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Genetic Variation in Irradiated and Control Populations of *Cnemidophorus tigris* (Sauria, Teiidae) from Mercury, Nevada with a Discussion of Genetic Variability in Lizards

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Summary. Whiptail lizards (*Cnemidophorus tigris*) were collected from fenced irradiated, fenced control, and unfenced areas near Mercury Nevada. No changes in allele frequencies at 26 allozyme loci could be ascribed to irradiation or fencing. This species is the most polymorphic and heterozygous lizard so far examined. - Heterozygosity estimates derived from electrophoretic studies on 20 additional species of lizards are compared with *Cnemidophorus*. A general trend seems to emerge. Fossorial lizards have uniformly low levels of heterozygosity (ca. 1%). Territorial "sit and wait" predators are intermediate (ca. 5%). Highly vagile apparently nonterritorial lizards are the most heterozygous (ca. 10%). Assuming that this trend does not reflect some of sampling error, two current, non-mutually exclusive hypotheses explain the observed situation: (1) the niche width variation hypothesis predicts higher variability in populations where individuals are exposed to large-scale environmental heterogeneity; and (2) the population size hypothesis predicts that, all other things being equal, vagility would tend to increase the effective population size by reducing inbreeding, which would promote higher levels of genetic variation.

Introduction

This study compares levels of genetic variability in allozymes among three populations of the teiid lizard *Cnemidophorus tigris* from the region of Mercury, Nevada, U.S.A. One population was free ranging and undisturbed, one was living in a fenced enclosure which reduced immigration and emigration to virtually zero, and one was living in a similar enclosure but subjected to long-term chronic gamma radiation. Thus we are able to compare both the effects of fencing (restricted mobility, potential restriction of effective population size) and the effects of irradiation on a part of the genome of an abundant lizard species. Finally we review levels of genetic variability amongst the undisturbed mainland lizard populations studied to date in a search for obvious historical or ecological patterns.

Between August 1962 and January 1964, three 9-ha enclosures were established in Rock Valley, at the U.S. Atomic Energy Commission's Nevada Test Site. These fenced areas were used in studies of the ecological effects of exposure to continuous low levels of gamma radiation (French 1964; French *et al.* 1974; Kaaz *et al.* 1971; Turner *et al.* 1973; Medica *et al.* 1973; Turner 1975). One of the enclosures (Plot B) was exposed to almost continuous gamma irradiation from a ^{137}Cs source atop a 15-m tower in the center of the area. The other two enclosures (Plots A and C) were nonirradiated and served as control areas.

In 1974 we examined genetic variability for allozyme morphs in populations from plots A (nonirradiated) and B (irradiated). Our free-living sample was comprised of individuals collected the previous year by Mr. P.A. Medica and co-workers and maintained in frozen condition for one year.

At the beginning of the experiment whiptail lizards occupying the irradiated area were exposed to approximately 2.5 R/day at the outer fence to around 11 R/day near the center of the plot (French 1964; French *et al.* 1966). The annual exposure of *Cnemidophorus tigris* was estimated with implanted lithium fluoride microdosimeters to be around 150-600 rads/year, depending on animals' locations within the area (Turner and Lannom 1968; Turner *et al.* 1973). Most animals probably received annual doses of 200 rads or less.

Radiation might affect allele frequencies in the population in two ways: by increasing the mutation rate and by imposing a new environmental stress to which the populations must adapt. Both the number of alleles present and the amount of heterozygosity might over the long run be increased by an increase in mutation rate. These effects would probably require many generations to be noticed. Since *C. tigris* is a long-lived species, with individuals reported to live

Table 1. Allozyme variation observed in three populations of *Cnemidophorus tigris* from Nevada

Population		Fenced Control	Fenced Irradiated	Free Ranging	Total	χ^2 (d.f.)
Sample size:		n=28	n=31	n=31	n=90	
Locus/allele		Frequency				
Idh	a)	0.982	0.855	0.935	0.922	
	b)	0.018	0.145	0.065	0.078	N.T.
Pgm-1	a)	0.018	0.065	0.032	0.039	
	b)	0.982	0.935	0.952	0.955	
	c)	0	0	0.016	0.006	N.T.
Pgm-2	a)	0.054	0.048	0.177	0.094	7.62*
	b)	0.946	0.952	0.823	0.906	(2)
Mdh-1	a)	0	0	0.032	0.011	
	b)	0	0	0.048	0.017	
	c)	1.0	1.0	0.920	0.972	N.T.
Mpi	a)	0.232	0.145	0.161	0.178	1.69^+
	b)	0.768	0.855	0.839	0.822	(2)
Ipo-1	a)	0.054	0.097	0.048	0.067	
	b)	0.946	0.903	0.952	0.933	N.T.
Ipo-2	a)	1.0	1.0	0.984	0.994	
	b)	0	0	0.016	0.006	N.T.
Adh	a)	0.982	1.0	1.0	0.994	
	b)	0.018	0	0	0.006	N.T.
Got-1	a)	1.0	0.968	0.952	0.972	
	b)	0	0.032	0.048	0.028	N.T.
Got-2	a)	0	0.016	0.032	0.017	
	b)	0.982	0.951	0.952	0.961	
	c)	0.018	0.032	0.017	0.022	N.T.
Pgi	a)	0.982	1.0	0.952	0.978	
	b)	0.018	0	0.048	0.022	N.T.
6-Pgd	a)	0.982	1.0	1.0	0.994	
	b)	0.018	0	0	0.006	N.T.
Xdh	a)	1.0	0.952	0.919	0.956	
	b)	0	0.048	0.081	0.044	N.T.

6-7 years (see Turner *et al.* 1969b), it is unlikely that such effects could be demonstrated in this study.

More likely, the environmental stress of radiation in the experimental enclosure might cause new selective pressures to act within the enclosure. If strong directional selection were imposed, we might observe a change in allele frequencies and possibly a decrease in variability.

Complete or partial sterility has been observed among females of four species of lizards occupying the experimental enclosure (Turner *et al.* 1973; Medica *et al.* 1973; Turner 1975). These animals exhibit complete regression of one or both ovaries and hypertrophy of pleuroperitoneal fat bodies. Some female *Uta stansburiana* are sterile at an age of 11 months; sterile females of other species *(Crotaphytus wislizenii, Cnemidophorus tigris* and *Phrynosoma platyrhinos)* are older. Females from nonirradiated enclosures showed no such effects.

Allozyme frequencies have been observed to change in response to environmental stress in several species (Johnson 1971; Hamrick and Allard 1972; Wills and Nichols 1971), though McKinney and Turner (1971) found no detectable effects of irradiation on allozyme frequencies of *Uta stansburiana*.

Materials and Methods

In June, 1974, approximately 30 *Cnemidophorus tigris* of mixed age classes were collected from an irradiated plot (plot B) and a control plot (plot A). Because of difficulties in obtaining a sample of free-ranging lizards, we used a sample collected by P. Medica during 1973 and maintained frozen for one year. Electrophoretic techniques and scoring of allozyme morphs

Table 1. (continued)

Population		Fenced Control	Fenced Irradiated	Free Ranging	Total	χ^2 (d.f.)
Sample size:		n=28	n=31	n=31	n=90	
Locus/allele						
Fum	a)	0.964	0.984	0.952	0.967	
	b)	0.036	0.016	0.048	0.033	N.T.
Pept-2	a)	0	0	0.065	0.022	6.85*
	b)	0.929	0.758	0.744	0.830	(2)
	c)	0.071	0 .242	0.162	0.161	
Es-1	a)	0.107	0.048	0.177	0.111	13.44**
	b)	0.857	0.952	0.694	0.834	(2)
	c)	0.036	0	0.129	0.056	
Es-2	a)	0.161	0.226	0.258	0.217	3.29^+
	b)	0.321	0.419	0.500	0.416	(2)
	c)	0.518	0.355	0.242	0.367	
Es-3	a)	0.018	0.129	0.177	0.111	7.62^+
	b)	0.089	0.048	0	0.044	(4)
	c)	0.607	0.613	0.726	0.650	
	d)	0.286	0.161	0.081	0.172	
	e)	0	0.048	0.016	0.022	
Es-4	a)	0.018	0.016	0.032	0.022	2.30*
	b)	0.107	0.097	0.177	0.128	(4)
	c)	0.125	0.032	0	0.050	
	d)	0.554	0.645	0.565	0.589	
	e)	0.196	0.210	0.161	0.189	
	f)	0	0	0.065	0.022	
Gp-2	a)	0.982	0.952	1.0	0.978	
	b)	0.018	0.048	0	0.022	N.T.

* and ** indicates the differences in allelic frequencies were statistically significant at the 0.05 and 0.01 probability levels, respectively.

$^+$ indicates tested, not significant.

The following loci were fixed in all three samples: Mdh-2, Ldh-1, Ldh-2, Gpd, Pept-1, Gp-1.

Abbreviations for loci follow Kim *et al.* 1976, Gorman *et al.* 1975, Yang *et al.* 1974.

The allele designated "a" is the least anodal.

N.T. indicates that no statistical tests were performed because of small sample size.

followed procedures elaborated in our recent papers (Kim *et al.* 1976; Gorman *et al.* 1975). For each lizard 26 presumptive gene loci were scored.

Results and Discussion

Allele frequencies for the three samples are presented in Table 1. Estimates of variability are summarized in Table 2. A total of 20 loci showed more than one allele segregating (77%), with individual samples ranging from 15 to 17 varying loci. Many of these were rare alleles. If we adopt the criterion that a locus is polymorphic if no allele has a frequency greater than 0.95, then 35% of the loci in the pooled estimate were polymorphic.

Significant differences in allozyme frequencies between the three populations were found at only three loci: Pgm-2, Pept-2, and Es-1. The χ^2 values and associated probabilities are indicated in Table 1. At the Pept-2 locus free-ranging and irradiated populations were alike but different from fenced controls. For Es-1 and Pgm-2 the free-ranging population differed from the fenced populations, which showed no difference between themselves. We conclude that the observed differences are not attributable to irradiation.

Estimates of the amount of heterozygosity did not differ significantly among the populations. For each of three pairwise comparisons, heterozygosity was computed from Hardy-Weinberg equilibrium at each locus and then the possibility that each population was more heterozygous than another was tested with a sign test (Siegel 1956). No significant differences

Table 2. Summary of genetic variation observed in three populations of *Cnemidophorus tigris* from Nevada

Population	Fenced Control	Fenced Irradiated	Free Ranging	Pooled
% loci polymorphic (most common allele ⩽ 0.99)	61.5	57.7	65.4	76.9
% loci polymorphic (most common allele ⩽ 0.95)	30.7	30.7	38.5	34.6
% heterozygosity	10.99	11.91	14.64	12.56
alleles/locus	1.88	1.88	2.08	2.27

Table 3. Levels of heterozygosity among lizard populations (island populations excluded)

Habitat Mode	Family Genus	No. Populations Studied (no. species)	Range of Heterozygosity (%)	Mean[a] Heterozygosity (%)	Reference
fossorial	Amphisbaenidae *Bipes*	3(3)	0 - 3.2	1.1	Kim *et al.* 1976
	Anniellidae *Anniella*	4(2)	0 - 2.2	1.1	Bezy *et al.* 1977
terrestrial or arboreal: territorial sit and wait	Iguanidae *Uma*	5(5)	0.3 - 2.9	1.3	Adest 1977
	Crotaphytus[b]	5(4)	2.1 - 4.6	3.2	Montanucci *et al.* 1975
	Uta	17(1)	0 - 10.0	4.8	McKinney *et al.* 1972
	Anolis	3(1)	3.6 - 5.7	4.9	Webster *et al.* 1972
	Callisaurus	4(1)	3.0 - 7.9	5.5	Adest, in prep.
	Sceloporus	5(2)[c]	1.7 - 13.4	6.2	Hall and Selander 1973; Tinkle and Selander 1973
vagile searcher	Lacertidae *Lacerta*	3(1)	5.9 - 12.9	9.1	Gorman *et al.* 1975
	Teiidae *Cnemidophorus*	1(1)	-	14.6	this study

[a] each population weighed equally, regardless of sample size.

[b] calculated from Montanucci *et al.* (1975) for populations with 8 or more individuals. Sample sizes for individual loci sometimes less than 8. Montanucci *et al.* recognized two genera (*Gambelia* and *Crotaphytus*) that are closely related and are most often treated as *Crotaphytus*.

[c] includes four chromosomal races (possible species) of *Sceloporus grammicus*.

were observed. Whatever the long-term genetic effects of irradiation and/or fencing might be, no trend is apparent from our data.

Though the estimates of variability provide little information about how organisms may respond genetically to radiation stress, they are of some interest in themselves. We have assembled all data available to us, published and unpublished, on the levels of heterozygosity among lizard populations. The estimate of heterozygosity in *Cnemidophorus tigris* is the highest so far observed. A clear trend emerges.

Table 3 summarizes the data on heterozygosity in mainland lizard populations. We are deliberately considering mainland populations only, since insular populations would theoretically and indeed in reality do, show various degrees of depressed levels of variability because of such factors as founder effect and/or other historical bottlenecks, and small effective population sizes on small islands. The effects of insularity on genetic variability in lizards have been dicussed in detail (Soulé and Yang 1973; Gorman *et al.* 1975, 1976; Gorman and Kim, 1975).

Levels of heterozygosity fall into three discrete classes. The first class is uniformly low hovering around 2% or less. In this class we find the genera *Bipes*[1] comprised of three species; *Anniella* with two species; and *Uma* with five named species. *Bipes* and *Anniella* are fossorial and not vagile. The genus *Uma* is exceptional. It is a sit-and-wait territorial iguanid which is restricted to sand dunes. This reduces it, effectively, to being comprised of a series of insular populations (Norris 1958). There might be reasonably high vagility within dunes, but there is probably little movement between dunes. It too has uniformly low heterozygosity.

The second class of variability estimates hovers around the 5% level. The ranges of heterozygosity in this group are high even within species (e.g. *Uta stansburiana*), and so too are the standard errors. The species that fall into this second class are all members of the lizard family Iguanidae, and could be categorized as territorial sit-and-wait predators. That is, they tend to have restricted home ranges, part of which they actively defend against conspecific intruders of the same sex. Their foraging strategy is such that little movement is involved until a prey item is seen.

The third class consists of lizards that are highly vagile. Rather than wait for visual signs of prey they expend much time and energy moving about searching for prey. Olfaction presumably plays a major role in their foraging strategy. Home ranges tend to be large, and true territoriality, in *Cnemidophorus* at least, apparently does not exist, although there may be agression shown when one individual violates the "individual distance" of another (Fitch 1967). The mean heterozygosity in the group is 9% or more.

Thus, the apparent correlation: with increasing vagility there is increasing heterozygosity. Why should we find such a striking correlation? Three answers come readily to mind. First, it may well be spurious. We have stated that the variance for heterozygosity estimates is high even among populations of a single species. The present data are based upon information from about one half of one percent of the world's lizard species. Clearly more data are needed.

If the correlation is biologically meaningful, there are two hypotheses, not mutually exclusive, that appear to fit the observed data.

The first might be termed the niche-width variation hypothesis. Individual, wide ranging, vagile lizards are presumably exposed to a greater amount of environmental heterogeneity than populations comprised of territorial individuals that are relatively sedentary. Fossorial forms are presumed to be the most sedentary. In reality comparison of "niche width" between organisms as diverse as *Bipes* and *Cnemidophorus* is difficult at best, and no such data are available.

The remaining hypothesis, termed by Soulé (1971, 1976) the gene flow variation hypothesis, or the population size hypothesis, assumes that vagility is related to effective population size which in turn affects levels of variability within populations.

There is no doubt that genetic variability in a population has to be some function of mutation rate, population age (i.e. time for mutations to accumulate and spread) and effective population size. This is true whether or not the new alleles are selectively neutral. Nonterritorial, highly vagile searchers are presumed to have a much wider choice of potential mates than species that defend restricted sites and which tend to be highly polygynous, a condition met by most iguanid lizards. A further presumption, although completely devoid of field data, is that burrowing forms have the lowest dispersal rates and highest degree of inbreeding.

Only further study will enable us to establish (1) the reality of the trend and (2) the most probable explanation.

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[1] The genus *Bipes* is in the family Amphisbaenidae. Some students of reptilian phylogeny treat the Amphisbaenians as a taxon of equal rank to lizards. Whether or not Amphisbaenians are true lizards is of no import in this discussion. Both lizards and Amphisbaenians are members of the reptilian order (or superorder) Squamata, as are snakes. There are not published data on variability in snakes.

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