

THE GENETIC BASIS OF PUPAL COLOR DIMORPHISM IN Papilio
polyxenes, AND ITS MAINTENANCE BY NATURAL SELECTION

by

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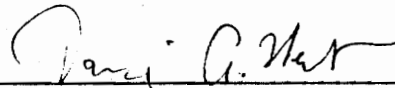
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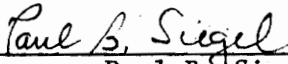
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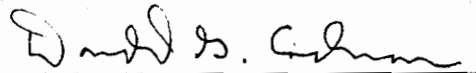
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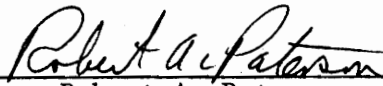
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INTRODUCTION

The genetic basis of environmentally controlled polymorphisms and their maintenance in nature by natural selection are poorly understood. Since the expression of the possible phenotypes is ultimately under the control of the environment, a thorough understanding of the environmental stimuli responsible for the expression of these phenotypes is necessary before any attempt can be made at investigating their genetic basis. Pupal color dimorphism in Papilio polyxenes is an environmentally controlled polymorphism for which at least some of the environmental stimuli important in determining the two phenotypes are known. The purpose of this thesis was to investigate the genetic basis of the pupal color dimorphism in this species and discuss the maintenance of this trait in nature by natural selection.

LITERATURE REVIEW

Environmentally controlled polymorphisms may evolve under conditions where there is a mosaic of distinct environments, and where the distribution of individuals in these environments is not correlated with that of their parents (Sheppard, 1967). These conditions may often be found in the sedentary phases of the life cycles of many plant and animal populations (Clarke and Sheppard, 1972).

In the insects, particularly in the Lepidoptera, some of the best known examples of environmentally controlled polymorphisms can be found (Merrifield and Poulton, 1899). In many species of Lepidoptera individuals of both the larval and pupal stages rely on crypsis for protection from predation. In these species the crypsis usually involves a distinct dimorphism, with the larvae and pupae being either green or brown, intermediates being rare or absent. Each morph is particularly well adapted to a distinct type of background. Thus, green pupae are usually found on green backgrounds while brown pupae are usually found on brown backgrounds (Clarke and Sheppard, 1972). The selective advantage of crypsis is obvious, and experiments have repeatedly shown the importance of crypsis to survival.

Hidaka, et al. (1959) conducted experiments designed to show whether the pupal color dimorphism in Papilio xuthus had any protective value. First, green and brown pupae were placed head down in green grass so that only the abdominal halves were visible. A pair of fowl that had been shown to have equal preference for the two morphs were then allowed to feed at leisure in the grass for a period of five

minutes. A similar experiment was performed in November when the grass was grey. In each experiment the cryptic pupae were better protected from predation by fowl.

Baker (1970) looked at bird predation on pupae of Pieris rapae, a species that has the ability to modify its pupal color according to that of its surroundings (Poulton, 1887, 1892). He found that the survival of pupae was enhanced both by their ability to modify their color to match the surroundings and by the choice of pupation sites, and he suggested that selective predation would affect the evolution of both traits (Baker, 1970).

Wiklund (1974) studied the net survival ("selective advantage") and the different rates of elimination ("adaptive fitness") of color-adapted and non-color-adapted pupae of Papilio machaon during the summer and diapausing generations in a Swedish population of the swallowtail that had been under investigation for fifteen years. Color-adapted and non-color-adapted pupae were placed throughout the habitat in pairs no more than half a meter apart. Care was taken that no two pupae were visible from the same point and that the visible angle (as previously described by Baker, 1970) was the same for each member of a pair. All pupae were checked periodically.

In the summer generation the net survival of the color-adapted pupae was 1.5 times as great as that of the non-color-adapted pupae, with the green color-adapted morph having a slightly greater net survival than that of the brown color-adapted morph. In the hibernating

generation there was no difference in net survival of the color-adapted and non-color-adapted pupae.

In the summer generation the rate of elimination of the non-color-adapted brown form was two to three times greater than that of the color-adapted brown form while the rate of elimination of the non-color-adapted green form was five to seven times greater than that of the color-adapted green form. In the hibernating generation the rate of elimination of the color-adapted and non-color-adapted brown form was approximately the same and was two to three times as great as that of the color-adapted green form.

Two conclusions can be drawn from these data: (1) that the importance of pupal color adaptation in Papilio machaon was considerably greater in the summer generation than in the hibernating generation, and (2) that the protective effectiveness of the green morph was substantially greater than that of the brown morph, even when both match their backgrounds. Thus there is considerable evidence of the importance of crypsis to the survival of butterfly pupae.

It was E. B. Poulton (1887) who first showed experimentally that the pupae of various members of the Lepidoptera were capable of adapting to the color of their background. Later work (Brecher, 1921) confirmed Poulton's observations that Pieris brassicae and Pieris rapae modify their pupal color by sensing the color of their surroundings and that the sensitive period coincides with the spinning period of the prepupa. It is during this period that variation in the wavelength of light striking the prepupa has its greatest effect on pupal

color. Recent studies (Kayser and Angersbach, 1974) have shown correlations between the degree of melanization, the level of bile pigment, and the wavelengths of light striking a prepupa of Pieris brassicae.

According to Gardiner (1974), who investigated the dimorphism in Pieris brassicae and in Papilio machaon, the mechanism by which pupae of these species adapt to the color of their backgrounds is very different. For this reason pupal coloration in the pierids will not be further discussed in this paper.

A good deal is known about the environmental and physiological determinants of pupal color in the papilionids. In contrast to the pierids, the dimorphism in the papilionids does not seem to be very dependent upon the color of the general environmental surroundings.

Sevastopulo (1948) placed larvae of Papilio polytes and Papilio demoleus in receptacles of different colors and allowed them to pupate. He observed that the pupae of Papilio polytes were dimorphic while those of Papilio demoleus were not, and further that pupal color in Papilio polytes was not correlated with the color of the environment. He concluded from these and other observations that the dimorphism in this species was not a result of the environmental condition but of how far the prepupal larva had walked before pupation. Thus to Sevastopulo a green pupa was green not because it was among green foliage but because the prepupal larva had walked only a short distance prior to pupation, while in contrast, a brown pupa was brown not

because it had pupated on a brown tree trunk, but because the prepupal larva had walked a long distance before pupation.

The Japanese species Papilio xuthus is trimorphic, with green, brown, and orange pupal colors. Ishizaki and Kato (1959) studied the environmental stimuli responsible for pupal color in this species. Their data seemed to justify the following conclusions: (1) low humidity and total darkness at the time of pupation will promote the formation of brown pupae, especially if the larvae pupate on paper as opposed to glass, (2) orange pupal color occurs most often when larvae are reared in conditions of constant darkness and low temperatures, at least to the end of the fourth larval instar, and (3) pupae of the orange type enter diapause without exception while those of the green and brown types eclose without entering diapause.

A similar situation appears to be true in the North American swallowtail Papilio polyxenes where photoperiod has an "overriding" effect on whether or not the pupae will be dimorphic (West, et al., 1972). Thus if larvae of this species are reared under short day conditions the result is an almost exclusive production of brown pupae, all of which enter diapause. Rearing larvae under long day conditions ". . . permits the expression of the green-brown alternative . . .", depending on the pupation substrate. Thus when larvae were reared on long day conditions, small twigs (either green or brown) and smooth plastic surfaces tended to promote the formation of green pupae, while rough logs (either green or brown) tended to promote the formation of brown pupae. These data suggested that the amount of light reaching

the underside of the prepupa might affect the color of the resulting pupa.

Wiklund (1972) demonstrated that the wavelength of light striking the underside of a prepupa of Papilio machaon reared under non-diapausing conditions has an effect on the resulting pupal color. Thus when larvae were exposed to wavelengths of 500 (green), 580 (yellow), and 620 nm (orange), green morphs were produced at frequencies of 94, 97, and 100 percent respectively as opposed to 55 percent of the green morph produced in total darkness.

Clarke and Sheppard (1972) initiated a comprehensive investigation into the factors influencing pupal color in swallowtail butterflies. A number of different types of data were collected and analyzed.

First, pupae of Battus philenor from the midsummer brood were collected in the wild from three types of pupation sites: thin twigs and shoots less than 12 mm in diameter, horizontal branches over 50 mm in diameter, and large vertical tree trunks. These data indicated that there was a significant association between background color and pupal color and between twig diameter and pupal color; so that green pupae were found more often on small twigs, even if the twigs were brown.

Second, adults of Papilio polytes were released in two greenhouses. Both greenhouses were supplied with the larval food plant, Citrus, and in one of them numerous small brown twigs (less than 12 mm in diameter) were placed between the plants. In both greenhouse populations the adults mated normally and laid eggs on the Citrus. The larvae fed on the Citrus and then pupated on the leaves or stems of the

plants and on the small brown twigs in the population supplied with them. After two months the twigs were removed from the one greenhouse and placed in the other greenhouse. From the data on pupal color and background color two conclusions could be drawn: first, there was a positive correlation between pupal color and background color, and the proportion of brown pupation sites affected the proportion of brown pupae appearing; and second, when pupal color did not match background color it was most often a green pupa on a small brown twig.

To investigate the possible effects of the environment during the last instar, last instar larvae of Papilio polytes from each of the two greenhouses were divided into two groups. One group was placed in a bowl containing many brown sticks (less than 12 mm in diameter), while the other group was placed in a bowl containing few of these sticks. Since the frequencies of brown and green pupae were the same in each bowl no matter which greenhouse population the larvae came from, Clarke and Sheppard concluded that "there is absolutely no evidence that the earlier environment in the greenhouses had affected the colour of the pupae which eventually pupated in the bowls, only the environment in the bowls."

Gardiner (1974) investigated possible environmental stimuli in the pupal color dimorphism of Papilio machaon. Larvae were reared under natural daylight conditions in England in June and July. This point is important in that all but two or three percent of the pupae entered diapause but were still dimorphic. In this study larvae were allowed to pupate on various substrates. It was found that most green

pupae were produced on the stalks of the food plant, while larvae which were forced to pupate on "Ostwald" paper of various colors gave a high frequency of brown pupae (121 browns:7 greens). Of the seven green pupae, four were produced on yellow paper and three on green paper. Also, transferring the larvae just prior to pupation demonstrated that the sensitive period for the production of dimorphic pupae was during the spinning period of the prepupal larvae. This confirms Clarke and Sheppard's observations on Papilio polytes. From these data Gardiner came to the conclusion "that pupae on a flat background are brown, whereas those formed on a stalk-like background give a very significant percentage of green pupae." The explanation for this was thought to be that when a prepupal larva pupates on a stalk as opposed to a flat surface the brain receives more light from the underside. Also, it seemed possible that there may have been an additional effect due to the texture of the pupation surfaces. At any rate, Gardiner's results on the "Ostwald" paper tend to disprove the idea that background color is a major factor affecting pupal color in the papilionids.

It now appears that the most important factor influencing the pupal color of Papilio polyxenes, and probably other dimorphic members of this family, is the texture of the pupation substrate (West and Hazel, unpublished). Larvae were reared on long days and allowed to pupate on various natural and artificial substrates of different textures. Thus, the larvae pupated on rough or smooth twigs of the same size, rough or smooth logs of the same size, and smooth plastic canisters with black construction paper either on the inside or the

outside. In all comparisons there was a significantly higher frequency of brown pupae on the rougher surfaces. For example, all (20) pupae formed on smooth logs were of the green type, while all (33) pupae formed on rough logs were of the brown type. Also, those larvae that were forced to pupate on black construction paper or on smooth plastic backed by black construction paper were split into groups, each group containing both treatments. One group was placed in constant darkness and the other group in constant light during pupation. The results from the light and dark groups were homogeneous, with the black construction paper producing a large percent of the brown morph and the plastic backed by black construction paper producing a large percent of the green morph. Although the numbers were small, these results suggest that texture is a major factor and that the amount of light reaching the underside of the prepupa is of minor importance in the determination of pupal color in Papilio polyxenes.

A reanalysis of previous studies supports the view that texture is the most important stimulus in the determination of pupal color. When larvae of Papilio xuthus were allowed to pupate in glass and paper chambers at two different relative humidities the overwhelming percentage of brown pupae were produced in the paper chambers, regardless of the two humidity treatments (Ishizaki and Kato, 1956). Also, when mature larvae of Papilio machaon were forced to pupate on "Ostwald" papers of various colors, brown morphs were very frequent regardless of the color of the paper (Gardiner, 1974). In the study of Clarke and Sheppard (1972) fourteen larvae of Papilio polytes pupated on red

plastic flowering pots, one on a red plastic watering can, and two on transparent polyethylene sheeting, all smooth surfaces. All seventeen of these pupae were green. All of these results can best be explained by the texture hypothesis.

There is also evidence that the type of texture may be important in the production of dimorphic pupae (West and Hazel, unpublished). Two examples illustrate this point. First, the texture of the smooth logs was not noticeably smoother to the human touch or eye than that of the black construction paper. The only difference in the two substrates was that the texture of the smooth logs was of a linear sort due to the structure of the wood, whereas the texture of the construction paper was of a random sort due to the dispersion of the paper fibers. Yet the smooth logs elicited a large percent of the green morph, while the construction paper elicited a large percent of the brown morph. Second, larvae were tested on a gradient of textures from smooth plastic through sandpapers of various grit sizes, with no corresponding increase in the frequency of the brown pupal morph. Thus, the type of texture must be of some importance.

Pupal color in Papilio xuthus and probably other dimorphic members of the genus is controlled by a peculiar endocrine mechanism which was studied by Hidaka (1961a, 1961b). Prepupal larvae were ligatured between the second and third segments. In those prepupae reared in conditions that would stimulate the production of brown pupae the result of this operation was that the segments anterior to the ligature were brown in color while those posterior were green. In addition, the

brain and the sub-esophageal, mesothoracic, and the prothoracic ganglia were dissected and then transplanted in various combinations into a pouch of intersegmental skin between the fifth and sixth segments. After pupation the larval skin was removed and the pupal color recorded. In all operations where the brain or ganglia were transplanted alone no brown color could be observed in the area of the transplants. Only those combinations that included the prothoracic ganglion caused browning in the area of the transplants. In an additional experiment transplants were not made until the middle of the prepupal period instead of at the beginning. Three types of transplants were made: the brain, or the sub-esophageal ganglion, or the prothoracic ganglion. Only the transplant of the prothoracic ganglion produced browning. The conclusion from these data is that the prothoracic ganglion is directly responsible for the production of brown pupal color, probably by the secretion of a hormone. However, there does seem to be some additional involvement of the cephalo-thoracic nervous system at the beginning of the prepupal period. It seems altogether likely that this system stimulates the release of the prothoracic hormone. The mode of action of this hormone is not completely understood, nor has it yet been identified. Ohnishi (1959) suggested that it may activate oxidative reactions taking place in the cuticle. He was able to show that the integumentary carotenoids of the brown pupa correspond to the oxidized form of the carotenoids of the green pupa. He also concluded that browning consists of the formation

de novo of the pigments melanin, astaxanthin, and papilioerythrin in the brown pupa.

Previous studies have indicated that those pupae that match their backgrounds are at a selective advantage relative to those that do not (Hidaki, et al., 1959; Baker, 1970; Wiklund, 1973). If this is indeed the case, then the obvious question is: why do mismatches occur?

Clarke and Sheppard (1972) discussed two alternative explanations for the occurrence of mismatches in their Papilio polytes populations. They thought their results could best be explained "by assuming that in the absence of an appropriate stimulus the pupa is green." This hypothesis explained the occurrence of green pupae on the red flower pots, the watering can, the polyethylene sheeting, and the occurrence of green pupae on small brown twigs in nature. Thus,

To produce a brown pupa on a green background would require a suitable environmental stimulus, which would not be present among green leaves. To produce a green pupa on a brown background would only require that the environmental stimulus was not of sufficient magnitude to trigger the mechanism producing brown pigment, hence it would be expected that green pupae would be particularly frequent when attached to very thin stems.

Therefore mismatches are an indication that there is genetic variation in the ability to produce brown pigment.

One explanation for the occurrence of mismatches may lie in the subjective judgment of what crypsis entails. A green pupa on a brown background may be considered cryptic if it is surrounded by green foliage, especially if it resembles a small green leaf. Since most of the mismatches observed by Clarke and Sheppard were of this sort they

suggested that

Selection may therefore have adjusted the environmental switch mechanism in such a way that it is not sensitive enough to produce many brown pupae on a green background and at the same time has minimised the number of green pupae on a brown background.

If this were to be the case,

The actual number of mistakes would be stabilised according to the relative disadvantage of a brown pupa on a green background as compared with a green pupa on a brown one. This explanation presupposes that the switch mechanism cannot be refined enough to make mistakes very rare.

And, ". . . that selection is likely to change in direction from time to time and the heritability therefore be high." Clarke and Sheppard believed this explanation to be unlikely as they considered the dimorphism old enough for natural selection to have refined the switch mechanism to a higher degree.

An alternative explanation for the occurrence of mistakes is dependent on the ability of apostatic (frequency dependent) selection to change gene frequencies. This type of selection may result from the formation of hunting images by birds. If indeed apostatic selection acts on butterfly pupae,

then the rarer morph will tend to be at an advantage, that is a green pupa on a brown background will be at an advantage when it is rare since the predators are unlikely to form a hunting image of it, but at a disadvantage when common [(Clarke and Sheppard, 1972)].

If this be the case, then according to Clarke and Sheppard, "selection will be stabilising and adjust the sensitivity of the environmental switch to give the optimum frequency of the four combinations of pupal colour and background colour." In this explanation the variation in

the ability to produce brown pupae, and thus the occurrence of mismatches, is due to apostatic selection.

To investigate the genetic basis of the dimorphism Clarke and Sheppard (1972) conducted an artificial selection experiment using their greenhouse populations of Papilio polytes. In one greenhouse only those pupae that matched their backgrounds were allowed to remain and breed, while in the other greenhouse only those pupae that failed to match their backgrounds were allowed to remain. Thus the criterion for selection was background matching. The selection program lasted for approximately five generations. Rather than affecting the frequencies of matches and mismatches, selection caused an alteration of the pupation sites chosen by the larvae. The apparent reason for this was that in the population selected for mismatching, most of the selected pupae were of the green morphs on small brown twigs. Thus selection resulted in an increased tendency to choose brown twigs as pupation sites. In the population selected for background matching most of the selected pupae were of the green-on-green category, and selection thus resulted in an increased tendency of the larvae to choose green pupation sites.

Although Clarke and Sheppard considered that mismatches were due to genetic variation in the amount of stimulus needed for the production of brown pupae, their selection program was not designed along these lines. They selected on the basis of background matching and mismatching, instead of for the ability or inability to produce brown pupae. Also, their selection program was conducted under poorly

controlled conditions. Since larvae were allowed to pupate on a variety of substrates and since pupal color must be considered a function of the intensity of the environmental stimuli and the genetic ability of the prepupal larva to interpret these stimuli and respond with the appropriate pupal color, then without control of the environmental stimuli (pupation site) the underlying genetic differences between individuals could not be discerned and selected upon. Thus, based on Clarke and Sheppard's explanations for mistakes, the expected result of selection should have been no response in the ability to match or mismatch the backgrounds, but rather an alteration in choice of pupation site.

With the exception of the unsuccessful selection experiment of Clarke and Sheppard (1972), all investigations into the factors influencing pupal color in the papilionids have been limited to the environmental and physiological determinants of pupal color. To investigate the genetic basis of pupal color dimorphism in Papilio polyxenes two experiments were conducted: one was designed to determine the genetic variation between families in the ability to produce brown pupae; while the other was a selection experiment, to determine whether this variation was genetic and responsible for the dimorphism.

MATERIALS AND METHODS

Rearing

Adult Papilio polyxenes females were collected in Montgomery, Giles, and Augusta counties of western Virginia during the summer of 1975. Within a few hours after their capture females were placed in laying chambers consisting of either a 5 1/2 X 4 inch chromatography beaker or a 7 1/2 X 8 inch plastic canister. In the former case the wings of the females were trimmed so that only a centimeter or so remained. Small pieces of the larval food plant, either wild carrot (Daucus carota) or fennel (Foeniculum vulgare) provided oviposition sites. The chambers were covered with cheese cloth and placed under constant warm bright light. The females were fed daily with sugar water and usually laid eggs readily. The females were usually allowed to continue laying for as long as they lived.

Eggs were collected at daily intervals and placed in round plastic rearing dishes (1 1/2 X 6 or 1 X 4 1/2 inches) with tight lids. Whatman No. 1 filter paper circles were placed in the bottom of the dishes to collect the frass. The larvae were reared in two Percival environmental chambers set for a 15 hour day and a nine hour night. The day temperature was set at 80 degrees Fahrenheit while the night temperature was set at 70 degrees. During the early instars as many as 15 larvae could be reared in one of the large rearing dishes without crowding. This number was quickly reduced as the larvae grew, so that by the fifth instar the larvae were maintained at one per dish. All first generation lab-reared larvae were fed fresh food plant, wild

carrot or parsley, with the exception of broods number 22 and 23, which were reared on the artificial diet developed by Wiklund (1971) for Papilio machaon. The larvae were fed in abundance often several times daily. Rearing dishes were cleaned as needed (usually every other day) and sterilized in dilute sodium hypochlorite solution with every cleaning. Broods were segregated as to parent.

Just before pupation the fifth instar larvae of Papilio polyxenes purge their gut of all digested and semi-digested food and begin a period of searching for a pupation site. This period may last for several hours. Once a larva was noticed to have emptied the gut contents it was placed in a pupation chamber consisting of two 9 X 11 inch sheets of Norton Tufbak Durite type 1 600 A black emery cloth taped together to form a cylinder with the grit side facing inward. This substrate was chosen because it had been shown to elicit comparable frequencies of each morph. The emery cloth cylinder was then placed in a 7 1/2 X 8 inch plastic canister and was topped with an additional piece of emery cloth, grit side down. Care was taken to avoid crowding on the inside of the pupation chambers. The pupation chambers were placed in a Sherer environmental chamber set at a 15 hour day and a 9 hour night cycle. The temperature was maintained at a constant 70 degrees Fahrenheit. When the larvae had finished pupating the numbers of green and brown pupae formed on the emery cloth were recorded. After the pupae had eclosed the adults were selectively hand paired after the method described by Clarke and Sheppard (1956). Females

were then allowed to lay eggs and the larvae reared as previously described.

The Selection Programs

The selection was initially designed according to the procedure developed by Wright and discussed by Kimura and Crow (1963) for the maximum avoidance of inbreeding. The plan of selection called for sixteen families at generation zero crossed in such a manner as to avoid inbreeding through the fourth generation of selection.

Since all prepupal larvae were reared and tested in like environments all differences in pupal color were considered to be genetic in origin. Thus, individuals that responded with brown pupal color were thought to be genetically more sensitive to stimulation from the pupation environment than those that responded with green pupal color. Therefore, only adults eclosing from like colored pupae were paired. Offspring from the brown X brown pairings were designated as the sensitive (brown) line, while offspring from green X green pairings were designated as the insensitive (green) line. The larvae produced from these matings were reared and tested as previously described, with the exception that the larvae were reared on the artificial diet developed by Wiklund (1971).

Unfortunately the selection program had to be abandoned after only one generation due to an unusually high mortality from the use of the artificial food. Thus, 37 successful pairings were made for the first generation of selection of which only five survived to pupation. These five lines survived because they were switched from artificial

food to natural food around the third larval instar. Because of the use of the artificial food the numbers were so reduced by this time that only one more generation of selection could be conducted in the insensitive direction (green line) and no more selection in the sensitive direction (brown line). Other possible effects of the artificial diet will be considered in the discussion.

RESULTS

Analysis of Genetic Variation in the Ability to Produce Brown Pupae

Table I shows the sample size, number of brown pupae, number of intermediate pupae, the percentage of brown pupae, and the transformed percent of brown pupae produced per brood for all 23 broods tested. Transformations were made by the method of Mosteller and Youtz (1961) for sample sizes less than 50 and by the angular transformation for sample sizes greater than 50 to stabilize the variance (Sokal and Rohlf, 1969). Also shown are the total number of pupae tested, and the overall percent of brown pupae produced. The average transformed percent of brown pupae per brood was 33.03. The transformed data were tested for goodness of fit against a normal distribution using the Kolmogorov statistic (Stephens, 1974) and were found not to differ significantly from the normal (P greater than 0.225). The transformed data, displayed in Figure 1 as a histogram, shows that the distribution of brown pupae definitely has one mode and two tails.

Analysis of the Results of the Selection Program

Selected line and midparent values in terms of percentage of brown pupae produced for both the insensitive (green) line and the sensitive (brown) line are shown in Table II for the first and in Table III for the second generation of selection. Thus from Table II, in pairing 9 x 10 of the insensitive (green) line, adults eclosing from green pupae in broods 9 and 10 were hand paired. The overall percentage of brown produced in these two broods was 43.3%; this comprises the

midparent value for this pairing. The percentage of brown pupae produced from this pairing comprises the selected line value. In the second generation of selection in the insensitive (green) line three replicate pairings were made between individuals of the two selected first generation broods. These pairings were therefore designated (14 x 15) x (9 x 10) A, B, and C.

The numbers of green and brown pupae produced in the selected lines were tested against the numbers of green and brown pupae produced in the parental broods using the G-statistic. The results of this test, the intensity of selection, and the direction of the response to selection for each pairing are shown for both generations of selection in Table IV. Thus, in pairing 9 x 10 in the insensitive line 57% of the pupae produced in the two parental broods were green, giving a 57% intensity of selection. From this pairing a non-significant increase in the frequency of the green morph was produced in the next generation, and thus the direction of the response for this pairing was green.

Table I: Number of brown pupae, sample size, percent brown pupae, and transformed percent brown pupae produced for 23 broods pupating in controlled conditions.

Brood	Number brown		Sample size	Percent brown	Transformed % brown
1	5		15	33.3	35.87
2	1		7	14.3	25.35
3	3		51	5.9	14.06
4	8	6*	49	16.3	24.34
5	6		46	13.0	21.82
6	13		48	27.1	31.66
7	6		32	18.8	26.33
8	23	1*	52	44.2	41.67
9	25		43	58.1	49.58
10	4		24	16.7	25.07
11	2	1*	20	10.0	20.09
12	14		32	43.7	41.52
13	12	1*	26	46.2	42.87
14	8		27	29.6	33.42
15	3	1*	21	14.3	23.46
16	3		12	25.0	31.20
17	7	1*	27	25.9	31.16
18	5		24	20.8	27.95
19	13		66	19.7	26.35
20	11		20	55.0	47.74
21	5		15	33.3	35.87
22	8		10	80.0	61.64
23	5		12	41.7	40.56
Totals	190	11*	679	27.9	

Mean transformed % brown = 33.03

* Intermediate color

TABLE II: The response to selection in the first generation.

A. The insensitive (Green) line

Pairing	Midparent values		Selected line values	
	Percent Brown	Sample Size	Percent Brown	Sample Size
14X15	23.4	47	54.5	66
9X10	43.3	67	41.7	12

B. The sensitive (Brown) line

Pairing	Midparent values		Selected line values	
	Percent Brown	Sample Size	Percent Brown	Sample Size
14X15	23.4	47	65.6	32
3X4	11.7	94	71.4	7
22x23	59.1	22	92.9	14

Table III: The response of the insensitive (Green) line to selection in the second generation.

Pairing	Midparent values		Selected line values	
	Percent Brown	Sample Size	Percent Brown	Sample Size
(14X15)X(9X10)A	52.6	78	11.8	17
(14X15)X(9X10)B	52.6	78	21.7	23
(14X15)X(9X10)C	52.6	78	45.4	11

TABLE IV: The intensity of selection, direction and G-test of the response to selection.

<u>The first generation</u>			
<u>The insensitive (Green) line</u>		<u>The sensitive (Brown) line</u>	
<u>Pairing</u>	<u>Intensity</u>	<u>Direction</u>	<u>G-test</u>
14X15	77%	brown	$p < 0.005$
9X10	57%	green	N.S.
		<u>Pairing</u>	<u>Intensity</u>
		14X15	23%
		3X4	12%
		22X23	59%
		<u>Direction</u>	<u>G-test</u>
		brown	$p < 0.005$
		brown	$p < 0.005$
		brown	$p < 0.005$
<u>The second generation</u>			
<u>The insensitive (Green) line</u>			
<u>Pairing</u>	<u>Intensity</u>	<u>Direction</u>	<u>G-test</u>
(14X15)X(9X10) A	47%	green	$p < 0.005$
(14X15)X(9X10) B	47%	green	$p < 0.005$
(14X15)X(9X10) C	47%	green	N.S.

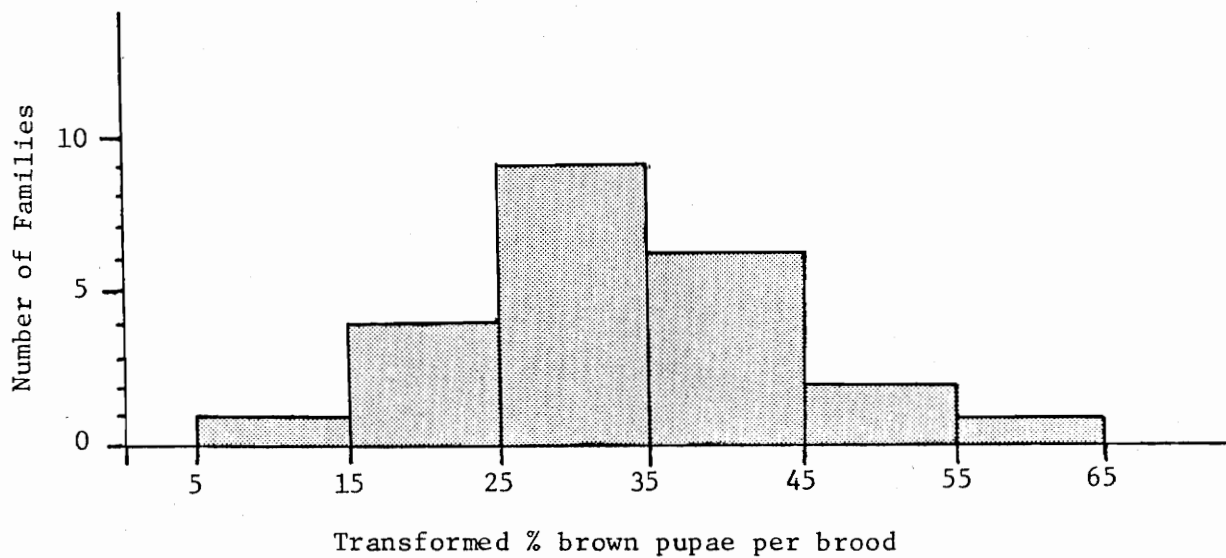


Figure 1: Distribution of sensitivities in a natural population of Papilio polyxenes plotted as a histogram.

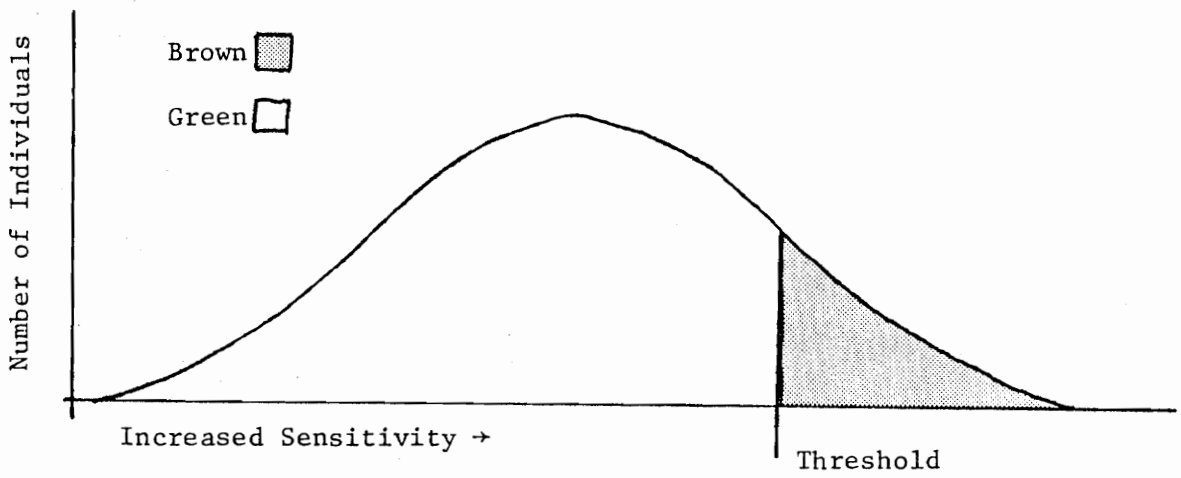


Figure II. Theoretical distribution of sensitivities showing threshold and two phenotypes.

DISCUSSION AND CONCLUSION

The genetic basis of the pupal color dimorphism in Papilio polyxenes can best be explained by considering the production of brown pupal color as a threshold trait (Falconer, 1965). If this were the case one would expect additive genetic variation around some population mean in the ability to produce a brown pupa, a trait that will be designated sensitivity. At some point in this scale of variation there is a threshold so that all individuals on one side, the sensitive side, of the threshold will be brown, while those individuals on the other side, the insensitive side, will produce green pupae. It should be noted that the threshold is an immovable point in the distribution of genetic sensitivities, separating a continuous distribution of genotypes into two discrete phenotypes. The data presented in this paper support this hypothesis.

The results from the 23 different broods reared and tested under uniform conditions indicate that there is considerable genetic variation in the mechanism responsible for the production of brown pupae in this species. Since sperm precedence is probably the case in this species (Clarke and Sheppard, 1962) in that the spermatophore from the last mating provides the sperm for all later fertilizations, the 23 broods sampled here represent a sampling of 46 haploid genomes. Thus, the percentage of brown pupae produced in each brood provides a measure of the average sensitivity of the two parents to the environmental stimuli responsible for brown pupal color. If the percentage of brown pupae formed in each brood is transformed, to stabilize the variance,

one can then obtain an estimate of the mean sensitivity of the population, which in this case was 33.03, this being the arithmetic mean of the transformed percentage of brown pupae produced in each brood.

Superimposed on this distribution of genotypes are two phenotypes. Of the 679 larvae from 23 broods, 190 or approximately 28% produced brown pupae. This would place the threshold at some point to the sensitive side of the mean so that all individuals to the sensitive side of the threshold make up the 28% of individuals having brown pupal color (See Figure II).

There must also be additive genetic variation within broods, hence the lack of uniformity in pupal color within any brood. For example, brood number 3 was the least sensitive of the broods tested, producing approximately 6% brown pupae. This would place it to the insensitive side of the population mean; yet there was still sufficient genetic variation within this brood for 3 individuals to be on the sensitive side of the threshold. Therefore the data indicate that there is indeed quantitative genetic variation within and among broods in sensitivity to the environmental stimuli responsible for the formation of brown pupae.

The results of the selection program confirm that the variation is genetic, and that it forms the basis for the two pupal color phenotypes. Since seven of the eight selected lines responded in the direction predicted by the threshold hypothesis, and since five of these seven lines responded significantly ($P < 0.005$), the threshold

hypothesis seems to be the best explanation for the genetic basis of pupal color dimorphism in this species.

The response of a threshold trait to selection will depend on the position of the threshold relative to the direction of selection. Since the position of the threshold controls the intensity with which one may select, it follows that the response of a threshold trait to selection in generation N will ultimately affect the intensity with which one may select in generation N+1. The results of selection in the green line illustrate this nicely. Broods 14 and 15 had a total of 77% green pupae. Therefore the selected individuals were taken out of the bottom 77% of the brood on the scale of sensitivity. The response to selection of the green line pairing from these broods was a significant change ($P < 0.005$) in the frequency of the two color forms but in a direction opposite to that predicted by the threshold hypothesis. This response can be explained by the fact that a 77% truncation for selection means that 27% of those individuals that had a possibility of being selected to pair for the next generation lay on the sensitive side of the mean. If by chance two such individuals were selected for the pairing and if the trait were highly heritable, then a significant response in the sensitive direction could be expected. I feel that the results of selection in pairing 14 x 15 do not represent a contradiction of the threshold hypothesis for the following reasons: (i) all other pairings responded in the direction predicted by the threshold hypothesis, (ii) the 77% truncation for selection in this line was the highest of all lines selected, thus the intensity of

selection in this line was the lowest of all selected pairings, and (iii) data from other selected lines indicate that this trait is quite heritable.

The result of the response of pairing 14 x 15 to selection was to increase the intensity of selection in the next generation so that the truncation for selection was 47%. Thus, in the second generation of selection in the insensitive direction two of the three replicate pairings moved significantly ($P < 0.005$) in the direction predicted by the threshold hypothesis, and the third non-significantly in the same direction.

The pairings in the brown line experienced the most intense selection. As a result, all brown lines responded significantly to selection ($P < 0.005$), and in the predicted direction.

Although the use of the artificial diet did reduce the numbers to a large extent it seems doubtful that this had any effect on the outcome of the selection experiment. Rothschild et al. (1975) have reported a lack of response to background color in Pieris brassicae pupae reared on a carotenoid-free diet. In that study all pupae reared on this diet were turquoise blue. The effects of the artificial diet on pupae of Papilio polyxenes were two-fold. The artificial diet did not seem to have an effect on the ability of pupae to modify their color, although those pupae that would have normally been considered green were turquoise blue. Also, pupae reared on the artificial diet experienced developmental difficulties so that most of them had severely deformed wing pads. Switching the larvae from artificial food

to natural food at around the third or fourth larval instars appeared to reverse the effects of the artificial diet completely, so that normally colored pupae were formed with no developmental difficulties. For this reason the effect of the artificial diet on the selection would be small or non-existent, unless the mortality was non-random with respect to sensitivity.

The effects of natural selection on a genetic system of the sort presented will be two-fold. First, selection will be stabilizing by eliminating those individuals that are extremely sensitive or extremely insensitive to environmental stimulation. Extremely sensitive prepupae will produce brown pupae with very little environmental stimulation, even on green surfaces. Extremely insensitive prepupae will produce green pupae even with stimulation that would normally be adequate for the production of brown pupae. Pupae of this type will tend to be green on brown backgrounds. Thus natural selection on the basis of background matching will be stabilizing by eliminating individuals from the tails of the sensitivity distribution. Second, selection will tend to maintain genetic variation in sensitivity. Since selection will be on the basis of background matching it may sometimes favor the extremely sensitive and extremely insensitive extremes of the distribution of sensitivities. The following example will clarify this point. Although an extremely sensitive prepupa will have a high probability of producing a brown pupa regardless of the substrate, in cases where it pupates on a brown surface it will still match its background and be selected for. The same will hold true for extremely

insensitive prepupae. If an insensitive prepupa pupates on a green surface it will be selected for since it will still match its background. Thus, natural selection on the basis of background matching will tend to stabilize the sensitivity distribution at the optimum level for the habitat, while at the same time maintaining genetic variation in the sensitivity of prepupal larvae.

Clarke and Sheppard (1972) have expressed the idea that a green pupa on a brown background may be more adaptive than a brown pupa on a green background. My data give no indication of skewness in the distribution of sensitivities, although the sample size is small and if there is any difference in the adaptiveness of the two extremes of the sensitivity distribution it would be small.

Clarke and Sheppard (1972) have also suggested that apostatic selection may play a part in maintaining genetic variation with regard to the pupal color dimorphism. In Papilio polyxenes, females lay eggs singly and the larvae feed in this manner. I have only found larvae of this species on rare occasions and have never found a pupa in the field, even though visits to the habitat were made twice daily during the summer for the collection of food plant and adult butterflies. Since apostatic selection is a form of density-dependent selection, in that without a high density of prey species of one morph a hunting image cannot be formed, it seems very unlikely that apostatic selection is an important selective force operating on the pupal color dimorphism in a widely dispersed species like Papilio polyxenes.

Pupal color in the machaon group of swallowtails, that is Papilio machaon, P. polyxenes, P. xuthus, and others, presents an interesting evolutionary problem. In this group the life history of a species plays a large part in determining whether or not it will be dimorphic. Thus in the multivoltine species, P. polyxenes and P. xuthus, short photoperiod has an overriding effect on all other stimuli so that all pupae enter diapause and are monomorphic (brown in P. polyxenes, orange in P. xuthus) (West et al., 1972; Ishizaki and Kato, 1959), while in P. machaon in Great Britain where it is univoltine, larvae never experience short photoperiods and the pupae are diapausing and dimorphic (Gardiner, 1974). It would be most interesting to investigate the effect of short photoperiod on pupal color in this species. It is possible that the overriding effect of photoperiod on the dimorphism is a newly evolved trait in the multivoltine members of this group. If this be true then the selective advantage of the dimorphism may be different in the diapausing generations of multivoltine and univoltine members of this group.

Baker (1972) found in the pupae of Pieris rapae that selection acted on both the choice of pupation site and on the matching between pupal color and background color. Also, Clarke and Sheppard (1972) suggested that in those species where the pupation site chosen by a larvae is not correlated with that chosen by its parents, selection will tend to favor the evolution of pupal color dimorphism.

Clarke and Sheppard (1972) have shown in Papilio polytes, a dimorphic species, that there is genetic variation in choice of

pupation site and that this variation can be selected upon, resulting in an alteration in the choice of pupation site. The data presented in this thesis show that there is genetic variation in the ability to modify pupal color in Papilio polyxenes. It therefore follows that there should be little additive genetic variation in the choice of a pupation site in a monomorphic species such as Papilio glaucus, since there should be a correlation in choice of pupation site from parent to offspring, and since strong selection should operate on the choice of a pupation site.

SUMMARY

It has been suggested that environmentally controlled polymorphisms may evolve under conditions where there is a mosaic of distinct environments, and where the distribution of individuals in these environments is not correlated with that of their parents (Sheppard, 1967).

It has been shown that the major stimulus responsible for the pupal color dimorphism in the swallowtail butterfly Papilio polyxenes is the texture of the pupation surface, with rough surfaces stimulating the production of a high frequency of the brown morph and smooth surfaces stimulating a high frequency of the green morph (West and Hazel, unpublished).

It has also been shown that in the absence of an appropriate stimulus a pupa of Papilio xuthus will be green (Hidaka, 1961a, 1961b).

When 23 broods of Papilio polyxenes were surveyed for their ability to produce brown pupae under controlled conditions, it was found that there was considerable variation among broods in the frequencies of brown pupae produced. Selection experiments were then conducted under controlled conditions. Individuals of like pupal color were considered to be genetically more similar than those with unlike pupal color. Thus, the criterion of selection was pupal color. Selection resulted in an alteration of the frequencies of the pupal morphs so that there was an increased frequency of brown morphs in the lines selected for brown pupal color, and an increased frequency of the green morphs in most of the lines selected for green pupal color.

From the results of the survey of variation and the selection experiment it is concluded that the genetic basis of the environmentally controlled pupal color dimorphism in Papilio polyxenes is of the threshold type described by Falconer (1965) for certain diseases. Underlying the two pupal color phenotypes is genetic variation in the ability to produce a brown pupa, a trait designated sensitivity. There is a threshold at some point in the distribution of genotypes, so that with controlled stimulation all individuals with genotypes giving them a sensitivity to the more sensitive side of the threshold will produce brown pupae, while those individuals with a sensitivity to the less sensitive side of the threshold will produce green pupae.

It is concluded that in the wild natural selection on the basis of background matching maintains the sensitivity at the optimum level for the habitat by eliminating the extremely sensitive and extremely insensitive individuals. However, because natural selection acts on background matching, it may never completely eliminate these extremes, thus maintaining genetic variation in sensitivity and thereby allowing some mismatches of pupal and background color.

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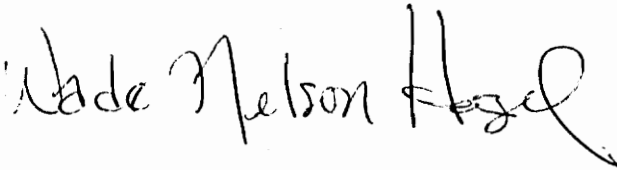
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A handwritten signature in cursive script that reads "Wade Nelson Hazel". The signature is written in dark ink and is positioned in the lower half of the page.

THE GENETIC BASIS OF PUPAL COLOR DIMORPHISM IN PAPILIO POLYXENES
AND ITS MAINTENANCE BY NATURAL SELECTION

by

Wade N. Hazel

ABSTRACT

A survey of the genetic variation in the ability to produce brown pupae was conducted using 23 broods reared from wild caught females. In addition a selection experiment was designed to investigate the genetic basis of the dimorphism. It was concluded that the genetic basis of the dimorphism is a threshold trait with quantitative genetic variation underlying the two phenotypes of green and brown pupal color. It was further concluded that the dimorphism is maintained in nature by weak stabilizing selection, thus explaining the occurrence of mismatches in pupal and background color.