ACTA ZOOL. MEX. (*ns*). 9. 1985

GENETIC RELATIONSHIPS OF THE SANTA CATALINA ISLAND RATTLELESS RATTLESNAKE, *Crotalus catalinensis* (SERPENTES: VIPERIDAE)

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ABSTRACT

The phylogenetic relationships of the Santa Catalina Island rattleless rattlesnake, *Crotalus catalinensis*, are investigated using allozyme data from 27 presumptive gene loci. These data indicate that *C. catalinensis* shared a most recent common ancestor with *C. ruber*, and suggest that convergence in morphological characteristics has resulted in conflicting hypotheses of phylogenetic affinities. Analysis of rates of allozyme evolution in other reptilian taxa indicates that *C. catalinensis* is of more recent origin than most other reptilian species on Isla Santa Catalina.

RESUMEN

Crotalus catalinensis es una especie de ofidio que habita solamente en la Isla Santa Catalina, B.C. Norte, México. Morfológicamente se distingue de las otras especies de *Crotalus* por la ausencia de cascabel en la cola.

Las relaciones evolutivas de *C. catalinensis* han sido objeto de controversia, ya que se le ha considerado afin con *C. atrox*, *C. ruber* y *C. scutulatus* En este trabajo se investigaron las relaciones filogenéticas de *C. catalinensis* por medio de información proveniente de aloenzimas de 27 loci genéticos presupuestos. Se encontró que *C. catalinensis* es una especie hermana de *C. ruber*, estando ligadas ambas especies por 3 estados de carácater derivados-compartidos: Acp - 1 (ab), Cat-A (ab) y S-Sod-A (ac). Estos resultados no apoyan hipótesis previas sobre las relaciones filogenéticas de la especie sustentadas en la similitud morfológica entre *C. catalinensis* y *C. scutulatus*, la

cual probablemente se debe a convergencia o a la retención de estados de carácter primitivos.

En comparación con el resto de la especies de reptiles de la Isla Santa Catalina, *C. catalinensis* parece tener un origen más reciente De acuerdo con el cálculo de divergencia genética basado en el "reloj bioquímico" es muy probable que la colonización de la isla por el ancestro de *C catalinensis* haya sido posterior a la de las demás especies, o bien que para *C catalinensis* el flujo genético entre la península de Baja California y la isla haya sido más prolongado, interrumpiéndose hace aproximadamente un millón de años.

INTRODUCTION

Crotalus catalinensis Cliff, a rattleless species of rattlesnake, is restricted in distribution to a single island, Isla Santa Catalina, in the Gulf of California, Mexico. The phylogenetic relationship of this species to other rattlesnakes has been debated. Klauber (1956), in the first edition of his monograph on the rattlesnakes, depicted *C. catalinensis* as being most closely related to *C. ruber*, the red diamond rattlesnake; justification for this belief was not detailed, possibly because *C. catalinensis* was only recently described. Brattstrom (1964) also believed *C. catalinensis* to be most closely related to *C. ruber.* Although skeletal material had not been examined, he believed the two species "so close on the basis of external characters that the osteology probably does not differ greatly" (Brattstrom, 1964:244).

In the second edition of his monograph, Klauber disclosed a change of opinion; he believed *C. catalinensis* to be most closely related to *C. scutulatus,* the Mojave rattlesnake, based on morphological similarities including head scales and coloration of diamond patterns, tail rings and rattle (Klauber, 1972:33). However, this belief was apparently not without some skepticism. He stated *C. catalinensis* to be "an obvious derivative of *C. atrox*" (the western diamondback rattlesnake) followed in the next paragraph by "its closest relative seems to be *C. scutulatus*" (Klauber, 1972:166). Nevertheless, we believe his preferred hypothesis to be *C. catalinensis* and *C. scutulatus* sharing the most recent common ancestor because of detailed notations (e.g., Klauber, 1972:33, 166) and depiction (Klauber, 1972:168).

Consideration of *C. catalinensis* and *C. scutulatus* as sister species results in a significant paleobiogeographic problem.

The rattleless rattlesnake occurs on a small island some 700 km south of the nearest contact with the range of the Mojave rattlesnake [the head of the Gulf of California at Bahia San Jorge, Sonora, Mexico (Klauber, 1972)]. How did the original island colonist(s) arrive on Isla Santa Catalina? Although the swimming ability of rattlesnakes is well documented (see Klauber, 1972), is a trans-Gulf of California dispersal of 700 km likely? Alternatively, was the Mojave rattlesnake previously distributed further south into Baja California or Sinaloa, Mexico, diminishing the required dispersal distance? These questions result from Klauber's belief that *C. catalinensis* and *C. scutulatus* shared a common ancestor–a belief based on overall morphological similarity, and not on the presence of shared derived character states. However, unless rates of change have been equal in all lineages, overall similarity will not reflect phylogenetic relationships. Because equal rates of morphological change have not been demonstrated, it is possible that *C. catalinensis* is phylogenetically more closely related to a species which is morphologically less similar.

While investigating the paleobiogeography of the Baja California herpetofauna, Murphy (1983a, 1983b) studied patterns of genetic (allozyme) differentiation between a broad variety of reptiles. He found *C. catalinensis* to be biochemically most similar to *C. ruber* (Murphy, 1983a, Table 11) and, at the same time, easily distinguishable from both *C. atrox* and *C. scutulatus* (unpublished data). Subsequent to Murphy's initial investigation, we examined additional specimens and have analyzed these data using a cladistic method, encoding the data as characters and states. We report our findings herein.

MATERIALS AND METHODS

Specimens of *C. catalinensis, C. ruber, C. atrox,* and *C. scutulatus* constituted our taxonomic in-group (TIG; *sensu* Watrous and Wheeler, 1981). Widely-distributed species of our TIG are represented by individuals from various parts of their range (Appendix 1). Our taxonomic out-group (TOG) included both *C. viridis* and *C. mitchellii*. [A cladistic analysis of allozyme data from preliminary investigations supports the above arrangement of taxa within our TIG and TOG (Murphy, unpublished data); seventeen species of *Crotalus* and one species of *Sistrurus* (the out-group) were investigated.]

Most specimens were sacrificed in the field shortly after capture by injection of sodium pentabarbitol. Heart and skeletal muscle, liver, and kidney tissues were dissected and initially maintained in liquid nitrogen with subsequent laboratory storage at $-42^{\circ}C$. Some specimens were taken into the laboratory alive, then sacrificed by freezing. Tissue samples were partially thawed, minced with scissors, diluted with an approximately equal volume of deionized water, and mechanically homogenized. Tissue homogenates were refrozen for 16 h, then thawed and centrifuged at 31,000 *g* for 20 min. at $2^{\circ}C$. Supernatant fractions were used for the electrophoretic analysis.

Enzymatic and non-enzymatic presumptive gene products were separated via horizontal starch gel electrophoresis (Selander *et al.*, 1971; Yang *et al.*, 1974) using a combination of 11.2% Electrostarch (lot No. 392; Madison, Wisconsin) and 2.8% Connaught starch (Ontario, Canada). Enzyme nomenclature follows the recommendations of the Nomenclature Committee of the International Union of Biochemistry (1979), and nomenclature of the presumptive gene loci follows Murphy and Crabtree (1985). Relative mobilities of gene products were scored from extracts of specific tissues using established histochemical staining procedures and multiple buffer systems as given in Crabtree and Murphy (1984). Allozyme data were treated as products of codominant alleles at a given gene locus; electromorphs sharing common electrophoretic mobility were scored as homologous allelic products of a given locus. From allelic frequencies (Table 1), genetic similarity (*I*) and genetic distance (*D*) coefficients were calculated (Nei, 1972) and compared with those of other reptilian species groups.

Phylogenetic evaluation of the allelic data is based on the out-group comparison method of observed character state distributions (Watrous and Wheeler, 1981) as clarified for allozyme analyses by Murphy *et al.* (1983). Here we add that total allelic composition should be considered the character state, while the locus represents the character. This procedure for hypothesizing the evolutionary polarity of allelic character states is similar to that of Patton and Avise (1983). However, in transforming their allelic data to characters and states, the latter authors considered the alleles to be characters, and presence or absence as states (see Michevich and Johnson, 1976). We concur with Michevich and Mitter (1981) in considering such "independant allele" models of data coding to be inappropriate.

RESULTS AND DISCUSSION

The products of 27 presumptive gene loci were resolved for the four species of our TIG and one species of our TOG, *C. viridis*, before exhausting available tissues of *C. catalinensis;* the products of 25 loci were resolved in the other species of our TOG, *C. mitchellii* (Table 1). Ten loci were found to be monoallelic for all specimens examined, these included Adh-A, Dlr-A, Gpi-A, M-Icdh-A, Ldh-A, Ldh-B, Dip-1, Dip-2, Dip-4, and M-Sod-A. Intraspecific comparison of widely distributed species in our TIG, *C. atrox* and *C. scutulatus,* failed to show unique alleles between specimens from the central Chihuahuan Desert of Mexico and those from southern California. Consequently, all individuals within these species were considered to represent a single taxonomic unit for purpose of data analysis. (Based on the small sample sizes used in this analysis, we cannot be certain that no statistically significant differences occur between these distantly distributed populations at the polymorphic loci surveyed.)

Significant allelic differences probably occur between the subspecies *C. v. viridis* from Montana, and *C. v. helleri* from southern California. Preliminary results from our ongoing electrophoretic investigations of rattlesnake phylogenetics indicate these two populations may have fixed allelic differences at three of 52 loci surveyed; however, again our sample sizes are too small to statistically test for significant differences. We note these problems because of our use of one specimen each of *C. viridis* from southern California and Montana in our out-group. For purposes of out-group comparison as applied herein, these allelic differences are inconsequential; we did not investigate the relationships of the species in our TOG. However, estimations of genic polymorphism and genetic similarity (*I*) should not be made for *C. viridis* from our allele frequency data (Table 1).

Estimates of allozyme variability within the four species of our TIG are given in Table 2. We believe sample sizes to be adequate for estimating allozyme variability, based on the criteria of Gorman and Renzi (1979), Nei (1978), and Nei and Roychoudhury (1974). Although *C. scutulatus* appears far more variable than *C. catalinensis, C. ruber,* and *C. atrox,* the estimates are not unlike those observed in Montana populations of *C. viridis* (unpublished data) and those reported

Table 1.
Allele frequencies at 17 polymorphic loci in 6 species of rattlesnakes, genus *Crotalus* (and sample sizes). NR = not resolved

Locus	*C. catalinensis* (4)	*C. ruber* (19)	*C. atrox* (9)	*C. scutulatus* (7)	*C. viridis* (2)	*C. mitchellii* (1)
Acp-1	a 0.50 b 0.50	a 0.83 b 0.17	b	b	b	NR
Cat-A	a 0.38 b 0.62	a 0.10 b 0.90	b	b	b	b
Dip-3	c	c	c	b 0.17 c 0.83	a 0.50 b 0.50	c
Est-1	b	b	b	a 0.93 b 0.07	a	b
Est-2	b	c	a 0.50 c 0.50	c 0.21 d 0.21 e 0.58	c 0.50 e 0.50	e
Fum-A	b	b	a	a	a 0.25 b 0.75	b
Gcdh-A	a	a	a	a	a	b
Iddh-A	a	a	a	b	b	b
S-Icdh-A	a	a	a	a 0.72 b 0.28	a	a
M-Mdh-A	b	b	a 0.06 b 0.94	a 0.14 b 0.86	b 0.50 c 0.50	b
S-Mdh-A	a	a	a 0.94 b 0.06	a	a	a
Me-1	b	a 0.04 b 0.96	b	b	b	NR
Me-2	b	a 0.08 b 0.92	b	a 0.10 b 0.90	b	b
Mpi-A	b	b	b	a 0.50 b 0.50	b 0.50 c 0.50	c
Pgm-A	b	b	b	a	b 0.50 c 0.50	c
S-Sod-A	a 0.12 c 0.88	a 0.03 c 0.97	b	c	c	c
Xdh-A	a	a	a	a	a	b

for leafnose snakes *(Phyllorhynchus decurtatus)* from Baja California, México (Murphy and Ottley, 1980). These estimates of relatively low levels of variability provide confidence that our analysis will not be significantly altered by effects of sample size. However, we caution that general comparisons of our variability estimates may be somewhat misleading. Various investigations usually survey different subsets of presumptive gene loci. The proportion of "rapidly-evolving" proteins (*sensu* Sarich, 1977), including esterases, some peptidases, and many general non-enzymatic proteins, vary between studies. These presumptive gene loci often contribute significantly to estimates of variability; estimates of genic variation may be affected more by choice and number of loci, and not so much by small samples.

Table 2.
Estimates of overall genic variability in sampled populations of *Crotalus*.

Species	*n*	Frequency of minor allele detection	% loci polymorphic*	Alleles per locus	% heterozygosity per individual**
C. catalinensis	4	0.125	11.1	1.11	2.0
C. ruber	19	0.026	18.5	1.19	1.8
C. atrox	9	0.056	11.1	1.11	3.1
C. scutulatus	7	0.071	25.9	1.30	5.5

* A locus was considered polymorphic if more than one allele was detected
** Based on the number of heterozygotes

Examination of overall genetic similarity (*I*) and genetic distance (*D*) (Table 3) shows *C. catalinensis* and *C. ruber* to be the two most similar species. These, in turn, are more similar to *C. atrox* than to *C. scutulatus*. Finally, *C. atrox* and *C. scutulatus* are distinguished by a value essentially equivalent to that observed between *C. scutulatus* and either *C. ruber* or *C. catalinensis*. Compared with other reptile species differentiations, *C. catalinensis* is very similar to *C. ruber;* most sister species

of lizards and snakes are separated by *I* values of about 0.8 or less (Adest, 1977; Avise and Ayala, 1976; Murphy, 1983a, Murphy *et al.*, 1983).

Table 3.
Matrix of Nei's (1972) genetic similarity *(I)* genetic distance *(D)* values for rattlesnakes of the genus *Crotalus* included in the taxonomic in-group. *I* values are to the right of the diagonal, *D*'s are left. Values of *D* are given ± one standard error

	C. catalinensis	*C. ruber*	*C. atrox*	*C. scutulatus*
C. catalinensis	—	0.954	0.883	0.753
C. ruber	0.047 ±0.042	—	0.888	0.753
C. atrox	0.124 ±0.070	0.118 ±0.068	—	0.788
C. scutulatus	0.283 ±0.110	0.284 ±0.110	0.238 ±0.100	

Although useful in summarizing patterns of allelic differentiation, and perhaps in providing guidelines for approaches to phylogenetic analysis, it may not be possible to use *I* and *D* values to construct phylogenetic hypotheses. Some controversy exists over the ability to "phylogenetically" analyze (cluster) distance coefficients. Farris (1981) believed that it may not be possible to cluster *I* and *D* coefficients phylogenetically, or any non-metric "distance" measure for that matter. Felsenstein (1984) believed that distance methods could be used if branch lengths were taken to be "expected distances" rather than path lengths. However, under his model two assumptions should be met: (1) additivity of distance along the (assumed) true tree, and (2) independence of statistical errors of distance measurements. He noted, however, that "these assumptions are dubious for many kinds of data often analyzed by distance methods" (Felsenstein, 1984:23). Unfortunately, these "kinds" of data include distance estimates calculated from allozyme frequencies;

Felsenstein (1984:20) notes that the distance measures of both Nei (1972) and Rogers (1972) may change nonlinearly with time–they may violate the assumption of additivity. Thus, we choose not to use these coefficients to hypothesize that *C. catalinensis* and *C. ruber* shared a most recent common ancestor. Because allozyme data are particulate, they may be divided into discrete characters and states. This attribute of allozyme data allows the application of phylogenetic methods of data analysis which seem to be free of the problems associated with clustering using distance measures. To hypothesize confidently that *C. catalinensis* and *C. ruber* shared a most recent common ancestor, it is necessary to demonstrate that synapomorphic character states unite them.

The out-group comparison method of character analysis produces three synapomorphic character states uniting *C. catalinensis* and *C. ruber:* Acp-1(ab), Cat-A(ab), and S-Sod-A(ac) (Table 1). In all three loci, the derived allele [e.g. Acp-1(a)] occurs with the plesiomorphic allele [Acp-1(b)]. Alternatively, Me-2(a) appears synapomorphic uniting *C. ruber* with *C. scutulatus* while excluding *C. catalinensis*. Me-2(a) is considered derived because it was not observed in the outgroup. However, Crabtree and Murphy (1984) report three alleles at Me-2 in Montana *C. viridis*. It is possible that one of these alternative alleles is homologous with the relatively rare Me-2(a) allele observed in both *C. ruber* (frequency 0.08) and *C. scutulatus* (frequency = 0.10). Because Me-2(a) is infrequently observed, it is not surprising that this rare allele, if present, was not observed in *C. viridis* (N = 2) or *C. catalinensis* (N = 4). Notwithstanding, consideration of *C. ruber* and *C. catalinenisis* as sister species is the more parsimonious hypothesis involving fewer homoplasious transformations.

Greater confidence in the hypothesis that *C. catalinensis* and *C. ruber* shared a most recent common ancestor would exist if the derived alleles were fixed in both populations. However, this allelic distribution was not observed. Additionally, demonstration of synapomorphic or autapomorphic character states in species exclusive of *C. catalinensis* and *C. ruber* would add greater confidence to the phylogenetic hypothesis. From Table 1 we deduce that three autapomorphic alleles occur in both *C. atrox* [S-Mdh-A(b); S-Sod-A(b); and Est-2(a)] and *C. scutulatus* [Pgm-A(a); Mpi-A(a), and S-Icdh-A(b)]. It does not seem likely that *C. catalinensis* shared the most recent common ancestor with either *C. atrox* or *C. scutulatus,* especially considering the occurrence of only a

single autapomorphic allele in both *C. ruber* [Me-1(a)] and *C. catalinensis* [Est-2(b)].

The hypothesis that *C. ruber* and *C. catalinensis* are sister species is contradictory to Klauber's (1972) conclusions. Klauber believed *C. scutulatus* and *C. catalinensis* to be more closely related because of similarities in color pattern and, perhaps more significantly, reduction in number of head scales. Many morphological characteristics, including number of head scales and particularly color patterns, vary along a continuum within populations, and overlap with those found within other populations and related species. Overall, there exists a relatively broad range of morphological variation from which the majority of individuals in a population may express one end of the cline. In the rattlesnakes, the morphological traits uniting *C. catalinensis* and *C. scutulatus* occur in other related species of *Crotalus*, including *C. ruber* (Klauber, 1972). Unlike continuously-measured morphometric characteristics, allozymes occur as units–discrete entities exhibiting only discrete alternative units as variation. [Note that the presence or absence of specific alleles is differentiated from the frequency of occurrence of alleles within a population. For a detailed discussion of the latter situation relative to metric differences see Lewontin (1984).] In *Crotalus*, and other reptilian species, the observed number of intralocus allelic variants appears to be less than that of most morphological quantitative characters, such as numbers of head scales or ventral scutes. Qualitative morphometric data (e.g., color and pattern, and proportional measurements) usually provide even greater variation. Both intrapopulation (and interpopulation) allelic alternatives (variants of a character) are not observed to be as numerous as variants observed for scale, color and other morphological characters. Infrequently, three alleles at a specific locus are observed within a population, and, rarely, four or more alternative, intrademic alleles. We believe convergence in color and scale patterns to be far more likely than convergence in allozymes. Based on the observed variability in morphological features, we believe the data more strongly argue for the hypothesis that *C. catalinensis* shared a most recent common ancestor with *C. ruber*. The overall morphological similarity between *C. scutulatus* and *C. catalinensis* likely reflects either convergence or the retention of primitive character states. Significantly, this is another situation in Baja California reptiles where morphological similarity does not reflect phylogenetic relationships (see Murphy, 1983a).

Aside from providing insights into the phylogenetic relationships of the rattleless rattlesnake, our allozyme data allow formation of a phylogenetic hypothesis for the remaining species in our TIG *inter se*. Using the out-group criterion of character state analysis, synapomorphic alleles uniting *C. atrox* and *C. scutulatus* [M-Mdh-A(a)], and linking *C. atrox* with *C. ruber* and *C. catalinensis* [Iddh-A(a)], are observed (Table 1). Preference of one of these two hypotheses over the other is uncertain because both result in the addition of a single homoplasious step. However, if intralocus variability is used as a criterion for choosing between the synapomorphies, then the hypothesis uniting *C. atrox* with *C. ruber* and *C. catalinensis* is preferable. We observed no intrapopulation variation at Iddh-A as we did at M-Mdh-A (Table 1). Significantly, the choice of either alternative evolutionary hypothesis does not disrupt the proposal that *C. catalinensis* and *C. ruber* shared a most recent common ancestor.

Paleobiogeographic considerations

Allozyme data may also be valuable for paleobiogeographic studies in providing estimation of dates of speciation events when used in conjunction with other species pairs. Our data are relevant to such a study on the paleobiogeography of the Baja California herpetofauna. Murphy (1983a) reported *I* values for *C. catalinensis* based on the evaluation of 36 presumptive gene loci. However, because of tissue age and depletion we were subsequently able to confidently resolve gene products at only 27 of the 36 presumptive gene loci.

Before using biochemical data to make a prediction of the geologic age of the specification event, it is first necessary to demonstrate equivalency in rates of allozyme evolution between species pairs. Our method of determining relative rates of allozyme evolution, follows Rosen and Buth (1980:302). The out-group comparison method is used to reconstruct the most likely genotype of the hypothetical TIG ancestor. Genetic similarly coefficients (*I*) are then calculated for all extant species-hypothetical ancestor pairs. The calculation of similar *I* values allows the proposal that relative rates of allelic substitution from the common ancestor have been essentially equal, as assumed by Nei (1972).

Along with monoallelic loci, the character states at Gcdh-A and Xdh-A were considered plesiomorphic. Polymorphiyc plesiomorphic character states were hypothesized to be Est-1(ab), Est-2(de), Fum-A(ab), M-Mdh-A(bc), and Dip-3(abc); the alternative plesiomorphic alleles at each locus were arbitrarily assigned equal frequencies of occurrence. Genetic similarity coefficients between each species of our TIG and the hypothetical ancestor were calculated and follow: *C. catalinensis*, $I = 0.87$; *C. ruber*, $I = 0.86$; *C. atrox*, $I = 0.86$; and *C. scutulatus*, $I = 0.91$. For the species pairs considered in this paleobiogeographic analysis, including *C. catalinensis* and *C. ruber*, and *C. ruber* and *C. atrox*, rates of allelic substitution appear to have been essentially equal.

In the paleobiogeographic scenario for the Baja California herpetofauna, *C. atrox* and *C. ruber* are thought to have speciated as a result of the Pliocene formation of the San Gorgonio Barrier–a seaway or mesic filter barrier at the head of the Peninsular Ranges (Murphy, 1983a). Several other xerophilic species pairs are thought to have been formed at the same time, including: collared lizards, *Crotaphytus insularis* and *C. collaris*; spiny lizards, *Sceloporus magister* and *S. rufidorsum;* and rattlesnakes, *Crotalus mitchellii* and *C. tigris*. *I* values for the first two species pairs were reported as $I = 0.85$ (calculated from data of Montanucci *et al.*, 1975) and $I = 0.75$, respectively. No allozyme data are available for the other rattlesnake species pair. The genetic similarity between *C. ruber* and *C. atrox* ($I = 0.89$) is slightly greater than anticipated ($I = 0.80$).

The *I* value for *C. ruber* and *C. catalinensis* ($I = 0.95$) is higher than the average *I* value calculated between insular and peninsular sister species ($I_{\bar{x}} = 0.86$) for Isla Santa Catalina. Although the ancestor of *C. catalinensis* is thought to have arrived by overwater dispersal (Klauber, 1972; Murphy, 1983a), the discrepancy between observed and expected *I* values for the *C. catalinensis* and *C. ruber* comparison could result from (1) a slower rate of genetic divergence (= allelic substitution) relative to most other reptilian species, (2) a relatively more recent disruption of gene flow, or (3) a more recent colonization event; the latter two scenarios cannot be differentiated in the absence of fossil data. (Because relatively equal rates of change have been demonstrated, the possibility of unequal rates of evolution among our rattlesnake species is unlikely.)

An application of the "biochemical clock" allows

further evaluation of the paleobiogeographic scenarios. The biochemical clock can be applied to the rattlesnake data by recalibrating such that the anticipated divergence time for *C. catalinensis* is essentially equivalent to that of other Santa Catalina island species. This results in a *D* of 1 being roughly equivalent to 41.4 million years (MY) of divergence. A similar calculation for the *C. ruber–C. atrox* species pairs estimates a *D* of 1 equal to 18.9 MY. Because this latter estimation is far more concordant with the previous calibration of genetic distance values for other Baja California reptiles (Murphy, 1983a), it seems more likely that either the progenator of *C. catalinensis* colonized Isla Santa Catalina after most other populations became established, or that gene flow between the peninsula and the island continued for a relatively longer period of time being disrupted about 1 MY ago. Considering the swimming ability of rattlesnakes (Klauber, 1972), we cannot confidently choose between these latter two scenarios.

ACKNOWLEDGMENTS

Many of the tissue samples used in this study were collected by the support and under the auspicies of the Instituto de Ecologia, Mexico City, Mexico. For field assistance and donation of specimens, we thank D. Adest, G. Adest, G. Aguirre L., R. Ayrey, J. Cram, A. Eisner, C. Lieb, D. Morafka, and particularly J. Ottley. M. Miyamoto (University of Miami) dissected many specimens and supplied tissues. T. Vaughn and family provided transportation to Isla Santa Catalina. G. Aguirre L. prepared the Spanish resumen. This study was supported by the National Science Foundation Grant DEB 77-03259 to George C. Gorman, the National Institute of Health Grant NIH RRO8156-08 to R. W. Murphy and D. J. Morafka, the Biomedical Research Support Grant and UCLA Committee on Research Grant U.R. 3674 to D.G. Buth, and by the UCLA Fisheries Program of the Department of Biology, UCLA through the generosity of D.G. Buth. D.G. Buth made his laboratory facilities available to us and, along with D.J. Morafka and G.A. Adest, provided valued criticism. All specimens were collected by permission from the Direccion General de la Fauna Silvestre, and appropriate U.S. State agencies, including Arizona, California, Montana, and Texas.

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APPENDIX I

Most voucher specimens are deposited, or are currently being maintained, in the herpetological collections of M. L. Bean Museum at Brigham Young University, Provo, Utah (BYU), the California Academy of Sciences, San Francisco (CAS), the Instituto de Ecología, Mexico City, Mexico (IE; currently maintained at California State University at Dominguez Hills, Carson, California 90747 U.S.A.), and the R.W. Murphy frozen collection (RWM, CBC; currently maintained at California State University at Dominguez Hills). In addition, some specimens were returned to the Direccion General de la Fauna Silvestre in Mexico City (FS-RWM).

Crotalus atrox (9): Mexico, Durango (IE 5236, 5442, 5526, 5557), Chihuahua (IE 5502), Zacatecas (IE 5608); USA, Arizona (IE 5500), California (RWM 2130, and voucher retained by W. Mautz). *C. catalinensis* (4): Mexico, Gulf of California (BYU 34614-15, 34642-43). *C. mitchellii* (1): USA, California (RWM 2133). *C. ruber* (19): Mexico, Baja California Sur (CAS 147685; BYU 34589, 34592, 34594, 34624, 24626, 24628-29, 24630, 34653, 34759, 34963, 34966; FS-RWM 979; no voucher-1); Baja California Norte (FS-RWM 547, 1842); USA, California (RWM 2156, 2132). *C. scutulatus* (7): Mexico, Chihuahua (IE 5501), Coahuila (IE 5521-22); USA, Arizona (IE 5497), California (RWM 1966), Texas (RWM 2154-55). *C. viridis* (2): USA, California (RWM 2133), Montana (CBC 82-20).