating is a risk for Amphion larvae then during summer there could be strong selection for them to rest in the coolest parts of their environment. If the coolest locations were sites where pink larvae were more cryptic than green larvae (e.g., stems rather than leaves), then becoming pink at high ambient temperature could be adaptive. The stronger temperature effect at the long photoperiod supports this hypothesis. Amphion is nearctic (Hodges 1971), and therefore larvae are unlikely to encounter high temperatures and short daylengths simultaneously. There would be stronger selection for temperature-correlated color change during the summer, when photoperiod is long, then during spring or fall.

Correlations between temperature and foodplant variation. The foodplants of Amphion vary in their phenotype both over time and among habitats. If (a) larval morphs are differentially cryptic on different plant variants (or have unequal survival for any other reason), and (b) there is a correlation between ambient temperature and plant phenotype,

then temperature may be a useful cue for a caterpillar to use. Grape, peppervine, and Virginia creeper grow both in the forest understory and in open, disturbed sites. Vines growing in the sun have pinker stems than vines growing in the shade (see Chapter 3), and differ in other characteristics including leaf size. At a given photoperiod, a larva at a higher ambient temperature is likely to be in a sunnier habitat than a larva at a lower temperature. The temperature-related color change in the caterpillars is consistent with the habitat-related color change in the plants.

Both the second and the third hypotheses for the adaptiveness of temperature-related color changes should be tested experimentally. They are not mutually exclusive, and both may be important. Temperature-related changes in resting behavior correlated with changes in the relative fitness of green and pink morphs, and latitudinal variation in the strength of the temperature effect, would support the temperature/behavior interaction hypothesis.

The problem of pseudoreplication. A complication in the interpretation of the temperature and photoperiod experiments is the pseudoreplication of the design (Hurlbert 1984). Although I controlled the temperature and daylength, the four incubators were from different manufacturers, and differed in light intensity and light quality (all fluorescent versus partially incandescent, directed versus diffuse, overhead versus lateral, inner surface of incubator white versus reflective metallic).

At least three aspects of light are known to affect pupal coloration in the Lepidoptera: wavelength (Angersbach & Kayser 1971;

Kayser & Angersbach 1974, A.G. Smith 1978, 1980, D.A.S. Smith et al. 1988), directionality (Angersbach 1975), and intensity (Kayser & Angersbach 1975). Light quality also affects coloration in a grasshopper Gastrimargus africanus, aphid Macrosiphum avenae, and mantid Mantis religiosa (Rowell 1970, Fuzeau-Braesch 1985). The differences in the incidence of pink Amphion among my treatments, therefore, could be partly or entirely attributed to effects of illumination, rather than to temperature and photoperiod. The best control would have been to use four identical environmental chambers, but they were not available. In retrospect, an appropriate control would have been to rear split broods in all four chambers, when all were set to the same temperature and photoperiod. The effect of temperature and photoperiod must be retested with appropriately controlled lighting, and light quality should be tested explicitly as a factor altering morph frequencies.

Light quality could be an adaptive cue for color determination in Amphion larvae, as it is for butterfly pupae and grasshoppers. Transmitted light varies qualitatively and quantitatively among habitats (Endler 1978, Hailman 1979), and the crypsis of an individual will be partly dependent upon the light conditions under which it is viewed (Endler 1978). On the same plant, therefore, the relative crypsis of two morphs could change if they were viewed in bright sunlight versus shade. In addition, the argument presented above with reference to potential correlations between temperature and foodplant variation probably holds even more strongly for correlations between ambient light conditions and foodplant variation.

Environmental effects in the controlled crosses

Why were so many offspring in the genetic crosses pink? Obviously my rearing procedure failed to control some important nongenetic source(s) of variation, that produced an overall increase in the percent pink between the two generations. The same potential sources of environmental variation that may have added to the interbrood variation among wild females are possibilities here: maternal effects and food quality.

Maternal effects. All of the mothers for these crosses were reared in the lab under warm/long daylength conditions (summer conditions). Perhaps these conditions produce mothers that produce many pink offspring; mothers reared under other conditions might produce fewer pink offspring. To test for an effect of maternal photoperiod or temperature on offspring color, Amphion larvae should be reared under two or more conditions (long and short daylength, for example), and then their eggs split and reared under all conditions. Maternal effects would be demonstrated if the maternal, but not the paternal, rearing environment affected the offspring brood composition.

Maternal conditions affect a diversity of offspring traits in arthropods (summarized in Dingle 1986). For example, in the milkweed bug (Oncopeltus fasciatus) and in members of at least four other insect orders, the photoperiod experienced by a female influences her offsprings' tendency to go into diapause (Saunders 1982, Groeters and Dingle 1987). In the lime aphid, Eucallipterus tiliae, color differences among individuals are partly attributable to the crowding conditions experienced by their mothers (Kidd 1979).

Maternal effects will be adaptive if the conditions of the female are a good indicator of the conditions that her offspring will encounter. The temperature and photoperiod that an Amphion mother experiences may correlate with seasonal changes in the foodplants, and with seasonal changes in the thermal environment her offspring will encounter.

Food quality. If summer leaves produce more pink larvae than spring leaves, this could explain the increase in percent pink in the crosses. The potential adaptiveness of foodplant variation will be discussed in Chapter 3.

Foodplant effects complicate any future experiments on genetic variance or maternal effects. One solution is to develop a standardized, artificial diet for Amphion, but my preliminary attempts were unsuccessful. Although a mass-rearing effort should be able to select for a strain that would feed on an artificial diet, this would not allow me to examine maternal effects in natural populations. An ecologically more realistic approach would be to identify the foodplant factors that affect morph determination, and then treat foodplant influences as a covariate in any further experiments.

Polyphenism allows an individual to respond adaptively to variability in its environment, and one of the most variable features of a caterpillar's environment is its foodplant (Denno and McClure 1983). If any characteristics of the foodplant are reliable indicators of the relative fitness of different color morphs, there will be selection on caterpillars to use those characteristics as environmental

cues. Chapter 3 will examine the relationship between plant variability and morph determination in Amphion and in Eumorpha fasciata.

Table 2-1. Fourth instar brood frequency for 35 wild-collected Amphion floridensis females.

Female[a]	Week[b]	Site[c]	Number of Fourth Instar			Per cent
			Green	Pink	Total	pink
1	1	w	43	3	46	7
2	1	n	51	1	52	2
3	1	w	26	2	28	7
4	1	n	36	38	74	51
5*	1	n	13	32	45	71
6	1	n	20	9	29	31
7	2	n	58	7	65	11
8	2	w	89	3	92	3
9	2	n	29	20	49	41
10	3	w	55	13	68	19
13*	4	n	27	20	47	43
14	4	n	34	21	55	38
15	4	w	52	8	60	13
16	4	n	44	19	63	30
17	5	n	49	9	58	16
18*	5	n	53	35	88	40
20	6	n	55	23	78	29
22*	6	w	31	1	32	3
23	7	n	38	2	40	5
25**	8	n	19	6	25	24
26	9	w	54	36	90	40
27	9	n	8	32	40	80
29*	10	n	11	15	26	58
30	10	n	10	40	50	80
33	11	n	15	10	25	40
36	12	n	20	2	22	9
37	12	w	16	12	28	43
41x	14	n	27	3	30	10
42x	14	n	51	7	58	12
43	14	n	30	25	55	45
44x	16	n	86	16	102	16
45x	17	n	12	2	14	14
46*	20	n	14	40	54	74
47	20	n	28	47	75	63
48	20	n	6	36	42	86

Females were collected between March and August 1986. Larvae were reared at 29 C, 16L:8D on Ampelopsis arborea.

[a] All females were mated once, as determined by post-mortem dissection, with the following exceptions:
* Female mated twice
** Female mated three times
x Number of matings unknown

[b] Week in which female was collected. Week 1 = 13 - 19 March 1986

[c] Site at which female was collected. w = west edge of Paynes Prairie; n = north edge of Paynes Prairie.

Table 2-2. Estimation of within-family and between-family components of the total variance in brood frequencies (% pink) of Amphion floridensis.

ANOVA TABLE

Source of variation	df	MS	Expected MS	F	P
Among families	14	632.67	$s^2 + n_o s^2_A$	11.62	0.0001
Within families	19	54.43	s^2		

Based on two to three samples per female, from fifteen wild-caught females. Data were arcsine transformed before analysis.

Partitioning the variance into between-family and within-family components (Sokal and Rohlf 1981):

Average sample size, $n_o = 2.261$

$$s^2_A = \frac{MS(broods) - MS(within)}{n_o} = 255.743$$

$$\text{Intraclass correlation} = r = \frac{s^2_A}{s^2 + s^2_A} = 0.825$$

Percent of variation among families = 82.5%
Percent of variation within families = 17.5%

Table 2-3. Effect of female collection site and week of collection on offspring brood frequency (% pink). ANCOVA on the arcsine transformed data.

Source	DF	Mean Square	F	P
Site	1	732.400	3.09	0.088
Season	1	681.596	2.87	0.100
Error	32	237.144		

Table 2-4. Changes in egg weight of Amphion floridensis.

Female	Weight of 5 eggs (mg) Day 1	Day 3	Day 5	Day 7
56-3c	6.19	*	4.44	
56-2e	5.57	*	5.24	
74-3	5.55	* 4.74 *	4.13	
55-5a	5.95	* 4.88		
53-4b	5.66	* 5.45		4.35
56-4b	6.69	* 5.61	5.56	
55-1g	5.85	* 5.46		
53-1b	5.97	* 5.46		
74-4	5.13	*	4.50	
56-4d	6.44	*	5.46	
55-5b	6.34	5.94 *	5.32	
55-5g	5.29	4.55 *	4.44	
55-5d	5.72	5.59		
Mean	5.87	5.30	4.89	4.35
s.d.	0.44	0.44	0.53	-

Eggs were collected on subsequent days from lab-reared females and weighed 24 hours after collection (up to 48 hours after laying). Asterisk indicates that female laid eggs on intervening days.

Table 2-5. Differences in larval color frequencies between early and late clutches from 13 female Amphion.

Female	Day 1 G:P (%P)		Day 2 G:P (%P)	# Days between clutches
7	39: 6 (13)	>	20: 1 (5)	6
16	17: 7 (29)	>	17: 5 (23)	1
20	14:11 (44)	>	26: 7 (21)	1
26	15:16 (52)	>	15:12 (44)	1
4	12:14 (54)	=	14:16 (53)	5
8	29: 1 (3)	=	32: 2 (6)	6
10	35: 9 (20)	=	20: 4 (17)	4
13	13:10 (43)	=	14:10 (42)	2
17	26: 5 (16)	=	23: 4 (15)	1
18	21: 6 (22)	<	26:22 (46)	3
30	7:23 (77)	<	3:17 (85)	3
42	30: 0 (0)	<	21: 7 (25)	3
44	25: 3 (11)	<	22: 5 (19)	4
Mean % pink	29.5		30.8	
Median % pink	22		23	

Data are number of green:pink fourth instar larvae and (% pink). =, <, and > compare the % pink.

Table 2-6 . Offspring brood frequencies (% pink in fourth instar) of inbred crosses of Amphion floridensis.

a. GREEN X GREEN CROSSES

Prediction: Offspring will be more green than parents

Brood	Parent Line	# 4th instar green	pink	Offspring % pink	Parent % pink	Direction of change (color G^2 significance+)
I1	1	106	67	39	7	Pink 21.02 ***
IVg6	4	23	126	85	51	Pink 26.67 ***
VIIg2	7	50	26	34	11	Pink 11.20 ***
VIIg3	7	45	31	41	11	Pink 16.91 ***
VIIg6	7	38	16	30	11	Pink 19.99 ***
VIIIg4	8	26	4	13	3	Pink 24.56 ***
IXg1	9	15	7	32	41	Green 0.52 ns
Xg1	10	44	12	21	19	-- 0.10 ns
Xg2	10	19	8	30	19	Pink 1.15 ns
Xg3	10	30	27	47	19	Pink 11.34 ***
Xg5	10	8	20	71	19	Pink 23.16 ***
XIVg1	14	36	5	12	38	Green 8.41 **
XIVg2	14	32	6	16	38	Green 5.64 *
XVg2	15	32	26	45	13	Pink 14.57 ***
XVIg1	16	30	18	38	30	Pink 0.65 ns
XVIIg4	17	19	25	57	16	Pink 19.27 ***
XVIIg6	17	16	21	57	16	Pink 17.46 ***
XXVIg4	26	44	5	10	40	Green 14.95 ***
			MEAN:	37.7	22.3	
Second generation:						
IX1a	IXg1	27	32	54	32	Pink 1.60 ns

Table 2-6--continued

b. PINK X PINK CROSSES

Prediction: Offspring will be more pink than parents

Brood	Parent Line	# 4th instar green	pink	Offspring % pink	Parent % pink	Direction of change (Color G^2 Significance+)	
IVp2	4	23	45	66	51	Pink	3.19 ns
IVp3	4	7	56	89	51	Pink	23.67 ***
VIp1	6	19	80	81	31	Pink	24.09 ***
VIp2	6	43	63	59	31	Pink	7.34 **
IXp3	9	4	24	86	41	Pink	15.59 ***
XVIp2	16	14	32	70	30	Pink	16.74 ***
XXp2	20	7	18	72	29	Pink	13.90 ***
XXp3	20	6	75	93	29	Pink	74.35 ***
			MEAN:	77.0	36.6		
Second generation:							
XXp3d	XXp3	1	5	83	93	Green	0.39 ns
XXp3e	XXp3	11	67	86	93	Green	1.83 ns
XXp3g	XXp3	7	70	91	93	--	0.15 ns

+ ns not significant ($p > 0.05$)
* $p < 0.05$ (G^2 crit = 3.84)
** $p < 0.01$ (G^2 crit = 6.635)
*** $p < 0.001$ (G^2 crit = 10.83)

Table 2-7. Offspring brood frequencies (% pink in fourth instar) of outbred crosses of Amphion floridensis.

a. GREEN X GREEN CROSSES

Prediction: Offspring will be more green than mid-parent value

Brood	Parents (mat/pat)	# 4th instar Offspring green	pink	% pink	Mid-parent[@] % pink	(mat/pat)	Direction of Change (Color G^2	Significance[+])
VIIg8	7/8	46	0	0	7	(11/3)	Green	2.45 ns[#]
XVIg3	16/17	31	44	59	23	(30/16)	Pink	43.52 ***
XVIg6	16/15	55	12	18	22	(30/13)	Green	0.66 ns
XVIIg1	17/16	11	15	58	23	(16/30)	Pink	14.05 ***
XXVIg3	26/30	60	6	9	60	(40/80)	Green	75.28 ***
XXVIg6	26/29	36	23	39	49	(40/58)	Green	2.36 ns
XXVIg7	26/29	65	20	24	49	(40/58)	Green	23.18 ***
XLIg1	41/42	32	31	49	11	(10/12)	Pink	56.79 ***
XLIg2	41/43	97	34	26	28	(10/45)	--	0.28 ns
XLIIg1	42/41	33	4	11	11	(12/10)	--	0.0 ns
XLIIg2	42/41	12	3	20	11	(12/10)	Pink	0.99 ns
XLIIg3	42/43	24	19	44	29	(12/45)	Pink	4.37 *
XLIIIg	43/41	8	9	53	28	(45/10)	Pink	4.43 *
			MEAN:	31.5	27.0			

Table 2-7--continued

b. PINK X PINK CROSSES

Prediction: Offspring will be more pink than mid-parent value

Brood	Parents (mat/pat)	# 4th instar Offspring green	pink	% pink	Mid-parent@ % pink	(mat/pat)	Direction of Change (Color	G^2	Significance+)
VIIIp1	8/14	24	30	56	21	(3/38)	Pink	30.64	***
XVp1	15/16	2	12	86	22	(13/30)	Pink	24.81	***
XVp2	15/16	34	39	53	22	(13/30)	Pink	33.74	***
XVIp4	16/17	16	51	76	23	(30/16)	Pink	84.09	***
XXVIp1	26/27	8	43	84	60	(40/80)	Pink	14.14	***
XXVIp2	26/27	14	64	82	60	(40/80)	Pink	17.51	***
XXVIp3	26/27	20	37	65	60	(40/80)	Pink	0.57	ns
			MEAN:	71.7	38.3				

@ mid-parent % pink = (maternal % + paternal %)/2

+ ns not significant ($p > 0.05$)
* $p < 0.05$ (G^2 crit = 3.84)
** $p < 0.01$ (G^2 crit = 6.635)
*** $p < 0.001$ (G^2 crit = 10.83)

This value was calculated using chi square, since the observed frequency of pink = 0, and ln 0, needed to calculate G, is not a real number.

Table 2-8. Mean percent pink of parent and offspring broods for four types of crosses.

	CROSS TYPE			
	Inbred		Outbred	
	gxg	pxp	gxg	pxp
Mean midparent % pink	22.3	36.6	27.0	38.3
Mean offspring % pink	37.7	77.0	31.5	71.7

Table 2-9. Effect of midparent brood frequency and parental color on offspring brood frequency (% pink). ANCOVA on the arcsine-transformed data from outbred crosses.

Source	DF	Mean Square	F	P
Color	1	5.240	14.12	0.0016
Midparent	1	0.330	0.89	0.36
Error	17	0.371		

Table 2-10. Effect of midparent brood frequency and parental color on offspring brood frequency (% pink). ANCOVA on the arcsine transformed data from inbred crosses.

Source	DF	Mean Square	F	P
Color	1	5.216	15.65	0.0006
Midparent	1	0.036	0.11	0.75
Error	23	0.333		

Table 2-11. First experiment on effect of temperature and daylength on brood frequencies in fourth instar Amphion larvae.

	Number of larvae (Green:Pink)			% Pink		
Female	WL[a]	WS	CL	WL	WS	CL
1	43:3	-	18:5	7	-	22
2	51:1	17:0	55:4	2	0	7
3	26:2	26:2	47:6	7	7	11
4	36:38	47:5	40:17	51	10	30
5	13:32	22:25	-	71	53	-
6	20:9	-	18:1	31	-	5
7	58:7	52:3	48:2	11	5	4
8	89:3	76:0	57:4	3	0	7
10	55:13	36:11	40:7	19	23	15
13	27:20	24:10	22:15	43	29	41
14	34:21	25:1	23:5	38	4	18
16	44:19	25:2	-	30	7	-
17	49:9	28:2	23:8	16	7	26
18	53:35	28:29	14:10	40	51	42
20	55:23	11:3	-	29	21	-
23	38:2	29:0	-	5	0	-
26	54:36	36:16	50:6	40	31	11
27	8:32	16:10	-	80	38	-
30	10:40	19:8	15:9	80	30	38
33	15:10	42:12	-	40	22	-
36	20:2	16:9	21:0	9	36	0
37	16:12	27:1	27:0	43	4	0
			MEAN	32	19	17
			MEDIAN	30.5	15.5	13

[a] WL = warm/long daylength (29 C, 16 h Light:8 h Dark)
WS = warm/short daylength (29 C, 12L:12D)
CL = cool/long daylength (19-21 C, 16L:8D)

Table 2-12. ANOVA of effect of temperature and daylength on brood frequencies in Amphion floridensis (first experiment).

Source of variation	df	MS	F	P
Family	13	407.47	3.78	0.002
Environment	2	375.44	3.48	0.046
Error	26	107.82		

Table 2-13. Second experiment on effect of temperature and daylength on brood frequencies in fourth instar Amphion larvae.

	Number of Larvae (Green:Pink)				% Pink			
Female	WL	WS	CL	CS[a]	WL	WS	CL	CS
IVg6	23:126	15:8	7:3	19:5	85	35	30	21
VIIIg4	26:4	15:0	17:4	14:3	13	0	19	18
Xg1	44:12	18:2	19:0	24:1	21	10	0	4
Xg2	19:8	20:11	22:0	25:4	30	35	0	14
Xg3	30:27	19:2	21:1	15:0	47	10	5	0
XIVg1	36:5	22:4	22:1	36:4	12	15	4	10
XIVg2	32:6	20:7	17:8	17:8	16	26	32	32
XVg2	32:26	29:11	51:2	27:1	45	28	4	4
XVp2	34:39	26:4	56:0	27:1	53	13	0	4
XVIg1	30:18	36:22	24:3	29:2	38	38	11	6
XVIg3	31:44	25:5	53:0	26:3	59	17	0	10
XVIp2	14:32	12:17	14:4	22:5	70	59	22	19
XVIp4	16:51	8:14	32:8	20:8	76	64	20	29
XVIIg1	11:15	18:9	27:3	5:20	58	33	10	80
XVIIg4	19:25	26:4	26:0	21:8	57	13	0	28
XXp2	7:18	2:23	12:4	5:16	72	92	25	76
XXp3	6:75	5:25	7:11	14:11	93	83	61	44
				MEAN	50	34	14	23
				MEDIAN	53	28	10	18

[a] Conditions as in previous table
CS = cool/short daylength (21 C, 12L:12D)

Table 2-14. ANOVA of effect of temperature and photoperiod on morph frequencies (second experiment--balanced, factorial design, without replication).

Source of variation	df	MS	F	P
Temperature (T)	1	4643.4	26.21	< 0.001
Photoperiod (P)	1	0.04	0.000	> 0.75
Family (F)	15	739.5	6.82	< 0.01
T x P	1	1452.8	13.40	< 0.005
T x F	15	177.2	1.63	> 0.1
P x F	15	111.5	1.03	> 0.25
Error	15	108.4		

Table 2-15. Effect of rearing density during the second through fourth instar on fourth instar morph frequency in Amphion.

	# Larvae per Cup			
Female	1	2	5	10
1	4:5[a]	7:2	6:4	9:1
2	4:6	6:3	8:2	10:0
3	3:7	8:2	4:6	8:2
4	3:6	5:3	9:1	5:4
7	0:10	3:7	2:7	7:2
10	4:6	5:3	7:3	8:2
11	2:8	3:7	3:7	2:8
13	1:8	5:5	4:5	7:3
16	7:3	7:3	10:0	9:1
17	1:8	7:3	8:2	9:1
18	5:5	7:3	10:0	10:0
Total	34:72	63:41	71:37	84:24
% Pink	68	39	34	22

[a] Data are number of green:pink larvae.

Table 2-16. "Density" effect is really due to differences in humidity and/or food desiccation.

Female	Group[a]		
	1W	10	1D
4	3:7	3:7	3:7
5	9:1	6:3	2:8
6	9:1	8:2	3:7
7	7:2	7:2	0:10
10	9:1	8:2	4:6
11	4:6	2:8	2:8
13	2:7	7:3	1:8
15	6:4	6:4	1:9
16	10:0	9:1	7:3
17	9:1	9:1	1:8
18	10:0	10:0	5:5
Total	78:30	75:33	29:79
% Pink	28	31	73

[a] Data are number of green:pink Amphion larvae.
Groups: 1W = 1 larva per cup, with damp filter paper and excess of leaves
10 = 10 larvae per cup, with excess of leaves
1D = 1 larva per cup, with smaller quantity of leaves and no filter paper.

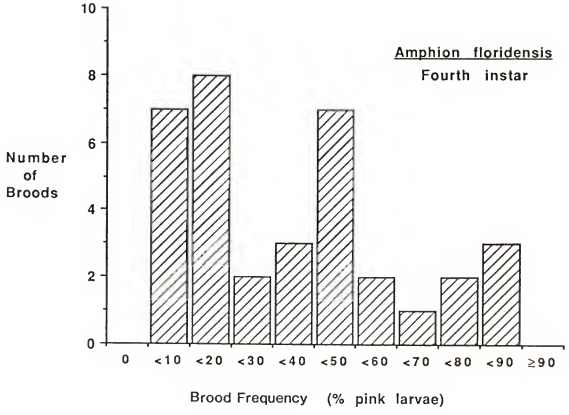

Figure 2-1. Histogram of the fourth instar brood frequencies (incidence of pink larvae) of Amphion floridensis. When brood frequencies are arcsine transformed, the distribution is not significantly different from normal.

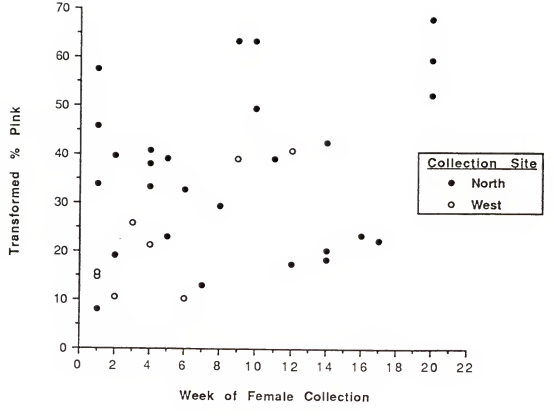

Figure 2-2. Seasonal change in brood frequencies of fourth instar Amphion floridensis. Week 1 = 13-19 March 1986; Week 20 = 24-30 July 1986. Open circles = females captured on the west edge of Paynes Prairie; closed circles = females captured on the north edge of Paynes Prairie. The regression equation: Transformed % pink = 30.0 + 0.79(Week); R^2 = 0.207.

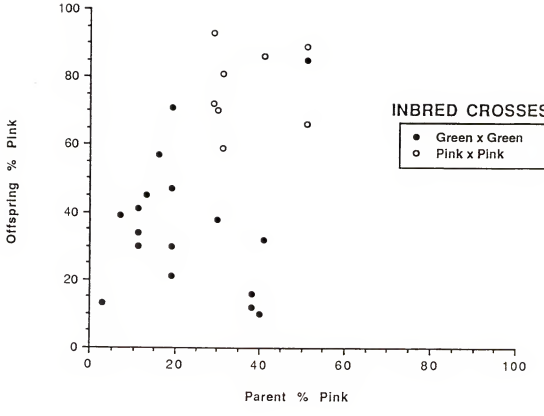

Figure 2-3. The incidence of pink larvae (% pink) in offspring broods plotted against the incidence of pink larvae in the families from which their parents were drawn, for inbred green x green and pink x pink crosses of Amphion floridensis. Offspring broods from green x green crosses are significantly less pink than from pink x pink crosses; there is a trend for offspring from parental broods with a high incidence of pink to be more pink than from parental broods with a low incidence of pink.

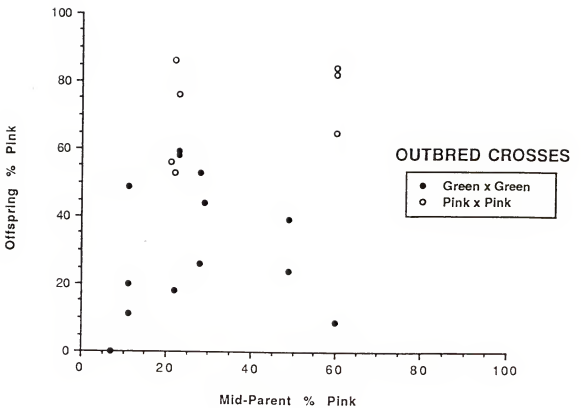

Figure 2-4. The incidence of pink larvae (% pink) in offspring broods plotted against the mid-parent incidence of pink larvae in the broods from which their parents were drawn, for outbred green x green and pink x pink crosses of Amphion floridensis. Mid-parent % pink = (maternal % pink + paternal % pink)/2. As for the inbred crosses, offspring from green x green crosses are significantly less pink than from pink x pink crosses.

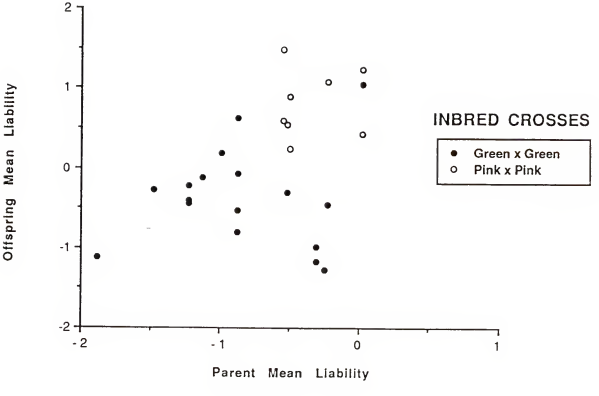

Figure 2-5. Data from Figure 2-3 (inbred crosses) re-plotted on a liability scale. Liability is measured in standard deviation units from the threshold. In broods with an incidence of pink larvae below 50%, therefore, most individuals have liabilities below the threshold, and the mean liability is negative. In broods with an incidence of pink greater than 50%, the mean liability is positive.

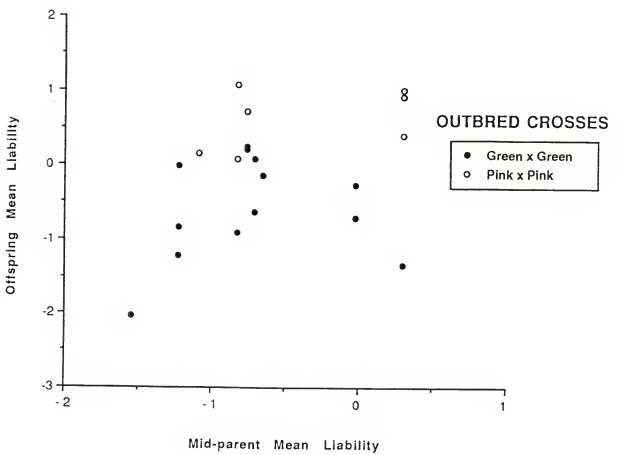

Figure 2-6. Data from Figure 2-4 (outbred crosses) re-plotted on a liability scale. Regression equation: Offspring liability = 0.70 + 0.24(Mid-parent liability); $R^2 = 0.524$, $p = 0.36$.

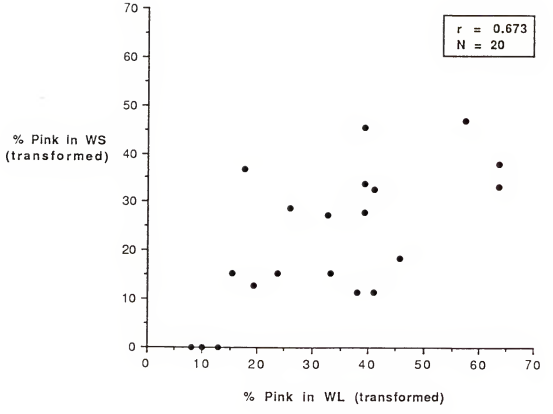

Figure 2-7. Correlation between brood frequencies of Amphion floridensis at two photoperiods: Warm/Long photoperiod (29 C, 16L:8D) versus Warm/Short photoperiod (29 C, 12L:12D). Each data point represents the fourth instar brood frequencies of a group of siblings reared in each environment.

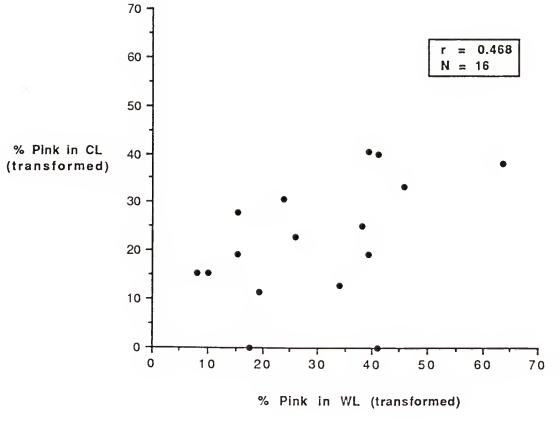

Figure 2-8. Correlation between brood frequencies of Amphion floridensis at two temperatures: Warm/Long photoperiod (29 C, 16L:8D) versus Cool/Long photoperiod (21 C, 16L:8D).

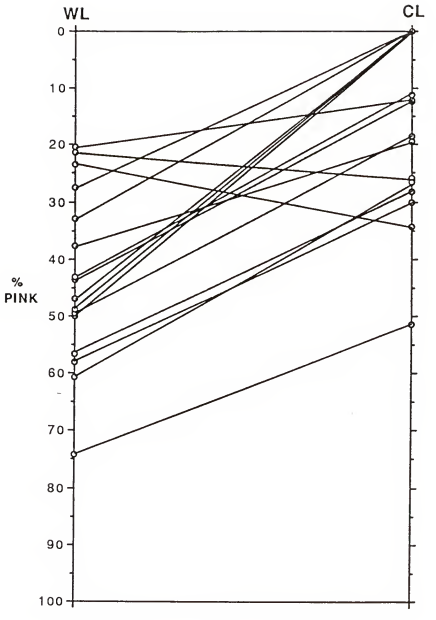

Figure 2-9. Family x environment interactions: WL vs CL. Each line represents the difference in the brood frequency ($\overline{\%}$ pink, arcsine transformed) in a single family when groups of siblings are reared at two temperatures under long photoperiod. Most families showed similar responses (lower percent pink at the lower temperature), but two families showed the opposite tendency.

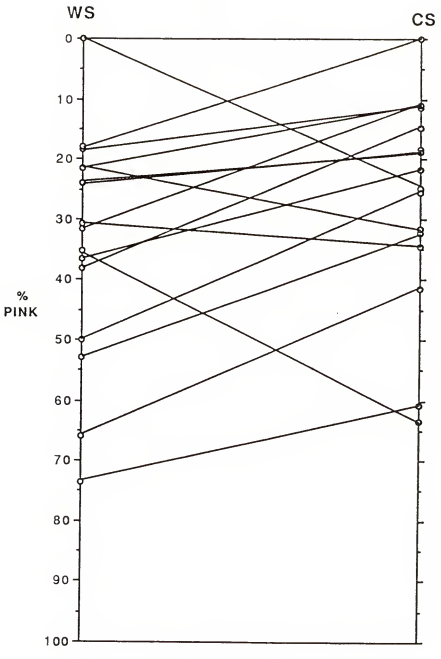

Figure 2-10. Family x environment interactions: WS vs CS. See Figure 2-9 for explanation. Larvae were reared at two temperatures under short photoperiod. The change in brood frequency with temperature is less uniform at this shorter photoperiod; some families showed little difference between the two temperatures, and four families showed a higher brood frequency at the lower temperature.

CHAPTER 3
FOODPLANT CORRELATES OF LARVAL COLOR

Introduction

Changes in color morph frequencies correlated with rearing density and season (Chapter 2) suggest that food quality may be an important determinant of larval color in Amphion floridensis. As Greene (1989) recently noted, dietary influences on morph determination in polyphenic species have received little attention, in comparison to such non-dietary environmental influences as temperature, daylength, and population density. This chapter will address the role of the foodplant in the color polymorphism of sphingid caterpillars.

McLachlan (1865), Gentry (1874), and Meldola (1873, 1882) were the first naturalists to suggest that color polymorphisms in caterpillars were controlled by their foodplants, and provided numerous examples of correlations between foodplant variation and caterpillar color variation. The degree of variation that has been attributed to foodplant effects ranges widely. In Smerinthus ocellata caterpillars, for example, variation in the size and intensity of inconspicuous lateral spots has been associated with foodplant species (Meldola 1882). Fawcett (1901) suggested that variation in the shade of green of some swallowtail larvae was due to variation in the color of the leaves on which the larvae fed. At the other extreme, foodplant-correlated morphs of some geometrid caterpillars differ in shape as

well as coloration, and are so dissimilar that they were originally considered to be separate species (Cockayne 1928; Greene 1989).

Although covariation of plant phenotype and caterpillar phenotype in the wild suggests that the plant itself may affect larval coloration, there are, in fact, at least five proximate explanations for such correlations. (1) A caterpillar's color is determined genetically and/or by environmental factors other than the foodplant, but due to selective predation, each morph survives better on a different kind of foodplant. This is the hypothesis given for a non-random distribution of two forms of Papilio demodocus larvae on two kinds of foodplants (Clarke et al. 1963; but see below). (2) Larvae may respond to an environmental cue that is correlated with plant phenotype. Among the Orthoptera, for example, many acridid grasshoppers are green at high humidity and brown at low humidity; in the natural environment this probably results in a match to green or brown vegetation (Rowell 1971). Thus different caterpillar color morphs found on fresh versus senescent plants, or on sunny versus shaded plants, could be the result of environmental cues independent of the foodplant variation. (3) Female oviposition preferences could become linked to genetic and/or strong maternal influences on color determination, so that eggs destined to become one morph are put on different plants from eggs destined to become another morph. This would be analogous to the European Cuckoo (Cuculus canoris), which has polymorphic eggs and is a brood parasite of many species of birds. Each female lays eggs that mimic one particular host, and she selects the correct nest for the kind of egg she produces (Ford 1975).

Although this is theoretically possible in the Lepidoptera, I do not know of any examples. (4) Caterpillars with high mobility can move between foodplants (e.g. Danaus plexippus, Rawlins and Lederhouse 1981); if plant heterogeneity is on a small enough spatial scale, morphs may select different plant types. This would be analogous to the differential selection of resting sites in the melanic and pale forms of some moths (Kettlewell 1955a). Because the energetic costs and predation risks of moving between foodplants are probably high, and because a caterpillar would have no way of assessing available plant phenotypes until in direct contact, this is an extremely unlikely explanation for non-random larval distributions among plants. It is, however, very important in the non-random distribution of larval resting sites within a plant, and will be discussed in Chapter 4. (5) Individual caterpillars are phenotypically plastic, and color is directly influenced by some quality of the foodplant.

This chapter examines the importance of the last hypothesis for sphingid caterpillars. As the list of alternative hypotheses emphasizes, the occurrence of covariation between larvae and plants in the wild is not an adequate demonstration that the plant itself is a direct causal agent; controlled feeding experiments are essential. Similarly, lab experiments alone do not demonstrate that foodplant influences are ecologically relevant; simply because foodplants can affect larval morphs does not demonstrate that such effects do occur in the wild. In experiments on two sphingid species, I will focus both on how hostplant variation influences caterpillar color variation, and on the evidence that such variation is ecologically important.

If foodplant quality affects larval color, either (a) foodplant quality is a reliable indicator of the environment the larva will encounter, so that larvae responding to the cue have higher fitness than larvae that are not responsive to the cue; or (b) the plant is manipulating the larva, in which case the fitness of larvae that do not respond would be higher than those that do. I will discuss the possible proximate mechanisms and the potential costs and benefits for a caterpillar of such a system of morph determination.

Foodplant Effects in Caterpillars

To demonstrate that food quality affects color morph determination, groups of larvae must differ in color when reared with different feeding regimes (for example on two plant varieties), while other environmental or genetic factors are held constant. Few caterpillars have been examined experimentally in this way, and among them the results are sometimes contradictory or inconclusive.

In Callophrys mossii bayensis (Lycaenidae) and Papilio demodocus (Papilionidae), there has been disagreement over the relative importance of genotype and foodplant variability in determining larval color forms. Callophrys mossii bayensis has red, orange, and yellow morphs in the final instar, and Brown (1969) speculated that the variation was due to choice of feeding sites on the Sedum foodplants: larvae matched either their red leaf or their yellow flower feeding sites. Later studies discounted foodplant effects and suggested that the forms were genetically determined (reviewed in Orsak and Whitman 1986). Recent experiments have supported both hypotheses: the major

color forms were determined genetically, but slight but significant changes in color occurred when larvae were restricted to feeding on either yellow flowers or red bracts (Orsak and Whitman 1986).

Papilio demodocus larvae are monomorphic in most of their range and feed on the Rutaceae (Citrus). In South Africa, however, a second, distinctive form has been found, and is non-randomly associated with umbellifer foodplants. Van Son (1949) assumed that the different forms were controlled by the foodplant, but Clarke et al. (1963) found no significant difference in morph frequencies when individuals were reared on the two foodplants. Instead Clarke et al. (l.c.) argued that the polymorphism was controlled by one pair of genes, and demonstrated that this genetic model was compatible with morph frequencies obtained from wild females and from controlled crosses. They presented indirect evidence of differential predation, and suggested that larvae of the umbellifer morph might be less cryptic on Citrus than larvae of the citrus morph, and therefore suffer higher mortality.

The evidence supporting differential predation in P. demodocus was that morph frequencies of mature larvae found in the wild differed from the morph frequencies found when eggs or young larvae were collected and reared in the lab. Since the reared larvae were exposed to different environmental conditions than the mature larvae, obviously Clarke et al.'s interpretation depends on an absence of environmental effects on morph determination. Their data sets are so small, however, that they are also compatible with a polygenic genetic model with environmental, including foodplant, effects on morph determination. The authors' rejection of foodplant effects was based on a sample of

only 10 larvae reared on Citrus and 14 larvae on fennel; in fact there was a slight excess of umbellifer-patterned larvae on the fennel.

Thus, although the P. demodocus system is often cited as an example of differential predation maintaining a polymorphism (cf. Edmunds 1974, Futuyma 1986), the alternative explanation, of environmental morph determination, cannot yet be rejected. Van Son's original postulation of foodplant effects merits further analysis.

In contrast to the ambiguous results in Callophrys mossii bayensis and Papilio demodocus, clear foodplant effects have been demonstrated in Plusia gamma (Noctuidae) and Nemoria arizonaria (Geometridae). P. gamma larvae vary continuously in color from whitish green to black with yellow stripes. Although population density is the major environmental determinant, foodplant species also significantly influences average larval color (Long 1953). The two morphs of N. arizonaria mimic either oak catkins or oak twigs. By feeding matched broods of larvae on homogenized artificial diets supplemented with different natural foods and plant extracts, Greene (1989) demonstrated definitively that food quality determined larval morph.

In summary, food quality has been shown to have both major (in N. arizonaria) and minor (in C. m. bayensis) effects on caterpillar color, but the generality of the effect within the Lepidoptera is unknown.

Foodplant Effects in Sphingid Caterpillars

In the Sphingidae, there are a large number of anecdotal observations that larval color morphs occur non-randomly with respect to foodplant quality (Table 3-1), but few controlled studies. At least

four different factors related to foodplant have been hypothesized or demonstrated to cause these correlations: plant species, plant color, damage levels, and seasonal changes in plant quality.

Foodplant species. In L. populi, S. ocellata, and S. ligustri, alternative caterpillar morphs are different shades of green, and numerous naturalists have found consistent differences among the larvae on different foodplant species in the wild. Grayson (1986) summarized six years of field censuses of Smerinthus ocellata (total N = 298 larvae on six foodplant species) and Laothoe populi (total N = 108 larvae on six foodplant species). For both moths, caterpillar morph frequencies were homogeneous on each foodplant across years, and heterogeneous among foodplants, indicating a non-random association of larval morphs and foodplant species. In addition, although the data sets are too small for statistical analysis, there is a suggestion that, on the plant species shared by the two caterpillars, their colors vary in parallel (Table 3-2). The plants with the most green (rather than grey) larvae of S. ocellata have the most yellow-green (rather than white-green or white) larvae of L. populi.

The field correlations between plant species and larval color were supported by controlled feeding experiments (Grayson 1986). Seven broods of L. populi were split and reared on both Salix fragilis and Populus alba. More yellow-green larvae were reared on the former plant species, and more white larvae on the latter (Grayson 1986).

The color adjustment in larvae of L. populi, S. ocellata, and S. ligustri is among shades of green. Such variation is common in the tribe Smerinthini; however, in most of the macroglossine sphingids

(tribes Macroglossini, Philampelini, Dilophonotini) the caterpillar morphs differ in hue or pattern rather than just shade. The effect of foodplant species on switching caterpillars between green and non-green morphs has been shown only in Erinnyis ello: larvae reared on Euphorbia cotinifolia were more likely to become brown in the final instar than larvae reared on Poinsettia pulcherrima (Schneider 1973).

Foodplant color. Correlations between plant color variation and larval color variation have been noted in the Smerinthini (Poulton 1885ab, Grayson 1986), Eumorpha fasciata (Gossington 1975) and Rhyncholaba acteus (Fogden and Fogden 1974). In all of these instances, however, the plants may have been different species rather than color morphs within a single species; this category, therefore, may be a subset of the first. Thus in Smerinthus ocellata the various foodplants that affect morph frequencies also differ in leaf color (see above). In a popular article, Gossington (1975) claimed that the foodplants of Eumorpha fasciata are green in the shade but have pink stems and seed capsules in the open sun, and that different larval morphs were found on the two. He did not, however, state whether these two plant types were variants of the same species, or were different species. Similar covariation in plant and larval color for Rhyncholaba acteus was mentioned in another popular reference (Fogden and Fogden 1974); again it is impossible to determine if the different plant morphs were conspecific or congeneric.

Foodplant damage levels. In controlled rearings, fresh Poinsettia plants produced more green larvae of E. ello than previously-defoliated

plants (Schneider 1973), but correlations with damage level have not been reported in wild populations of E. ello.

Seasonal changes in plant quality. Plants change phenotypically as the season progresses, and the proportions of larval forms may also change over the season. Gentry (1874) suggested that changes in Nicotiana tabacum (tobacco) plants from summer to fall resulted in a higher incidence of melanic Manduca quinquemaculata larvae later in the season, but provided no proximate or ultimate explanation for the change. An unusual dark form of Manduca sexta has been found in recent years in North Carolina, from late summer through late fall (E. Lampert, North Carolina State University, pers. comm.). The tobacco plants at this time are sprouting from suckers after having been cut back in mid-summer. Thus if the foodplant is responsible for the production of the dark larvae, the foodplant quality may be some factor that normally changes seasonally in the plants, or it may be a factor related to the cutting and regrowth.

Although Table 3-1 suggests that correlations between larval morphs and plant morphs are found in all five sphingid tribes, the actual evidence for foodplant determination of color morphs in sphingids is meager. Members of only two tribes have been examined experimentally. Only for S. ocellata and L. populi (Smerinthini) are data from field censuses available which confirm that the distribution of wild larvae is non-random. Only for these two species plus E. ello (Dilophonotini) have controlled rearings verified that the plant itself is an important factor. For all of the other examples in Table 3-1 the

correlations are potentially due to covariation between plant and caterpillar, differential predation, or other factors.

This Study

I measured the importance of foodplant quality in color determination in representatives of the tribes Macroglossini (Amphion floridensis) and Philampelini (Eumorpha fasciata). With lab experiments, I tested food effects directly while holding other environmental factors constant; with field censuses and experiments, I tested whether these effects were relevant to wild larvae.

Foodplant Effects in Eumorpha fasciata

Eumorpha fasciata is a highly variable sphingid, with color polymorphism occurring in the second through fifth larval instars. Caterpillars feed on Ludwigia (Onagraceae; formerly Jussiaea), which are herbaceous or semi-woody shrubs, patchily abundant in damp or wet habitats. E. fasciata is larger than Amphion; mature larvae may be up to 9 cm long, and adult wingspan is about 10 cm.

In all instars one morph is bright green. In instars 2-4 the non-green morphs are bright pink, yellow, or pink and yellow; in instar 5 the major non-green morph is boldly patterned with black or brown, yellow, white, green, and rust. A rare final-instar form is primarily yellow. In this chapter morphs will be designated green, pink and yellow, or black. Excellent figures of many morphs, including early instars, are given in Moss (1912, plate XI).

E. fasciata is common throughout its neotropical range (breeding from northern Argentina to South Carolina [Hodges 1971]), and larval variability has been noted in many populations (Surinam [Sepp 1852], Argentina [Berg 1875], Peru [Moss 1912], Brazil [Moss 1920], Florida [Gossington 1975]). Some authors have recorded only the dark form of the mature larva (Boisduval 1874; Burmeister 1879; Gundlach 1881); this may be due to the green form being rare or absent in some geographic areas, or to a longstanding confusion of the larvae and adults of this species with those of Eumorpha vitis (Rothschild and Jordan 1903).

Moss and Gossington both commented on non-random distributions of larval forms. Moss (1912, 1920) observed that green larvae predominated in Brazil and non-green larvae in Peru, and that they occurred on different Ludwigia species in the two regions. He considered the higher proportion of green larvae in Brazil to be a "nice adaptation to a greener and more flourishing vegetation." As noted above, Gossington (1975) found green larvae on shaded Ludwigia plants and non-green larvae on Ludwigia growing in full sun. He suggested that the difference might be related to anthocyanin pigments accumulating in the sun plants. Neither author provided quantitative data to support their observations.

I conducted field censuses of wild populations of Eumorpha fasciata on several foodplant species over four years. Finding that caterpillar morph frequencies differed among the plant species, I conducted lab rearings to determine whether the differences were, in fact, due to qualities of the plants themselves.

Methods

Field censuses

In the fall of 1985-1988, I censused Ludwigia stands for E. fasciata eggs and larvae. Instars are distinct, and can be identified with certainty from changes in their tails and markings. I found eggs in Gainesville from late August through mid-October in 1985, 1986, and 1987, and until mid-November in 1988. Census effort varied, with more time spent in 1985-86 than in 1987-88. In 1985-86 all censuses were done at sites in or around Gainesville, FL; in 1987 and 1988 local populations were very small, and therefore additional sites were censused as far south as Sebring, FL. The sites and sampling dates are given in the Appendix.

Larval color morphs in the lab

Foodplant-correlated morph frequencies found in the field led to a lab experiment on the role of foodplant in color determination. Eggs were collected from L. peruviana, L. leptocarpa, and L. octovalvis in 1986 and reared in the lab on leaves from all three species. Eggs were re-distributed so that some from each foodplant were reared on all three species. Larvae were reared individually in clear plastic containers (11.5 cm diameter x 3.5 cm high) in a constant-environment chamber at 29 C, 16L:8D. Cups were cleaned and larvae given fresh leaves daily, and larval color at each instar was recorded. Leaves were collected daily or every other day; if the latter, extra leaves were refrigerated in a plastic bag with a damp paper towel.

Statistical analyses

Most samples were analyzed with G tests, using Williams' correction (Sokal and Rohlf 1981). When overall G tests found significant heterogeneity, a simultaneous test procedure (STP) was used to compare subsamples. Because multiple tests were performed, care was taken to keep the experiment-wise error rate at 5% (Sokal and Rohlf 1981). For 2 x 2 tables with small sample sizes Fisher's Exact Test was used instead of the G test.

Results

From 1985 to 1988, 897 E. fasciata eggs and 698 larvae were found in the field (Table 3-3). Eggs, varying in color from green to white to pale orange, are almost always on leaf undersurfaces, and can be found fairly easily when branches are turned upside down. E. fasciata larvae rest under leaves or stems in all instars, and do not move far from feeding sites until they are ready to pupate. Because small larvae (first through third instar) cause little feeding damage and are most difficult to find, they are underrepresented in my samples. In contrast, the obvious feeding damage of fourth and fifth instar larvae often reveals their location. By searching initially for feeding damage and then for the larvae I avoided biasing the samples in favor of less cryptic morphs.

Correlations between larval color and foodplant species

More than 20 Ludwigia species occur in north Florida (Godfrey and Wooten 1981). Species on which I have found E. fasciata eggs or larvae include (in decreasing local plant abundance) L. peruviana (L.) Hara,

L. octovalvis (Jacq.) Raven (two morphs), L. leptocarpa (Nutt.) Hara, and L. decurrens Walt. The two forms of L. octovalvis differ in the size of the flowers and seedpods, and were treated initially as different species, which will be referred to as L.oct (bigflr) and L. oct (smallflr). Voucher specimens of all four species, and both morphs of L. octovalvis, have been deposited in the University of Florida Herbarium (specimen accession numbers FLAS 168046 - 168050). Two unidentified Ludwigia species had five additional E. fasciata larvae.

Figure 3-1 suggests that year-to-year variation in morph frequencies within plant species was small, but that variation among foodplant species was large. Fifth instar samples ranged from 89% green on L. peruviana to 13% green on L. octovalvis (smallflr). The pattern of decreasing percent green with instar was found on each plant species (Table 3-4).

Within plant species, fifth instar morph frequencies were homogeneous across years on both L. peruviana and L. leptocarpa (L.p. 1985-88: $G = 1.609$, $df = 3$, $p > 0.5$; L.l. 1986-88: $G = 0.046$, $df = 2$, $p > 0.95$). Because the two forms of L. octovalvis (smallflr and bigflr) did not differ in the frequency of black larvae in either 1986 or 1987 (1986: $G = 2.273$, $df=1$, $p > 0.1$; 1987: Fisher's Exact Test, $p = 0.339$), data for the two forms were combined for analysis. Unlike the samples on L. peruviana and L. leptocarpa, significant heterogeneity was found ($G = 12.762$, $df=2$, $p < 0.005$), with significantly more black larvae in 1986 than in 1987 and 1988.

In contrast to the general homogeneity within plant species, the frequency of black larvae among the foodplant species was highly

variable ($G = 152.12$, $df=4$, $p < 0.001$). Because the samples from L. octovalvis were not homogeneous, the data for this species were treated as two samples, one from 1986 and one from 1987-1988; within each sample smallflr and bigflr data were combined. The frequency of black larvae, therefore, was compared among the following five groups: L. peruviana, L. leptocarpa, and L. decurrens, all years; L. octovalvis (1986); and L. octovalvis (1987-88). Three significantly different groups consist of (1) L. peruviana with the highest proportion of green larvae; (2) L. octovalvis (87-88) and L. leptocarpa with intermediate proportions of green larvae; and (3) L. octovalvis (86) with the lowest proportion of green larvae. The small sample of larvae on L. decurrens ($N = 8$) was not significantly different from the second or third groups. If the L. octovalvis data from 1986-88 are lumped despite their heterogeneity, then the interplant variation is still highly significant ($G = 138.78$, $df=3$, $p < 0.001$), and the STP analysis shows that only L. peruviana differs significantly from L. leptocarpa, L. octovalvis, and L. decurrens.

Fourth instar results parallel the fifth instar: year-to-year variation in morph frequencies was not significant for any plant species, but variation among plant species was significant (Figure 3-2; L.p. homogeneous by inspection; L.l. $G = 1.601$, $df=2$, $p > 0.1$; L.o. $G = 0.321$, $df=2$, $p > 0.5$; overall interplant heterogeneity $G = 38.645$, $df=2$, $p < 0.001$). The simultaneous test procedure found that larvae on L. peruviana differed from those on L. leptocarpa and L. octovalvis. The sample on L. decurrens was too small ($N = 5$ larvae) to include in the analysis, and the two forms of L. octovalvis were combined.

Lab rearings

The correlations between foodplant species and larval color may be due to the foodplants themselves, but may also result from environmental differences not related to the foodplants (habitat differences), selective mortality of green larvae on L. octovalvis and L. leptocarpa and non-green larvae on L. peruviana, or female oviposition preference linked with strong maternal or genetic effect on offspring color. I planned to test for habitat effects with an analysis of variance of the morph frequencies on Ludwigia species co-occurring at sites. Unfortunately, even when several Ludwigia species were found at one site, larvae were found predominantly on a single species. For example, although the three major Ludwigia species were all abundant at site NEa in 1986, 55 of 59 fifth instar larvae were found on L. octovalvis. Thus my census data are not sufficient to discriminate among potential explanations for the observed pattern. By rearing larvae in the lab on the three major plant species I tested directly if any of the variation in morph frequencies was due to plant quality.

In the fifth instar, lab-reared samples ranged from 30% green on L. peruviana to 0% green on L. octovalvis (Figure 3-1; overall heterogeneity $G = 14.476$, $df=2$, $p < 0.001$). Samples reared on L. peruviana and L. octovalvis differed significantly, but the sample on L. leptocarpa was not significantly different from either (L. peruviana versus L. leptocarpa $G = 3.334$, $p > 0.05$; L. leptocarpa versus L. octovalvis, Fisher's exact test $p = 0.118$). In the fourth instar all three samples differed significantly (overall $G = 20.473$, $p < 0.001$;

for pairwise comparisons $X^2_{crit} = 3.84$: L. peruviana versus L. leptocarpa G = 5.826; L. leptocarpa versus L. octovalvis G = 4.827). The relative order of the three foodplants (L. peruviana producing the most green larvae and L. octovalvis producing the fewest) was identical to their relative order in the field censuses.

On all three foodplants the frequency of green larvae in the fifth instar was lower in the lab than in the wild during the same season (Figure 3-1). The only significant difference in proportion green between field and lab, however, was for larvae reared on L. peruviana ($G = 29.387$, $p < 0.001$). Among the fourth instar larvae only the sample reared on L. octovalvis had a lower proportion green in the lab (Figure 3-2), although this difference was not significant ($G = 0.911$, $p = 0.34$).

Individuals reared on the three foodplants differed significantly in pupal weights, and the lightest pupae were found on the plant that produced the most nongreen larvae (Table 3-5; all three pairwise-comparisons among plant species were significant, Tukey's studentized range test, alpha = 0.01). The lightest pupae were reared on L. octovalvis (mean = 2.5 g), and the heaviest pupae on L. peruviana (mean = 3.9 g). Within the L. peruviana sample, there was a trend for black larvae to have higher pupal weights than the green larvae, but the difference was not significant (Table 3-6). Because so few green larvae were produced in the lab, it is not possible to factor out the contributions of sex, color, and foodplant with a three-way ANOVA, or to compare the pupal weights of green and black morphs on L. octovalvis or L. leptocarpa.

Seasonal changes in plants and in larval morph frequencies

All Ludwigia species increased the red pigmentation on their stems, leaves, and seed pods as the autumn progressed, with the most dramatic changes shown by L. leptocarpa. In the 1987 field censuses I attempted to correlate the color of larvae with the color of the individual plants on which they were found, to investigate effects of intraspecific foodplant variation. My ratings of plant color were unsuccessful, however, because intraplant color heterogeneity was very high. Plant color does not change evenly: for example, adjacent to a fully-green leaf may be a bright crimson leaf, or a seed pod that is crimson above and bright green below.

As a rough test for effects of intraspecific foodplant variation on larval color, I looked for a seasonal change in larval color on each Ludwigia species. A change in the proportion of non-green larvae later in the season could be due to seasonal changes in the plants (although further experiments would be required to rule out the role of temperature and daylength). I calculated the median dates on which I found green and non-green larvae on each foodplant species in each year, and compared these dates with a Wilcoxon test.

Table 3-7 lists the median dates for fifth instar larvae of each color, and Table 3-8 for fourth instar larvae. Non-green larvae were not collected consistently later than green larvae, and the Wilcoxon test was not significant for the fifth instar ($p > 0.05$; the sample size for fourth instar larvae is too small for statistical analysis). For two fifth-instar samples, however, the median dates for black larvae were two and four weeks later than the median dates for green

larvae (L. peruviana 1988 and L. octovalvis 1988). I do not know why these two samples differ from the rest. Although the ratio of green to non-green larvae does not change uniformly over the fall, the 1988 samples indicate that a more careful comparison of intraspecific plant variation is warranted.

Other sources of color variation in Eumorpha fasciata

The lab rearings demonstrate that some of the difference in morph frequencies found among foodplants in the wild is due to qualities of the foodplants themselves. Foodplant quality, however, is obviously not the only determinant of larval color morph in Eumorpha, since different morphs are often found on the same individual plants. During censuses, on 38 occasions at least two fourth instar or at least two fifth instar larvae were found on one plant or continuous clump of plants. In 13 of these instances (34%) the larvae were of different morphs. A dramatic demonstration of the co-occurrence of morphs was found in October 1988. One L. octovalvis plant, approximately 1 m high and 1-1/2 m in diameter, had fifteen fourth instar larvae on it. Nine larvae were green, five yellow and pink, and one pink.

Foodplant Effects in Amphion floridensis

For Amphion floridensis, I could not collect field evidence to measure correlations between foodplant quality and caterpillar color. Although adults are abundant and easily captured, I found only 11 eggs and one fourth instar larva during concerted searching of foodplants in the spring and summer of 1985. Moreover, in collecting countless

bushels of wild foodplant from 1985 through 1988, I recorded only 53 Amphion eggs and eight larvae. This contrasts sharply with my observations of more than 250 Enyo lugubris larvae on the same plants in the same period, and almost 700 larvae of E. fasciata.

I therefore reared broods of Amphion outdoors on two foodplant species in two habitats, and looked for variation in larval morph frequencies. Laboratory rearings tested the effect of both interspecific and intraspecific foodplant variation under controlled conditions.

Lab Experiment: The Effect of Plant Species

The few eggs and larvae of Amphion I have found in Gainesville have been on representatives of three genera of the Vitaceae: Ampelopsis arborea (peppervine), Parthenocissus quinquefolia (Virginia creeper, woodbine), and Vitis rotundifolia (muscadine grape). I reared larvae from wild females on all three foodplant species.

Methods

Larval rearing followed the protocol described in the Chapter 2. All larvae were fed Ampelopsis in the first instar and put in experimental groups in the late first to early second instar. A split-brood design was used: from each brood, fifteen larvae were reared on each foodplant, in three groups of five. Rearings were done at room temperature (approx. 23 C). Larvae were kept on a shelf adjacent to a window, and experienced natural daylength. The experiment was run from 8 May to 28 July 1988, with broods from nine wild-caught females.

Food was collected daily. Usually all three plant species were collected from the same partly-shaded site. All species had some pink color on leaf undersides or petioles, but no bright red. On a few occasions Ampelopsis was obtained from a separate, sunnier site. Only young leaves of Vitis were picked, since this species becomes very tough in the summer, and larval survival is poor (pers. obs.), but leaves of all ages were used for Ampelopsis and Parthenocissus.

Results

For seven of the nine broods the fewest pink larvae were produced on Ampelopsis (Table 3-9). Using my criterion for ties, the Friedman test is very conservative, and the difference between foodplants is not significant (see Methods: Statistics in Chapter 4) ($X^2_r = 4.22$, df=2, $p = 0.154$). However, when the data for all nine females are combined and compared with a contingency table, the foodplant differences are significant ($G=11.49$, df=2, $p < 0.005$).

The number of ties was high partly because the occurrence of pink larvae was low: median % pink for Ampelopsis-reared groups was only 7% (range = 0% - 69%) in this experiment, compared with medians of 30% and 41% in offspring of wild females in 1986 and 1987 (under different rearing conditions; see Chapter 2).

Pupal weights were lowest on Vitis and highest on Ampelopsis (Table 3-10). Pairwise comparisons among the foodplant means found that Vitis differed from each of the other two species, but that Ampelopsis and Parthenocissus did not differ from each other (Tukey's

studentized range test, alpha = 0.01). Thus the foodplant species that produced the most pink larvae (Vitis) produced the lightest pupae.

Lab Experiment: The Effect of Plant Color

All of the local Vitaceae show great phenotypic variability in leaf size, shape, and color. As in many tropical plants (Lee et al. 1987), non-woody parts of the vines accumulate a layer of anthocyanin pigments when growing in the sun. The pigment accumulation is local: on the same vine shaded leaves will lack the pigments, while exposed leaves may develop brilliant scarlet rachises or petioles (pers. obs.). To test whether this intraspecific phenotypic variation affected larval color, larvae from wild females were reared on A. arborea leaves differing in their redness.

Methods

The pigment accumulation in Ampelopsis was assessed visually, but not quantified. Each day I collected leaves with no pink or red pigmentation and leaves with bright red rachises. Several local Ampelopsis sites were used, but on a given day all leaves came from the same location; green leaves were collected from shady areas within a generally sunny patch. Whenever possible, sun and shade leaves were collected from the same vine. Only fresh, healthy leaves were used.

I used a split-brood design. For six wild females, three groups of 15 larvae were reared in each of the treatments, and for two females two groups of 15 larvae were reared. All larvae were given Ampelopsis (not controlled for color) on the day of hatching, and broods were divided when larvae were one day old. Of the initial groups of 30 or

45 larvae, 23-45 per treatment per female survived through color determination in the fourth instar. Larvae were reared in an environmental chamber at 29 C, 16L:8D.

Results

In seven of the eight broods, the groups reared on red leaves had more pink larvae than the groups reared on green leaves (Table 3-11; Wilcoxon test, 1-tailed, $p = 0.025$). The mean frequency of pink larvae was 57% on red-pigmented leaves (s.d. = 18.7%) and 39% on leaves lacking red pigment (s.d. = 29.6%).

Field Experiments

The laboratory experiments demonstrated that foodplant quality significantly affects larval color in Amphion floridensis. Laboratory rearings tell us how an insect is capable of responding under simplified, highly controlled conditions, but cannot demonstrate that the same response occurs in the wild. In addition, intact plants differ significantly in physical and chemical factors from excised leaves, and results obtained with the two are not always comparable (Scriber 1977; Wolfson 1988).

In the first field experiment, I compared morph frequencies of caterpillars reared on potted Ampelopsis and Parthenocissus that I maintained under sunny or shady conditions. This experiment simultaneously measured effects of plant species and of intraspecific plant variation. If the foodplant effects demonstrated in the lab are also important in the field, then more pink larvae should occur on Parthenocissus than on Ampelopsis, and more pink larvae on the pink,

sunny plants than the green, shady plants. Since foodplant color and habitat covary (pinker plants growing in sunnier habitats), however, any differences in larval morph frequencies between the sites could be due either to foodplant variation or to variation in the general environment. Therefore, the second experiment was designed to separate foodplant effects from non-foodplant environmental factors. I continued to maintain the plants in either the sunny or the shady environment. The environment the caterpillar experienced was uncoupled from the environment in which the plant was reared, by placing sun-reared foodplants in the shade, just for the duration of the larval feeding.

Methods

I made cuttings of Ampelopsis and Parthenocissus from vines along SW 35th Drive in Gainesville. Lengths of vine with roots were planted in potting soil in 1-gallon plastic pots in early March 1987. All pots were placed in partial shade for two weeks until the cuttings became established, at which time they were randomly assigned to a Sun or a Shade site (see below). Thirty-one Ampelopsis and 28 Parthenocissus were used in the Sun site, and 30 Ampelopsis and 26 Parthenocissus in the Shade site. Plants were fertilized with Osmocote 14-14-14 (Sierra Chemical Company) in mid-April and late May.

To minimize desiccation and root overheating, I buried empty 1 gallon plastic pots in the ground and put the potted plants into them. This kept the roots cooler and allowed the plants to be moved within or between sites. Both sites were cleared of ground vegetation, and a

layer of cypress mulch several centimeters deep was added between pots to retain moisture and inhibit weeds. Plants were watered every one to three days. They were moved around within each site haphazardly every 1 to 2 weeks, both to even out potential effects of within-site variation, and to prevent plants from rooting into the ground.

Qualitative assessments of plant color were made 8-9 May and 13-16 July. Two stems and the petioles (Parthenocissus) or rachises (Ampelopsis) of three leaves (one each from the lower, middle, and top third of the plant, each > 2cm long) were categorized as green, greenish-pink, pink, or red at their midpoint. In addition, I recorded the color of the new growth, and whether any bright red color occurred anywhere on the plant.

Sites. Vines of each species were grown in two environments. The Sun site was a rectangular patch 2 m wide by 10 m long, within a larger clearing that in mid-May was in full sun from 1130 to 1530, and partial sun from 0850 to 1130 and from 1530 to 1700. The Shade site, approximately 25 m away, was in the understory of a live oak hammock. Dappled sunlight reached the site throughout most of the day, with full sun coming through a gap for less than an hour in the mid-afternoon.

In the Sun site, plants were placed in a regular array, four pots by 15 pots, with about 25 cm between pot edges. In the Shade site, a rectangular array was avoided, to prevent opening a large gap and defeating the purpose of being in the understory. Instead, pots were placed in an irregular, sinuous path. Approximately the same distance between pots was used.

Procedure. The first experiment ran from 30 April to 27 June 1987, with offspring of 14 wild females. The second experiment ran from 9 July to 13 September 1987, with offspring of 8 wild females.

Since first instar larvae are easily damaged by handling, and face high risk of drowning in rainstorms, caterpillars were reared in the lab until the mid- to late- second instar (3 to 4 days after hatching). Larvae were reared in the lab at 29 C, 16L:8D, on Ampelopsis arborea. Then in the field, the second-instar larvae were placed by hand on stems and leaflets of plants. Each plant was protected with a cylindrical screen cage (23 cm diameter x 60 cm high) staked to the ground outside the pot. Larvae molted to the third instar, spent the entire third instar, and molted to the fourth instar, in the field. Once larvae reached late third instar (about four to six days after placement) the plant was thoroughly searched daily. New fourth instar larvae were removed and kept in plastic cups overnight before scoring for color, since pink color does not develop for several hours after ecdysis. (Because the color is determined prior to ecdysis, bringing newly-molted larvae indoors did not affect their classification.)

First experiment. Twenty larvae per female were placed in each experimental group, in two groups of 10. Thus for each female, 10 siblings were placed on each of two Ampelopsis in the Sun, two Ampelopsis in the Shade, two Parthenocissus in the Sun, and two Parthenocissus in the Shade. Since eighty larvae per female were rarely available on a given day, the order in which they were assigned to experimental groups was randomized, with the constraint that one set

of larvae was placed in each experimental group before the second set was assigned to any group.

Shade plants grew more slowly and had less leaf tissue than sun plants. While 10 third instar larvae rarely caused heavy defoliation of sun plants, they were able to decimate some shade plants. To prevent this, I relocated larvae from small plants every 1-2 days. As a result, most Sun larvae fed on one foodplant, but most of the Shade groups fed on two or three plants. The smaller size of shade plants also meant that, on average, shade plants were re-used more frequently than sun plants, and at shorter intervals. Sun plants were allowed to recover for an average of 23 days (minimum = 11 days) and shade plants for an average of 15 days (minimum = 5 days) between uses.

Overall, 827 of the 1160 larvae (71%) used in the first experiment survived to the fourth instar. The 29% disappearance was due to rain, ants, spiders, and Polistes wasps. Mortality did not differ among the four experimental groups, ranging from 25% on Parthenocissus in the sun to 32% on Parthenocissus in the shade (Friedman test, N = 14 broods, $X2r = 0.92$, $df = 3$, $p > 0.80$). Mortality increased over the season both in the sun and the shade: total mortality for the first 5, middle 4, and last 5 broods was 19%, 33%, and 34%. Some groups of 10 larvae were destroyed entirely; if additional eggs from the same female were available, a replacement group of 10 would be put in the same treatment group.

Second experiment. To separate plant from non-plant effects, groups of larvae were reared on shade plants in the Shade site and sun plants in the Sun site as before, but also on sun plants in the Shade.

These last plants were grown and maintained in the Sun site, but moved into the Shade site for the five to six days needed to rear a group of larvae. The fourth treatment, shade plants in sun, could not be run because of a shortage of shade-grown plants. Larval rearing sites will be designated SU (sun) and SH (shade) and plants will be designated R or G (R = sun-grown, reddish plants; G = shade-grown, green plants). The three experimental groups, therefore, will be designated R/SU (reddish plants in sun), G/SH (greenish plants in shade), and R/SH (reddish plants in shade). Both foodplant species were used, but larvae from a given female were reared entirely on one plant species.

Results

Assessment of plant color in Sun and Shade sites. Relative plant color was compared for each plant species between the sites, at the beginning of each experiment. Three measures of plant color were calculated: petiole (Parthenocissus) or rachis (Ampelopsis) color; stem color; and new growth color. Petioles were scored at a point one third of the distance from stem to leaflets, rachises were scored between the two basal sets of leaflets, and stems were scored halfway up the plant.

My original 4-point scale for color (green = 1, greenish-pink = 2, pink = 3, and red = 4) was modified because a large proportion of stems, petioles, and rachises were bicolored, with the upper side (facing the sun) considerably pinker than the lower (shaded) side. To take this variation into account, each datum was recorded on an 8-point scale, which was the sum of the measurements for upper and lower surfaces. Thus, a stem that was pink above and greenish-pink below was

scored a 5 (= 3 + 2), but a stem that was pink all around was scored a 6 (= 3 + 3).

Three leaves per plant were scored, and their sum was taken as the color rating for leaves for that plant. The color rating for stems was the sum of the scores for two stems. Possible scores ranged, therefore, from 6 to 24 for leaves, from 4 to 16 for stems, and from 2 to 8 for new growth. Ratings were compared for plants grown in the sun and plants grown in the shade with a Mann-Whitney U-test, corrected for ties. Sample sizes vary, because plants that were in use on the day of surveying were not scored, and because not all plants had large leaves at three distinct heights, or two main stems.

For all twelve comparisons the mean scores were higher for sun-reared plants than for shade-reared plants, indicating that plants in the sun were pinker than plants in the shade (Table 3-12). For Ampelopsis, all differences were significant (leaves, stems, and new growth, both in May and in July). For Parthenocissus, stems were significantly pinker on sun plants during both surveys, but the differences in leaf color and in new growth color were not significant. Based on this scoring system, Ampelopsis showed a greater pigment response to the rearing environment than did Parthenocissus.

First experiment. Table 3-13 shows the number of green and pink larvae from each brood produced in each of the four treatments (Ampelopsis and Parthenocissus, in Sun and in Shade). The treatment totals suggest that larvae reared in the Shade site on both foodplants were more likely to be pink than larvae reared in the Sun site. Using a Friedman test, I found that this difference is non-significant (X^2_r =

3.58, df = 3, $p > 0.3$), indicating that the treatment groups did not differ in their frequency of pink larvae.

The occurrence of pink larvae in this experiment was very low (53/827 larvae = 6%), however. Only 18 of the 55 brood x treatment groups produced any pink larvae; four of the broods produced no pink larvae in any treatment. This incidence of pink is significantly lower than in siblings reared concurrently in the lab (final column of Table 3-13; median = 41% pink). In all 10 broods in which larvae were reared both in lab and in the field, the proportion pink was higher in the lab (Wilcoxon test, $p < 0.01$). Because of the low incidence of pink larvae in the field, an additional statistical analysis was performed.

Ignoring inter-family variation, I examined only the treatment totals, with a 3-factor log-linear analysis (Bishop et al. 1975, Sokal and Rohlf 1981). This is analogous to a 3-factor ANOVA, for categorical data. Three variables are included in the models: rearing environment (Sun versus Shade), plant species (Ampelopsis versus Parthenocissus), and larval color (Green versus Pink). The question is whether larval color is independent of rearing environment, plant species, and their interaction. Initial examination of Table 3-14a suggests that rearing environment has an effect on color, with Sun producing fewer pink larvae than Shade, but that plant species is not important, since proportion pink is similar on Ampelopsis and Parthenocissus.

The first analysis tests whether a 3-way interaction exists: is the frequency of pink affected by both rearing environment and plant species, with the effect of plant species differing in the two

environments? To test this model, a goodness-of-fit test is performed with the interaction missing. A large value for G indicates that the expected values calculated without the interaction are not similar to the observed values, and therefore the interaction is significant. If G is small, then a model including the interaction does not fit the observed values significantly better than a model without the interaction. For the data in Table 3-14a, omitting the interaction term yields a very small value of G ($G = 0.262$, $p > 0.5$; Table 3-14b); there is no three-way interaction.

Two-way interactions between rearing environment and color, and between plant species and color, are then tested in the same way: goodness-of-fit tests are performed with the interactions missing. A large G indicates that the model with the interaction missing is a poor fit, and therefore the interaction is significant. For rearing environment x color, $G = 30.88$ ($df = 2$, $p < 0.001$); for plant species x color, $G = 1.30$ ($p > 0.1$).

Thus, the initial interpretation of Table 3-14a is supported by the log-linear analysis (although not by the Friedman test): the percent pink is affected by the rearing environment but not by the plant species.

Second field experiment. This experiment was designed to test whether the difference in morph frequencies between groups reared in the sun and in the shade was due to differences in (1) the non-plant environment, (2) the plants, or (3) a combination of plants and the non-plant environment. The three treatment groups were G/SH ("green"

plants in the shade) and R/SU ("red" plants in the sun), as in the previous experiment, but also R/SH ("red" plants in the shade).

If only the non-plant environment were responsible for the difference found in the previous field experiment, then the frequency of pink larvae should be similar in G/SH and in R/SH; if only the plants themselves were responsible for the difference, then the frequency of pink larvae should be similar in R/SU and R/SH. If both plant and non-plant environment are important, however, the prediction is unclear, because the results of the first experiment were opposite my expectation.

When the experiment was initially planned, I had predicted that if differences between sites were found in the first experiment, then more pink larvae would be found in the Sun site (R/SU) than in the shade (G/SH) (see p. 108). The original predictions for the second experiment, therefore, had been that if both plant and non-plant environment were important, then the relative frequency of pink should have been R/SU > R/SH > G/SH.

Despite my expectation, in the first experiment there were more pink larvae on shade plants in the shade (G/SH) than on sun plants in the sun (R/SU). This does not change the predictions if only the plant or only the non-plant environment is important. The prediction if both plant and non-plant influences are important, however, is reversed. If both foodplant and non-foodplant habitat differences are influencing morph frequencies, the proportion pink should be highest on green plants in the shade, intermediate on red plants in the shade, and lowest on red plants in the sun (G/SH > R/SH > R/SU).

Both plant quality and the non-plant environment affected larval color in the second field experiment. The proportion pink in the three experimental groups differed significantly (Friedman test, $X^2_r = 12.25$, df = 3, $p < 0.001$). When plant quality was held constant (R/SH versus R/SU), larvae experiencing the Shade environment were more likely to become pink than larvae experiencing the Sun environment. Similarly, when the non-plant environment was held constant (R/SH versus G/SH), larvae feeding on sun-reared plants were more likely to become pink than larvae feeding on shade-reared plants.

Contrary to both predictions (the a priori prediction that R/SU should have the most pink larvae, and the prediction from the first experiment that G/SH should have the most pink), for all eight broods the proportion pink was highest on sun plants in the shade (R/SH) (Table 3-15). At least one pink larva occurred in each brood in R/SH, while in five R/SU groups and four G/SH groups no pink larvae occurred.

In contrast to the first field experiment, the proportions pink in R/SU and G/SH did not differ significantly ($G = 0.14$, 1 df, $p > 0.5$), although the trend was similar (the proportion pink was slightly higher in the shade). Thus the difference between larvae reared on sun plants in the sun and shade plants in the shade was much greater in the spring than in the late summer. A 3-way log-linear analysis indicated that the three-factor interaction among Rearing Environment, Season, and Color is significant (Table 3-16; $G = 5.14$, df = 1, $p < 0.025$). Whether this difference is due to changes in the plants, the non-plant environment, or the samples of caterpillars cannot be assessed without additional data.

Discussion of foodplant effects in Amphion floridensis

In Eumorpha fasciata, a straightforward correlation between plant species and caterpillar color was found in the field, and then confirmed with laboratory rearings. In Amphion, correlations were demonstrated in laboratory rearings, but could not be confirmed in the field experiments. Unlike the data on foodplant effects on Eumorpha fasciata, therefore, the field and laboratory data for foodplant effects on Amphion are contradictory.

The laboratory experiments demonstrated that both interspecific and intraspecific variation in foodplants significantly affected the frequency of pink larvae: fewer pink larvae were reared on Ampelopsis than on Parthenocissus or Vitis, and fewer pink larvae were reared on Ampelopsis leaves without red pigment than on leaves with red pigment. The field experiments, however, failed to find significant differences in the morph frequencies of groups reared on Ampelopsis and Parthenocissus, and in the first experiment significantly fewer pink larvae were found on the plants with significantly more red pigment. Nevertheless, the second experiment demonstrated that some aspect of plant quality affects morph frequencies in the field: morph frequencies differed for larvae on sun-plants and shade-plants when both were placed in the shade (R/SH versus G/SH, 20.0% pink versus 6.4% pink). Because the non-plant environment for these two groups was identical, the difference had to be caused by qualitative differences in the plants themselves.

The original prediction that in the first experiment sun plants would produce more pink larvae than shade plants was based on three

assumptions: (a) that leaves with more red pigment would produce more pink larvae than leaves with less red pigment, as demonstrated in the laboratory rearings; (b) that other differences in the plants grown in sun and shade would not also affect larval color, in the opposite direction; and (c) that if non-plant environmental factors were important, they would work in the same direction as plant color--the sun environment would produce more pink larvae than the shade environment. The third assumption was partly based on the fact that the incidence of pink larvae was higher at 29 C than at 21 C (Chapter 2). Clearly the second and third assumptions are false: other differences besides color between sun and shade plants are also influencing larval color, and the shade environment produces more pink larvae than the sun environment, independent of the foodplant.

Although the shade plants had significantly less pink color than sun plants, they must also have differed in other chemical and physical qualities. Qualities in which sun-reared and shade-reared plants, or sun and shade leaves, have been demonstrated to differ include water content, toughness, total nitrogen, soluble sugars, and phenolic levels (Schultz 1983a, Collinge and Louda 1988, Mole et al. 1988). Controlled experiments in which the variation in specific physical and chemical qualities of leaves is measured will be required to determine the importance of individual factors on larval color.

Why, however, did the excised red leaves in the lab produce more pink larvae than the excised green leaves? One potential explanation is that not all aspects of leaf chemistry are affected equally when leaves are removed from their plants. Another possible explanation is

that in the lab experiment, both red and green leaves came from sunny sites, with the green leaves coming from shaded parts of the vines. Pigment accumulation is local; adjacent leaves can differ in color if one is shaded and the other exposed. Other factors may respond to insolation at the level of the entire plant: shaded leaves on generally-sunny plants may be more similar to the exposed leaves on the same vines than they are to shaded leaves on shaded vines. To test this, larvae should be reared in the lab on green leaves taken both from the shaded regions of sunny Ampelopsis vines, and from understory Ampelopsis vines.

An important difference between the sun and shade plants in this experiment that was not due to insolation was in how much damage they experienced: shade-reared plants were more severely defoliated than sun-reared plants. As noted in the Methods, plants were re-used multiple times, and because Shade plants had fewer leaves, they were used more often, and at shorter intervals, than Sun plants. Herbivore damage can induce significant changes in plant chemistry that affect the growth and survival of herbivores attacking the plant at a later time (Rhoades 1983, Ryan 1983, Baldwin 1988), and Schneider (1973) demonstrated that in the sphingid Erinnyis ello previously-damaged plants produced a higher frequency of brown larvae than undamaged plants. I therefore examined the data from the first field experiment to see whether the higher use of already-damaged plants can partly explain the higher frequency of pink larvae in the shade groups.

I evaluated the effect of re-using plants by dividing the groups of 10 larvae from the first field experiment into three categories: (1)

groups in which all plants were being used for the first time; (2) groups in which at least one plant was used for the first time, and at least one plant for the second or later time; and (3) groups in which all plants had been used previously one or more times. Table 3-17 tabulates the frequency of groups with and without any pink larvae in the three categories. Groups in the third category (re-used plants) were more likely to have pink larvae than groups in the first category (previously undamaged plants) ($G = 13.98$, $df = 2$, $p < 0.001$). This pattern holds for both Ampelopsis and Parthenocissus. Thus the data suggest that the higher frequency of pink larvae on the shade-grown plants may, in fact, be due to damage-induced changes in the plants.

General Discussion--Foodplant Effects in Caterpillars

With these experiments, foodplant variation has now been shown to affect morph frequencies in five sphingid species representing four different tribes (Smerinthini: S. ocellata [Poulton 1885a, 1886; Grayson 1986], L. populi [Grayson 1986]; Dilophonotini: E. ello [Schneider 1973]; Philampelini: E. fasciata [this study]; Macroglossini: A. floridensis [this study]). In the lab rearings, therefore, foodplant effects have been demonstrated in every species tested. Many more of the anecdotal reports in Table 3-1 will probably be confirmed when examined quantitatively. The ability to respond to foodplant variation by changing color is a common trait among larval Sphingidae.

The widespread nature of the plant effects implies that they are adaptive, but alternative hypotheses are that the foodplant effects

have no effect on larval fitness, or are due to manipulation of the caterpillar by the plant. For any species, no matter how 'adaptive' the color change seems, the fitness of individuals must be measured experimentally. For the foodplant effect to be adaptive, then the average fitness of color morph A must be higher than the average fitness of morph B, on the kind of foodplant that biases development towards morph A (e.g., green E. fasciata have higher fitness than nongreen on L. peruviana).

How and why does foodplant variation influence larval coloration? What is the potential adaptiveness of using plant traits as cues, and would larvae responding to such cues have higher average fitness than larvae that do not respond?

Why use foodplant quality as a cue?

I have not demonstrated the function of the color polymorphisms in either Amphion or E. fasciata. Since my preliminary evidence suggests that the body temperatures of color morphs do not differ (see Chapter 4), the major adaptive function of the polymorphism must lie in a reduction of predation risk. Caterpillars of both species are palatable to birds, and Amphion are taken frequently by Polistes wasps (pers. obs.). Therefore, there will be strong selection for a larva to be sensitive to any cue indicating which morph would have a lower predation risk in the next instar.

Because foodplant cues cannot give the larva information on the local frequency of caterpillar morphs, a caterpillar cannot use foodplant cues to gain any frequency-dependent advantage. Plant traits

may, however, provide a reliable indication of the average crypsis of each morph. If there is high interplant variation in the shape, size, or color of leaves and other plant parts, then different color morphs may be more cryptic on different plants. The color of the leaves used by L. populi and S. ocellata varies among plant species (Grayson 1986), and stems of grape vines used by Amphion larvae range in color from green to bright scarlet. To the human eye, pink Amphion larvae are more cryptic on the purplish woody stems, and on non-woody stems of an intermediate pink, than on either green or bright scarlet stems. A larva may increase its crypsis by selecting stems that it matches (Chapter 4), but also by using information from the plant when it decides which color to become.

In many of the species in Table 3-1, the direction of change induced by the foodplant seems as if it could increase the average crypsis of the caterpillars. This has been assumed explicitly by the naturalists correlating leaf color and larval color in L. populi and S. ocellata (Poulton 1885a,b, 1886; Grayson 1986), and it is tempting to generalize to the other species described. Care must be taken, however, to avoid broad adaptationist scenarios. Differential crypsis of larval morphs among plant morphs, or among resting sites on a plant, must be assessed experimentally (Endler 1978, 1984), as must the fitness of each morph on each kind of foodplant. With the available data I can only raise the hypothesis that the crypsis of Amphion and Eumorpha fasciata larval morphs will correlate with the foodplant biases in their morph determination. Thus, on Ludwigia peruviana, green fifth-instar larvae should have a lower risk of predation than

non-green larvae, and on L. leptocarpa and L. octovalvis, the relative risk should be reversed. Field experiments, in which the relative survival of each morph on each foodplant is measured, are needed.

What cues can larvae use?

A caterpillar's foodplant is also its immediate environment. Poulton (1885a,b, 1886) recognized the importance of separating the effects of plant-as-habitat from plant-as-food. He called effects requiring ingestion "phytophagic" (a term originally used less restrictively by Walsh [1864]) and later termed the external effect of leaf color "phytoscopic" (Poulton 1892). Although "phytoscopic" implies the importance of the visual traits of the plant, other kinds of non-nutritional traits, such as texture, may also be relevant. Either ingestive or non-ingestive characteristics (or both) of the plant are influencing morph determination in sphingids. What are the advantages of sensitivity to each type of cue, if the major function of the sensitivity is improving crypsis?

The color in an instar is determined by the juvenile hormone titer at a critical period during the molt into that instar (Truman et al. 1973, Ikemoto 1983; unpublished data). Larvae with JH titers above a threshold are green; larvae with lower JH titers are non-green. Juvenile hormone synthesis is affected by a variety of environmental effects, including both nutrition and visual cues (reviewed in Feyereisen 1985, Hardie and Lees 1985). Juvenile hormone synthesis increased one day after mating in the cockroach Periplaneta americana, and one to two days after resumption of feeding in previously-starved

P. americana (Feyereisen 1985). If this delay between an environmental stimulus and increased juvenile hormone synthesis is a physiological constraint, then any cue used by the caterpillar must be present at least a day before the molt. For the fourth-instar color, therefore, the cue probably must be available during much or all of the third instar, and possibly during earlier instars as well.

Phytoscopic cues. From instar to instar, current leaf or stem color is probably a good indicator of future leaf or stem color. For a caterpillar that rests on the same substrate across instars, therefore, visual or texture cues from its current resting sites will be reliable cues about the appearance of its future resting sites. If, however, use of resting sites changes during development, then the physical cues available to a caterpillar may not provide useful information about future sites. A caterpillar resting under a leaf has little information about the colors of non-leaf resting sites. Under such conditions, use of phytoscopic cues would not be expected.

Phytophagic cues. To be a useful phytophagic cue, an ingested compound must reliably indicate the appropriate morph. Tannin content is lower in oak catkins than in oak leaves, and N. arizonaria use an absence of dietary tannins as a cue to become catkin mimics rather than twig mimics (Greene 1989).

Occurrence of phytophagic and phytoscopic effects. Both nutritional and phytoscopic control of polymorphism are known from other systems. Food quality affects the ratio of alate and wingless Myzus persicae aphids (Mittler 1973, Harrewijn 1978), and the ratio of long- and short-winged Zonocerus variegatus grasshoppers (McCaffery and

Page 1978). Among many of the social insects, nutrition is a major determinant of caste (Lüscher 1976, Hardie and Lees 1985).

Phytoscopic and substrate-texture cues are extremely important in the green-brown color dimorphisms of butterfly pupae, and the homochrome responses of orthopterans. For butterflies, available pupation sites are often variable. The pupae of many swallowtail butterflies are dimorphic green or brown (Smith 1978; Hazel 1980), those of Danaus chrysippus (family Danaidae) are green or pink (Smith et al. 1988), and pupae of many pierids vary continuously from green to brown (Angersbach and Kayser 1971; Smith 1980). Numerous field and lab studies have demonstrated (1) that color is under environmental control (Angersbach and Kayser 1971; Smith 1978, 1980, and refs. therein; Hazel 1980; West and Hazel 1985), (2) that pupae tend to match the background on which they pupate (Clarke and Sheppard 1972;Sims and Shapiro 1983a), and (3) that mortality of non-matching pupae is higher than mortality of matching pupae (Baker 1970; Wiklund 1975; Sims and Shapiro 1983b).

In pierid butterflies light quality is the most important environmental influence on pupal color (summarized in Smith 1980). Color determination in swallowtails and D. chrysippus, in contrast, involves interactions of many environmental cues, including photoperiod, humidity, substrate color, light quality, and substrate texture, with the relative importance of factors varying among species (West et al. 1972; Smith 1978; Hazel and West, 1979; Hazel 1980; Smith et al. 1988). Because the wavelengths of transmitted light, and reflectance of backgrounds, differ in green versus brown sites, and substrate texture is different for non-woody stems, bark, or rocks,

which are typical pupation sites, these cues provide reliable information about the pupal color that will be more cryptic.

Many grasshoppers demonstrate a homochrome response, in which they undergo a slow color change (taking several days, and expressed after a molt), until they match the general coloration of their habitat (reviewed in Rowell 1971, Fuzeau-Braesch 1985). For example, many grasshoppers in recently-burned areas become more melanic (Hocking 1964). The change is mediated by the grasshopper visual system. In Gastrimargus africanus, grasshoppers respond both to the albedo of their environment, by becoming darker on light-absorbing surfaces, and to the wavelength of the background, by changing the amount of orange pigmentation (Rowell 1970).

Demonstrating Phytophagic versus Non-Ingestional Effects in Sphingids

Because the plant is both resting site and food, manipulating the two factors independently is methodologically difficult. Schneider (1973) and I offered whole, unaltered leaves to larvae, and therefore cannot differentiate leaf nutrition from leaf texture, color, or toughness. Poulton (1885a,b, 1886) and Grayson (1986) conducted experiments in which they attempted to separate phytophagic and phytoscopic effects on color in two sphingid caterpillars.

Based on a hypothesis put forth by Meldola (1882), Poulton (1885a,b) originally predicted that the differences in larval color of Smerinthus ocellata were due to phytophagic influences. After extensive experimentation, he concluded instead that the larvae were influenced by "that part of the environment which is so close to them

as to be almost or quite in contact" (1886, p. 160). For many of S. ocellata's foodplants (willow species and apple varieties with large leaves) Poulton assumed that this environment was the leaf undersurface. In foodplant species with the upper and lower surfaces of leaves differing in color, field censuses demonstrated that larval color generally matched the leaf undersurface. In an ingenious and labor-intensive experiment Poulton (1886) had his wife sew leaves together so that either the upper or the lower surfaces were exposed. If larval color were due primarily to ingestive qualities, both groups of larvae should be similar. In contrast, if larval color were due directly to leaf color or reflected light quality, then larvae exposed to leaf upper surfaces should differ from larvae exposed to lower surfaces. Another group of larvae were fed upon leaves of one Salix individual which lacked the species' typical white "bloom" on the lower surface. Finally, Poulton covered larval ocelli to compare the color of blinded and normal larvae.

With these experiments Poulton asserted that "conclusive experimental proof has been afforded of the theory ... that the colour of the leaf, and not its substance when eaten, is the agent which influences the larval colours" (1886 p. 154). Unfortunately Poulton's larvae suffered massive mortality, and those surviving belonged to many experimental groups with small sample sizes. His primary evidence for phytoscopic rather than phytophagic effect was that five larvae offered unsewn leaves of Salix viminalis were "slightly on the white side of intermediate" while one larva given sewn leaves exposing only the underside was "strongly white". Poulton was an ardent selectionist

(Kimler 1986); lacking any knowledge of statistics (antedating R.A. Fisher) he therefore gave a posteriori explanations for the coloration of each individual in each experiment that did not support his theory, based on differences in rearing conditions, plant quality or 'inherited tendencies'. In sum, despite Poulton's confidence that he had explained the cause of foodplant-correlated variation in S. ocellata (1890), the issue was actually left unresolved.

A century later Grayson (1986) again attempted to identify the foodplant qualities affecting larval morphs in Smerinthus ocellata, and also in Laothoe populi. She found strong evidence that visual characteristics of the foodplant are important in morph determination in L. populi. Larvae reared in complete darkness on Salix fragilis and Populus alba did not differ in morph frequencies, whereas under day/night conditions the two foodplants produced significantly different morph frequencies. The samples on P. alba were small, however, and the larvae on both plants that were reared in darkness had atypical color. Since insects reared in complete darkness often do not feed well (Rowell 1971; Scriber and Slansky 1981) the convergence of forms may be indicative of a shared abnormal response to constant darkness, rather than an absence of necessary visual cues.

Better evidence came from an experiment in which L. populi larvae were fed in darkness, and given white, green, or black paper backgrounds during the day, in the absence of leaves. Larvae in green or black cages became yellow-green or dull-green; in contrast, larvae in white cages were almost all white. Grey paper that matched the green paper in reflected light intensity was then used, and the same

divergence from white paper was found, suggesting (but not proving) that larvae were using reflected light intensity in assessing their background.

In the second species she examined (S. ocellata), Grayson's experiments, like Poulton's, were unable to separate phytoscopic from phytophagic effects. Because larvae reared on two foodplants did not differ in color, Grayson concluded that nutritional quality did not affect caterpillar color. Virtually all of her lab reared larvae, however, were grey; the green morph, common in the wild, was not obtained under any food or lighting condition. Larvae reared outdoors in white muslin sleeves on the two foodplants all developed into a white-green morph never found in wild larvae; Grayson interpreted this odd morph as demonstrating that light quality was a critical cue, but in fact did no direct tests of the effect of light. Grayson's conclusion, that neither textural nor phytophagic cues were important to L. populi or S. ocellata, was not supported by adequate empirical tests. No experiment, therefore, has adequately compared the relative importance of ingestive (phytophagic) and non-ingestive (phytoscopic) foodplant variation in sphingid color determination.

In summary, the experiments of Poulton and Grayson suggest that physical characteristics of the foodplants are important in determining larval morphs in smerinthine sphingids. Their experiments are not adequate, however, to determine whether or not nutritional quality of foodplants also affects larval color. A convincing proof of nutritional effects is to use artificial diets to which various nutrients, secondary compounds, or plant extracts are added. My

several attempts to coerce Amphion floridensis to feed on artificial diets were completely unsuccessful. Further exploration of artificial diets for sphingids will be an important step in allowing the controlled examination of nutritional effects on color determination.

Phytophagic versus Non-Ingestional Effects in Geometrids

Experiments on geometrid caterpillars have demonstrated definitively that both types of foodplant influences can affect larval coloration, but like the experiments on sphingids, the relative importance of the effects has not been compared in any species.

Having "expended a vast amount of unproductive labour" (1892, p. 294) upon testing foodplant effects on S. ocellata, Poulton abandoned this species in favor of geometrid and noctuid larvae (1890, 1892, 1903). In contrast to his ambiguous results with S. ocellata, his experiments on the latter families provide convincing evidence that non-ingestive qualities of the foodplants can affect larval color. The species he tested typically rest on twigs or lichen-covered trunks, rather than under leaves, and he was able to vary the quantity and quality of available resting sites without varying the food. In many species larvae reared without exposure to any wood were paler than those provided with twigs, and larvae exposed to different kinds of wood or artificial backgrounds often developed appropriate variation in coloration. Because the food was kept constant, the experiments demonstrated that color change was not caused by phytophagic effects.

Greene (1989) was able to feed the dimorphic geometrid Nemoria arizonaria on artificial diets. Catkins mixed with diet produced

catkin morphs, at two photoperiods and two temperatures; oak leaves mixed with diet produced twig morphs. As with Grayson's experiments, demonstrating that one factor affects morph determination does not prove that another factor is not also important. Although Greene demonstrated that temperature and photoperiod did not affect morph determination, he did not publish any tests of the effect of physical characteristics of the foodplant; Poulton's evidence that such factors are important in geometrids suggests that they should be examined in N. arizonaria. (Similarly, because Greene did not demonstrate that other chemicals in oak leaves did not affect morph variation, his conclusion that dietary tannins are the primary trigger is premature.) Greene (pers. comm.) did raise caterpillars on artificial diets under yellow or green light, attempting to mimic the color of catkins and leaves, and found no effect. Incident light and background color, however, are distinct phenomena. To test effects of background color, larvae should be reared on contrasting backgrounds under the same light regime. To test effects of incident light, a wider range of wavelengths is needed. In both Papilio machaon and Pieris brassicae, green and yellow light do not differ in their effect on pupal color, but differ from both longer and shorter wavelengths (Wiklund 1972, Kayser and Angersbach 1974).

The geometrids are obviously an intriguing group in which to examine the effects of foodplant. Both phytophagic and phytoscopic effects occur, as do both continuous and discontinuous variation, and seasonal and simultaneous polymorphism. Poulton's suggestion that species with continuous variation were more sensitive to phytoscopic cues than species with strong discontinous dimorphism needs testing

and, if supported, explanation. Overall the response of geometrids to foodplant cues, whether nutritional or non-ingestional, seems to be stronger than the responses of sphingids.

Predictions

This study, combined with Greene (1989), demonstrates that foodplant influences on facultative polymorphisms merit further attention. Under what conditions should foodplant quality affect polymorphism, and under what conditions would it be more adaptive for caterpillars to disregard foodplant cues? What kinds of chemicals can be used as cues? How specific are phytophagic responses to particular compounds? When should phytophagic cues be used, and when should phytoscopic? I list four predictions. I will not be surprised if they do not hold, but if they stimulate any comparative studies of foodplant effects on phenotypic variation in insects, they will have served their purpose.

A. Phytoscopic effects should be more important in species that use the same resting site throughout development than in species that change resting sites as they grow. Obviously they should be more prevalent in species in which there is natural variation in the appearance of that resting site (e.g., leaf color variation among the willow and poplar hosts of L. populi and S. ocellata).

B. The chemical cues that are important in phytophagic effects will vary among species. Tannins are a reliable cue for N. arizonaria, because they are lower in catkins than in leaves; in other species, different chemicals will be reliable indicators of the most appropriate morph.

C. Phytophagic cues should be more prevalent in specialized than in generalized feeders, because for the former, it is more likely that there will be a chemical that correlates reliably with the appropriate response. Geometrids would be the appropriate group with which to test this prediction, because both generalized and specialized species occur (Covell 1984). Among polymorphic species, are phytophagous effects more important in the specialists?

Sphingids tend to be specialized feeders, but a few species, such as Hyles lineata, feed on a large number of plant families. These polyphagous species should not have phytophagic determination of color morphs.

D. If caterpillars that use phytophagic cues are tested with hosts outside of their normal range, the response, on average, should be less adaptive than when they are tested with their normal hosts. If the chemical cue which they used was also present in the new host, it might not correlate with the most adaptive response. (For this reason, when the correlation between the direction of a foodplant effect and larval fitness is tested, the plant-caterpillar pairings should not include introduced species.)

Table 3-1. Reported correlations between foodplant variation and sphingid caterpillar morphs.

Species	Kind of change[a]	Suggested correlate[b]	Evidence[c]	Reference
Tribe Sphingini				
Manduca quinquemaculata	H	T	F	Gentry 1874
Manduca sexta	H	T&/orD	F	E. Lampert, pers. comm.
Sphinx ligustri	S	S	F	Meldola 1882
	S	S	FE	Poulton 1885
Acherontia atropos	S	S	F	Meldola 1882
Tribe Smerinthini				
Libyoclanis punctum	H	T&/orC	F	Pinhey 1962
Laothoe populi	S	S	F	Boisduval et al 1832
	S	S	FE	Grayson 1986
Mimas tilia	S	S	?	Poulton 1890
Smerinthus ocellata	S,M	S	F	Boscher 1879
	S,M	S	F	Meldola 1882
	S	S	FE	Grayson 1986
	S	S	FE	Poulton 1885a,b, 1886
Paonias myops	M	C	?	Baynes-Reed 1882
Protambulyx strigilis	H,M	S	F	Moss 1920
Tribe Dilophonotini				
Aellopos ceculus	S,M	S	F	Moss 1920
Erinnyis ello	H	S,D	E	Schneider 1973
Tribe Philampelini				
Eumorpha fasciata	H,M	C	F	Gossington 1975
		O	F	Moss 1920
		S	FE	This study
Tribe Macroglossini				
Rhyncholaba sp.	H	C	?	Fogden & Fogden 1974
Rhagastis olivacea (ins. 1)	H	C	?	Sevastopulo 1946
Amphion floridensis	H	S,C,D	E	This study

All data refer to final instar unless noted after species name.

[a] Kind of Change: S = change in shade of color, hue unchanged
H = change in hue
M = change in markings

[b] Suggested correlate: S = foodplant species, C = plant color morphs, D = foodplant damage levels, T = time (seasonal change in food quality), O = other: more green in Brazil than Peru as "adaptation to the greener and more flourishing vegetation"

[c] Evidence: F = based on field observations
E = verified experimentally
? = not stated; presumably anecdotal observations

Table 3-2. Relative frequency of different morphs of Smerinthus ocellata and Laothoe populi final instar larvae found on five species of willow.

	S. ocellata			L. populi		
	Number				Number	
Willow species	Green	Grey	% Green	% YG	YG	DG/WG
Salix alba	2	30	6	20	1	4
S. viminalis	50	43	54	50	1	1
S. caprea	12	4	75	100	6	0
S. atrocinerea	96	7	93	67	2	1
S. fragilis	12	0	100	100	31	0

Data from Grayson 1986

YG = yellow-green; DG/WG = dull green/white-green.

Table 3-3. Field observations of eggs and larvae of Eumorpha fasciata in north Florida, 1985-1988.

	INSTAR					
	egg	1	2	3	4	5
Total number	897	48	64	83	189	314
% Green		100	95	81	76	54

Table 3-4. Eumorpha fasciata field censuses tabulated by foodplant species and year.

		Instar[a]								
		Third			Fourth			Fifth		
Foodplant species	Year	G	Pi/Y		G	Pi/Y		G	Bl	Y
Ludwigia peruviana	1985	23	0		30	2		49	3	1
	1986	3	1		19	0		36	5	0
	1987	2	0		9	0		11	0	1
	1988	7	0		18	1		25	5	0
	TOTAL	35	1		76	3		121	13	2
	% Green	97			96			89		
L. leptocarpa	1986	4	1		11	5		6	12	1
	1987	3	3		11	10		8	15	0
	1988	4	6		8	3		7	14	0
	TOTAL	11	10		30	18		21	41	1
	% Green	52			63			33		
L. oct (smallflr)	1986	9	3		13	10		7	50	0
	1987	0	0		2	0		1	4	0
	1988	0	1		0	1		0	0	0
	TOTAL	9	4		15	11		8	54	0
	% Green	69			58			13		
L. oct (bigflr)[b]	1986	2	0		3	1		0	11	0
	1987	4	0		3	3		9	8	1
	1988	3[2]0			11	9		5	8	0
	TOTAL	9[2]0			17	13		14	27	1
	% Green	91			57			33		
L. decurrens	1986	1	0		4	1		2	6	0
	% Green							25		
Ludwigia spp.		1	0		1	0		3	0	0
GRAND TOTAL		66[2]15			143	46		169	141	4

[a] Caterpillar morphs: G = Green; Pi/Y = Pink, Yellow, and Pink and Yellow combined; Bl = 'Black' (boldly patterned); Y = Yellow. [] = larvae with intermediate phenotypes.

[b] L. oct (smallflr) and L. oct (bigflr) are different morphs of Ludwigia octovalvis; these were recorded separately, but are combined for most statistical analyses (see text).

Table 3-5. Pupal weights of Eumorpha fasciata reared on three species of Ludwigia.

	MALES			FEMALES		
Foodplant Species	Mean	s.d.	N	Mean	s.d.	N
L. peruviana	3.813	0.362	17	3.956	0.588	15
L. leptocarpa	3.321	0.520	19	3.456	0.436	13
L. octovalvis	2.424	0.375	10	2.573	0.189	13

ANOVA Table

Source	df	MS	F	P
Plant species	2	12.765	65.52	0.0001
Sex	1	0.423	2.17	0.14
Plant x Sex	2	0.0003	0.00	0.998
Error	81	0.195		

Table 3-6. Pupal weights of green versus black Eumorpha fasciata reared on Ludwigia peruviana.

Fifth instar color	N	Pupal Weight	S.D.
Green	9	3.710 g	0.569
Black	20	3.936 g	0.464

Unpaired t-test, $t = 1.1334$, $p = 0.27$

Table 3-7. Median dates for finding green and black (or yellow) fifth instar Eumorpha fasciata larvae in the wild.

Year	Plant species	(#G/#non-G)	Green	Black
1985	L. peruviana	(46/4)	14 OCT	9 OCT
1986	L. peruviana	(36/5)	26 SEP	5 OCT
1986	L. leptocarpa	(6/13)	8 OCT	5 OCT
1986	L. octovalvis	(7/61)	9 OCT	9 OCT
1987	L. peruviana	(not included - just 1 non-green larva)		
1987	L. leptocarpa	(8/15)	10 OCT	10 OCT
1987	L. octovalvis	(10/13)	21 SEP	17 SEP
1988	L. peruviana	(25/5)	6 OCT	19 OCT
1988	L. leptocarpa	(7/14)	8 OCT	8 OCT
1988	L. octovalvis	(5/8)	27 SEP	28 OCT

Median dates for finding green and non-green larvae do not differ: Wilcoxon test, $p > 0.05$

Table 3-8. Median dates for finding green and pink or yellow fourth instar Eumorpha fasciata larvae in the wild.

Year	Plant species	(#G/#non-G)	Green	P/Y
1986	L. leptocarpa	(11/5)	2 OCT	5 OCT
1986	L. octovalvis	(16/11)	2 OCT	26 SEP
1987	L. leptocarpa	(11/10)	10 OCT	17 OCT
1987	L. octovalvis	(5/3)	17 OCT	10 OCT
1988	L. leptocarpa	(8/3)	8 OCT	8 OCT
1988	L. octovalvis	(11/10)	6 OCT	6 OCT

Table 3-9. Fourth-instar color ratios of Amphion floridensis larvae reared in the lab on three foodplant species (G:P = number of green: number of pink larvae).

Female	Foodplant Species[a] Vitis	Parth	Ampel
	G:P[b]	G:P	G:P
88-2	14: 1	12: 3	14:0
88-4	4:11	9: 6	11:4
88-5	7: 8	10: 5	10:4
88-7	12: 3	12: 2	16:0
88-8	14: 1	14: 1	14:0
88-10	13: 1	9: 6	15:0
88-12	8: 7	4:11	4:9
88-13	8: 7	7: 8	14:1
88-17	11: 3	15: 0	12:1
TOTAL	91:42	92:42	110:19
% Pink	31.6	31.3	14.7

[a] Parth = Parthenocissus
Ampel = Ampelopsis

Table 3-10. Pupal weights of *Amphion floridensis* reared on three species of Vitaceae.

	MALES			FEMALES		
Foodplant Species	Mean	s.d.	N	Mean	s.d.	N
Vitis rotundifolia	1.641	0.209	56	1.844	0.212	43
Parthenocissus quinquefolia	1.681	0.151	46	1.916	0.182	61
Ampelopsis arborea	1.722	0.142	45	1.975	0.179	55

ANOVA Table

Source	df	MS	F	P
Plant species	2	0.3181	8.97	0.0002
Sex	1	4.1841	117.99	0.0001
Plant x Sex	2	0.0076	0.22	0.81
Error	301	0.0355		

Table 3-11. Brood frequencies of fourth instar Amphion larvae when reared in the lab on two forms of Ampelopsis leaves.

	Na	% PINKb	
		Foodplant Colorc	
Female	Red/Green	Red	Green
32	42/44	74	66
33	37/37	54	49
34	45/43	44	63
35	43/44	51	18
36	37/41	49	15
37	29/26	31	0
38	30/31	63	19
39	23/26	91	81
	MEAN	57.1	38.9
	MEDIAN	52.5	34.0

[a] N = number of larvae from each brood reared on each color leaf
[b] % Pink = % of larvae that were pink in the fourth instar
[c] 'Red' leaves had bright red rachises and variable reddish shading on leaf undersides. 'Green' leaves were all green.

Table 3-12. Average color scores of Ampelopsis and Parthenocissus plants reared outdoors in two environments (Sun and Shade).

		PLANT PART		
		LEAVES	STEMS	NEW GROWTH
Ampelopsis				
May	SUN	10.5*** (21)	7.5*** (11)	3.8* (25)
	SHADE	6.2 (19)	4.0 (10)	2.7 (15)
July	SUN	12.8*** (31)	7.9*** (31)	4.5**(30)
	SHADE	6.2 (22)	4.2 (20)	3.4 (21)
Parthenocissus				
May	SUN	10.3+ (18)	8.8* (10)	3.3NS(18)
	SHADE	8.6 (17)	5.0 (5)	2.8 (16)
July	SUN	10.7NS (18)	9.5* (17)	3.6NS(18)
	SHADE	9.6 (21)	6.4 (20)	3.0 (23)

Table values are mean score - significance level of difference between Sun and Shade - (sample size). High values indicate more red pigmentation. Data analyzed with Mann-Whitney U-test.

*** = p = 0.0001
** = p <= 0.001
* = p < 0.05
+ = p = 0.06
NS = Not Significant (p > 0.06)

Table 3-13. First field experiment.

Female	Sun/Amp	Sun/Parth	Sh/Amp	Sh/Parth	Lab[a]
86-21	16:1	12:2	10:0	15:0	75:9
86-26	17:0	19:0	20:0	16:0	39:29
86-27	18:0	14:0	18:0	17:0	-
86-30	17:0	19:0	18:0	15:0	17:13
86-31	9:1	16:0	16:0	18:0	15:16
86-32	15:0	13:0	2:6	9:6	0:23
86-35	19:0	18:0	18:0	16:0	61:18
86-39	11:0	20:0	13:5	9:3	-
86-42	- [b]	10:0	9:6	13:1	-
86-46	11:0	16:0	14:1	14:0	24:16
86-47	8:0	14:0	8:1	8:1	-
86-50	17:2	16:1	14:5	14:4	31:40
86-52	19:0	16:0	10:0	10:5	26:7
86-53	16:1	13:0	11:1	10:0	27:7
TOTAL	193:5	216:3	181:25	184:20	Median =
% Pink	2.5	1.4	12.1	9.8	41%

Data are number of fourth instar Amphion larvae (Green:Pink).

[a] Lab = brood-mates reared indoors at 29 C, 16L:8D on Ampelopsis arborea.

[b] No larvae survived in this group. Brood 42 was excluded from the Friedman test, but included in the goodness-of-fit analysis.

Table 3-14. First field experiment.

Table 3-14a. Effect of rearing environment and plant species on number of green and pink fourth instar Amphion larvae. Data tested with log-linear analysis.

Rearing Environment	Plant Species	Caterpillar Color Green	Pink
SUN	Amp	193	5
	Parth	216	3
SHADE	Amp	181	25
	Parth	184	20

Table 3-14b. Log-linear analysis of data in Table 3-11a.

Model	G	DF	P
Complete interaction	0.26	1	> 0.5
Conditional independence			
a. Environment x Color	30.88	2	< 0.001
b. Plant species x Color	1.30	2	> 0.1

Table 3-15. Second field experiment.

Female	R/Su[a]	G/Sh	R/Sh	Lab[b]
86-62	10:0[c]	8:0	7:1	-
86-63	16:2	7:1	8:2	6:25
86-64	16:1	12:1	8:3	10:1
86-66	9:0	16:0	14:1	8:37
86-67	13:3	12:1	11:4	-
86-68	18:0	11:3	3:2	36:28
86-74	17:0	11:0	7:2	11:11
86-82	10:0	11:0	10:2	-
TOTAL	109:6	88:6	68:17	Median =
% pink	5.2	6.4	20.0	43.8%

[a] R/Su = Sun-raised (Red) plant/Larvae reared in sun
G/Sh = Shade-raised (Green) plant/Larvae reared in shade
R/Sh = Sun-raised (Red) plant/Larvae reared in shade

[b] Lab = brood-mates reared indoors at 29 C, 16L:8D on Ampelopsis arborea.

[c] Data are number of fourth instar larvae (Green:Pink).

Table 3-16. Effect of rearing environment (of both plants and larvae) and season on the production of green and pink Amphion caterpillars.

Rearing Environment	Season[a]	Caterpillar Color Green	Pink	% pink
Sun	Expt.1	409	8	1.9
	Expt.2	109	6	5.2
Shade	Expt.1	365	45	11.0
	Expt.2	88	6	6.4

[a] Expt.1 was conducted 30 April to 27 June 1987
Expt.2 was conducted 9 July to 13 September 1987

Table 3-17. The frequency of groups with at least one pink larva, when reared entirely on previously-unused plants, partly on previously-used plants, or entirely on previously used plants.

Plant Status	Ampelopsis		Parthenocissus		TOTAL		
	-[a]	+	-	+	-	+	%-
Previously unused	11	1	12	2	23	3	88
Partially used	3	4	3	0	6	4	60
Entirely used	4	6	5	8	9	14	39

G-test on total: $G = 13.978$, $df = 2$, $p < 0.005$.

[a] - = number of groups with no pink larvae
+ = number of groups with at least one pink larva.

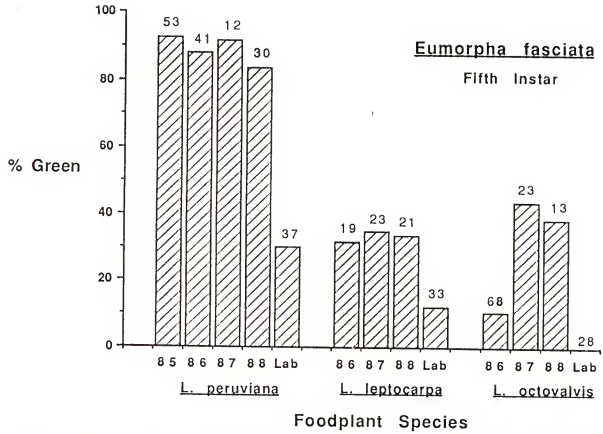

Figure 3-1. Frequency of green fifth instar Eumorpha fasciata on three Ludwigia species. Numbers below x-axis = year (1985-1988); "Lab" = Larvae reared at 20 C, 16L:8D, in 1986. Numbers above bars = sample size. Morph frequencies are homogeneous within foodplant species across years, and heterogeneous among foodplant species. Lab-reared larvae showed a similar correlation between plant species and morph frequency.

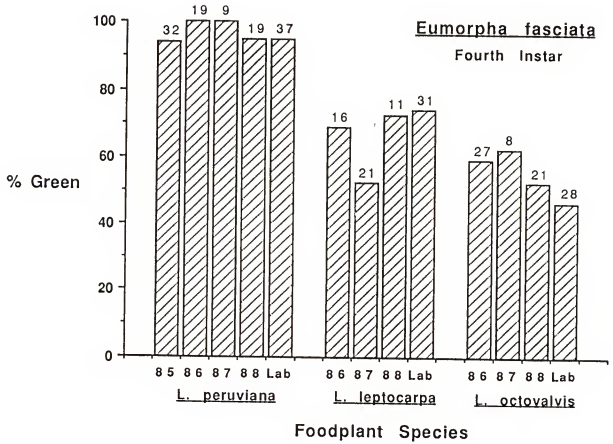

Figure 3-2. Frequency of green fourth instar Eumorpha fasciata on three Ludwigia species. See Figure 3-1 for explanation.

CHAPTER 4
BEHAVIORAL CORRELATES OF COLOR

Introduction

When offered a choice, many animals show preferences for resting sites on which they are cryptic (summaries in Edmunds 1974, Endler 1984). The costs and benefits of resting site choices, however, include numerous factors in addition to crypsis. By moving to a particular resting site, for example, an animal may reduce one source of predation, but increase predation or parasitism from other sources (Curio 1970a,b). Resting sites that differ microclimatically may lead to variation in development rates (Rawlins and Lederhouse 1981). The resting sites used should be those which afford the highest average fitness.

In polymorphic species, alternative morphs using the same resting sites may be equally cryptic to a predator, if each morph has a color pattern that represents a random sample of its background (Endler 1978). If, however, morphs are more cryptic on different substrates, some visual predators will select for covariation of color and resting site preference (Kettlewell 1955a,b, Endler 1978). In addition to predators, the thermal properties of color morphs could theoretically lead to differences in their resting-site preferences, although the effect of surface color on insect body temperatures is still unclear (Casey 1981, May 1985). Some studies have failed to detect differences

in equilibrium body temperatures among color morphs (e.g. Schistocerca gregaria green versus dark red, Stower and Griffiths 1966), but differences in wing melanization significantly affect the equilibrium body temperatures of butterflies (Watt 1968, reviewed in Casey 1981).

There have been few explicit tests of resting-site selection in polymorphic animals. Correlations between color morphs and their observed resting sites (e.g. Panolis flammea moths, Majerus 1982) do not demonstrate that the morphs make different behavioral decisions: without experiments, alternatives such as differential predation or environmental influences on morph determination cannot be ruled out as explanations (see Chapter 3). This chapter will look for behavioral variation among the larval morphs of four sphingid species, and begin to address the costs and benefits of the resting-site decisions made by pink and green Amphion floridensis caterpillars.

Experimental evidence of behavioral differences in polymorphic species

Convincing evidence of substrate preference differences have been obtained for several polymorphic orthopterans. When tested in an arena, the red and greenish-grey morphs of the grasshopper, Circotettix rabula rabula, preferred red and green substrates, respectively (Gillis 1982). The grasshoppers visually compare their appearance and their background: by painting different colored masks around their eyes, Gillis (l.c.) significantly changed their preferences. Morph-related differences occur in the grasshoppers Acrida turrita and Oedipoda miniata (Ergene 1950a,b) and in the mantid Miomantis paykullii (Edmunds 1974), but not in the mantid Sphodromantis lineola (Edmunds 1974).

The experiments on melanic and typical moths have produced ambiguous results, and none has found differences as clear-cut as those with orthopterans. In the peppered moth, Biston betularia, significant differences in the selection of dark and light backgrounds were found by Kettlewell (1955a), Boardman et al. (1974), Kettlewell and Conn (1977), Steward (1985), and Grant and Howlett (1988, in B. betularia cognataria), but not by Mikkola (1984), Howlett and Majerus (1987), or Grant and Howlett (1988, in B. betularia). In the genus Phigalia (Geometridae), P. titea morphs selected different substrates in one experiment (Sargent 1969) but not in another (Sargent 1985), and Lees (1975) found no difference among morphs in P. pilosaria. In other species, behavior differences were found by Sargent (1966) and Kettlewell and Conn (1977), but not by Steward (1976, 1977, 1985) or Mikkola (1984). Some of the variation among studies may represent true differences in species' tendencies to select different substrates, but much is due to the designs of the experiments (discussed by Mikkola 1984, Grant and Howlett 1988). Limited evidence indicates that individual differences in resting site preferences have a genetic basis, and that moths are not making visual comparisons between their own coloration or reflectance and that of their background (Sargent 1968, Grant and Howlett 1988).

In polymorphic caterpillars, experiments have compared resting behavior only in Nemoria arizonaria (Geometridae, Greene 1989) and in Phlogophora meticulosa (Noctuidae, Majerus 1978). When N. arizonaria larvae were placed on oak branches in the lab, the catkin morph chose catkins as resting sites, and the twig morph chose twigs or leaves.

All 40 larvae placed on their preferred substrates did not move in 30 minutes, but 66 of 80 larvae placed on a non-preferred substrate moved to a preferred site within 30 minutes.

Ford (1955) suggested that green and brown morphs of P. meticulosa each rested where they were more cryptic on their foodplant. Majerus (1978) found no difference in the choice of color substrates among phenotypes, but he tested larvae under highly artificial conditions, in plastic boxes with piles of leaves. The only significant behavioral variation he found was among nongreen and green third-instar larvae. In tests with clear boxes that were half covered with black paper and placed in the sun, the green larvae were found in the uncovered area more often than the nongreen larvae. This demonstrates differences in the response to light and/or heat. When larvae were placed outdoors on vegetation, and protected from predators, green third-instar larvae were found higher in the vegetation than were nongreen larvae.

Measuring the consequences of behavioral differences among morphs

Kettlewell (1955b) measured the relative risk from bird predation for cryptic and non-cryptic B. betularia, but his experiments do not reveal much about the costs and benefits of natural behavioral decisions. First, it is not clear that B. betularia morphs differ in behavior (see above), and second, the moths were pinned to tree trunks, which are not their natural resting sites (Mikkola 1979, 1984).

The only attempt to measure the costs and benefits of natural resting sites in a polymorphic insect is that of Curio (1970a,b,c) for Erinnyis ello caterpillars (Sphingidae). Curio assumed that

differences he observed (1965, 1966) in the resting sites of caterpillars were due to behavioral differences among morphs (but he never confirmed this experimentally). Through the early fifth instar, all color morphs of E. ello rested in Poinsettia foliage. The large green larvae remained in the foliage, but the brown were found primarily on trunks. Curio asked what selective factors were responsible for the large brown larvae moving away from the foliage. By measuring the rates of disappearance of groups of larvae, he concluded that mortality in the foliage was eight times higher than on trunks, but that no differences existed in the disappearance rates among the color morphs. He assumed that mortality was due to wasps, Polistes crinitus, and that the wasps limited their foraging to the foliage. Looking for a factor that could be responsible for the difference among color morphs, Curio (1970a) tested whether Anolis lineatopus lizards, which hunted on trunks, exerted selection that kept the green larvae in the foliage. In feeding trials, naive wild Anolis ate small E. ello larvae of all colors. Large larvae, however, were treated differently depending on their color: green or blue caterpillars were attacked and eaten, but brown caterpillars were not attacked. Overall, Curio concluded that brown larvae benefitted from reduced predation by both wasps and lizards, and that additional selective factors must explain the high proportion of larvae that remained green in the final instar (73%). Unfortunately Curio's experiments were not properly controlled, some of his assumptions may have been false, and the design and statistical analyses are inadequate. His conclusions, therefore, are premature.

Resting site differences in sphingid caterpillars

In addition to E. ello (Curio, l.c.) resting site differences have been reported in wild caterpillars of Xylophanes tersa (green versus brown, Moss 1920), Acherontia atropos (green or yellow versus brown, Sevastopulo 1971), Herse convolvuli (green versus purple-and-white, Edmunds 1974, 1975), and Sphecodina abbottii (green-and-brown versus brown, Heinrich 1979). In all cases the green morph was reported to be more common under leaves or on green vegetation than the nongreen morph. The differences were quantified only by Curio (1966) for Erinnyis ello, and Heinrich (1979) for Sphecodina abbottii, and none of these observations has been confirmed with controlled experiments.

Resting Behavior of Sphingid Caterpillars

I examined the resting behavior of caterpillars of Amphion floridensis (fourth instar, green vs. pink), Xylophanes tersa (fifth instar, green vs. brown), Eumorpha fasciata (third and fourth instar, green vs. pink-and-yellow), and Enyo lugubris (fifth instar, green vs. green-with-purple-blotches, and purple vs. green-with-purple-blotches). Pairs of larvae matched for size and rearing conditions but differing in color were placed outdoors on potted hostplants, and their behavior was monitored at regular intervals over several hours. (Plants were caged or placed in a screened enclosure to exclude predators.) The hypothesis tested was that green and non-green larvae selected different sites on which to rest, and that the green larvae would remain closer to leaves than would the non-green larvae.

Resting Behavior of Amphion floridensis

The pink and green morphs of fourth instar Amphion are well-suited to their foodplants. Each morph matches colors found on some parts of their foodplants, since all non-woody plant parts in the local Vitaceae range in color from green to pink or red, particularly when grown in full sun (pers. obs.). The foodplant experiments (Chapter 3) demonstrated that larval morphs could be non-randomly distributed both among foodplant species and among foodplant color variants. This experiment tested whether larval morphs distributed themselves nonrandomly on the same plant.

Methods

I reared Amphion larvae in the lab on Ampelopsis through the fourth instar, or on Ampelopsis or Parthenocissus in the field from mid-second through fourth instar. Pairs of fourth instar larvae (one green, one pink) were matched for rearing conditions and size. Fourth-instar larvae are approximately three centimeters long. The behavior experiments were run outdoors in a sunny site between 5 May and 7 June 1987, with twenty-one pairs on Ampelopsis, and 18 pairs on Parthenocissus. I made observations between 0830 and 1700.

Ampelopsis arborea and Parthenocissus quinquefolia are woody vines with compound alternate leaves, long petioles, and wiry tendrils at the leaf bases. Beyond these family characteristics, however, the species differ considerably in plant architecture when grown under identical conditions. Larvae were tested on both species to determine if differences in the plant morphology affected their behavior.

Ampelopsis. Potted plants were 1/3 to 1/2 meter high. The compound leaves are spaced alternately along a vertical or slanted stem, with blades slanted up or parallel to the ground. Expanded Ampelopsis leaflets are larger than a fourth-instar larva, which can rest completely concealed beneath. On smaller leaves the larva rests under the rachis. These plants have a very open architecture: leaves are widely spaced, and all plant surfaces can be searched easily.

Parthenocissus. Potted plants were shorter than the Ampelopsis, with the main stem creeping along the ground. Closely-spaced leaves rise like umbrellas, with the leaflets drooping radially from vertical petioles. Fourth instar larvae can rest under individual leaflets of fairly small leaves. As a consequence of the creeping habit, closer leaf spacing, and drooping leaflet orientation, Parthenocissus vines were much denser and more difficult for me to search.

Behavioral observations. On Ampelopsis I placed a matched pair of larvae opposite each other on the main stem halfway up the plant. On Parthenocissus I placed the matched pair on the vertical petiole of a fully-developed leaf. Larvae were left undisturbed for 50 - 120 minutes before censusing began. I searched each plant at 30 minute intervals over five to seven hours, and recorded the substrate and behavior of each larva. Behavior categories were eating, resting, and moving. Substrate included woody stem, non-woody stem, petiole, rachis, leaflet, or ground. Larvae half under a leaflet and half on the rachis or petiole were recorded as under the leaflet. Although I recorded substrate color for non-woody tissue as green, greenish-pink, or pink, this was not satisfactory because color varied continuously

from pure green to bright crimson. In addition the stems and rachises were often bicolored, with upper surfaces red and lower surfaces green. I therefore analyzed the data only with respect to plant part, and not substrate color.

The matched design ensured that each larva of a pair had exactly the same available substrates. I obtained nine to 14 census points per pair of caterpillars, and compared the proportion of time they spent in different activities or resting sites.

Non-parametric statistics. For many analyses in this chapter, I compared the percentage of censuses spent in different locations, or in different activities, by the pink and green members of a pair. Care must be taken in comparing percentages, since they are discontinuous for small sample sizes. For example, with 10 censuses, the percent spent on any substrate can only be a multiple of 10. In comparing percentages based on unequal sample sizes, therefore, exact tied values may be impossible--for example, 8/10 censuses on a pink larva (80%) versus 9/11 on a green larva (82%) is as close to a tie as is possible. The total number of censuses for each member of a pair was equal, but some of the comparisons look only at subsets of censuses, which were often unequal: for example, the censuses which were not spent feeding, or the censuses which were not spent under leaves.

In nonparametric tests using proportions or percentages, therefore, I considered pairs to be tied if the addition of one point to any category would make the proportions equal. Thus in the previous case, one more observation could have made the data for the pink larva 9/11; therefore I would count the two samples as tied. In contrast, a

pair with values 8/11 and 9/10 would not be considered tied, because the addition of one census to either brood could not alter their relative rank.

Log-linear analyses were performed using the methods of Sokal and Rohlf (1981) and Bishop et al. (1975).

Results

Feeding. Seventy-five of the 78 larvae fed during at least one census. Individual larvae fed on zero to four censuses, and green and pink larvae spent an equal proportion of censuses feeding (Figure 4-1; green larvae: mean = 1.7 censuses, s.d. = 1.0; pink larvae: mean = 1.8, s.d. = 0.7). Leaflets were the primary plant part eaten, but I also saw larvae eating stems (4 observations), petioles (6), and rachises (2).

Walking. Eight green and nine pink larvae were observed walking during a census. Larvae moved frequently, because they were found in different positions at sequential censuses, but they must have spent only a short time in movement on each occasion. This low number of observed movements (1.7 % of total observations) may be due to larvae freezing when I approached the plants, but I was careful not to touch the plants, and I tried to locate the caterpillars and assess their behavior from a distance.

Resting Sites . A non-feeding caterpillar has three decisions to make: (1) To rest on or off of its feeding site (under a leaf versus not under a leaf). If resting off of its leaf, the larva must decide (2) how far to go, and (3) what kind of substrate to select. I did not

measure the distances that larvae moved between censuses, so I cannot address the second decision. I will examine the first and third decisions separately.

Resting site data were examined in three ways. (1) What is the proportion of total observations spent on a substrate (leaf, non-woody stem, or woody stem)? This is comparable to field data that would be obtained if the identities of individual larvae were not recorded. If differences are found in the use of substrates, they may be due to larvae selecting different kinds of sites, or to larvae partitioning their time differently among the same sites, or both. To separate these possibilities, data were examined further: (2) How many larvae selected a particular substrate for at least one census? (Do pink and green larvae differ in the kinds of substrates they select?) (3) In pairs with both larvae using a particular substrate, do larvae differ in the amount of time spent on it?

First decision: Resting Under Leaves. Ampelopsis: (1) Of the 232 observations when green caterpillars were resting (i.e., excluding censuses when feeding and walking), they were under leaves 68.5% of the time; in contrast. of the 233 observations when pink larvae were resting, they were under leaves only 15.5% of the time ($G = 142.48$, 1 df, $p < 0.001$). (2) Nineteen of 21 green larvae rested under leaves on at least one census; in contrast, only 10 of 21 pink larvae rested under leaves (Figure 4-2; $G = 88.5$, 1 df, $p < 0.001$). Therefore green larvae are more likely to select leaves as resting sites than are pink larvae. (3) Of the 10 pairs in which both green and pink larvae rested under leaves, the green larva spent more censuses under leaves in 7

pairs, less time in one pair, and equal time in two pairs (Sign test, $p=0.035$). Therefore, even when the same sites are chosen, the pink larvae spend less time under leaves.

Parthenocissus: (1) Green larvae were under leaves 51.0% of the 194 resting observations; only one pink larva (representing 0.5% of the 191 resting observations) spent any time resting under a leaf ($G = 158.83$, $p < 0.001$). (2 & 3) In contrast to the one pink larva found under a leaf, 14 of the 18 green larvae spent at least one census under a leaf (Figure 4-3). This difference is highly significant ($G = 21.21$, 1 df, $p < 0.001$). The 14 green larvae spent an average of 7 censuses under leaves.

Thus the first decision concerning a resting site--stay on a leaf or move away--is made differently by green and pink larvae. A green larva is more likely to select a resting site under a leaf than a pink larva, and will spend more time there.

Second Decision: Resting on different non-leaf substrates. If a larva does not rest under a leaf, the second decision concerns how far to move. I analyzed two possible decisions the larvae might face: (a) the type of substrate (non-woody [including rachis, petiole, and non-woody stem] versus woody), and (b) the position on the plant (near the leaves versus near the ground, regardless of the type of substrate).

Since a larva spending most of its time under a leaf has less time available to spend elsewhere on the plant, the analysis includes only the censuses that were not spent under leaves. The seven pairs in which the green larva spent all of its time under leaves have been excluded from further analyses.

(a) Woody versus Non-Woody. More pink larvae rested on wood than green larvae. Among the 17 remaining pairs on Ampelopsis, 11 pink larvae and 5 green larvae rested on wood for at least one census ($G = 4.20$, 1 df, $p < 0.05$). Among the 15 remaining pairs on Parthenocissus, 10 pink and 4 green larvae rested on wood for at least one census ($G = 4.76$, 1 df, $p < 0.05$).

For green larvae, however, the probability of resting on wood increased as the number of non-leaf resting censuses increased (Figure 4-4). Of 18 green larvae spending one to four censuses off leaves, only two were found on wood; in contrast, of 14 green larvae spending six or more censuses off leaves, 7 rested on wood ($G = 5.66$, 1 df, $p < 0.025$). No such pattern was found among pink larvae; however, only one pink larva spent fewer than eight censuses off leaves (Figure 4-5).

This suggested that the difference between green and pink larvae in probability of resting on wood could be due to the amount of time spent off leaves, rather than to different preferences for non-woody versus woody tissue. I re-analyzed the data, using only the pairs in which both green and pink larva spent at least six censuses not under leaves. Because the sample size is reduced, I combined data from Ampelopsis ($N = 5$ pairs) and Parthenocissus ($N = 9$ pairs). (1) Of the total non-leaf observations on these 14 pairs of larvae, the green larvae spent 38% on wood and 62% on non-wood. The pink larvae spent 55% on wood and 45% on non-wood. This difference is significant ($N = 130$ observations of green larvae and 147 observations of pink larvae; $G = 7.65$, 1 df, $p < 0.01$). (2) Seven green and twelve pink larvae rested on wood for at least one census ($G = 4.00$, 1 df, $p < 0.05$). (3) In the

seven pairs with both green and pink larvae on wood for at least one census, the green larva spent more time on wood than the pink larva in one pair, equal time in three pairs, and less time in three pairs.

Thus the initial conclusion is supported: when larvae are not resting under leaves, green and pink larvae distribute themselves differently between non-woody and woody tissue. Pink larvae are more likely to select woody resting sites than green larvae.

(b) Near the ground versus not near the ground. When the data from these 14 pairs are re-sorted into resting sites within 2 cm of the ground versus higher on the plant, rather than into woody versus non-woody sites, no significant differences are found between green and pink larvae. (1) The green larvae spent 44% of the total observations near the ground, and the pink larvae 50%. This difference is not significant ($G = 0.90$, 1 df, $p > 0.10$). (2) Eight green and eleven pink larvae rested near the ground for at least one census ($G = 1.41$, 1 df, $p > 0.10$). (3) In the seven pairs with both the green and pink larvae near the ground on at least one census, the green larva spent proportionately more censuses near the ground in six pairs, and equal time in one pair (Sign test, $p = 0.032$, but in the direction opposite the prediction).

Summary: Amphion floridensis. Both color morphs used leaves, non-woody stems, and woody stems as resting sites. Fewer pink than green caterpillars spent any censuses under a leaf. Among larvae that spent at least six censuses off of leaves, more pink larvae used woody stems as resting sites. Pink and green larvae did not differ in the frequency with which they rested within 2 cm of the ground.

Resting and Feeding Behavior of Xylophanes tersa

Xylophanes tersa is monomorphic green in instars one through four, and dimorphic green and brown in the fifth instar. Moss (1920) reported that in Brazil, the green larvae rested in the foliage, and brown larvae rested on trunks and stems near the ground. In two congeners that remained green in the final instar (X. porcus and X. chiron) mature larvae stayed in the foliage near feeding sites during the daytime. In three congeners that were monomorphic brown in the fifth instar (X. guianensis, X. anubus, and X. loelia), mature larvae rested during the day near or on the ground.

Methods

The methods and analyses were similar to those used to compare the morphs of Amphion floridensis, with modifications dictated by differences in the plant and caterpillar species.

Hostplants for Xylophanes spp. are in the family Rubiaceae. Recorded hostplants for X. tersa include Spermacoce glabra, Pentas, and Manettia (Moss 1912, 1920; Hodges 1971). In Florida larvae are found on Spermacoce and Richardia (Kimball 1965; J. Watts pers. comm.). Experiments were conducted on Pentas lanceolata, which is not native to the United States, but is a popular garden plant in Florida. Eggs are often found on Pentas in Gainesville, and larval survival is high.

The plants were 2/3 - 1m high, with 2-4 major vertical stems, approximately 1 cm in diameter. Stems were grey-brown wood for a few centimeters at the base, then green-woody (green, but with lenticels, a rough texture, and the beginnings of brown coloration) for 5-15 cm, and

then green and non-woody. All non-woody tissue is bright green. Leaves are elliptic, up to 18 cm long and 7 cm wide.

Observations on 33 pairs of larvae were conducted outdoors in a screened enclosure between 0900 and 1700, from 19 August to 13 September 1987. Xylophanes tersa larvae were collected from outdoor Pentas plants in instars 1-4 and reared in plastic containers on excised leaves at 29 C, 16L:8D until the fifth instar, when they were about six or seven centimeters long. Brown and green fifth instar Xylophanes larvae were matched for size. Members of a pair were placed opposite one another, head-up, midway up a vertical stem. Larvae were allowed to settle for at least 20 minutes, and then censused at 20 - 30 minute intervals. Nine to 12 census points were obtained per pair. At each census the behavior (resting, walking, or feeding) and location were recorded. Location was described by substrate (leaf, stem, woody stem), and height on the plant (bottom, middle, or top third). Larvae that were partly under a leaf and partly on a stem were excluded from the analyses of substrate choice (but not from the analyses of height). This eliminated 30 observations of green larvae, and 23 observations of brown larvae. For 28 of the pairs, when larvae were on the same third of the plant, I recorded which was closer to the ground.

Results

Feeding and walking. Xylophanes caterpillars fed during 38% of the observations; individual larvae were seen feeding on one to nine censuses. Brown and green larvae did not differ in the frequency of feeding: of the 33 pairs, the green larva fed more often in 11 pairs,

the brown fed more in 11 pairs, and both fed the same amount in 11 pairs. Leaves were eaten on 91% of the feeding observations; larvae rarely fed on petioles (n = 11 observations), stems (10), and flowers (4). Only three green and five brown larvae were observed walking on stems during censuses.

Resting and feeding sites: Leaf vs. non-leaf. For Amphion larvae, which did not feed on many censuses (14% of observations), I was able to compare the proportional use of resting sites within each pair. Because X. tersa larvae were eating on so many of the censuses, however, I did not have enough resting censuses for each larva to make within-pair comparisons. I therefore combined the data for all pairs (Table 4-1), and conducted a 3-factor log-linear analysis to test for interactions among larval color, behavior (resting versus feeding), and location (under leaf versus not under leaf).

The analysis confirmed that (a) green and brown larvae do not differ in the proportion of time spent feeding (color x behavior), and (b) green larvae spend more time under leaves than brown larvae (color x location). Green larvae spent 20.1% of the observations under leaves, and brown larvae spent only 6.9%. In addition, (c) larvae of both colors spend more time feeding when under leaves than when not under leaves (behavior x location). Larvae fed half of the time they were under leaves, and only one third of the time they were not under leaves.

More green larvae than brown larvae spent at least one census (resting or feeding) under a leaf (19 green versus 8 brown, $G = 7.580$, $df = 1$, $p < 0.01$). For resting sites only, 15 green vs. 5 brown larvae

were entirely under leaves on at least one census ($G = 7.220$, 1 df, $p < 0.01$). Every caterpillar spent at least one census on the stem.

Resting and feeding sites: Woody tissue. The previous analysis divided sites into leaf versus non-leaf. Stems, however, varied from green to green-woody to woody. Are brown larvae found proportionately more often than green larvae on green-woody and woody stems? I divided stem sites into green versus green-woody or woody, and found a non-significant excess of brown larvae on wood. Eleven green and 16 brown larvae spent at least one census (resting or feeding) on green-woody stems ($G = 1.537$, 1 df, $p > 0.1$); 0 green and 3 brown larvae spent at least one census on woody stems.

Resting and feeding sites: Height. Moss (1920) reported that brown larvae rested nearer the ground than green larvae. For 26 pairs I recorded the height on the plant (top, middle, or bottom third) on which each larva rested at each census. The combined data for all larvae of each color show no difference in the number of censuses in which green and brown larvae were found at each height (Table 4-2). For each member of a pair, I averaged the resting heights (bottom of plant = 1, middle = 2, top = 3), and compared them with a Wilcoxon test ($N = 21$ pairs, $T = 106.5$, $p >> 0.05$).

Behavior of Eumorpha fasciata

Third and fourth instar Eumorpha fasciata larvae were tested on potted Ludwigia octovalvis. In addition, I examined the resting sites of wild E. fasciata larvae encountered during field censuses in 1986 and 1987 (see Chapter 3).

Field censuses (see Chapter 3 for methods).

In the third and fourth instar, I found green larvae under leaves more often than pink and yellow (Table 4-3; G-test on fourth instar totals, $G = 7.705$, $p < 0.01$). This difference, however, is attributable to the correlation between plant species and larval color. Most of the green larvae were found on L. peruviana, which has the largest leaves; I therefore am comparing green larvae on large-leaved plants with nongreen larvae on smaller-leaved plants. The proper comparison, to look for behavioral differences, is between color morphs on the same foodplant, but my samples for any one foodplant species are too small for statistical analysis (Table 4-3). In the fifth instar, there was no significant difference between colors in resting sites, when plant species were combined ($G = 3.39$, 2df, $p > 0.1$). Few fifth instar larvae rest under leaves on any Ludwigia species. These are large caterpillars (up to 10 cm long), and except for L. peruviana the leaves will probably not support their weight.

Behavior experiments

Methods

I had a limited number of third and fourth instar larvae available for behavioral observations on Ludwigia octovalvis plants. Eggs and young larvae were collected in the wild and reared in the lab. I compared ten green and pink (or pink-and-yellow) pairs in the third instar, and 17 pairs in the fourth instar.

Some pairs were tested on potted plants, about 30-50 cm high; others were tested on wild plants nearby. Leaves were dark green above

and lighter green below, stems ranged from green to bright red to brown (woody), and seedpods ranged from green to bright red. Many stems and pods were bright red on their upper surface and bright green on their lower surface.

Results

Feeding. Green and nongreen larvae did not differ in the frequency with which they fed during censuses. Third instar: All of the pink and eight of the ten green larvae ate on at least one census. The green larva ate more often than the pink larva in two pairs, and less often in four pairs. Fourth instar: All of the larvae ate on at least one census (green larvae: range = 1-9 censuses; pink larvae: range = 2-8 censuses). Green larvae ate more often than pink larvae in eight pairs, and less often in four pairs (Sign test, $p = 0.39$).

Resting sites. In the third instar, larvae showed no difference in their selection of leaf and non-leaf resting sites, but in the fourth instar, green larvae stayed under leaves more often than nongreen larvae. Third instar: For both color morphs, seven of the ten larvae rested under leaves on at least one census. Combining feeding and resting sites for all pairs, there is no difference in the total number of censuses spent under leaves and not under leaves (Table 4-4a; $G = 0.78$, $p > 0.1$). Fourth instar: Fewer fourth instar larvae were found under leaves; six green and four pink larvae spent at least one census under a leaf. Green larvae spent more censuses under leaves than pink larvae (Table 4-4b; $G = 15.50$, $p < 0.001$).

Resting and Feeding Sites of Fifth-Instar Enyo lugubris

Methods

I collected wild females, and reared their offspring in the lab on Ampelopsis arborea through the first day of the fifth instar. In the fifth instar, the six-centimeter long larvae are green, purple, or green with large purple blotches ("blotched"). I observed eight triplets of green, blotched, and purple larvae on the same plant, two pairs of green and blotched, and twenty-one pairs of blotched and purple. Because the sample size of triplets is too small for three-way comparisons, I compared blotched with purple larvae, using the 21 pairs, and green with blotched larvae, using the two pairs plus the eight triplets. The eight purple larvae in the triplets were omitted from the comparisons. Larvae within each pair were siblings.

Results

I found no differences in the behavior of green, blotched, or purple E. lugubris caterpillars.

Sixty-one of the sixty-two larvae ate on at least one census. There was no significant difference in the proportion of time each color spent feeding. Green vs Blotch: Green larvae ate on 52% of the censuses, and blotched larvae on 60% of the censuses. Members of each pair did not differ significantly in how often they ate (Wilcoxon test, $p > 0.05$). Purple vs Blotch: Purple larvae ate on 45%, and blotched larvae on 51% of the total censuses (Wilcoxon test, $p > 0.05$).

The larvae spent virtually all of their feeding or resting censuses under the Ampelopsis leaves, and there were no obvious

differences between the color forms. Because these larvae were large relative to the size of individual leaflets, their substrates were difficult to characterize. They tended to be partly under a rachis, and partly under a leaflet.

On the few censuses when larvae were not feeding or resting under leaves, the color morphs did not show any apparent differences. Green vs Blotch: Four green and three blotched larvae walked on one census; one blotched larva walked on two censuses. Three green larvae rested on woody stems (for one, two, and three censuses), and two blotched larvae rested on woody stems (for one census each). Purple vs Blotch: Six blotched and seven purple larvae walked on one to four censuses (one census per larva, except for one of each color walking on three censuses, and one blotched larva walking on four censuses). One blotched larva and three purple larvae rested on wood for a single census, and three blotched larvae rested on wood for two censuses.

Summary of Behavior Experiments

In Amphion and X. tersa, significantly more green larvae rested under leaves than nongreen larvae. In the fourth instar larvae of E. fasciata, I had too few larvae to detect a difference in the number using different substrates, but overall, green larvae spent significantly more censuses under leaves. The limited data on E. lugubris and the third-instar larvae of E. fasciata did not show behavioral differences among morphs. In E. fasciata, the natural resting sites of wild larvae varied among foodplant species, probably reflecting differences in plant architecture, but there was not

evidence of strong differences between the morphs found on the same plants (larger sample sizes are needed).

Weight Gain of Pink and Green Amphion Larvae

Selection of different resting sites on plants may affect a caterpillar's growth rate, in three ways. (1) Caterpillars at different sites on a plant may have significantly different body temperatures (Rawlins and Lederhouse 1981, Casey 1976); feeding and growth rates of many Lepidoptera are temperature-dependent (Rawlins and Lederhouse, l.c., Scriber and Slansky 1981, Stamp and Bowers 1988). (2) Choice of resting site may determine the quality of food that is eaten. The caterpillars of Omphalocera munroei develop more rapidly on young than on old leaves (as is true for most Lepidoptera, Scriber and Slansky 1981), yet larvae select old leaves as feeding and resting sites, because of reduced predation risk (Damman 1987). Similarly, Hemileuca lucina caterpillars fed on a higher proportion of mature leaves after disturbances by Polistes wasps caused them to move from branch tips to the shaded interior of their plants (Stamp and Bowers 1988). (3) Although a frequent generalization is that cryptic caterpillars feed primarily or exclusively at night (e.g., Herrebout et al. 1963), it has also been suggested that cryptic species resting under leaves are more likely to feed diurnally than cryptic species resting away from leaves (Heinrich 1979, Heinrich and Collins 1983, Schultz 1983a). Thus differences in resting sites may affect temporal feeding rhythms, and thereby alter growth rates.

In all four species I tested above, there was considerable feeding during the day, but no difference among morphs in the proportion of censuses spent feeding (Table 4-5). My observations did not extend over the entire day, however, and my presence may have affected feeding rates. In addition, differences in growth may result from differences in body temperature or in the quality of food that is eaten. I therefore conducted an experiment to compare biomass gains of green and pink Amphion larvae in their natural environment over a 24-hour period. I tested whether pink larvae did proportionately more of their feeding at night, and if they gained biomass more slowly than green larvae.

Methods: Biomass gain of green and pink larvae

I ran the experiment outdoors so that larvae experienced normal temperature and light fluctuations; the experiment was conducted from 7 May to 7 June 1987. Larvae were reared in the lab on Ampelopsis through the fourth instar, or on Ampelopsis or Parthenocissus in the field from mid-second through fourth instar. Larvae within each pair were siblings, reared under identical conditions. The larvae varied widely in initial weights (range = 135 to 399 mg, mean = 229 mg), and I tried to match each pink and green pair within 30 mg. Larvae were placed on potted Ampelopsis or Parthenocissus plants, one pair per plant, in a sunny site. Each plant was enclosed in a screen cage (23 cm diameter x 60 cm high) to exclude predators.

"Dawn" larvae were weighed and placed on the plants soon after dawn (0645-0810, n=18 pairs), and re-weighed at dusk and the following dawn. "Dusk" larvae were weighed and placed on plants just before dusk

(2000-2025, n=19 pairs), and re-weighed at the following dawn and dusk. For each weighing, larvae were off of the plants for a maximum of 30 minutes.

The number of larvae was limited, and I was not able to match all pairs within 30 mg. Since the amount of leaf material assimilated per time would not be constant throughout the instar, even under controlled conditions (Scriber and Slansky 1981), I cannot compare the amount of weight gained by each larva within the poorly-matched pairs. For statistical analyses, therefore, I used only the well-matched pairs. For measurements of 24-hour weight gains, this sample consisted of 14 pairs started at dawn, and 8 pairs started at dusk. For the daytime and nighttime weight gains, however, different subsets were used. Some of the larvae matched at dawn were no longer matched at dusk, and vice versa; in addition, some larvae that were poorly-matched at dawn were well-matched at dusk, and vice versa. The sample for daytime weight gain, therefore, consisted of 20 pairs, and the sample for nighttime weight gain consisted of 14 pairs.

Results: Weight gain of green and pink larvae

The data set was heterogeneous in three ways. (1) Because experiments were run on 10 different days over a one-month period, pairs experienced very different temperature regimes over 24 hours. (2) Because larvae were not weighed with empty guts, most weighings are overestimates. I weighed 5 green and 5 pink larvae that had been food-deprived for 12 hours. After 30 minutes of feeding, their weights increased by 6.0 - 11.6 % due to leaf material in the gut (mean gain =

9.9%). (3) Some pairs fed on Ampelopsis, and other pairs on Parthenocissus, because the number of available plants was limited. These three sources of heterogeneity do not bias the direction of the data; they simply increase the variance and make it less likely that I would find significant differences.

How important is daytime versus nighttime weight gain? On average, more than half of each larva's 24-hour biomass increase occurred during daylight (Table 4-6). Therefore, daytime feeding is important for fourth-instar Amphion larvae in Gainesville in early summer.

Do pink larvae gain less weight than green larvae? For the 22 pairs that were matched for initial weight, the green larvae gained significantly more weight than the pink larvae over 24 hours (paired t-test, $t = 2.16$, $p = 0.042$). I therefore tested whether a difference occurred just during the day, or both day and night. There is a strong suggestion that the difference in weight gain between the green and pink larvae is due only to the diurnal component of their feeding. For the pairs that were well-matched at dawn, the difference in daytime weight gains approached significance ($p = 0.06$, Table 4-7), but for the pairs well-matched at dusk, the difference in nocturnal weight gain was not significant ($p = 0.24$).

Temperatures of Caterpillars in the Field

The absence of strong seasonal changes in color morph frequencies in Amphion and E. fasciata in Florida (see Chapters 2 and 3), and the

co-occurrence of green and black E. fasciata over a large geographic range (see Chapter 3), suggest that thermal biology is not the major factor selecting for color polymorphism in these species. Nevertheless, thermal biology may affect the costs and benefits of being each color, and of choosing different resting sites. I therefore looked for temperature differences among color morphs of Amphion caterpillars.

Two questions are of interest: (a) Do green and nongreen caterpillars have different equilibrium body temperatures under full insolation? (Does color affect body temperature?) If green and nongreen caterpillars attain different body temperatures under the same conditions, then thermoregulation may be an important factor selecting for color polymorphism. (b) Do green and nongreen caterpillars have different body temperatures on their natural resting sites? (Do the differences in resting sites used by pink and green color morphs lead to differences in their average body temperatures?) I made preliminary measurements of the body temperature of green and pink caterpillars, both when exposed to full sunlight and when on natural resting sites.

I also measured the body temperatures of E. fasciata caterpillars encountered during censuses in 1986. Among insects with non-metallic colors, a comparative survey demonstrated that the dark brown or black species tended to have lower reflectances and slightly higher temperature excesses than paler species (Willmer and Unwin 1981). In addition, the effect of reflectance on body temperatures was greater for large species. Since the major fifth-instar morphs of E. fasciata are green and patterned black, and E. fasciata is the largest species I

studied, it is the most likely species in which I might find morph-related differences in body temperatures.

Methods

I measured the temperatures of pink and green Amphion caterpillars that were placed on Ampelopsis plants in a sunny site. Caterpillars were put on plants at least two hours before their temperatures were measured. Temperatures were measured at midday on 27 September 1987, and at 1400 on 29 September 1987. Both days were sunny; ambient shade temperature ranged from 29 to 32 C.

All temperature measurements were taken using a BAT-12 digital thermometer (Bailey Instruments), with an MT-26/2 hypodermic-needle microprobe. Caterpillar temperatures were taken by pressing the probe firmly against the caterpillar, so that at least 5mm was in contact with the thorax. [Casey (1976) found that surface temperatures of sphingid caterpillars were not significantly different from temperatures measured by puncturing the body wall.] Caterpillars were not removed from their plants; they were momentarily shaded, and temperatures taken on the side facing away from the sun. Only resting caterpillars were used. Ambient temperatures were obtained by shading the microprobe and holding it at the same height as the caterpillar, a short distance away from the plant.

To compare the temperature of caterpillars exposed to full sunlight, I matched pink and green siblings for weight, and attached each to a wooden dowel (14.5 cm x 2.1 mm diameter) with a drop of superglue. Caterpillars were glued ventrally, with their posteriors at

one end of the dowel. Any caterpillar with excess superglue coating it laterally was not used. The free ends of the dowels were inserted into a line of holes on a hinged board, which was placed on the edge of a table in a grassy clearing, and oriented so that the dowels were perpendicular to the sun's rays. Each caterpillar therefore had one lateral side fully exposed to the sun, and one lateral side shaded (Figure 4-6). The matched pairs of larvae were taken from the shade and placed in full sunlight for 10 minutes, and then their temperatures were recorded by pressing the probe against their shaded sides. I alternated which color caterpillar had its temperature taken first. Measurements were taken on 17 pairs of caterpillars in early August 1987, during sunny periods between 1000 and 1500. I limited the exposure to 10 minutes, because of unpredictable cloud and wind patterns.

I obtained temperature measurements on E. fasciata caterpillars during censuses between 25 September and 24 October 1986. I measured the temperature of 30 fourth-instar and 60 fifth-instar larvae. Censuses were conducted at various times during the day, under both overcast and sunny conditions.

Results

All three data sets have small sample sizes, and these results must be considered preliminary.

Amphion floridensis on foodplants. When pink and green caterpillars were free to select their own resting sites, they did not differ in their temperatures, or in the difference between their body

temperature and the ambient temperature (Table 4-9; body temperature: t-test, $p = 0.56$; temperature excess: t-test, $p = 0.33$).

Amphion floridensis on dowels. When caterpillars were exposed to full sunlight for 10 minutes, there was a slight but consistent difference in the temperatures of green and pink individuals. The mean temperature of the green caterpillars was 34.25 C (s.d. = 0.46), and the mean temperature of the pink caterpillars was 34.62 C (s.d. = 0.49) (paired t-test, $p = 0.049$).

Eumorpha fasciata. Caterpillar temperature paralleled ambient temperature, but the temperatures of the green and nongreen caterpillars in each instar did not differ (fourth instar: Figure 4-7 and Table 4-8a; fifth instar: Figure 4-8 and Table 4-8b).

General Discussion

In Amphion floridensis, Xylophanes tersa, and fourth instar Eumorpha fasciata, green larvae spent significantly more time resting under leaves than nongreen larvae. If these behavioral differences are adaptive, then under natural conditions the costs and benefits of choosing particular resting sites must differ between the color morphs. Nongreen larvae must have higher average fitness when they move away from leaves to rest, and green larvae must have higher average fitness when they rest under leaves. Behavior differences are not universal in sphingid caterpillars, however; none was found in Enyo lugubris or in third-instar larvae of Eumorpha fasciata. Because I have the most

information on the biology of Amphion caterpillars, I will limit my discussion to this species.

The temperature difference between the pink and green Amphion exposed to full sun was less than 0.5 C, and the temperatures of caterpillars on their natural resting sites did not differ. Color morphs on equivalent substrates, therefore, will have equivalent body temperatures. Since thermoregulation will not differ among color morphs, predators or parasitoids must be the major factors selecting for behavioral differences.

Which enemies are selecting for behavioral differences? In tests with captive animals, pink and green Amphion caterpillars were eaten readily by Carolina wrens (Thryothorus ludovicianus), red-winged blackbirds (Agelaius phoenicius), rice rats (Oryzomys palustris), shrews (Blarina sp.), and predacious pentatomid and reduviid bugs. Unfortunately, Amphion caterpillars are virtually impossible to find in the wild, so information on natural predators will have to come from placing reared larvae in their appropriate setting. During my field experiments (Chapter 3), I regularly observed vespid wasps, Polistes annularis and P. exclamans, hunting the caterpillars I put on potted plants. Unless larval densities are similar to those that occur naturally, however, such observations will not provide accurate estimates of the intensity of predation, because predators may show atypical numerical and functional responses. Because larvae will have to be put out at low densities, the number that can be observed simultaneously will be low, and information on predators will accumulate slowly. Switching to another sphingid to obtain

quantitative measures of predation intensity is a possibility, but neither of the two caterpillars that I found regularly in the wild, Enyo lugubris and Eumorpha fasciata, showed large behavioral differences among morphs.

Manipulative field experiments are necessary to measure the survival of green and pink Amphion larvae when they are confined to leaves, nonwoody stems, and woody stems. The mortality from the predator(s) responsible for the behavioral difference will show an interaction between color and substrate: mortality will be lower on green caterpillars that are on leaves, and lower on pink caterpillars that are off of leaves. Insectivorous birds as a group obviously have the capacity to act as such selective agents. Field experiments have demonstrated higher bird predation on conspicuous snails and moths in the wild (Sheppard 1951, Cain and Sheppard 1954, Kettlewell 1955b), and laboratory experiments have demonstrated better discrimination by blue jays of moths on cryptic versus noncryptic backgrounds (Pietrewicz and Kamil 1977). Just because birds can act as such selective agents, however, does not mean that they do. Are green Amphion more cryptic to any of their Florida bird predators than pink, when both rest under leaves?

In addition to birds, vespid wasps have the potential to select for behavioral variation among color morphs. In other systems in which vespid wasps are important predators, behavioral features of caterpillars have been identified as antipredator adaptations. Barathra brassicae larvae (Noctuidae) stop moving or drop off of their foodplants when they detect wing vibrations from Dolichovespula wasps

(Markl and Tautz 1975, Tautz and Markl 1978). Intact caterpillars that showed such responses suffered lower mortality than caterpillars which had their sensory hairs removed and did not react to wasp approaches. Omphalocera munroei larvae (Pyralidae) protect themselves against vespid and sphecid wasps by taking shelter within tied leaves (Damman 1987), and Hemileuca lucina caterpillars (Saturniidae) move from sunny feeding sites on new leaves to shaded areas with older leaves, in response to harassment by Polistes wasps (Stamp and Bowers 1988).

Could the correlation between larval color and substrate choice be an adaptation to reduce wasp predation? In the vespid Polybia sericea, olfactory cues are more important than visual in eliciting landing behavior, but visual cues definitely play a role in the initial approach to a prey item (Richter and Jeanne 1985). This is similar to the sequence documented by Tinbergen (1972) in the sphecid wasp, Philanthus triangulum, which uses visual cues during its initial approach to a prey item, but requires olfactory cues before it will attack. If Polistes are attracted to caterpillar-sized patches of colors or reflectances, then a caterpillar that contrasts less with its background might be less attractive to them. It is not obvious that pink caterpillars would be more cryptic to a wasp on Ampelopsis stems than under leaves, or that green would be more cryptic under leaves, but the hypothesis is testable. By extracting prey in alcohol, Tinbergen (1972) and Richter and Jeanne (1985) assumed that they produced odorless prey possessing typical visual features. Odorless green and pink caterpillars can be attached to different substrates on Ampelopsis vines, and wasps' behavior can be quantified. Wasps should

take longer to approach green larvae under leaves and pink larvae on stems, or approach them less frequently, than green larvae on stems and pink larvae under leaves.

Because the wasp will also use olfactory and tactile cues while hunting, it is unlikely that an Amphion caterpillar can avoid an attack simply by resting on a particular part of the vine (I have observed Polistes foraging at all levels on a trellis of Ampelopsis, from ground level to the top.) By slowing the wasp's approach, however, an Amphion caterpillar may gain sufficient time to escape. Like Barathra brassicae, Amphion larvae seem to be sensitive to airborne wing vibrations: they sometimes drop off of plants when a wasp lands nearby (pers. obs.), and on one occasion I saw a caterpillar drop when the wasp was still in the air, several centimeters away. Reducing its contrast with its background may give the Amphion caterpillar a few extra seconds in which to perceive a wasp before it is perceived.

Other costs and benefits of resting site selection. The best resting sites will be those that maximize a caterpillar's fitness, and will depend on tradeoffs among many factors in addition to the selective predator(s). Strong directional selection by other factors could result in all individuals using one particular resting site; strong disruptive selection could lead to all individuals using wide arrays of substrates.

Predation/parasitism. Predation and parasitism rates will vary spatially for at least three reasons, regardless of a caterpillar's color. (1) Predation and parasitism risks differ among microsites on a hostplant (Price et al. 1980). By attaching gypsy moth caterpillars to

different substrates at different heights on trees, Weseloh (1982) demonstrated that the risk of tachinid fly parasitism differed for larvae on leaves versus bark, and for larvae near the ground versus higher in the canopy. (2) If the specialization of predators or parasites on particular kinds of substrates is either learned or density-dependent, there will be a frequency-dependent advantage for individuals resting on less-used substrates (Clarke 1962, Croze 1970, Ayala and Campbell 1974). (3) Caterpillars that rest farther from feeding sites may spend more time walking, and movement is a major cue used by many predators in locating prey (Robinson 1969, Iwao and Wellington 1970, Boer 1971). Future experiments should quantify each predator's importance in selecting (a) for particular resting sites, independent of color and frequency; (b) for background-matching, independent of site; and (c) for frequency-dependent variation in resting sites.

Development rate. The Amphion weight-gain experiment must be replicated, with better controls and larger sample sizes. If my preliminary result is confirmed, however, the difference in daytime weight gain of green and pink larvae (Table 4-7) demonstrates a cost that pink larvae suffer because of their distance from leaves. Over a single dawn-to-dusk period the average weight gain of the green larvae was 16% higher than of the pink larvae. Faced with a lower growth rate, a pink caterpillar may pupate at a smaller size, or prolong its development. [In addition to demonstrating that green and pink larvae differ in weight gain in the field, it is also necessary to compare their weight gain in the lab, under uniform conditions. If pink larvae

feed less than green larvae during the day when there is no difference in the availability of food, then the field results represent a cost associated with being pink, and not a cost associated with resting away from leaves.]

The major cost of increased development time for herbivorous insects is often assumed to be a higher risk from predation or parasitism (Feeny 1976; Price et al. 1980; Moran and Hamilton 1980). Using published data for gypsy moth larvae, for example, Schultz (1983b) calculated that a 3% increase in development time could lead to a 20% increase in tachinid fly parasitism. Empirical data addressing this hypothesis are scarce, and recent studies have demonstrated that the interactions between development time and larval mortality are complex (Clancy and Price 1987, Damman 1987). In a leaf-galling sawfly (Pontania sp), slow-growing individuals suffered less total parasitism than faster-growing individuals (Clancy and Price 1987). Damman (1987) demonstrated that interactions among several factors resulted in lower predation on slower-developing larvae of the pyralid moth, Omphalocera munroei. Development is faster on new than on old leaves, and at small group sizes, mortality on young leaves is lower. On old leaves, however, groups of larvae can construct shelters that protect them from predators, and Damman found the lowest mortality rates in larger groups of larvae feeding on old leaves. As Clancy and Price (1987) caution, the magnitude and direction of the interaction between development time and mortality cannot be assumed in any herbivore-prey system, and must be tested empirically.

Thermoregulation and humidity. Although temperature regulation does not select for differences between the morphs, spatial variation in temperature and humidity will have an important effect on the cost of selecting particular sites for all caterpillars. Similar microclimatic requirements may lead to smaller behavioral differences than would be predicted on the basis of predation alone.

Under many temperature conditions, pink and green larvae may be able to maintain an optimal body temperature on their preferred resting site; i.e., green larvae may be able to find leaf sites allowing them to maintain that temperature, and pink larvae may be able to find stem sites. Comparisons of the variances of both temperature and humidity at different resting sites throughout the day and season will reveal whether larvae can choose the sites that are optimal with respect to predation, or whether they have to make compromises due to the microclimate.

Other tradeoffs. Schultz (1983a,b) proposed two additional costs for larvae that travelled long distances, but there are no empirical data to address their importance. He suggested that walking may be energetically expensive for a caterpillar, and that caterpillars moving longer distances risked higher contact rates with pathogens. If pink Amphion travel farther than green larvae during their development, these hypothetical additional costs could be important. Although I have demonstrated that color morphs use different resting sites, however, I have not demonstrated that they travel different distances. All individuals may move similar distances from feeding sites, but simply move to different kinds of substrates.

Conclusion. The behavioral differences found in Amphion floridensis, Xylophanes tersa, and fourth instar Eumorpha fasciata confirm the anecdotal reports of nongreen larvae moving away from leaves to rest. For green and nongreen larvae the relative costs and benefits of resting on particular substrates must differ. Further experiments are necessary to identify the enemies selecting for the behavioral variation, and to measure the fitness of caterpillars that make different resting site decisions. Preliminary evidence that pink Amphion larvae gained less weight than green larvae suggests one cost of resting away from leaves; if the fitness of green and nongreen larvae is equal, then there must be some additional advantage for a pink larva in moving away from leaves.

Table 4-1. Relationship of larval color to feeding rate and resting sites, for fifth instar Xylophanes tersa feeding on Pentas lanceolata.

Larval Color	Behavior	Location		Total
		Under Leaf	Not Under Leaf	
Green	Feeding	34	93	127
	Resting	32	169	201
	Total	66	262	328
Brown	Feeding	12	110	122
	Resting	11	201	212
	Total	23	311	334

Log-linear analysis

Model	G	DF	P
Complete interaction	0.318	1	> 0.5
Conditional independence			
a. Color X Behavior	0.004	2	> 0.99
b. Color x Location	25.48	2	< 0.001
c. Behavior x Location	8.11	2	< 0.025

Table 4-2. Position of resting sites of Xylophanes tersa larvae.

Larval Color	Position of Resting Site on Plant		
	Bottom	Middle	Top
GREEN	22	102	48
BROWN	24	108	47

Data are number of observations of 26 green and brown larvae resting on the bottom, middle, or top third of Pentas plants (3 to 10 observations per larva). Green and brown larvae do not choose different heights on which to rest ($G = 0.128$, $df = 2$, $p > 0.9$).

Table 4-3. Resting sites of wild Eumorpha fasciata caterpillars.

a. Third and Fourth Instar

Plant Species	Third Instar				Fourth Instar			
	Green		Pink/Yellow		Green		Pink/Yellow	
	UL	NUL	UL	NUL	UL	NUL	UL	NUL
L. peruviana	8	1	1	0	15	12	-	-
L. octovalvis	4	3	0	2	0	12	1	6
L. oct bigflr	2	3	-	-	1	3	0	3
L. leptocarpa	4	0	0	1	2	15	0	13
L. decurrens	1	1	-	-	1	4	0	1
Total	19	8	1	3	19	46	1	23

UL = under leaf
NUL = not under leaf

b. Fifth Instar Larvae (all foodplants combined)

Resting Site	Green	Black
Under leaf	2	4
Vertical stem	22	18
Horizontal stem	31	58

Table 4-4. Number of observations of third and fourth instar Eumorpha fasciata caterpillars under leaves and not under leaves of Ludwigia octovalvis.

a. Third instar (cumulative data from 10-11 censuses per pair, for 10 pairs of larvae)

Larval color	Under Leaves	Not under Leaves
Green	38	65
Pink/Yellow	32	71

$G = 0.78$, $p > 0.1$

b. Fourth instar (cumulative data from 10-11 censuses per pair, for 17 pairs of larvae)

Larval color	Under Leaves	Not under Leaves
Green	31	143
Pink/Yellow	8	165

$G = 15.50$, $p < 0.001$

Table 4-5. Probability of feeding during daytime censuses in four species of sphingid caterpillars.

Species, instar	Number feeding/ Total larvae	Percent of censuses seen feeding	
		Green	Nongreen
Amphion floridensis, 4	75/78	14	14
Xylophanes tersa, 5	66/66	39	37
Eumorpha fasciata, 3	18/20	23	34
E. fasciata, 4	34/34	46	39
Enyo lugubris, 5	61/62	52	51

Table 4-6. Weight gain of green and pink fourth instar larvae of Amphion floridensis during day and night.

Begin experiment at DAWN (N=18 pairs)

	MEAN WEIGHT in mg (+sd)	
	Green larvae	Pink larvae
Dawn 1 (W1)	252 (+73)	253 (+70)
Dusk (W2)	458 (+129)	447 (+123)
Dawn 2 (W3)	624 (+179)	577 (+136)
Dawn-to-dusk weight gain*	57%	60%

Begin experiment at DUSK (N=19 pairs)

	MEAN WEIGHT in mg (sd)	
	Green larvae	Pink larvae
Dusk 1 (W1)	215 (+41)	198 (+48)
Dawn (W2)	297 (+52)	299 (+79)
Dusk 2 (W3)	409 (+75)	387 (+70)
Dawn-to-dusk weight gain*	54%	47%

* Dawn-to-dusk weight gain = mean proportion of the 24 hour weight change that was gained from dawn to dusk.
For DAWN larvae = (W2-W1)/(W3-W1)
For DUSK larvae = (W3-W2)/(W3-W1)

Table 4-7. Mean weight gain of Amphion caterpillars during 24 hours, day (dawn to dusk), and night (dusk to dawn).

	WEIGHT GAIN			
	Green	Pink	paired t	p
DAWN-TO-DAWN (N=14)	397 (+123)	327 (+97)	2.57	0.023
DUSK-TO-DUSK (N=8)	202 (+86)	206 (+72)	-0.23	0.82
DAY (N = 20)	193 (+80)	167 (+72)	1.98	0.063
NIGHT (N = 14)	136 (+62)	110 (+43)	1.24	0.237

Only pairs which were matched for initial weight within 30 mg were included in each data set. Data are in mg (+ sd).

Table 4-8. Temperatures of green and pink fourth-instar Amphion floridensis caterpillars on their natural resting sites.

	Green larvae	Pink larvae
Sample size	32	30
Mean weight (s.d.) in mg	374 (109)	371 (117)
Mean temperature (s.d.) in C	37.7 (2.0)	38.0 (2.0)
Mean temperature excess* (s.d.)	2.1 (1.5)	1.8 (1.5)
Temperature range	32.0 - 42.1	32.9 - 40.7

Data were collected between 1200 and 1400 on 27 and 29 September 1989.

* Temperature excess = Caterpillar temperature - ambient temperature. Ambient temperature was measured adjacent to each plant, at the same height above the ground as the caterpillar, in the shade. Air temperature 1m above the ground, in the shade, ranged from 29 to 32 C.

Table 4-9. Analysis of covariance of temperatures of green and nongreen caterpillars of Eumorpha fasciata in the field.

A. Fourth instar

Source of variation	DF	MS	F	p
Larval color	1	0.0910	0.03	0.87
Ambient temperature	1	526.7	170.51	0.0001
Error	27	3.089		

B. Fifth instar

Source of variation	DF	MS	F	p
Larval color	1	6.501	1.41	0.24
Ambient temperature	1	587.5	127.36	0.0001
Error	57	4.613		

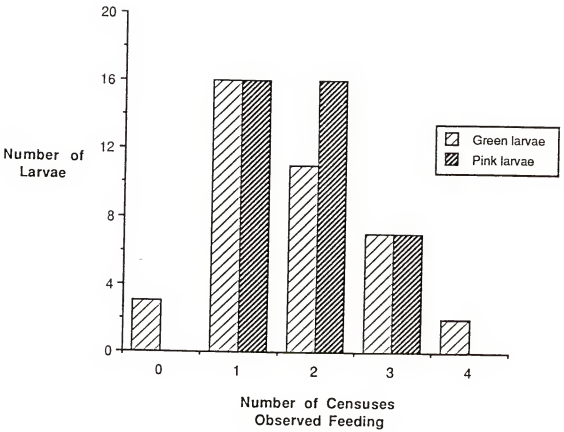

Figure 4-1. Frequency of feeding in green and pink fourth instar Amphion floridensis larvae. Pairs of green and pink larvae were censused on potted plants at half hour intervals, for nine to fourteen censuses per pair.

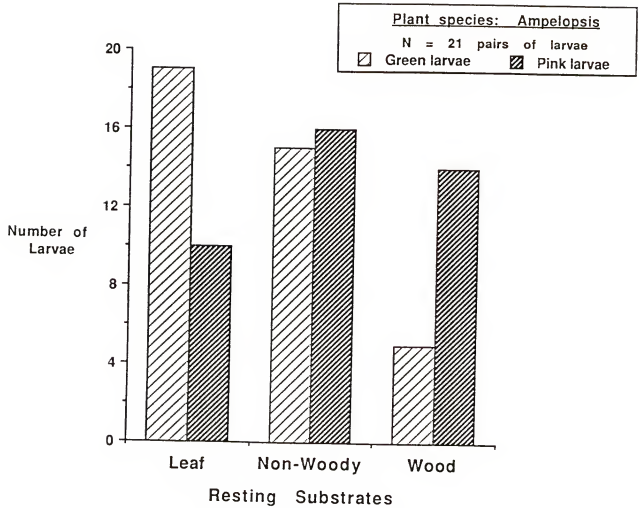

Figure 4-2. Resting sites used by green and pink fourth instar Amphion floridensis larvae on Ampelopsis arborea. The bars represent the number of larvae (out of 21) that rested on a substrate on at least one census. An individual larva used one, two, or three different substrates. Most green larvae rested under leaves, and few rested on wood. Fewer pink larvae rested under leaves, and more rested on wood.

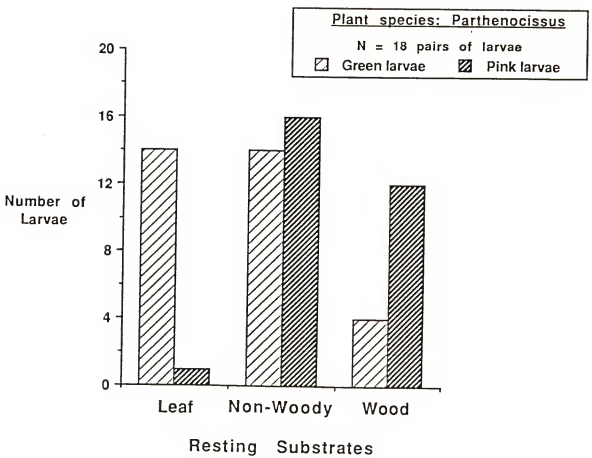

Figure 4-3. Resting sites used by green and pink fourth instar Amphion floridensis larvae on Parthenocissus quinquefolia. See Figure 4-2 for explanation.

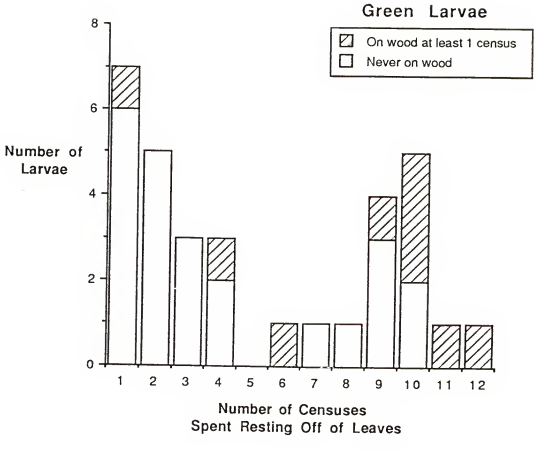

Figure 4-4. Use of woody versus non-woody resting sites by green larvae of Amphion floridensis. Open bars represent the number of larvae spending all of their non-leaf censuses on non-woody stems; hatched bars represent larvae spending at least one non-leaf census on wood. Most larvae that were away from leaves for only 1-4 censuses rested only on non-woody stems; larvae that were away from leaves for more than five censuses were more likely to spend at least one census resting on wood.

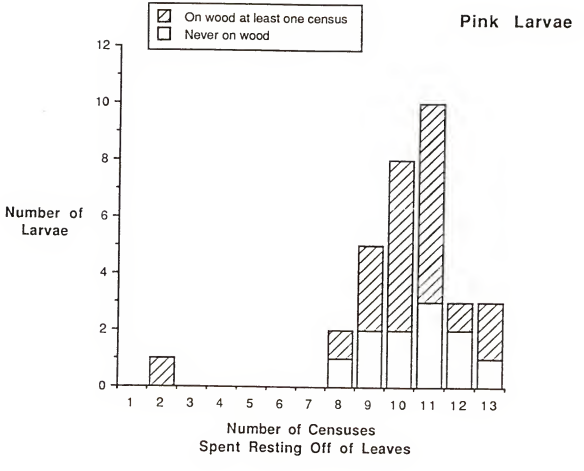

Figure 4-5. Use of woody versus non-woody resting sites by pink larvae of Amphion floridensis. See Figure 4-4 for explanation. Unlike green larvae, there is no change in the tendency of pink larvae to rest on wood as the number of censuses spent away from leaves increases.

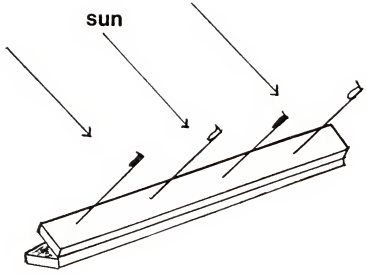

Figure 4-6. Apparatus used to measure the body temperature of green and pink fourth instar Amphion floridensis larvae in full sunlight. Caterpillars were glued to dowels, and positioned with a lateral side perpendicular to the sun.

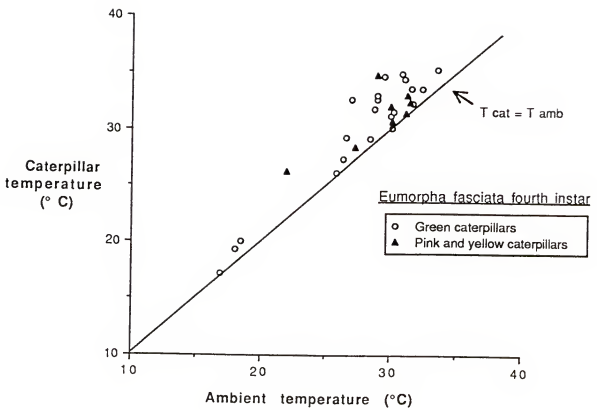

Figure 4-7. Body temperatures of green and nongreen (pink, yellow, or pink-and-yellow) fourth instar larvae of Eumorpha fasciata. Temperature measurements were taken in the field at various times of the day, between late September and late October 1986.

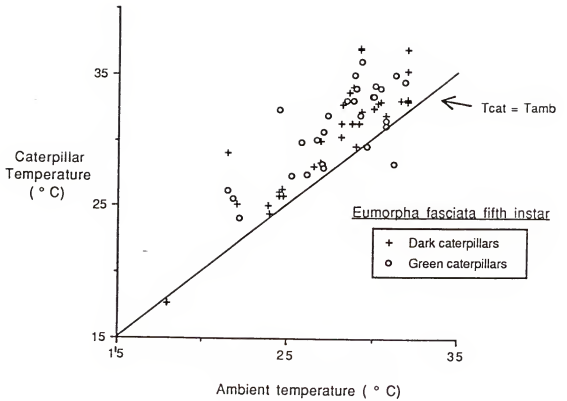

Figure 4-8. Body temperatures of green and black fifth instar larvae of Eumorpha fasciata. Temperature measurements were taken in the field at various times of the day, between late September and late October 1986.

CHAPTER 5
DEVELOPMENTAL CORRELATES OF COLOR IN AMPHION FLORIDENSIS

This chapter examines covariation between color and four developmental traits in Amphion floridensis. As vocal a critic of adaptationists as Stephen Jay Gould has suggested that animal color patterns, "often less subject than morphology to developmental covariance, represent one promising domain" in which to "seek optimality" (1986, p. 66). The large number of studies demonstrating the adaptiveness of animal coloration (Cott 1940, Edmunds 1974, Owen 1980), however, must not obscure the fact that some features of coloration may not be optimal. Even if constraints on color patterns are less stringent than on morphology, we should not assume, without evidence, that they are minimal.

Developmental correlates of larval color have been examined in two sphingids: Manduca sexta (Safranek and Riddiford 1975) and Cephonodes hylas (Sasakawa and Yamazaki 1967). Although developmental differences were found among morphs in both of these species, the black M. sexta are laboratory mutants, and the C. hylas morphs differed in rearing conditions as well as color. In M. sexta, development time from egg to the beginning of the fifth instar was one day longer in black larvae than in green. Black larvae became lighter pupae and adults, and had lower fecundity. The differences in fecundity have persisted in the laboratory for over a decade (L. Riddiford, pers. comm.). In C. hylas,

isolated, green larvae became heavier pupae and survived longer than crowded, brown larvae. Sasakawa and Yamazaki (l.c.) did not state whether green and brown individuals reared under the same conditions also showed these differences, and did not measure fecundity.

Methods

These data were collected during the rearings of Amphion larvae discussed in Chapter 2. The pupal weight, sex, and development time data are from the offspring of subsets of the 35 wild females. The fecundity data are from the lab-bred females used as mothers in the green x green and pink x pink crosses. All larvae were reared at 29 C, 16L:8D, on Ampelopsis arborea, as described in Chapter 2.

Pupal weight. I tried to weigh five pupae of each sex and color for each of seventeen families. Because many broods had few pink or few green larvae, however, and a number of larvae became malformed pupae, I obtained weights of at least one individual of each sex and color for only eight broods. Data were sorted by sex and larval color and subjected to a three-way analysis of variance, with family treated as a random variable.

Development time and sex. Development time is defined as the number of days between egg-laying and adult eclosion. Because larvae were reared in groups, and broods were not censused daily after reaching the fifth instar, I cannot compare the duration of larval instars for the pink and green individuals. I used data from 14 families in which I had development times for at least one individual of each sex and color. Data were subjected to a three-way analysis of

variance, with family treated as a random variable. The pupae in these fourteen families were also used to look for sex differences in the proportions of pink and green individuals.

Fecundity. I measured the fecundity of 34 females that had been green larvae and 16 females that had been pink larvae. Each female was mated to a single like-colored male. Moths were fed daily, and allowed to oviposit until they died (see Chapter 2 Methods). Fecundity is defined as the number of eggs laid.

Results

Pupal weight. Pupae from pink larvae were 7% heavier than pupae from green larvae in both males and females (Table 5-1). The difference between pupal weights of pink and green individuals was significant (Table 5-2, $p < 0.005$).

Development time. Green and pink larvae did not differ in development time (Table 5-3). Although the effects of sex and family on development time were significant, the effect of color was negligible (Table 5-4).

Sex. There were no sex differences in color expression in Amphion: male and female larvae were equally likely to be pink in the fourth instar. Of 291 female and 332 male pupae from 14 females, 31.3% of the females and 34.0% of the males were pink (G-test, $G = 0.52$, 1 df, $p > 0.25$).

Fecundity. The pink females had lower fecundity than the green females (Figure 5-1; Green: mean = 306, sd = 168; Pink: mean = 216, sd = 125). If the data points from the 50 females are considered to be

independent, then the difference is significant (1-tailed t-test, $t = 1.90$, $df = 48$, $p < 0.05$). Some of the females, however, were full siblings. For example, six females (four green and two pink) were siblings from family 16, and three females (one green and two pink) were siblings from family 4. Fecundities of sisters are likely to be correlated, due both to genotype and to common rearing environment, and therefore relatedness should be taken into account.

I re-analyzed the data, treating family line as a class variable. I used only the family lines with sisters of both colors represented, to prevent empty cells in the ANOVA; the data set contains 15 green and 12 pink females from seven lines. Mean fecundity for the green females was 297 eggs (s.d. = 187), and for the pink females, 174 eggs (s.d. = 100). With this analysis, the fecundity difference approached statistical significance ($0.05 < p < 0.10$, Table 5-5).

Fecundity is a function of both female longevity and daily egg production. The pattern of egg deposition in the lab varied widely, with some females laying eggs fairly evenly, and others showing high variances among days. Some of the longest-lived females continued laying eggs until they died, but others stopped five to seven days before their death. As a measure of the duration of the oviposition period, I compared the number of days on which each female produced at least 10 eggs, and found no difference between the green and pink females (Green: range = 1-11 days; Pink: range = 1-9 days; Kolmogorov-Smirnov 2-sample test, $X^2 = 0.902$, $df = 2$, $p > 0.50$). As a measure of daily egg production, I compared the maximum number of eggs produced on a single day, and again found no difference between the green and pink

females (Green: mean = 104, s.d. = 30, range = 33-185; Pink: mean = 88, s.d. = 38, range = 24-148; t-test, t = 1.57, df = 48, p = 0.12). Although neither test was significant, the data suggest that the asymmetry in fecundity is due to lower rates of egg production, rather than to shorter survival.

Discussion

Because the fecundity difference between pink and green Amphion was not quite significant statistically, it must be confirmed with a new experiment. Nevertheless, in combination with the pupal weight data, the trend is even more striking. In many Lepidoptera fecundity is related to body size (Chew and Robbins 1984, Slansky and Scriber 1985). Thus in M. sexta, Safranek and Riddiford (1975) attributed the lower fecundity of black females to their smaller size; the fecundity of green and black females of similar size apparently did not differ. If fecundity in Amphion is related to size, then pink females should have laid more eggs, since they were significantly heavier as pupae.

A reduction in fecundity is clearly not an adaptive trait for pink Amphion; lower fecundity represents a cost of becoming pink. The pink larvae are not making a tradeoff between fecundity and early reproduction; they did not develop more rapidly than the green. For the average fitness of green and pink individuals to be equal, therefore, the increase in survival gained by becoming pink must be substantial.

If just the females experience a reduction in reproductive success by becoming pink, then I would expect sex differences in color

expression: under identical environmental conditions males should be pink more often than females. The lack of such a difference suggests that pink males also have lower reproductive success, for example through a mating disadvantage or lower fertility (Thornhill and Alcock 1983). In fact, since the pairings from which I obtained fecundity data were assortative with respect to color, differences in male spermatophore quality could be responsible for some of the observed difference. In future experiments, therefore, in addition to comparing the fecundity of green and pink females, the components of male reproductive success should be examined.

If the phenotypic correlation has a genetic basis, then selection favoring an increase in the incidence of pink larvae will be opposed by selection for high fecundity; the reduction in fecundity would act as a constraint on the evolution of color polymorphism. Since selection should act to decrease the genetic correlation between two traits under such conditions (Lande 1982), however, it is possible that the correlation is almost entirely phenotypic. Once the correlation between color and fecundity is confirmed, therefore, quantitative genetic experiments are needed to partition the correlation into its genetic and environmental components (Falconer 1981, Hegmann and Dingle 1982).

Both egg development and sphingid caterpillar color are under juvenile hormone control (egg development: Engelmann 1983, Koeppe et al. 1985; color: Fain and Riddiford 1975, Ikemoto 1981, unpublished data). If juvenile hormone titer at the critical period for color determination can vary independently of the hormone titer at other

points in development, then selection acting on color would not produce correlated changes in other JH-controlled events. The covariation between color and fecundity suggests that hormonal differences between pink and green Amphion extend beyond the critical periods for color determination, and persist at least into the pupa. When techniques for measuring juvenile hormone titers become sensitive enough to obtain repeated samples from single individuals, covariation of JH titers during the larval molt and during vitellogenesis should be measured.

Table 5-1. Mean pupal weights (g) of lab-reared Amphion floridensis.

	FEMALES			MALES		
	Weight	s.d.	n	Weight	s.d.	n
GREEN	1.676	0.266	69	1.508	0.216	72
PINK	1.796	0.306	38	1.610	0.218	51

Table 5-2. Effects of color, sex, and family on Amphion floridensis pupal weight.

SOURCE	DF	SS	MS	Appropriate MS for calculating F	F	P
Color (C)	1	0.9806	0.9806	Color/CxF	26.68	<0.005
Sex (S)	1	0.9226	0.9226	Sex/SxF	16.14	<0.02
Family (F)	7	4.7397	0.6771	Family/Error	16.63	<0.001
C x S	1	0.0038	0.0038	CxS/CxSxF	0.31	>0.50
C x F	7	0.2573	0.0368	CxF/Error	0.90	>0.50
S x F	7	0.4001	0.0572	SxF/Error	1.40	>0.20
C x S x F	7	0.0851	0.0122	CxSxF/Error	0.30	>0.50
Error	198	8.0623	0.0407			

Table 5-3. Development time for Amphion floridensis, measured as number of days from egg laying through adult emergence, for moths reared on Ampelopsis arborea at 29 C, 16L:8D.

	FEMALES			MALES		
	# Days	s.d.	n	# Days	s.d.	n
GREEN	39.70	2.00	200	38.68	1.76	219
PINK	40.00	2.06	91	38.83	1.87	113

Table 5-4. Effects of color, sex, and family on Amphion floridensis development time.

SOURCE	DF	SS	MS	Appropriate MS for calculating F	F	P
Color (C)	1	0.1023	0.1023	Color/CxF	0.04	>0.75
Sex (S)	1	97.4074	97.4074	Sex/SxF	31.24	<0.001
Family (F)	13	898.7750	69.1365	Family/Error	39.22	<0.001
C x S	1	4.9151	4.9151	CxS/CxSxF	2.95	>0.10
C x F	13	33.7106	2.5931	CxF/Error	1.47	>0.10
S x F	13	40.5387	3.1184	SxF/Error	1.77	<0.05
C x S x F	13	21.6534	1.6656	CxSxF/Error	0.94	>0.50
Error	567	999.4605	1.7627			

Table 5-5. ANOVA table of fecundity of green versus pink Amphion floridensis females.

Source	DF	SS	MS	F	P
Family (F)	6	280023.51	46670.59	3.48	0.028
Color (C)	1	134945.68	134945.68	4.48 @	<0.10
F x C	6	180864.02	30144.00	2.25	0.10
Error	13	174240.58	13403.12		

@ Family line is a random variable. Therefore, the F-value for color is the color mean square divided by the interaction mean square (Sokal and Rohlf 1981).

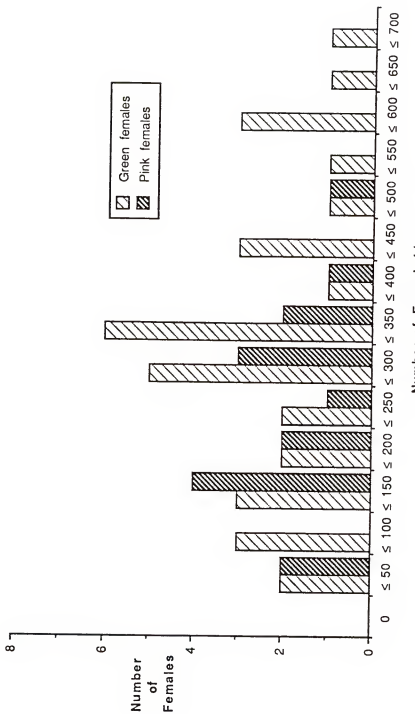

Figure 5-1. Fecundity of green and pink Amphion floridensis females.

CHAPTER 6
CONCLUSIONS AND FUTURE RESEARCH

Caterpillars of Amphion floridensis and Eumorpha fasciata are phenotypically plastic; the color of an individual is influenced by the environment in which it develops. In Amphion, morph frequencies were significantly affected by temperature, with more pink larvae at 29 C than at 21 C, but not by photoperiod (16L:8D versus 12L:12D). In both Amphion and E. fasciata, foodplant quality had a significant effect on morph determination. Not all of the difference among Amphion individuals, however, is due to environmental influences: the genetic crosses demonstrated that pink and green siblings were genotypically distinct.

In contrast to the color polyphenisms exhibited by many adult Lepidoptera (Shapiro 1976), the morph frequencies in Amphion and E. fasciata did not show strong seasonal shifts. High variation was maintained under constant laboratory conditions: every wild Amphion female produced a polymorphic brood, and a diversity of color forms of E. fasciata were reared simultaneously. The sensitivity to foodplant cues in both species allows larvae to respond to heterogeneity on a very fine spatial and temporal scale. These observations suggest that sphingid color polymorphisms are primarily an adaptive response to variation within any one environment, rather than to variation among environments. In addition, the absence of temperature differences

between color morphs suggests that climatic selection is not directly selecting for the polymorphisms. Predators and parasitoids must be the primary agents selecting both for color variation and for behavioral variation, but obviously direct experimental evidence is needed.

Behavioral variation among color morphs was found in Amphion, Xylophanes tersa, and fourth instar Eumorpha fasciata, but not in Enyo lugubris or third instar E. fasciata. In all species showing behavior differences, the green larvae rested under leaves significantly more than the nongreen larvae. In Amphion, the pink larvae gained less weight during the day than the green larvae, suggesting that by resting away from leaves they limited their opportunities to feed. Resting off of leaves, therefore, has a potential developmental cost.

Until lifetime survival and reproductive success are measured, it is not possible to determine if pink and green larvae have equal fitness at their natural frequencies. With respect to development, pink larvae are at a disadvantage relative to green larvae. In addition to the slower weight gain in the field, pink females in the lab were less fecund than their green sisters. Predation experiments will be crucial to determine (1) if pink larvae survive better than green larvae, (2) if frequency-dependent selection increases the fitness of each morph when it is less common, and (3) the adaptiveness of the correlation between behavior and phenotype.

If the pink larvae do not have an additional advantage to compensate for their lower fecundity and slower weight gain, selection should reduce the incidence of pink among Gainesville Amphion. The fact that all females produced both green and pink individuals under a

range of conditions suggests that under some conditions, at least, the fitness of green and pink individuals must be similar.

The search for the ideal sphingid

Progress in biological research depends on finding the right organism. The two major species I have studied, Amphion floridensis and Eumorpha fasciata, are a reciprocal pair: the strengths of one are the weakness of the other. Amphion is an ideal species in which to examine the mechanisms of color determination. It is easy to culture and its polymorphism is relatively simple. Because larvae cannot be sampled in the wild, however, I cannot determine natural densities and frequencies, and cannot design realistic predation experiments. Eumorpha fasciata, in contrast, is an ideal field organism, abundant and easy to sample. Correlations can be made between morph frequencies and hostplants, climate, and predation intensity, to provide preliminary evidence for functional hypotheses. Field experiments can test the adaptive significance of color. Unless females can be induced to oviposit, however, laboratory studies of the control of color are severely constrained, and the reproductive components of fitness cannot be measured. The ideal sphingid would combine the assets of E. fasciata and A. floridensis.

Future Research

Geographic variation

As selective pressures vary, populations should vary in morph frequencies, phenotypic responses to environmental cues, and behavior.

(a) The decision rules regarding the conditions under which to change color are likely to differ, both because environmental cues that are reliable in one population may not be reliable in another (Hoffmann 1978), and because the fitness tradeoffs for each morph will be different. In Florida, for example, Amphion color frequencies shifted with rearing temperature. As suggested in Chapter 3, a potential adaptive explanation is that at different temperatures the optimal resting sites change, and each color morph is more cryptic on a different kind of substrate. If for Amphion in the northern United States the optimal morph frequency does not change with temperature in a similar way, northern populations should show a different response to temperature. Geographic studies of the responses to foodplant quality will probably not show consistent trends, but on a smaller scale, populations that differ in hostplant utilization may show different degrees of susceptibility to foodplant cues.

(b) Resting site differences should vary among populations in response to the local costs and benefits. In Florida, my experiments suggest that pink Amphion have higher fitness resting primarily away from leaves. In another habitat, with different predators, climate, and food quality, the fitness of pink larvae may be higher if they remain under leaves. If caterpillars from different populations are tested under the same conditions, will they make different behavioral decisions, and can these be correlated with their respective selective pressures? Are there some populations in which all larvae tend to rest under leaves, and other populations in which all tend to rest away from leaves?

On the Galapagos, Curio (1965, 1966) reported differences in the resting sites of green versus grey and brown Erinnyis ello larvae in the third through fifth instar. On Jamaica, in contrast, he reported differences in resting sites only in the fifth instar (1970a,b,c), although larvae were also polymorphic in earlier instars (Schneider 1973). These differences have numerous potential explanations, including variation in plant architecture and in sampling procedures. If, however, these differences persist when individuals from the two populations are tested under identical conditions, then (a) what are the differences in the selective pressures on the two islands, and (b) how rapidly can resting site preferences change in response to changes in selective pressures?

Developmental rules

I have ignored the complexity that arises when a species has multiple alternative forms, and when it is polymorphic in more than one instar. The polymorphism in Amphion floridensis is simple. Dimorphism is limited to two instars, and both morphs are common only in the fourth instar (Figure 6-1). Although Eumorpha fasciata has a very complex polymorphism (Figure 6-2), in my analyses I treated it simply as dimorphic green/nongreen in the fourth and fifth instar.

For Eumorpha fasciata, field experiments must address the fitness consequences not only of being green or black in the fifth instar, but of being green, pink, pink-and-yellow, or yellow-with-pink in the second through fourth instars, and of occasionally being yellow in the fifth instar. The environmental cues that trigger color changes at

earlier instars must be examined as well. Is a change from green to pink in the third molt triggered by the same environmental cues as a change from green to black in the fourth molt?

Colors do not follow one another at random, and both mechanistic and functional studies are needed of the relationships among the colors at sequential instars. In the developmental sequences of lab-reared samples, general patterns appear (Figures 6-1 to 6-4). In species with polymorphism in more than one instar, individuals retain some flexibility over several instars. Fourth instar color is not completely determined by the third instar color, nor fifth instar by the fourth. Thus, a pink second instar Eumorpha fasciata caterpillar has at least three color options at its next molt (pink, pink and yellow, or yellow with pink), and each of these will have several options when it molts to the fourth instar. Nevertheless, not all colors have the same probability of being followed by every other color: nongreen forms rarely become green in any species; pink Enyo rarely become blotched; and in E. fasciata the shifts between pink and pink-and-yellow, and between pink-and-yellow and yellow-with-pink, are more common than shifts between pink and yellow-with-pink. Although decisions about color may be made several times in an individual's life, therefore, an individual's future color is not independent of its present color.

Do these asymmetries result from past selection for the most adaptive developmental sequences, or do they represent limitations on the plasticity of individuals? Do underlying developmental or genetic constraints prevent plasticity from being maximal at all instars

simultaneously? Why, especially, are color reversals from nongreen to green so rare?

The absence of reversals from nongreen to green may be a general rule in the family. Reversals do not occur in Eumorpha typhon, which can become nongreen in instars two through five (J.O. Schmidt, pers. comm.). In Erinnyis ello, brown larvae never shifted back to green under a variety of rearing conditions, although limited reversals of bluegreen to green were found when larvae were shifted from crowded to isolated conditions (Schneider 1973). In reading hundreds of descriptions of sphingid caterpillars, I found only one other explicit statement of nongreen larvae molting into green (Cephonodes hylas hylas, Sevastopulo 1939).

Brown-to-green and black-to-green color changes are not uncommon in insects (Rowell 1970, Robinson and Hartley 1978, Meyer 1979, Johnson 1984). Among Lepidoptera, many swallowtail butterfly caterpillars, for example Papilio polyxenes and P. xuthus, are brown-and-white or black-and-white at early instars, but green at later instars. Color reversals after one or more molts have been observed in orthopterans when conditions changed from those favoring nongreen to those favoring green individuals (Robinson and Hartley 1978, Meyer 1979).

Along with analyzing the genetic correlations between sphingid color and fecundity (Chapter 5), future experiments should measure the genetic correlations between larval colors at sequential instars. One approach to testing whether the absence of reversals from nongreen to green is due to developmental limitations would be a genetic assimilation experiment (Waddington 1961). If sphingid larvae were

reared under environmental conditions favoring nongreen intermediate instars, and then shifted to conditions favoring green in the fifth instar, the number of reversals should be increased. These individuals would form the parents for the next generation. Over several generations, is it possible with such a technique to select for lines that are nongreen at intermediate instars and green at later instars, or does selection for green final instars always lead to a correlated increase in green at intermediate instars? If a correlated response is found, then the juvenile hormone control of color determination should be probed. Are there genetic correlations between the juvenile hormone titers at subsequent instars? Is it possible to select for higher-than-average titers at one point in development, and lower-than-average titers at another point?

Comparisons among Sphingids, Geometrids, and Noctuids

The Sphingidae contains more than 1000 species, and offers rich opportunities for comparative studies. They are a fairly homogeneous family, however, in some respects, and if comparative studies reveal similar patterns among species, it may be difficult to separate selective and historical explanations. The rarity of reversals from nongreen to green, for example, may be widespread within the family because species are constrained by the same underlying developmental system, or because species have been exposed to similar selective pressures. Variable coloration is also a common strategy of caterpillars in the Geometridae and Noctuidae. Comparative studies

among the families will reveal patterns that are shared by all three, and patterns that are distinct to each.

Within each family, what are the major cues used to trigger color changes? Is the variation within each family in the control of polymorphism as large as the variation among families? How prevalent is behavioral variation in each family, and are the mechanisms similar?

The recent demonstration of foodplant effects in the geometrid Nemoria arizonaria (Greene 1989) provides an intriguing contrast with my results. The response of Nemoria caterpillars to their diet was extremely uniform: all individuals fed catkins became catkin morphs, and all individuals fed leaves became twig morphs. In contrast the effect of foodplant on both E. fasciata and A. floridensis was weaker. Individuals on a particular diet did not respond uniformly in any of the tests (plant species and leaf color in Amphion, and plant species in E. fasciata). How and why do the responses to foodplants differ?

Majerus (1978, 1983a,b) found that a complex set of morphs in the later instars of Phlogophora meticulosa (Noctuidae) were not responsive to any environmental cues, but were entirely controlled by genotypic differences. Many noctuid caterpillars show density-related color changes, so environmental control of color occurs within the family. What are the consequences for P. meticulosa of an absence of phenotypic plasticity? Has plasticity been selected against?

Epilogue

The simple question, "Why are sphingid caterpillars polymorphic?" does not have a simple answer. I end with the recognition that this

question is really three:

What is the adaptiveness of sphingid color polymorphism?

What is the adaptiveness of plasticity of sphingid color determination, and how is plasticity controlled?

What is the adaptiveness of the association between color and behavior, and how does this assocation evolve?

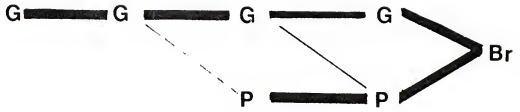

Figure 6-1. Observed transitions between instars in Amphion floridensis. Each letter indicates a different color; each column represents an instar, beginning with instar 1. Lines indicate transitions between colors in successive instars. G = green; P = pink; Br = brown. Thick lines: > 75% of the transitions from a preceding morph; medium lines: 34-75%; thin lines: 10-33%; dotted lines: < 10%. Based on 1259 larvae reared in the lab on Ampelopsis arborea at 29 C, 16L:8D.

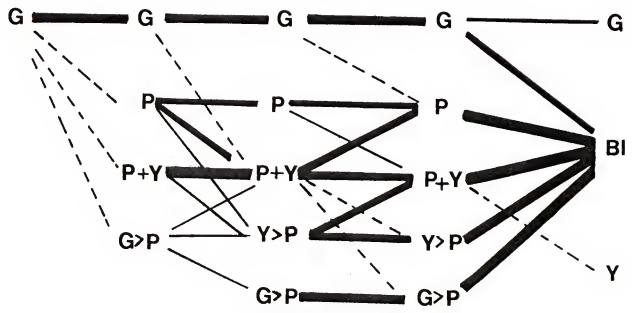

Figure 6-2. Observed transitions between instars in Eumorpha fasciata. Based on 155 larvae reared in the lab on three species of Ludwigia. G = green; P = pink; P+Y = pink and yellow; Y>P = yellow with pink; G>P = green with pink; Bl = black with multicolored markings; Y = yellow with pale markings.

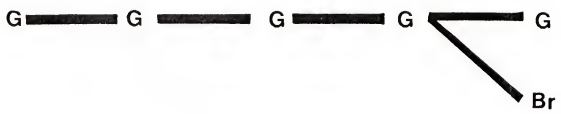

Figure 6-3. Observed transitions between instars in Xylophanes tersa. Based on more than 150 larvae reared in the lab on Pentas lanceolata.

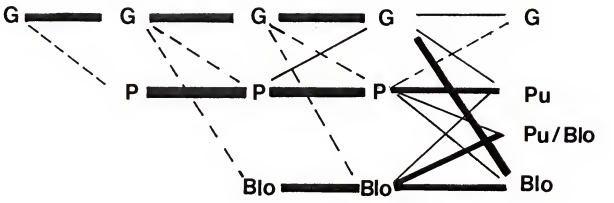

Figure 6-4. Observed transitions between instars in Enyo lugubris. Based on 826 larvae reared in the lab on Ampelopsis arborea under several temperature and photoperiod conditions. Blo = green with purple blotches; Pu = purple; Pu/Blo = identity uncertain.

APPENDIX
EUMORPHA FASCIATA CENSUS SITES AND SPRING BROOD

In 1985 I censused one large stand of Ludwigia peruviana on Paynes Prairie State Preserve in Gainesville at 1-5 day intervals from 17 September through 1 November. From 18 to 30 September all eggs and larvae were collected for rearing in the lab. In 1986 I censused four sites with L. peruviana, L. octovalvis, and/or L. leptocarpa weekly between 26 August and 7 November. Numerous other sites in Gainesville, Micanopy, McIntosh, and Hawthorne were searched once or twice. In 1987 Eumorpha fasciata was less abundant in Gainesville. Two sites sampled in 1986 were destroyed, and at the two remaining sites few larvae were found during searches between 27 August and 16 October. Consequently I made two trips to sample Eumorpha populations further south, near Inverness and Lake Placid, Florida. In 1988 irregular censuses were made in Gainesville and Inverness from 2 September to 15 November.

Gainesville Census Sites

Paynes Prairie North (PPN) (1985-1988). The north entrance of Paynes Prairie State Preserve, Gainesville. A large stand of Ludwigia peruviana along the main dike and to the east. Smaller stands of L. leptocarpa, L. octovalvis, and an unidentified Ludwigia species (it never flowered) were also censused in 1987.

River Styx (1986-1988): Along the edge of County Road 346. A small stand of Ludwigia leptocarpa, and a few plants of L. octovalvis. Caterpillars abundant in 1986 and 1988. Site mowed in 1987.

Northeast (1986-1988). Two sites in northeast Gainesville near the intersection of SR 24 and CR 225.

NEa: Roadside ditches along CR 225 0.5 km north of the intersection. Large stands of L. octovalvis, L. leptocarpa, and L. peruviana. Small amounts L. decurrens and L. maritima. Larvae abundant 1986 and 1988; site mowed in 1987. Larvae were almost exclusively on L. octovalvis.

NEb: 1.5-3 km west of the intersection of SR24 and CR225, along CR 232. Deep roadside ditches with dispersed plants of L. octovalvis (bigflr), L. leptocarpa, L. peruviana, and L. octovalvis (smallflr). Larvae occurred on all foodplants, but primarily on L. oct bigflr.

Other Census Sites

Inverness. In 1987 and 1988 I censused Ludwigia leptocarpa and L. octovalvis (bigflr) along Henderson Lake on SR 44 in Inverness (80 km south of Gainesville), and along McKethan Lake in the Withlacoochee State Forest.

Highlands County. On 16-18 October 1987 I censused sites in the vicinity of the Archbold Biological Station in Lake Placid, Florida.

a. Sebring on US 27, along Lake Jackson (L. octovalvis (bigflr)).

b. Price Memorial Tract of the Archbold Biological Station, on Lake Placid (L. peruviana, L. leptocarpa, L. decurrens).

c. Lake Placid Public Boat Ramp (L. leptocarpa, L. decurrens, L. octovalvis (bigflr)).

d. Bishop Park, Lake June-in-Winter (L. leptocarpa, L. decurrens, L. peruviana).

e. Lake Kissimee State Park (L. peruviana).

Spring Broods

Although Eumorpha fasciata larvae occur primarily from August through November in Gainesville, there is a small spring brood in at least some years. In May 1986 I found six eggs and 28 larvae on unidentified Ludwigia species, and in August 1986 I found a few fourth and fifth instar larvae, when most of the population was eggs or first instar larvae. No early eggs or larvae were found at the same site in the spring of 1987 or 1988. The 28 larvae were distributed as follows: first instar green, 5; second instar green, 1; second instar green-pink, 2; fourth instar green, 1; fourth instar pink, 2; fifth instar green, 3; fifth instar black, 14.

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Linda Susan Fink received her early education in Great Neck, New York. She was a member of the first coed freshman class at Amherst College, and received her B.A. summa cum laude in 1980. Her Honors thesis investigated bird predation on overwintering monarch butterflies in Mexico. Aside from her research, her major accomplishment at Amherst was performing in a full-length ancient-Greek production of Aeschylus's Agamemnon. After entering the Department of Zoology at the University of Florida in 1982, she received her M.S. degree in December 1984, for work on the maternal guarding behavior of the green lynx spider. Upon completion of her Ph.D., she will become a Visiting Assistant Professor of Biology at Middlebury College.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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