THE EVOLUTION AND ECOLOGICAL GENETICS OF PUPAL COLOR DIMORPHISM IN SWALLOWTAIL BUTTERFLIES (LEPIDOPTERA: PAPILIONINAE)

by

Wade Nelson Hazel

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APPROVED:

D. A. West, Chairman

Paul B. Siegel
P. B. Siegel

E. F. Benfield
E. F. Benfield

Robert A. Paterson
R. A. Paterson

J. L. Eaton

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Blacksburg, Virginia

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CHAPTER 1

Introduction

An environmentally-cued dimorphism involving the production of green or brown pupae has evolved in many species of swallowtail butterflies (Merrifield and Poulton 1899, Sheppard 1958). As a result of the environmental influence, the pupal phenotypes are cryptic. Therefore it has been assumed, with some experimental support, that the adaptive significance of the trait is that it decreases the probability of pupae being attacked by visual predators (Hidaka _et_ _al_. 1959, Baker 1970, Wiklund 1975).

Although the pupal color is to an almost overwhelming extent under environmental control, the ability of the prepupal larvae to respond to specific environmental cues has a genetic basis, and selection can alter this ability (Hazel 1977).

In some multivoltine species the presence or absence of the dimorphism may be seasonal and related to diapause, so that larvae reared on a short photophase produce mostly brown diapausing pupae, regardless of the pupation site (West _et_ _al_. 1972, Smith 1978, Owen 1971, Ishizaki and Kato 1956). However, when the dimorphism is expressed, environmental cues associated with color of the pupation site control pupal color (Sheppard 1958, Ishizaki and Kato 1956, Hidaka 1961a, Clarke and Sheppard 1972, Wiklund 1972, West _et_ _al_. 1972, Gardiner 1974, Smith 1978, Hazel and West 1979). The two most important cues appear to be the texture and color of the pupation site. However, different species do not respond in the same way to these two cues and differences between species are the subject of some debate (Smith 1978, Hazel and West 1979).

Sheppard (1958) and Clarke and Sheppard (1972) suggested that the evolution of pupal color dimorphism may be causally related to the types of pupation sites that a species utilizes in nature. This hypothesis predicts that species monomorphic for pupal color should choose relatively stereotyped pupation sites while dimorphic species should choose more variable sites. Unfortunately, because the pupae are indeed cryptic, there is little information on the natural pupation sites of swallowtail butterflies, and certainly no quantitative data. This has made hypotheses concerning the evolution of the dimorphism, as well as the significance of differences between species in the environmental cues affecting pupal color, difficult to evaluate.

This dissertation examines the existing hypotheses concerning the evolution and ecological genetics of pupal color dimorphism in the swallowtails. In general, three types of data will be presented: (1) the characteristics of the natural pupation sites of six species of swallowtails (two species monomorphic and four species dimorphic for pupal color) will be given in Chapter IV and discussed with respect to the evolution of the dimorphism; (2) studies of the environmental control and seasonality of the dimorphism in the four dimorphic species will be presented in

Chapters V and VI, respectively, and the data will be discussed in relation to the ecology of the species and the characteristics of their pupation sites[1]; (3) a selection experiment designed to investigate the genetics of the dimorphism will be presented in Chapter VII and will be discussed in relation to the proposed genetic basis of the trait. A summary of all data will be presented in Chapter VIII.

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[1] Data for the natural pupation sites of three species and the environmental control of pupal color in two species has been published elsewhere and will only be summarized in Chapters VI and V, respectively (see West and Hazel 1979 and Hazel and West 1979).

CHAPTER II

Review of Literature

Although it had been known since 1867 (Wood 1867) that pupal color in some butterflies varies with the color of the pupation site, it was not until 32 years later, and after considerable debate (see Poulton 1892), that investigators were able to show that the dimorphism in the swallowtails was determined by the characteristics of the pupation site (Merrifield and Poulton 1899). This led Poulton to write, concerning pupal color in _Papilio_ _machaon_ L., "It is a great pleasure to me to see this species, upon which so much doubt has been thrown...now finally proved beyond doubt to be susceptible to the colours of its environment." (Merrifield and Poulton 1899, p. 389). In the years between Woods paper (1867) and Merrifield and Poulton's report (1899), most investigations had concentrated on the environmental control of pupal color in the Pierids and Papilionids, and the determination that pupal color was controlled by the color of the pupation site had been much easier in the Pierids (Poulton 1887, 1892).

As investigations continued, it became apparent that pupal color in Pierids and Papilionids differed in a number of aspects. Pupal color in the pierids varies continuously from dark green to dark grey in response to the color of the pupation site (Brecher 1921, Angersbach and Kayser 1971, Gardiner 1974), while in the Papilionids pupal color is distinctly dimorphic, usually green or brown, with rare intermediates (Merrifield and Poulton 1899). In addition, the physiological mechanisms that determine pupal color are different in the two groups (Smith 1980, Hidaka 1961a, 1961b).

Some experimental work suggests that the adaptive significance of the environmental influence on pupal color lies in the crypsis that it provides the sedentary and exposed pupa. Hidaka (1959) placed green and brown pupae of *Papilio xuthus* head-down in grass and a pair of fowl were allowed to feed in the grass. During the summer when the grass was green, green pupae were more likely to escape predation. In November, when the grass had turned "grey", brown pupae were eaten less often.

Baker (1970) investigated bird predation on the pupae of *Pieris rapae*. In discussing the rate of disappearance of the pupae Baker notes that, "...the better the pupa matches the colour of its surroundings and the more it is protected by virtue of its position then the less chance it has of being taken." (Baker 1970, p. 55).

Wiklund (1975) investigated the evolutionary significance of pupal dimorphism in the swallowtail *Papilio machaon* L. in southern Sweden. Cryptic and non-cryptic pupae were placed in pairs in the vegetation of *machaon*'s natural habitat. Care was taken that members of a pair were equally visible. The survival of the pupae was then monitored periodically throughout both the summer and diapausing generations. In the summer generation the net survival of cryptic pupae was 1.5 times that of non-cryptic morphs. In addition, the rates of elimination of non-cryptic pupae were significantly higher. No differences were detected in the diapausing generation.

The seasonality of pupal color has been investigated or discussed in several species (West *et al.* 1972, Shapiro 1976, Owen 1971, Smith 1978). West *et al.* (1972) were able to show in *Papilio polyxenes* that rearing larvae on short day photoperiods caused the almost exclusive production of brown pupae which entered diapause. Rearing larvae on long day conditions "...permits the expression of the green-brown alternative...", depending on the pupation site. Smith (1978) also found that daylength during rearing influenced pupal color in *Papilio polytes* so that significantly more brown pupae were produced when larvae were reared on short days. Even though some pupae entered diapause Smith found no relationship between it and brown pupal color. In *Papilio xuthus* an "orange" morph is produced which always enters diapause (Ishizaki and Kato 1956). Therefore, as Shapiro (1976) has suggested, it may be that pupal color and diapause in some species are physiologically or genetically linked. Other cues, such as humidity and temperature,which seem to play minor roles in pupal color determination and possibly diapause, may also be important in relation to seasonal changes (Ishizaki and Kato 1956, West *et al.* 1972, Owen 1971, Smith 1978).

The influence of the pupation environment on pupal color has been most extensively investigated by Smith (1978). In both *Papilio polytes* and *Papilio demoleus* he found that the texture of the pupation site was the most important environmental cue: rough surfaces promoted the production of green pupae. Background color was also found to have an effect in both species, with yellow surfaces and yellow light enhancing the frequency of green pupae and blue surfaces and light increasing the frequency of brown pupae. In general, the influence of these two colors on pupal color is consistent with the results obtained using other species of swallowtails (Wiklund 1972, Merrifield and Poulton 1899) as well as in several Pierids (Okamoto 1960, Poulton 1887, Harrison 1928).

Perhaps the most interesting result of Smith's study was that texture and background color did not affect *polytes* and *demoleus* in the same manner. Texture had a greater effect on pupal color in *polytes* than in *demoleus*. Smith interpreted this difference in terms of the time of day that the larvae of these species select their pupation sites. Because *polytes* larvae tended to choose pupation sites during the hours of darkness, when color would be of little value as a cue, they are more influenced by texture. The larvae of *demoleus* often choose pupation sites during the day and then are more influenced by color.

Hidaka (1961a, 1961b) made detailed studies of the physiological mechanism underlying the production of green and brown pupae in P. xuthus. Using ligatures, organ transplants, the severing of nerves and ablation, he was able to show that the production of a brown pupae was dependent on the release of a hormone from the prothoracic ganglion prior to the pupal molt. The mode of action of this hormone probably involves the activation of oxidative reactions in the cuticle. This hypothesis has been suggested by Onishi (1959), who found integumentary carotenoids in green pupae and their oxidized forms in brown pupae, as well as melanin, astaxanthin, and papilioerythrin. Hidaka was also able to show that release of the browning hormone occurs only after the prothoracic ganglion has been stimulated by the brain. Smith (1978), who has investigated the physiology of pupal color in P. polytes and demoleus also found brown pupal color to depend on the presence of a hormone, but his data suggested that the hormone was secreted from a source in the head rather than from the prothoracic ganglion.

There have been two attempts to investigate the genetic basis of pupal color dimorphism in the swallowtails (Clarke and Sheppard 1972, Hazel 1977). Both investigations utilized artificial selection and the results have proved intriguing.

Clarke and Sheppard (1972) were particularly troubled by pupal color--background mismatches (usually green pupae on thin brown twigs) in their greenhouse populations of _P. polytes_. They theorized that there might be genetic variation in the ability of individuals to match their backgrounds. Therefore they choose background matching and mismatching as their criterion of selection. After five generations of selection no differences could be detected in frequencies of matching and mismatching pupae, but the pupation sites in both the matching and mismatching lines had been altered. Since most pupae that had mismatched their backgrounds had been formed on small brown twigs, their selection had resulted in an increased preference for that sort of pupation site. Most pupae that had matched their backgrounds had been formed on green stems and foliage, and their selection had resulted in an enhanced tendency to choose these types of sites.

Hazel (1977) proposed that the occurrence of mismatching pupae might be a reflection of genetic differences in the tendency of individuals to produce brown (or green) pupae. Pupal color would then behave as a threshold trait, with each individual having a genetically determined liability of becoming a brown pupa in much the same way that an organism may have a genetic liability of contracting a certain disease(Falconer 1965). Therefore, the criterion of selection was simply pupal color, and the pupation environment was held constant. Eight pairings were made using the offspring of wild caught females of _Papilio_ polyxenes. In five pairings only adults eclosing from green pupae were selected. Four pairings resulted in an increase in the production of green pupae in the next generation, and in two the increase was significant. In three pairings adults were selected which eclosed from brown pupae and each of these pairings resulted in a significant increase in the frequency of grown pupae in the next generation. These results are consistent with a threshold hypothesis. This hypothesis allows one to make two predictions. The first is that because natural selection favors background matching, and because the pupal color phenotypes are enormously affected by the environment, selection will be inefficient at eliminating the genetic variation underlying the trait, and mismatches will be expected to occur. The second prediction is that the mean tendency of a population to produce brown pupae is correlated with the frequency of brown pupation sites utilized by that population. In other words, an increase in the availability of brown pupation sites in a population should select for an increase in the tendency to produce brown pupae, as these individuals will more often match their backgrounds. The overall tendency to produce brown pupae would eventually become balanced by stabilizing selection depending on the relative availability (or perhaps use) of green and brown pupation sites and the relative survival of pupae on these sites.

CHAPTER III

General Materials and Methods

Six species of swallowtails were used or will be discussed in this study. Of these, two species are monomorphic for pupal color and the remaining four are dimorphic. All six are multivoltine in the mountains of southwest Virginia, where these experiments were carried out.

Papilio glaucus L. produces monomorphic brown pupae, sometimes with small amounts of green speckling laterally on the abdomen and elsewhere. Its most common food plants are tulip tree (*Liriodendron tulipifera* L.) and wild cherry (*Prunus serotina* Ehrhart).

Papilio cresphontes Cramer produces monomorphic brown pupae with greenish-grey saddles across parts of the thorax and abdomen. Larvae feed on hop tree (*Ptelea trifoliata* L.).

Papilio troilus L. produces green and brown pupae and its larvae feed on spice bush (*Lindera benzoin* (L.) Blume) and sassafras (*Sassafras albidum* (Nutt.) Ness).

Papilio polyxenes Fabr. produces both green and brown pupae. Its natural food plant is wild carrot (*Daucus carota* L.).

Battus philenor (L.) also produces dimorphic, green and brown pupae. Its larvae feed on Dutchman's pipevine (*Aristolochia macrophylla* Larmarck).

Eurytides marcellus (Cramer) produces green and brown pupae and its larvae feed on pawpaw (*Asimina triloba* (L.) Dunal).

Unless otherwise noted, all experiments were carried out in the spring and summer months of 1977, 1978, and 1979, and all butterflies were reared from larvae collected in the field or from eggs obtained in the lab from wild caught females collected in Montgomery and Giles counties, Virginia. For most experiments larvae were reared in environmental chambers on long day conditions (15 h photophase, 24° C) by methods previously employed (Hazel 1977). Any differences in the rearing conditions will be given in the materials and methods of the individual chapters. For all experiments larvae were fed fresh food plant as needed: parsley (*Petroselinum crispum* (Miller) Mansfield) or wild carrot for *P. polyxenes*, pipevine for *B. philenor*, pawpaw for *E. marcellus*, spicebush or sassafras for *P. troilus*, tulip tree or wild cherry for *P. glaucus* and hop tree for *P. cresphontes*.

CHAPTER IV

The Natural Pupation Sites of Swallowtail Butterflies

Introduction

Sheppard (1958) suggested that environmentally cued polymorphisms would be evolved "...in situations in which there is a mosaic of distinct environments and the distribution of offspring with respect to them is un-correlated with those experienced by their parents..." (Clarke and Sheppard 1972, p. 1; see also Wiklund 1972, and Clark 1976). West and Hazel (1979) have attempted to extend this generalization by relating the habitat of the larval food plants to the evolution of pupal color dimorphism in swallowtail butterflies. To test this hypothesis they located the natural pupation sites of three species, one of which was monomorphic for pupal color. While the results were generally consistent with Sheppard's hypothesis, there were exceptions to our generalization that the evolution of the trait is related to the habitat of the larval food plant. Thus, we concluded "...that the primary determinants of whether a population is monomorphic or dimorphic are the food plant(s) and the relative pressures of various predators." (West and Hazel 1979, p. 392).

In this chapter, data on the natural pupation sites of two species dimorphic and one species monomorphic for pupal color are presented. The results will be discussed in relation to the hypothesis of Sheppard (1958) and its generalization by West and Hazel (1979).

Materials and Methods

Larvae of *Eurytides marcellus*, *Papilio troilus* and *P. cresphontes* were used in this study. The latter species produces monomorphic pupae, while the other species produce both green and brown pupae. All three species feed and pupate in the forest or in the ecotone at its edge. Larvae were reared on natural mid-summer daylength and temperature conditions (approximately 15 h photophase) as described in West and Hazel (1979).

The experimental methods and study areas were the same as those employed by West and Hazel (1979). In general, larvae were marked dorsally or laterally with UV-fluorescent vinyl-latex paint (Ultra-violet Products, Inc., San Gabriel, California 91778) and released after gut evacuation in one of three study areas. The larvae were located on their pupation sites later the same or the next evening by the fluorescence of the paint marks in the presence of UV light (Plate I). The study areas were designated 'meadow', 'edge' and 'forest'. The vegetation of the 'meadow' study area was dominated by tall grasses and weeds while that of the 'forest' was composed of trees with a ground cover of dead sticks and leaves and some sparce vegetation. The 'edge' study area was similar to the 'meadow' area except with more woody vegetation and was in fact on the ecotone between the 'forest' and 'meadow' study areas.

The success in finding the marked insects varied with the species, study area and intensity of bird predation on wandering larvae. For *marcellus* and *troilus* it was about 70 percent and for *cresphontes* about 35 percent.

Results

Papilio cresphontes

Larvae are brown with light grey, green-tinged saddles across the dorsal thorax and abdomen. When released on the tip of a branch, larvae moved slowly to the ground. Once on the ground larvae showed no initial interest in pupation and moved up to 508 cm from the release point before settling on a site. The larvae suffered heavy bird predation and this accounts for the relatively low recapture rate. The characteristics of the pupation sites are shown in Figure 1.

Papilio troilus

Last instar larvae are green with large 'pupiled' thoracic eye-spots. After cessation of feeding larvae change color to yellow green or yellow. When released on the tip of a branch after gut evacuation they moved down the branch to the ground. Movement was jerky with frequent pauses, and larvae appeared to have difficulty holding on to broad or flat surfaces and would often fall from leaves and stems to the ground. Once on the ground they showed no initial interest in pupation; sometimes wandering up to 6.5 m before settling on a

Plate I. Prepupal larva of _Papilio cresphontes_ on its eventual pupation site. Light colored marks on the lateral abdomen and thorax were made with UV paint.

pupation site. In general brown pupae were formed when pupation occurred amongst brown surroundings and green pupae were formed in green surroundings. Two pupae did not appear to match their backgrounds. One was a green pupa on the inside of a rolled brown leaf and the other was a brown pupa on a dead weed stalk in the 'meadow' study area. The latter pupa was formed in late July and entered diapause. The characteristics of the pupation sites are shown in Figure 2.

Eurytides marcellus

Last instar larvae are continuously variable in color from green to black, with intermediates being disruptively banded with blace, yellow and white bands. These color are maintained throughout the pupal stage. When released on a branch tip larvae moved quickly towards the trunk and down to the ground. On the ground larvae moved slower but showed no initial interest in pupation sites. Larvae reached their eventual pupation sites in variable amounts of time, some within several hours after gut evacuation and others not until the next day. Pupae appeared to match their backgrounds; green pupae on the undersides of green leaves or amongst green foliage and brown pupae on the undersides of brown leaves. One brown pupa was formed on the inside of a rusted curtain rod. The characteristics of the pupation sites are shown in Figure 3.

A summary of these data, together with those of West and Hazel (1979) is presented in Table 1. This summary indicates that each species prefers to pupate within a given range of heights and on surfaces within a specific range of sizes or with specific characteristics. Photographs of the pupation sites are shown in Plates II-XI.

Discussion

The hypothesis of Sheppard (1958), that environmentally cued polymorphisms evolve in situations where individuals are distributed on a mosaic of distinct environments such that the distribution of parents is un-correlated with that of their offspring, is supported by the observations on *Papilio troilus* and *Eurytides marcellus*. However, like the data of West and Hazel (1979), my data are not consistent with an extension of Sheppard's hypothesis to include the habitat of the larval food plant as it affects the availability of similar pupation each generation. Both species feed on forest plants, yet like *Papilio polyxenes*, which feed in a successional habitat, *marcellus* and *troilus* choose pupation sites that are generally above the litter where both 'green' and 'brown' sites are available, and where selection should favor background matching.

The pupation sites of *Papilio cresphontes* which are sometimes above the leaf litter are an apparent exception to Sheppard's hypothesis. However, the concepts of monomorphism and dimorphism are not altogether appropriate

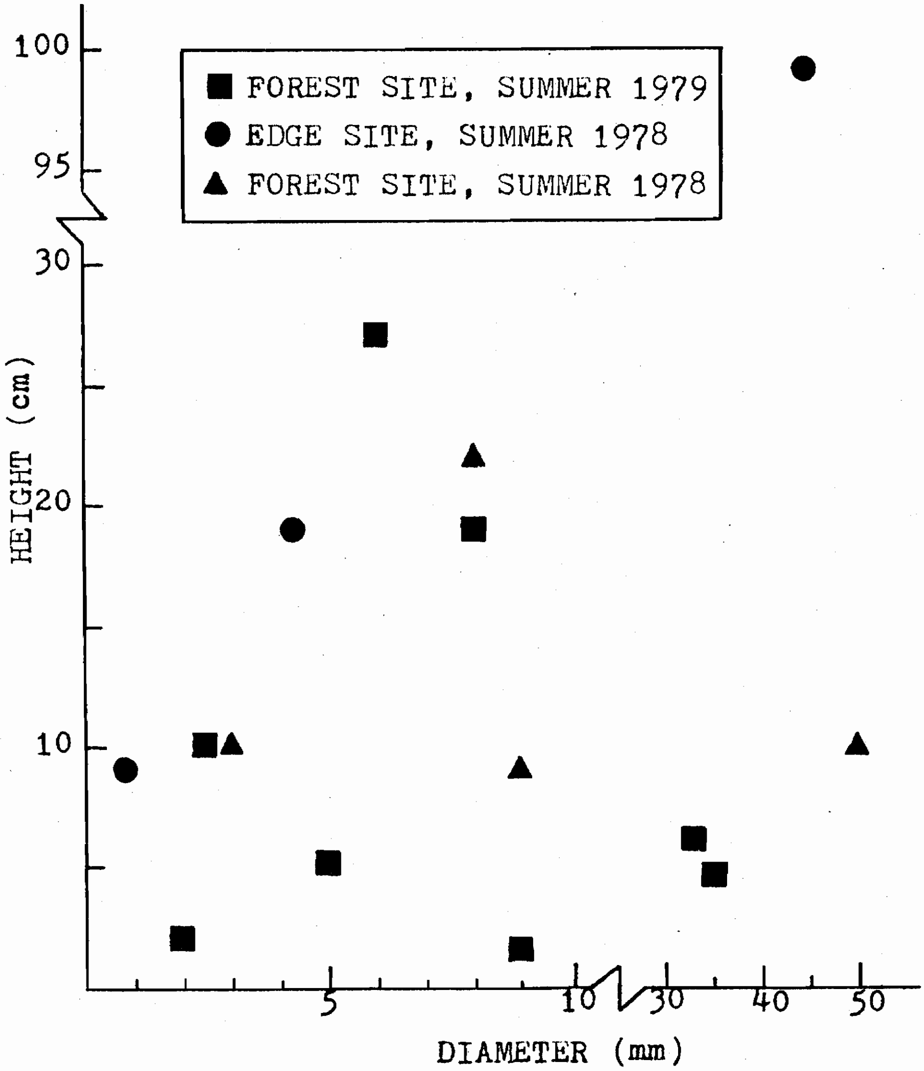

Figure 1. Distribution of natural pupation sites of *Papilio cresphontes* by height above ground and diameter of substrate. Note broken scale.

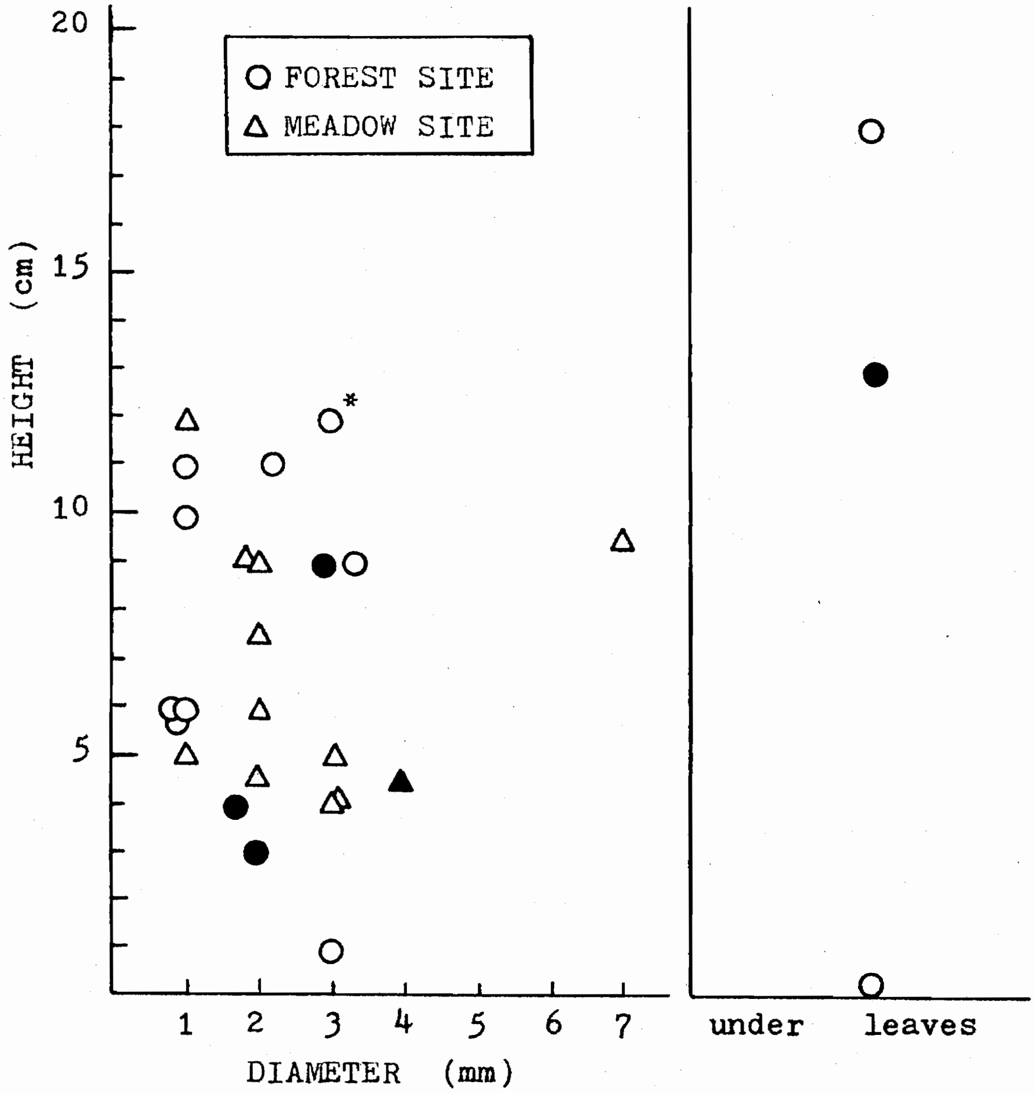

Figure 2. Distribution of natural pupation sites of _Papilio troilus_ by height above ground and diameter of substrate. Shaded symbols refer to brown pupae. *predicted before color could be determined.

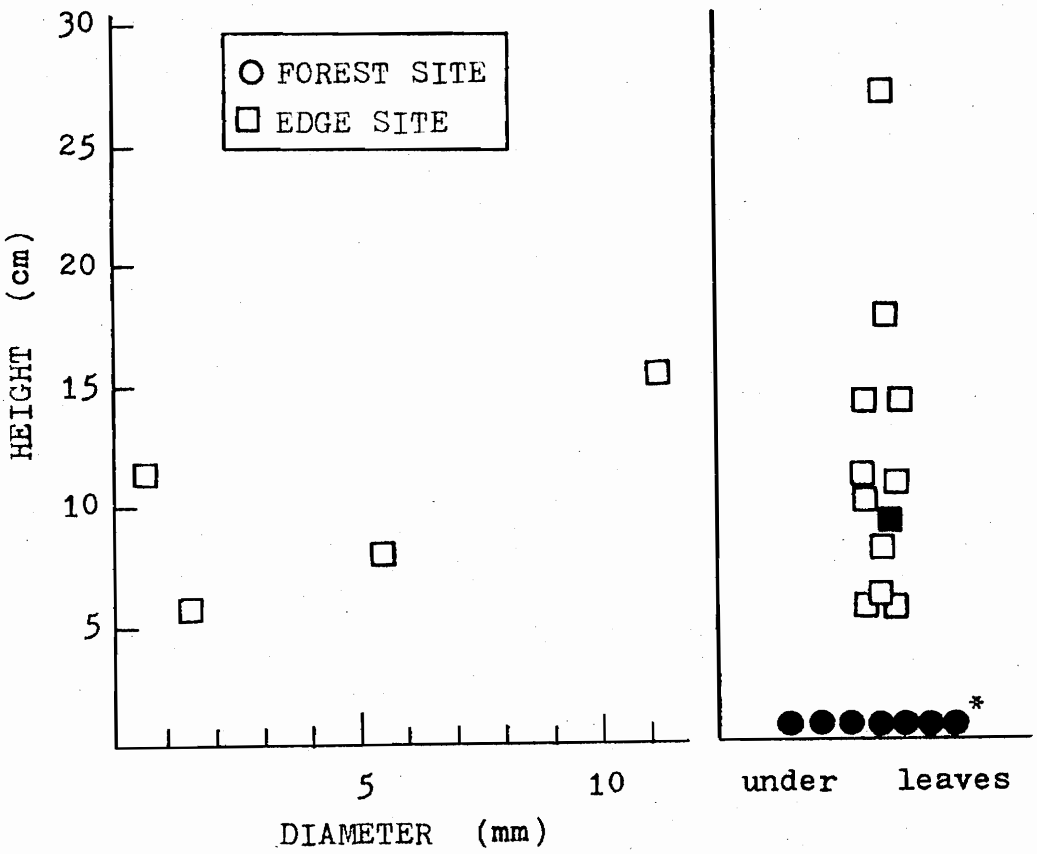

Figure 3. Distribution of natural pupation site of <u>Eurytides marcellus</u> by height above ground and diameter of substrate. Shaded symbols refer to brown pupae. *pupation was on inside of a curtain rod.

Table 1: Comparisons of the natural pupation sites of six species of swallowtail butterflies

	LARVAL HABITAT	PUPATION SITE
A. Monomorphic species		
P. cresphontes	forest forest-meadow ecotone	generally pupates above the leaf litter on broader sites than any other species, with the exception of *philenor*; pupae may be positioned vertically or horizontally.
P. glaucus	forest forest-meadow ecotone	pupates horizontal in the leaf litter, nearly always less than 5 cm above the ground.
B. Dimorphic species		
E. marcellus	forest forest-meadow ecotone	generally pupates above the leaf litter in a horizontal position on the undersides of leaves; green pupae on green leaves, brown pupae on brown leaves
B. philenor	forest	pupation occurs well above the leaf litter on the trunks and limbs of trees and on cliffs; pupation may be vertical or horizontal; green pupae are rare and are only formed on thin stems or petioles.
P. polyxenes	meadow	green pupae are formed on vertical weeds and grasses, brown pupae are formed on tree trunks and fence posts; pupation is generally vertical and above the litter.
P. troilus	forest forest-meadow ecotone	pupation is usually just above litter on slender stems, vines and petioles; pupae may be vertical or horizontal.

Plate II. Brown Eurytides marcellus pupa on vertical brown leaf.

Plate III. Green pupa of _Eurytides_ _marcellus_ on the underside of a green leaf.

Plate IV. Brown pupa of Papilio troilus on a small twig amongst brown leaves.

Plate V. Green pupa of *Papilio troilus* on green leaf petiole

Plate VI. Pupa of <u>Papilio</u> <u>cresphontes</u> on dead stick.

Plate VII. Brown _Papilio_ _polyxenes_ pupa on tree trunk.

Plate VIII. Green pupa of <u>Papilio polyxenes</u> on grass stem.

Plate IX. Brown pupa of <u>Battus</u> <u>philenor</u> on cliff.

Plate X. Green pupa of <u>Battus</u> <u>philenor</u> on poison ivy petiole.

Plate XI. Pupa of _Papilio glaucus_ on the underside of a dead stick.

descriptions for the pupae of *cresphontes*. These pupae are more accurately described as being bi-colored; both greenish-grey and brown. This sort of coloration is probably adaptive on the species' natural sites in the sense that the pupae are able to blend with almost any environment. In Endler's terms (1978), such pupae resemble a random sample of the background where these colors are randomly distributed. The preference of this species for broader pupation sites than the other species may account for the resemblence of its pupae to a dead, lichened stick.

Since neither these nor earlier data (West and Hazel 1979) on the natural pupation sites of swallowtails is supportive of an extension of Sheppard's (1958) hypothesis to include the habitats of the larvae as a factor influencing the type of pupation site that a species uses, it is necessary to inquire as to why in similar habitats different species choose different pupation sites. The most obvious example of such a situation is the contrasting pupation sites of *Battus philenor* and *Papilio glaucus*, both of which feed and pupate in forest habitats; *philenor* well above the ground on tree trunks or cliffs and *glaucus* in the leaf litter (West and Hazel 1979). Of the eastern North American swallowtails only *philenor* is believed to be distasteful to some types of predators, particularly birds (Brower 1958). Larvae of *philenor* also occur at densities in nature that are far greater than those of any other species. *Papilio* *glaucus*, on the other hand, is probably edible to all predators and occurs at much lower densities than does *philenor*. Differences in larval density and palatability as they affect the relative impact of predation in the two species may provide the necessary selection pressure to account for differences in site choice. An experimental test of this possiblility is now in progress and the preliminary results indicate that *glaucus* is highly susceptible to avian predation when on '*philenor*' sites and *philenor* highly susceptible to rodent predation when on '*glaucus*' sites.

Even considering these differences in the natural pupation sites of *glaucus* and *philenor* it is still difficult to account for the evolution of dimorphism in the latter species, as green pupae are rarely produced in nature. Even in the laboratory, *philenor* produces higher frequencies of brown pupae than any other species on the same substrates (Hazel and West 1979 and Chapter V). The greater tendency of *philenor* to produce brown pupae suggests that it is more 'sensitive' to the environmental cues affecting pupal color (see Hazel 1977 and Chapter VII). Greater 'sensitivity' in *philenor* would be expected if it has been selected for background matching in habitats where brown pupal color was adaptive on most pupation sites. According to the model for the genetic basis of pupal color (Hazel 1977), selection for the elimination of genetic variation in 'sensitivity' would be inefficient even though relatively few pupation sites where green is the more adaptive pupal color are utilized. It is also possible that apostatic selection favoring greens because of their relative rarity has resulted in the retention of the dimorphism (Clarke and Sheppard 1972, Clark 1962, Allen 1976). The high dinsity of *philenor* in nature would provide the precondition for apostatic selection to occur. Similiarly, because so few individuals choose pupation sites where green is the more adaptive pupal color, the choice of such a site may be itsèlf favored by apostatic selection.

Aside from these difficulties in the swallowtails, Sheppard's hypothesis for the evolution of pupal color dimorphism gains additional support in the members of the satyrine genus *Heteronympha*, the Australian browns (Common and Waterhouse 1972). Three of the seven species comprising the genus are dimorphic and pupate above the ground suspended by the cremaster. Three other species produce monomorphic brown pupae and pupate loose in the leaf litter. A seventh species produces monomorphic green pupae and pupates suspended by the cremaster above ground in swampy habitats. It is clear that different species of swallowtails and Australian browns show different pupation site preferences that are related to the presence of monomorphic or dimorphic pupae. Even within some dimorphic swallowtails species-specific preferences for different pupation sites have evolved, such as a preference for pupation on leaves as opposed to stems, but the selection pressures responsible for these differences are unknown.

An alternative explanation of these data is that the presence of the dimorphism in some species and its absence in others is the result of historical accidents. Unfortunately the six species represent three genera of swallowtails and two groups within one genus, and very little is known about pupal color in their relatives. Nevertheless, there is evidence of both monomorphism and dimorphism among the relatives of Battus philenor and Papilio glaucus. In the philenor group, B. polydamus (L.) is dimorphic (Moss 1919), while B. polystictus (Butler) may be monomorphic green (Burmeister 1879). In the belus group, B. belus (Cramer) is dimorphic (Moss 1919) while B. lycidas (Cramer) and B. crassus are evidently monomorphic green (Moss 1919), though B. crassus may have a non-green form as well (Burmeister 1879). Although Papilio glaucus is monomorphic brown, its relative P. eurymedon apparently can produce green pupae as well (Brower, personal communication). Even though these facts say nothing about the evolution of the dimorphism in the species investigated in this dissertation, they do suggest an evolutionary plasticity in the dimorphism.

The available data suggest that the primary determinant of whether a population is monomorphic or dimorphic for pupal color is the degree of variability in the pupation sites that it uses and the lack of a parent-offspring correlation in this variation. In addition, since Clarke and Sheppard (1972) have shown that the choice of a pupation site has a genetic basis, the way in which natural selection affects the choice of a pupation site will be related to the evolution of an environmentally-cued dimorphism in pupal color.

CHAPTER V

The Environmental Control of Pupal Color in Swallowtail Butterflies

Introduction

The two most important characteristics of the pupation site controlling pupal color in swallowtails appear to be the texture and color of the site and its surroundings, but there is variation between species in the relative importance of these cues (Smith 1978, Hazel and West 1979). Smith (1978) has proposed that this variation is related to the time of day that prepupal larvae are sensitive to the environmental cues. Accordingly, species which have a high probability of passing through the sensitive period at night should rely on texture as the major environmental cue, while those which have a high probability of passing through this period during daylight should rely on color as the major environmental cue. Alternatively, Hazel and West (1979) have suggested that differences between species are related to differences in the ecology of their pupation sites and the effect that this has on the crypsis of their pupae.

In this chapter the relative effects of color and texture on pupal color are investigated in two species dimorphic for pupal color. These data will be discussed as they apply to the hypotheses of Smith (1978) and Hazel and West (1979).

Materials and Methods

These experiments were carried out during the spring and summer of 1979 and the rearing conditions were as described in Chapter III. Eggs of Eurytides marcellus came from 11 females; between 17 and 63 mature larvae were reared from each female. A single female of Papilio troilus produced 37 mature larvae. These larvae pupated in the conditions described below, and when adults eclosed nine pairings were made between the siblings. This resulted in between 5 and 49 mature larvae per pairing. Both species were maintained as described in Chapter III. Larvae were transfered to the appropriate conditions for pupation within 30 minutes after gut evacuation.

The experiments were designed to examine the affects of texture and color, and their interaction on pupal color. Larvae were therefore forced to pupate on one of four colors within two textures. The rougher substrates were cylinders of red, green, blue or yellow construction paper (approximately 7 cm in diameter by 7.5 cm high), placed in the bottom of a clear plastic rearing dish and topped by a square piece of the same color of paper held in place by the lid of the plastic dish. This allowed light to enter around the edges of the square piece of paper while at the same time preventing larvae from escaping. The whole chamber was placed on top of another rearing dish above a white surface, so that light could also enter from below. The smooth substrates were clear round plastic rearing dishes (15 cm in diameter by 4 cm high) covered outside on top and sides with one of the four colors of paper and raised above a surface of the same color to allow light to enter from below. Pupation occurred under constant fluorescent illumination supplemented during the day (14-15 h) by natural light from an adjacent window. The temperature ranged from 22° to 26° C. The offspring of the *marcellus* females were tested on all eight surfaces. Those of the single *troilus* female were tested on rough yellow and rough green surfaces, while these of the sib pairings were tested on the remaining six surfaces. Larvae were assigned randomly to all test surfaces. Intermediate colored pupae were rare (two in *marcellus* and none in *troilus*), and these were omitted from the analyses.

The pupation conditions used here were generally comparable to those used for *Papilio polyxenes* and *Battus philenor* (Hazel and West 1979), except that the chambers used for *marcellus* and *troilus* were somewhat smaller and the rough surfaces had construction paper tops.

Results

The results for *E. marcellus* are shown in Table 2 and *P. troilus* in Table 3, together with tests of independence in 2 x 2 tables by chi-square or Fisher's exact test. In *marcellus* pupal color is little affected by texture. Comparisons of rough and smooth textures within colors give only one significant result (between rough and smooth green). However, comparisons of colors within textures indicate a large effect of substrate color. Within either texture, red, blue, and green produce significantly more brown pupae than does yellow.

The behavior of *troilus* is very similar to that of *marcellus*. Comparisons of rough and smooth textures within colors again give one significant difference (between rough and smooth green). Comparisons of colors within textures indicate that pupal color in *troilus* is also affected by color: red, blue, and green produce significantly more brown pupae than does yellow. However, on smooth surfaces green elicits fewer brown pupae than do red or blue, while producing more browns than does yellow.

Figure 4 and Table 4 show graphic and statistical comparisons of the four dimorphic species which have been studied in this manner The results for *Battus philenor* and *Papilio polyxenes* are those of Hazel and West (1979). These comparisons indicate that the chief differences between these species is in the relative effect that texture has on pupal color. Although the results presented in Table 4 show more significant differences between *polyxenes* and *philenor* than between any other pair of species, Figure 4 indicates that their patterns of response to the eight environments is quite similar. Both species are affected by texture (although texture has more of an effect in *philenor*). When pupation occurred on a smooth surface the color affected both species in a similar manner; *philenor* simply produced more brown regardless of the environment.

On the other hand, comparisons of marcellus and troilus show only one significant difference (Table 4), and their patterns of response on the eight environments are very similar (Figure 4). *Papilio troilus* is more affected by texture than marcellus but less so than *polyxenes* or *philenor*.

Discussion

The results presented here together with those of Hazel and West (1979) show species-specific differences in the environmental control of pupal color most of which are due to differences in the relative importance of texture as an environmental cue, or as in *philenor*, to a tendency to produce relatively high frequencies of brown pupae regardless of the pupation environment. The response of all four species to color is generally consistent, with yellow light enhancing the production of green pupae (in *polyxenes* green also favors the production of the green morph). This is not unreasonable since it has been shown that the light transmitted through green leaves contains a higher proportion yellow and green wavelengths than does direct sunlight scattered by the atmosohere (Moss and Loomis 1952, Gates 1962, 1965, Rackham 1965). Thus, when pupation occurs amongst green foliage the incident light probably contains a large component of yellow light. The response of these species to texture is not so consistent, however, *P. polyxenes* and *B. philenor* are greatly affected by the

Table 2: Per cent brown, sample sizes, and 2 x 2 comparisons of the numbers of green and brown pupae of *E. marcellus* pupating on surfaces of different texture and colour.
+, $p<0.05$; ++, $p<0.01$; -, not significant

Substrate	Percent Brown	Sample Size	2 x 2 comparisons						
			2	3	4	5	6	7	8
1. Rough red	100	44	-	-	++	-	+	++	++
2. Rough blue	89	53		-	++	-	-	-	++
3. Rough green	98	56			++	-	+	++	++
4. Rough yellow	0	44				++	++	++	-
5. Smooth red	95	37					-	-	++
6. Smooth blue	84	44						-	++
7. Smooth green	78	46							++
8. Smooth yellow	0	50							

Table 3: Per cent brown, sample sizes, and 2 x 2 comparisons of the numbers of green and brown pupae of *P. troilus* pupating on surfaces of different texture and colour.
+, $<p$ 0.05; ++, $p<0.01$; -, not significant

Substrate	Percent Brown	Sample Size	2 x 2 comparisons						
			2	3	4	5	6	7	8
1. Rough red	91	33	-	-	++	-	-	++	++
2. Rough blue	97	33		-	++	-	-	++	++
3. Rough green	81	16			++	-	-	++	++
4. Rough yellow	0	17				++	++	++	-
5. Smooth red	86	37					-	++	++
6. Smooth blue	83	41						++	++
7. Smooth green	13	46							++
8. Smooth yellow	0	38							

Table 4: The results of 2 x 2 comparisons of numbers of green and brown pupae produced by four different species of swallowtails pupating in similar environments (* = $p<0.05$; -- = not significant).

	SPECIES COMPARED					
PUPATION SUBSTRATE	philenor & polyxenes	philenor & troilus	philenor & marcellus	polyxenes & marcellus	polyxenes & troilus	troilus & marcellus
Rough red	--	--	--	--	--	--
Rough blue	--	--	--	--	--	--
Rough green	*	--	--	*	*	--
Rough yellow	*	*	*	--	--	--
Smooth red	*	*	*	--	--	--
Smooth blue	*	--	--	*	*	--
Smooth green	*	*	--	*	--	*
Smooth yellow	*	*	*	--	--	--

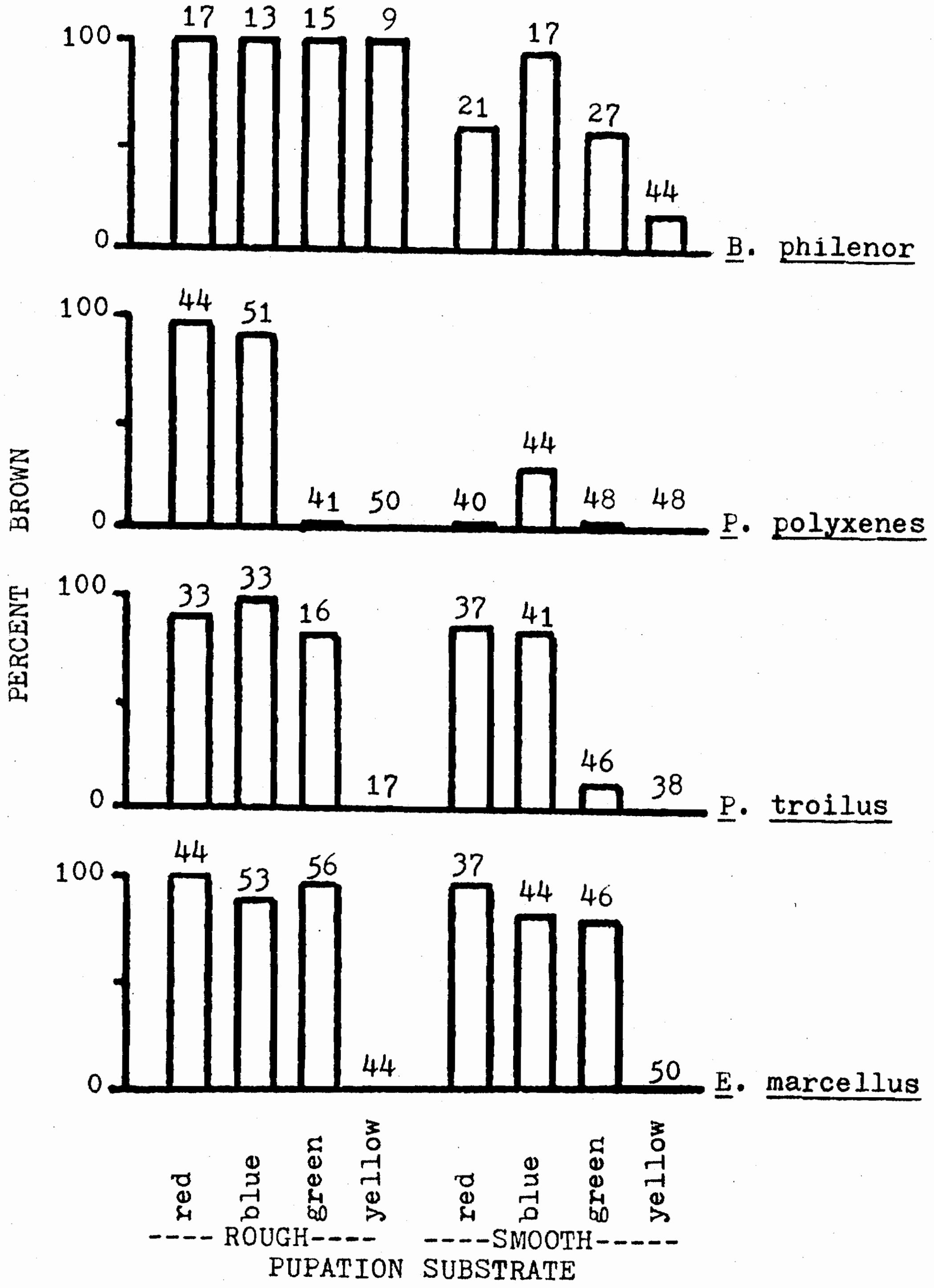

Figure 4. Comparisons of pupal color responses of Papilio polyxenes, P. troilus, Battus philenor and Eurytides marcellus on similar substrates. Sample sizes are shown for each substrate.

texture as an environmental cue is also not unreasonable, since in nature the colors and textures of some surfaces are related, with rough surfaces being brown and smooth surfaces being green (Smith 1978). So both types of cues provide information bearing on the appropriateness of being a green or a brown pupa.

Hazel and West (1979) have suggested that differences between species in the relative effects of color and texture in determining pupal color are related to differences in the information that the two cues provide and relative appropriateness of this information in determining the more adaptive pupal color on their natural pupation sites. Since texture and color provide different sorts of information--texture providing information that at best concerns the color of the pupation site alone, and color providing information about the color of the surroundings as well--their significance in determining the appropriate pupal color is likely to be related to the pupation site and how that affects a potential predator's view of the pupa. Thus, texture should be an appropriate cue only in species which pupate on sites where the color and texture are related and where crypsis often involves blending with the pupation site as opposed to the general surroundings. We have argued that this is the case in _polyxenes_ and _philenor_ since the latter species often pupates on broad tree trunks where to be cryptic the pupa must blend with its pupation site, while polyxenes often pupates on thin weed stalks where crypsis involves blending with the surroundings.

The increased use of color as an environmental cue determining pupal color in E. marcellus and P. troilus coupled with their preference for slender pupation sites or the undersides of leaves is consistent with the hypothesis of Hazel and West (1979). Since the textures of thin stems and leaves are not likely to vary in relation to their color, texture is not a relevant cue in determining pupal color. The overwhelming use of color as a cue in these species is in marked contrast to other swallowtails in which texture is of greater importance (Smith 1978, Hazel and West 1979).

As was mentioned previously, Smith (1978) found that pupal color in P. demoleus and P. polytes was influenced by texture and color, but in polytes texture had a greater effect. He proposed that this was an adaptation to the high probability that polytes passes through its sensitive period during darkness. Unfortunately, the precise timing of the sensitive period is not known for any swallowtail, but in P. polyxenes there is some evidence that it occurs very shortly before a larva starts spinning on the pupation site. That is, just around the time of commitment to that particular spot as a pupation site. This is, of course not unreasonable in an insect that wanders over a great variety of substrates before settling on a pupation site. However, observations of wandering larvae in nature suggest that some larvae which have evacuated the gut late in the day do not commit themselves to a pupation site until the next morning, although the elapsed time between gut evacuation and committment is in these cases longer than usual. Thus, there may be some flexibility in the timing of the sensitive period. Individuals completing gut evacuation late in the day may not in fact be going through the sensitive period at night. This possibility is supported by evidence that _polyxenes_ larvae kept on a 15 hr photophase and which pupate on yellow paper almost invariably produce green pupae, even though the time of gut evacuation may vary from morning to late afternoon. If kept in darkness throughout the prepupal period these insects almost invariably produce brown pupae (West _et_ _al_. in preparation).

In addition, it is difficult to see how the use of two sorts of cues for pupal color determination would evolve if some individuals could only use one cue because they pass through the sensitive period in the dark. For these reasons it is unlikely that differences in the use of color and texture as environmental cues by different species have evolved as a result of differences in the timing of the sensitive period. It is more likely that species-specific differences in the environmental cues affecting pupal color have evolved as a result differences in the pupation sites which these species utilize.

CHAPTER VI

Photoperiodic Control of Pupal Color in Swallowtail Butterflies

Introduction

Shapiro (1976) has defined seasonal polyphenism as "as annually repeating pattern of changing phenotypic ratios in successive generations, under some kind of environmental control". In some species of swallowtails both pupal color and diapause fit this definition and in most temperate zone species the environmental control is photoperiodic. This chapter investigates the photoperiodic control of pupal color and diapause in four species.

Materials and Methods

Papilio polyxenes

Larvae were reared from eggs in four photophases (14 h, 13.5 h, 13 h and 12.5 h) at a constant temperature of 26° C. Pupation was on surfaces that elicit almost exclusive production of green pupae when rearing takes place in a 15 h photophase. Larvae pupated on the same photophases as those in which they were reared. The determination of whether the pupae of a brood had entered diapause was made at least two weeks after the last eclosion in that brood.

Papilio troilus

Larvae were reared on two photophases (11 and 15 h) and pupated on yellow construction paper in constant light, as described in Chapter V.

Battus philenor

Larvae were reared from eggs on three photophases (11, 13.5 and 15 h). Data for the 11 and 15 h photophases are those of Hazel and West (1979) for pupation on yellow plastic. Larvae reared on the 13.5 h photophase pupated on the leaves of the larval food plant or on the tops and sides of plastic rearing dishes. Diapause data were recorded only for those pupae reared on the 13.5 h photophase.

Eurytides marcellus

Larvae were reared from eggs on either a 15 or 11 h photophase at a constant temperature of 26° C. Those reared on the 15 h photophase pupated on the surfaces described in Chapter V, while those reared on 11 h photophase pupated on yellow construction paper as described in Chapter V. Diapause data was recorded for both photophases as described for polyxenes.

Results

Papilio polyxenes

The results for each brood at each photophase are given in Table 5. The pooled results for each photophase for percent green and percent development are shown in Figure 5. On photophases of 14 and 13.5 h there was significant heterogeneity among broods for both pupal color and diapause (on 14 h: $\chi^2_{(5)}=32.1$, $p<0.005$ for pupal color, and $\chi^2_{(5)}=28.9$, $p<0.005$ for diapause; on 13.5h: $\chi^2_{(5)}=26.0$, $p<0.005$ for pupal color, and $\chi^2_{(5)}=30.8$, $p<0.005$ for diapause). On photophases of 13 and 12.5 there was no heterogeneity among broods. Heterogeneity among broods for development within brown pupae was significant at both 14 and 13.5 h photophases ($\chi^2_{(5)} = 17.3$, $p < 0.005$ at 14 h and $\chi^2_{(5)} = 20.5$, $p < 0.005$ at 13.5 h). There was no heterogeneity among broods for development in green pupae at any photophase. Spearman's rank correlation of percent green pupae per brood on 14 and 13.5 hour photophases was significant ($R_s = 0.88$, $p < 0.05$, $N = 6$). Rank correlations for overall percent diapause and percent diapause within pupal color morphs on 14 and 13.5 h photophases were not significant. Although a rank correlation between diapause and percent brown per brood at 14 and 13.5 h photophases was not significant, a Chi-square test shows a significant association between the two ($\chi^2_{(2)} = 80.9$, $p < 0.005$ on 14 h; $\chi^2_{(2)} = 39.0$, $p < 0.005$ on 13.5 h).

<u>Eurytides</u> <u>marcellus</u>

The pupal colors of <u>marcellus</u> reared on 11 and 15 h photophases are shown in Table 6, and the ratios are identical. All pupae reared on the 11 h photophase entered diapause. Those reared on the 15 h photophase showed variation among broods in the frequencies of pupae entering diapause, and these data are shown in Table 7. Spearman's Rank Correlation of percent diapause in green pupae with percent diapause in brown pupae in each brood was significant ($R_s = 0.71$, $p < 0.05$, $N = 11$). The same correlation of percent brown with percent diapause in each brood was not significant. However, a chi-square test on the pooled results of all eleven broods shows a significant positive association between the two phenotypes ($x^2_{(2)}=31.82$, $p<0.005$). In addition there is variation between broods in the strength of this association (contrast broods 6-79 and 10-79).

Papilio troilus

The effects of photoperiod on pupal color and diapause in troilus are shown in Table 8. Although the sample size on the 11 h photophase is small the results are still significantly different from thsoe of the 15 h photophase ($p<0.05$). In addition, all pupae that had been reared on a 15 h photophase developed, regardless of color, while the two brown pupae produced on the 11 h photophase entered diapause; and this difference is also significant ($p<0.05$).

Battus philenor

The results for philenor reared on the 11 and 15 h photophases are shown in Table 9, and the ratios of green and brown pupae on the two photophases are not significantly different ($p>0.5$). Unfortunately diapause data could not be gathered for these photophases, but observations in the lab and field indicate that nearly all pupae reared on midsummer photophases eclose, while those reared on short day conditions enter diapause. Data from the pupae reared on the 13.5 photophase (Table 10) show no association of pupal color and diapause ($p>0.5$).

Table 5: Effect of photophase on pupal color & diapause in _P. polyxenes_ with pupation on surfaces which elicit 100% green pupae & development on a 15 h photophase.

Photophase	Brood	Sample Size	Percent Green	Percent Green Develop	Percent Brown Develop	Overall Percent Develop
14 h	1-78	20	80	100	0	80
	3-78	39	90	100	100	100
	1-79	31	42	100	33	61
	3-79	31	48	100	13	57
	4-79	14	93	100	0	93
	6-79	21	67	100	86	95
	Total	156	70	100	34	79
13.5 h	1-78	17	59	80	0	53
	3-78	41	44	94	55	72
	1-79	50	12	83	16	24
	3-79	9	0	0	11	11
	4-79	4	75	100	0	75
	6-79	12	25	100	67	75
	Total	133	30	90	29	48
13 h	1-78	3	0	0	0	0
	3-78	42	12	100	32	38
	1-79	5	0	0	0	0
	3-79	19	0	0	5	5
	4-79	10	0	0	0	0
	Total	79	6	100	17	21
12.5 h	1-78	4	0	0	0	0
	3-78	24	11	33	22	24
	1-79	20	0	0	0	0
	3-79	43	0	0	0	0
	4-79	14	0	0	0	0
	6-79	11	0	0	9	9
	Total	116	2	33	5	6

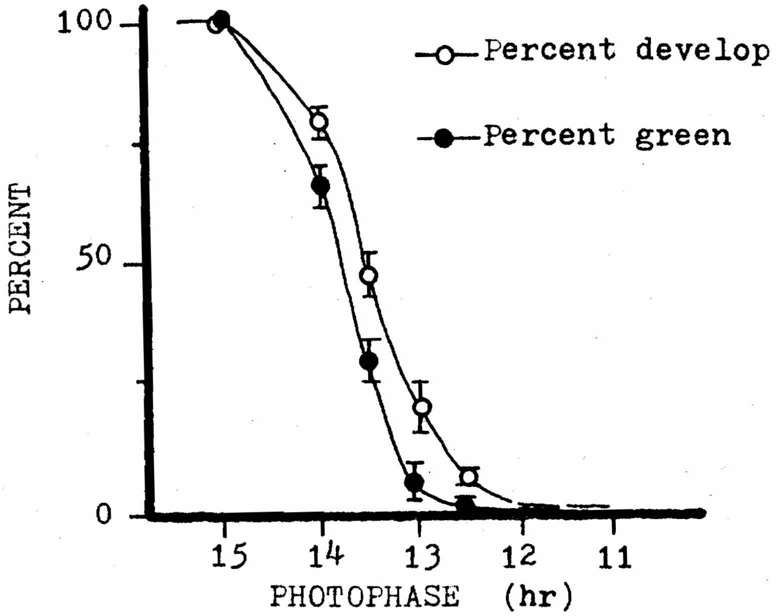

Figure 5. The effect of photophase on pupal color and diapause in Papilio polyxenes. (Brackets indicate standard errors.)

Table 6: Effect of photophase on pupal color in *Eurytides marcellus* for pupation on yellow construction paper.

	Photophase	
	11 h	15 h
Number green pupae	25	50
Number brown pupae	1	0
Total	26*	50

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*all of these pupae entered diapause; see Table 7 for diapause data for the 15 h photophase.

Table 7: Variation in diapause and diapause within broods and pupal color morphs for Eurytides marcellus reared on a 15 h photophase with pupation in conditions described in Chapter V.

Brood	Number Green	Number Brown	Percent Green Diapause	Percent Brown Diapause	Overall Percent Diapause
1-79	11	5	18	80	37
2-79	15	25	87	96	92
3-79	18	33	72	97	88
4-79	10	9	50	67	58
5-79	22	46	50	91	78
6-79	26	58	54	62	60
7-79	13	35	46	83	73
8-79	7	15	71	100	91
9-79	5	10	40	90	73
10-79	13	22	100	100	100
11-79	9	37	56	81	76
TOTAL	149	295	60	84	76

Table 8: Effect of photophase on pupal color and diapause in *Papilio troilus* with pupation on yellow construction paper in constant light.

	Photophase	
	11 h	15 h
Number green pupae	0	17
Number brown pupae	2	0
Number diapausing	2	0
Total	2	17

Table 9: Effect of photophase on pupal color in _Battus philenor_ with pupation on smooth yellow plastic in constant light.*

	Photophase	
	12 h	15 h
Number green	23	13
Number brown	2	6
Total	25	18

*data copied from Hazel and West (1979) page 379, Table 4.

Table 10: the relationship between pupal color and diapause for _Battus philenor_ reared on a 13.5 h photophase.

	Number Diapause	Number Develop
Green pupae	16	1
Brown pupae	13	3

Discussion

If it is adaptive for diapausing pupae to be brown in a species which is multivoltine and which produces both green and brown pupae in the summer, then such a situation could evolve in any, or a combination of three ways. First, selection could favor an alteration of pupation site choice in the autumn so that only brown pupation sites were chosen. A second way would be for selection to favor the evolution of a photoperiodic control of pupal color so that only brown pupae are produced in the autumn, regardless of the pupation site. A final way would be for a phenotypic correlation to evolve between pupal color and diapause so that brown pupae would diapause regardless of the time of year. However, for this to evolve in a multivoltine species, delayed reproduction must also be adaptive since brown diapausing pupae formed in late spring and early summer would miss several opportunities for reproduction before autumn.

Battus _philenor_ rarely produces green pupae in nature, even in the midsummer generation, so it is not entirely surprising that it has not evolved a relationship between pupal color and diapause. It may be that there has been insufficient selection pressure for the evolution of such a relationship. In addition, if apostatic selection operates on pupal color in _philenor_, it would oppose the evolution of a relationship between the two traits.

The results for *E. marcellus* are at first puzzling. Although it potentially has the shortest generation time of any of the swallowtails in this study, it also is the only species which does not reproduce as often as is possible during the summer: brown pupae, and some green pupae, enter diapause regardless of the time of year. In the population along the North Fork Roanoke River from which these *marcellus* were obtained, adults were most common in the field in early May and increasingly rare throughout the summer. From an r- and K-selectionist point of view this is a paradox: short generation time is a characteristic of a r-selected species and delayed maturity (early onset of diapause) a characteristic of K-selected species (for review see Stearns 1976, 1977).

It may be that marcellus larvae suffer heavily from density dependent predation and parasitism. Observations in the field suggest that *marcellus* larvae occur at higher densities in the spring than those of any of the other dimorphic species in the area except *philenor*. However, philenor larvae are gregarious and aposamatic. Such high densities might be expected to facilitate development of searching images in potential predators, expecially if the prey were palatable. Such a situation would favor variation in larval coloration (*marcellus* larvae vary in color from green, through green with black bands, to black), a short larval stage and midsummer diapause in pupae because of the advantages of delayed reproduction in the face of high larval mortality.

Although brown pupal color and diapause are associated in marcellus the association is not complete. Some brown pupae develop and some greens enter diapause. Variation between broods in the degree of association between the two traits might be taken as evidence for a genetic basis for the association. During the summer, selection should be expected to favor some digree of diapause (delayed reproduction) due to the probability of high larval mortality. But during these generations selection for the correlation between brown pupal color and diapause is relaxed, since within the diapausing and non-diapausing classes cryptic green and brown pupae should be equally viable. Also, by autumn, when selection would be expected to favor an association between the two traits, the leaves on which marcellus often pupates fall to the ground, and because of the sudden build-up of leaf litter at this time the pupae would soon become invisible to predators above the litter. Selection would favor the association of brown pupal color and diapause only on pupation sites that remain visible throughout the winter.

The seasonal control of pupal color and diapause is perhaps most highly evolved in P. polyxenes. The photoperiod on which the larvae are reared strongly affects both traits. Fifteen hour photophase allows a flexibility of pupal colors depending on the characteristics of the pupation sites and nearly all pupae develop. On short day photophases (13 h or less) predominantly brown diapausing pupae are produced. On intermediate photophases (13.5 and 14 h) there is an association between brown pupal color and diapause. The association is adaptive because in years when the summers are shorter than usual some individuals will be destined to diapause and these will be brown. Since *polyxenes* pupates on vertical surfaces which probably remain above the litter throughout the winter, selection favors the association on intermediate and short photophases. On long photophases *polyxenes* behaves as a r-selected species, but on intermediate photophases it employs the same sort of 'bet-hedging' strategy that *marcellus* employs on long photophases. For *polyxenes* this is an adaptation to environmental uncertainty with respect to the length of the summer while for *marcellus* it is an adaptation to environmental uncertainty in the intensity of predation on larvae during the summer.

The significant correlation between percent brown pupae per brood on a 14 h photophase and percent brown per brood on a 13.5 h photophase in *polyxenes* suggests that the heterogeneity among broods on these daylengths is the result of genetic differences between broods in their average tendencies to produce brown pupae ("sensitivity", Hazel 1977). It might be expected that diapause would behave in the same way, although the correlation for diapause was not significant ($R_s = 0.81$, $p<0.1$), probably because the sample size was to small. Generally, broods producing a high frequency of diapausing pupae on a 14 h photophase also produced high frequencies on a 13.5 h photophase.

The sample size for _P._ _troilus_ was small but the results suggest that it behaves like _polyxenes_.

These experiments reveal a large amount of flexibility in the seasonal control of pupal color and diapause in the species examined. Variation in the degree of association of brown pupal color and diapause and its photoperiodic control are believed to have a genetic basis, and species specific differences are thought to result from differences in the relative palatabilities, pupation sites, intensities of larval predation, and possibly the strength of apostatic selection in maintaining genetic variation.

CHAPTER VII

The Genetic Basis of Pupal Color in Swallowtail Butterflies

Introduction

Three kinds of evidence, physiological (Hidaka 1961a, b, Smith 1978), ecological (West and Hazel 1979, Hazel and West 1979) and genetic (Hazel 1977) are consistent with the hypothesis that pupal color in swallowtails is the all-or-none expression of a genetic tendency to produce a brown pupa, and that genetic variation in this tendency in a population is maintained by weak stabilizing selection. This chapter examines this evidence and provides a further test of this hypothesis by selecting for pupal color in _Eurytides marcellus_.

Materials and Methods

Pairings were made to form a base population, without regard to the color of the pupae, from individuals eclosing from pupae in the broods shown in Table 7. The rearing conditions were the same as those described in Chapter V and the selected generation was reared on these conditions as well.

Within 30 minutes of gut evacuation larvae were moved to clear plastic rearing dishes (2.5 cm high and 10 cm diam.) and placed on a white surface which was illuminated as described in Chapter V.

Pupal color in _E. marcellus_ is distinctly dimorphic in nature and also in the laboratory on most artificial surfaces. However, when pupation occurs on clear plastic, pupae vary continuously in color from green to brown. Thus, in order to conduct a selection experiment a system had to be developed to classify pupae. The categories used are described in Table 11 and pictured in Plate XII.

Positive assortative mating was practiced and inbreeding was avoided in the formation of three selected lines: green 1, green intermediate and brown. A total of 10 green 1 pairings, three green intermediate and five brown pairings were made. Selection was for one generation.

Results

Phenotypic frequencies of the parental and selected generation are given and compared in Table 12. In both the green 1 and brown lines selection of these phenotypes resulted in a significant increase in their frequencies in the next generation as measured by chi-square tests. The green intermediate line showed no response to selection.

It is also possible to make brood-by-brood comparisons using chi square tests. Of the 10 broods which made up the green 1 line, six showed a significant ($p<0.05$) increase, one showed a significant decrease and three showed no significant change in the frequency of the green 1 phenotype in the selected generation. Of the five broods making up the brown line three showed a significant increase and two showed no significant changes in the frequency of the brown phenotype in the selected generation. None of three

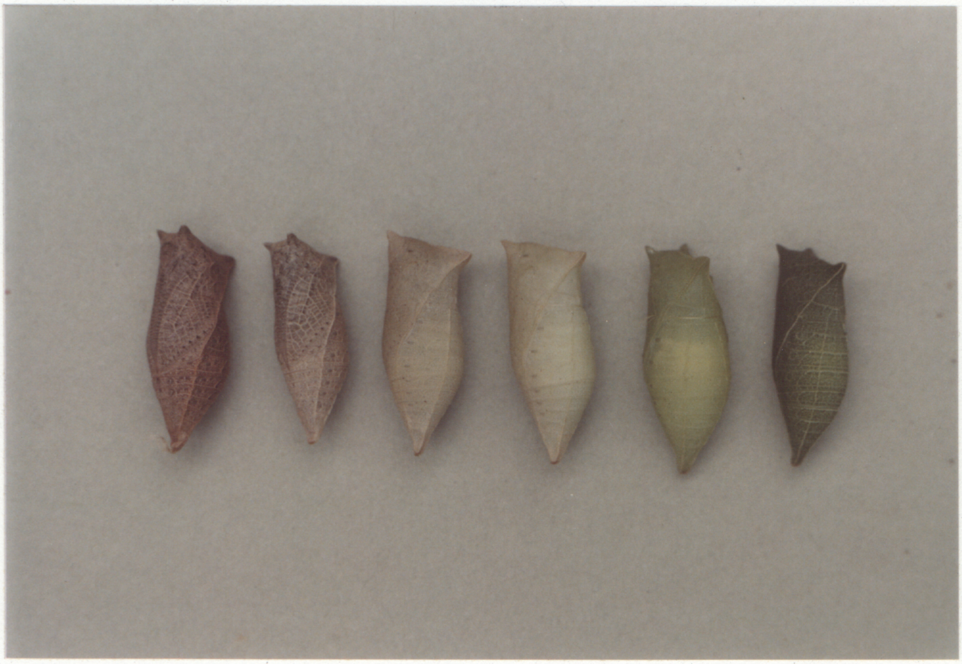

Plate XII. Variation in Eurytides marcellus pupal colors for pupation on clear plastic. Pupal color categories from left to right are brown, brown (lighter shade), brown intermediate, green intermediate, green-2 and green-1.

broods comprising the green intermediate line showed a significant change in the frequency of that phenotype.

Discussion

The selection experiments described in this chapter were designed to test the hypothesis that pupal color is a threshold trait. That is, the discontinuous expression of a quantitative trait. However, for Eurytides marcellus pupating on clear plastic in constant light the pupal color phenotypes are not discontinuous but vary from green to brown. The reason for the continuous expression of the pupal color phenotypes in some situations and their discontinuous expression in other situations may be the result of the physiological mechanism responsible for the production of brown pupae. It is clear that the production of a brown pupa results from the release of a substance from the central nervous system of the insect (Hidaka 1961 a, b, Smith 1978). It is possible that the amount of this substance which is released is related to the degree of brown pigmentation in the cuticle. If this were the case, most pupation environments would cause the release of either a large amount of the browning substance, in which case the pupa would be brown; or a small amount of the substance, in which case the pupa would be green. As a result, intermediately colored pupae would be rare and the trait would appear to be discontinuous. However, when pupation occurs on clear plastic the environ-

Table 11: Classification of pupal color phenotyped of *Eurytides marcellus* pupating on clear plastic in constant light.

Phenotype	Description
Green 1	green; cuticle opaque
Green 2	green; cuticle translucent
Green intermediate	pale green; brown in thicker areas of cuticle; cuticle opaque or translucent
Brown intermediate	tan; lacking brown spots on dorsal abdominal segments; cuticle opaque or translucent
Brown	tan to brown, with brown spots on dorsal abdominal segments; cuticle opaque

Table 12: The effect of selection on the pupal color phenotypes of Eurytides marcellus (int.= intermediate).

Selection Criteria	Pupal Color Phenotypes	Parental Generation Number	Parental Generation Percent	Selected Generation Number	Selected Generation Percent
Green 1	Green 1	322	33	236	44 **
	Green 2	47	5	21	4
	Green int.	146	16	76	14
	Brown int.	124	13	41	8
	Brown	325	34	159	30
	Total	964		533	
Green int.	Green 1	90	34	52	40
	Green 2	0	0	6	5
	Green int.	51	20	26	20
	Brown int.	48	18	16	12
	Brown	72	28	29	22
	Total	261		129	
Brown	Green 1	262	32	52	25
	Green 2	33	4	6	3
	Green int.	67	8	21	10
	Brown int.	87	11	13	6
	Brown	366	45	113	55 **
	Total	815		205	

** significant response ($p < 0.025$)

ment probably has little effect on pupal color so that genetic differences are no longer masked and pupal color is expressed as a continuously varying trait.

This hypothesis is supported by two sorts of evidence. First, since marcellus alone among the swallowtails studied is unaffected by texture it should be the only species capable of exhibiting continuous variation in pupal color in the absence of color cues. In the other swallowtails textural cues chould be sufficient to cause the continuous genotypic differences to be expressed discontinuously at the phenotypic level. Second, the results of selection indicate that the differences in pupal color are genetic. Both brown and green-1 lines gave significant responses to selection. Selection of the green intermediate phenotype produced no change in its frequency. This would be expected if the green-1 and brown phenotypes represented the more extreme genotypes and the green intermediate phenotype represented more intermediate genotypes. The results of selection on the discontinuous phenotypes in Papilio polyxenes (Hazel 1977) is also consistent with the results of selection on the continuous phenotypes of Eurytides marcellus since in polyxenes a significant response to selection only occurred when selection was sufficently intense.

There is an important difference between an artificial selection experiment and the way in which selection acts in nature. In the laboratory selection was for differences in sensitivity as reflected by differences in pupal color phenotypes, while in nature selection is for the pupal color phenotypes as they relate to a variable pupation environment (degree of crypsis). As a result selection in nature in inefficient at eliminating the the extreme, less fit genotypes because of the large environmental component affecting their phenotypic expression. Therefore, selection is only weakly stabilizing. However, the mean sensitivity of the population should be maintained at a level correlated with the relative use of pupation sites where green and brown are the adaptive pupal colors. This prediction is borne out in the results of West and Hazel (1979), Hazel and West (1979) and in Chapters IV and V. *Battus philenor*, which undoubtably chooses more pupation sites where brown is the more adaptive pupal color than do any of the other dimorphic species, also produces higher frequencies of brown pupae on artifical substrates.

CHAPTER VIII

Conclusion

The overall results of this dissertation show the large effect of the kind of pupation sites which a population utilizes has: (1) on the liklihood that an environmentally-cued dimorphism will evolve, (2) on what environmental cues will influence the expression of the dimorphism, and (3) the level at which the mean genetic tendency of the population to produce brown pupae will be stabilized.

Variation in the color of the pupation site, in the sense that on some sites it is more adaptive for a pupa to be green and on others brown, apparently provides the necessary selection pressure for the evolution of the dimorphism. Variation in the type of pupation site, in the sense that on certain sites it may be more adaptive to be cryptic with the surroundings and on others the actual pupation substrate, is related to differences between species in the environmental cues controlling the expression of the pupal color phenotypes. And variation in the frequencies of pupation sites where brown is the more adaptive pupal color is related to the mean genetic tendency of a population to produce brown pupae.

Since the type of pupation site which species utilize has played such an important role in the evolution and maintenance of the dimorphism, as well as in its environmental control, it is important to ask what factors have played a part in the evolution of differences in pupation site preferences in swallowtails. This is especially important since it is known that the choice of a pupation site can be altered by by artifical selection (Clarke and Sheppard 1972). West and Hazel (in preparation) have investigated the evolution of differences in site choice between *Papilio glaucus* and *Battus philenor* and have found that differences, such as whether pupation was in trees or in the leaf litter, were due to differences in the susceptability of the species to different kinds of predators. The more subtle differences in pupation site preferences in other species, such as a preference for the undersides of leaves as opposed to stems, have yet to be investigated. Although these differences are important in relation to the environmental cues controlling the expression of pupal color.

The fact that pupation site choice has a genetic basis is at first glance not consistent with Sheppard's hypothesis, which stipulates that for the evolution of the dimorphism the pupation sites should be uncorrelated between parent and offspring. A genetic basis would by definition imply such a correlation. However, larvae may display genetically based preferences for a particular type of pupation site without regard to its color. This would explain the differences in pupation site preferences between species without violating Sheppard's stipulation.

Bradshaw (1972, 1973) and others (for review see Shapiro 1976) has considered the evolution of seasonal polyphenisms, which are conceptually similar to environmentally-cued polymorphisms. And these ideas when combined with Sheppard's hypothesis allows one to reconstruct the events which possibly played a part in the evolution of the dimorphism. If the choice of monomorphic (stereotyped) pupation sites can be considered ancestral, then the first step in the evolution of the dimorphism would be for selection to favor the choice of more variable (dimorphic) pupation sites. This would create a situation that would then favor the evolution of dimorphic pupae and a means of correlating pupal color with that of the relevant surroundings. It is likely that these events would have to occur almost simultaneously, since the choice of variable pupation sites and the ability to produce variably colored pupae would not in themselves be adaptive without a means of correlating pupal color and the color of the pupation site.

The dependence of the evolution and environmental control of pupal color on the evolution of variable pupation site preferences strongly hints at the possibility that the evolution and maintenance of genetic variation in one trait is tied to the evolution and maintenance of genetic variation in another trait.

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Vitae

Wade Nelson Hazel

May 27, 1980

Place of birth: Portsmouth, Virginia

Date of birth: November 13, 1951

Martial status: single

Early schooling: Westhaven Elementary School, Portsmouth, VA (1958-64); Harry A. Hunt Jr. High School, Portsmouth, VA (1964-66); Woodrow Wilson High School, Portsmouth, VA (1966-70).

College: Virginia Polytechnic Institute and State University B.S. 1974 (Biology).

University of Virginia (Mountain Lake Biological Station), 1974 (summer).

VPI & SU, M.S. 1976 (Zoology, David A. West, major professor. Thesis entitled "The genetic basis of pupal color dimorphism in *Papilio polyxenes* and its maintenance by natural selection").

VPI & SU, Ph. D. 1980 (Zoology, David A. West, major professor. Dissertation entitled "The evolution and ecological genetics of pupal color dimorphism in swallowtail butterflies (Lepidoptera: Papilioninae)").

Teaching experience: VPI & SU, 1974-78, Graduate Teaching Assistantships: General Biology Labs (non-majors, majors, and biochemistry majors sections), Honors

Biology Lab, Histology Lab, and Human Anatomy Lab.

Rearch:

Published Papers:

1. Hazel, W. N. 1977. The genetic basis of pupal color dimorphism and its maintenance by natural selection in Papilio polyxenes (Papilionidae: Lepidoptera). Heredity, 38(2):227-236.
2. West, D. A. and Hazel W. N. 1979. Natural pupation sites of swallowtail butterflies (Lepidoptera: Papilioninae): Papilio polyxenes Fabr., P. glaucus L. and Battus philenor (L.). Ecological Entomology, 4:387-392.
3. Hazel, W. N. and West, D. A. 1979. Environmental control of pupal colour in swallowtail butterflies (Lepidoptera: Papilioninae): Battus philenor (L.) and Papilio polyxenes Fabr. Ecological Entomology, 4:393-408.

Published Abstract:

Hazel, W. N. and West, D. A. 1978. Ecological genetics of pupal color and pupation site choice in swallowtail butterflies. Genetics, 88(4) part 2:s40.

Papers Presented at International, National, and Regional Meetings:

"The genetic basis of pupal color dimorphism in the swallowtail butterfly Papilio polyxenes and its maintenance by natural selection." Conference of the Southeastern Ecological Genetics Group (SEEGG) (Mountain Lake Biological Station), August 1976.

"Genetics and ecology of pupal color dimorphism in the swallowtail butterflies." Society for the Study of Evolution, Annual Meeting (Ithaca), June 1977.

"Predation on the pupae of a distasteful butterfly." Conference of SEEGG (Georgia Center for Marine Studies, Skidaway Island, GA), August 1977).

"Ecological genetics of pupal color and pupation site choice in swallowtail butterflies." Genetics Society of America Annual Meeting (Columbia), June 1978, (poster session).

"Environmentally-cued traits and the maintenance of genetic variation." Conference of SEEGG (Clemson), September 1978.

"Environmental control of pupal color in two species of swallowtail butterflies in relation to their pupation sites." Society for the Study of Evolution, Annual Meeting (Boulder), June 1979.

"Environmental cues responsible for pupal color dimorphism in swallowtail butterflies and their adaptive significance." Conference of SEEGG (Duke University Marine Lab, Beaufort, N.C.), August 1979.

Grants and Awards:

1. Graduate research assistantship as well as assistance in preparation on NSF grant to David A. West (2978-80).

2. Sigma Xi "Excellence in Research" award for research leading to a Master of Science degree (1977).

3. Service Award (approx. $400) at Mt. Lake Biological Station (1974).

4. "Excellence in Teaching" award, College of Arts and Sciences, VPI & SU (1978)

Membership in Professional Societies:

Genetics Society of America
Society for the Study of Evolution
Southeastern Ecological Genetics Group
(honorary: Phi Sigma)

W N. Hazel

THE EVOLUTION AND ECOLOGICAL GENETICS OF PUPAL COLOR DIMORPHISM IN SWALLOWTAIL BUTTERFLIES (LEPIDOPTERA: PAPILIONINAE)

by

Wade Nelson Hazel

(ABSTRACT)

Data bearing on the evolution, environmental control and genetic basis of pupal color dimorphism was presented or discussed for six species of swallowtail betterflies. _Papilio glauces_ and _P. cresphontes_ produce only brown pupae while _P. polyxenes_, _P. troilus_, _Battus philenor_ and _Eurytides marcellus_ produce both green and brown pupae.

Natural pupation sites of the species were located and results were generally consistent with the hypothesis of Sheppard (1958) which relates the evolution of the environmentally-cued dimorphism to environmental variation.

The relative importance of texture, color and photoperiod as environmental cues controlling the expression of pupal color was investigated and the results were discussed in relation to differences in the pupation sites of the species.

The genetic basis of pupal color was investigated in E. marcellus by selecting for an increase in the tendency of larvae to produce green or brown pupae. The results were found to be consistent with the genetic basis of the trait as proposed by Hazel (1977).

It was concluded that the primary factor responsible for the evolution of the dimorphism and the environmental cues which control its expression is nature of the pupation sites that a species utilizes.