Herpetologica, 47(1), 1991, 54–68

DISTRIBUTION AND GENETIC VARIATION IN VENOM A AND B POPULATIONS OF THE MOJAVE RATTLESNAKE (*CROTALUS SCUTULATUS SCUTULATUS*) IN ARIZONA

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ABSTRACT: Two geographically distinct venoms (venom A and venom B) are produced by the Mojave rattlesnake (*Crotalus scutulatus scutulatus*) in Arizona. The purpose of this investigation was to define further the distribution and genetic divergence between the two venom populations. Seventy-one specimens were collected from central Arizona for immunological and biochemical venom assays and allozyme electrophoretic assays of divergence. Venom assays indicate that snakes of venom B type are restricted to central Arizona, occupying the region from east and northeast of Phoenix, south to near Tucson. Snakes of venom B type overlap with those of venom A type to the north, west, and south of this region, and they intergrade with snakes of venom A type to produce a third venom type (A+B). The results of starch gel electrophoresis of 55 different tissue enzymes indicate high gene flow between the two venom types with a low genetic divergence (Nei's D = 0.003), indicating that the two venom types are conspecific. Environmental and ecological isolating mechanisms that could have been responsible for the origin and distribution of these venom populations are discussed.

Key words: *Crotalus scutulatus scutulatus*; Venom types; Geographic variation; Ouchterlony; Protease activity; Starch gel electrophoresis; Tissue allozymes; Arizona

THE Mojave rattlesnake, *Crotalus scutulatus scutulatus* (Kennicott), is an inhabitant of the Mojave, Sonoran, and Chihuahuan deserts of the southern United States and northern Mexico (Gloyd, 1940; Klauber, 1972).

The venom composition of *C. s. scutulatus* differs markedly in Arizona, where two distinct venom types have been described (Glenn and Straight, 1978). One type (venom A) contains Mojave toxin (a Phospholipase A_2 complex toxin) but does not contain specific proteolytic and hemorrhagic activities, and another (venom B) contains proteolytic and hemorrhagic activities but lacks the Mojave toxin (Glenn and Straight, 1989; Glenn et al., 1983). Geographic variation in venom composition has also been reported in *C. basiliscus basiliscus*, *C. lepidus klauberi*, *C. viridis viridis*, and *C. horridus atricaudatus* (Bober et al., 1988; Glenn and Straight, 1985, 1987).

Glenn and Straight (1989) reported intergradation between the two venom populations of *C. s. scutulatus*, extending in a more or less northwest–southeast arc from Phoenix to Tucson. The venom B population appears to be surrounded by the venom A population from the northwest to the southeast of its range. The northeast barrier to the venom B distribution is the Colorado Plateau's Mogollon Rim (Glenn and Straight, 1989).

No obvious morphological differences are known to distinguish individuals from each venom race (Glenn et al., 1983), although differentiation of venoms could also have been accompanied by ecologic, physiologic, and/or genetic divergence in these populations. The objectives of this study were to define further the geographic

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boundaries of the venom A and B populations, with special emphasis on areas of potential contact, and to determine if allozyme data from tissue protein electrophoresis would reveal any diagnostic genetic markers for the two populations.

Materials and Methods

Collection of Snakes

Snakes were collected along roadways entering into and transecting the central Arizona section of the range of *C. s. scutulatus*, with extensive collecting in locations in and around the inferred contact zone and the population range of venom B (Fig. 1). We drove roads from just prior to dusk throughout the night until just prior to dawn. Six separate surveys were completed during the summer months (May–August) of 1986 and 1987. The specimens were identified at the time of capture by size, particular head or body markings, and number of tail bands. The time of capture, road mileage, air temperature, altitude, weather conditions, phase of moon, and relative humidity were also recorded. Collection success was best on cloudy nights or nights with no moon, high temperatures (above 26 C), and high humidity during August.

Certain geographic areas within the range of *C. s. scutulatus* were difficult to collect and/or yielded few specimens. This was especially true along the eastern and northeastern fringe of the range where there is much uplift in the landscape. Also, certain other areas were not accessible (Indian reservations, military ranges), and they were omitted from this study. A total of 71 snakes was obtained for venom examination, but because of deaths due to a viral outbreak in captivity, only 60 specimens were available for tissue sampling and starch gel electrophoresis of proteins. All voucher specimens were fixed in 10% formalin and were permanently preserved in 70% ethanol in the Monte L. Bean Life Science Museum, Brigham Young University. Catalogue numbers and locality data for the specimens are presented in Appendix I.

Venom Assays

Specimens were housed at the Venom Research Serpentarium, Veterans Administration Medical Center, Salt Lake City, Utah. Individual venoms were collected using manual extraction techniques, and the venoms were centrifuged at 1500 *g* for 15 min. The supernatant was lyophilized and stored at 4 C.

Venom proteolytic activity was assayed using a modification of the method of Rinderknecht et al. (1968), with Remazol brilliant blue hide-powder azure (Cal-Biochem) as the substrate; 100 μg of venom (1 mg/ml 0.1 M sodium phosphate buffer, pH 8.5) was added to 5.0 ml of hide-powder suspension, stirred in a 37 C water bath for 15 min, filtered, and the proteolytic activity was measured at 520 nm. The activity was compared to the proteolytic activity of a venom pool of *Crotalus atrox*. Optical densities (O.D.) were recorded, and O.D. readings of <0.015 were considered "no activity" above background.

Mojave toxin presence was assayed using antigenicity (Ouchterlony Immunodiffusion) of each venom sample versus antiserum (rabbit) to Mojave toxin as described by Glenn et al. (1983). Twenty microliters of (50 mg/ml) antiserum to Mojave toxin were placed in a center well of pre-cast agarose discs (Miles Laboratories, Inc.), and 20 μl samples of venom of *C. s. scutulatus* (40 mg/ml) were placed in the outer wells. The discs were incubated at 37 C for 72 h and were observed every 24 h for immunoprecipitin reactions.

The results of this assay were compared with the proteolytic activity assay previously described. The presence of proteolytic activity and immunological absence of the Mojave toxin characterizes venom B. Conversely, the immunological presence of Mojave toxin and the absence of the proteolytic activity characterizes venom A, and the presence of both Mojave toxin and the proteolytic activities indicate an A+B type.

Tissue Protein Electrophoresis

Because morphological characters were previously shown to be uninformative for

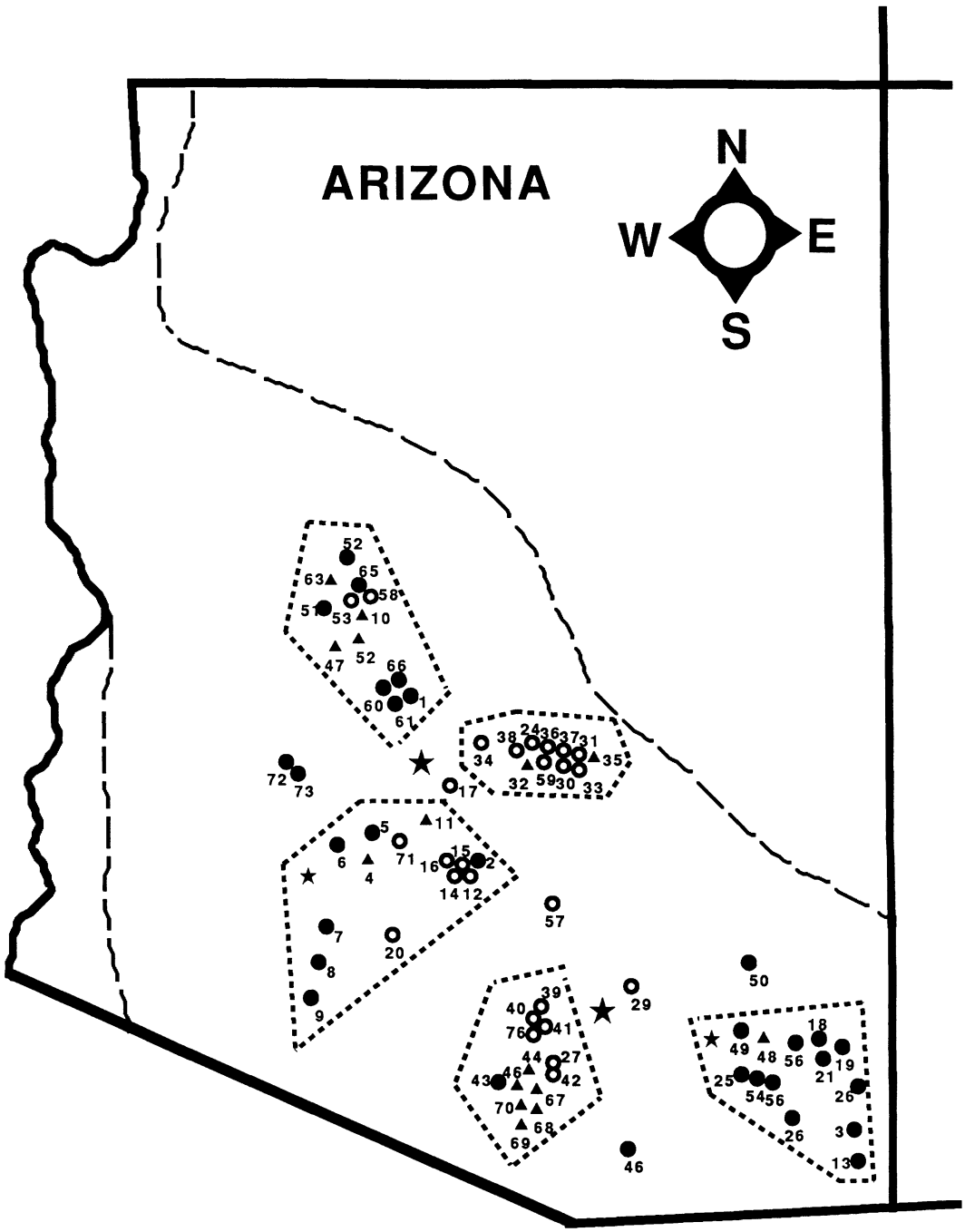

FIG. 1.—Locality in Arizona of each specimen of *Crotalus s. scutulatus* collected and venom typed for this study. Closed circles represent venom A, open circles represent venom B, and triangles represent venom A+B. Stars represent, from north to south, the cities of Phoenix and Tucson. Minor stars represent Gila Bend to the west and Willcox to the east. Numbers correlate with numbers for each specimen listed in Appendix I. Dotted lines group specimens postulated to be members of single interbreeding units, while prominent dashed lines define total range of *C. s. scutulatus* in Arizona.

markers between venom types (Glenn et al., 1983), divergence at the allozyme level was investigated. Sixty specimens were sacrificed for extraction of blood and tissues for protein electrophoretic analysis. After decapitation, blood was collected in sodium citrate tubes and was centrifuged into plasma and hemolysate fractions. Ventral dissections were performed, and samples of heart, liver, duodenum, skeletal muscle, and kidney were taken. Samples of blood and tissue were stored at −80 C.

Tissue samples were added to approximately equal volumes of pH 6.8 Tris-EDTA buffer (Selander et al., 1971), were homogenized, and then were centrifuged at 12,000 *g* for 20 min at 7 C. The supernatant was stored frozen at −80 C. Blood plasma and hemolysate fractions were used without modification. Horizontal electrophoresis (Murphy et al., 1990) was performed with 12.5% (w/v) starch gels (1:1 Sigma Starch-Electrostarch [Lot 392]) at 5–7 C. Histochemical staining procedures followed Murphy et al. (1990), and enzyme and buffer systems and tissues screened are listed in Table 1.

Enzyme names were those recommended by the International Union of Biochemistry (1984), and locus abbreviations followed Murphy and Crabtree (1985*a*) with some modifications. Electromorphs at specific loci were considered homologous if their mobilities were the same. Those with differing mobilities were given different alphabetic designations in parentheses: the most anodal allele denoted "a" and successively more cathodal ones denoted "b–d". Different mobilities of alleles at a particular locus were verified through side-by-side comparisons.

Each venom type (A, B, and A+B) of *C. s. scutulatus* and comparatives of *C. atrox* were considered to be separate operational taxonomic units (= OTU's) for purposes of data analysis. Allele frequency data were summarized with BIOSYS-1 (Version 2.0: Swofford and Selander, 1981). The average locus heterozygosity by direct count (H), percent of loci polymorphic (P), and the mean number of alleles per locus were computed as genetic variability estimates for each sample. Coefficients of genetic distance of Nei (1978) and Rogers (1972) for all possible pairwise sample comparisons were calculated and then clustered by the UPGMA algorithm (Sneath and Sokal, 1973). A contingency Chi-square analysis of Workman and Niswander (1970) was performed to test for allozyme frequency heterogeneity between the three OTU's of *C. s. scutulatus*.

Results

Venom Protease and Immunochemical Analysis

Protease activity and immunodiffusion analyses of the venom samples for the presence or absence of proteolytic activity and/or the Mojave toxin are listed in Table 2. Thirty specimens were of venom A type with a protease activity range of O.D. 0.000–0.013 ($\bar{x} = 0.003$), 25 specimens were of venom B type, with a protease activity range of O.D. 0.024–0.301 ($\bar{x} = 0.125$), and 16 specimens were of venom A+B type, with a range of O.D. 0.022–0.223 ($\bar{x} = 0.088$). The geographic distribution based on these and earlier (Glenn and Straight, 1989; Glenn et al., 1983) assays is shown in Fig. 2. Individuals with venom B were taken from just southwest of Prescott, Yavapai Co., to south of Tucson. The east–west extension of the venom B type appears narrower, with the eastern-most individual from the vicinity west of Safford, Graham Co. Venom B extends southwest from this area to Tucson and then loops around in a narrow northwest arc from Tucson to the east-northeast of Phoenix, and then to near Prescott. The presence of A+B types suggests that pure venom B individuals may range as far east as Willcox, Cochise Co., approximately 80 km from the Arizona-New Mexico border. This race may possibly extend northward below the Mogollon rim and south to or beyond the Arizona-Mexico border. However, the western-most extension appears limited to the northwest arc previously mentioned (Fig. 2). The intergradation zone, as defined by the presence of snakes with venom A, venom B, and venom A+B, appears approximately 72 km wide at its widest point in Cochise and Graham counties, with

TABLE 1.—Enzymes and electrophoretic conditions used in surveys of all samples of *Crotalus*. Enzyme names and enzyme commission numbers follow the Nomenclature Committee of the International Union of Biochemistry (1984). Locus abbreviations follow Murphy and Crabtree (1985*a*) where homologous systems were resolved, and tissue symbols are: D = duodenum, H = heart, K = kidney, L = liver, M = skeletal muscle, P = blood plasma, and R = red blood cell fraction.

Enzyme	Enzyme commission number	Locus	Tissue	Buffer conditions[1]
Aconitate hydratase	4.2.1.3	Acon-A	L	C
Adenosine deaminase	3.5.4.4	Ada-1	D	D
Albumin	—	Alb	P	A
Alcohol dehydrogenase	1.1.1.1	Adh-A	H	D
Alkaline phosphatase	3.1.3.1	Alp-A	H	C
Aspartate aminotransferase	2.6.1.1	mAat-A	L	A
Aspartate aminotransferase	2.6.1.1	sAat-A	L	A
Calcium-binding protein	—	Cabp-1	H	C
Catalase	1.11.1.6	Cat-A	L	A
Creatine kinase	2.7.3.2	Ck-A	H	H
Esterase	—	Est-1, -2	P, L	A
Esterase	3.1.1.–	Est-D-[1], -[2]	H	D
Fructose-bisphosphatase	3.1.3.11	Fbp-1, -2	L, M	D
Fructose-bisphosphate aldolase	4.1.2.13	Ald-1, -2	H, K	B
β-Glucuronidase	3.2.1.31	β-Gluc-1	L	F
Glucose dehydrogenase	1.1.1.47	Gcdh-A	L	A
Glucose-6-phosphate dehydrogenase	1.1.1.49	G6pdh-A	L	E
Glucose-6-phosphate isomerase	5.3.1.9	Gpi-A	L	I
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	Gapdh-1, -2	L	F
Glycerate dehydrogenase	1.1.1.29	Glydh-A	H	A
Glycerol-3-phosphate dehydrogenase	1.1.1.8	G3pdh-B	K	I
Guanine deaminase	3.5.4.3	Gda-A	L	B
Guanylate kinase	2.7.4.8	Guk-1	K	A
Hexokinase	2.7.1.1	Hk-A	K	E
L-Iditol dehydrogenase	1.1.1.14	Iddh-A	L	C
Isocitrate dehydrogenase	1.1.1.42	mIcdh-A	L	D
Isocitrate dehydrogenase	1.1.1.42	sIcdh-A	L	D
L-lactate dehydrogenase	1.1.1.27	Ldh-A, -B	L	C
Malate dehydrogenase	1.1.1.37	mMdh-A	L	I
Malate dehydrogenase	1.1.1.37	sMdh-A	L	I
Malic enzyme	1.1.1.40	mMe-A	M	F
Malic enzyme	1.1.1.40	sMe-A	H	F
Mannose-6-phosphate isomerase	5.3.1.8	Mpi-A	L	D
Mannosidase	3.2.1.24	Man-A	L	H
Peptidases	3.4.13.9	Pep-A, -B, -F, -S	K, L	H
	3.4.13.11	Pep-D	K	H
Phosphoglucomutase	5.4.2.2	Pgm-A	L	I
Phosphogluconate dehydrogenase	1.1.1.44	Pgdh-1, -2	K, H	D
Purine-nucleoside phosphorylase	2.4.2.1	Pnp-A	L	B
Thiosulfate sulfurtransferase	2.8.1.1	Tst-1, -2	H	E
Superoxide dismutase	1.15.1.1	mSod-A	L	G
Superoxide dismutase	1.15.1.1	sSod-A[1]	R	H
Superoxide dismutase	1.15.1.1	sSod-A[2]	L	H
Xanthine dehydrogenase	1.1.1.204	Xdh-A	L	H

[1] A: Lithium hydroxide pH 8.2, 14 h at 35 ma; B: Phosphate citrate pH 7.0, 21 h at 25 ma; C: Poulik pH 8.7, 16 h at 25 ma; D: Sodium citrate pH 8.0, 8 h at 35 ma; E: Sodium citrate (NADP) pH 8.0, 8 h at 35 ma; F: Tris-citrate pH 8.0, 22 h 25 ma; G: Tris-citrate EDTA pH 8.0, 25 h at 35 ma; H: Tris-HCl pH 8.5, 22 h 25 ma; I: Tris-citrate pH 7.0, 21 h 35 ma (buffers from Murphy et al., 1990).

areas of constriction occurring west of Phoenix and south and southwest of Tucson. These restrictions are likely artifacts of human intervention due to the loss of habitat associated with expansion of both cities within the intergradation zone. A finger of the zone may enter east into the range of venom B just north of Phoenix, because two venom A+B animals were found northeast of Phoenix among nine

TABLE 2.—Antibody against Mojave toxin (MTAb, + or − means presence or absence, respectively, of precipitate reaction to the Mojave toxin), protease activity measured at 520 nm on a spectrophotometer, and venom type of 71 specimens of *Crotalus s. scutulatus*. Numbers correlate with localities plotted in Fig. 1 (exact localities are given in Appendix I).

Specimen number	MTAb	Protease activity 100 μg/15 min O.D.	Venom type	Specimen number	MTAb	Protease activity 100 μg/15 min O.D.	Venom type
1	+	0.004	A	17	−	0.229	B
2	+	0.000	A	18	+	0.004	A
3	+	0.003	A	19	+	0.008	A
4	+	0.118	A+B	20	−	0.112	B
5	+	0.000	A	21	+	0.005	A
6	+	0.000	A	24	−	0.204	B
7	+	0.008	A	25	+	0.000	A
8	+	0.010	A	26	+	0.013	A
9	+	0.000	A	27	−	0.110	B
10	+	0.223	A+B	28	+	0.005	A
11	+	0.192	A+B	29	−	0.173	B
12	−	0.208	B	30	−	0.272	B
13	+	0.001	A	31	−	0.100	B
14	−	0.194	B	32	+	0.107	A+B
15	−	0.136	B	33	−	0.097	B
16	−	0.301	B	34	−	0.053	B
35	+	0.022	A+B	55	+	0.004	A
36	−	0.037	B	56	+	0.000	A
37	−	0.049	B	57	+	0.029	A+B
38	−	0.106	B	58	−	0.060	B
39	−	0.024	B	59	−	0.087	B
40	−	0.040	B	60	+	0.003	A
41	−	0.128	B	61	+	0.001	A
42	−	0.069	B	62	+	0.006	A
43	+	0.000	A	63	+	0.071	A+B
44	+	0.094	A+B	65	+	0.005	A
45	+	0.045	A+B	66	+	0.000	A
46	+	0.000	A	67	+	0.116	A+B
47	+	0.070	A+B	68	+	0.118	A+B
48	+	0.043	A+B	69	+	0.035	A+B
49	+	0.000	A	70	+	0.035	A+B
50	+	0.004	A	71	−	0.099	B
51	+	0.003	A	72	+	0.007	A
52	+	0.084	A+B	73	+	0.006	A
53	−	0.111	B	76	−	0.152	B
54	+	0.001	A				

individuals with venom B (Fig. 2). No specimens were found north of this easterly extension of the zone where the landscape rises extensively. The intergradation zone may be wider southeast of Tucson; only a single specimen (venom A) was taken from this area and thus does not adequately represent this region. Southeastern Cochise Co. (south of Interstate 10) and southwestern New Mexico were inhabited by only snakes with venom A. Also, only snakes with venom A were found south of Gila Bend, Maricopa Co. in southwestern Arizona, and north and west throughout the remaining western extension of the species range (Glenn and Straight, 1989; Glenn et al., 1983).

Starch Gel Electrophoresis of Tissue Proteins

Fifty-five presumptive loci were electrophoretically assayed for 58 *C. s. scutulatus* and two *C. atrox* from Arizona. Thirty-nine of these were monomorphic for the same allele in all individuals studied including: Adh-A, Ald-1, Ald-2, Alp-A, mAat-A, sAat-A, Cabp-1, Cat-A, Ck-A, Est-2, Est-D^2, Fbp-1, Gcdh-A, Gapdh-2,

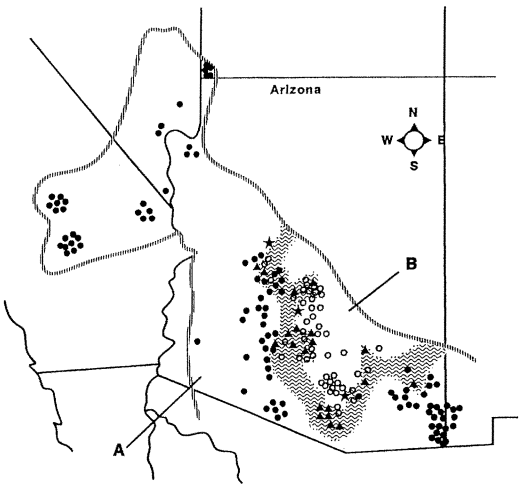

FIG. 2.—Range of venom type A, and B of *Crotalus s. scutulatus* in the southwestern U.S.A. from this and previous studies combined (Glenn and Straight, 1989; Glenn et al., 1983). Horizontal hatching represents the zone of intergradation between the two venom types. Stars represent, from north to south, the cities of Prescott, Phoenix, and Tucson.

Glydh-A, G3pdh-B, Gda-A, β-Gluc-1, Gpi-A, Guk-1, Hk-A, Iddh-A, Ldh-A, Ldh-B, mMdh-A, sMdh-A, Pep-A,B,D,F,S, Pgdh-2, Pnp-A, mSod-A, sSod-A^1, sSod-A^2, Tst-1, Tst-2, and Xdh-A. The allele frequencies found for the remaining polymorphic loci are summarized in Table 3. *Crotalus atrox* is fixed or nearly fixed for electromorphs that are rare or absent in all samples of *C. s. scutulatus* at four loci: Ada-1, Mpi-A, Pgdh-1, and Pgm-A. Also, *C. atrox* has a relatively higher frequency (0.75) of sMe-A(c) electromorphs that are present at low to medium frequency (0.167–0.571) in samples of *C. s. scutulatus*. At Gapdh-1, *C. atrox* was fixed for the b allele present in all samples of *C. s. scutulatus*, and within the latter, the venom B sample exhibited a higher frequency (0.417) for this allele than venom A (0.286).

Samples of venom A possessed minor alleles at four loci [Est-1(c), sIcdh-A(b), mMe-A(b), and Mpi-A(c)] that were absent from samples of snakes with venom B. Conversely, snakes with venom B possessed minor alleles at three loci [Alb(b), Fbp-2(b), and Man-A(b,c)] which were absent from snakes with venom A. Three loci (sAcon-A, G6pdh-A, and Pgm-A) contained minor alleles confined to the A+B type individuals. The mean number of alleles per locus was the same among all three OTU's of *C. s. scutulatus*, but the percentage of polymorphic loci varied, being highest in snakes with venom B ($P = 20.4$) and lowest in snakes with venom A+B ($P = 16.7$: Table 3). The mean locus heterozygosity (H) ranged from 0.038 for snakes with venom B to 0.045 for snakes with venom A+B.

Pairwise comparisons of two genetic distance coefficients (Nei, 1978; Rogers, 1972), calculated over all 55 loci, are presented in Table 4. In both distance measures, the highest values consistently occurred between the OTU's of *C. atrox* and *C. s. scutulatus*. Within the venom types of *C. s. scutulatus*, the highest values (0.003 for Nei and 0.022 for Rogers) were between type A and type B. These values indicate a relatively recent divergence of venom types and/or gene flow between populations of the two types of venom.

To determine if there is evidence for a possible restriction of gene flow, a contingency Chi-square analysis (Workman and Niswander, 1970) was performed to test for homogeneity of electromorph frequencies across all three OTU's within *C. s. scutulatus*, because individuals from scattered localities were initially pooled to form the venom class OTU's used above. The common electromorphs at three loci showed significant heterogeneities in frequencies (Acon-A(a), $\chi^2 = 7.8$, df = 2, $P = 0.020$; sMe-A(b), $\chi^2 = 20.3$, df = 4, $P = 0.000$; and Mpi-A(a), $\chi^2 = 11.6$, df = 4, $P = 0.021$). Significant heterogeneities may result from three possible causes; inadequate sample sizes, genetic differences in allele frequencies between the venom types, or geographic variation in allele frequencies within the venom types. Even though small sample sizes cannot be ignored (28 individuals for venom type A, 18 for venom type B, and 12 for venom type A+B), there is also a possibility that the combined venom OTU's may not represent single breeding units, but may consist of geographically differentiated demes with different allele frequencies. There-

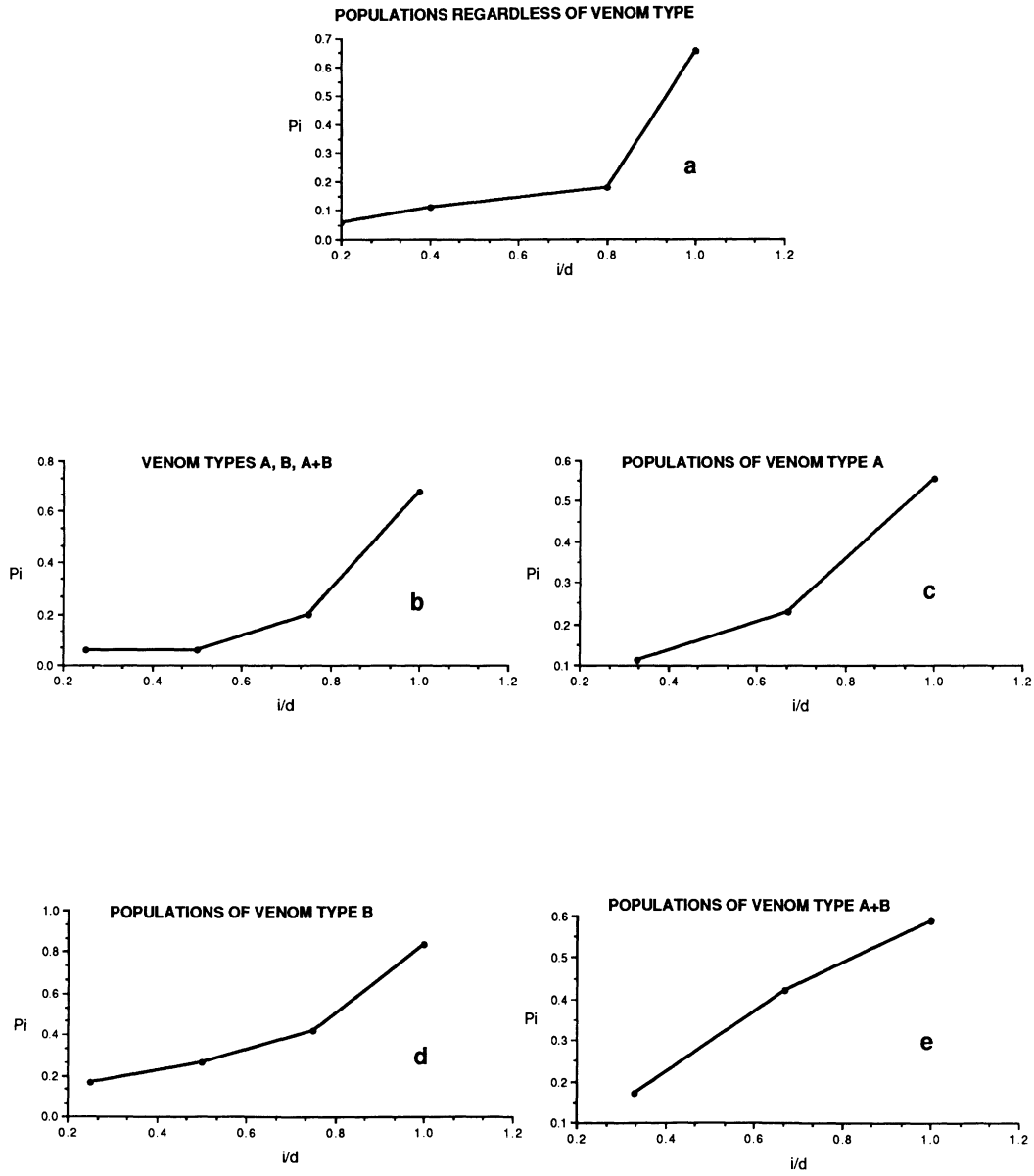

FIG. 3.—Relationship between conditional average allele frequency, P_i (the average frequency of an allele in all populations in which it is found), and its incidence, i/d (the number of populations in which the allele is observed, i, divided by the total number of populations examined, d), for samples grouped (a) geographically regardless of venom types, (b) venom types A, B, A+B, (c) geographic populations within venom type A, (d) venom type B, and (e) venom type A+B. The points plotted represent the average value of P_i for all alleles having the same i/d value for each group being measured.

fore venom A was subdivided into three geographically distinct groups (Yavapai Co., $n = 6$; Maricopa Co., $n = 8$; Cochise Co., $n = 14$), venom B into 4 groups (McDowell Mountain, $n = 7$; Maricopa Co., $n = 5$; Yavapai Co., $n = 2$; Pima Co., $n = 2$), and venom A+B into three groups (Yavapai Co., $n = 4$; Maricopa Co., $n = 2$; Pima Co., $n = 6$). All samples were analyzed as above for significant allele fre-

TABLE 3.—Summary of allele frequencies for 16 polymorphic loci in all *Crotalus* available for electrophoresis. For loci with a pair of alleles, only first allele is indicated. Numerical designations for OTU's are as follows: 1 = venom A; 2 = venom B; 3 = venom A+B; 4 = *Crotalus atrox*. Collecting sites are shown in Fig. 1 and listed in Appendix I, and locus abbreviations are explained in Table 1. Sample size for each OTU is in parentheses.

	C. s. scutulatus			*C. atrox*
Locus	1 (n = 28)	2 (n = 18)	3 (n = 12)	4 (n = 2)
Acon-A				
a	1.00	1.00	0.917	1.00
Ada-1				
a	0.911	0.972	0.917	0.00
Alb				
a	1.00	0.972	1.00	1.00
Est-1				
a	0.768	0.833	0.75	1.00
b	0.179	0.167	0.208	0.00
c	0.018	0.00	0.00	0.00
d	0.036	0.00	0.042	0.00
Est-D[1]				
a	0.115	0.219	0.227	0.00
b	0.635	0.656	0.364	1.00
c	0.250	0.125	0.409	0.00
Fbp-2				
a	1.00	0.972	1.00	1.00
G6pdh-A				
a	1.00	1.00	0.958	1.00
Gapdh-1				
a	0.714	0.583	0.50	0.00
mIcdh-A				
a	0.982	0.972	1.00	1.00
sIcdh-A				
a	0.946	1.00	1.00	1.00
mMe-A				
a	0.964	1.00	1.00	1.00
sMe-A				
a	0.071	0.25	0.333	0.00
b	0.357	0.583	0.333	0.25
c	0.571	0.167	0.333	0.75
Mpi-A				
a	0.786	0.972	1.00	0.00
b	0.179	0.028	0.00	0.00
c	0.036	0.00	0.00	1.00
Man-A				
a	1.00	0.889	1.00	1.00
b	0.00	0.083	0.00	0.00
c	0.00	0.028	0.00	0.00
Pgm-A				
a	1.00	1.00	0.958	0.00

TABLE 3.—Continued.

Locus	*C. s. scutulatus* 1 (n = 28)	2 (n = 18)	3 (n = 12)	*C. atrox* 4 (n = 2)
Pgdh-1				
a	0.179	0.167	0.083	0.00
b	0.268	0.306	0.292	0.00
c	0.357	0.389	0.485	0.00
d	0.196	0.139	0.167	1.00
% loci polymorphic*	18.5	20.4	16.7	1.9
$\bar{x}$ no. alleles per locus (±SE)	1.3 (0.1)	1.3 (0.1)	1.3 (0.1)	1.0 (0.0)
$\bar{x}$ heterozygosity** (±SE)	0.043 (0.15)	0.038 (0.015)	0.045 (0.016)	0.009 (0.009)

* 0.99 criterion.
** Mean locus heterozygosity by direct count.

quency heterogeneity within each venom type. Results showed significant heterogeneities in allele frequencies at four loci (Est-D^1(a), χ^2 = 45.5, df = 18, P = 0.000; Man-A(a), χ^2 = 18.8, df = 9, P = 0.027; sMe-A(b), χ^2 = 34.7, df = 18, P = 0.010; and Pgm-A(a), χ^2 = 27.2, df = 9, P = 0.001). Again, small sample sizes do not permit firm conclusions, but at least some of this heterogeneity may be due to geographic structuring within venom types.

In order to qualitatively assess gene flow within and among venom races, the Slatkin (1981) conditional allele model was used to display graphically gene flow patterns. This procedure is based on strong dependence between the overall level of gene flow among a defined set of populations, and the average frequency of an allele, conditioned on the number of populations in which that allele occurs. A "J" shaped curve is characteristic of high gene flow among the populations (Slatkin, 1981). The samples of *C. s. scutulatus* were subdivided and analyzed in three ways: (1) samples grouped geographically regardless of venom types (n = 5; Figs. 1, 3a), (2) venom types A, B, A+B (Fig. 3b), and (3) geographic populations within venom types (same as above for heterogeneity χ^2 test and presented as Fig. 3c–e). Except for samples representing venom type A+B (Fig. 3e), all patterns are typical of groups with high gene flow. Again, because of the small sample sizes of populations with venom A+B, this might not be a true representation of the samples, especially because all other groupings reflected a high pattern of gene flow.

DISCUSSION

Noncorrelation of Venom and Allozyme Characters

Venom analyses indicate that there is geographic variation in venom properties of the Mojave rattlesnake in central Arizona. The allozyme and venom data are not correlated. Though venom races and their intergrades showed distinct geographical patterns of distribution, the allozyme data provided no fixed differences between the two types of venom. The independent evolution of tissue protein characters from other characters has been shown to occur in other species of organisms. For example, Johnson et al. (1972) were able to differentiate between sibling species of cotton rats (*Sigmodon*) using allozyme data, which were morphologi-

TABLE 4.—Matrix of Nei (1978: above diagonal) and Rogers (1972: below diagonal) genetic distance coefficients for all pairwise combinations of venom types of *Crotalus s. scutulatus* and *C. atrox*.

Venom types	A	B	A+B	*C. atrox*
Venom A	—	0.003	0.002	0.072
Venom B	0.022	—	0.001	0.080
Venom A+B	0.024	0.022	—	0.077
C. atrox	0.092	0.100	0.100	—

cally indistinguishable. Yet in other studies, morphologically distinct races and even species of a wide range of organisms demonstrated little if any allozymic differentiation (Avise et al., 1975, 1979; Rosenfeld and Wilkinson, 1989; Turner, 1974; Zink, 1986; Zink et al., 1989). Independent evolution of the venom characters and the allozyme characters may reflect selection of venom proteins (Wilkinson, 1989), as has been suggested for morphological characters in other reptiles (Murphy and Crabtree, 1985*b*), compared to relatively neutral allozyme variation (Kimura, 1982; Nei, 1983).

Existence of a Zone of Intergradation

Buth (1984) stated that hybrids may be revealed through protein electrophoresis by their higher-than-expected levels of variability and by their shared allozyme components with the parental types. Murphy et al. (1984) stated that these components should be autapomorphies from both parental types. The venom intergrades showed no significant increase in any indicators of variability (percent loci polymorphic, mean number of alleles per locus, and mean heterozygosity) above the A and B venom types separately (Table 3). Yet, the allozyme data support the existence of an intergradation zone between the venom types based on two phenomena. First, seven of the 16 polymorphic loci (Alb, Est-1, Fbp-2, sIcdh-A, mMe-A, Mpi-A, and Man-A) have minor alleles which occur only in one venom type (or in one venom type and the intergrade venom A+B type) but not in the other type. Greenbaum (1981) suggested that these "marker alleles" indicate an inhibition of gene flow across a hybrid zone even though overall allozymic differentiation among populations is slight at best. Hafner (1982) did not concur with this interpretation but did suggest that the expression of these minor alleles within a hybrid zone may be the result of the zone itself, which is an indication of the second phenomenon.

Three of the 16 polymorphic loci (Acon-A, G6pdh-A, and Pgm-A) have minor alleles only in individuals with venom A+B. The appearance of rare alleles within hybrid zones is considered a common phenomenon (Woodruff, 1989). Barton et al. (1983) suggested that these alleles may be deleterious and are maintained in the hybrid zone by a balance between their occurrence through intragenic recombination or mutation and their elimination by natural selection, but another explanation based on neutrality was proposed by Woodruff (1989). The high level of allozyme similarity between the venom types, indicated by low genetic distances (Table 4), their indistinguishable morphological characters, the presence of rare alleles in the intergrade zone, the clear geographic separation of the type A and B venoms, and the relatively narrow intergrade zone between populations of venoms A and B are good examples of introgressive hybridization (Hafner, 1982).

Primary or Secondary Contact

The zone of intergradation between snakes of type A and type B venoms is the result of either primary or secondary contact (Mayr, 1969). To be the result of primary contact implies that the present ecological environment is involved in segregating the types of venoms. Most reported intergradation zones of primary contact involve clines in characters of an organism due to adaptive differentiation along an environmental gradient in the absence of a physical barrier (Endler, 1977). There are no apparent ecological differences between the habitats of venom type A and venom type B, even though a more detailed study, possibly involving an analysis of stomach contents (e.g., Beaver, 1976) may reveal a difference in availability and selection of prey between the two types. An intergrade zone resulting from secondary contact involves the reassociation of once allopatric populations. While in allopatry, the populations differentiated from each other because of adaptation to distinct specific habitats, or merely by genetic drift while separated by a physical barrier (e.g., woodland habitat: Van Devender and Mead, 1978). Endler (1982) stated that one cannot determine whether intergradation resulted from pri-

mary or secondary contact simply by examining the parameters of the zone of contact, because the effects of the two may yield the same patterns. Yet, in the absence of any environmental patterning between the two types of venom, the appearance of high gene flow as expressed in the Slatkin graphs (Fig. 3), and the very low genetic distances (0.003) between the venom types A and B, these populations appear to be conspecific, with the intergrade zone resulting from a geologically recent secondary contact following relatively brief allopatric isolations.

Paleobiogeography of the Venom Types

If the two types of venom of *C. s. scutulatus* have diverged in allopatry, and now are in a state of parapatric hybridization (Glenn and Straight, 1989; this report), venom differences may simply have resulted from the fixation of mutations during isolation of the ancestral population of one or both venom types. This is especially likely if neither type of venom has an advantage over the other, and the venom divergence may simply be a pleiotropic by-product of historical geographic isolation.

Because there are no apparent present geological or physiographical barriers between the two types of venom, our hypothesis is that isolating events during the Pleistocene glacial periods may be the origin of their differentiation (Rand, 1948). For example, Hubbard (1973) proposed that during the Wisconsin Glaciation (which began approximately 120,000 yr ago) the southwestern regional deserts and other xeric habitats drastically decreased in total area and separated into three major refugia, east to west, where xerophilous animals (those adapted to arid conditions) survived. This refugial scenario is similar to that of Savage (1960), Ballinger and Tinkle (1972), and Morafka (1977). However, recent paleoecological data examining macrofossil assemblages from packrat middens indicate that there may not have been any major refugia of xerophilic flora in the southwestern desert region during the late Pleistocene (Van Devender and Burgess, 1985). Instead, the desert scrub communities remained in situ where local conditions allowed (Van Devender and Mead, 1978), and some desert animals did not track the contraction of plant communities but remained in place by acclimating to the advancing woodland habitat (Mead et al., 1983; Murphy, 1983; Van Devender et al., 1976; Van Devender and Mead, 1978).

There is some evidence that east–west differences in the composition of venom of *C. s. scutulatus* occur from Texas to Arizona (Glenn and Straight, 1989; Rael et al., 1984; Zepeda et al., 1985). These geographic differences could be the result of either of the above paleoecological scenarios but do not distinguish between them. Also, the in situ precursors or internal mechanisms causing the venom divergences (with or without any "refugia" scenario) are not known (e.g., hybridization or other selective forces within a given population).

Finally, because the extent or direction of any introgression could not be established with certainty, more sensitive biochemical assays of rapidly evolving parts of the genome (i.e., mitochondrial DNA: see Avise et al., 1987) will be necessary to infer the population genetic interactions of these types of venom. Both Phoenix and Tucson are on the edge of the venom B range and near the middle of the intergrade zone, where many heavily traveled roads intersect the region. This and many other human activities create unquestionable barriers of high mortality to the snakes, and the influence of humans on these populations could eclipse their evolutionary outcome.

Acknowledgments.—We thank C. Schwalbe and T. Johnson, Arizona Game and Fish Department, for the Arizona collecting permits and for providing valuable collecting advice. We also thank R. Babb, K. Aitkin, L. Simons, and L. Nienaber of Arizona State University for field assistance. H. McCrystal and H. Lawler of the Arizona Sonoran Desert Museum, Tucson, Arizona; B. Tomberlan of Portal, Arizona; and A. Wilkinson, Phoenix, Arizona, also provided valuable collecting assistance. We would also like to thank R. Jaeger, H. Shaffer, R. Murphy, J. Cadle, and an anonymous reviewer for their comments regarding this manuscript. This study was partially supported

by the Associate Students of Brigham Young University grants for research, BYU Zoology Department research awards, and the Venom Research Laboratory, Veterans Administration Medical Center, Salt Lake City, Utah.

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Accepted: 3 July 1990
Associate Editor: H. Bradley Shaffer

APPENDIX I

Localities of Specimens

Localities of specimens of *Crotalus s. scutulatus* and *C. atrox* used in this study (numbers correspond to those in Table 2 and Fig. 1) are given below.

Crotalus s. scutulatus.—Cochise Co.: #3 = BYU-39477, 1.6 km S of Portal on Stateline Rd.; #13 = BYU-39485, 0.8 km W of AZ 80, 0.8 km N of Rueker Canyon Rd.; #18 = BYU-39490, outside of Bowie on Bowie Rd.; #19 = BYU-39488, outside of Bowie on Bowie Rd.; #21 = BYU-39491, outside of Bowie on Bowie Rd.; #25 = BYU-39493, outside of Willcox; #26 = BYU-39494, 16 km S of San Simon on Portal Rd.; #28 = BYU-39496, 31.4 km N of Turkey Creek Rd., AZ 186 junct. on AZ 186; #48 = BYU-39514, 1.2 km N of I-10, AZ 666 junct. on W fork of AZ 666; #49 = BYU-39515, 1.9 km N of I-10, AZ 666 junct. on W fork of AZ 666; #54 = BYU-39519, 8 km SE of Willcox on AZ 186; #55 = BYU-39520, 14.5 km SE of Willcox on AZ 186; #56 = BYU-39521, 3.2 km W of Bowie on I-10.

Graham Co.: #50, 32.8 km N of I-10, AZ 666 junct. on AZ 666.

Maricopa Co.: #1 = BYU-39475, 4.7 km N of AZ 74 on Lake Pleasant Rd.; #2 = BYU-39476, 27.4 km E of Maricopa on E/W Maricopa Rd.; #4 = BYU-39478, 58 km W of Maricopa on E/W Maricopa Rd.; #5 = BYU-39479, 58 km W of Maricopa on E/W Maricopa Rd.; #6 = BYU-39480, Gila Bend junct. on E/W Maricopa Rd.; #7 = BYU-39481, 29 km S of Gila Bend on AZ 85; #8 = BYU-39482, 38.6 km S of Gila Bend on AZ 85; #17, 45 km N of Maricopa on N/S Maricopa Rd.; #20 = BYU-39489, Vekol Valley at eastern foot hills of Sand Tank Mts.; #24 = BYU-39492, 15.8 km W of Rio Verde Rd., Forest Rd. junct. on Rio Verde Rd.; #30 = BYU-39498, 1.5 km from junct. of McDowell Mt. Rd., McDowell Mt Park Dr. on McDowell Mt. Rd.; #31 = BYU-39499, 0.5 km W of Forest Rd., Rio Verde Dr. junct. on Rio Verde Dr.; #32 = BYU-39500, 10.3 km W of Rio Verde Dr., Forest Rd. junct. on Rio Verde Dr.; #33 = BYU-39501, 4.7 km W of Rio Verde Dr., Forest Rd. junct. on Rio Verde Rd.; #34 = BYU-39502, 4.2 km W of Scottsdale on Pinnacle Peak Rd.; #35, 0.1 km W of Rio Verde Dr., Forest Rd. junct. on Rio Verde Dr.; #36 = BYU-39503, 8.4 km W of Rio Verde Dr., Forest Rd. junct. on Rio Verde Dr.; #37 = BYU-

39504, 7.4 km W of Rio Verde Dr., Forest Rd. junct. on Rio Verde Dr.; #38 = BYU-39505, 13.5 km E of Rio Verde Dr., Happy Valley Rd. junct. on Rio Verde Dr.; #47 = BYU-39513, 0.8 km S of AZ 60, Vulture Mine Rd. junct. on Vulture Mine Rd.; #57 = BYU-39522, 53 km N of Oracle, AZ 89 junct. on AZ 89; #59 = BYU-39524, 3.5 mi W of Rio Verde Dr., Forest Rd. junct. on Rio Verde Dr.; #60 = BYU-39525, AZ 74, AZ 93 junct.; #61 = BYU-39526, AZ 74, AZ 93 junct.; #66 = BYU-39530, AZ 74, AZ 93 junct.; #72 = BYU-39535, 12.9 km S of I-10 on Salome Hwy.; #73 = BYU-39657, 12.9 km S of I-10 on Salome Hwy.

Pima Co.: #9, 64 km S of Gila Bend on AZ 85; #27 = BYU-39495, Hilltop Rd., Serrietta Rd. junct.; #29 = BYU-39497, Oracle, Coronado Ntnl. Forest; #39 = BYU-39506, 1.6 km W of Mile Wide Rd., Sandereo Rd. junct. on Mile Wide Rd.; #40, 13.5 km S of Mile Wide Rd., Sandereo Rd. junct. on Sandereo Rd.; #41 = BYU-39507, 2 km W of Mile Wide Rd., Sandereo Rd. junct. on Mile Wide Rd.; #42 = BYU-39508, 9.8 km N of Serrietta Rd., Diamond Bell Ranch Rd. junct. on Serrietta Rd.; #43 = BYU-39509, 25.4 km S of Sasabe Rd., Ajo Way junct. on Sasabe Rd.; #44 = BYU-39510, 10.9 km S of Sasabe Rd., Ajo Way junct. on Sasabe Rd.; #45 = BYU-39511, 13.5 km S of Sasabe Rd., Ajo Way junct. on Sasabe Rd.; #46 = BYU-39512, 14.6 km N of AZ 83, AZ 82 junct. on AZ 83; #67 = BYU-39531, 12.7 km S of Ajo Way, Sasabe Rd. junct. on Sasabe Rd.; #68 = BYU-39532, 24.8 km S of Ajo Way, Sasabe Rd. junct. on Sasabe Rd.; #69 = BYU-39533, 24.3 km S of Ajo Way, Sasabe Rd. junct. on Sasabe Rd.; #70, 16 km S of Ajo Way, Sasabe Rd. junct. on Sasabe Rd.; #76, 8 km S of Mile Wide Rd., Sandereo Rd. junct. on Sandereo Rd.

Pinal Co.: #11, 6.3 km N of Maricopa on N/S Maricopa Rd.; #12 = BYU-39484, 10.3 km W of Casa Grande on E/W Maricopa Rd.; #14, 25.1 km E of Maricopa on E/W Maricopa Rd.; #15 = BYU-39486, 20.7 km E of Maricopa on E/W Maricopa Rd.; #16 = BYU-39487, 19.3 km E of Maricopa on E/W Maricopa Rd.; #71 = BYU-39534, 35.4 km E of Gila Bend on E/W Maricopa Rd.

Yavapai Co.: #10, 26.7 km N of AZ 74, AZ 93 junct. on AZ 93; #51 = BYU-39516, 12.4 km S of AZ 93, AZ 71 junct. on AZ 93; #52 = BYU-39517, 6.3 km S of AZ 93, AZ 71 junct. on AZ 93; #53 = BYU-39518, 4.2 km E of AZ 93, AZ 71 junct. on AZ 71; #58 = BYU-39523, 6.1 km E of AZ 93, AZ 71 junct. on AZ 71; #62 = BYU-39527, 53 km E of AZ 97, AZ 96 junct. on AZ 96; #63 = BYU-39528, 51.3 km E of AZ 97, AZ 96 junct. on AZ 96; #64, 37.7 km N of AZ 93, AZ 71 junct. on AZ 93; #65 = BYU-39529, 3 km E of AZ 93, AZ 71 junct. on AZ 71.

Crotalus atrox.—#22 = BYU-39660, outside of Bowie on Bowie Rd.; #23 = BYU-39661, 1.6 km S of Bowie on Bowie Rd.