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VARIATION IN THE AGGRESSIVE BEHAVIOR OF THE PARTHENOGENETIC LIZARD (*Cnemidophorus uniparens*, Teiidae)

Mark Grassman
Memphis State University

David Burton
University of Wyoming

David Crews
The University of Texas

ABSTRACT: The desert-grassland whiptail (*Cnemidophorus uniparens*) is an all-female lizard species that reproduces clonally by parthenogenesis. Here we report marked geographic variation in the aggressive behavior of paired individuals in which pairs consisted either of individuals from within study sites or individuals representing each of two different study sites. Although we document allozyme variation within each study site, this did not differ significantly between sites. Previously reported restriction endonuclease analysis of mitochondrial DNA indicates that the two lizard populations used in the present study arose from the same or closely related maternal ancestors by interspecific hybridization. Gross climatological differences do not appear to explain the behavioral variation between sites. The possible roles of polygenic effects not detected by the biochemical analyses and laboratory studies of environmental effects on the development of aggressive behavior await further investigation.

Unisexual organisms provide an important model system that allows investigators to address persistent questions in behavioral biology in a novel way. To what extent is an individual's behavior shaped by its genotype versus its environment? How plastic are the responses of genotypes to environmental variation and how does genotypic plasticity vary among individuals? Reproduction by parthenogenesis is rare in vertebrates but relatively common in a few genera including the lizard genus *Cnemidophorus* (for an overview of unisexuality in vertebrates see Dawley, 1989 and Vrijenhoek, Dawley, Cole, & Bogart, 1989). Evidence

Address correspondence to Mark Grassman, Department of Biology, Memphis State University, Memphis, TN 38152, USA.

from studies involving chromosomal and electrophoretic analysis indicates that the parthenogenetic *Cnemidophorus* sp. arose by hybridization (Dessauer & Cole, 1989). Some parthenogenetic *Cnemidophorus* sp. may have arisen from a single set of parents and are close to being genetically uniform (e.g., *C. neomexicanus,* Parker & Selander, 1984) whereas others may have arisen from multiple hybridization events and are quite diverse (e.g., *C. tesselatus,* Parker & Selander, 1976).

Several models have been proposed to describe variation in clonally reproducing species (Vrijenhoek, 1984). The frozen niche variation model proposes that diversity in clonal species arises primarily as a result of multiple hybridized zygotes from the same sexual ancestral species (Vrijenhoek, 1979, 1984) and is supported by a variety of field and laboratory data based on *Poeciliopsis* sp. fishes (Schenck & Vrijenhoek, 1989; Schultz & Fielding, 1989; Vrijenhoek, 1984, 1989; Wetherington, Schenck, & Vrijenhoek, 1989b; Wetherington, Weeks, Katora, & Vrijenhoek, 1989a). Secondarily, after one or more clones are produced by hybridization, recombination and/or mutation may produce additional variation within phyletic clones (Vrijenhoek, 1979). Thus, polyphyletic clones may be secondarily composed of cryptic clones or genotypically variable individuals having arisen from the same ancestral hybrid zygote. Although significant advances have been made in understanding the biology of clonally reproducing fishes, substantial deficits in understanding the biology of parthenogenetic lizards led Parker, Walker, and Paulissen (1989) to comment that ". . . the evolutionary study of cloned hybrid complexes of *Cnemidophorus* should serve as a guide to further genetic and/or morphological [we would add ecological, physiological and behavioral] study of contradictory cases rather than yielding robust conclusions of a mature theory."

Polyphyletic variation resulting from multiple hybrid origins and variation apparently from mutation or recombination have been documented for the *C. velox* complex, i.e., the named species *C. uniparens* and *C. velox* are both derived from the hybrid combination *C. burti* × *C. inornatus* × *C. inornatus.* Mitochondrial DNA analysis indicates that although *C. uniparens* and *C. velox* arose by hybridization involving the same species, the maternal ancestor of *C. uniparens* is *C. inornatus* and that of *C. velox* is *C. burti* (Moritz et al., 1989). Interestingly, *C. uniparens* and *C. velox* differ in several ways. *Cnemidophorus uniparens* inhabits desert grasslands south of the range of *C. velox* in Arizona, New Mexico, and Mexico. *Cnemidophorus velox* inhabits pinyon-juniper woodlands between 1,350–2,450 m associated with the Colorado Plateau in Colorado, Arizona, and New Mexico. Additional variation, apparently resulting from mutation and/or recombination has been reported in *C. uniparens* (Dessauer & Cole, 1986, 1989).

Although genetic variation for *C. uniparens* seems to be quite limited, there is considerable variation in aggressive dominance (Grassman &

Crews, 1987). Also, while collecting *C. uniparens* at two of our study sites for behavioral, reproductive and genetic studies (Moritz et al., 1989) we observed differences in average snout-to-vent length, minimum body size at which animals contained oviductal eggs, and the tendency of individuals to bite the fingers of their captors (unpublished field observations). Ecological studies of *C. uniparens* near Rodeo, New Mexico (Hulse, 1981), and our records based on hundreds of individuals collected over a 5-year period, indicate that these animals are phenotypically different from some *C. uniparens* collected near Willcox, Arizona. Rodeo and Willcox are approximately 121 km apart and separated by the Chiricahua Mountains. Near Rodeo, the smallest individual with either vitellogenic follicles or oviductal eggs ever recorded was 59 mm SVL (snout–vent length). Near Willcox, the smallest animal to date to have either vitellogenic follicles or oviductal eggs was 54 mm SVL. This reported variation in behavior and geographical differences in minimum body size at first clutch raise some interesting questions. For example, do individuals from near Willcox differ from those from near Rodeo in terms of other phenotypic characteristics such as aggressive and dominance behavior? Are differences between these populations a result of multiple independent origins for *C. uniparens* and can individuals from some populations dominate others with different parental origins?

The impetus for the present study was to document geographic variation in aggressive behavior between *C. uniparens* captured near Willcox and Rodeo, and to determine whether these two study populations are different phyletic clones based on mtDNA (Moritz et al., 1989) and allozyme analyses. Also, these populations were compared using protein electrophoresis and restriction endonuclease analyses of mtDNA (Densmore, Moritz, Wright, & Brown, 1989; Moritz et al., 1989; Moritz, personal communication). Here the purpose was to determine whether geographic variation in behavior was correlated with genetic diversity as estimated using state of the art biochemical methods. To determine whether environment might explain the geographic variation in behavior, average annual temperatures and precipitation covering 36 years, and average monthly temperatures (maximum, minimum, and mean) and precipitation for the 42 months prior to collecting the animals were compiled and analyzed for differences between the two study sites.

GENERAL METHODS

Animals

Populations of *C. uniparens* are found on the desert flats both east and west of the Chiricahua Mountains in southeastern Arizona (AZ) and southwestern New Mexico (NM). Our study sites were near Willcox, AZ and Rodeo, NM (121 km to the southeast of Willcox). Reproductively

mature *C. uniparens* were collected during the breeding season. Animals used in Experiments I and II were collected in June 1986. Experiment I was replicated with different animals collected in June 1987. Animals were collected using a portable drift fence, toe-clipped for permanent identification, returned to the Southwestern Research Station, American Museum of Natural History, where they were weighed, measured, palpated to determine reproductive condition, and housed until being transported to the University of Texas.

Housing and Maintenance

Animals were maintained in a computer-controlled environmental chamber. The microprocessor was programmed to simulate the changes in photoperiod, temperature, and humidity characteristic of southeastern AZ during the lizard's reproductive season based on weather data from previous years. The temperature was 27 °C at 0900 hr central standard time and gradually increased to 33 °C by 1200 hr where it remained until 1300 hr. For a complete description of animal capture and care see Moore, Whittier, and Crews (1984). Lizards were housed in 75 × 30 × 30 cm glass terraria with a 5 cm deep sand substrate. Three lizards all from the same collecting site were housed together in each terrarium. Radiant heat was provided by a 50-W heat lamp suspended 24 cm above the sand. In addition to the room lights, each cage received illumination from one General Electric ultraviolet light (F20T12/BL) and a Chroma 50 fluorescent light suspended 61 cm above the sand. Lizards were fed calcium and phosphate dusted crickets or mealworms three times weekly and were provided water in bowls ad libitum.

Quantification of Aggression

An ethogram for *C. uniparens* documenting aggressive behavior has been developed (Crews, Gustafson, & Tokarz, 1983), and agonistic encounters have been observed both in the laboratory (Crews et al., 1983; Gustafson & Crews, 1981) and field. *Cnemidophorus uniparens* will establish dominance hierarchies in the laboratory (Grassman & Crews, 1987; Gustafson & Crews, 1981).

In keeping with previous studies (Grassman & Crews, 1987; Gustafson & Crews, 1981), charges directed at cagemates were the behavior of interest. A charge was operationally defined as a rapid approach by one individual directed at another individual that resulted in the recipient either fleeing, chasing, or biting the instigator (Grassman & Crews, 1987). These encounters are often accompanied by other behaviors characteristic of aggression in *C. uniparens*: head nodding, bobbing, armwaving, and aggressive mouth gape (Crews et al., 1983; Gustafson & Crews, 1981).

All behavioral observations were made using a television camera and remote monitoring and recording systems. Observations were conducted between 1000 and 1300 hr, the lizards' period of peak activity in the field and laboratory. Lizards were observed for 30 min on each of 2 days. The test cage was a 75 × 30 × 30 cm glass terrarium divided in half with poster board. This formed two 37.5 × 30 × 30 cm test cages.

DESIGN OF BEHAVIORAL STUDIES

The intensity of aggression exhibited between individuals paired within inbred strains can differ from that in pairs comprised of individuals representing different strains (Bakker, 1986). Thus, separate experiments involving pairing animals within and between study sites were conducted.

Experiment I

In Experiment I, conducted in 1986 and replicated in 1987, a pair of lizards both from Willcox or Rodeo was placed in a test cage, and charges by each individual were recorded. Individuals forming the pairs had not been housed together previously. Pairs of lizards representing each site were observed simultaneously to eliminate temperature effects between sites. This experiment was designed to determine if there were differences in aggressiveness between Willcox pairs versus Rodeo pairs. The purpose of replicating Experiment I in 1987 with different individuals was to determine if differences in responses between populations were repeatable.

Animals collected from the Willcox study site are smaller in terms of average SVL compared to those from Rodeo (Grassman & Crews, unpublished data). Thus a sample of lizards from the Willcox population was naturally apt to be smaller in terms of SVL than a sample from Rodeo. This was not viewed as a problem with regard to interpreting behavioral differences between Willcox and Rodeo lizards for several reasons. First, two previous studies using *C. uniparens* demonstrated no relationship between SVL and aggressive dominance (Grassman & Crews, 1987; Gustafson & Crews, 1981). Second, in Experiment I the behavior of Willcox animals was independent of that for Rodeo animals. Third, size differences between the two populations could provide one explanation for any observed differences in aggression and could be examined further.

In Experiment I and the replicate experiment the pairs consisted of individuals from within the respective study sites; thus, this was a nested design. Observing each pair for 30 min on each of 2 days constituted two replicates for the nested design.

Experiment II

Experiment II involved pairs of lizards in which one individual from Willcox and one individual from Rodeo were placed in the test cage. This experiment was designed to determine if individuals from one population were dominant over those of the other population. Animals having similar SVLs were paired. Half of the Willcox animals in the Willcox–Rodeo pairs were larger than their cagemates and the Willcox animals were not significantly smaller than the Rodeo animals (Willcox, SVL = 62.9 ± 1.3, Rodeo, SVL = 64.4 ± 0.9; paired comparisons $t = 0.93$, df = 14, $p > .10$, 1-tailed, $p > .25$, 2-tailed). Thus, if SVL was an important variable behind differences observed in Experiment I, this effect would be eliminated from Experiment II. Alternatively, if SVL was not important the results of Experiment I should be corroborated by those of Experiment II. In Experiment II the 30 min observations made on 2 days were pooled. Pairs consisted of an individual from each study site; thus, this was a randomized blocks (paired comparisons) design.

Statistical Analysis of Behavior

Initially, data were analyzed using analysis of variance (ANOVA, Hicks, 1973). For each experiment geographic variation between study sites was the variable of interest. The pair effect was included in the models primarily to control variation and to account for the fact that behavior of individuals within pairs was not independent. Although animals from Willcox actively moved about in both experiments, few or no aggressive interactions were recorded and the data contained zero values. This raised concerns regarding the reliability of the tests of hypotheses. Therefore, assumption free bootstrap methods (Efron, 1982) were used to generate probability estimates (p') for comparison with the tabular p values. Briefly, this involved comparing F statistics generated using ANOVA performed on the observed data with the frequency distribution of F statistics generated by randomly sampling the data set with replacement and recalculating the ANOVA table (iterations = 10,000 for each experiment). The bootstrap likelihood for the observed outcomes (p') was estimated by comparing the observed F with the iteratively generated distributions of the F statistics. In all cases the tests of hypotheses based on bootstrap methods corroborated those based on tabular F statistics. The bootstrap estimates (p') are reported in Tables 1–3 for comparison with the tabular values.

GENETIC ANALYSIS

Although restriction endonuclease analysis of mtDNA did not distinguish *C. uniparens* collected near Willcox from those collected near

Rodeo (Densmore et al., 1989), protein electrophoretic analyses were performed to test the hypothesis that the two populations were genotypically distinct. The electrophoretic analysis was based on liver, muscle, and kidney extracts from 20 individuals each from the Rodeo and Willcox populations. Tissues were removed and stored in liquid nitrogen (−196 °C), and preparation and storage of tissue homogenates followed the protocol of Sites et al. (1988). The supernatant fractions of these homogenates were run on horizontal gels of starch (12.5%) containing equal parts of Sigma (lots 83F-0612, 44F-0619, 94F-0537) and electrostarch (lot 392).

Thirty-three enzyme systems encoded by 42 presumptive gene loci were resolved for both samples (Willcox and Rodeo). Enzymes used were as follows (Nomenclature follows recommendations of the Nomenclature Committee of the International Union of Biochemistry (1984). Suffixes "1" and "2" designate loci of multilocus systems in order of decreasing anodal mobility. Superscripts k, l, m refer to kidney, liver, and skeletal muscle, respectively): aconitate hydratase (Acon-1,2)^{l}, adenosine deaminase (Ada-A)^{k}, alcohol dehydrogenase (Adh-A)^{l}, aldehyde dehydrogenase (Aldh-A)^{l}, aspartate aminotransferase (Aat-1,2)^{l}, calcium-binding protein (Cbp-A)^{m}, creatine kinase (Ck-A)^{m}, dihydrolipoamide dehydrogenase (Dldh-A)^{l}, esterase (Est-1,2)^{l}, fructose biphosphotase (Fbp-A)^{l}, β-galactosidase (β-Gal-A)^{l}, general protein (GP-1,2)^{l}, glucose dehydrogenase (Gcdh-A)^{l}, glucose-6-phosphate dehydrogenase (G6pdh-A)^{l}, β-glucosidase (β-Gluc-A)^{l}, glycerate dehydrogenase (Glydh-A)^{l}, glycerol-3-phosphate dehydrogenase (G3dph-A)^{l}, hexokinase (Hk-A)^{l}, D-2-hydroxyacid dehydrogenase (2-Hadh-A)^{l}, L-iditol dehydrogenase (Iddh-A)^{l}, isocitrate dehydrogenase (Icdh-1,2)^{l}, L-lactate dehydrogenase (Ldh-A and Ldh-B)^{l}, malate dehydrogenase (Mdh-1,2)^{l}, malic enzyme (Me-1)^{l}, mannose phosphate isomerase (Mpi-A)^{l}, α-mannosidase (α-Mann-A)^{l}, peptidase (L-leucylglycylglycine) (Pep(lgg))^{l}, peptidase (phenylalanyl-L-proline) (Pep(pap))^{k}, phosphoglycerate kinase (Pgk-A)^{l}, phosphoglucomutase (Pgm-1,2)^{l}, phosphogluconate dehydrogenase (Pgdh-A)^{l}, superoxide dismutase (Sod-A)^{l}, uridine kinase (Uk-A)^{m}, xanthine dehydrogenase (Xdh-A)^{l}. Staining protocols were from Selander, Smith, Yang, Johnson, and Gentry (1971), Harris and Hopkinson (1976), and Richardson, Baverstock, and Adams (1986). Allozyme data were statistically summarized with the BIOSYS-1 program of Swofford and Selander (1981). Individual genotypes were used to calculate allele frequencies and the genetic distance coefficient (Hillis, 1984; Nei, 1978).

CLIMATOLOGICAL ANALYSIS

To test the hypothesis that climatic conditions differed between the Willcox and Rodeo study sites monthly averages of the high temperature, average daily temperature, low temperature, and precipitation for Jan-

TABLE 1
Experimental Design and Analysis of Variance Involving the Number of Charges Against Cagemates when *Cnemidophorus uniparens* from Two Study Sites, Willcox, AZ and Rodeo, NM, Were Paired with Individuals From Within Their Respective Localities (Animals Collected June 1986)

Site:	Willcox					Rodeo				
Pair:	1	2	3	4	5	6	7	8	9	10
Replicate 1:	0	0	0	0	0	17	0	38	8	36
2:	0	0	0	0	0	56	21	23	22	50

ANOVA:

Source	df	SS	MS	*F*	*p'**
Site	1	3,672.05	3,672.05	28.48	0.001
Pair	8	1,549.40	193.68	1.50	0.250
Error	10	1,289.50	128.95		
Total	19	6,510.95			

*Bootstrap result. See the text for explanation.

uary 1983–June 1983 were statistically explored using ANOVA. *Cnemidophorus uniparens* individuals probably live a maximum of 3 or 4 years (Hulse, 1981). Additionally, annual average temperatures and precipitation for 1950–1986 were examined. Data were taken from National Oceanic and Atmospheric Administration summaries from stations closest to the study sites. For the monthly summaries data were for Willcox, AZ (8 km from the Willcox study site) and Animas, NM (20 km from the Rodeo study site) stations. With respect to the annual summaries, incomplete data were available for Rodeo, New Mexico (2 km from the Rodeo study site).

RESULTS

From a sample of 20 *C. uniparens* captured near Willcox, in 1987, four individuals smaller than 59 mm SVL were found to contain either vitellogenic follicles or oviductal eggs as determined by palpation. For Experiment I conducted in 1986, and for the replicate of the experiment conducted in 1987, Willcox lizards were significantly smaller than those from Rodeo (Willcox 1986, mean $\pm$ standard error SVL = 62.2 $\pm$ 1.4, Rodeo 1986, SVL = 65.9 $\pm$ 0.68, $t = 2.26$, df = 18, $p < .025$; Willcox 1987, SVL = 62.4 $\pm$ 1.0, Rodeo 1987, SVL = 69.2 $\pm$ 0.9, $t = 5.04$, df = 18, $p < .0005$).

TABLE 2

Experimental Design and Analysis of Variance Involving the Number of Charges Against Cagemates When *Cnemidophorus uniparens* from Two Study Sites, Willcox, AZ and Rodeo, NM, Were Paired with Individuals from Within Their Respective Localities (Replicate of the Experiment Outlined in Table 1 Using Animals Collected June 1987)

Site:	Willcox	Rodeo
Pair:	1 2 3 4 5	6 7 8 9 10
Replicate 1:	0 0 0 0 0	20 43 13 6 43
2:	0 0 0 0 0	17 50 60 0 36

ANOVA:

Source	df	SS	MS	*F*	*p'**
Site	1	4,147.20	4,147.20	35.72	0.001
Pair	8	2,517.60	314.70	2.68	0.081
Error	10	1,176.00	117.60		
Total	19	7,840.80			

*Bootstrap result. See the text for explanation.

Behavioral Analysis

In Experiment I lizards in pairs consisting of two individuals from Rodeo charged each other more frequently than did lizards in pairs from Willcox (Table 1). Animals from Willcox exhibited no agonistic behavior. This finding was repeated in the replicate experiment with individuals from Rodeo charging each other more frequently than did lizards in pairs from Willcox (Table 2). Again, as in Experiment I, animals from Willcox exhibited no agonistic behavior. Pooling the data for Experiment I and the replicate experiment yielded a nonsignificant site by year interaction ($F(1,16) = .06$, $p = .8112$) indicating that the site effect was the same for both years. In Experiment I, variation comparing pairs nested within study sites was not significant (Table 1). In the replicate experiment variation comparing pairs nested within study sites was marginally nonsignificant (Table 2). Individuals from Rodeo charged each other an average of 54.6 (range 21–88) and 57.6 (range 6–93) times per hour in Experiment I and the replicate experiment, respectively. Also, there was a significant correlation in the number of charges between cagemates (one-tailed Spearman rank correlation $r = 0.59$, $p \leq .05$).

In Experiment II (one individual was from Willcox, and the other from Rodeo), lizards from Rodeo again charged more often than the lizards from Willcox (Table 3). One Willcox animal charged its Rodeo cagemate

TABLE 3

Experimental Design and Analysis of Variance Involving the Number of Charges Against Cagemates When *Cnemidophorus uniparens* from the Willcox, AZ Study Site Were Paired with Those from the Rodeo, NM Study Site (Animals Collected June 1986)

Pair (block):	1	2	3	4	5	6	7	8
Site								
Willcox, AZ	0	9	0	0	0	0	0	0
Rodeo, NM	4	21	28	9	0	6	6	3

ANOVA:

Source	df	SS	MS	*F*	*p'**
Site	1	289.00	289.00	7.66	0.0095
Pair	7	468.72	66.96	1.78	0.1460
Error	7	264.00	37.71		
Total	15	1,021.75			

*Bootstrap result. See the text for explanation.

on its second day test. In each experiment individuals from both collecting sites actively moved about and approached each other. However, the individuals from Willcox (with the exception of the single animal in Experiment II) did not charge their cagemates. An average of only 9.6 (range 0–28) charges per hour by Rodeo animals was recorded.

Genetic Analysis

Twenty-seven of the 42 loci examined in samples from both sites were monomorphic for the same electromorph. A chi-square test of allele frequency heterogeneity between samples (Workman & Niswander, 1970) showed Pgdh-A to be the only locus with a significant intersample (Willcox vs. Rodeo) difference in allele frequency (chi-square = 8.06, df = 1, $p \leq .01$). Taken together, all variable loci showed no significant intersample allele frequency differences (chi-square = 15.20, df = 16, $p = .51$). The Nei (1978) genetic distance between these two samples was less than 0.01, and similar values were obtained for all other D estimates.

Individuals were delineated into eight genotypes (three collected from the Rodeo study area, and seven from the Willcox study area with two genotypes in common) on the basis of variation at six loci (Table 4). Representatives having the most common genotype accounted for 62% of the individuals collected and were found in samples from both field study areas (80% and 42% for Rodeo and Willcox, respectively). Representatives of the second most common genotype accounted for 18% of the individuals collected (10% and 26% for Rodeo and Willcox, respec-

TABLE 4
Eight Genotypes Based on Variation at Six Presumptive Loci for Triploid *Cnemidophorus uniparens* Collected Near Willcox, AZ and Rodeo, NM

	Enzymes					
Site (N)	*Pgdh-A*	*Dldh-A*	*Est-2*	*Fpb-A*	*aMann*	*Mdh-2*
Rodeo, NM						
(16)[a]	aaa	aaa	aaa	aaa	aaa	aaa
(2)[b]	abb	aaa	aaa	aaa	aaa	aaa
(2)	aaa	ab?	aaa	aaa	aaa	aaa
Willcox, AZ						
(8)[a]	aaa	aaa	aaa	aaa	aaa	aaa
(5)[b]	abb	aaa	aaa	aaa	aaa	aaa
(1)	bbb	aaa	aaa	aaa	aaa	aaa
(2)	abb	aaa	aaa	aaa	aaa	abb
(1)	aaa	aaa	abb	aaa	aaa	aaa
(1)*		aaa	aaa	aab	aaa	aaa
(1)*		aaa	aaa	aaa	abb	aaa
(1)* **		aaa	aaa	aaa	aaa	aaa

[a,b]Genotypes with the same superscripts are represented at both study sites.
*Pgdh-A was unresolved for these individuals.
**This individual is indistinguishable from genotypes varying only at Pgdh-A.

tively). The two remaining individuals from the Rodeo study area (10% of the Rodeo sample) represented a genotype unique to that site. Of the remaining seven individuals captured at the Willcox, AZ study area, two had the same genotype (11% of the Willcox sample), four other individuals each exhibited unique genotypes (each representing 6% of the Willcox sample) and one individual was unresolved for the Pgdh-A locus.

Climatological Analysis

The results concerning maximum, minimum, and average temperatures were similar and too extensive to detail; thus, only some general observations are presented here. For the years 1983–June 1986 Willcox was 0.72 °C warmer than Animas. For those years for which annual data were available, Willcox and Rodeo had the same average temperature (16.1 °C) while that of Animas was 15.5 °C. Although the 0.72 °C difference between Willcox and Animas was statistically significant, it does not seem that this difference alone could explain the behavioral differences observed. Most of the temperature difference was a result of higher winter temperatures in Willcox and there were significant month by site interactions. Stations did not differ in terms of precipitation.

DISCUSSION

The complete lack of aggression between individuals from the Willcox study site, as well as the high degree of variation in aggressiveness comparing sites was unexpected. Animals from Willcox did not interact aggressively. Because the results were nearly identical for separate years, the responses are very unlikely to be a result of sampling or experimental bias. Animals from Rodeo interacted aggressively and dominated those from Willcox when housed together in Willcox–Rodeo pairs. Rodeo animals attacked Rodeo cagemates at a higher rate than they attacked Willcox cagemates. It appears that aggressive individuals facilitate attacks by their cagemates. The positive correlation in charges between cagemates suggests that charges facilitate charges by cagemates. Thus, the immediate social context influences the level of aggression. That one Willcox individual charged its Rodeo cagemate, after being attacked repeatedly, indicates that Willcox animals are capable of aggressive behavior. Tissues from this animal were included in the mtDNA analysis and found to be indistinguishable from those of the other Willcox and Rodeo *C. uniparens* included in the analysis (Densmore et al., 1989). Variation in the aggressiveness of animals from the Rodeo study area has been documented in other studies dealing with dominance (Grassman & Crews, 1987; Gustafson & Crews, 1981).

Several loci reported as fixed heterozygotes by Dessauer and Cole (1986) were screened in our anaylyis and found to be heterozygous in the 40 specimens surveyed (Aat, Ada, Aldh, Icdh-1, Mdh-1, Mpi-A, two peptidases, Pgm-2, and Sod-A). Further, many of the heterozygote phenotypes displayed a 2:1 ratio of staining intensity on our gels typical of the dosage effect apparent in this and some other triploid parthenogenetic *Cnemidophorus* and the Mdh-1 locus displayed the same 3-allele phenotype (abc) as reported by Dessauer and Cole (1986). Despite the levels of allozyme variability within the Willcox and Rodeo samples, the difference between samples was not statistically significant.

Genetic variation based on protein electrophoresis has been previously reported for *C. uniparens* by Dessauer and Cole (1986). Both protein electrophoresis and restriction endonuclease analysis of mtDNA (Densmore et al., 1989; Moritz et al., 1989; Moritz, personal communication) suggest that the *C. uniparens* populations used in this study are not significantly distinct from each other and may have descended from the same or closely related parental stock. It should be kept in mind that because polyphyletic clones arise from multiple hybrid zygotes, perhaps even from a single hybrid mating (Parker et al., 1989), it should be possible to have multiple hybrid clones which are identical in terms of mtDNA. Although there was no statistical difference between the Willcox and Rodeo populations, eight distinct clones were identified and seven of these were represented in the Willcox sample. Because all but two clones differed at a single locus most of this diversity presumably rep-

resents divergence following hybridization within one or possibly two phyletic clones (Parker et al., 1989). In contrast to the situation at Willcox, the majority of individuals from the Rodeo study site were indistinguishable. Clonal variation at the Willcox study site appears to be high compared to the Rodeo study site. Yet, the animals from the Rodeo study site are more variable in terms of aggressive behavior.

Variation in aggressive behavior observed between *C. uniparens* populations at Rodeo and Willcox, AZ can be accounted for by the frozen niche variation model. Parker et al. (1989) argued that a lack of correlation between color pattern and electrophoretic markers violates an assumption of the frozen niche model; polyphyletic clones which are morphologically distinct also will be genetically "recognizable." Perhaps polyphyletic clones may be genetically distinct but not always recognizable in terms of allozyme variation. In the hermaphroditic killifish, *Rivulus marmoratus*, variation based on histocompatibility tests has been reported among individuals collected in Florida (Harrington & Kallman, 1968; Kallman & Harrington, 1964) whereas allozyme data indicate that genetic homogeneity predominates (Massaro, Massaro, & Harrington, 1975; Vrijenhoek, 1985). In contrast, recent DNA fingerprinting analyses reveal substantial genetic variation and significant mutational distances even among sympatric *R. marmoratus* clones (Turner, Elder, Lauchlin, & Davis, 1990; Turner, Elder, & Lauchlin, 1991). Furthermore, sympatric clones distinguishable using DNA fingerprinting can differ in terms of aggressive behavior (Grassman, unpublished data). If electrophoretic results for individuals are thought of as a phenotypic character like color pattern, one might not predict that these must be correlated across polyphyletic clones. Suites of loosely linked characters should be necessarily related only in so far as they are or evolutionarily, developmentally, and/or functionally related. The frozen niche model takes into account the complexities of the multidimensional niche and predicts that clones should vary phenotypically along multiple axes (Vrijenhoek, 1984). Thus, different clones are likely to be similar in some characters whereas they may vary in others.

In addition to genetic factors, environmental conditions and genotype–environment interactions during development may play some role in the development of aggressive behavior in lizards. Recently, it has been demonstrated that incubation temperatures of lizard eggs influences aggressive behaviors in both males and females (Gutzke & Crews, 1988). Results from the analysis of the monthly and annual data did not seem to explain the observed differences in behavior. The significant month by site interactions indicated that one site was not consistently warmer than the other. Temperature differences between the study sites were small, and although Willcox was warmer than Animas in terms of the monthly analyses, based on annual data Willcox is more like Rodeo than Animas.

In examining the gross climatological data we assumed that this may

correlate with the local environment of the egg during development. However, the possibility that microclimatic differences between study sites could result in differences in aggression between adults cannot be ruled out. While it would be desirable to measure the environment of the nests themselves, the nesting sites for these animals are not known. One might propose that reflectance, moisture content and chemical composition of the soil (the Willcox site is near a large playa), or the kinds of oviposition sites available may influence the development of behavior in these animals. Other factors not related to climate such as density effects on the development of behavior could be important.

Some investigators have argued that individuals of parthenogenetic species may be less aggressive than those of closely related gonochoristic species. Differences in aggressiveness comparing parthenogenetic lizards (*C. tesselatus* and *C. neomexicanus*) and females of gonochoristic *C. sexlineatus* reported by Leuck (1985) were statistically nonsignificant. However, Leuck (1985) concluded that reduced genetic variation in parthenogenetic lizards resulted in reduced aggression (increased altruism). Alternatively, reduced genetic variation may result in reduced heritable variation in behaviors (including altruism) on which natural selection might act. Also, one might expect to find reduced variation in fitness eliminating benefits that would accrue to supposedly altruistic lizards. Species differences not related to reproductive mode (parthenogenetic vs. gonochoristic) and geographic variation must be taken into account in order to determine the effect of reproductive mode on behavior. Thus, it seems unlikely that reduced aggression would apply to parthenogenetic lizards as a general principle.

It must be noted here that a statistically significant correlation between behavioral and biochemical diversity would not demonstrate a causal link between genes and behavior. Conversely, a lack of correlation between allozymes and behavior does not rule out heritability in aggressive behavior. However, a failure to demonstrate a correlation between the genetic analyses and geographic variation in behavior is not meaningless "negative data" but is important information suggesting either that the genetic analyses are not measuring behaviorally relevant genetic diversity (there may be polygenic variation in behavior undetected by the biochemical analyses used), or that clonal genotypes may be quite plastic in terms of behavior. Either way the results raise interesting questions worthy of consideration. In order to demonstrate behavioral variation resulting from multiple hybrid origins, one must identify the genetic basis of the behavior in question. In some gynogenetic fish, for example, ecological and behavioral variation are correlated with variation in certain enzyme markers (Vrijenhoek, 1984). This supports the conclusion that behavior varies among clones. However, in parthenogenetic *Drosophila* individual variation in reproductive output within lineages is as variable as that among different lineages (Crews, Teramato, & Carson,

1985). The present study, taken together with those of Dessauer and Cole (1986) and Crews et al. (1985), indicates that genotypic similarity based on the analysis of a small proportion of structural genes at least in some cases may not accurately reflect the potential genetic basis of variation in behavior, ecology, and reproduction. Laboratory investigations involving *C. uniparens* with documented parental and sibling relationships reared under controlled environmental conditions are needed to explain the relative roles of genes and environment in the development of aggressive behavior in these animals.

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