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MITOCHONDRIAL-DNA ANALYSES AND THE ORIGIN AND RELATIVE AGE OF PARTHENOGENETIC LIZARDS (GENUS *CNEMIDOPHORUS*). IV. NINE *SEXLINEATUS*-GROUP UNISEXUALS

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Abstract.—Mitochondrial DNAs (mtDNAs) from nine morphologically distinct unisexual species and five bisexual species of lizards, all from the *sexlineatus* species-group of *Cnemidophorus*, were compared using restriction endonucleases. The unisexual lizards have mtDNAs that are identical at all or nearly all of the 128 sites cleaved. Although differing little in sequence, some mtDNAs differed in size due to the presence of tandem sequence duplications. Phylogenetic analysis of cleavage maps indicates that the mtDNAs of the unisexuals are most similar to that of the bisexual species *C. inornatus*. Considerable mtDNA diversity exists among *C. inornatus* populations, and one geographically restricted subspecies, *C. i. arizonae*, was identified as the most probable maternal ancestor of all nine unisexuals. All but one of these are triploid, and all have at least one *C. inornatus* gene complement. This, together with the homogeneity of their mtDNAs, suggests that all stem from one or a small number of allodiploid females (presumably parthenogenetic) that originated in a restricted geographic area in the recent past. These data, when combined with those from allozyme studies, preclude the possibility that most of the triploid unisexuals could have arisen via fertilization of an unreduced diploid ovum from one species by a haploid sperm from a different species.

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Among vertebrates, parthenogenetic (all-female) reproduction is rare and is strongly correlated with hybridization. The hypothesis that vertebrate parthenogenesis is a direct consequence of interspecific hybridization arose out of this correlation (Lowe and Wright, 1966; reviewed by Cole [1975], Dessauer and Cole [1989], and Moritz et al. [1989*a*]) but has been opposed on theoretical grounds (Cuellar, 1974, 1977, 1986; Darevsky et al., 1985). Unisexual species of whiptail lizards (genus *Cnemidophorus*) exemplify the hybrid origin of parthenogenesis. These comprise one-third of the approximately 50 species in the genus and are associated with each of its six karyologically defined species groups (Lowe et al., 1970). By various combinations of karyotypic, biochemical, and morphological criteria, all of the unisexual species appear to be hybrid in origin (see reviews listed above; Wright, 1978; Cole, 1985; Dessauer and Cole, 1986; Walker, 1986; Cole et al., 1988).

The *sexlineatus* group of *Cnemidophorus* includes both bisexual and unisexual species. Chromosomal studies of *sexlineatus*-group unisexual species revealed that some are diploid (Bickham et al., 1976), that others are triploid (Pennock, 1965; Lowe and Wright, 1966; Cole, 1979), and that all of the chromosome sets in the unisexual species are derived from bisexual species in the *sexlineatus* group (Lowe et al., 1970). However, because of their karyotypic uniformity, the identity of the bisexual species involved in the genesis of the unisexuals cannot be determined by chromosomal analyses alone (Neaves, 1969; Wright, 1978). The hybrid ancestry of most *sexlineatus*-group unisexual species has been resolved by allozyme comparisons (summarized in Dessauer and Cole [1989]). However, a better understanding of the genetic basis of vertebrate parthenogenesis requires an even more precise knowledge of the parentage of these taxa than the allozyme studies have provided.

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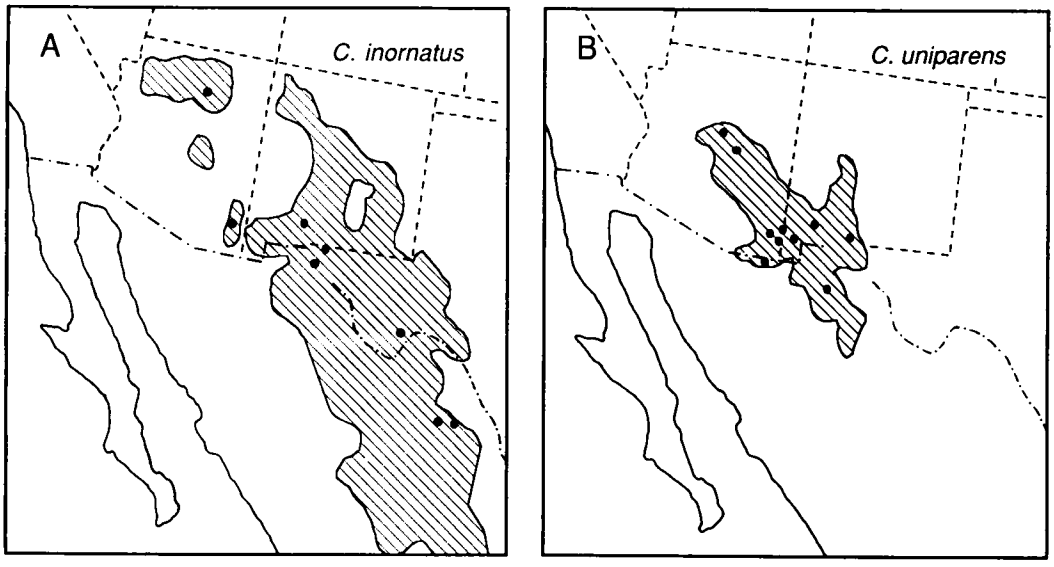


FIG. 1. Map of the southwestern United States and adjacent parts of Mexico, showing geographic ranges and sampling points of *C. inornatus* and *C. uniparens*.

Restriction-endonuclease cleavage-site comparisons of mitochondrial DNAs (mtDNAs) from unisexuals and their bisexual relatives provide information on the maternal ancestry and relative ages of the unisexual species (Brown and Wright, 1979). Previous studies have identified three maternal ancestors for unisexual *Cnemidophorus*: *C. tigris marmoratus* for *C. tesselatus* and *C. neomexicanus* (Brown and Wright, 1979; Densmore et al., 1985, 1989); *C. gularis* for *C. laredoensis* (Wright et al., 1983); and either *C. costatus* or *C. burti* for *C. velox* and *C. exsanguis* (Moritz et al., 1989*b*). The mtDNA within each of these five unisexual species is remarkably homogeneous, attesting to the recent formation of these taxa. The mtDNA analysis of *C. velox* (Moritz et al., 1989*b*) also provides unequivocal evidence for its formation via an allodiploid hybrid, rather than via a nonhybrid female spontaneously capable of parthenogenetic reproduction, as hypothesized by others (Cuellar, 1974; Darevsky et al., 1985).

The focus of this paper is on the *sexlineatus*-group unisexual species other than *C. velox, C. exsanguis,* and *C. laredoensis.* These include *C. uniparens, C. opatae, C. sonorae, C. flagellicaudus,* and five other morphologically distinct but undescribed species. Cleavage-site variation in mtDNA was examined among 18 individuals from one species (*C. uniparens*) and among representatives of the other eight. Also, mtDNAs from these and several bisexual species in the *sexlineatus* group were compared to resolve the maternal ancestry of the unisexual species.

MATERIALS AND METHODS

The taxonomy of bisexual and unisexual *Cnemidophorus* continues to be unsettled (e.g., Cole, 1985; Walker, 1986; Frost and Wright, 1988; Dessauer and Cole, 1989). We analyzed mtDNAs from several morphologically distinct but as yet unnamed unisexual taxa (J. W. Wright, unpubl.), which are here designated as "sp. C," "sp. N," "sp. O," "sp. P," and "sp. S." Some of these may be analogous to the pattern classes of *C. tesselatus* (Zweifel, 1965). The geographic ranges and sampling points for *C.uniparens* and *C. inornatus* are shown in Figure 1. Museum numbers for voucher specimens are given in the Appendix.

The mtDNAs were prepared from field-collected lizards (Fig. 1, Appendix) and analyzed as described in Densmore et al. (1985, 1989). DNA fragment sizes were determined by electrophoresis through agarose and polyacrylamide gels, using *Mbo* I-digested Hela mtDNA, *Hae* III-digested

TABLE 1. Mitochondrial-DNA cleavage types detected among nine unisexual *Cnemidophorus* species. For all enzymes, the common restriction pattern is designated "A." Site gains are indicated by uppercase letters, and site losses are indicated by lowercase letters. The species and number of individuals (*N*) in which each cleavage type was found are given. Cleavage-type A occurred in all unisexual species except *C. opatae* and "sp. P."

Cleavage type	Species (*N*)	Restriction enzyme					
		Hinf I	*Hin*P I	*Mbo* I	*Msp* I	*Rsa* I	*Scrf* I
1	*C. uniparens* (2)	A	b	B	A	B	A
2	*C. uniparens* (2)	b	A	A	A	A	A
3	*C. uniparens* (1)	A	A	c	b	C	A
4	*C. uniparens* (1)	A	A	c	b	A	A
5	*C. uniparens* (1)	A	A	c	A	A	A
6	*C. uniparens* (1)	A	A	D	A	A	A
7	*C. uniparens* (1), *C.* "sp. S" (2)	A	A	A	A	D	A
A	*C. flagellicaudus* (1), *C. uniparens* (9), *C. sonorae* (1), *C.* "sp. C" (1), *C.* "sp. N" (1), *C.* "sp. O" (1), *C.* "sp. S" (1)	A	A	A	A	A	A
8	*C.* "sp. C" (1)	C	A	A	A	A	A
9	*C.* "sp. C" (2)	—	—	A	A	E	—
10	*C. opatae* (2), *C.* "sp. P" (2)	A	A	e	A	A	A

ΦX174 RF-DNA, and *Hin*d III digested λ-phage DNA as size standards. Statistical analyses were performed using PAUP (version 2.4; available from D. L. Swofford, Illinois Natural History Survey, Champaign) and PHYLIP (version 2.9; Felsenstein, 1985) as described in Moritz et al. (1989*b*).

RESULTS

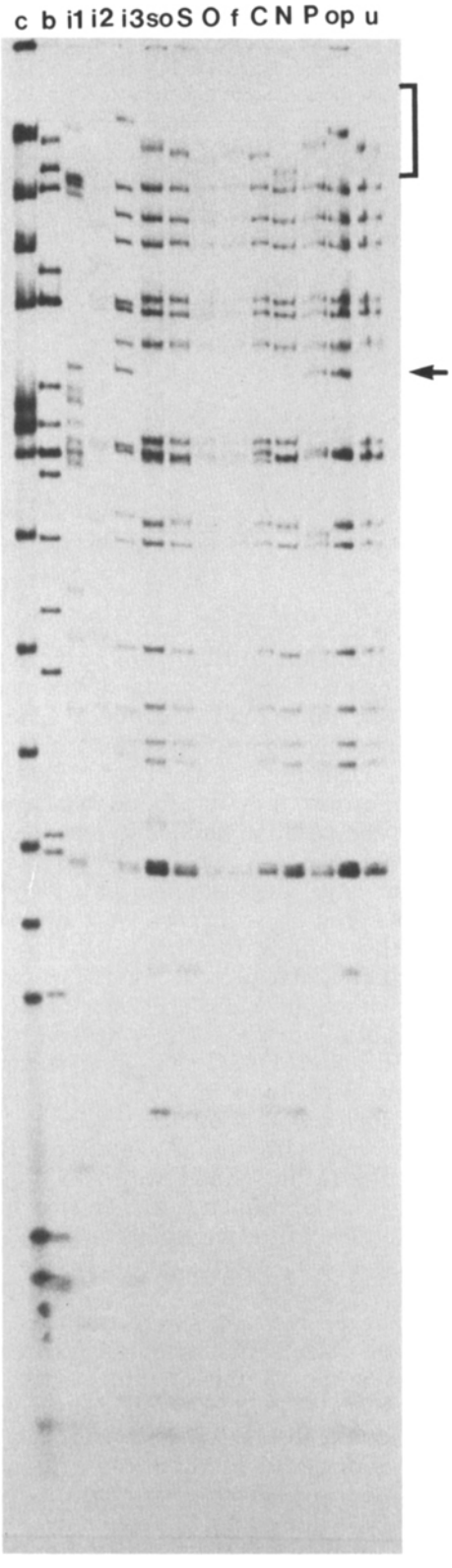
Mitochondrial-DNA Variation Among Unisexual Cnemidophorus

Fifty-three mtDNAs were analyzed with up to 18 restriction enzymes that recognize 4-, 5-, or 6-bp sequences (Table 1; also see Fig. 5). Two classes of sequence variation were detected: changes in the length of restriction fragments due to size variation and changes in the number or location of cleavage sites due (presumably) to base substitutions.

The size variation observed was due to 1) differences in copy number of short, tandemly repeated sequences, 2) large (>900 bp) sequence duplications, and 3) a sequence deletion. As in previous studies of *Cnemidophorus* mtDNAs (Densmore et al., 1985, 1989; Moritz et al., 1989*b*), the length of one fragment (typically the largest; see Fig. 2) varied by up to 600 bp. Numerous fragment-size classes were evident, and within-individual fragment-size heterogeneity (heteroplasmy) was evident in approximately 20% of the individuals. These size variants are attributed to variation in the copy number of short tandem repeats. However, unlike the *C. exsanguis, C. tesselatus,* and *C. velox* mtDNAs (Moritz et al., 1989*b*; Densmore et al., 1985, 1989), no restriction enzymes were found that cleaved within the putative, tandemly repeated sequences.

Four large duplications that included mtDNA coding sequences were observed (see Moritz and Brown [1986, 1987] for details). Three were present in mtDNAs from either single individuals or specific populations of *C. uniparens* and were 0.9 kb, 1.5 kb, and 6.8 kb in size (Fig. 3). The fourth (4.8 kb) was found in a single *C. opatae* individual and was unusual in being heteroplasmic with unduplicated mtDNA molecules (Moritz and Brown, 1987). Finally, a 3.9-kb sequence was deleted from at least 50% of the molecules in one ("sp. N") mtDNA (C. Moritz and W. Brown, unpubl.).

Cleavage-site changes were much less frequent than size variation; only 12 were noted among the 53 unisexual mtDNAs. For an analysis of within-taxon cleavage-site variation, we focused on the most geographically widespread of these unisexuals, *C. uniparens.* Eighteen *C. uniparens* mtDNAs from 10 localities (Fig. 1) were analyzed using six enzymes that cleave at 4-bp sites



(Table 1). Nine of the 18 mtDNAs were identical at the 128 sites cleaved. This common cleavage type, designated "A," is shared among most of the unisexuals and is presumed to be the ancestral state (Fig. 3). Among the remaining, non-A *C. uniparens* mtDNAs there were nine site changes: three were in single individuals, and six were shared by two or more individuals (Table 1, Fig. 3). Two of the shared changes (the *Hinf* I site loss [cleavage types 2a and 2b; Fig. 3] and the *Mbo* I site loss [cleavage types 3, 4, and 5; Fig. 3]) were found in geographically proximal populations. Variation was also detected within populations (e.g., each of three individuals from one locality had a distinct mtDNA [cleavage types 3, 4, and A; Table 1, Fig. 3]).

The amount of sequence variation in *C. uniparens* mtDNAs was summarized in two ways. The mean sequence divergence among the eight cleavage types listed in Table 1 was 0.27%, with a range from 0.1% to 0.6% (Table 2). The mean sequence divergence for all pairwise comparisons of the 18 mtDNAs was 0.16%.

Thirty-five mtDNAs representing the other eight unisexual species were assayed with three enzymes that recognize 4-bp sites (*Mbo* I, *Msp* I, and *Rsa* I), and 14 of the 35 were assayed with three additional enzymes (*Scr*F I, *Hin*P I, and *Hinf* I). The most common type, found in six mtDNAs, contained 128 cleavage sites. This type was identical to cleavage-type A of *C. uniparens* (Table 1). Four different site changes were detected in this series of digests (Table 1, Fig. 4). An *Mbo* I site loss was shared by all mtDNAs of *C. opatae* and *C.* "sp. P" (see also Fig. 2). An *Rsa* I site gain was shared by four of

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FIG. 2. Fragment patterns produced by *Mbo* I digestion of mtDNAs from each of nine unisexual and five bisexual species. The uniquely sized *Mbo* I fragment that is due to a site change shared among "sp. P," *C. opatae,* and some *C. inornatus* (from Willcox Playa) is indicated by the arrow. The size range for the largest (CV) fragment (see text) is indicated by the bracket. Abbreviations: c = *C. costatus*; b = *C. burti*; i1, i2, and i3 = *C. inornatus* from Coahuila, Canutillo (El Paso Co., TX), and Willcox Playa, respectively; so = *C. sonorae*; S = *C.* "sp. S"; O = *C.* "sp. O"; f = *C. flagellicaudus*; C = *C.* "sp. C"; N = *C.* "sp. N"; P = *C.* "sp. P"; op = *C. opatae*; u = *C. uniparens*.

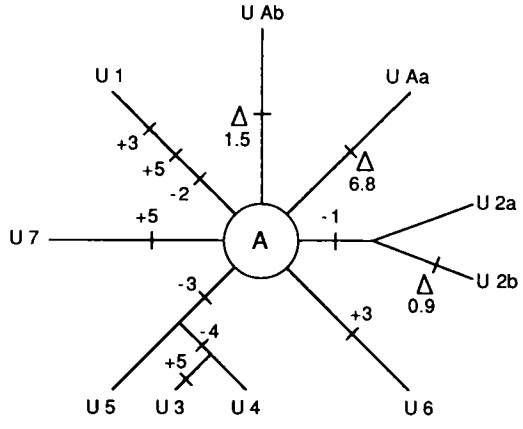

FIG. 3. Minimum-length network of mtDNA cleavage types from *C. uniparens*. The circled A represents the most common cleavage type, presumed to be ancestral. Restriction-site changes (+ = gain; − = loss) are indicated by bars and are identified by the numbers corresponding to restriction enzymes listed in Table 1. Duplications are indicated by triangles, with numbers indicating sizes in kb. Numbers at the branch termini correspond to mtDNA cleavage types, as defined in Table 1; each number is prefixed by a U to indicate that the mtDNA source was *C. uniparens*.

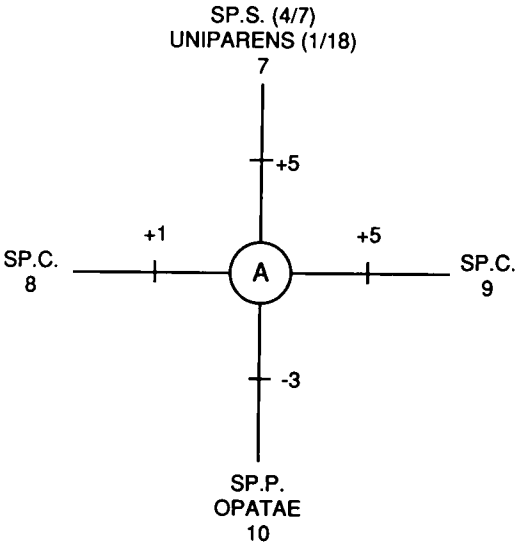

FIG. 4. Minimum-length network of mtDNA cleavage types from nine unisexual *Cnemidophorus*. Only one *C. uniparens* variant (U7) has been included. The circled A represents the most common cleavage type, presumed to be ancestral. Restriction-site changes (+ = gain; − = loss) are indicated by bars and are identified by numbers corresponding to restriction enzymes listed in Table 1. Numbers at the branch termini correspond to mtDNA cleavage types, as defined in Table 1.

the seven *C.* "sp. S" mtDNAs and one of the *C. uniparens* mtDNAs. The other changes were found in *C.* "sp. C," in which an additional *Rsa* I site occurred in two mtDNAs and an additional *Hinf* I site in a third. The sequence divergence among these four cleavage types ranged from 0.1% to 0.2%, with a mean of 0.15% (Table 3). The mean sequence divergence among the 14 mtDNAs assayed with all six restriction enzymes (excluding *C. uniparens*) was 0.08%.

Comparisons Between Unisexuals and Their Bisexual Relatives

Analysis of mtDNAs digested with enzymes that recognize 4-bp sites revealed that those from the Willcox Playa (Cochise Co., AZ) population of *C. inornatus* were very similar to those of the unisexual species, while those from other species of *Cnemidophorus* and other populations of *C. in-*

TABLE 2. Sequence variation among eight *C. uniparens* mtDNA cleavage types. The number of cleavage sites in each comparison appears (in bold type) on the diagonal. Estimates of percentage divergence are below the diagonal, and the numbers of site changes are above the diagonal. Cleavage-type designations are the same as in Table 1.

Cleavage type	Cleavage type							
	1	2	3	4	5	6	7	A
1	**129**	4	5	6	4	4	4	3
2	0.39	**127**	3	4	2	2	2	1
3	0.49	0.30	**126**	1	1	3	3	2
4	0.59	0.40	0.10	**127**	2	4	4	3
5	0.39	0.20	0.10	0.20	**127**	2	2	1
6	0.39	0.20	0.30	0.40	0.20	**129**	2	1
7	0.39	0.20	0.30	0.40	0.20	0.20	**129**	1
A	0.29	0.10	0.20	0.30	0.10	0.10	0.10	**128**

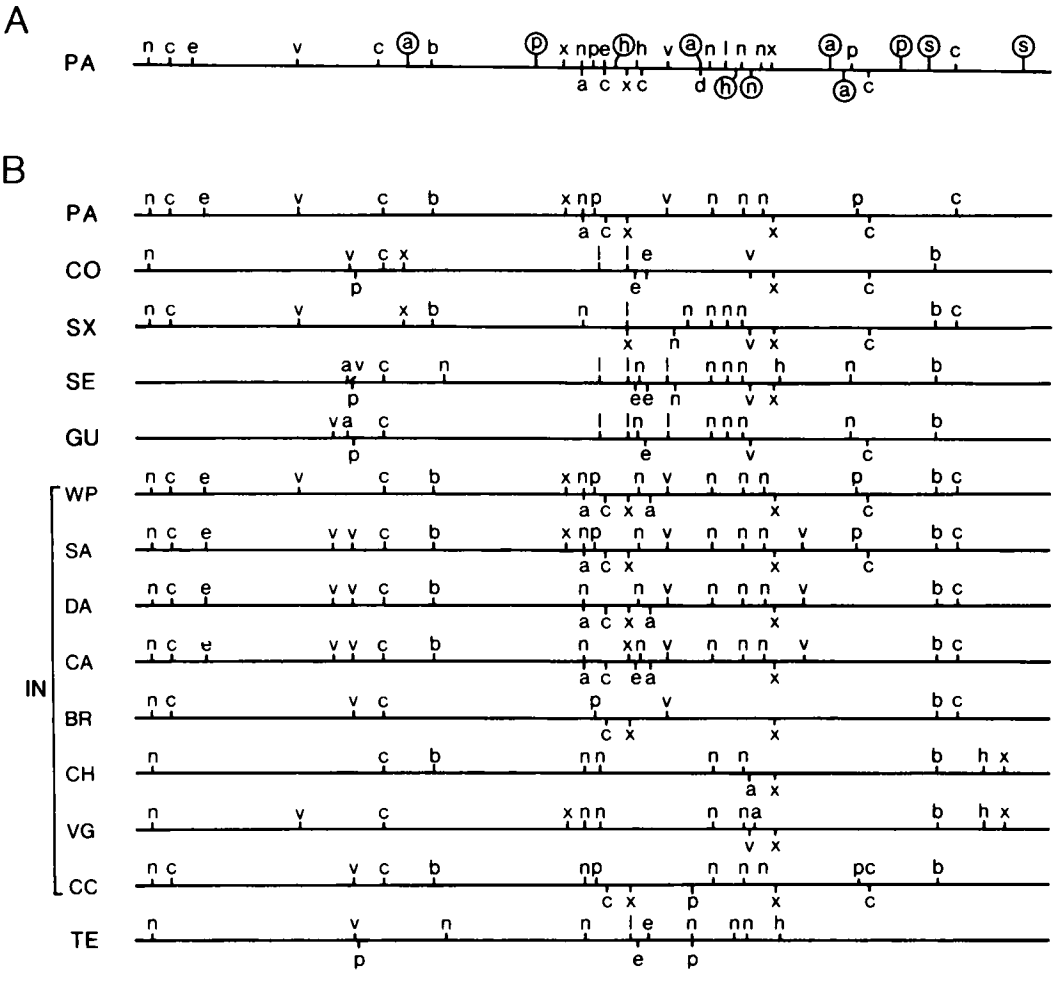

FIG. 5. Comparison of mtDNA cleavage sites among unisexual and bisexual *Cnemidophorus*. A) Cleavage-site map for the mtDNA of the unisexual species. Sites found in all *Cnemidophorus* mtDNAs studied are circled. The circular mtDNA has been linearized at the origin of replication, as determined by Brown and Wright (1979). B) Locations of phylogenetically informative cleavage sites in mtDNAs from the unisexual species and their bisexual relatives. PA = unisexual cleavage-type A. Species abbreviations: CO = *C. costatus*; SX = *C. sexlineatus*; SE = *C. septemvittatus*; GU = *C. gularis*; IN = *C. inornatus*; TE = *C. tesselatus*. Locality abbreviations for *C. inornatus* (IN): WP = Willcox Playa (= Cochise Co., AZ); SA = Samalayuca, Chihuahua, Mexico; DA = Dona Ana Co., NM; CA = Canutillo, El Paso Co., TX; BR = Brewster Co., TX; CH = Coahuila, Mexico; VG = Villa de Garcia, Nuevo Leon, Mexico; CC = Coconino Co., AZ. Cleavage-site abbreviations: a = *Ava* I; b = *Bam*H I; c = *Bcl* I; d = *Xho* I; e = *Eco*R I; h = *Hind* III; l = *Sal* I; n = *Nci* I; p = *Pvu* II; s = *Sst* II; v = *EcoR* V; x = *Xba* I.

ornatus were very different. A representative gel, in which *Mbo* I digests of mtDNAs from several of the taxa are compared, is shown in Figure 2.

The relationships among these mtDNAs were quantified by comparing the locations of cleavage sites for enzymes that recognize 5- or 6-bp sites (Fig. 5). This was done by comparing DNA fragment sizes after electrophoresis of single and double enzyme digests of the mtDNAs in adjacent lanes of the same gel. The goal of this analysis was to identify, if possible, the mtDNA from the bisexual population that is most similar to that from the unisexuals.

Initially, we compared cleavage-type-A mtDNAs from unisexual species with mtDNAs from their possible bisexual ancestors (*C. costatus, C. gularis, C. inornatus, C. septemvittatus,* and *C. sexlinea-*

TABLE 3. Sequence variation among four mtDNA cleavage types from unisexual *Cnemidophorus*. Only the commonest cleavage type (A) of *C. uniparens* has been included. Cleavage-type 9 has been excluded from these comparisons because no mtDNA of this type was digested with all six enzymes (Table 1). The number of cleavage sites in each comparison appears (in bold type) on the diagonal. Estimates of the percentage divergence are below the diagonal, and the numbers of site changes are above it. Cleavage types are as designated in Table 1.

Cleavage type	Cleavage type			
	A	7	8	10
A	**128**	1	1	1
7	0.10	**129**	2	2
8	0.10	0.20	**129**	2
10	0.10	0.20	0.20	**127**

TABLE 4. Percentage sequence divergence among six *sexlineatus*-group mtDNAs. Divergence estimates were derived from pairwise cleavage-map comparisons among mtDNAs from five bisexual species and the most common mtDNA (cleavage type A) in the nine unisexual taxa examined. PA = unisexual cleavage-type A; CO = *Cnemidophorus costatus*; SX = *C. sexlineatus*; SE = *C. septemvittatus*; GU = *C. gularis*; IN = *C. inornatus*. The number of cleavage sites in each comparison appears (in bold type) on the diagonal. Divergence estimates appear below the diagonal, and their standard errors are given above it.

Cleavage type	Cleavage type					
	PA	CO	SX	SE	GU	IN
PA	**36**	2.3	1.3	2.2	2.2	0.5
CO	10.3	**27**	1.9	1.6	1.8	1.0
SX	5.3	8.1	**38**	1.8	1.8	1.3
SE	10.9	6.5	8.3	**41**	1.2	2.0
GU	10.2	7.0	8.2	4.3	**34**	2.0
IN	0.9	8.9	5.1	9.8	9.0	**39**

tus). Among the 69 cleavage sites detected, 11 were shared by all mtDNAs (Fig. 5). The estimates of sequence divergence yielded by these comparisons varied from 0.9% (between the unisexual species and the Willcox Playa population of *C. inornatus*) to 10.3% (between the unisexual species and *C. costatus*) (Table 4). Clustering by UPGMA (Sneath and Sokal, 1973) indicated that the mtDNAs from the unisexual species were most similar to those of *C. inornatus*, with *C. sexlineatus* mtDNA being the next most similar (Fig. 6A).

The cleavage sites were treated as characters for phylogenetic analysis, using *C. tesselatus* as the outgroup (Densmore et al., 1985, 1989; Moritz et al., 1989*b*). Of the 69 cleavage sites, 37 had states (present/absent) that were shared by two or more mtDNAs (Fig. 5B) and were, thus, phylogenetically informative. Wagner parsimony analysis with exhaustive branch swapping produced a single shortest tree with 52 steps and a consistency index of 0.71 (Fig. 6B). This tree has the same topology as the UPGMA phenogram (Fig. 6A). The node uniting the mtDNAs from the unisexual species and *C. inornatus* was strongly supported and was present in all 100 bootstrap replicates. The next node, which defined *C. sexlineatus* mtDNA as sister to the mtDNAs from *C. inornatus* and the unisexuals, was also present in a high proportion of the bootstrap replicates (Fig. 6B). In order to maximize convergent site losses over convergent site gains (DeBry and Slade, 1985), the data were also analyzed using Dollo parsimony. The shortest tree in this case had the same topology as the Wagner parsimony tree but was five steps longer.

The above analyses identified *C. inornatus* as the maternal ancestor for these nine unisexual lineages. To resolve further the maternal ancestry of the unisexual species, cleavage sites were compared between unisexual cleavage-type A and mtDNAs from eight morphologically and geographically diverse populations of *C. inornatus* (Fig. 1). The mtDNAs differed considerably among the *C. inornatus* populations, with only 17 of the 57 cleavage sites shared by all. The estimates of sequence divergence ranged from 0.22% (between the mtDNAs from Dona Ana Co., NM, and Canutillo, El Paso Co., TX) to 6.8% (between those from Brewster Co., TX, and Coahuila or Nuevo Leon, Mexico) (Table 5). The UPGMA cluster analysis (Fig. 7A) identified the Willcox Playa *C. inornatus* mtDNA as the most similar to that of the unisexual species. The other *C. inornatus* mtDNAs from southern New Mexico, adjacent Texas, and northern Chihuahua (i.e., Dona Ana Co., NM; Canutillo, El Paso Co., TX; and Samalayuca, Chihuahua, Mexico) also resembled the unisexual mtDNA. The southern mtDNAs (from Coahuila and Nuevo Leon, Mexico) were similar to each other but quite different

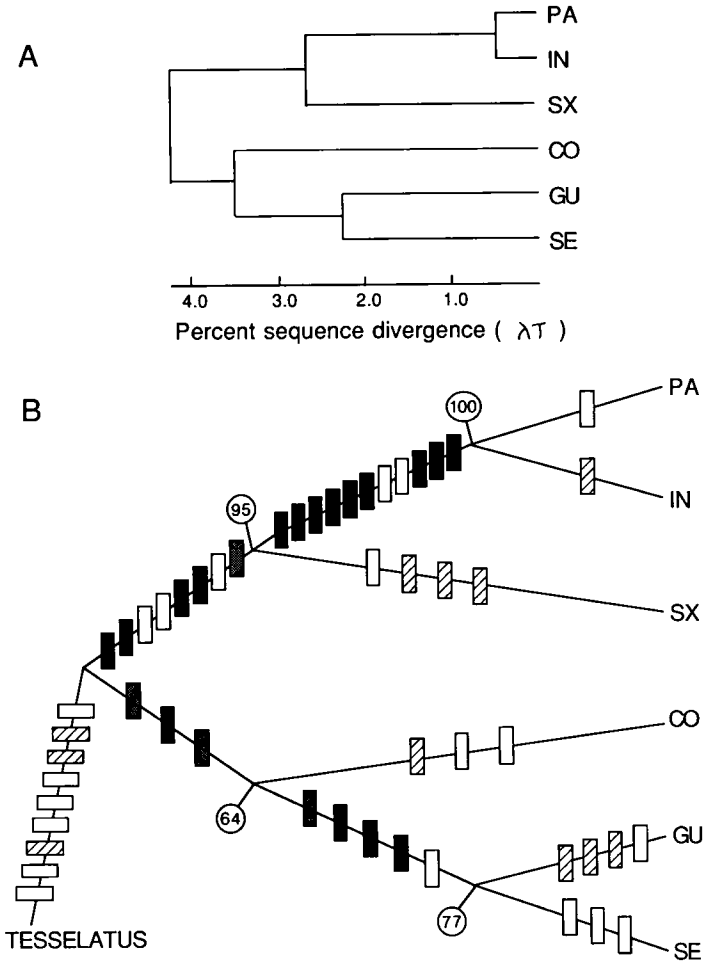

FIG. 6. Similarity and relationships among mtDNAs from the unisexual species and related bisexual species of *Cnemidophorus*. A) UPGMA clustering of sequence-divergence estimates; B) phylogeny based on maximum-parsimony analysis of character data. The numbers at each node indicate the percentage of bootstrap replicates in which the taxa to the right were monophyletic. Symbols: ■ = site gain without homoplasy, ▩ = site loss without homoplasy, ▨ = site gain with homoplasy, □ = site loss with homoplasy. PA = unisexual cleavage-type A. Species abbreviations: IN = *C. inornatus*; SX = *C. sexlineatus*; CO = *C. costatus*; GU = *C. gularis*; SE = *C. septemvittatus*.

from the northern *C. inornatus* and the unisexual mtDNAs. Indeed, the unisexual mtDNA differs as much from the southern *C. inornatus* mtDNAs as from *C. sexlineatus* mtDNA (see Tables 4, 5).

Of the 57 sites cleaved, 25 were informative when treated as characters for phylogenetic analysis. At this level of analysis, *C. sexlineatus* mtDNA was used as the outgroup. Among the mtDNAs studied, this was the immediate sister group to the clade of mtDNAs from *C. inornatus* and the unisexuals (Fig. 6B). The single shortest tree produced by Wagner parsimony (Fig. 7B) required 41 steps to explain the 25 characters and had a consistency index of 0.63. The topology of this tree was the same as that of the UPGMA phenogram (Fig. 7A) and of the shortest tree produced using Dollo parsimony. However, the node uniting the Willcox Playa *C. inornatus* and the unisexual species was only supported by three characters, none of which was unique. Not surprisingly, this node was relatively unstable in the bootstrap analyses (Fig. 7B). At 42 steps there were four topologies, and a

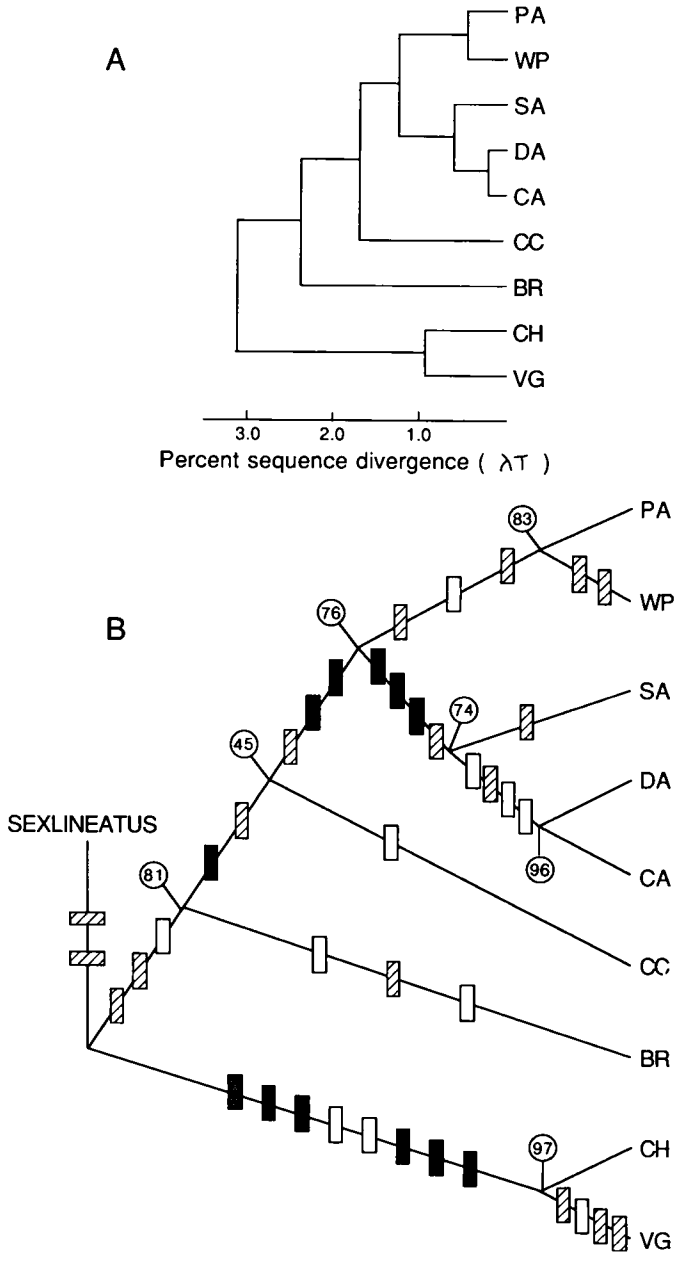

FIG. 7. Similarities and relationships of mtDNAs from the unisexual species and different geographic populations of *C. inornatus*. A) UPGMA clustering of sequence-divergence estimates; B) phylogeny based on maximum-parsimony analysis of character data. PA = unisexual cleavage-type A. Abbreviations for *C. inornatus* populations: WP = Willcox Playa (Cochise Co.), AZ; SA = Samalayuca, Chihuahua, Mexico; DA = Dona Ana Co., NM; CA = Canutillo, El Paso Co., TX; CC = Coconino Co., AZ; BR = Brewster Co., TX; CH = Coahuila, Mexico; VG = Villa de Garcia, Nuevo Leon, Mexico. The numbers at each node indicate the percentage of bootstrap replicates in which the taxa to the right were monophyletic. Symbols: ■ = site gain without homoplasy, ◘ = site loss without homoplasy, ▨ = site gain with homoplasy, □ = site loss with homoplasy.

TABLE 5. Percentage sequence divergence among mtDNAs from eight *C. inornatus* populations and the most common mtDNA (cleavage-type A) in the nine unisexual taxa examined. Divergence estimates were derived from pairwise cleavage-map comparisons. Abbreviations for *C. inornatus* populations: WP = Willcox Playa, AZ; SA = Samalayuca, Chihuahua; DA = Dona Ana Co., NM; CA = Canutillo, TX; CC = Coconino Co., AZ; BR = Brewster Co., TX; CH = Coahuila; VG = Villa de Garcia, Nuevo Leon. PA = unisexual cleavage-type A. The number of cleavage sites in each comparison appears (in bold type) on the diagonal. Divergence estimates appear below the diagonal, and their standard errors are given above the diagonal.

	Cleavage type								
	PA	WP	SA	DA	CA	CC	BR	CH	VG
PA	**36**	0.5	0.6	0.9	0.9	0.8	1.3	1.5	1.5
WP	0.9	**39**	0.6	0.8	0.8	0.9	1.2	1.5	1.4
SA	1.6	1.5	**41**	0.5	0.6	0.9	1.2	1.6	1.6
DA	2.9	2.4	1.1	**38**	0.2	1.1	1.3	1.5	1.6
CA	3.2	2.6	1.3	0.2	**39**	1.1	1.3	1.5	1.6
CC	2.3	2.7	2.9	3.8	4.1	**35**	1.3	1.4	1.5
BR	4.5	5.1	4.5	4.4	4.6	4.3	**27**	1.8	1.8
CH	6.0	5.8	6.5	5.8	6.0	5.1	6.8	**30**	0.8
VG	6.0	5.1	6.5	6.5	6.8	5.8	6.8	1.8	**30**

consensus of these included the mtDNAs from Dona Ana County, Canutillo, and Samalayuca as possible sister groups to the unisexual mtDNA. As in the UPGMA analysis, the *C. inornatus* mtDNAs from Coahuila and Nuevo Leon were closely related to each other but distantly related to the remaining mtDNAs (Fig. 7B).

Cleavage-site diversity was examined in the mtDNAs of Willcox Playa *C. inornatus* by digestion with six enzymes that recognize 4-bp sites. Six cleavage types were identified among the 14 mtDNAs, and the mean pairwise sequence divergence among them was 0.9%. The mean sequence divergence among all 14 mtDNAs was 0.14%.

DISCUSSION

Maternal Ancestry of the Unisexual Taxa

The above analyses clearly demonstrate that the mtDNAs present in all nine unisexual species (*C. flagellicaudus, C. sonorae, C. opatae, C. uniparens,* and the five undescribed species) were derived from *C. inornatus*. Side-by-side comparisons of *Mbo* I-digested mtDNAs (Fig. 2) illustrate how similar the unisexuals are to each other and to some *C. inornatus*, and how different they are from two other *sexlineatus*-group species. Phylogenetic analysis of cleavage sites for 12 enzymes that recognize 5- and 6-bp sites verifies the close relationship between the mtDNAs from *C. inornatus* and the unisexuals (Fig. 6). Furthermore, the mtDNA in all of these unisexuals is remarkably similar to that found in a single subspecies of *C. inornatus*. *Cnemidophorus inornatus* occurs across much of southwestern North America (Fig. 1) and includes four nominate subspecies (*C. i. inornatus* Baird, *C. i. arizonae* Van Denburgh, *C. i. heptagrammus* Axtell, and *C. i. paulus* Williams), as well as several unnamed morphologically distinctive populations (J. Wright and C. Lowe, unpubl.). Considerable mtDNA divergence exists among the eight geographically and morphologically diverse populations of *C. inornatus* examined here. Both phylogenetic analysis of cleavage sites (Fig. 7) and side-by-side comparison of *Mbo* I patterns (Fig. 2) indicate that the predominant cleavage type in the unisexuals is most similar to that present in the Willcox Playa (Cochise Co., AZ), population of *C. inornatus*, one of the few remaining populations of *C. i. arizonae* (Wright and Lowe, 1965). The geographic range of this subspecies has recently contracted, presumably due to habitat destruction and the concomitant spread of *C. uniparens* (Wright and Lowe, 1968). The slight differences in mtDNA between the unisexuals and the Willcox Playa *C. inornatus* population could thus be due to sequence polymorphism or geographic variation that existed in a previously more widespread *C. i. arizonae* or to mutations that have occurred since the unisexual and bisexual lineages split.

Mitochondrial-DNA analysis has now been used to determine the maternal ancestry of all of the *sexlineatus*-group unisexual species (this study; Wright et al., 1983; Moritz et al., 1989*b*). Eight of the nine unisexual lineages in the present study are triploids that appear to stem from matings between male *C. costatus* or *C. burti* and female allodiploid hybrids that, like the ninth lineage (diploid *C.* "sp. P"), arose by hybridization between a male *C. costatus* or *C. burti* and a female *C. inornatus*. It is possible that the two groups of unisexuals represented by *C. velox* and *C. exsanguis* and by the nine species in this study are the products of reciprocal initial hybridization events, as originally suggested by Good and Wright (1984).

Mitochondrial DNA and the Origin of Unisexuality

Studies of morphology and allozymes indicate that all of the unisexual species within the *sexlineatus* group are hybrids. Where such lineages are either diploid (*C. laredoensis*; McKinney et al., 1973) or triploid with chromosome sets derived from three different species (*C. exsanguis*; Good and Wright, 1984), it is likely that their parthenogenetic mode of reproduction is a consequence of the initial hybridization. However, the remaining *sexlineatus*-group unisexuals are triploids with two of the three sets of nuclear genes derived from one bisexual species. These could have arisen via fertilization of unreduced oocytes produced either by a nonhybrid diploid ("spontaneous origin" hypothesis) or by a hybrid diploid ("hybrid origin" hypothesis) (Cuellar, 1974; Darevsky et al., 1985). This aspect of the spontaneous-origin hypothesis can be falsified by mtDNA analysis if the bisexual that is represented by two sets of nuclear genes in the unisexual is found to be the paternal parent species. Of the triploid lineages considered in this study, all but two (*C. uniparens* and *C. opatae*; Neaves, 1969; Dessauer and Cole, 1989; J. Wright and M. Simovich, unpubl.) have a single set of genes from *C. inornatus* and a double set of genes from a second species, probably *C. burti* or *C. costatus*. The mtDNA analysis demonstrates that *C. inornatus* was the maternal parent of all of these unisexual lineages. Thus, for at least seven of these unisexual species, a nonhybrid origin that involved an unreduced ovum is excluded (also see Moritz et al. [1989*b*]). The most probable sequence of events in the genesis of these unisexuals was hybridization between a *C. inornatus* female and either a *C. costatus* or a *C. burti* male to produce one or more allodiploid (probably parthenogenetic) females, followed by matings of the allodiploid females with *C. costatus* or *C. burti* males. The plausibility of this sequence is also supported by the existence of the diploid unisexual *C.* "sp. P" and by the possibility that it is related to the triploid *C. opatae* in exactly this way (see Fig. 8). While it is possible that all of these unisexuals stem from the same allodiploid ancestor, the morphological and allozymic diversity of the separate lineages suggests that several subsequent matings occurred which may have been separated in time and space (Dessauer and Cole, 1989; J. Wright and M. Simovich, unpubl.).

Mitochondrial-DNA Diversity in Unisexual Taxa

In contrast to the considerable variation in external morphology, allozymes (Neaves, 1969; Dessauer and Cole, 1986, 1989; J. Wright and M. Simovich, unpubl.), ecology, and extent of geographic distribution (Wright and Lowe, 1968) of the unisexual species examined here, there is little variation in mtDNA. The observed cleavage-site variation could have been inherited from the maternal ancestor(s) or it could have arisen subsequently, by mutation. Those variants that occur in both the unisexuals and extant representatives of their maternal ancestors can be explained by multiple hybrid origins of the unisexual species. Those variant sites that are present in the unisexual species but absent in an adequately sized sample of individuals from the maternal parent species are more difficult to assess. However, if these variants are geographically restricted, it seems reasonable to attribute them to mutation. In the latter case, the number of such variant sites should be roughly correlated with the ages of the unisexual lineages.

Among the unisexuals, mtDNA heterogeneity was assessed in greatest detail for *C.*

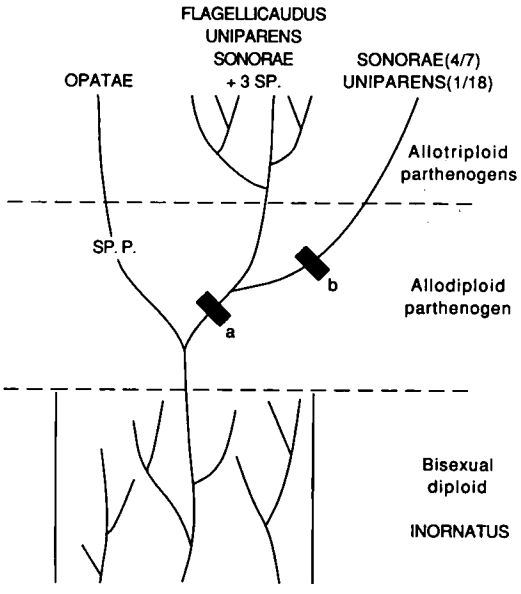

FIG. 8. Possible evolutionary history of the mtDNAs from the nine unisexual species analyzed here. The mtDNAs represent a small sample of the cleavage types that presumably existed among bisexual *C. inornatus*. The predominant mtDNA lineage among all of the unisexual species except *C.* "sp. P" and *C. opatae* is characterized by an *Mbo* I site gain (a) that may have occurred in their shared allodiploid ancestor. One *Rsa* I site gain (b) is shared by some *C.* "sp. S" mtDNAs and one *C. uniparens* mtDNA. This could have arisen in their shared allodiploid ancestor (as indicated) or independently in each of the two taxa.

uniparens. This is the most widespread of the unisexual lineages considered here (Fig. 1), and it is still extending its range at the expense of its maternal progenitor, *C. inornatus* (Wright and Lowe, 1968). Analysis of nuclear gene diversity (histocompatibility loci; Cuellar, 1976) indicates that there is a low level of clonal diversity for this species. Eleven different mtDNA types (including cleavage and duplication variations) were detected among the 18 individuals, although the amount of sequence divergence between any two individuals was minimal. The variation was distributed within and between localities. In two cases, a particular mtDNA variation was shared between nearby localities, but most changes, including each of the duplications, were restricted to a single individual or to a local population. Since all the low-frequency variations were absent from the Willcox Playa population of *C. inornatus*, we tentatively attribute them to mutation arising subsequent to the initial hybridization. The average mtDNA sequence divergence between individual *C. uniparens* was 0.16%, a value similar to that found for *C. tesselatus* (0.06%; Densmore et al., 1989) and for *C. velox* and *C. exsanguis* (0.22%; Moritz et al., 1989*b*). These divergence levels are among the lowest reported for natural populations of animals (reviewed by Avise and Lansman [1983], and Avise [1986]).

Perhaps the most remarkable finding of this and the two companion studies (Densmore et al., 1989, and Moritz et al., 1989*b*) is the extremely low level of mtDNA cleavage-site variation within multilineage groupings of unisexual taxa. The level in this study is comparable to that in *C. tesselatus* and *C. neomexicanus*, where several morphologically diverse lineages may have arisen from as few as 2–3 separate hybridizations (Densmore et al., 1989). The mtDNA data for these *sexlineatus*-group unisexuals indicate that they could be derived from one or a few closely related, allodiploid, presumably parthenogenetic females (Fig. 8). Only two cleavage-site differences were shared by multiple unisexual lineages. The first was the shared absence of an *Mbo* I site among *C.* "sp. P," *C. opatae* (Fig. 1), and the Willcox Playa *C. inornatus* (Fig. 2). If the polarity inferred for this character is correct, then the presence of the *Mbo* I site in the mtDNAs of the other unisexuals is a derived state. Presumably, the site was gained in a shared allodiploid precursor (Fig. 8), or else it was present in an unknown population of *C. i. arizonae*. The second shared difference is an *Rsa* I site that was present in the mtDNAs of four of the seven *C.* "sp. S" individuals and one of 18 *C. uniparens* individuals but absent from the other unisexual and the Willcox Playa *C. inornatus* mtDNAs. The distribution of this character can also be explained in two ways. The site gain could have been independently acquired by mutation in one lineage of each of the two unisexual species. Alternatively, some of the *C. uniparens* and some of the *C.* "sp. S" could stem (by different backcrosses) from an allodiploid with the additional *Rsa* I site (Fig. 8). This interpretation requires that each of these unisexuals

resulted from more than one backcrossing event. Given the uniformity of histocompatibility loci in two populations of *C. uniparens* (Cuellar, 1976), this seems unlikely. However, more comprehensive analyses of nuclear gene variation in the two forms are required before the latter hypothesis can be adequately evaluated.

Regardless of the exact number of *C. inornatus* females that participated in the origin of these unisexual species, the contrast between the homogeneity of the unisexual mtDNAs and the heterogeneity of *C. inornatus* mtDNAs and the great similarity between the mtDNAs from the unisexuals and those from the Willcox Playa *C. inornatus* suggest that the allodiploid precursor(s) of the unisexuals originated within a restricted geographic region over a short time. The hybridization events presumably took place somewhere in the former range of *C. i. arizonae,* i.e., in the region where Arizona, New Mexico, Chihuahua, and Sonora abut. This coincides with the approximate center of the distribution of the unisexual species (Fig. 1). If *C. velox* and *C. exsanguis* stem from one or two reciprocal allodiploids, as suggested above, they may also have originated in this region.

Ages of the Unisexual Lineages

The approximate age of a unisexual lineage is an important variable about which there is little information (Maynard Smith, 1986). Both allozyme and mtDNA analyses of other unisexual *Cnemidophorus* have suggested that they are very recently evolved (Parker and Selander, 1976, 1984; Brown and Wright, 1979; Wright et al., 1983; Densmore et al., 1985, 1989; Moritz et al., 1989*b*). The sequence divergence between the mtDNAs from Willcox Playa *C. inornatus* and the unisexual lineages analyzed here (<1%) is considerably less than that found among different geographic populations of *C. inornatus* (up to 6.8%). Some of the diversity between the bisexual populations may be the result of the sorting of polymorphisms through founder events (Birky et al., 1983) or stochastic sorting of lineages (Avise et al., 1984; Neigel and Avise, 1986), processes that could not apply to the unisexuals if their mtDNAs were initially homogeneous. However, it seems unlikely that the above effects could create the observed differences in mtDNA heterogeneity unless the radiation of *C. inornatus* predated the origin of the unisexuals. This, together with the remarkable homogeneity of mtDNAs within and among the unisexual lineages, suggests that they were formed relatively recently, probably no earlier than the Pleistocene.

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Appendix

All *Cnemidophorus* used in these analyses have been deposited as voucher specimens in the herpetological collections of either the Natural History Museum of Los Angeles County (LACM) or the University of Michigan Museum of Zoology (UMMZ). Abbreviated locality data and catalog numbers are presented in the table below; except where specifically indicated as UMMZ, catalog numbers are for LACM specimens. More complete specimen data may be requested from J.W.W. (LACM numbers) or W.M.B. (UMMZ numbers).

Taxon	Collection locality	*N*	Voucher specimens
C. inornatus	Cochise Co., AZ	15	137177–137191
	Coconino Co., AZ	3	137269–137271
	Chihuahua, Mexico	1	122408
	Coahuila, Mexico	1	130634
	Dona Ana Co., NM	1	134339
	Nuevo Leon, Mexico	1	131732
	Brewster Co., TX	2	130636, 130638
	El Paso Co., TX	1	137193
C. sexlineatus	Otero Co., CO	1	128302
	San Miguel Co., NM	1	128309
	Woods Co., OK	2	128316–128317
	Robertson Co., TX	1	128325
C. flagellicaudus	Yavapai Co., AZ	1	130657
	Catron Co., NM	5	134318–134322
C. opatae	Sonora, Mexico	2	131724, 134402
C. sonorae	Pima Co., AZ	4	124371–124374
C. uniparens	Cochise Co., AZ	8	131826; UMMZ 182963–182969
	Yavapai Co., AZ	3	130659, 134780, 137200
	Chihuahua, Mexico	1	137201
	Dona Ana Co., NM	1	137199
	Hidalgo Co., NM	2	128372, 134395
	Luna Co., NM	1	134397
	Sonora, Mexico	2	137204–137205
C. "sp. C"	Cochise Co., AZ	6	134324, 134327–134329, 131748 134836
C. "sp. N"	Sonora, Mexico	6	131580–131581, 134720–134721, 134723–134724
C. "sp. O"	Pima Co., AZ	3	134792, 134795–134796
C. "sp. P"	Sonora, Mexico	3	137195–137197
C. "sp. S"	Cochise Co., AZ	4	134370, 131778, 131781, 134368
	Grant Co., NM	1	137198
	Sonora, Mexico	3	131794, 131796–131797