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.

T. D. SARGENT

Department of Zoology,
University of Massachusetts,
Amherst 01002

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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.

simultaneously with the last phenylhydrazine injection on the 19th day, the hemoglobin was reduced only to 0.7 g per 100 ml on the 26th day. We suspect that T_3 has stimulated the formation of certain new frog-like cells, although this remains to be confirmed.

In the lower section of Table 2 we also show data on *R. catesbeiana* froglets (recently metamorphosed frogs). A considerable reduction in hemoglobin, equivalent to the reduction in tadpoles, was observed in froglets after a single injection of phenylhydrazine, with relatively little recovery under these experimental conditions even after 14 days. Adult bullfrogs did not show as marked response to the hemolytic agent as tadpoles and froglets. A single injection of phenylhydrazine (25 μg/g) reduced the erythrocyte count from 506,000 to 102,000 and hemoglobin from 5.0 to 1.7 g per 100 ml after 3 days. Seven days after four daily 12.5 μg/g injections, the erythrocyte count was lowered to 44,000 and the hemoglobin to 0.97 g per 100 ml. Most of these animals did not survive these experiments. However, *R. pipiens* tolerated four daily doses of phenylhydrazine (12.5 μg/g) even though the erythrocyte count dropped from 682,000 to 93,000 and the hemoglobin from 7.2 to 1.7 g per 100 ml on the 5th day. Eight of 16 frogs survived the 9th day with an average cell count of 21,000 and 0.11 g of hemoglobin per 100 ml of whole blood.

Bullfrog tadpoles which had less than 0.2 g of hemoglobin per 100 ml of whole blood for 1 month gradually recover their hemoglobin over a subsequent 2- to 3-month period and survive without difficulty. During their anemic period, CO_2 production of the tadpole did not differ appreciably from the controls. Urea excretion was depressed during the period immediately following phenylhydrazine injection, but ammonia production was not appreciably affected. All anemic tadpoles seemed to be fully capable of moving freely, comparable to normal tadpoles.

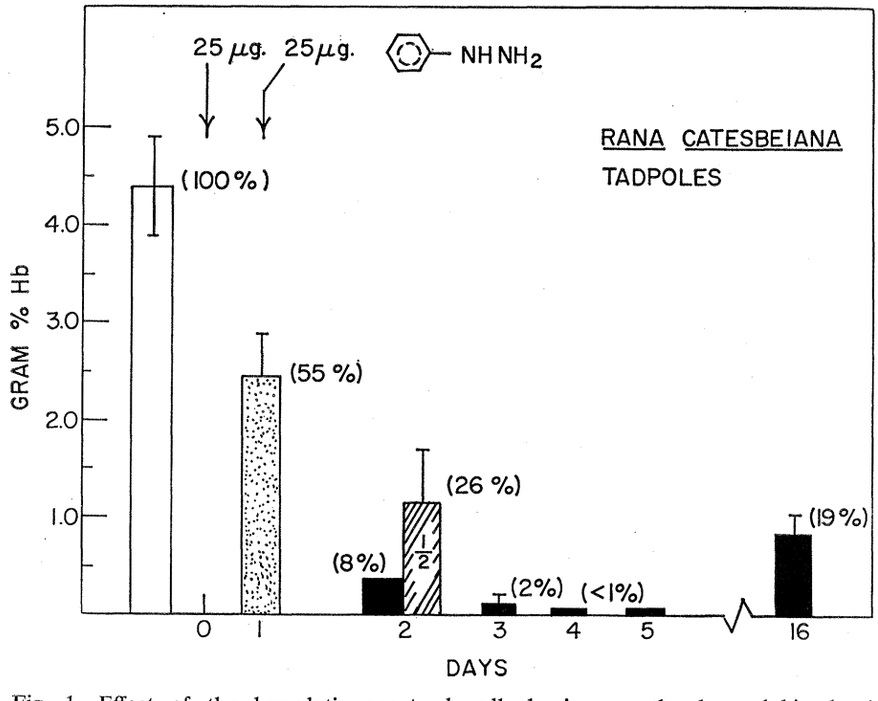

Fig. 1. Effect of the hemolytic agent phenylhydrazine on the hemoglobin levels (grams of hemoglobin per 100 ml whole blood) of *Rana catesbeiana* tadpoles. Phenylhydrazine was injected intraperitoneally. The experimental group indicated by the stippled bar received a single injection (25 μg/g of body weight) at zero time, and the hemoglobin was determined 1 day later. The groups represented by solid bars received like injections at zero time plus a second identical dose on day 1, and the hemoglobin was determined on the days indicated. The group represented by a shaded bar received a dose of 12.5 μg/g of body weight at time zero and a second such dose on day 1, and the hemoglobin was determined on day 2. The control group (white bar) did not deviate from 4.4 g of hemoglobin per 100 ml of whole blood during the 16-day experiment. Vertical lines represent the standard deviation from the average. Figures in parentheses represent the percent of hemoglobin relative to the control group.

The ability of the tadpole to survive for several weeks without the aid of an oxygen-carrying pigment may be rationalized in terms of its oxygen needs. The absence of hemoglobin in certain cold-water fish, in certain fish larvae, and even in an occasional adult frog has been noted earlier (*6, 7*). The normal tadpole utilizes about 65 ± 15 μl of oxygen per hour per gram of wet weight, at 23° ± 2°C (*1, 8*). Air-saturated water is 0.25 m*M* in oxygen in the same temperature range, or 55 μl O_2/ml. Thus, if tadpole fluids equilibrate with air within 1 hour, minimal amounts of oxygen should be available for normal respiration. It is recognized that the availability of hemoglobin may be crucial to the tadpole and the frog under conditions of elevated oxygen demand. In contrast, a mammal such as the rat consumes 15 times as much oxygen (900 μl/hr per gram) (*7*) and thus is always dependent on an auxiliary mechanism for extra oxygen in the form of the blood oxygen carrier, hemoglobin. If these correlations are valid, many classes of animals, including poikilothermic vertebrates (amphibians, many fish, and reptiles) and numerous invertebrates (especially worms and molluscs) with low oxygen utilization (*7*) may be able to survive without hemoglobin. This suggests the further study of representative animals from these classes, using appropriate hemolytic agents and following hemoglobin levels, overall metabolism, and survival.

The sensitivity of human erythrocytes to hemolytic agents such as phenylhydrazine has been associated with the inability of these cells to maintain adequate levels of glutathione (*9*). This, in turn, has been correlated with reduced intracellular levels of the reduced form of nicotinamide-adenine dinucleotide phosphate and glucose-6-phosphate dehydrogenase activity. While no corresponding studies in the tadpole or frog are yet available, it is of interest to speculate as to the possible impact of tadpole hemoglobin which completely lacks —SH groups (*3*). Presumably, the total lack of a protein "—SH buffer" might make the tadpole red cell especially susceptible to a hemolytic agent, as has been observed.

GUSTAVO FLORES
EARL FRIEDEN
Department of Chemistry and
Institute of Molecular Biophysics,
Florida State University, Tallahassee

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Avena magna: An Important New Tetraploid Species of Oats

Abstract. Avena magna *is a new tetraploid species morphologically similar to the hexaploid* A. sterilis, *having a high concentration of protein, large caryopses, and outstanding resistance to crown rust. One genome in* A. magna *appears homologous to the* A_s *genome present in hexaploid, tetraploid, and one group of diploid species.* Avena magna *is a possible ancestor of cultivated oats.*

We found a new tetraploid [2*n* (diploid number) = 28] species of *Avena* among collections obtained from the Mediterranean region by Rajhathy, Zillinsky, and Hayes (*1*). This species may have been significant in the evolution of hexaploid *Avena*. It is also of special interest because it has large caryopses (groats) with high protein content, outstanding resistance to crown rust, and thick culm wall.

The hexaploid (2*n* = 42) species *A. sterilis* L. is indigenous to the Mediterranean region. Murphy and coworkers (*2*) and Zillinsky and Murphy (*3*) have recently reported finding resistance to the major oat diseases among collections of *A. sterilis* obtained from Israel (*2*) and other Mediterranean countries (*1*).

While screening collections of *A. sterilis* for protein content at Beltsville, Maryland, we discovered that CW-525, C.I. 8330 (accession numbers of Canadian Department of Agriculture and U.S. Department of Agriculture, respectively) possessed unusually large caryopses with high protein content. The percentage of protein ranged from 23.4 to 30.0 percent, depending on the environment in which the plants were grown. The percentages of protein among cultivars of *A. sativa* L. ranged from 15 to 21. When CW-525 was grown in irrigated fields at Aberdeen, Idaho, in 1967, the weight of 100 caryopses was 3.5 g compared to 2.8 g for the cultivar Garland, C.I. 7453, and 0.9 to 2.4 g for several hundred lines of *A. sterilis* entries. The seedlings of CW-525 were resistant to race 264 of crown rust at Beltsville, Maryland, and Ottawa, Ontario; adults were resistant to race 264 in the cooperative oat-rust nursery in Puerto Rico (see *4*).

We crossed CW-525 with *A. sativa* and *A. sterilis* as a part of our inheritance and breeding studies. Reciprocal crosses between CW-525 and *A. sativa* and *A. sterilis* were obtained, but all F_1 plants were sterile. As a result of the unexpected sterility, we studied the taxonomy and chromosome number of several plants from the collection CW-525. All of the plants sampled were tetraploid.

Although CW-525 resembles *A. sterilis* more than it does any other species, it is markedly different in several characters. Like *A. sterilis*, its spikelets articulate only at the base of the lowest floret, leaving a dissemination unit of three tightly attached florets (Fig. 1). We believe that morphological and chromosomal differences in CW-525 favor its recognition as a new species. The herbaria of the U.S. National Museum, Smithsonian Institution, Washington, D.C., and Royal Botanic Gardens, Kew, England, were searched unsuccessfully for previous collections resembling CW-525.

AVENA MAGNA Murphy *et* Terrell, new species

Avenae sterili *affinis, internodorum parietibus incrassatis, florum characteribus, chromosomatum numero differt.*

Rachillae segmentum infimum latum compressum. Articuli cicatrix 3.3 ad 4.0 mm longa, 1.5 ad 2.0 mm lata. Lemmatum I et II partes inferiores ⅔ ad ⅚ densissime longo-villosae, pilis usque ad 8 mm longis; lemmatum partes superiores ⅙ ad ⅓ pilis usque ad 1 mm longis indutae; lemmatum apices obtusi vel truncati, bidentati. Lemma III dense villosum. Aristarum segmenti basales densissime pilis circa 1 mm (raro 2 mm) longi armati. Paleae praecipue apices versus pubescentes. Chromosomatum numerus: 2n = *28.*

Type: Plants grown in greenhouse (Beltsville, Maryland, U.S., August 1967) from seeds collected by F. J. Zillinsky from a roadside population in the Rabat region of western Morocco between villages Oulmes and Tiflet (approximately 30 km southeast of Tiflet) at an elevation between 1000 and 1300 m, 27 May 1964 (holotype K; isotopes NA, US). (Sixty plants of *A. magna* grown under field conditions at Aberdeen, Idaho, in 1967, were identical for all taxonomic characteristics and chromosome number with the plants grown in the greenhouse.)

In contrast, *A. sterilis* has a hollow peduncle; lowest rachilla segments are rounded; articulation scars are usually about 2 mm long and 1 mm wide; and the lower two-thirds of lemmas of first and second florets are rather densely long-villous with hairs up to 7 mm long (hairs not concealing the lemma surface in contrast with *A. magna*). The upper one-third of lemmas are glabrate to short-pubescent; lemma apices are long-attenuate to acute and bidentate (lemma width just below apices is less than 1 mm, but that of *A. magna* is 1 to 2 mm); and lemmas of tertiary florets are glabrous to sparsely villous. Basal segments of awns are glabrous or in subspecies *macrocarpa*, pubescent with hairs up to 1 mm long; paleae are glabrous and ciliolate. The somatic chromosome number is 42.

In addition, *A. magna* has slightly larger florets and caryopses, slightly thicker awns, and slightly longer pedicels. Glumes are about as large as the largest ones in *A. sterilis*. Density of lemma hairs is perhaps twice that in *A. sterilis*. Hairs in *A. magna* are either dark brown or whitish. In making comparisons between CW-525 and *A. sterilis* sens. lat., we took into account the hairiest extremes, particularly *A. sterilis* subsp. *macrocarpa* var. *setosissima* Malzew subvar. *maxima* (Perez-Lara) Malzew as illustrated by Malzew (*5*) in his Plate 87. Other differences include the internode walls, especially of the upper part of culms, which are much thicker in CW-525 than is usual in *A. sterilis*. Presumably, this characteristic may be of agronomic benefit in preventing breakage of culms. Finally, our plants of CW-525 have a