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Patterns of Genetic Variation in the Cricket Frog, *Acris crepitans*, in Kansas

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Geographic variation and population structure of 16 populations of *Acris crepitans* along a transect in Kansas were examined using the gene products of seven electrophoretically polymorphic protein loci. The pattern of allelic frequency change for adult and juvenile age classes was stable between the years of sampling. A significant difference in allelic frequencies between adult and juvenile age classes was found for two loci. There was a general decrease in genetic variation westward, a pattern which could be explained by the probable frog dispersal route. However, the seven loci differed in variation patterns across the state. Although stochastic events certainly play a role, this differential pattern of allelic frequency change, combined with temporal stability, suggests a major role of selection in shaping the observed geographic variation patterns. In a regression model of environmental variables on gene frequencies of seven loci, six loci differed in significant variables that entered the regression model, and variables that measured fluctuations in temperature and moisture rather than annual averages explained the most variation in allelic frequencies. Variation patterns of the seven loci contributed differentially to aspects of population structuring, e.g., age-class structure, habitat differentiation, and differentiation between geographic areas. The very strong population differences in allelic frequencies between pond and stream habitats suggests local adaptation due to selection.

THE cricket frog, *Acris crepitans*, is a small semi-aquatic frog that is common over much of the eastern half of the United States. Previous studies show that across its geographic range, *A. crepitans* exhibits variation in a diversity of characters: electromorphs (Dessauer and Nevo, 1969; Salthe and Nevo, 1969), morphology (Nevo, 1973a), color morphs (Nevo, 1973b), supernumerary chromosomes (Nur and Nevo, 1969), and mating call characteristics (Capranica et al., 1973). These studies, however, covered large geographic areas, and did not provide detail on gene flow patterns, temporal stability, or historical aspects which are pertinent to the evaluation of possible causal mechanisms of observed genetic variation patterns.

The state of Kansas spans a western segment of the range of *A. crepitans*, yet its length incorporates a variety of habitats and climatic regimes. Climatic and physiographic patterns in Kansas are relatively simple because there is considerable contrast longitudinally but not latitudinally. The major river drainages flow almost directly west to east, a result of the Mississippi River system downcutting westward during the Pleistocene (Metcalf, 1966). Subsequently, dispersal and gene flow in *Acris* in the Kansas River drainage may have been restricted to a linear pattern of westward movement along a climatic and physiographic gradient. This study examined patterns of allozymic variation in populations of *A. crepitans* along an east–west transect in Kansas, in relation to probable dispersal patterns and environmental influences. Patterns of variation between age classes and sample years, and genetic differentiation in two habitat types, ponds and streams, in eastern Kansas were also examined.

MATERIALS AND METHODS

Sixteen sampling localities in Kansas were chosen for study. Thirteen were distributed along a 30°N latitude transect within the Kansas River drainage from the western to the eastern border (Fig. 1), and represent a possibly continuous distribution of frogs. Three southern localities (numbers 7–9) were in the Arkansas River system.

Sampling localities were grouped into four geographical areas based on geographic location or drainage system—western, central, eastern, and southeastern Kansas (Fig. 1). All sam-

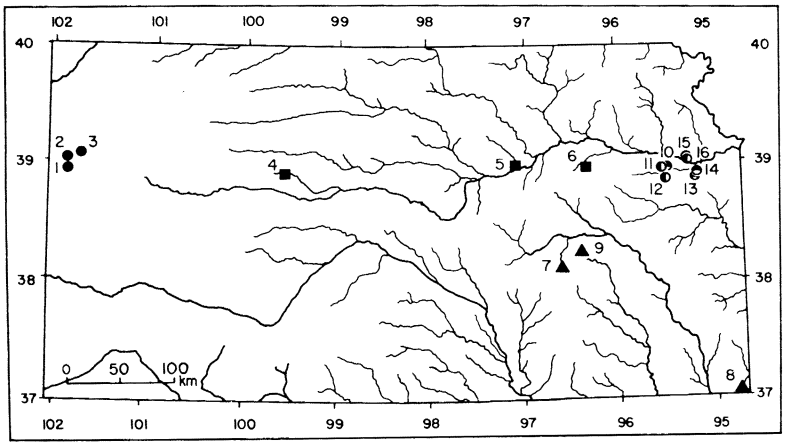

Fig. 1. Sampling localities in Kansas. Solid circles = the western Kansas group, squares = the central, triangles = the southeastern, and circles divided horizontally and vertically = the eastern ponds and streams, respectively. Locality names corresponding to numbers are: 1, Willow Creek; 2, Goose Creek; 3, Lake Creek; 4, Big Creek; 5, Chapman Creek; 6, Mill Creek; 7, Cottonwood River (Thurman Creek); 8, Shoal Creek; 9, Jacobs Creek; 10, Collins Pond; 11, Deer Creek; 12, Rock Creek; 13, Womaker Pond; 14, Quarry Pond; 15, Ditch Pond; 16, Mud Creek.

pling localities were stream habitats, except the eastern group where pond localities were included. Three pairs of eastern localities in close proximity (0.1–7 km) were chosen: two pairs consisted of a pond and a stream and the third, two ponds (Fig. 1).

Temperature and moisture variables were recorded for each locality. Variables were chosen to measure yearly averages as well as within-year variation (following Bryant, 1974). Climatic data were taken from Anonymous (1972–1978) and Flora (1948). The coefficients of variation of mean monthly precipitation and temperature and mean daily temperature range were from months the frogs were generally active (April–Oct.).

Frogs in eastern Kansas were collected every 4–6 wk from late 1978 through 1980. Western and central localities were sampled once in the fall of 1979 and 1980, and spring of 1980. The southeastern localities were sampled once. Frogs were caught by hand along the water's edge and approx. 100 m were covered for each locality. Attempts were made to collect 20–30 frogs at each locality, otherwise the collecting period was terminated after 45 min.

Blood, liver, and heart tissues were taken from anesthetized frogs and stored at −20 C. Liver and heart tissues were frozen in equal volumes of frog Ringer's solution. During the tissue removal process color morph, reproductive condition, sex, and age (adult or juvenile) were recorded for each frog. Due to high mortality (Burkett, 1969; Gray, 1983), few adults were caught after late July when the juveniles metamorphosed. The adult age class, therefore, primarily represents those frogs caught between April and Aug. and juveniles between Aug. and Oct.

Seventeen loci were initially surveyed using horizontal starch gel electrophoresis and seven consistently scorable polymorphic loci were chosen for study. Procedures generally followed those of Selander et al. (1971) and Shaw and Prasad (1970). Phosphoglucomutase (PGM), glucose phosphate isomerase (GPI), and superoxide dismutase (SOD) from liver tissue were run in a tris EDTA borate buffer, pH 8.1. Two esterases, using the substrates 4-methylumbelliferyl acetate (UMEST) and a-naphthyl acetate (aNAC) also were scored from liver but were run in a tris citrate buffer, pH 7.5. Leucine amino peptidase (LAP) and general protein (GPRO) from blood plasma were run in lithium hydroxide buffer, pH 8.1, and heart lactate dehydrogenase (LDH) was run in tris citrate, pH 7.5.

Allelic frequencies were calculated separately for adults, juveniles, and for each sampling date. Heterogeneity Chi-square analyses determined age-class differences and differences between sample years within each age class.

Several measures of genetic variation were used to compare populations by localities and geographic areas. Percent polymorphic loci is the percent of the seven loci that were polymorphic at the 95% level. The polymorphic index, PI, is the equivalent to the mean heterozygosity per locus under Hardy-Weinberg equilibrium (Hamrick and Holden, 1979). The PI's within a geographical area were averaged for an area PI. The average effective number of alleles, $\tilde{N}_e$, for each locality was calculated by the methods of Hartl (1980), and an area $\tilde{N}_e$ was determined as for area PI. To determine inbreeding, Wright's F_{IS} value ($F_{IS} = 1 - H_o/H_e$) (Wright, 1965) was calculated for each locality and for each locus, where H_o is the observed heterozygosity and H_e is the expected.

Population structuring was characterized using Nei's genic diversity index (Nei, 1973). Nei's G_{ST} is equivalent to Wright's F_{ST} but accommodates more than two alleles and several levels of structuring. Total genic diversity or heterozygosity (H_T) may be partitioned into diversity within and among geographic areas, within and among localities in a geographic area, and within and between age classes in a locality. Significance of G_{ST} values was tested with a heterogeneity Chi-square for unequal sample sizes (Workman and Niswander, 1970).

Genetic similarities among localities were summarized by: 1) a UPGMA phenogram (Sneath and Sokal, 1973) based on a matrix of Nei's genetic distance, uncorrected for sample size (Gorman, 1983); and 2) a principal components analysis (BMDP/4M81) of the covariance matrix derived from arcsine-transformed allelic frequencies. In the case of multiple alleles (e.g., GPI) the less common alleles were combined to yield two alleles per locus for both of the above methods.

Finally, a multiple stepwise regression analysis (BMDP/2R81) of environmental variables on each locus after arcsine transformation of frequency of the most common allele was run to determine which variables statistically explained the greatest geographic variation in allelic frequencies.

Results

Significant year-to-year differences in allelic frequencies occurred in both adults and juveniles; however, there was little consistency to the pattern of differences among these population subgroups. For adults the only locus that exhibited a consistent (but statistically nonsignificant) pattern of temporal gene frequency change among localities was SOD, where the frequency of the common allele increased between 1979 and 1980. The juvenile pattern of temporal change was different from that of adults in that GPRO was the only locus with a consistent pattern among localities of a decrease in allelic frequency from 1979–80 and for three localities these differences were significant. The general lack of consistent temporal changes in allelic frequency among localities for either adults or juveniles suggests that there is not a strong yearly component to allelic frequency variation.

Significant allelic frequency differences occurred between the adult and juvenile age classes, 93% of which were accounted for by three loci, UMEST, LAP, and GPRO. For all localities but one (Deer) the frequency of the common allele in juveniles was lower than that of adults at the UMEST locus (four localities significantly lower). For LAP the common allele frequency increased in juveniles (five localities significantly). For GPRO half of the significant differences were attributed to an increase in frequency of the common allele in juveniles and in half a decrease. These age-related differences suggest that there may be differential selection acting on the terrestrial vs aquatic life stages.

When adult and juvenile allelic frequencies were combined for each locality, a general decrease in the level of polymorphism from east to west was observed (Table 1). For five of seven loci (GPI, SOD, UMEST, aNAC, and LAP, but not GPRO or LDH) there was an increase in the average frequency of the common alleles from east to west. Wright's inbreeding coefficient, F_{IS}, was slightly lower in western Kansas, which suggests that the clinal decrease in genetic variation from east–west was not due to increased inbreeding. Loci showed differential patterns of variation westward, however, when examined individually. Unlike SOD, UMEST, and aNAC which showed a moderate increase in the frequency of the common allele from east to west (Table 2), LAP and GPI exhibited only a slight westward increase in the common allele, from 0.911 and 0.891, respectively, in eastern Kansas to 0.960 and 0.995, respectively, in western Kansas. The pattern for LDH, however, was very different. The common allele in eastern Kansas decreased westward and eventually was

TABLE 1. MEASURES OF POLYMORPHISM AND INBREEDING COEFFICIENTS FOR INDIVIDUAL LOCALITIES AND AVERAGES FOR GEOGRAPHIC AREAS. All values are unweighted for sample size. PI is polymorphic index (see text) and $\bar{N}_e$ is the effective number of alleles for seven loci.

Locality	N	% Poly loci (95%)	Area % poly	PI	Area PI	$\bar{N}_e$	$\bar{N}_e$	Freq.[a] common allele	Area freq.	F_{IS}	Area F_{IS}
Willow	82	.429		.117		1.18		.960		−.039	
Goose	41	.429		.110		1.17		.966		.139	
Lake	153	.571		.147		1.22		.939		.235	
			.476		.125		1.19		.955		.122
Big	48	.571		.289		1.56		.803		.033	
Chapman	92	1.00		.343		1.58		.758		.248	
Mill	99	1.00		.327		1.52		.815		.235	
			.857		.320		1.55		.792		.172
Cottonwood	27	1.00		.349		1.60		.801		.187	
Shoal	7	.833		.270		1.45[b]		.857		.153	
Jacobs	7	.714		.335		1.64		.714		.243	
			.849		.318		1.56		.791		.194
Collins	95	.857		.348		1.63		.739		.315	
Deer	44	1.00		.369		1.69		.730		.128	
Rock	152	1.00		.366		1.63		.749		.310	
Womaker	179	.857		.309		1.54		.781		.168	
Quarry	235	.857		.334		1.58		.755		.301	
Ditch	161	.857		.353		1.63		.733		.097	
Mud	28	1.00		.372		1.63		.742		.030	
			.918		.350		1.62		.747		.193

[a] Average for five of seven loci.
[b] For six loci.

eliminated. The common allele of GPRO in eastern Kansas also decreased westward, though not to the same extent as that of LDH.

Nei's genic diversity index indicated that loci differed in levels of variation and that most of the observed variation was within-population and not due to population structuring (Table 3). Individual loci differed most from one another with regard to the among-area component, G_{ST}, i.e., loci differed in amount of geographic variation (Table 3). While LDH varied the most geographically, heterogeneity Chi-square tests indicated significant geographic heterogeneity ($P < 0.001$) for each of the seven loci. LAP, GPI, and LDH had the strongest among-locality component, G_{LS} (Table 3), and even though LAP and GPI had the lowest amount of geographic variation, they differentiated localities within areas. GPRO had the strongest between-age-class component, G_{AL} (Table 3). Recall from previous heterogeneity Chi-square tests between age classes that UMEST, LAP, and GPRO accounted for 93% of the significant differences, but GPRO did not display a consistent pattern.

The principal components analysis (adult and juvenile frequencies combined) also revealed that the seven loci differentially explained geographic variation. The first three factors of the analysis explained 83% of the variance. Factor 1 explained 51% of the variance and generally grouped the localities in their proper east-west geographical positions (Fig. 2). The highest factor loadings for Factor 1 were LDH and aNAC. LAP had the highest factor loading of Factor 2, which separated pond samples from stream samples in the eastern Kansas cluster. SOD had the highest loading of Factor 3, which separated the clusters for central and southeastern Kansas samples. Factors 2 and 3 explained 19% and 13% of the variance, respectively. These results concur with results from Nei's diversity index analysis (Table 3) where LDH and aNAC explained the highest among-area variation and LAP explained the highest among-locality variance.

TABLE 2. ALLELIC FREQUENCY DISTRIBUTION[a] OF SEVEN POLYMORPHIC LOCI OF *Acris crepitans* FROM 16 SAMPLING LOCALITIES ACROSS KANSAS. Only one of two allelic frequencies is indicated for all loci but GPI, for which all four are indicated. Numerical designation of localities as in Figure 1.

Locus	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
UMEST	.927	.903	.917	.479	.625	.752	.796	.929	.500	.611	.732	.663	.664	.645	.587	.661
aNAC	.970	.988	.964	.573	.538	.688	.685	.714	.429	.469	.670	.608	.564	.515	.503	.661
LAP	1.00	.940	.940	.975	.901	.894	.768	1.00	1.00	1.00	.730	.929	.958	.924	.984	.952
GPRO	.336	.357	.371	.474	.759	.590	.286	—	.286	.436	.567	.539	.541	.507	.474	.589
LDH	.000	.000	.000	.375	.683	.769	.554	.500	.571	.733	.833	.813	.890	.848	.785	.848
SOD	.909	1.00	.882	.100	.793	.854	.852	.857	.643	.700	.568	.707	.773	.736	.708	.786
GPIa	.006		.010	.010	.051	.089	.019	.214		.053	.049	.104	.052	.029	.116	.250
b	.994	1.00	.990	.990	.932	.886	.904	.786	1.00	.916	.951	.837	.948	.953	.884	.750
c					.017	.011	.019			.031		.029		.018		
d						.011	.058									

[a] Combined adult and juvenile data for all sampling periods.

TABLE 3. RESULTS OF NEI'S GENE DIVERISTY INDEX. H_T is total expected heterozygosity, G_{AL} is gene diversity between age classes within localities, G_{LS} is gene diversity among localities within geographic areas, and G_{ST} gene is diversity among geographic areas within Kansas.

Locus	Diversity index[a]			
	H_T	G_{AL}	G_{LS}	G_{ST}
GPI	.1337	.002	.031	.018
SOD	.3410	.004	.016	.041
UMEST	.4192	.007	.014	.055
aNAC	.4608	.002	.014	.114
LAP	.1319	.011	.049	.007
GPRO	.5000	.038	.014	.041
LDH	.4796	.008	.026	.423
Average	.3523	.010	.023	.100

[a] $G_{AL} = D_{AL}/H_T$ where, for expected heterozygosities, $D_{AL} = H_{Locality} - H_{Age}$, $G_{AL} = D_{LS}/H_T$ where $D_{LS} = H_{Subpopulations} - H_{Locality}$ and $G_{ST} = D_{ST}/H_T$ where $D_{ST} = H_{Total} - H_{Subpopulations}$.

The UPGMA analysis generally grouped localities according to geographic area, with the western localities forming a very distinct cluster from the rest (Fig. 3). The two pairs of closely related eastern localities were ponds, yet streams were in closer proximity to Ditch and Collins ponds (Fig. 1). Thus, genetic similarities among eastern localities was not a function of the geographic distance between them.

Environmental variables that entered the regression models differed among loci (Table 4). Moisture variables explained most of the variance for LDH and SOD, whereas temperature variables did so for aNAC, UMEST, GPRO, and GPI. Of the variables entered, those that measured variation within sample years entered the model first for 5 of 6 loci. This suggests that within-year variation in temperature or precipitation was perhaps more important biologically than annual averages. The regressions were significant ($P < 0.001$) for all loci but LAP.

DISCUSSION

Temporal stability of allelic frequency of populations of *A. crepitans* throughout the state of Kansas indicates that stabilizing selection is strong enough to overcome yearly fluctuations due to drift. Temporal stability is apparent even though the average lifespan for *Acris* is 1 yr (Burkett, 1969; Gray, 1983), and each year represents a new generation. Furthermore, *A. crepitans* has manifested a stable allelic frequency

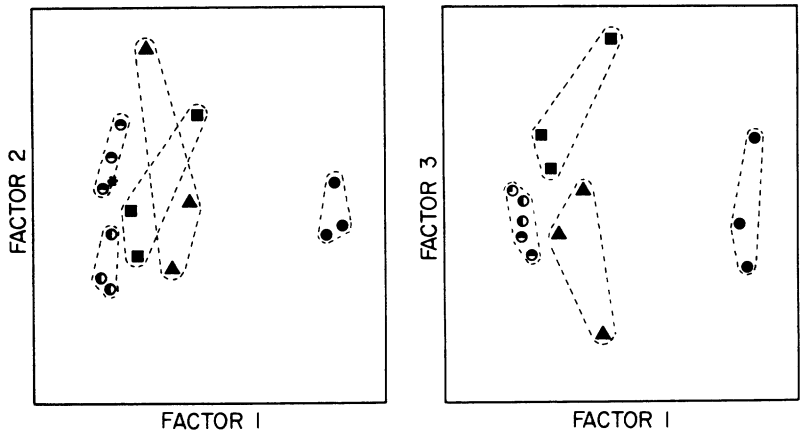

Fig. 2. Principal components analysis of arcsine-transformed gene frequencies of 7 polymorphic loci of *Acris crepitans*. Key to symbols is described in Figure 1 except that the asterisked circle = two ponds, and the quartered circle = two ponds and one stream. For interpretation of factors, see text.

for at least one locus (LDH) for 12 yr. Salthe and Nevo (1969) reported a frequency in eastern Kansas of 0.80 for the common allele at the heart LDH locus, while we found an average frequency of 0.84. Two loci (UMEST and LAP) differed significantly between adult and juvenile age classes. However as juveniles became adults, allelic frequencies shifted and were not significantly different from the previous adult age class. This shift in allelic frequencies between age classes indicates different selective pressures between life stages in a complex life cycle (Samollow, 1980) but supports the notion of overall stabilizing selection from year to year.

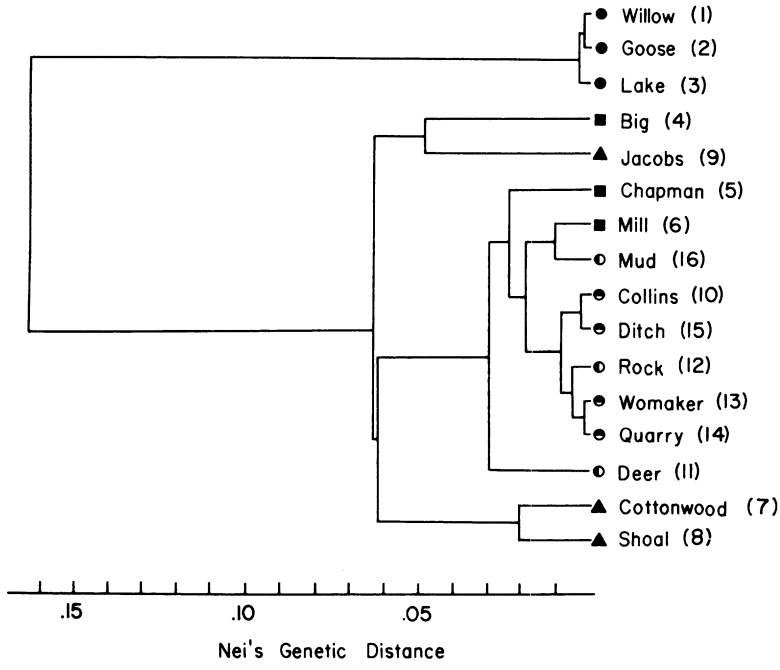

Fig. 3. Phenogram of *Acris crepitans* populations based on UPGMA clustering (Sneath and Sokal, 1973) of Nei's genetic distance. Key to symbols is described in Figure 1. Numbers in parentheses correspond with locality numbers in Figure 1.

TABLE 4. RESULTS OF MULTIPLE LINEAR REGRESSION ANALYSIS OF ENVIRONMENTAL VARIABLES ON ARCSINE-TRANSFORMED GENE FREQUENCIES OF INDIVIDUAL LOCI. Contributed R^2 of significant (F to enter $P < .05$) variables and total variance explained by each model are indicated.

Environmental variables	Locus						
	LDH	aNAC	UMEST	GPRO	SOD	GPI	LAP
Temperature							
Ave. Jan. temp.		.0471	.0259				
Ave. July temp.		.0219					
Annual temp.	.0425	.0812		.4966			
Ave. daily temp. range		.7590	.4000	.0322		.1059	
Coefficient of variation of monthly temp.			.4975	.0779		.5063	
Ave. frost-free period							
Moisture							
Ave. annual precip.							
Ave. July evap.					.5639		
Ave. relative humidity	.0203			.0928			
Coefficient of variation of monthly precip.	.9425			.2256	.0585	.0747	
Yearly occurrence of 30 consecutive days with 0.064 cm of rain		.0381	.0280		.0564		
Total R^2	.9873[a]	.9473[a]	.9514[a]	.9261[a]	.7698[a]	.6869[a]	

[a] Significant at the $P < .001$ level.

Even though populations of *A. crepitans* manifested temporal stability, they differed geographically in allelic frequency and genetic variability. The general decrease in genetic variability of frogs from eastern–western Kansas is consistent with patterns predicted by westward dispersal. In the process of westward dispersal of frogs, new founder populations, by chance, could have an altered gene pool. Along a linear dispersal route, such as a stream drainage system, differences in gene pools would most likely reflect an increase in frequency of the more common allele in the westward localities and result in the decrease in genetic variability. However, the fact that the rare alleles of LAP and GPI were never lost with westward dispersal suggests that the western-most founder populations were large enough to incorporate much of the ancestral (eastern) genome and that selection may have played a role in maintaining allelic frequencies.

The seven loci differed greatly in their patterns of geographic variation. Differential patterns of geographic variation among loci have been suggested as evidence of natural selection (Dessauer and Nevo, 1969; Lewontin, 1974; Singh et al., 1982). However, genetic drift may partially explain heterogeneity of allelic frequency patterns in some situations such as range expansion (Easteal, 1985). Although effects of genetic drift cannot be discounted for *Acris* in Kansas, the large difference in variation patterns among loci in established populations and temporal stability suggest evidence of natural selection.

All loci displayed clinal variation, although to varying degrees. However, no single environmental variable or suite of variables (temperature or moisture) accounted for the clinal variation of allelic frequencies in a regression analysis. What explained the most variation was within-year fluctuations in temperature and moisture rather than annual averages. Bryant (1974) found this relationship to be true for poikilotherms in general. It is difficult to ascertain whether fluctuations in temperature or moisture is potentially more stressful to *Acris*. The locus with striking geographic variation (LDH) had 95% of the variation explained by a single moisture variable (coefficient of variation of monthly precipitation). For no other locus was so much of the variation explained by a single environmental variable. The fixation in western Kansas of an LDH allele with an average frequency of approximately 0.20 in eastern Kansas suggests that correlations of allelic frequency variation with environmental variation may not be spurious, and also suggests natural selection as a causal agent.

Acris are poikilotherms and require moist

conditions. Their close proximity to water allows them to regulate body moisture and temperature except under low temperature or moisture conditions. Because western Kansas experiences the greatest fluctuations in both temperature and moisture, western *Acris* may be specialized both behaviorally and enzymatically for these stressful conditions. The genetic composition at the margin of the range seen in this study and that of Dessauer and Nevo (1969) may be indicative of directional selection to specific environmental conditions (Soule, 1973; McClenaghan and Gaines, 1980). Nei's distance measure and phenogram indicated that the three western populations, which have probably been genetically isolated for at least 20 yr due to lack of water flow (Jordan, 1982), were as similar to each other as were the eastern Kansas populations. Enzyme kinetic studies that measure enzyme activity under varying but controlled environmental conditions (Koehn, 1969; Merritt, 1972; Graves and Somero, 1982) are necessary before specific selective agents can be determined.

On a smaller geographic scale, populations of *Acris* in eastern Kansas differed genetically between two habitat types, ponds and streams. Genetic differentiation between pond and stream habitats was apparent even though some ponds and streams were in close proximity. Populations in closer proximity should experience more gene flow and be genetically more similar. Except for some natural sloughs, ponds in Kansas are man-made, and therefore pond populations were necessarily derived from stream stock. Present differences, then indicate that either gene flow is more restricted than expected between pond and stream habitats or the effects of gene flow are offset by selection. Genetic differences between localities or habitat types in eastern Kansas due to drift can be ruled out because allelic frequencies are temporally stable both within and among localities and populations sizes are large (Gorman, 1986). Gray (1983) found that for *Acris* in Illinois, a 7.1% migration rate between a large and small farm pond 0.8 km apart was sufficient to influence color morph frequencies at the small pond. Gray's results indicate that genetic differentiation between habitat types in eastern Kansas is probably not due to limited gene flow, and the degree of similarity among pond habitats in eastern Kansas, regardless of geographic distance between them, is indictive of selection. *Acris* color morph frequency (Gorman, 1986) and tadpole tail spot patterns (Caldwell, 1982) also differ among ponds and streams in Kansas and both have been attributed to predator-mediated selection. Color morphs of adults do not differ genetically with regard to the seven allozymic loci and therefore allozymic frequency patterns in *Acris* are independent of color frequency patterns (Gorman, 1986). Thus, differentiation in allozymic frequencies between habitat types is probably not due to predation-mediated selection, at least on adults, but may be due to direct selection on the physiology of the frogs.

Results of this study are in agreement with previous results on a larger geographic scale (Dessauer and Nevo, 1969; Salthe and Nevo, 1969), where the eastern populations of *A. crepitans* are genetically more variable than the western populations. A greater variety of habitats are available to these frogs in eastern Kansas and, in accordance with the niche width variation hypothesis (Van Valen, 1965), populations in eastern Kansas are genetically more variable. Random events due to dispersal cannot entirely account for the decrease in genetic variability westward and allelic frequencies appear temporally stable in all popualtions sampled. As an extension of this study, transplantation experiments might be useful in distinguishing factors which influence genetic variation patterns, both on a macrogeographic scale as well as microgeographic scale among habitat types.

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