

ENVIRONMENTALLY INDUCED COLOR POLYMORPHISM IN  
CATERPILLARS OF *BISTON BETULARIA COGNATARIA*

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## ABSTRACT

A developmental color polymorphism occurs in the larvae of *Biston betularia cognataria*, the American subspecies of the peppered moth. These larvae derive protection from predators by resembling twigs of the food plants on which they feed. They range in color from bright green to dark brown affording them an opportunity to utilize many types of food plants and still benefit by imitating the color of the plant's twigs. This study is an attempt to determine the environmental cue or cues which induce the development of body color in the larvae. By manipulating diet and background colors, it was shown that visual cues are the major factors determining caterpillars' body colors, and that diet is a minor contributing factor.

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## INTRODUCTION

Color polymorphisms have been known in caterpillars for at least two centuries (Harris, 1766). It is especially well documented that a member of the family Geometridae, *Biston betularia*, exhibits striking color polymorphisms in the larval stages (Dimmock, 1888; Poulton, 1892). Larval color can range from bright green to dark brown and has no subsequent effect on the color of pupae or adults. These caterpillars apparently derive protection from predators by their resemblance to twigs of the food plants on which they feed (DeRuiter, 1952). They normally remain motionless throughout the day while attached, camouflaged, to a twig, and at night move about while feeding. The ability to be green, brown, or various shades in between affords an opportunity for the larvae to utilize many types of food plants and still benefit by imitating the color of the plant's twigs.

Much work was done in this area in the late 19th century by E. B. Poulton (1884, 1885, 1890, 1892) who studied both larvae and pupae of many species of lepidopterans. The family Geometridae appears to have the most sensitive and variable larvae with regard to color polymorphism, and of these *Biston betularia* appears to be the most versatile. There is no evidence to suggest in this species that caterpillar color in

one generation has any effect on the color of caterpillars in subsequent generations; that is, it does not appear to be genetically determined. Instead, the color of individual caterpillars is developmentally induced. Based on extensive studies, Poulton concluded that the phenotypic plasticity in development of several lepidopteran larvae he examined is induced by phytoscopic effects (visual stimuli) and not related to diet. This general conclusion has been confirmed for hawkmoth larvae (Sphingidae) by Grayson and Edmunds (1989) who add that for certain color morphs brood differences suggest genetic influence.

In a different study using another geometrid species, *Nemoria arizonaria*, Greene (1989) determined that a seasonal dimorphism in color and morphology was induced by seasonal differences in diet related to materials in ingested leaves and catkins of the host plant. Greene's results differ from Poulton's findings; and, perhaps unaware of that early work, Greene failed to consider the possibility of visual cues in contributing to the development of cryptic form in caterpillars.

As independent workers reached separate conclusions about environmental factors promoting larval polymorphisms within different geometrid species, the purpose of this work is to examine more closely the roles of both vision and diet as stimuli initiating color development. Various background colors and foods were manipulated to determine their effects

on the body colors developed by caterpillars of the geometrid, *Biston betularia cognataria*, a North American subspecies of the peppered moth.

## PROCEDURES AND RESULTS

*Biston betularia* is widespread throughout the Northern Hemisphere, including the United States, Japan, and Great Britain. Larvae of the North American subspecies, *Biston betularia cognataria*, were used in this study. The caterpillars were produced by a breeding stock which was established from adult moths captured during the summer of 1990 at the Mountain Lake Biological Station in southwestern Virginia. Newly hatched larvae were available in continuous supply from March to November of 1991. The female lays hundreds of eggs following a single mating. Because of the large number of progeny it is usually easy to have all siblings of about identical age available for experimental treatments.

Immediately following hatching from eggs, larvae from the same brood were placed in a clear plastic container (4 liters) with crab-apple (*Malus sp.*) leaves for food. Within a week they were transferred to experimental environments. All caterpillars used in different treatments of the same experiment were generally from the same brood, or when too few from a single brood were available, equal numbers from different broods were apportioned to the treatments to

eliminate effects of broods. The caterpillars were provisioned with fresh leaves daily throughout the experimental period, and within given treatments diet was restricted to a single food source; namely, crab-apple, willow (*Salix sp.*), or birch (*Betula nigra*). In certain experiments leaves were stripped from the branches, and in others the leaves remained attached. Except where noted, all experiments were performed in a humidified laboratory with overhead fluorescent lighting set on a 12 hour L:D cycle.

After four to five weeks of development in controlled surroundings, the caterpillars were visually evaluated for body color. Scoring was done by assigning individual caterpillars as "best fits" to one of four categories: 1 = bright green, 2 = green intermediate, 3 = brown intermediate, and 4 = very dark brown (See Plate I). To assess subjectivity in assigning categories, two people generally worked together to ensure that the scoring was consistent. In all, nearly three thousand caterpillars were assigned to one of the four categories.

### *Experiments*

#### 1. Intact Host Plants

To determine the extent to which *cognataria* responds to the experimental host plants selected for this study,

caterpillars were placed in two grey plastic trash barrels (128 liters); one containing willow branches with attached leaves and the other containing birch branches with attached leaves. The tops of the barrels were covered with black muslin cloth to keep larvae from escaping. Results show a very highly significant difference between the distributions of body color phenotypes among the caterpillars raised on intact willow compared to those raised on intact birch (Table 1, Figure 1,  $G = 744.64$ ,  $p < .001$ ). This experiment does not, however, indicate whether ingested materials or visual cues trigger the pronounced differences in body color development.

## 2. Effect of Branches

To test the effect of different color backgrounds on body color development, food was controlled. In both treatments all caterpillars were fed only crab-apple leaves from the same tree. Two yellow waste baskets (48 liters) covered with white muslin were used. In one container green willow and bamboo branches stripped of leaves were used to produce a green environment. In a second container brown birch branches stripped of leaves were used to produce a dark brown background. Results show that even with exactly the same diet there is again a very highly significant difference in the distribution of body colors of the caterpillars between the treatments (Table 2, Figure 2,  $G = 501.70$ ,  $p < .001$ ). These

results indicate that phytoscopy might be a major factor determining larval color in *cognataria*.

### 3. Artificial Backgrounds

The next experiment was similiar to the previous one except an attempt was made to simulate green and brown surroundings by using colored pipe cleaners and containers. This method eliminates any effect the real branches might have had on the caterpillars' colors; that is, perhaps tactile or chemical cues rather than strictly visual cues could have confounded previous experiments. Here, green pipe cleaners were placed in a yellow plastic wash tub (20 liters) which was covered with white muslin to create a bright environment, and brown pipe cleaners were placed in a brown plastic tub (identical dimensions) covered with black muslin to produce a dark enclosure. Both of the treatments were fed *only* crab-apple leaves. The G-test once again shows a very highly significant difference between the treatments using colored artificial branches (Table 3, Figure 3,  $G = 214.30$ ,  $p < .001$ ). Nearly 90 % of the caterpillars raised in the brown containers were brown (phenotype 4), and none were green. This distribution contrasts sharply with the wide range of phenotypes developed by caterpillars in the bright environment.

#### 4. Reflectance or Hue

In the first three experiments, the visual cues provided were attempts to simulate natural surroundings by using green and brown branches, both real and artificial. However, it was not clear whether the caterpillars were responding to actual differences in hue, requiring color discrimination, or simply differences in background reflectance, i.e., dark versus light. To test whether green or brown color phases result from a caterpillar's experience in light versus dark backgrounds, this next experiment compares caterpillar responses to black versus white environments.

Again, the caterpillars were fed only crab-apple leaves stripped from their branches. Half the caterpillars were placed in a white plastic waste basket covered with white muslin and the other caterpillars were placed in black plastic waste baskets covered with black muslin. The results are presented in Table 4 and Figure 4. The number 1 phenotype in this experiment was not bright green as in the preceding experiments but, instead, a pale grey closer to white than to green, and, the number 4 phenotype was nearly black rather than brown. There was a highly significant difference in the distribution of body color phenotypes between the two treatments ( $G = 61.26, p < .001$ ). Although this experiment used many fewer caterpillars than the previous three, it is clear that a caterpillar's ability to mimic the color of its

host tree's branches is based on actual color discrimination rather than simpler responses to light or dark surfaces.

## 5. Effect of Diet

While experiments 2 through 4 clearly demonstrate that visual stimuli can trigger body color differentiation among caterpillars raised on a single food source, the following experiments were designed to test the effects of different diets on caterpillars deprived of visual cues; that is, diet was the only factor varied.

To prevent color vision from having an effect, the experiments were conducted in nearly total darkness. In the first experiment (5-A) the caterpillars were raised in a dark room. A photographer's red lamp was used for illumination during brief periods, normally lasting only five to ten minutes a day, while new leaves were added to the culture barrels. It later became apparent that the caterpillars could see during these brief periods as they exhibited positive phototaxis directed toward the red light source. To eliminate visual cues altogether a second experiment (5-B) was run in complete darkness throughout, without any light source used at any time. The experiments were set up with two grey trash barrels (128 liters) with black muslin covering the tops. In 5-B, lids were also placed on the barrels to further reduce any chance of light entering experimental enclosures. One

barrel received willow branches with leaves intact and the other barrel received birch branches with leaves intact. The results from these two experiments shown in Table 5 are very different. All 63 caterpillars on willow, under red light, were invariably bright green. Those on birch, under red light, showed a range of variation, perhaps as a result of visual cues picked up during the brief periods when the red light was turned on. In total darkness however, both groups showed a wide range of body color phenotypes, yet the willow fed group was shifted more strongly toward green, and the birch fed group was closer to the brown end of the color scale (Figure 5). In fact the difference between the phenotypic distributions was surprisingly even more pronounced in experiment B (total darkness) than experiment A (red-light).

Experiment 5-B indicated non-visual cues contribute to color differentiation; however, it remains unclear whether actual ingested materials or tactile cues induce developmental responses. In an attempt to minimize possible confounding effects of textural differences between birch and willow branches and leaves, the following experiment was designed to assess the effect of ingested materials on color differentiation. To ensure uniform visual and textural experiences, the caterpillars were maintained in six identical clear plastic containers (4 liters) placed within a larger white waste basket. Three of the groups were fed willow leaves stripped from branches, shredded, and suspended on

clear polyethylene nets. The other three groups were fed birch leaves stripped from branches, shredded, and suspended on nets. The results, pooled within treatments, are presented in Table 6 and Figure 6. The distribution of body colors between willow-fed and birch-fed caterpillars are significantly different ( $G = 9.79, p < .025$ ), suggesting that diet, too, is a factor in color determination. However, because even the shredded leaves differ in color and texture, particularly as they dry out after being stripped from their branches, this conclusion must be accepted with caution.

#### 6. Interaction of Diet and Visual Cues

Because vision plays a major role in how a caterpillar responds developmentally to its surroundings, and that diet also appears to have some effect, the following experiment was conducted to test for possible synergistic interactions between diet and visual cues. Ten waste baskets, six white and four black, were used in this experiment. A clear plastic container (4 liters) was placed inside the black or white containers giving the caterpillars a view of the white or black background. The caterpillars in three of the white tubs and two of the black tubs were fed willow leaves which were stripped from branches, and the remaining black and white tubs were provisioned with birch leaves stripped from their branches. The results of this experiment are shown in Table

7 along with statistical comparisons between treatments by G-tests. A two-way ANOVA does not indicate an interaction between color and leaf source, but each treatment taken singly (i.e., black versus white, or birch versus willow) does confirm that vision and diet each contribute to body color differentiation.

## DISCUSSION

*Biston betularia* adult females lay eggs in large masses. Immediately following hatching, first instar larvae are extremely active, moving rapidly in an inchworm motion. The caterpillars at this stage are very tiny with a body length of only 2 mm and a head width of .5 mm. At this early age they are active during the day as well as at night. They also exhibit strong positive phototaxis (Grant, pers. com.). Thus, in a natural forest environment the caterpillars probably move well into the canopy shortly after hatching.

In the laboratory caterpillars climb to the top branches of their host plants, spin silk threads, and hang individually suspended underneath the leaves. This behavior in a natural setting could result in the young caterpillars being blown by the wind to different trees, and in a mixed deciduous forest a clutch of caterpillars may be distributed widely over a variety of tree species.

After the dispersal phase, the caterpillars become quite sedentary and most of their movements are restricted to nighttime feeding activities. During the daylight hours they remain motionless, and continue this activity pattern throughout larval development until they reach the final instar stage before pupation. Mature larvae may grow to be 60

mm long with head widths approaching 5 mm.

The caterpillars bear a striking resemblance to twigs, and in order to gain effective protection from visual predators the caterpillars must have the proper behavior in addition to appropriate color and morphology. Very small larvae clasp onto the edges of leaves, but as they grow larger they must move back from the leaves onto the petioles, and in later instars they typically attach to twigs of approximately the same diameter as their bodies. They grasp the bark with their hind prolegs, and extend their bodies away from the supporting branch at angles closely mimicking natural twig bifurcations. This posture is actively maintained throughout the day, and, especially for larger larvae, the pose is often supported by a slender silk guy. They also frequently span the interaxil distance separating the main stem from its branch.

The body is also twig-like in form, and the head region is shaped like a terminal leaf scar. In addition, mottling and pigment around the spiracles resemble lenticels along the bark of the host plant. Most relevant to this study is that the body color closely matches the twigs of the particular host plant individual caterpillars feed upon. Tietz (1972) has documented that *Biston betularia* is polyphytophagous in the wild; thus, in the laboratory there are many potentially usable host plants. The caterpillars are capable of ranging in color from pale grey to bright green to shades of brown to

nearly black. This study indicates that the color developed by individual caterpillars is facultative. Such phenotypic plasticity is obviously highly adaptive in maximizing crypsis for a species that feeds on a variety of tree species spanning a wide range of bark coloration. The proximal cues triggering color differentiation appear, according to this study, manifold, involving at least two non-interactive components.

A century ago Poulton (1892) concluded that color vision and not diet was the proximal environmental cue which *Biston betularia* larvae used to regulate body color. It is clear from the several experiments reported here that color vision is, indeed, the major factor in the regulation of color in the *cognataria* subspecies as well. These experiments show that caterpillars placed in containers with various background colors, but fed the same diet, developed different body colors indicating that color vision is involved. Poulton also wrote that caterpillars can only be influenced by a substrate with which they actually make physical contact. His conclusion is not correct. My experiments using transparent containers placed within larger colored containers showed significant effects of surrounding colors even when the caterpillars were kept at a distance of at least one body length from the outer walls. The results, contrary to Poulton's report, demonstrate that actual contact with the stimulus is not a requirement for body color adjustment in this species. Specifically, a white or black background which the caterpillars could only see with

their stemmata but not touch was still effective in promoting body color differentiation.

In addition to assessing the role of visual cues on the facultative development of body colors, this study also indicates that diet is a relevant factor in body color development of *cognataria*. The experiment conducted in total darkness comparing caterpillars fed different foods showed a significant difference between treatments (Table 5B). Thus, even in the absence of any visual cues, different host plants resulted in different distributions in body colors among the larvae. However, while showing an effect of host plant, this experiment should be interpreted with caution because other non-visual factors were not controlled and might have served as cues. For example, perhaps the larvae are capable of responding to difference in texture of the bark between the two tree species, or branch geometry. In experiments designed to eliminate branches altogether in an attempt to control for color, texture, and spatial configurations, caterpillars raised on different diets still showed significant differences in body colors (Tables 6 and 7). While this study certainly confirms that color vision or phytoscopy has a major effect on the color *Biston* caterpillars develop, it is now also clear that diet appears to be a contributing factor.

While one hundred years separate the reports of researchers claiming that vision (Poulton, op. cit.) or diet (Greene, op. cit.) serve as the sole proximal cues in

environmentally induced polymorphisms in those geometrid caterpillars studied, the present research assigns a role for both. It remains for future studies to assess their relative importance in natural environments, and the possible roles of other cues contributing to this remarkable phenotypic plasticity.

TABLE 1

Comparison of color distribution among caterpillars fed willow branches with intact leaves and caterpillars fed birch branches with intact leaves in a simulated natural condition. The score was based on 1 = green, 2 = green intermediate, 3 = brown intermediate, and 4 = brown. The G-statistic tests for significant differences between groups.

Treatment	Score				G-statistic	p*
	1	2	3	4		
Willow fed	152	176	56	20	744.64	<.001
Birch fed	0	15	69	364		

Note: For 0 observations, .00001 was substituted as log transformations are required for this analysis.

# Figure 1

## Intact Host Plants

### Willow vs. Birch

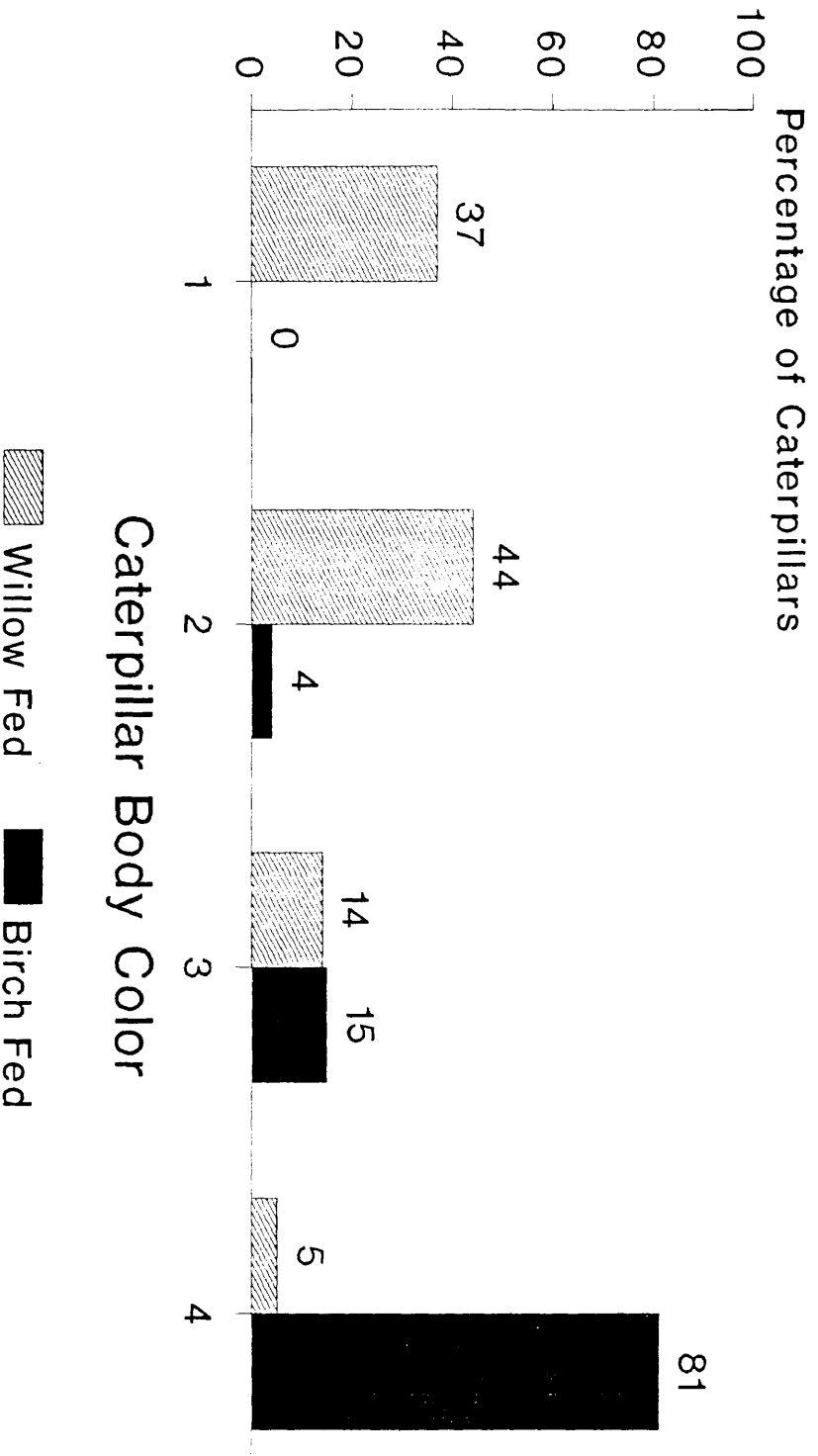


TABLE 2

Results of two treatments of caterpillars both fed only apple leaves; one treatment included green willow/bamboo branches stripped of leaves and the other contained brown birch branches stripped of leaves. The color distributions among the caterpillars in the two treatments differ by the G-test. See text for details.

Treatment	Score				G-statistic	p*
	1	2	3	4		
Willow branches	44	120	139	124	501.70	<.001
Birch branches	0	2	13	420		

# Figure 2

## Effect of Branches

### Apple Fed

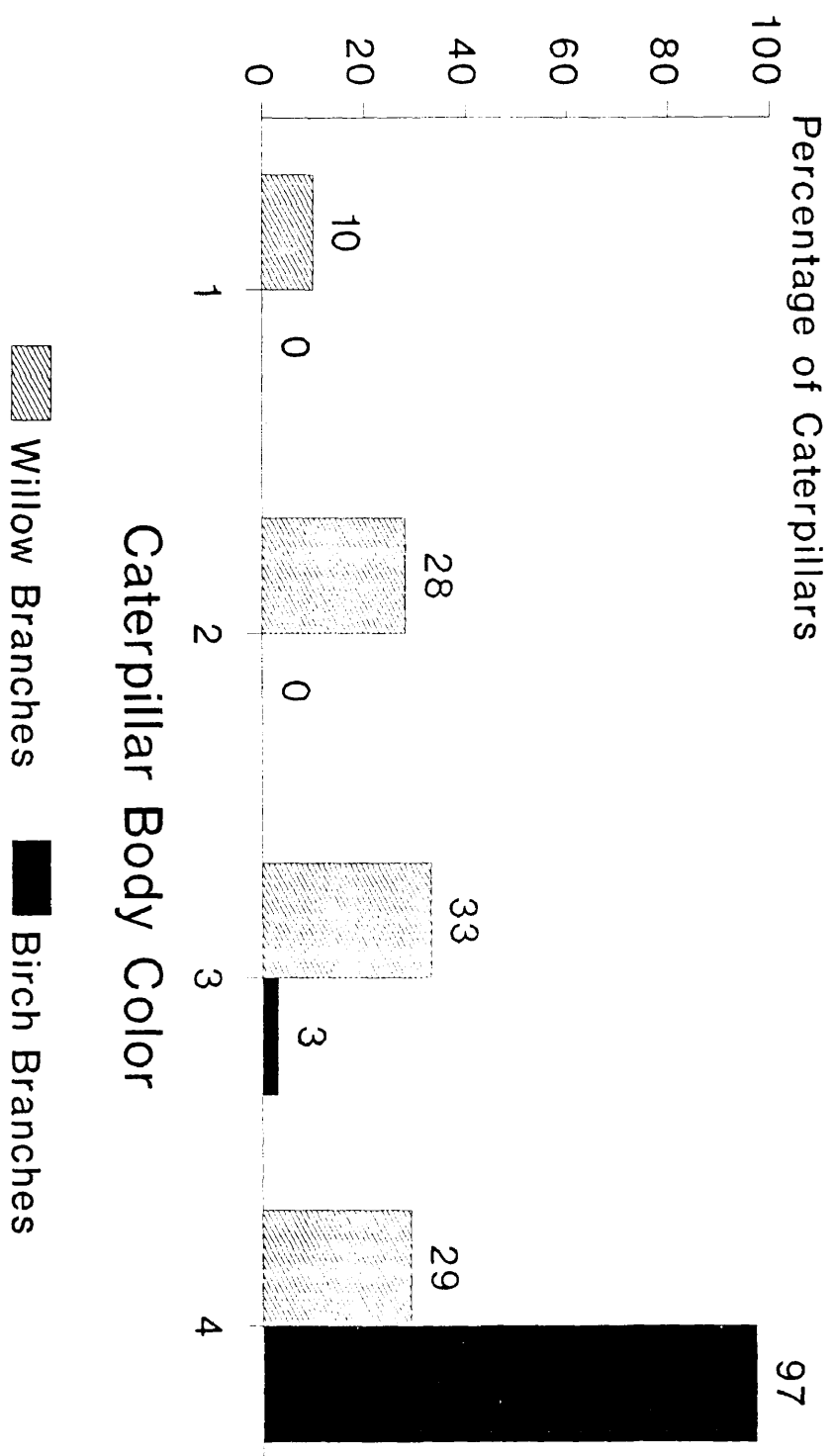


TABLE 3

Effect of brown artificial branches in a brown tub versus green artificial branches in a yellow tub on the color distributions among caterpillars. Both treatments were fed only apple leaves stripped from their branches. The G-statistic tests for significant differences between groups.

Treatment	Score				G-statistic	p*
	1	2	3	4		
Green/Yellow	17	51	55	15	214.30	<.001
Brown/Brown	0	6	16	153		

# Figure 3

## Artificial Branches

### Apple Fed

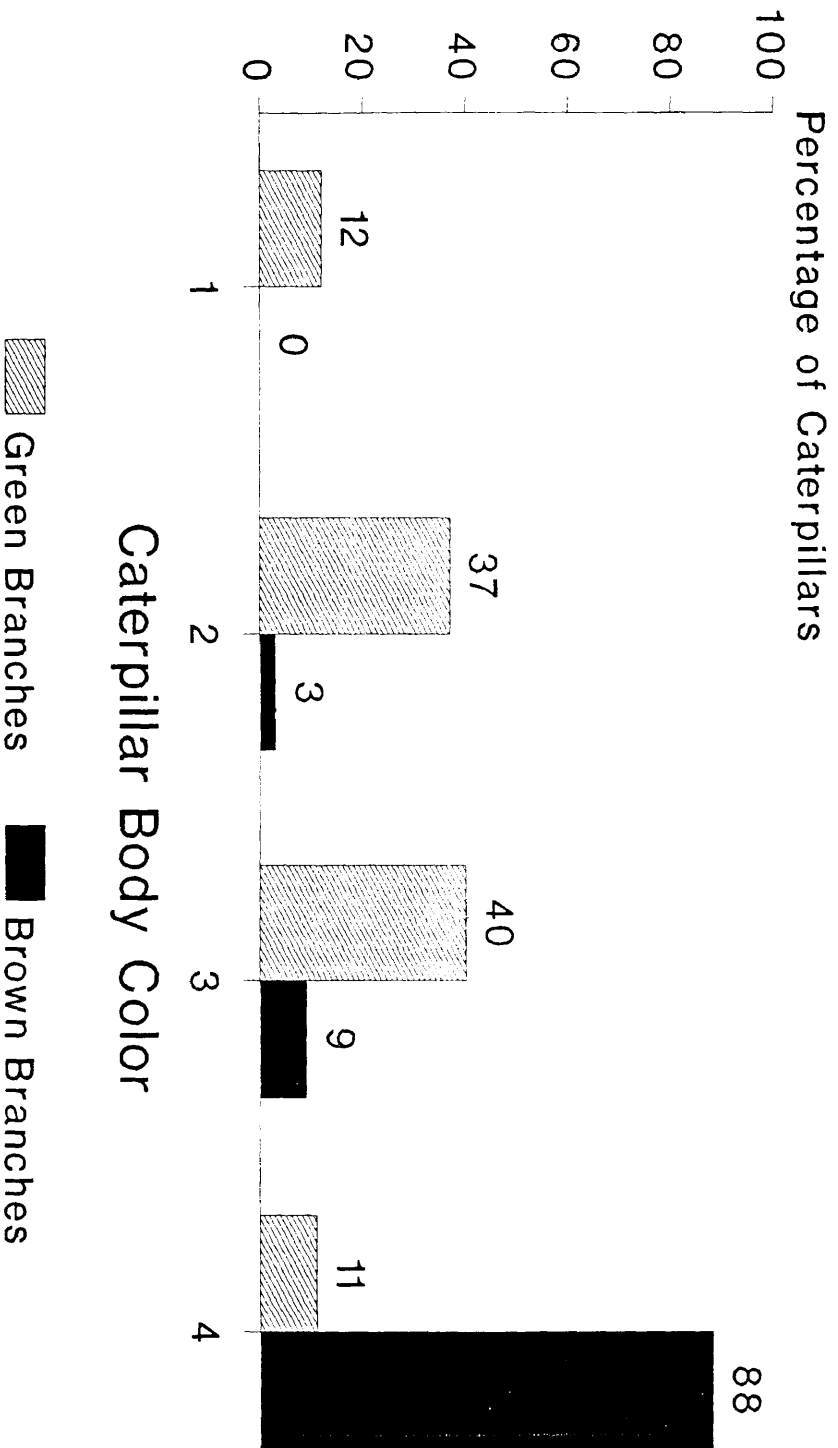


TABLE 4

Comparison of color distributions among caterpillars on white and black backgrounds. Both treatments were fed apple leaves stripped from branches. The G-statistic tests for significant differences between groups.

Treatment	Score				G-statistic	p*
	1	2	3	4		
White	16	6	11	4	61.26	<.001
Black	0	1	2	35		

# Figure 4

## White Versus Black

### Apple Fed

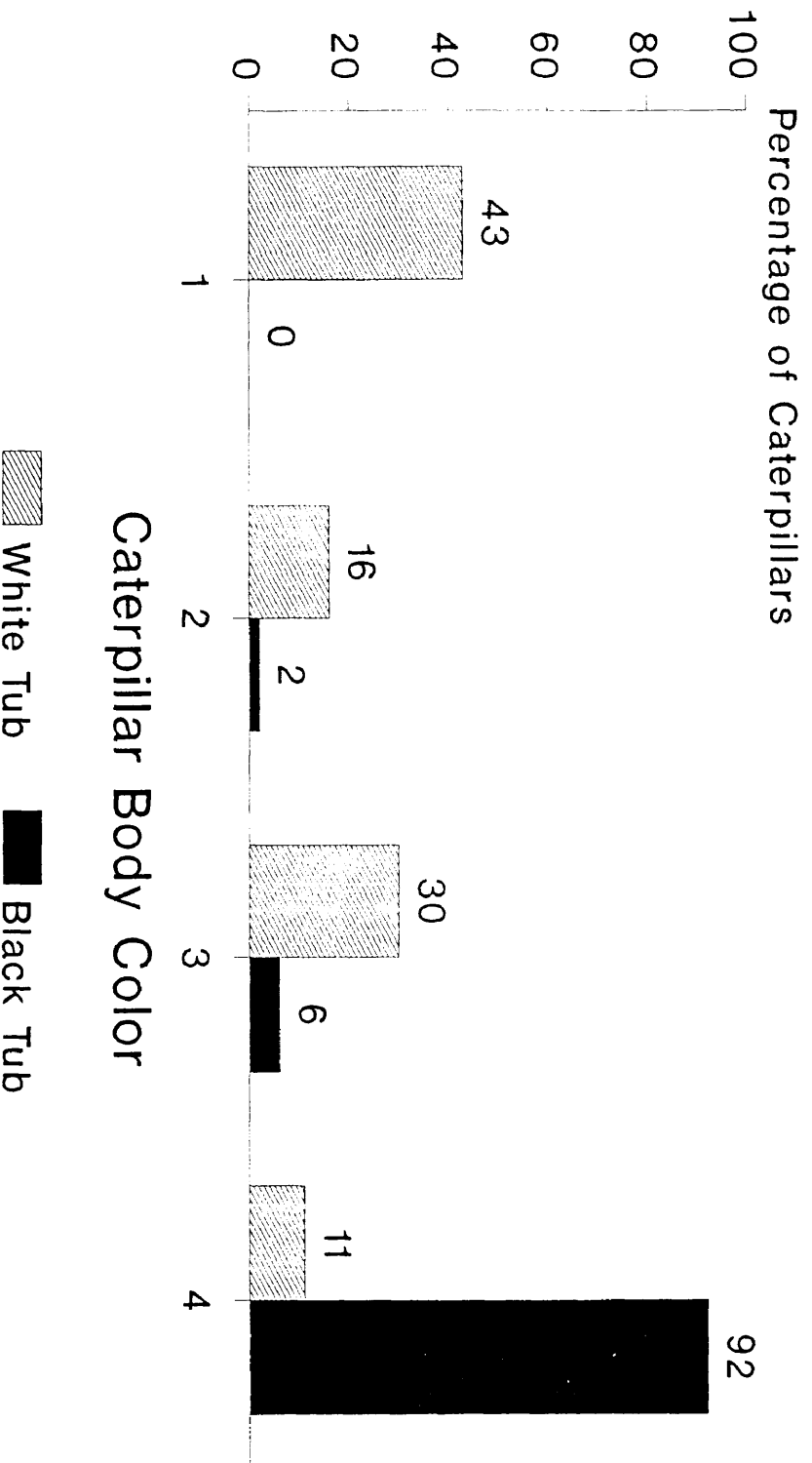


TABLE 5

Comparison of color distribution among caterpillars raised in nearly total darkness with a photographer's red lamp used briefly during feeding (RL), and a comparison of color distribution among caterpillars raised in total darkness (TD). One treatment in each was fed willow branches with intact leaves and the other treatment was fed birch branches with intact leaves. G-statistics test for significant differences between groups in each experiment.

Treatment	Score				G-statistic	p*
	1	2	3	4		
A: Willow fed (RL)	63	0	0	0	39.53	<.001
Birch fed (RL)	56	17	13	1		
B: Willow fed (TD)	93	46	14	2	66.39	<.001
Birch fed (TD)	11	22	38	4		

# Figure 5

## Total Darkness

### Willow vs. Birch

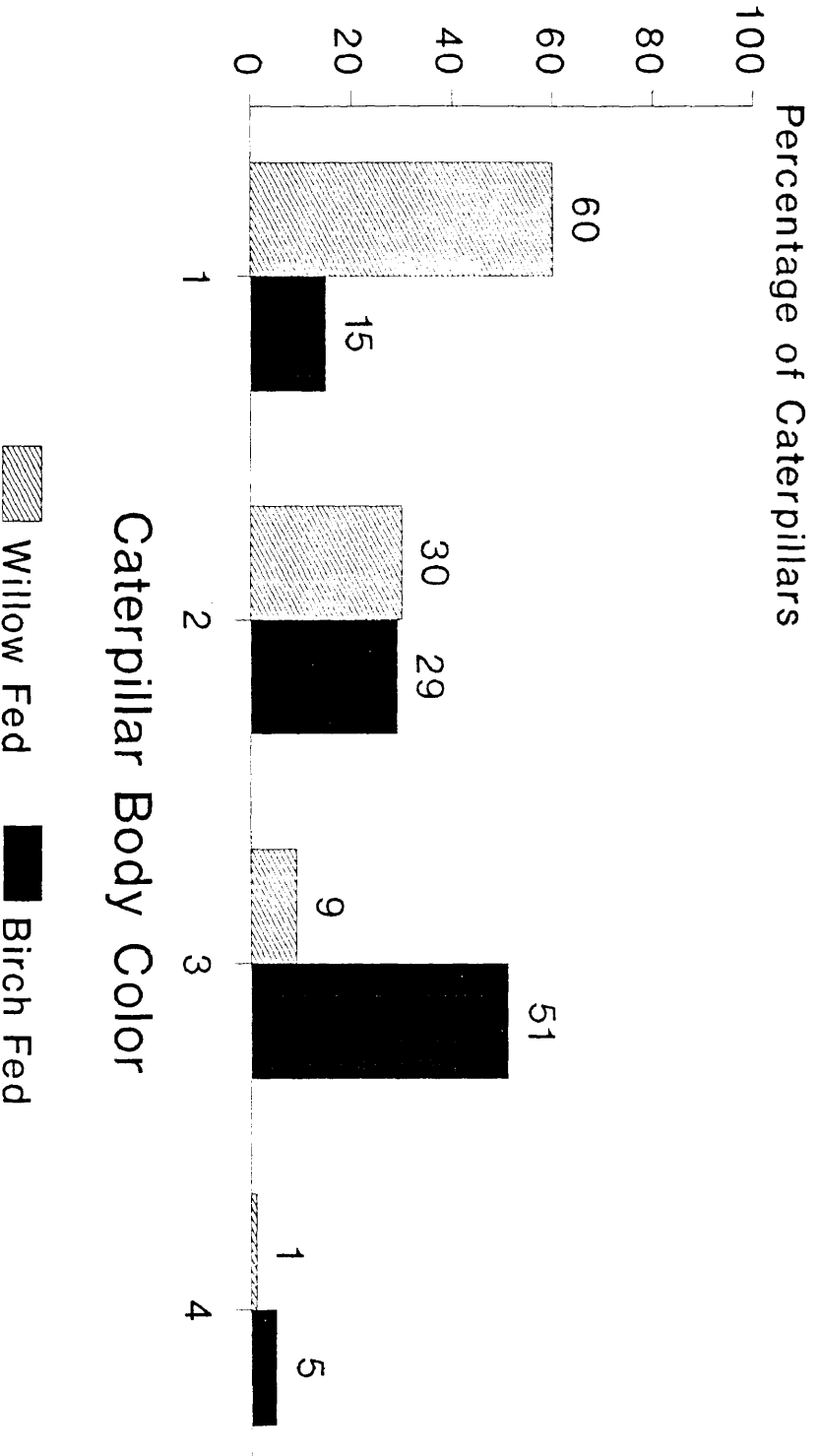


TABLE 6

Comparison of color distributions among caterpillars fed either willow or birch in clear containers placed in larger white tubs. Six containers were used. Three were fed willow and three birch. The data within treatments were pooled (homogeneous by G-test). Shown here the G-test indicates significant differences between treatments.

Treatment	Score				G-statistic	p*
	1	2	3	4		
Willow fed	0	9	36	16	9.79	<.025
Birch fed	0	6	41	47		

# Figure 6

## Effect of Diet

### Willow vs. Birch

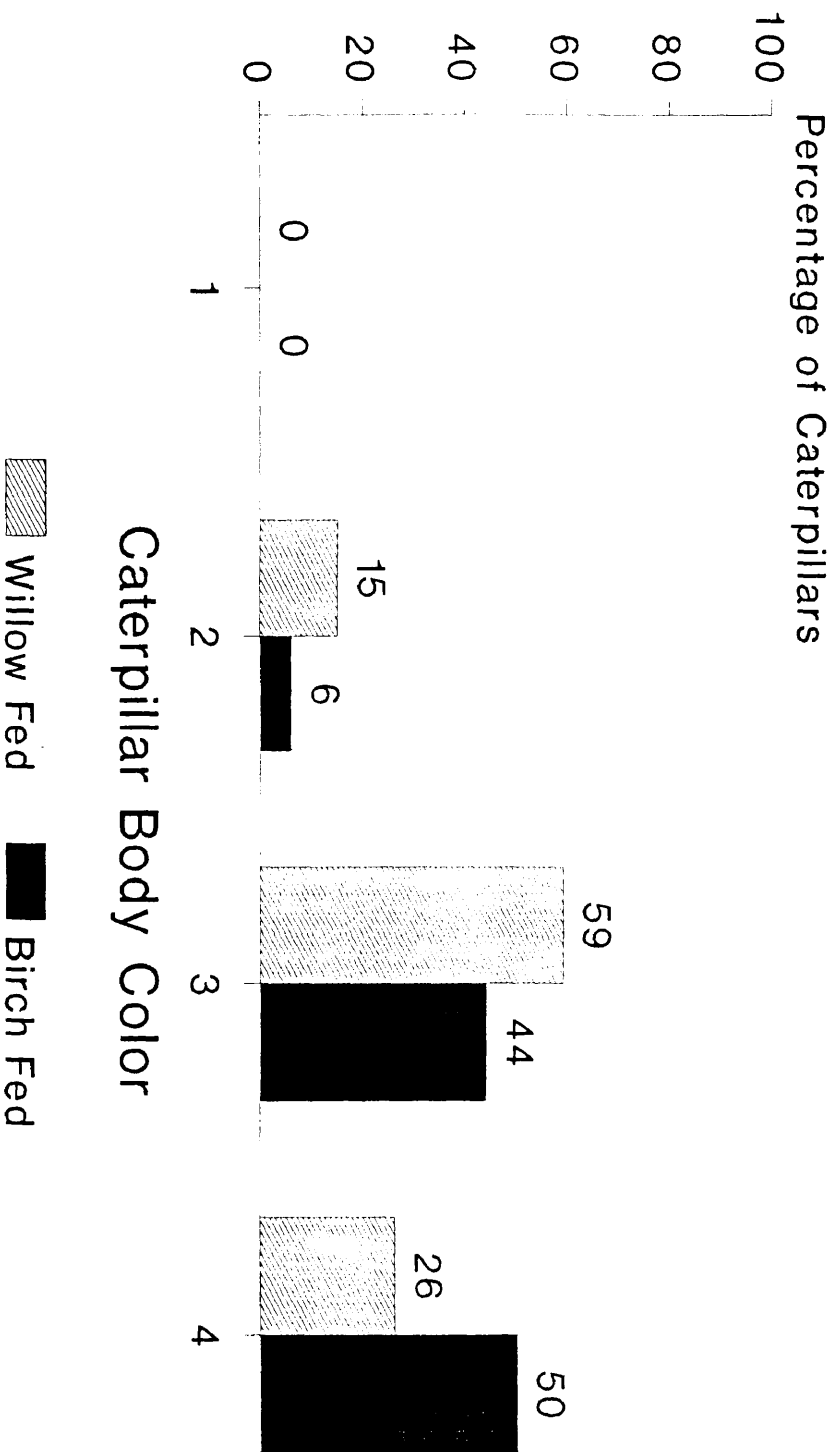


TABLE 7

Comparison of color distributions among caterpillars fed either willow or birch in clear containers placed in larger white or black tubs. Ten containers were used (6 white, 4 black). Half were fed willow and the other half fed birch leaves within each color group. G-tests determined data within treatments were homogeneous, and a Two-Way ANOVA and G-statistic tested for significant differences between the treatments.

Treatment	Score			
	1	2	3	4
White/Willow	24	34	14	0
White/Birch	8	21	26	13
Black/Willow	3	19	21	12
Black/Birch	0	3	15	70

Two-Way ANOVA	DF	SS	F	Probability
Color	1	69.96	125.08	<.001
Food	1	54.72	97.83	<.001
Color by Food	1	.77	1.38	>.241

G-Statistic Tests comparing treatments		
Treatment	G-statistic	p*
White/Willow Black/Willow	38.66	<.001
White/Birch Black/Birch	69.71	<.001
White/Willow White/Birch	33.04	<.001
Black/Willow Black/Birch	55.85	<.001

Plate I. *Biston betularia cognataria* larvae. The caterpillar to the left, posed on willow, is an example of phenotype 1; the one to the right, posed on birch, illustrates phenotype 4.



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