chromosome homology, such as banding, is needed to further illuminate chromosome evolution within the Xantusiidae.

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FREEZE TOLERANCE AS AN OVERWINTERING ADAPTATION IN COPE'S GREY TREEFROG (*HYLA CHRYSOSCELIS*).—Many temperate zone ectotherms are confronted with severe environmental challenges during winter. Aquatic forms usually are protected from freezing temperatures owing to the high thermal buffering capacity of water. Terrestrial vertebrate ectotherms generally avoid extreme winter temperatures by hibernating within insulated refuges below the frostline; however, some may be exposed to potentially lethal environmental temperatures. Those overwintering above the frostline must survive either by extensive supercooling or by tolerating the formation of ice within body tissues.

Deep and prolonged supercooling is a major overwintering adaptation of many terrestrial invertebrates (Lee, 1989). However, the biophysical constraints of large body mass (i.e., water volume) preclude this strategy for most verte-

brate ectotherms (Costanzo et al., 1988; Costanzo and Claussen, 1990). Many polar fishes avoid freezing by supercooling, but these produce specific antifreeze proteins that inhibit the growth of ice crystals (DeVries, 1983).

At least several species of amphibians and reptiles survive the repeated freezing and thawing of extracellular fluids. Natural freeze tolerance has been conclusively demonstrated in four species of frogs: *Rana sylvatica* (Lotshaw, 1977), *Hyla crucifer*, *H. versicolor* (Schmid, 1982), *Pseudacris triseriata* (Storey and Storey, 1986); two species of turtles: *Chrysemys picta* (Storey et al., 1988), *Terrapene carolina* (Costanzo and Claussen, 1990); and one snake: *Thamnophis sirtalis* (Costanzo et al., 1988). Laboratory experiments involving freezing in other species, including toads (Storey and Storey, 1986), aquatic frogs (Weigmann, 1929; Lotshaw, 1977; Schmid, 1982), plethodontid and ambystomid salamanders (Storey and Storey, 1986; our unpubl. data), and lizards (Weigmann, 1929; Spellerberg, 1972; Claussen et al., 1990), have resulted in death, irreparable injury, or ecologically negligible survival rates.

We hypothesized that Cope's gray treefrog (*H. chrysoscelis*) is tolerant of body freezing because this species is similar to *H. versicolor*, a known freeze-tolerant form, in morphological, genetic, and ecophysiological characteristics (Ralin, 1977). The two species differ primarily in chromosome compliment (*H. chrysoscelis* is diploid, whereas *H. versicolor* is tetraploid) and in the pulse frequency of mating calls (Ralin, 1977, 1981). The *H. chrysoscelis–versicolor* complex is distributed widely over eastern North America and ranges from the Gulf Coast of the United States to Manitoba and Nova Scotia, Canada (Conant, 1975). Geographically these species may be widely separated although they are sympatric over some parts of their ranges (Ralin, 1977). We investigated freeze tolerance in a population of *H. chrysoscelis* from southwest Ohio, located only 32 km from a breeding population of *H. versicolor*, individuals that are known to be freeze tolerant (Layne and Lee, 1989).

Materials and methods.—Cope's gray treefrogs (*H. chrysoscelis*) were collected as they called from a small breeding pond in Butler County, Ohio, during June 1989. Eight adult males [$\bar{x}$ mass ± standard error of the mean (SEM) = 11.2 ± 0.4 g] were kept on damp moss inside an environmental chamber. Frogs were exposed to 23 C under a 12:12 (L:D) photoregime and were fed crickets and flesh fly larvae ad libitum. On 15 Oct., the frogs were habituated to 15 C, 12:12 (L:D) for 2 wk during which time food was withheld. Subsequently, the frogs were exposed to hibernative conditions [4 C, 0:24 (L:D)] until they were tested for freeze tolerance (3–8 Jan. 1990).

Eight frogs, held individually within plastic (50 ml) centrifuge tubes, were cooled inside an insulated jar submerged in a cold (−6.5 C) bath. The airspace in each tube was insulated with plastic foam to reduce the rate of ice formation. A 30-gauge thermocouple positioned against the abdomen provided a continuous recording of body temperature on a data logger (OM 501-C, Omega Electronics).

Ice formation in supercooled frogs was induced by briefly applying aerosol coolant to the exterior of each tube. The onset of ice formation was clearly indicated by the release of the latent heat of fusion. Freezing proceeded until the following criteria were met: (1) a minimum of 24 hr had elapsed, and (2) body temperature had declined to −2.5 C or below. The frogs were then transferred to a cold room (4 C) and allowed to thaw. Following a 3 d habituation period they were transferred to 22 C and tested for recovery criteria. We judged that the frogs had recovered fully if they fed (caught and ingested live crickets), maintained normal body postures, and locomoted spontaneously.

The blood of nine additional frogs (collected in Butler County, Ohio, during June 1990 and exposed to hibernative conditions for 30 d) was analyzed to determine whether glucose or glycerol, cryoprotectants utilized by freeze-tolerant frogs (Storey, 1990), are mobilized by *H. chrysoscelis*. Blood was obtained from six rapidly thawed frogs previously frozen to −2.9 C (±0.1 C, SEM) for 53.5 h and from three unfrozen (control) frogs; it was collected directly from the ventricle and centrifuged to separate the plasma. The plasma was assayed for glucose using a colorimetric procedure (no. 510, Sigma Chemical Company, St. Louis, Missouri) and for glycerol using established methods (Baust et al., 1983) for high-performance liquid chromatography (HPLC). The osmotic concentration of plasma was measured using a Wescor 5500 vapor pressure osmometer.

Although *H. chrysoscelis* and *H. versicolor* are morphologically indistinguishable, each species can be identified on the basis of its karyotype. One drop of blood from two unfrozen frogs was separately mixed with 0.5 ml buffered formalin

(pH 6.8) and examined at 100× on a phase contrast microscope. Cell dimensions (length and width) were measured for random samples of erythrocytes using an ocular reticule. Erythrocyte measurements of our frogs (19.5 × 13.4 μm) were much smaller than those (22.3 × 16.1 μm) from sympatric *H. versicolor* (J. Vaughn, unpubl.). Thus, the frogs used in our experiments were undoubtedly the diploid form, *H. chrysoscelis*.

Results.—The eight frogs tested in winter cooled to a mean (±SEM) temperature of −1.8 ± 0.2 C before body freezing was induced. Following ice nucleation they cooled slowly ($\bar{x}$ = 0.13 ± 0.01 C/h), remained frozen for 24.0–40.0 h ($\bar{x}$ = 28.3 ± 1.7 h), and reached final body temperatures of −2.5 to −2.9 C ($\bar{x}$ = −2.7 ± 0.1 C). Upon their removal from the cooling chamber, the limbs and skin of the frogs were rigid indicating that much ice had formed within their bodies. Respiratory activity was not detected. Most frogs regained the righting response and the ability to retract their limbs within 48 h of thawing. One individual appeared dead following thawing; however, by 72 h, it resumed a normal posture and eventually recovered fully. Feeding did not commence until >7 d post-thawing, but all frogs eventually accepted food and remained healthy for at least 6 wk.

The marked elevation in plasma glucose concentration in frozen vs unfrozen frogs tested in summer (Table 1) was highly significant ($t = 4.9$, $df = 7$, $P = 0.002$; t-test). The osmotic concentration of plasma from frozen frogs was significantly ($t = 4.4$, $df = 7$, $P = 0.003$; t-test) greater than that from control frogs (Table 1); correlation analysis showed that plasma osmolality was directly related ($r^2 = 0.85$, $df = 5$, $P = 0.009$) to plasma glucose concentration. Glycerol concentrations in all plasma samples were low (<0.1 μmol/g) and, in some samples, below the level of reliable detection.

TABLE 1. FREEZING-INDUCED CHANGES IN BLOOD GLUCOSE AND OSMOLALITY IN COPE'S GRAY TREEFROG, *Hyla chrysoscelis*, TESTED DURING SUMMER. Means are shown ± 1 SEM.

Group	Plasma glucose (μmol/ml)	Plasma osmolality (mOsm)	Mass (g)	n
Unfrozen	1.0 ± 0.2	249 ± 2	9.7 ± 0.6	3
Frozen	24.9 ± 3.3	274 ± 4	8.2 ± 0.5	6

Discussion.—Natural freeze tolerance is an important adaptation of vertebrate ectotherms that potentially are exposed to subzero temperatures during hibernation (Schmid, 1982; Storey and Storey, 1988) or early and late in the activity season (Costanzo et al., 1988). Freeze tolerance, as an effective physiological adaptation, must be a genetically based characteristic of a population. Thus, very high survival rates should result from freezing trials conducted under ecologically relevant temperatures and exposure durations. In the present study, all *H. chrysoscelis* recovered fully from rigorous tests of their ability to survive ice formation in body tissues. Freeze tolerance in this species is likely an important adaptation promoting overwinter survival. W. D. Schmid (1986) reported that *H. chrysoscelis* survived freezing in the laboratory, but did not provide details of the freezing protocol (e.g., cooling rate, equilibrium temperature, duration), cryoprotectant concentrations, or survival criteria.

The ice contents of treefrogs were not measured because a limited number of specimens were available for experimentation. However, the dynamics of ice formation in *H. chrysoscelis* probably are similar to those in sympatric *H. versicolor* (Layne and Lee, 1989). Presumably, about 40–50% of the total body water in *H. chrysoscelis* would have frozen under our experimental conditions; this amount is less than the lethal ice content (52–62%) for *H. versicolor* during winter (Layne and Lee, 1989). Subsequent research on *H. chrysoscelis* should address the physiological limits to, and the effect of season on, its capacity for freeze tolerance. Several investigators (Schmid, 1982; Layne and Lee, 1989) have reported a diminished freeze tolerance in treefrogs during late spring relative to autumn and winter.

The cryoprotectants, glucose and glycerol, utilized by freeze-tolerant frogs are produced via glycogenolysis and are rapidly mobilized from the liver in direct response to ice formation in tissues. Elevated blood concentrations of glycerol (6–42 μmol/ml) and glucose (4–60 μmol/ml) were reported by Storey and Storey (1985) for juvenile *H. versicolor* from Ontario, Canada. Much higher glycerol concentrations in tissues (0.3 M, Schmid, 1982; 465 μmol/g, Storey and Storey, 1985) have been measured in adult *H. versicolor* from northern locales. However, glycerol was not detected in conspecific adults from southeastern Indiana (Layne

and Lee, 1989) nor in our *H. chrysoscelis* collected 32 km distant; thus, glucose is apparently the sole cryoprotectant in both hylids from this region. Because seasonal differences in cryoprotectant synthesis occur in treefrogs (Storey and Storey, 1988), our data for summer-tested *H. chrysoscelis* are not conclusive. Nevertheless, the mean concentration of blood glucose in our summer *H. chrysoscelis* (25 μmol/ml) was very similar to that (23 μmol/ml) reported for sympatric, winter *H. versicolor* (Layne and Lee, 1989).

The available cryoprotectant data for *H. versicolor* from Ontario and Indiana suggest the northern form generates more cryoprotectant in response to freezing. This trend coincides with seemingly adaptive geographic differences in the capacity for freeze tolerance: Ontario frogs (which presumably encounter relatively colder winter temperatures) tolerate freezing at −7.5 C for >2 wk (Storey and Storey, 1985), whereas those from Indiana cannot survive freezing at −7.0 C for as few as 24 h (Layne and Lee, 1989). Whether such latitudinal differences in freeze tolerance and cryoprotectant concentrations exist among *H. chrysoscelis* populations remains to be determined.

The formation and removal of ice within the body invokes a number of physiological perturbations (see review by Storey and Storey, 1988). Because freezing results in the progressive accumulation of ice (i.e., pure water) in the abdominal cavity and beneath the skin, to survive animals must contend with subzero temperature, an increased osmotic concentration, and a decrease in the hydration state of tissues and organs (Lee et al., 1990). *Hyla chrysoscelis* readily endures both cold (Layne and Romano, 1985) and dehydration (Ralin, 1981), attributes which likely enhance its capacity for freeze tolerance. Generally, dehydration tolerance in amphibians is related to the environmental characteristics of their habitats, and many hylids, particularly the arboreal *Hyla* spp., survive a substantial loss of body water (Farrell and MacMahon, 1969). This quality may be an important preadaptation promoting freeze tolerance in treefrogs. Additional research on the ability of hylids to tolerate body freezing, from both ecophysiological and phylogenetic perspectives, should prove rewarding.

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SHORT- AND LONG-TERM MOVEMENTS OF THE FROG *ELEUTHERODACTYLUS JOHNSTONEI* IN BARBADOS, WEST INDIES.—Movements of individuals influence social organization and genetic relationships within and between populations. Following daily movements of individuals can elucidate differences between the sexes and age/size classes in foraging and mating behavior. Movements of adults and juveniles over longer time spans influence genetic structure of populations. Several studies have addressed movements of pond-breeding anurans during the reproductive season (e.g., Fellers, 1979; Given, 1988; Perrill and Shephard, 1989), but little information exists on terrestrial frogs without breeding migrations.

I examined movements by the terrestrially breeding frog *Eleutherodactylus johnstonei* nightly and over several months at two sites in Barbados, West Indies. Males are territorial and defend their calling sites against intruders (Ovaska, 1992). Therefore, I predicted that movements of adult males are confined to the vicinity of their calling sites and that males exhibit site-tenacity and move shorter distances than females. I also predicted that movements of both adults and juveniles are shorter at high than low population densities. Woolbright's (1985) study on movement patterns of *E. coqui* in Puerto Rico provided a basis for interspecific comparisons.

Methods.—Two study sites that differed in vegetation type and structure were approx. 1.5 km apart in St. James, Barbados, West Indies. The Bellairs site, located near the grounds of the Bellairs Research Institute of McGill University, was an untended flower bed (25 m long, 3–7 m wide) separated by lawns and paved areas from other suitable habitats for frogs. Dense ground vegetation included bromeliads (*Billbergia* spp.), which provided retreats for the frogs. The Greenwich site was in a forested gully along a seasonal stream near the village of Greenwich. Ground vegetation was sparse, and frogs sheltered under rocks rather than in vegetation.

To document nightly movements of adult males and females, I followed frogs dusted with fluorescent pigments (Woolbright, 1985) and located their positions hourly all night using a portable ultraviolet light source. I caught frogs (calling males and adult females) soon after dark and, for each frog, recorded sex and snout–vent length (SVL) and sprinkled its dorsum with pigments (red, orange, or green) from a small vial. I released each frog at its original location minutes after capture. At each subsequent sighting of a marked frog, I placed a small numbered tag within 5 cm of the frog to aid in mapping its positions the following day. I measured the distance (a) between subsequent sightings, (b) between two farthest sightings, and (c) total distance moved by each individual. I marked 10–18 frogs on seven nights during the rainy season between 16 July and 20 Sept. 1988 at Greenwich site. On several occasions, however, heavy rains after midnight washed the pigments off. Data for 20 females and 19 males resighted all night and at least five times were obtained from four nights (16, 22 July; 8 Aug.; 20 Sept.). I was less successful in relocating marked frogs at Bellairs site due to dense vegetation and obtained comparable data for only nine males and three females (22, 24, 29, June 1988). I used the Mann-