Pupal Color Dimorphism and its Environmental Control in *Papilio polyxenes asterius* Stoll (Lepidoptera: Papilionidae)

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Abstract: The color dimorphism of pupae of the swallowtail butterfly *Papilio polyxenes* is thought to be an adaptation to variable surroundings in which the insect pupates. Laboratory experiments suggest that pupal coloration is determined by an interaction between photoperiod during larval development and background color immediately before and during pupation. Short photoperiod evokes brown pupal color regardless of background, a result consistent with the situation in nature, where short photoperiod presages a long overwintering period. Long photoperiod permits flexibility of pupal color development, allowing green or brown pupal color depending on the background color, or on the intensity of light reaching the underside of the larva just before pupation. Some "mistakes" occur, and the failure of natural selection to have eliminated all such inappropriate responses may be due to their selective advantage in some years.

DISCUSSION

The pupae of many species of Lepidoptera, especially butterflies, are variable or polymorphic in color, often matching to a high degree the color of the substrate to which they are attached. The concealing coloration has variously been attributed to a direct adaptation of the developing pupa to its background, or to a developmental response triggered by temperature, humidity, or photoperiod during late larval or prepupal life (Poulton, 1890, 1892; Brecher, 1921; Ford, 1953; Sheppard, 1958; Wiltshire, 1958; Hidaka, 1961b; Clarke and Sheppard, 1972). The pupae of swallowtail butterflies are often dimorphic, being either green or brown in the North American species *Battus philenor* (L.), *Papilio troilus* L., *P. polyxenes*, *P. brevicauda* Saunders, *P. bairdii* Edwards, and *Graphium marcellus* (Cramer), among others (Forbes, 1960; our unpublished observations), in *P. machaon* L. of Europe (Clarke, 1954; Cribb, 1970) and

in *P. protenor demetrius* Cr. of Japan (Ohnishi and Hidaka, 1956), while the Japanese species *P. xuthus* has an orange form as well (Ishizaki and Kato, 1956).

Although a photoperiodic influence could act over the whole of the larval period (about two weeks in *P. polyxenes*), a larva chooses its attachment site only about a day before pupation. If background color is to influence pupal color it must act rapidly. There is no evidence of a genetic basis to the color dimorphism in *P. machaon* (Clarke, 1954) or in *P. polytes* (Clarke and Sheppard, 1972).

The proximate factor responsible for brown pupal color in *P. xuthus* (Hidaka, 1961*a*) is the action of a hormone released by the prothoracic ganglion during the prepupal period. Without this hormone, the release of which is controlled by stimuli mediated by the brain, a pupa will be green.

The two experiments reported here attempted to identify the environmental factors responsible for determining pupal coloration in the North American species *P. polyxenes*. This swallowtail has two or three broods between April and October in the central Appalachians and overwinters as pupae. The larvae are thus exposed to two contrasting sets of environmental variables: long day length and the availability of food plant after a short pupation (May–August); and shorter day length and an uncertain availability or absence of food plant except after a long pupation (September, October). Furthermore, overwintering pupae are exposed to predation against brown or gray backgrounds, while summer pupae are usually in green surroundings, but may have green or brown immediate backgrounds. Considering how poorly camouflaged a green pupa is against the backgrounds of winter, while in summer such a pupa would often be more cryptic than would a brown one, we expected to find some environmental factor(s) that would trigger development of the appropriate color, whether the factor be temperature or photoperiod, or perhaps the color of the substrate to which the prepupal insect attaches. This expectation is based on the assumption that background matching is of selective value. Although this seems the simplest assumption, there is in fact very little direct evidence (e.g., Hidaka, Kimura and Onosaka, 1959).

In Experiment 1 we tested the effects on pupal color of photoperiod and temperature, using ranges of both that were comparable to, or slightly more extreme than, those encountered in nature. Stimulated by Prof. Sheppard's observations in *B. philenor* (Clarke and Sheppard, 1972), we tested the effect of substrate color during the prepupal period in Experiment 2.

MATERIALS AND METHODS

Experiment 1. The eggs came from three females, two of them (681-1 and 681-2) sibs from a brood reared in variable conditions in October 1968. That brood contained 19 green and 29 brown pupae. Female 681-1 came from a green pupa, 681-2 from a brown one. Both females were hand-paired to one

Table 1. Experiment 1: effects of temperature and photoperiod on pupal coloration in *Papilio polyxenes*.

			Pupal Color	
Photoperiod	Temperature	Female Parent	green	brown
		681-1	15	0
	Warm	681-2	15	0
		691	15	0
Long				
		681-1	8	0
	Cool	681-2	16	2
		691	20	0
		681-1	2	13
	Warm	681-2	2	13
		691	2	13
Short				
		681-1	0	15
	Cool	681-2	0	16
		691	0	15

wild-caught male (method of Clarke and Sheppard, 1956). The third female (691) came from the wild after insemination in May 1969, and all materials originated from Montgomery and Giles Counties, Virginia. At this latitude day length reaches nearly 15 hours in June and is about 11 hours in mid-October.

Within three days of hatching we distributed the larvae haphazardly to the four treatments. The larvae were kept in round, clear plastic dishes (15 × 3.8 cm) with tight lids and filter paper bottom liners and were given fresh leaves of wild carrot (*Daucus carota* L.) in abundance daily. We kept 15 larvae per dish at first but thinned to 5 per dish in the last two larval instars. The dishes were sterilized every other day in dilute sodium hypochlorite solution. Few larvae died in the warm treatments, but as many as a third of those in the cool treatments died in late larval life or as prepupae. They were not obviously diseased but seemed to have suffered some sort of arrested development because of the cool temperature.

Our treatments were of two photoperiods, Long (16 hr) and Short (8 hr), and of two temperature ranges, Warm (18°C night, 29°C day) and Cool (7°C night, 18°C day); all larvae, however, were on the same daily temperature cycle, i.e., 16 hr high, 8 hr low, the larvae on short days being placed in lighttight boxes during their "night." The humidity of the dishes was not controlled but was always close to saturation because of the fresh food plant and the tight lids. We used Percival environmental chambers, illuminated by eight 40-watt cool white high-output fluorescent lamps and four 25-watt incandescent lamps. As an insect pupated it was scored and removed.

Experiment 2. Eggs were collected from three wild-caught females taken in Montgomery Co., Va., in May 1970, and all larvae were reared in the Warm temperature regime used in Experiment 1, but the temperature and day-night cycles were synchronous. Other conditions were essentially the same as those in Experiment 1. When a larva started to wander in late 5th instar it was removed from the rearing dish and assigned for pupation randomly to one of the treatment canisters. These were round, clear plastic containers (18 × 18 cm) with fairly tight opaque lids. They contained either green twigs, brown twigs, a green log, or a brown log, the green objects being spray-painted flat forest-green and aged in the sun for a few days. The brown objects were left natural, and all had intact bark. The logs were 6 to 8 cm in diameter, 15 to 18 cm long, of black locust or apple; the twigs were about 0.5 cm in diameter, generally branched, and of wild cherry. Some food had to be added for a last feeding, but the larvae soon settled on pupation sites, either on the objects or on the walls of the canisters. Pupae were scored and removed to plastic dishes in the same environmental chamber in which they had been kept throughout the experiment. Some of the larvae were left in the rearing dishes for a direct comparison with the results of Experiment 1.

RESULTS AND DISCUSSION

Effect of temperature and photoperiod. Experiment 1 (Table 1) suggests an overwhelming importance of photoperiod, with all three broods responding in the same way, and Long day length evoking predominantly green pupae. Using the percent green pupae, transformed by the method of Mosteller and Youtz (1961), an analysis of variance and the F-test reveals a weak interaction between temperature and photoperiod (P just less than 0.05); but taking the two temperatures separately and testing by chi square, there is a highly significant effect of photoperiod in each temperature range (P < 0.001 in each). In Experiment 2 (Table 2) the comparable effect of photoperiod can be tested for those pupae forming on canister walls and in the rearing boxes. The influence of day length is again striking ($\chi^2_1 = 48.7$; P < 0.001). On Short days the responses on the clear plastic backgrounds are homogeneous in the two experiments (Fisher's exact test, P = 0.46), with nearly all pupae being brown. On Long days, however, the proportion of green pupae was slightly higher in Experiment 1 than in Experiment 2 (Fisher's exact test, P = 0.045).

Effect of background. In Experiment 2 (Table 2) nearly all larvae on short days developed into brown pupae (68/73) whatever the background of pupation. On Long days, however, there was a striking difference between those pupating on clear plastic dishes and canister walls or slender twigs on the one hand (60 green:13 brown), and on logs on the other (no green:13 brown). There is homogeneity between twig colors (Fisher's exact test, P = 0.22) and between

Table 2. Experiment 2: effects of photoperiod and background color on pupal coloration in *Papilio polyxenes*.

Photoperiod	Background	Pupal Color		
		green		brown
Long	Green twigs	10		1
	Brown twigs	14		8
	Green log	0		1
	Brown log	0		12
	Canisters	29		2
	Rearing dishes	7		2
Short	Green twigs	0		6
	Brown twigs	0		11
	Green log	0	1*	15
	Brown log	1		4
	Canisters	0		7
	Rearing dishes	2	1*	25

* Intermediate color.

twigs and clear plastic ($\chi^2_1 = 2.6$; $P > 0.10$). Pooling the results on plastic and on twigs, and comparing them to the results on logs, reveals a strong difference ($\chi^2_1 = 31.6$, $P < 0.001$). The similarity of results on clear plastic and on twigs may be accounted for by the importance of bright light reaching the undersides; on plastic or on slender twigs much light comes through, or by, the substrate. There is, in fact, a suggestion that brown twigs stimulated a slightly larger proportion of brown pupae than did green twigs. It may be that the effective background of a pupa on a slender twig is not the twig itself (see below). Clarke and Sheppard (1972) found in *Battus philenor* that twigs under 12 mm in diameter influenced pupal color in favor of that matching twig color, but brown twigs did so only to a degree. The twigs in our experiments were all considerably thinner than 12 mm, but Clarke and Sheppard do not give a lower limit to twig size, so that the question of the importance of light passing by the twig to the underside of the prepupa must remain open.

Although nearly all pupae that formed after exposure to Short day length were brown, there was a great deal of variation in the shade of brown, and some pupae had greenish patches; in fact there was often an excellent match to the human eye between the shade of brown of a pupa and that of its immediate background. This was in marked contrast to brown pupae formed after Long photoperiod, in which the shade of brown was nearly uniform.

Pupal color and diapause. Among pupae forming after Long photoperiod there were 60 green and 26 brown; for those eclosions that were recorded, 42 green

and 13 brown pupae completed development after a short pupation (7 to 13 days, with a mean of 9.3 days). For the remainder, precise eclosion times were not recorded, and some died, but none entered diapause. The proportions of the colors in these two groups are the same ($\chi^2_1 = 0.43$; P > 0.50). Among pupae formed after Short photoperiod only 2 eclosed after brief pupation, and both were green. The only other green pupa evidently died, and the remaining 68 brown or intermediate pupae entered diapause and had not eclosed a month after pupation. In *P. xuthus* (Ishizaki and Kato, 1956), the orange form of brown always enters diapause, while the other forms of brown and green usually do not. Thus in these two species there seems to be a diapause phenotype, orange in *P. xuthus* and variable brown in *P. polyxenes*. In our experiments green pupae did not enter diapause, nor did brown pupae that formed under "summer" photoperiod; the only pupae that entered diapause were brown ones reared in "autumn" photoperiod.

These results suggest a complex adaptive response of larvae about to pupate; in autumn Short day length is overriding and stimulates the development of brown pupae regardless of background color, but the insects are able to vary the shade of brown. In midsummer, Long day length permits the expression of a brown-green alternative, but there is little flexibility in the development of the shade of brown. (In fact, we cannot say whether it is day length or night length that is critical.) The selective advantage of brown pupae in autumn presumably comes from their long exposure, against dull backgrounds, to predators during the overwintering diapause. In midsummer, however, the backgrounds are more varied and the duration of pupation short. Selection might be expected to favor a matching to immediate background, since the background will not change over the 10-day pupal period, but there may be little to gain, from a selective point of view, in matching the shade of that background on which an insect pupates.

There remains the problem of those insects that make "mistakes." Three pupae out of 73 that were reared on Short photoperiod were green. In fact, as pointed out above, these "mistakes" did not enter diapause. Clarke and Sheppard (1972) suggested that such mistakes may be favored by apostatic selection. The results of experiments with artificial baits support this suggestion (Allen and Clarke, 1968). In addition, in *P. polyxenes*, the success of these late season "mistakes" will vary from year to year, but in some seasons the insects will be able to complete another generation before winter. Thus environmental variation may promote the maintenance of Short day length "mistakes" in *P. polyxenes*.

The corresponding "mistakes" among pupae formed after Long photoperiod raise the question of cryptic coloration in a swallowtail or other butterfly pupa. A green pupa on a green leaf is clearly "protected," as is a brown one on a brown tree trunk. On slender stems, however, the value of matching the color of the stem is less obvious, since the background against which a predator views

a pupa may be more the mass of surrounding vegetation than the stem itself. For example, resemblance to a green leaf, even on a brown stem, may lower the risk of predation under some circumstances, but until the predators are identified this must be speculative. As suggested above, it may be the intensity of light reaching the underside of a prepupa that determines whether or not it develops into a green or brown pupa, but this must also remain a speculation until the critical experiments have been done.

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