endorsement by the U.S. Department of Agriculture over those not named.

5. The hexane used in these investigations was purified to the equivalent of spectral grade by percolation of reagent-grade hexane through silica gel and subsequent distillation. The ethyl ether was distilled and stored over sodium. All other solvents used were reagent grade.
6. The chromatography was carried out on an F & M Model 500 instrument equipped with a Model 1609 flame ionization attachment with stainless steel columns packed with 5 percent SE-30 on Chromosorb W (3.05 m by 0.31 cm) at 150°C and with 10 percent diethylene glycol succinate (DEGS) on Chromosorb W (3.7 m by 0.31 cm) at 185°C. Nitrogen flow rate on the SE-30 column was 25 ml/min; that on the DEGS column was 14 ml/min.
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9. We thank Dr. J. Ruth and Mr. E. L. Gooden, USDA, Beltsville, Maryland, for determining the mass spectra and nuclear magnetic resonance spectra, respectively. We thank Dr. M. T. Ouye (Brownsville, Texas), Mr. L. F. Steiner (Honolulu, Hawaii), and Mr. J. C. Keller (Phoenix, Arizona), all of the U.S. Dept. of Agriculture, for supplying the insects used in this investigation and for carrying out the bioassays, and Dr. C. N. Smith, USDA, Gainesville, Florida, for supplying the results of repellency test.

20 September 1967

Cryptic Moths: Effects on Background Selections of Painting the Circumocular Scales

Abstract. *A dark moth* (Catocala antinympha) *and a light moth* (Campaea perlata) *rested on appropriate backgrounds in an experimental apparatus allowing a choice between black and white backgrounds. The selections remained unchanged when the circumocular scales of the moths were painted either black or white. These results suggest that selections of background by cryptic moths, with respect to background reflectance, are genetically fixed. Background selections by melanic forms in various species are interpreted in the light of this conclusion.*

Several geometrid and noctuid moths select appropriate backgrounds when presented with a choice of backgrounds differing in reflectance (*1*, *2*). These selections, if one assumes that they are visually based, could result from either of two mechanisms: (i) genetically fixed responses to particular background reflectances, and (ii) matching responses, in which the moths compare certain parts of their bodies with their backgrounds. Kettlewell (*1*) and Ford (*3*) have suggested a matching mechanism, involving the circumocular scales, to explain the different background preferences of typical and melanic *Biston betularia*. In an attempt to distinguish between the two alternatives, I altered the reflectances of two moths—a dark noctuid, *Catocala antinympha*, and a pale geometrid, *Campaea perlata*—by painting their circumocular scales. The results provided evidence of genetically fixed selections of background in these species.

The moths were collected near 150-watt bulbs in Leverett, Massachusetts, during the summer of 1967. Individuals to be painted were placed in a cyanide killing jar until their flutterings ceased; this treatment rendered the moths inactive for 3 to 5 minutes. They were then painted with either black or white Flo-Paque paint; I used a very fine red sable brush and viewed them through a × 2 binocular loop. Paint was applied to all scales of the head, of the collar on the thorax, and of the bases of the forewings (Fig. 1). These scales, because of their reflectance (which appeared similar to that of the forewings) and their position around the eyes, were assumed to include any that the moths might use in a reflectance-matching process.

The experimental apparatus consisted of two black and two white pieces of painted blotting paper, each 27.9 by 48.3 cm, formed into a cylinder of alternating black and white sections. The cylinder was set in a plywood box (35.6-cm square by 48.3-cm high) which was covered with a pane of window glass and a double layer of cheesecloth. The entire apparatus was placed in a wooded area where a thick canopy excluded direct sunlight.

Each morning, between 0600 and 0800 hours E.S.T., the background selections of the moths that had been collected and painted the previous evening were noted, and the moths were taken for later determinations of reflectance.

The percentage-reflectance values for the backgrounds and the moths' forewings were determined (*4*). For each species of moth, the forewings of 12 individuals were glued as montages onto black construction paper, and percentage reflectance was measured over a circle, 2.85 cm in diameter, within each montage. The altered reflectances were obtained from montages of painted forewings. The reflectance of the construction paper was 7.33 percent.

The reflectances and background selections of control and experimental groups of both species are shown in Fig. 2. Every group within each species differed significantly from a random distribution on the black and white backgrounds (chi-square tests), but no groups within either species differed significantly from one another (Fisher exact probability tests). These results suggest that selections of background by these moths, with respect to background reflectance, are genetically fixed.

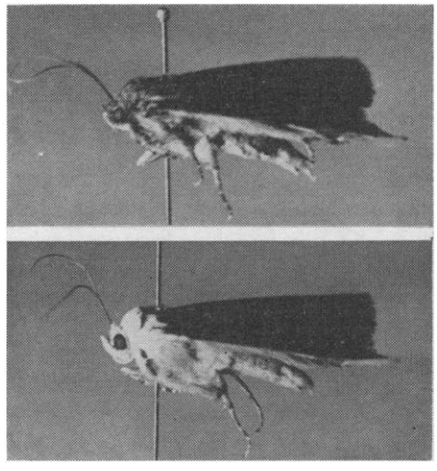

Fig. 1. Extent of the painted area on experimental moths, as shown by comparison of an unpainted (top) and a white-painted (bottom) *Catocala antinympha*. Moths are slightly enlarged.

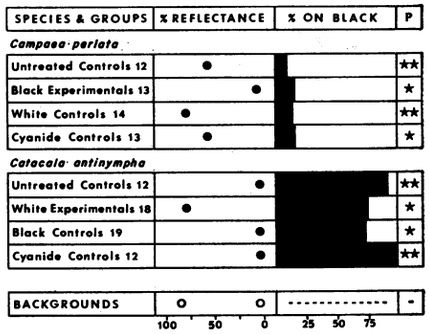

Fig. 2. Reflectances and background selections of experimental and control moths of two species, with reflectances of the experimental backgrounds. The number of individuals tested of each group is given. Significant deviations from chance selections of black and white backgrounds are indicated by stars: one star, $P < .05$; two stars, $P < .01$.

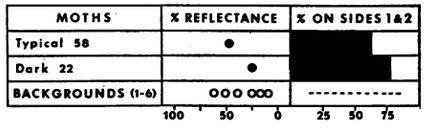

Fig. 3. Reflectances and background selections of two forms of *Cosymbia pendulinaria*, with reflectances of the experimental backgrounds. The number of moths on the lightest two backgrounds was significantly greater than by chance in both forms ($P < .001$), but the two distributions did not differ from one another ($P > .20$, chi-square tests).

These results are of further interest in the light of previous findings regarding melanic forms in two species. Melanic individuals of *Biston betularia* (*1*) and *Catocala ultronia* (*2*) select darker backgrounds than do nonmelanic individuals. If one assumes that genetically fixed selections of background are the rule in cryptic moths, the different forms of these species must differ genetically with respect to selections of backgrounds. Such genetic differences would be expected only in species characterized by long and continuous polymorphism. *Biston betularia* is certainly such a species (*5*), and my own collecting over several years indicates that melanic *Catocala ultronia* consistently comprise 5 to 10 percent of the local population.

In species in which melanics appear sporadically, genetic differences in selection of backgrounds may not become established. Such a situation apparently prevails in *Cosymbia pendulinaria*, a typically pale geometrid: during the summer of 1967 a number of distinctively dark individuals of this species were collected, while none were taken during three previous summers of extensive collecting. The spring of 1967 was abnormally cold (*6*), and, as exposure of moth larvae and pupae to low temperatures is known to produce dark adults in various species (*7*), this fact may account for the dark individuals taken during 1967. At any rate, selections of background by the typical and dark moths of this species did not differ (Fig. 3). This result provides additional evidence of genetically fixed preference of backgrounds in cryptic moths.

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20 October 1967

Induction and Survival of Hemoglobin-Less and Erythrocyte-Less Tadpoles and Young Bullfrogs

Abstract. *Injection of two 25-microgram-per-gram doses of the hemolytic agent phenylhydrazine reduced the hemoglobin level and the erythrocyte count to less than 1 percent of normal tadpole and young bullfrog blood. These anemic animals survive for weeks with little change in overall metabolism. A slow recovery of hemoglobin levels was observed. The implications of this observation for comparative biochemistry are considered.*

During anuran metamorphosis, tadpole hemoglobins undergo a dramatic extensive alteration in molecular composition and mechanism of biosynthesis (*1–3*). This results in hemoglobins of very different properties, particularly as to oxygen binding and other aspects of its chemistry (*1–3*). It has even been possible to attribute adaptive significance to this change (*4*). In a continuing effort to study hemoglobin biosynthesis in the tadpole and frog, we recently attempted to alter the red cell population and, in particular, to increase the number of reticulocytes with an injection of a hemolytic agent (*5*). Unexpectedly, we were able to produce a complete anemia in these animals with no apparent serious metabolic distress to the tadpole. In this report we wish to present the evidence for this finding and briefly consider its implications.

We injected *Rana catesbeiana* tadpoles intraperitoneally with the hemolytic agent phenylhydrazine (25 μg/g of body weight). Figure 1 shows the hemoglobin levels of the tadpoles 1 and 2 days, respectively, after injection, at 20° ± 1°C (temperature used for all experimental periods reported). In 1 day the hemoglobin is reduced from 4.41 to 2.5 g per 100 ml of whole blood, a reduction of 45 percent. After the second phenylhydrazine injection, the remaining hemoglobin declines to 0.3 g per 100 ml, and, finally, to an almost undetectable level after the 4th and 5th days. Partial recovery can be achieved in 16 days from the start of the experiment. Evidently, the dose of 25 μg/g is crucial for the maximum effect in this brief period. After two 12.5 μg/g doses, the hemoglobin was lowered to 1.2 g per 100 ml. Since the fall in hemoglobin is due to the destruction of red cells, the changes in tadpole blood cells were studied, with the results shown in Table 1. The number of erythrocytes drops from 230,000 to less than 100 per cubic millimeter. The count of other types of cells drops considerably, except for the lymphocytes, which become the predominant cell type. Another species of bullfrog tadpole, *R. grylio*, also can be made completely anemic 3 days after one injection of phenylhydrazine (25 μg/g). Additional representative data on the effect of T_3 (triiodothyronine) on this process are shown in Table 2. When three 25 μg/g injections of phenylhydrazine were given over a 19-day period and the blood tested 7 days later, the hemoglobin dropped from 5.0 to 0.18 g per 100 ml of whole blood. When T_3 was given

Table 1. Effect of phenylhydrazine on cell distribution in *Rana catesbeiana* tadpole blood. Experimental tadpoles were injected with phenylhydrazine (25 μg/g) twice, at 24-hour intervals. Blood was analyzed on the 5th day.

Cells	Cell count (thousands of cells per mm³)	
	Control*	Phenylhydrazine†
Erythrocytes	232	0.07
Erythroblasts	5.0	1.3
Lymphocytes	9.0	9.1
Leukocytes	4.0	2.2

* Controls had 4.41 ± .06 g of hemoglobin per 100 ml of whole blood. † Experimentals had 0.056 ± .015 g of hemoglobin per 100 ml.

Table 2. Effect of phenylhydrazine on hemoglobin levels in bullfrog tadpoles and young frogs. Standard deviations are in parentheses. Gram % indicates grams of hemoglobin per 100 milliliters of whole blood.

Experimental group	Hemoglobin (gram %)
*R. catesbeiana tadpoles**	
Control	5.0 (0.6)
7 days after 3 doses of phenylhydrazine	0.18(0.03)
7 days after 3 doses of phenylhydrazine and T_3	.70(0.2)
9 days after 3 doses of phenylhydrazine	.25(0.04)
R. catesbeiana froglets†	
Control	5.8 (0.80)
1 day after phenylhydrazine	0.13(0.03)
7 days after phenylhydrazine	.01(0.04)
14 days after phenylhydrazine	.19(0.09)

* One dose (25 μg/g) of phenylhydrazine given on 1st, 12th, and 19th days. In the case of 3,5,3′-triiodothyronine (T_3), the injection (0.5 nmole/g) was given simultaneously with the last phenylhydrazine injection, producing a 50 percent decrease in tail length 7 days later, on the 26th day. † The dose was 25 μg/g.