

LACK OF PHYSIOLOGICAL DIFFERENTIATION IN
THREE COLOR MORPHS OF THE CRICKET FROG
(Acris crepitans) IN ILLINOIS

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ABSTRACT

Physiological differences among color morphs on which selection might act to maintain the balanced polymorphism in the cricket frog (Acris crepitans) were evaluated. CTM, desiccation rates, resistance to prolonged stress and thermal preference, showed no significant differences among red, green or gray striped frogs from the same populations in Illinois.

INTRODUCTION

The genetic basis of inheritance is known for several cases of color and/or pattern polymorphism in amphibians. However, factors which determine morph frequency have been the subjects of much speculation including: 1) Mayr's (1942) founder principle and genetic drift (Goin, 1947), 2) visual selection by predators (Goin, 1947; Pyburn, 1961a; Mathews, 1971; Nevo, 1973; Stewart, 1974) including apostatic selection (Milstead et al., 1974), 3) selection for a behavioral characteristic associated with a particular morph (Wendelken, 1968), 4) selection for an associated physiological factor (Goin, 1947; Test, 1955; Main, 1961, 1965; Merrell and Rodell, 1967; Dasgupta and Grewal, 1968; Williams et al., 1968; Nevo, 1973), or 5) a combination of chance, visual selection by predators and physiological factors acting in concert (Jameson and Pequgnat, 1971; Nevo, 1973). Mathews (1971) pointed out that during the aquatic larval stage selection would have to act at the physiological or behavioral level, since adult color phases have not developed. Thus, the trend has been to attribute the adaptive value of amphibian color morphs to certain cryptic physiological or behavioral differences which may be controlled by pleiotropic genes. However, there is little or no conclusive evidence that adaptive differences actually exist.

The cricket frog, Acris crepitans, exhibits a genetically based color polymorphism in which green stripe is dominant to the nongreen condition (i.e., red stripe or gray) and red stripe is dominant to gray (Pyburn, 1961a, 1961b). The species has a geographical distribution extending from northeastern Mexico to southern Wisconsin and east to the Atlantic Coast. Thus, it is adapted to many different local conditions of temperature and moisture ranging from semidesert to swamp. Any demonstration of the adaptive or nonadaptive (neutral) value of the

three color morphs of Acris would provide interesting insights into the concept of amphibian polymorphism. This study investigated possible physiological morph differences upon which selection might act to maintain the balanced polymorphism in Acris crepitans. Frogs were collected from central and southern Illinois and critical thermal maxima (CTM), desiccation rates, resistance to prolonged stress, and thermal preference of the three morphs determined.

MATERIALS AND METHODS

Critical Thermal Maxima

CTM was determined for each morph using a modification of the method of Brattstrom (1968). Frogs of similar size were collected in fall from the La Rue Pine Hills Swamp (southern Illinois) in Union County, 8 miles southeast of Grand Tower, and the Mackinaw River (central Illinois) in McLean County about 10 miles northeast of Normal, and maintained at room temperature (22-23°C) on a diet of fruit flies for 1 week. After acclimation, a Yellow Springs Instrument Company (YSI) temperature probe (Model 43 TZ) was inserted into the cloaca of each frog (8-20 of each morph, Table 1). The animal was then placed in a glass bowl (80 cm dia, 3 cm deep) filled with 5 mm of water. The bowl was covered with a glass lid to prevent desiccation. The chamber was heated (about 1°C/min) by an overhead heat lamp 30 cm above the bottom of the bowl. The "onset of spasms" (Hutchison, 1961; Hutchison et al., 1966) or temperature where control of normal body movements was lost (i.e., behavioral or ecological death) was recorded as the CTM. The frog was then immediately placed in cool water and revived. Unrevivable frogs were considered to have reached physiological death and were not included in the results. Thus, the CTM reported is not to be confused with upper lethal temperature, as it precedes physiological death.

TABLE 1. COMPARISON OF PHYSIOLOGICAL ATTRIBUTES OF ACRIS CREPITANS MORPHS FROM CENTRAL AND SOUTHERN ILLINOIS

Collection Site	Red	Gray	Green	F Values ¹
Critical Thermal Maxima (°C)				
Mackinaw River	38.8±1.2 (20) ²	38.8±1.2 (20)	39.5±1.2 (10)	0.70
Pine Hills	39.0±1.0 (10)	39.0±0.8 (10)	39.1±0.9 (10)	0.06
Desiccation Rates (mg H ₂ O lost/min/g tissue)				
Dawson Lake	1.30±0.11 (10)	1.19±0.11 (10)	1.26±0.11 (10)	0.42
Pine Hills	1.39±0.12 (10)	1.38±0.11 (10)	1.46±0.10 (10)	0.21
Thermal Preference (°C)				
Dawson Lake	26.9±2.2 (16)	27.2±2.8 (19)	28.0±2.8 (15)	0.65
Pine Hills	27.5±2.8 (10)	28.5±3.7 (15)	28.2±3.1 (15)	0.31

¹ not significant.
² (sample size).

Desiccation Rates

Desiccation rates for each morph (10 frogs/morph/location, Table 1) were obtained at room temperature using acclimated frogs collected during spring at Pine Hills and Dawson Lake (central Illinois) in McLean County, 15 miles southeast of Bloomington, IL. Two half-pint bottles filled with desiccant (Filtrol Corporation Desiccite No. 25) were placed in the corners of a closed weighing chamber (Sartorius balance, Type 2703) to absorb moisture. Urinary bladders were emptied and frogs were placed in a small screen cage (5 x 2.5 x 2.5 cm), suspended from the hook which supports the weighing pan of the balance. After an equilibrium period of 15 min, an initial weight reading was taken. This was followed by six subsequent readings 5 min apart. The weight of water lost in each 5 min period was determined by subtraction and converted to mg water lost/min/g of tissue.

Resistance to Prolonged Stress

The ability of morphs to withstand prolonged exposure to five types of stress (heat, cold, desiccation, moisture, and starvation) was tested in six 10-gallon aquaria arranged as follows: The bottom of the control aquarium (No. 1) was covered with 3 cm of soil and a small glass bowl (15 cm dia, 3 cm deep) of water was buried to its rim in the center. Sixty *Acris crepitans* (14 red, 26 gray and 20 green) were toe clipped according to color as a check against metachrosis (Gray, 1972) and placed in the aquarium, which was covered with cheesecloth. Frogs were supplied fruit flies every other day, and maintained at room temperature (22-23°C) on a 8:16 light:dark cycle, during the experiment. The remaining five aquaria were set up in essentially the same fashion using the same numbers of each morph but differed as follows: Aquarium No. 2 and 3 were maintained at 37 and 2°C respectively. Aquarium No. 4 contained two half-pint bottles filled with desiccant (Filtrol Corporation Desiccite No. 25) and no water. Aquarium No. 5 was covered with a plastic sheet which maintained the relative humidity at 86-93% (measured with an Atkins Thermistor Psychrometer, Model 3202B). Frogs in aquarium No. 6 were not fed. The aquaria were checked at least four times daily until all frogs were dead. The time of death of each animal was recorded to the nearest hour.

Adequate numbers of all color morphs could not be obtained from the same area for these experiments. Red striped frogs were collected at Six Mile Creek in Woodford County, 8 miles northwest of Normal, IL, and gray and green striped frogs were collected at Dawson Lake, both in central Illinois. After results from aquarium No. 3 were known, the experiment was repeated using 20 red and 20 gray striped frogs from Six Mile Creek to determine if the initial differences found were among morphs or between populations.

Thermal Preference

Thermal preference of each morph was tested in an elongated metal box (86 x 10 x 10 cm) with plastic top. The box floor was lined with moist paper towels to prevent frog desiccation. Thermometers were inserted through the top at 3 cm intervals with the bulbs resting on the paper towels. A 100 watt bulb was placed in one end of the box, which was placed over three hot plates to establish a heat gradient from 20 to 49°C. Frogs (not used in previous experiments) from Pine Hills and Dawson Lake were placed on the towels and after 15 min, the temperature of the thermometer nearest an animal was recorded to the nearest 0.5°C.

Statistical Analysis

Values obtained for CTM, desiccation rate, resistance to prolonged stress and thermal preference, were grouped according to color class. Differences among and between morphs were tested for statistical significance by analyses of variance and the student's t test respectively.

RESULTS

Results of CTM, desiccation rate and thermal preference studies are summarized in Table 1. In each experiment, mean values reported are based on sample sizes of 10 or more. No significant differences were found among morphs from central or southern Illinois populations.

Results of prolonged stress studies (i.e., heat, cold, desiccation, humidity and starvation) also failed to reveal significant differences among morphs from the same population (Table 2). However, the cold stress experiment initially revealed significant differences and the Newman-Keuls Test (Winer, 1962) indicated death times of red striped frogs were higher than those of green striped frogs. When the experiment was repeated (Table 2, aquarium 2a), using red and gray morphs from the same population, no significant difference was apparent. Since death times of gray and green striped frogs from Dawson Lake were similar, the initial differences were probably between populations and not among morphs.

TABLE 2. RESISTANCE TO PROLONGED STRESS OF *ACRIS CREPITANS* MORPHS FROM CENTRAL AND SOUTHERN ILLINOIS

Aquarium No. ¹	Type Stress	Mean Survival (hr)			F Values
		Red	Gray	Green	
1	Control	483±387	248±181	344±496	1.94
2	Heat (37°C)	703±208	697±309	561±205	1.93
3	Cold (2°C)	1061±482	808±358	702±346	3.65 ²
4	Desiccation	176±13	184±12	180±12	2.38
5	Humidity	589±387	687±480	522±233	0.93
6	Starvation	289±132	264±301	307±350	0.13
2a	Cold (2°C) Repeat	807±211	749±233		0.24 ³

¹ sample size in aquaria 1-6 was 14 red, 26 gray and 20 green; sample size in aquaria 2a was 20 red and 20 gray.

² p<.05.

³ students t test--not significant.

DISCUSSION

In *A.crepitans*, Pyburn (1961a) suggested visual selection by predators directly determined color pattern frequencies. Wendelken (1968), disagreed with Pyburn's visual selection hypothesis and stated selection may favor some behavioral characteristic associated with a particular morph. Nevo (1973) favored the visual selection hypothesis but presented

evidence that selection may also act on morph associated physiological factors. Milstead et al. (1974) hypothesized apostatic selection as another means of maintaining the balanced polymorphism in A.crepitans.

Although Nevo (1973) found gray morphs from Texas were more resistant to desiccation than red morphs I could find no differences in desiccation rate and other physiological parameters in Illinois populations. It seems unlikely genes controlling Acris color polymorphism are nonadaptive or of neutral survival value. However, the lack of morph differences in CTM, desiccation rate, resistance to various types of prolonged stress and thermal preference eliminates a number of factors that might act differentially to maintain the balanced color polymorphism of Acris in Illinois. Different selective mechanisms may be operating in different geographical regions. Selective value of color and pattern variations and the extent of physiological and behavioral differences among morphs must be ascertained throughout the range of A.crepitans to understand the adaptive value of this polymorphism.

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