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EVOLUTION IN CONSTANT AND FLUCTUATING ENVIRONMENTS: THERMAL TOLERANCES OF DESERT PUPFISH (*CYPRINODON*)

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What are the evolutionary and ecological consequences of inhabiting constant, as opposed to fluctuating, environments? Students of evolution since Lamarck and Darwin have recognized that characteristics of organisms which are no longer functional tend to be eliminated (e.g. many cave dwelling species have lost their eyes and pigment). This suggests that organisms which once inhabited fluctuating environments should lose their tolerance to those conditions that they no longer encounter during their subsequent evolution in less variable environments. In ecological terms this means that the tolerance of a population for a particular parameter should correspond to the breadth of the niche in that parameter. In an ecological context tolerance usually has two components, a genetically determined capacity to withstand a certain range of a parameter and a phenotypic capacity, frequently termed acclimation, to shift tolerance within this range as a result of previously experienced conditions. Levins (1969) has recently found a fairly good correspondence between these two components of thermal tolerance and the breadth of the thermal parameter of the niche in Puerto Rican *Drosophila*.

The present paper compares the thermal tolerances of populations of desert pupfish that have been isolated for varying periods of time in thermally constant environments with those of populations inhabiting waters that fluctuate tremendously in temperature. Pupfish (*Cyprinodon nevadensis* and related species) of the Death Valley region of southern California and adjacent Nevada present ideal material for such a study. The present populations are descended from an ancestral stock that, by the early Pleistocene, had invaded the extensive drainage system supplying Lake Manly (which filled Death Valley). At intervals thereafter populations were isolated in permanent springs and streams as the water level in the drainage system fluctuated in response to climatic conditions. The ancestral *Cyprinodon* undoubtedly inhabited streams, marshes, and shallow lakes where the water temperatures fluctuated considerably on a daily and seasonal basis. Some populations are still exposed to drastic thermal fluctuations in shallow streams. However, several populations are isolated in thermal springs where water temperature is essentially constant. The geographic isolation of many of these springs is absolute and the time of isolation varies from more than 30,000 years to a few thousand years or less. Most of the isolated populations have differentiated morphologically, some sufficiently to be recognized as distinct species or subspecies (Miller, 1948).

In the present paper we compare the short-term thermal tolerances to both high and low temperatures of fish from six thermally constant springs and three thermally fluctuating streams or marshes. We have also performed "natural" and laboratory acclimation experiments in order to separate and evaluate the genetic and phenotypic components of thermal tolerance.

Materials and Methods

The populations.—The populations of *Cyprinodon* used in this study and the springs where they occur are identified and

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TABLE 1. *Description of the populations of* Cyprinodon *studied and the temperatures of their environments.*

Population	Locality	Taxonomic designation	Temperature[a]
Amargosa River	Dealth Valley, San Bernardino Co., California	*C. nevadensis amargosae*	fluctuates; 0 to > 40 C
Salt Creek	Death Valley, Inyo Co., California	*C. salinus*	fluctuates; 0 to > 40 C
Pupfish Marsh (Cottonball Marsh)	Death Valley, Inyo Co., California	*C.* sp.[b]	fluctuates; 0 to > 40 C
Forest Spring	Ash Meadows, Nye Co., Nevada	*C. nevadensis mionectes*	relatively constant; 26.4 C (21.2–27.9)
Big Spring	Ash Meadows, Nye Co., Nevada	*C. nevadensis mionectes*	constant; 27.3 C (27.0–28.0)
Saratoga Spring	Dealth Valley, San Bernardino Co., California	*C. nevadensis nevadensis*	relatively constant; 27.5 C (26.0–28.3)
Point-of-the-Rocks Spring	Ash Meadows, Nye Co., Nevada	*C. nevadensis mionectes*	relatively constant; 31.4 C (30.8–33.1)
Scrugg Spring	Ash Meadows, Nye Co., Nevada	*C. nevadensis pectoralis*	relatively constant; 32.5 C (31.0–33.1)
Devil's Hole	Ash Meadows, Nye Co., Nevada	*C. diabolis*	constant; 33.9 C (32.8–34.0)
Jed's Motel	Tecopa Hot Springs, San Bernardino Co., California	*C. nevadensis amargosae*	source 40.2 C, thermal gradient in outflow
Tecopa Bore	Tecopa Hot Springs, San Bernardino Co., California	*C. nevadensis amargosae*	source 47.5 C, thermal gradient in outflow

[a] Based on our own measurements and data in Miller (1948). Values in parentheses indicate the recorded range.
[b] Probably specifically distinct from the other named populations but most similar to *C. salinus*.

described briefly in Table 1. We have sampled three types of habitats with respect to variation in water temperature: 1) shallow streams or marshes, where the water temperature varies 15 to 20 C on a normal day and yearly extremes range from 0 to at least 40 C; 2) thermal springs with large source pools of essentially constant temperature; these springs either have no surface outflow (Devil's Hole and Forest Spring) or else nearly the entire breeding population of *Cyprinodon* is confined to the source pools; and 3) hot artesian wells with no source pool and a large population of pupfish in the outflow; in the two examples (Tecopa Bore and Jed's Motel) considered here the source is hot (above 40 C) and of constant temperature and the water cools as it moves down the outflow. The steepness of the thermal gradient in the outflow is variable and dependent upon local weather conditions. Both wells were dug within the last five years. Their outflows reach to the headwaters of one of the permanent flows of the Amargosa River and fish from the river (where temperatures fluctuate tremendously) have apparently colonized the outflows of the wells.

Temperature tolerance.—With the exception of one experiment (described later) in which fish were acclimated to constant temperatures in the laboratory, all measurements of thermal tolerance were made on fish within 36 hours of their capture in the field. Fish were captured by netting or trapping, placed in insulated containers, and transported to the laboratory. By the time the tests were begun the temperatures of the samples from all populations had converged to approximately 20 C. For measuring thermal tolerances approximately 20 fish from each population were placed in wide-mouth gallon jars containing fresh water from their own springs or stream. Temperatures within the jars were controlled by placing them in a water bath

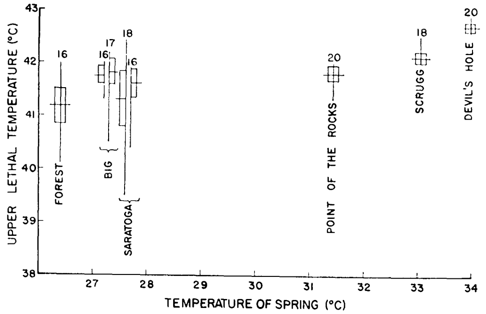

FIG. 1. Relationship between upper lethal temperature and temperature of spring for populations of *Cyprinodon* inhabiting thermally constant springs. Vertical lines represent ranges; horizontal lines, means; rectangles, 95 per cent confidence intervals; numbers above each diagram indicate sample size. Replicate samples for Big and Saratoga Springs were tested in January and April.

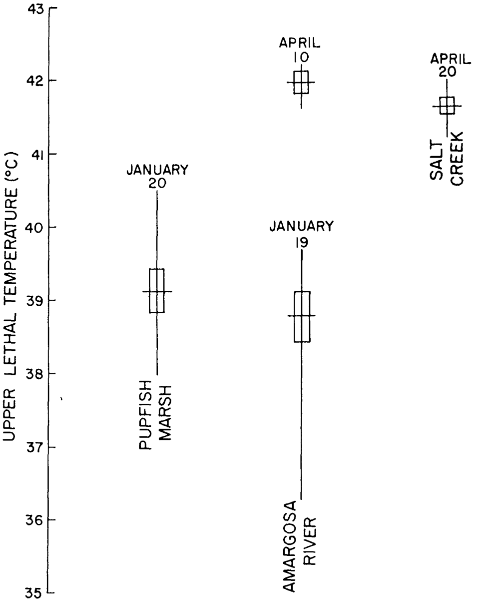

FIG. 2. Seasonal variation in the upper lethal temperatures of populations of *Cyprinodon* inhabiting thermally fluctuating waters. Symbols as in Figure 1. All three localities are at similar elevations in the floor of Death Valley and their inhabitants are exposed to similar temperature regimes.

that was heated or cooled at the desired rate. To measure tolerance to high temperatures the temperature was raised rapidly (5° per hour) to 30 C and then the rate of heating was controlled at 2 C per hour. Each container was aerated constantly. The temperature at which each fish died was recorded. This was reliably determined by noting when all respiratory ventilation ceased. The procedure for measuring tolerance to low temperatures was similar. Fish were cooled at 5 C per hour to 15 C and then the rate of cooling was reduced to 2 C per hour and carefully controlled. It was difficult to determine exactly when fish died at low temperatures, but an accurate index of cold tolerance was found to be the temperature at which fish lose equilibrium. This occurs at temperatures 1 to 2° above the lower lethal temperature.

To test for the effects of acclimation the following experiment was performed. Fish from a thermally constant (Saratoga Spring) and a thermally fluctuating (Amargosa River) habitat were brought into the laboratory and each population was divided into two groups. One group from each population was kept in a large aquarium at a constant temperature of 25 C for 29 days. The other groups were kept for the same period at 15 C. After this period of acclimation the temperatures of all groups were allowed to converge to 20 C overnight and tolerance to high and low temperatures were measured as before.

RESULTS

The upper lethal temperatures of fish freshly captured from thermal springs are correlated ($r = 0.86$) with the temperatures of their habitats (Fig. 1). Populations from the warmer springs had higher and less variable lethal temperatures than fish from cooler springs. No populations showed significant deviation from the general pattern. Replicate tests on two populations (Saratoga Spring and Big Spring) indicated the excellent reproducibility of the method and the absence of seasonal variation.

In sharp contrast to the thermal tolerances of fish from thermally constant springs, the upper lethal temperatures of

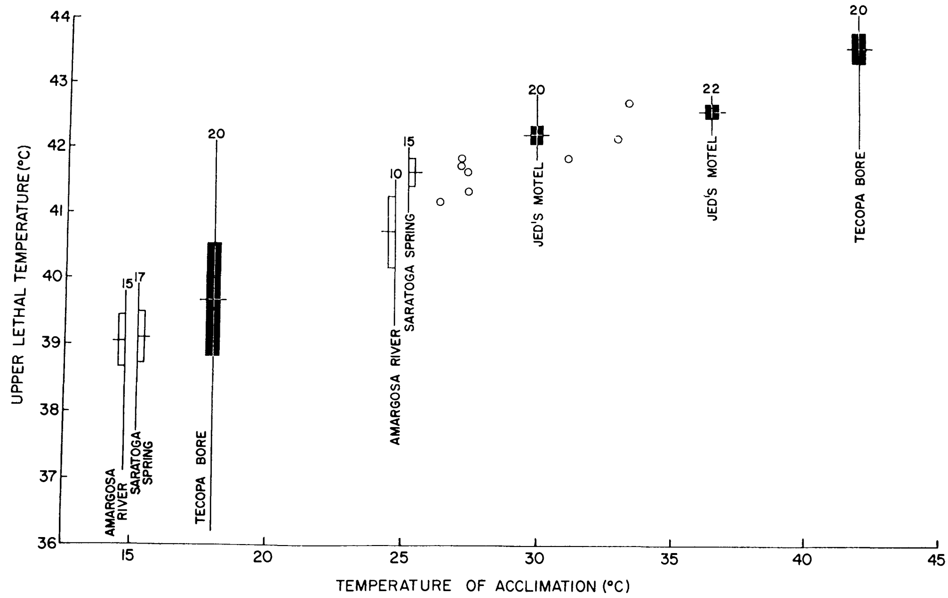

FIG. 3. Effects of thermal acclimation on the upper lethal temperatures of several populations of *Cyprinodon.* Circles indicate populations naturally acclimated in thermally constant springs. Shaded diagrams depict the results of "natural" experiments; unshaded diagrams, laboratory experiments. Symbols as in Figure 1.

fish from thermally fluctuating habitats showed significant seasonal variation (Fig. 2). Higher lethal temperatures during the warmer months of the year suggest that these fish have acclimated to the water temperatures that they have recently experienced. These data suggest that acclimation, rather than genetic differences, may account for some or all of the differences in thermal tolerance between the populations inhabiting springs of different temperatures.

We used two kinds of acclimation experiments to assess the quantitative effect of thermal acclimation on the upper lethal temperatures of pupfish. First, we took advantage of a "natural" acclimation experiment and measured the lethal temperatures of two samples from different temperatures in the cooling outflows of the two hot artesian wells (Tecopa Bore and Jed's Motel). These experiments were based on the assumption that fish captured at a given point in a thermal gradient were acclimated to the temperature at that point. Secondly, we kept fish from Saratoga Spring (constant) and Amargosa River (fluctuating) in the laboratory at two different temperatures and then measured their lethal temperatures after 29 days of acclimation. The data from these experiments (Fig. 3) indicate that the relationship between acclimation temperature and upper lethal temperature is identical to that between the temperatures of thermally constant springs and the lethal temperatures of their inhabitants. Thus acclimation is sufficient to account for the differences in the upper lethal temperatures of all populations of *Cyprinodon* that we have examined.

The data on tolerance of low temperatures, while less extensive, indicate a similar pattern (Table 2). The temperatures at which fish occur has an important influence on their ability to withstand extreme cold, but acclimation accounts for the entirety of this effect.

TABLE 2. *Tolerance of* Cyprinodon *to low ambient temperatures.*

Population	Previous thermal history	Loss of equilibrium
Devil's Hole	field acclimated; 33.9 C	6–7 C
Scrugg Spring	field acclimated; 32.5 C	6–7 C
Point-of-the-Rocks Spring	field acclimated; 31.4 C	4–5 C
Saratoga Spring	field acclimated; 27.5 C	2–3 C
Saratoga Spring	laboratory acclimated; 15 C	< 1 C
Amargosa River	laboratory acclimated; 15 C	< 1 C
Amargosa River	field acclimated in winter; < 20 C	< 1 C
Salt Creek	field acclimated in winter; < 20 C	< 1 C

In summary, the data on tolerance to both high and low temperatures indicate that differences between populations in their ability to withstand extreme temperatures are dependent solely on the temperatures to which the fish have recently been exposed and become physiologically acclimated. Fish from both constant and fluctuating thermal environments have similar capacity to acclimate to temperatures and there is no evidence of genetic differences in short-term thermal tolerances between any of the populations tested. Thus there is no indication of evolutionary change in thermal tolerance that can be related either to the thermal environment where a population occurs or the length of time it has been isolated in that environment.

DISCUSSION

All populations of *Cyprinodon* studied are tolerant of a wide range (about 40°) of environmental temperatures, and are able to shift within this range by means of thermal acclimation. These attributes are of obvious advantage to several contemporary populations which inhabitat streams and marshes where temperatures fluctuate as much as 20° daily and more than 40° seasonally. However, it is surprising that populations inhabiting thermally constant springs have equally broad tolerances and similar abilities to acclimate. From this it may be inferred that the ancestral *Cyprinodon*, which was widely distributed in the Death Valley region during the early and wetter parts of the Pleistocene, inhabited shallow lakes, marshes, and streams where temperatures fluctuated greatly. However, we assumed that some of the populations inhabiting thermal springs were isolated in their present habitats for tens of thousands of years and we predicted that they would have lost some of their ability to tolerate extreme temperatures. In attempting to account for our unexpected results it is first necessary to reexamine two important assumptions on which the present study is based.

We assumed that the springs have been isolated long enough for significant evolutionary change to have occurred in the pupfish populations. Both geological and biological evidence support this assumption. The geological evidence for the antiquity of the isolation of Devil's Hole is particularly strong. Devil's Hole is located high on the side of a limestone mountain (Fig. 4); it has no surface outflow and there is no evidence that it ever had one. *Cyprinodon* apparently colonized this spring when the Pleistocene lake that covered Ash Meadows was high enough to flood Devil's Hole. This must have occurred very long ago (between 30,000 and 100,000 years) because geological traces of the lake that covered the area have been almost entirely obliterated by subsequent erosion (Miller, 1948). The other springs in Ash Meadows are all at lower elevations and all but one have significant surface outflows (Fig. 4). The absolute duration of their isolation is unknown, but at least some of the outflows may have been confluent with each other and with the Amargosa River whenever conditions in the area were significantly

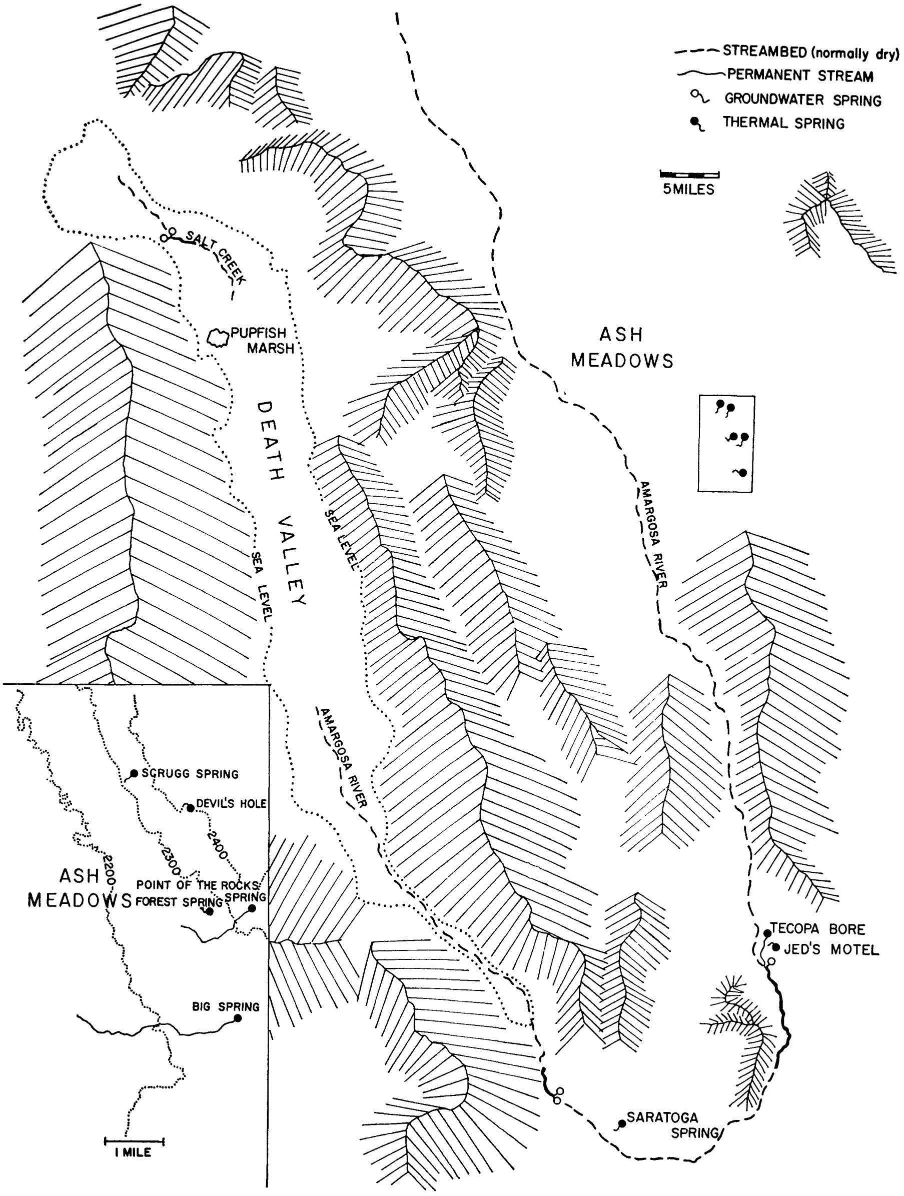

FIG. 4. A map of the Death Valley region of California and Nevada indicating the location and isolation of the populations of *Cyprinodon* sampled for the present study. Inset at lower left shows the springs in Ash Meadows, Nevada in greater detail.

more mesic than at present. Likewise, in the southern end of Death Valley, the isolation of Saratoga Spring from the Amargosa River is relatively recent.

There is direct biological evidence that considerable evolutionary differentiation has occurred in the isolated populations of *Cyprinodon.* Miller (1948, 1950) has made an extensive analysis of the variation among the populations in the Death Valley region and has found sufficient divergence to describe several species and subspecies. Of these we have studied three (or four) species of *Cyprinodon* and four subspecies of *C. nevadensis* (Table 1). The species are distinct in size, shape, and coloration (Fig. 5). The most divergent form, *C. diabolis* from Devil's Hole, is greatly dwarfed and lacks the pelvic fins and barred pigment pattern characteristic of its congeners. The subspecies of *C. nevadensis* are distinguished primarily on the basis of meristic characters (fin ray and scale counts), and these are often sufficient to distinguish populations of the same subspecies from different springs. Miller (1948) has concluded on the basis of breeding experiments and other evidence that the major differences between populations are genetic.

We also assumed that those springs which have virtually constant temperatures at the present time have maintained relatively stable temperatures throughout their isolation. This assumption is difficult to document, but even harder to disprove. Two kinds of circumstantial evidence support this assumption. First, the water supplying the thermal springs (at least those in Ash Meadows) is drawn from an extensive system of water-filled, limestone caverns that extends to great depths. As long as the springs have been in their present positions they have received their water from the same source. The temperature of the water in such a vast, underground reservoir could never have fluctuated drastically. Within the last 30 to 40 years the temperatures of the most constant springs (Big Spring and Devil's Hole) have varied only about 1° (Miller, 1948; present study). Secondly, it is unlikely that *C. diabolis* could have persisted in Devil's Hole, had the temperatures ever differed greatly from what they are at present. The present temperature (33.9 C) is only 9 or 10° below lethal temperature, but is high enough to permit sufficient algal growth to support the pupfish population. During the winter no direct sunlight falls on Devil's Hole and there is only enough primary production to support a population of 200 to 500 *C. diabolis*. Significantly lower winter temperatures would almost certainly have reduced primary production sufficiently to cause the extinction of this endemic species.

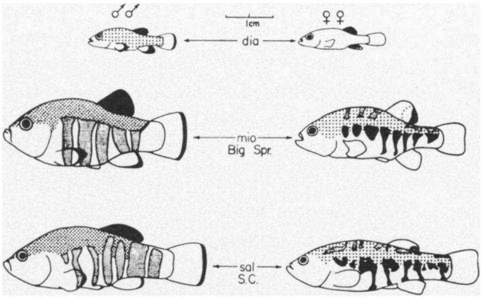

FIG. 5. Morphological differentiation of three species of *Cyprinodon* utilized in the present study. A male and female of *C. diabolis* (dia), *C. nevadensis mionectes* (mio), and *C. salinus* (sal) are illustrated.

We conclude that populations of *Cyprinodon* have been isolated in their present habitats for sufficient time to undergo many evolutionary changes, yet their thermal tolerances have not been modified. These results refute our original prediction that the range of thermal tolerance should be correlated with the degree of thermal fluctuation in the habitat and the length of isolation in that habitat. In making this prediction we had assumed that the ability to tolerate temperatures which the organism never encounters would have been eliminated by natural selection as are other attributes of organisms that no longer contribute to fitness. However, there is a fundamental difference between the ability to tolerate extreme temperatures which no longer are encountered and those attributes that evolutionary biologists cite as having been lost through disuse. Examples of the latter include the photoreceptors and pigments of organisms inhabiting the depths of caves (Poulson and White, 1969), the wings of birds and insects on small, remote islands (Carlquist, 1965), and many biosynthetic pathways of microbes and parasites (Zamenhof and Eichhorn, 1967). In each of these latter cases the organism has experienced a significant qualitative change in its environment and it is easy to imagine that the nonfunctional attribute actually confers a disadvantage (because of energetic considerations, if for no other reason) in the new environment. Zamenhof and Eichhorn (1967) have elegantly demonstrated that this is the case with some metabolic changes in bacteria. *E. coli* mutants that are incapable of synthesizing certain normally essential amino acids are competitively superior to wild types in environments where these amino acids are supplied. However, in the case of the pupfish, it is not immediately obvious that it is disadvantageous for a fish encountering a narrow range of temperatures to be tolerant of a much wider range. This should only be so if increased fitness within the narrow range must normally be attained at the expense of broad tolerance. Our data on *Cyprinodon* indicate that mutations having such effects must be rare, but unfortunately we are not sufficiently versed in biochemistry or biochemical genetics to suggest why this should be so (see Somero, 1969 for a discussion of some suggested mechanisms of thermal tolerance).

Two previous studies have examined the relationships between relative constancy of the thermal environment and thermal tolerance and acclimation. Brattstrom (1968) found that tropical frogs and toads were less tolerant of extreme temperatures than temperate ones and were also unable to acclimate to as wide a range of temperatures. More recently Levins (1969) reported that species of Puerto Rican *Drosophila* that are widely distributed and active during more of the day and the year are tolerant of a greater range of temperatures and better able to acclimate than congeners with more restricted patterns of activity. Although Levins obtained one anomalous result, both of these studies support the generalization that thermal tolerance and the ability to alter it by acclimation are correlated with the variability in temperature that the animal normally encounters. The seemingly contradictory results of the present study indicate that historical factors and phylogenetic relationships may sometimes produce significant exceptions to this generalization. In particular, they suggest that extremely homeostatic systems, perhaps because of their underlying genetic mechanisms, may be highly resistant to evolutionary change.

SUMMARY

We compared the thermal tolerances and ability to acclimate to environmental temperature of populations of desert pupfish from thermally constant springs and thermally fluctuating streams and marshes. All populations had similar abilities to withstand extreme temperatures and to acclimate. Even *Cyprinodon diabolis*, which has been isolated in a thermally constant spring for at least 30,000 years and undergone many morphological changes, is capable of tolerating as wide a range of temperatures as *C. salinus* and populations of *C. nevadensis* which encounter temperature fluctuations from 0 to 40 C in their present habitats. These results suggest that some homeostatic systems may be highly resistant to evolutionary change and that the usually predicted correspondence between the range of an environmental parameter which an organism encounters and the range of that parameter which it can tolerate does not always occur.

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ANNOUNCEMENT

FOURTH INTERNATIONAL CONGRESS OF THE INTERNATIONAL PRIMATOLOGICAL SOCIETY

The Fourth International Congress of the International Primatological Society will be held in Portland, Oregon, from 15 through 18 August, 1972. The Oregon Regional Primate Research Center (ORPRC) has been asked to organize the Congress and to act as the host institution. Three full-day symposia are planned, with invited papers to be given on primate behavior, primate reproduction, and medicine/pathology; and a half-day of panel discussion on three specific topics in primate odontology will be held. Short papers are invited in these general areas, as well as in paleontology, haemotology and genetics, histology, and neurology/biology; final date for submission of abstracts is 11 January 1972. Please address all inquires to Dr. William Montagna, General Chairman of the Congress, at ORPRC, 505 N. W. 185th Avenue, Beaverton, Oregon 97005, U.S.A.