trum varies widely in ungulates, depending in part on the efficiency of transfer and the vigor and specificity of the doe's immune response (*14*). Other variables to be considered are the dose of infecting virus, variations in the efficiency of the immune response, nonimmunologic controls over intracellular virus expression, and perhaps virus strain differences that may affect tropism.

Encephalomyelitis in caprine arthritis-encephalitis resembles visna of sheep (*7*), and immunodiffusion data indicate antigenic similarity but not identity between CAEV and visna virus. This is consistent with a recent observation of antibody to visna virus in goat serum (*15*). It seems likely that CAEV is part of a family of antigenically related ungulate retroviruses that cause chronic degenerative disease of several organ systems. The connective tissue component of the caprine arthritis-encephalitis syndrome is apparently unique, in that it represents the only reported viral-induced chronic arthritis of mammals.

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Genetic Differences in Physiological Tolerances of Amargosa Pupfish (*Cyprinodon nevadensis*) Populations

Abstract. *Amargosa pupfish from a constant-temperature spring have a narrower range of temperature and oxygen tolerances than pupfish from the much more variable Amargosa River, indicating that the two populations have diverged genetically since their isolation. The reduced tolerances of the fish inhabiting a constant environment support the predictions of evolution theory.*

Approximately 10,000 years ago, the lowering of water levels in the Death Valley area of the western United States caused the isolation of populations of desert pupfish (genus *Cyprinodon*) into different habitats. A major question for evolutionary biologists concerns the extent to which the several species and populations of pupfish (*1*, *2*) have differentiated genetically (*2*, *3*). Despite the morphological divergence among these fishes (*2*), investigators studying other aspects of pupfish biology (biochemistry, behavior, and physiology) have been unable to demonstrate genetic differentiation among the populations (*3*).

Of particular interest is the extent of differentiation in physiological tolerances. Pupfish habitats differ widely in temperature, salinity, and dissolved oxygen. For example, some populations inhabit springs with constant temperatures, while others inhabit rivers with variable temperatures. This latter observation led Brown and Feldmeth (*4*) to compare temperature tolerances of pupfish inhabiting variable and constant environments. They argued that natural selection should produce fish with reduced thermal tolerances in constant springs, compared to those experiencing variable temperatures. They concluded, however, that "there is no evidence of genetic differences in short-term thermal tolerances between any of the populations tested." The fish they compared were collected directly from the field, rather than raised in the laboratory in similar conditions from egg to adult. In addition, their methods of analysis could not have detected slight, but real, differences (*5*).

In the course of experiments to evaluate genetic differences between two subspecies of *Cyprinodon nevadensis*, *C. n. mionectes* and *C. n. amargosae*, we determined their physiological tolerances to temperature and oxygen. These two populations inhabit very different environments: *C. n. mionectes* is found in Big Spring, a constant temperature (27.3°C) desert spring, while *C. n. amargosae* is found in the Amargosa River, which varies in temperature from near 0° to 40°C. Populations of these two subspecies have been isolated for perhaps 400 to 5000 years (*1*, *6*). We predicated that *C. n. mionectes* would show a narrower range of thermal tolerances than *C. n. amargosae* because of the lack of temperature fluctuations in Big Spring. In addition, we expected that *C. n. mionectes* would not tolerate oxygen levels as low as *C. n. amargosae* could because the latter must frequently face nearly anoxic conditions in the summer when flow in the river is reduced and the fish are isolated in side pools.

All experiments were performed on fish that had spent their entire lives under identical conditions. Adults of both subspecies were collected in the field and acclimated to 27°C for 1 month before eggs were collected. Ten males and 50 females of each subspecies were the source of the fish tested. Offspring were isolated before hatching and maintained under identical conditions (temperature, water, and feeding regimes). Some experiments were performed on the F_2 offspring of these fish, also isolated and reared under identical conditions. Any differences persisting after one or two generations under identical conditions are considered to be genetically based. Hybrids between the F_1 fish were available for some tests. Hybrid intermediacy also may constitute evidence of genetic divergence of stocks (*7*). Several experiments were carried out to determine oxygen and temperature tolerances:

1) For oxygen shock experiments (*8*), fish were transferred from their aquariums to tanks with oxygen levels at 1.00 or 1.45 ppm, and time until loss of equilibrium was recorded for each individual.

2) For determining critical oxygen minima (*9*), ten fish were placed in test aquariums; the oxygen concentration was reduced until the fifth fish lost equilibrium and was recorded at that point.

3) In thermal shock experiments (*10*),

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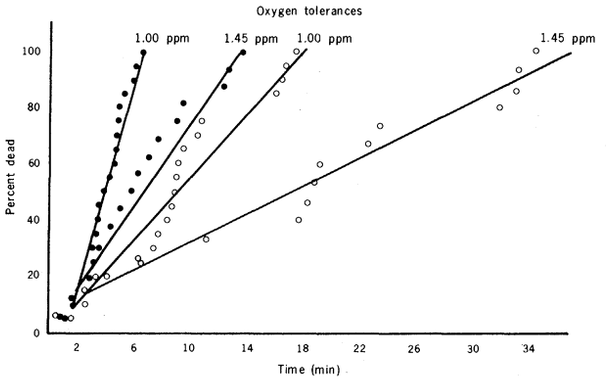

Fig. 1. Mortality of individual pupfish at low oxygen concentrations as a function of time. Each point represents the time at which one fish lost equilibrium. Solid circles, *C. n. mionectes*; open circles, *C. n. amargosae*. (The lines are drawn to aid discrimination of points.)

fish of both subspecies (maintained at 25° ± 1°C after hatching) were added to test aquariums at 8°C, and time until loss of equilibrium was recorded. In another set of similar experiments the water in test aquariums was 38°C.

4) Critical thermal maxima and minima (*11*) were determined for individual F_1 fish (isolated from the egg stage; maintained at 27° ± 1°C) by heating or cooling the water and recording the temperature at death or loss of equilibrium.

The median time to loss of equilibrium was substantially longer for *C. n. amargosae* than for *C. n. mionectes* at both low oxygen levels (Fig. 1; $P < .005$ for both, two-tailed W test) (*12*). At oxygen concentrations of 1.00 ppm, the mean time to loss of equilibrium was 4.03 minutes for *C. n. mionectes*, 8.76 minutes for hybrids, and 9.09 minutes for *C. n. amargosae*. Critical oxygen minima were 1.52 ppm for *C. n. amargosae* and 1.65 ppm for *C. n. mionectes*; hybrids again showed an intermediate value of 1.60 ppm.

The cold shock tests showed that *C. n. amargosae* survived significantly longer than *C. n. mionectes* ($N = 20$, $P < .002$; two-tailed W test of the median time of survival). Hybrids showed intermediate tolerances; the mean time to loss of equilibrium was 1.26 minutes for *C. n. mionectes*, 1.88 minutes for hybrids, and more than 3.68 minutes for *C. n. amargosae*. In the heat shock tests, eight of ten *C. n. mionectes* died within 6 minutes, while two lived longer than 15 minutes (at which point the experiments were terminated). None of ten *C. n. amargosae* died within 15 minutes. (Proportions different at $P < .01$; binominal confidence limits for proportions). Three of ten hybrids died within 15 minutes.

Critical thermal maxima and minima of the two populations also differed (Fig. 2) ($P < .05$, two-tailed W test). Since maxima and minima were run on the same fish, individual thermal ranges could be calculated. These also differed significantly (two-tailed W test, $P < .001$).

The two populations have thus diverged genetically since they have been isolated. The amount of differentiation is not great, however; acclimation temperatures certainly play a more important role in determining their tolerances than do genetic differences. Nevertheless, the genetic differences could be important to fish under natural conditions. Suppose some *C. n. mionectes* found themselves washed into the Amargosa River, for example. Pupfish have frequently been observed swimming within 1° or 2°C of their incipient lethal temperature (*1, 13*); the reduced tolerances of *C. n. mionectes* could, therefore, lead to their elimination. Similarly, pupfish often respond to predators by diving into the low-oxygen-content mud of the river bottom (*1*). The ability to tolerate low oxygen levels for 10 minutes rather than 5 might be critical to an individual in such circumstances.

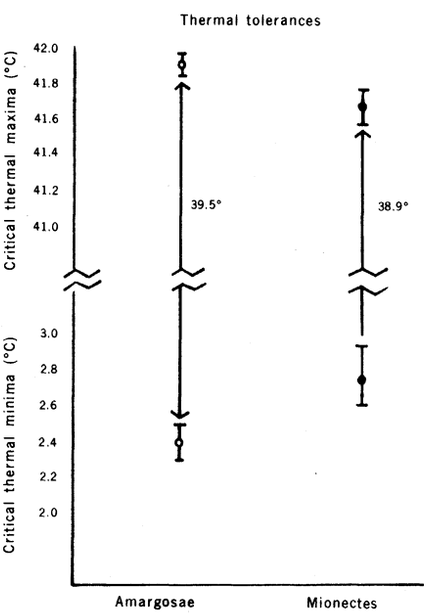

Fig. 2. Critical thermal maxima and minima (means ± S.E.) for pupfish, reared at 27°C. Open symbols, *C. n. amargosae* ($N = 23$); closed symbols, *C. n. mionectes* ($N = 12$). Values adjacent to vertical lines are average thermal ranges of individual pupfish.

Shrode and Gerking have shown that the thermal tolerance limits for oogenesis and egg development of pupfish are much narrower than those for adult survival (*14*). They predicted that, for a variety of species, reproductive limits should be approximately one-seventh of the critical thermal range, implying that fishes with narrower critical thermal tolerances should also show narrow reproductive tolerances. If this is true, then *C. n. mionectes* might well have a further disadvantage in the thermally variable Amargosa River.

The results support the hypothesis that organisms from constant habitats should show reduced ability to deal with environmental variability, and that such evolutionary changes can take place relatively rapidly. Powers *et al.* (*15*) have demonstrated that differences in lactate dehydrogenase allozymes in *Fundulus heteroclitus* (a closely related fish) affected oxygen saturation curves for hemoglobin, and presumably oxygen tolerances as well. Such results suggest that the evolution of physiological tolerance differences may not be as unlikely as has been thought in the past (*3, 4*).

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10. F_2 fish were used. Plastic test aquariums were immersed in a thermostatically controlled water

bath. Temperatures were monitored with a Honeywell 24-channel recording thermograph verified by a Schultheis mercury thermometer. For validation and discussion of this technique, see B. Bradley, *Limnol. Oceanog.* **21**, 596 (1976).
11. Individually reared F_1 fish in 180-ml plastic containers with holes to allow water circulation were placed in a large (about 100 liters), thoroughly aerated, water bath. Water temperatures were lowered approximately 10°C during the first hour and approximately 5°C per hour thereafter. Temperature at loss of equilibrium was recorded for each fish to 0.1°C. The temperature was raised slowly to 27°C after the experiment; 2 days later, the experimental procedure was repeated, raising the temperature approximately 8°C the first hour and 3°C per hour thereafter. The temperature at cessation of respiratory ventilation was recorded.
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Temperature Sensitivity of Tone in the Rabbit Facial Vein: Myogenic Mechanism for Cranial Thermoregulation?

Abstract. *Intrinsic myogenic tone in the buccal segment of the rabbit facial vein is exquisitely sensitive to small changes in temperature in the range 33° to 44°C. This particular venous segment also exhibits a preponderance of β-adrenergic receptors and receives a dense, medial sympathetic innervation. This area of the vein is proposed to act as a temperature-sensitive sphincter that distributes cooled nasal venous blood between superficial and deep venous drainage systems in the head and neck. Deviation of cool blood to deeper venous sinuses has been shown to be an important thermoregulatory mechanism.*

The buccal segment of the rabbit facial vein exhibits a number of uncommon properties. Isolated rings from this 10- to 14-mm length of vessel dramatically develop myogenic tone in response to stretch (*1*) and display a β-adrenergic receptor-mediated relaxation to sympathomimetic stimulation (*1*, *2*). The smooth muscle cell layers are endowed with a dense, three-dimensional adrenergic innervation (*2*). These characteristics are not observed in more proximal or distal segments of the vein nor in the adjacent part of the facial artery. A physiological role for this segment has not been proposed.

We have observed that the intrinsic myogenic tone of the rabbit facial vein responds to very small changes in ambient temperature. This tone is maintained as long as stretch is applied and is independent of both the sympathetic innervation and known endogenous vasoactive autacoids (*1*). We hypothesize that the temperature-sensitive changes in tone that occur in this restricted vascular segment are responsible for influencing the direction of venous return from the nose, either allowing cooled venous blood to course through the facial vein en route to the external jugular vein or shunting the flow of blood through deep cranial sinuses. These temperature-induced changes in venous flow assist in maintaining brain homothermia.

The right and left facial veins of New Zealand White rabbits (2.0 to 2.6 kg) were excised distal to their confluence with the deep facial vein. Two cleaned 4-mm rings were prepared from each vein and set up in tissue baths so that changes in vessel contractility could be recorded (*2*). They were equilibrated for 1 hour (without tension) in a modified Krebs physiological saline solution of the following millimolar composition: Na^+, 144.2; K^+, 4.9; Ca^{2+}, 1.6; Mg^{2+}, 1.2; Cl^-, 126.7; HCO_3^-, 25; SO_4^{2-}, 1.19; glucose, 11.1; and ethylenediaminetetraacetic acid, 0.024, bubbled with 95 percent O_2 and 5 percent CO_2. Water circulated from a heated reservoir around the tissue bath to maintain temperature normally at 37.5°C. Tissues were washed every 15 minutes during this equilibrium. After 1 hour, rings were stretched so that approximately 0.5 g of tension was applied to the vessel wall. The resulting developed tone was quantified by measuring the extent of relaxation following the addition of sodium nitrite ($5 \times 10^{-2} M$) (Fig. 1A). The temperature of the Krebs saline solution was increased or decreased by 1°C intervals by adjusting the temperature of the reservoir. Bath temperature was monitored by a thermistor probe (Yellow Springs Instrument model 421) suspended in the tissue bath and connected to a telethermometer bridge (Y.S.I. model 425c). The amount of tone recorded with each change in temperature was expressed as a percentage of the maximum tension produced by exogenously added histamine at 37.5°C (*3*).

Figure 1A illustrates changes in intrinsic tone that occur in response to small changes in bath temperature; the relationship between the equilibrium level of tone and bath temperature is shown in Fig. 1B. A fall of only 1°C from the control level of 37.5°C reduced the level of tone by 50 percent; a rise of 1°C

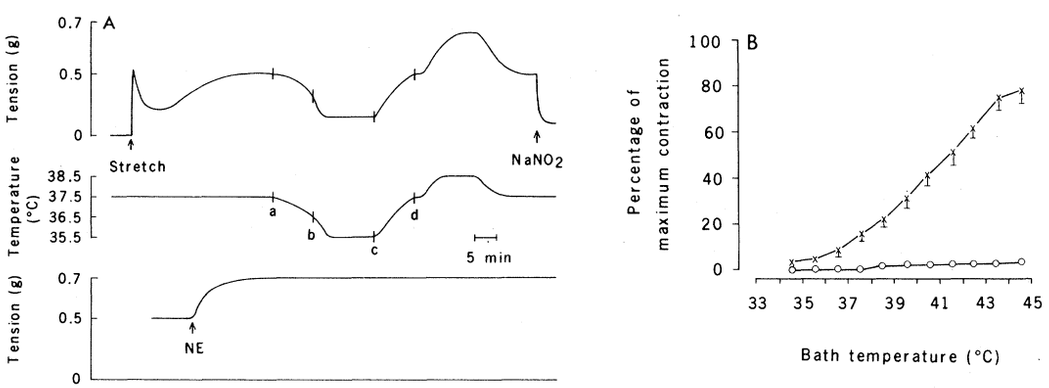

Fig. 1. (A) Typical in vitro tension recording of a rabbit facial vein (top trace). A maintained tone develops after the vein is stretched, but decreases if the tissue bath temperature (middle trace) falls by 1°C (*a*). There is a corresponding increase in the decline of tone if the ambient temperature falls faster (*b*). Tone returns to control levels as the temperature readjusts to 37.5°C (*c*). There is a reversible doubling of the level of tone accompanying a 1°C rise in ambient temperature (*d*). The total amount of developed tone is indicated by the addition of a maximal dose of sodium nitrite. Venous segments distal to the facial vein sphincter failed to show this temperature sensitivity when tone was induced by the addition of *l*-norepinephrine ($10^{-7} M$) (bottom trace). (B) Tone present in control (X) and sodium nitrite-treated ($5 \times 10^{-2} M$) (○) facial vein rings in a 12°C range of ambient temperature, expressed as a percentage of the maximal contraction elicited by histamine at 37.5°C. Data points are means ± standard errors for the control curve.

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