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MITOCHONDRIAL-DNA ANALYSES AND THE ORIGIN AND RELATIVE AGE OF PARTHENOGENETIC LIZARDS (GENUS *CNEMIDOPHORUS*). II. *C. NEOMEXICANUS* AND THE *C. TESSELATUS* COMPLEX

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Abstract.—Restriction-endonuclease analyses of mitochondrial DNAs from all six color-pattern classes (A–F) of the parthenogenetic lizard *Cnemidophorus tesselatus* yield estimates of nucleotide divergence that are extremely low (π = 0.06%). In digests of 75 *C. tesselatus* mtDNAs with 20 different restriction enzymes, only four cleavage-site differences were noted, three of which were found only in pattern class F. The near-identity of these mitochondrial DNAs with those from *C. tigris marmoratus* shows unequivocally that *C. t. marmoratus* was the species to which the maternal parent(s) of all *C. tesselatus* belonged. Mitochondrial-DNA analyses of another unisexual species, *C. neomexicanus*, led to the same conclusion. Mitochondrial DNAs from 96 individuals of these three species were extensively analyzed for cleavage-site differences; only 13 were found. The low interspecific sequence diversity found within *C. neomexicanus* and the *C. tesselatus* complex suggests a recent origin for both. Based on diversity data for mitochondrial DNA and allozymes, we estimate that a minimum of two hybridizations were required to produce all diploid *C. tesselatus* (C–F), followed by at least two more to generate the triploids (A and B). These data and those presented in the two accompanying papers indicate that events leading to parthenogenesis in *Cnemidophorus* are rare and strengthen the hypothesis that interspecific hybridization is a necessary, causal event in its establishment.

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The lizard genus *Cnemidophorus* consists of about 50 species, one-third of which are unisexual and consist exclusively of parthenogenetically reproducing females (see reviews by Cole [1975]; Wright [1978], Darevsky et al. [1985], Maslin and Secoy [1986], and Dessauer and Cole [1989]). The *C. tesselatus* complex, perhaps the best known of the unisexual species, is where parthenogenesis was first recognized in *Cnemidophorus* (Minton, 1958; Tinkle, 1959; Maslin, 1962). Zweifel (1965) analyzed geographic variation in *C. tesselatus* morphology and recognized six color-pattern classes, designated A–F. All but one of these had a unique geographic distribution. He suggested that classes A–F represented a transformation series, in which A, the simplest color pattern, was primitive, while B, C, and D were intermediates in the gradual evolution of the most complex pattern classes, E and F. This hypothesis was rejected when analysis of karyotypes showed that *C. tesselatus* originated from interspecific hybridization between the bisexual species *C. tigris* and *C. septemvittatus* and that classes A and B were triploids whose "simple" color patterns were due to the presence of a haploid genome from a third species, *C. sexlineatus* (Wright and Lowe, 1967; see Wright [1978] for discussion and additional references). While these findings explained the origin of the ploidy differences in *C. tesselatus*, they did not explain the origin of the differences in color pattern. Karyotype analyses also demonstrated that *C. neomexicanus* was of hybrid origin, and it was inferred, based on these and other analyses, that its bisexual parent species were probably *C. tigris* and *C. inornatus* (Lowe and Wright, 1966).

Neaves and Gerald (1968), Neaves (1969), and later Parker and Selander (1976) confirmed the hybrid origin of *C. tesselatus* using allozyme electrophoresis. The variation from a standard, highly heterozygous genotype was quite restricted, and no pattern class had a distinctive genotype; however, Parker (1979) felt that the presence of some of the allelic variation within two pattern classes (C and E) could be explained best by

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invoking multiple independent hybridization events. The hybrid origin of *C. neomexicanus* was also confirmed by allozyme electrophoresis (Neaves and Gerald, 1968; Neaves, 1969; Parker and Selander, 1984; Cole et al., 1988).

Mitochondrial DNA (mtDNA) is especially useful for studying the evolution of unisexual species because of its rapid rate of sequence evolution and maternal inheritance (Dawid, 1972; Dawid and Blackler, 1972; Brown et al., 1979; reviewed by Avise and Lansman [1983], Brown [1983, 1985], Avise [1986], and Moritz et al. [1987, 1989*a*]). Comparisons of mtDNAs from the unisexual *Cnemidophorus* and their bisexual relatives can accurately resolve the maternal ancestry of the former (Brown and Wright, 1979; Wright et al., 1983; Densmore et al., 1985, 1989; Moritz et al., 1989*b*). Similar studies have provided important insights about the formation of hybridogenetic frogs (Spolsky and Uzzell, 1984, 1986) and gynogenetic fish (Avise and Vrijenhoek, 1987; Goddard et al., 1989; Echelle et al., 1989). Detailed comparisons of mtDNA cleavage-site variation among unisexual and bisexual lineages can also provide estimates of their relative ages and of the minimum number of hybridizations involved in their formation.

Brown and Wright (1979) conducted a preliminary analysis of the mtDNA of *C. tesselatus* E and of *C. neomexicanus*, another unisexual species with a much smaller geographic range (see Parker and Selander, 1984). They found that the mtDNAs in both unisexuals came from the same ancestral taxon, *C. tigris marmoratus*. Their data also supported Parker and Selander's (1976) hypothesis that the *C. tesselatus* complex was of recent origin and extended the hypothesis to include *C. neomexicanus*. Because the *C. tesselatus* analysis was confined to class-E individuals, questions about the differences in color pattern and geographical distributions among the diploid pattern classes C, D, and F remained. Were several hybridizations involving mitochondrially distinct populations of *C. t. marmoratus* responsible for these differences? Had reciprocal crosses, involving *C. septemvittatus* instead of *C. t. marmoratus* females occurred?

To answer these questions, we extended our comparative restriction-endonuclease analyses of mtDNA to all six color-pattern classes of *C. tesselatus*, to additional *C. neomexicanus*, and to the bisexual species *C. septemvittatus*, *C. sexlineatus*, and *C. tigris* (including *C. t. marmoratus*).

Materials and Methods

The taxonomy of several unisexual and bisexual *Cnemidophorus* is still controversial (e.g., Cole, 1985; Hendricks and Dixon, 1986; Walker, 1986; Frost and Wright, 1988; Dessauer and Cole, 1989). In this paper, we have arbitrarily and without prejudice continued to follow Burger (1950) and Zweifel (1962) in recognizing *marmoratus* as a subspecies of *Cnemidophorus tigris* and Duellman and Zweifel (1962) in recognizing *C. septemvittatus*.

Preparation and analysis of mtDNAs from individual *Cnemidophorus* were performed as described in Wright et al. (1983), as modified by Densmore et al. (1985). The taxa analyzed included all six *C. tesselatus* pattern classes, *C. neomexicanus*, *C. septemvittatus*, *C. sexlineatus*, *C. tigris gracilis*, *C. t. marmoratus*, and *C. t. variolosus*. See the Appendix for details.

The following 16 restriction endonucleases were used in preliminary analyses of the mtDNAs and for constructing cleavage maps: *Ava* I, *Bam*H I, *Bst*E II, *Eco*R I, *Eco*R V, *Hin*d III, *Kpn* I, *Nci* I, *Pst* I, *Pvu* II, *Sal* I, *Sma* I, *Sst* I, *Sst* II, *Xba* I, and *Xho* I. To increase the sensitivity of the assay, digests of all *C. tesselatus*, *C. t. marmoratus*, and *C. neomexicanus* mtDNAs with the enzymes *Mbo* I, *Msp* I, *Rsa* I, and *Taq* I were also compared electrophoretically. To test for reciprocity, *Mbo* I-digested *C. septemvittatus*, *C. sexlineatus*, *C. tigris gracilis*, and *C. t. variolosus* mtDNAs were compared to selected *Mbo* I-digested *C. tesselatus* and *C. t. marmoratus* mtDNAs.

For maximum accuracy, restriction-endonuclease digests of mtDNAs were compared by electrophoresis in the same gel. Analyses performed in this manner are capable of resolving fragment-size differences as small as 1%. Sequence divergence was estimated (Nei and Li, 1979; Nei and Tajima, 1983) from cleavage-site changes inferred from the fragment patterns produced by *Mbo* I, *Msp* I, *Rsa* I, and *Taq* I. Phylo-

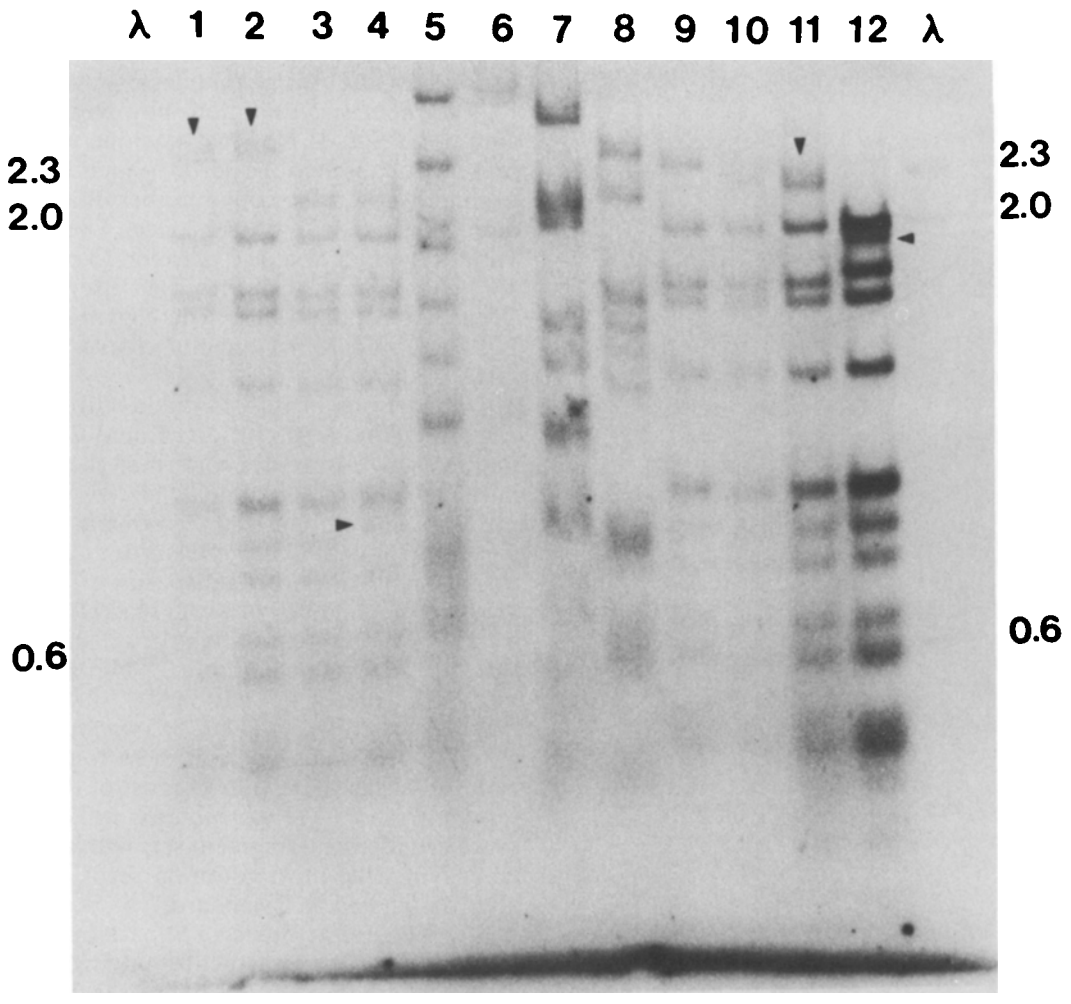

FIG. 1. Autoradiogram of *Mbo* I-digested *Cnemidophorus* mtDNAs after electrophoresis in a 1.2% agarose gel. Lanes labeled λ contain *Hind* III fragments of bacteriophage λ DNA as size standards. Numbers at the sides indicate fragment sizes in kilobase pairs. Lanes 1–4 and 10–12 contain mtDNAs representative of all color-pattern classes of *C. tesselatus*: 1) A, 2) B, 3–4) C, 10) D, 11) E, 12) F'. Lanes 5–9 contain mtDNAs from the three bisexual species implicated in hybridizations that generated the various *C. tesselatus*: 5) *C. septemvittatus*, 6) *C. sexlineatus*, 7) *C. tigris gracilis*, 8) *C. t. variolosus*, 9) *C. t. marmoratus*. The fragments in lanes 5–8 differ markedly from those in lanes 1–4 and 9–12. Comparing only lanes 1–4 and 9–12, lane 12 contains two novel fragments (of 1.91 kb and 1.77 kb; see horizontal arrow), but lacks the larger (>2.0 kb) fragment, and lane 4 contains an 845-bp fragment (horizontal arrow) that is 35 bp larger than (but which otherwise corresponds to) the 810-bp fragment in the other lanes (DV fragment; see Densmore et al., 1985). Differences in size, number, and relative intensity of the fragments larger than 2.0 kb in lanes 1–4 and 9–11 (vertical arrows) are due to copy-number variation of a tandem repeat and to heteroplasmy (see text and Densmore et al. [1985] for details).

genetic analysis was performed by treating each restriction site as a binary character and analyzing the characters using Wagner parsimony (PAUP, Version 2.4 available from D. L. Swofford, Illinois Natural History Survey, Champaign).

RESULTS

Analyses of selected *C. tesselatus* and *C. tigris marmoratus* mtDNAs with 16 restriction endonucleases that cleave at 5- and 6-bp sites revealed no within-taxon variation and

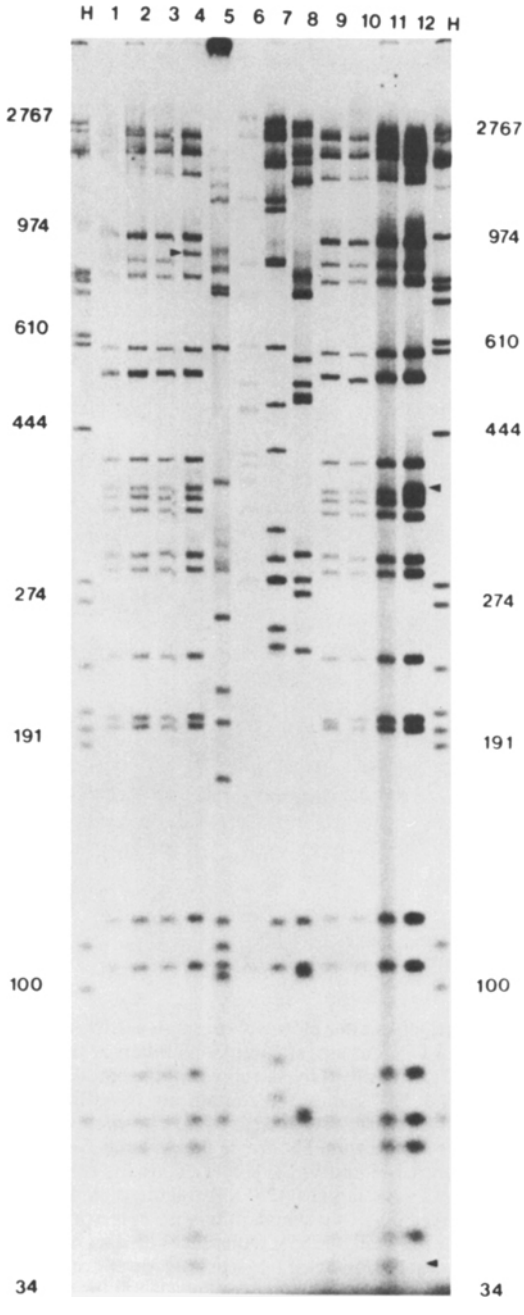

FIG. 2. Autoradiogram of *Mbo* I-digested *Cnemidophorus* mtDNAs after electrophoresis in a 4% polyacrylamide gel. Lanes labeled H contain *Mbo* I fragments of human (HeLa) mtDNA as size standards. Numbers at the sides indicate fragment sizes in base pairs. Contents of numbered lanes are as in Figure 1: Lanes 1–4 and 10–12 contain mtDNAs representative of all color-pattern classes of *C. tesselatus*: 1) A, 2) B, 3–4) C, 10) D, 11) E, 12) F'. Lanes 5–9 contain mtDNAs from the three bisexual species implicated in hybridizations that generated the various *C. tesselatus*: 5) *C. septemvittatus*, 6) *C. sexlineatus*, 7) *C. tigris gracilis*, 8) *C. t. variolosus*, 9) *C. t. marmoratus*. Comparing only lanes 1–4 and 9–12, lane 12 contains a novel 380-bp fragment and lacks a 39-bp fragment (arrows), and lane 4 contains the novel 845-bp fragment (arrow) that corresponds to the 810-bp fragment in the other lanes (see legend to Figure 1).

only one difference, at an *Eco*R V site, between six *C. t. marmoratus* from Hidalgo Co. and seven *C. tesselatus* (classes A–C and F). Fragment size variation, however, was common (Figs. 1, 2). Most was due to the presence or absence of a 35-bp sequence and to differences in the copy number of a tandemly repeated 64-bp sequence. The size variation and a cleavage map for 13 of the 16 enzymes have been presented elsewhere (Densmore et al., 1985). The enzymes *Sst* I, *Sma* I, and *Kpn* I did not cleave these mtDNAs.

Comparisons of digests made with four enzymes (*Mbo* I, *Msp* I, *Rsa* I, and *Taq* I) that recognize 4-bp sites confirmed the similarity of *C. tesselatus* and *C. t. marmoratus* mtDNAs and emphasized their distinctness from mtDNAs of *C. septemvittatus, C. sexlineatus,* and two other subspecies of *C. tigris* (*gracilis* and *variolosus*). This is illustrated by the representative *Mbo* I digests shown in Figures 1 and 2. Ninety-six mtDNAs from *C. tesselatus* ($N = 72$), *C. t. marmoratus* ($N = 21$), and *C. neomexicanus* ($N = 3$) were analyzed with these four enzymes. Because digestion of most of the *C. tesselatus* and *C. t. marmoratus* mtDNAs produced identical fragment sets with a given enzyme, the most common set is hereafter designated as "Standard," or St.

Mbo I digestion yielded a St pattern ($N = 83$) consisting of 28 fragments that migrated as 26 bands in the gels. The fragments ranged in size from 0.029 to 2.21–2.6 kb (Table 1). Two pairs of fragments (of 0.94 kb and 0.53 kb) comigrated, producing two bands of double intensity. All *C. tesselatus* A–E mtDNAs ($N = 67$), two *C. neomexicanus* mtDNAs, and all Hidalgo County *C. t. marmoratus* mtDNAs ($N = 14$) were St (see lanes 1–4 and 9–11 in Figs. 1 and 2). A total of six *Mbo* I site differences from St were observed in the remaining 13 mtDNAs from these species. All five *C. tesselatus* F had an

additional *Mbo* I site that yielded fragments of 1.91 and 0.38 kb instead of the 2.30-kb fragment found in St, and one of these (F') lacked a site that was present in St, resulting in a *Mbo* I fragment of 1.77 kb that corresponded to the St fragments of 1.73 kb and 0.04 kb. The *C. neomexicanus* mtDNA from Albuquerque lacked one *Mbo* I site (1.43-kb fragment = 1.33-kb + 0.11-kb fragments of St). Within *C. t. marmoratus*, the mtDNA from Brewster County lacked two *Mbo* I sites (1.46-kb fragment = 0.94-kb + 0.53-kb fragments of St; 0.38-kb fragment = 0.32-kb + 0.06-kb fragments of St), and the six mtDNAs from El Paso had an additional *Mbo* I site, which produced fragments of 0.48 kb and 1.91–2.12 kb. These came from the largest fragment of St (2.39–2.60 kb), which contains a region with a variable number of 64-bp tandem repeats (Densmore et al., 1985).

The St pattern for *Msp* I ($N = 76$) consisted of a minimum of 27 fragments migrating as 24 distinct bands. These fragments ranged in size from 0.02 kb to 2.27 kb (Table 2). Three of the bands contained pairs of comigrating fragments (0.58 kb, 0.38 kb, and 0.315 kb). When the estimated number of 64-bp tandem repeat copies (3–9 [Densmore et al., 1985]) is included, the maximum number of fragments in St increased to 34. With respect to inferred base substitutions, only the three *C. neomexicanus* mtDNAs and the *C. t. marmoratus* mtDNA from Brewster County differed from St. These each lacked one *Msp* I site; the 2.04-kb fragment in *C. neomexicanus* corresponds to the 1.74-kb + 0.27-kb fragments in St, and the 1.46-kb fragment from the Brewster Co. *C. t. marmoratus* corresponds to the 1.09-kb + 0.37-kb fragments in St.

The St pattern for *Rsa* I ($N = 73$) consisted of 35 fragments migrating as 33 distinct bands. The fragments ranged in size from 0.035 kb to 1.65–2.00 kb (Table 3). Two pairs of fragments (0.98 kb and 0.092 kb) comigrated. Most *C. tesselatus*, all *C. t. marmoratus*, and one mtDNA of *C. neomexicanus* were St. All pattern-class-F mtDNAs had one less *Rsa* I site, which resulted in the presence of a 1.46-kb fragment instead of the 1.25-kb and 0.21-kb fragments found in St. One mtDNA of pattern-

TABLE 1. *Mbo* I fragment sizes (in kb) of mtDNAs from *Cnemidophorus tesselatus*, by pattern class (A–F), and from *C. neomexicanus* and *C. tigris marmoratus*, by locality. (LU = Luna Co., NM [*C. neomexicanus*]; AL = Albuquerque, NM [*C. neomexicanus*]; BR = Brewster Co., TX [*C. tigris marmoratus*]; EP = El Paso Co., TX [*C. t. marmoratus*]; HD = Hidalgo Co., NM [*C. t. marmoratus*]) (see Appendix). A "+" indicates the presence of one fragment, and "++" indicates the presence of two fragments of equal size. A "−" indicates the absence of a fragment that is present in St (see text). An asterisk (*) indicates the presence of the 35-bp size variation in one or more of the mtDNAs (DV fragment of Densmore et al. [1985]). *N* is the number of mtDNAs examined.

Fragment (*kb*)	Samples					
	A–E, LU, HD	F	F'	AL	BR	EP
2.23–2.60[g]	+	−[a]	−[a]	+	+	−[f]
1.99	+	+	+	+	+	+
1.91		+[a]	+[a]			+[f]
1.77			+[b]			
1.73	+	+	−[b]	+	+	+
1.63	+	+	+	+	+	+
1.46					+[d]	
1.43				+[c]		
1.33	+	+	+	−[c]	+	+
0.94	++	++	++	++	+[d]	++
0.81	+*	+	+	+	+	+*
0.74	+	+	+	+	+	+
0.575	+	+	+	+	+	+
0.530	++	++	++	++	+[d]	++
0.480						+[f]
0.409	+	+	+	+	+	+
0.382					+[e]	
0.380		+[a]	+[a]			
0.377	+	+	+	+	+	+
0.366	+	+	+	+	+	+
0.354	+	+	+	+	+	+
0.322	+	+	+	+	−[e]	+
0.302	+	+	+	+	+	+
0.246	+	+	+	+	+	+
0.210	+	+	+	+	+	+
0.205	+	+	+	+	+	+
0.121	+	+	+	+	+	+
0.106	+	+	+	−[c]	+	+
0.076	+	+	+	+	+	+
0.063	+	+	+	+	+	+
0.060	+	+	+	+	−[e]	+
0.044	+	+	+	+	+	+
0.039	+	+	−[b]	+	+	+
0.029	+	+	+	+	+	+
N:	83	4	1	1	1	6

[a] Site loss: 2.30 kb → 1.91 kb + 0.38 kb.
[b] Site gain: 1.73 kb + 0.039 kb → 1.77 kb.
[c] Site gain: 1.33 kb + 0.106 kb → 1.43 kb.
[d] Site loss: 0.94 kb + 0.53 kb → 1.46 kb.
[e] Site loss: 0.322 kb + 0.060 kb → 0.382 kb.
[f] Site gain: 2.39–2.60 kb → 1.91–2.12 kb + 0.480 kb.
[g] Variable fragment (CV fragment; see text and Densmore et al. [1985]).

TABLE 2. *Msp* I fragment sizes (in kb) of mtDNAs from *Cnemidophorus tesselatus*, by pattern class (A–F), and from *C. neomexicanus* and *C. tigris marmoratus*, by locality (LU = Luna Co., NM [*C. neomexicanus*]; AL = Albuquerque, NM [*C. neomexicanus*]; BR = Brewster Co., TX [*C. tigris marmoratus*]; EP = El Paso Co., TX [*C. t. marmoratus*]; HD = Hidalgo Co., NM [*C. t. marmoratus*]) (see Appendix). A "+" indicates the presence of one fragment, and "++" indicates the presence of two fragments of equal size. A "−" indicates the absence of a fragment that is present in St (see text). An asterisk (*) indicates the presence of the 35-bp size variation in one or more of the mtDNAs (DV fragment of Densmore et al. [1985]). An "M" indicates the presence of a multiplicity of fragments of equal size. *N* is the number of mtDNAs examined.

	Samples		
Fragment size	A–F, EP, HD	LU, AL	BR
2.72	+	+	+
2.15	+	+	+
2.04		+[a]	
1.75	+	+	+
1.74	+	−[a]	+
1.46			+[b]
1.09	+	+	−[b]
1.08	+	+	+
0.72	+	+	+
0.59–0.73[c]	+	+	+
0.580	++	++	++
0.540	+	+	+
0.380	++	++	++
0.367	+	+	−[b]
0.315	++	++	++
0.270	++	−[a]	+
0.255	+	+	+
0.244	+	+	+
0.222	+	+	+
0.191	+	+	+
0.114	+*	+	+
0.064	M	M	M
0.052	+	+	+
0.041	+	+	+
0.024	+	+	+
0.018	+	+	+
N:	76	3	1

[a] Site loss: 1.74 kb + 0.270 kb → 2.04 kb.
[b] Site loss: 1.09 kb + 0.367 kb → 1.46 kb.
[c] Variable fragment (CV fragment; see text and Densmore et al. [1985]).

class E (designated E′ in Table 3) and two *C. neomexicanus* mtDNAs (LU2 and AL) also lacked an *Rsa* I site, resulting in the presence of a 2.28-kb fragment instead of the 1.29-kb and 0.98-kb St fragments.

The St pattern for *Taq* I ($N = 80$) consisted of 28 fragments, migrating as 25 distinct bands. The fragments ranged in size from 0.05 kb to 1.86–2.25 kb (Table 4).

TABLE 3. *Rsa* I fragment sizes (in kb) of mtDNAs from *Cnemidophorus tesselatus*, by pattern class (A–F), and from *C. neomexicanus* and *C. tigris marmoratus*, by locality (LU = Luna Co., NM [*C. neomexicanus*]; AL = Albuquerque, NM [*C. neomexicanus*]; BR = Brewster Co., TX [*C. tigris marmoratus*]; EP = El Paso Co., TX [*C. t. marmoratus*]; HD = Hidalgo Co., NM [*C. t. marmoratus*]) (see Appendix). A "+" indicates the presence of one fragment, and "++" indicates the presence of two fragments of equal size. A "−" indicates the absence of a fragment that is present in St (see text). An asterisk (*) indicates the presence of the 35-bp size variation in one or more of the mtDNAs (DV fragment of Densmore et al. [1985]). LU1 and LU2 are two individual *C. neomexicanus* from Luna County, NM. *N* is the number of mtDNAs examined.

	Samples		
Fragment	A–E, LU1, BR, EP, HD	E′, LU2, AL	F
2.28		+[a]	
1.65–2.00[c]	+	+	+
1.75	+	+	+
1.56	+	+	+
1.46			+[b]
1.29	+	−[a]	+
1.25	+	+	−[b]
1.16	+	+	+
0.98	++	+[a]	++
0.72	+	+	+
0.580	+	+	+
0.508	+*	+	+
0.459	+	+	+
0.417	+	+	+
0.404	+	+	+
0.390	+	+	+
0.379	+	+	+
0.277	+	+	+
0.255	+	+	+
0.229	+	+	+
0.209	+	+	−[b]
0.201	+	+	+
0.147	+	+	+
0.143	+	+	+
0.135	+	+	+
0.132	+	+	+
0.122	+	+	+
0.113	+	+	+
0.106	+	+	+
0.095	+	+	+
0.092	++	++	++
0.079	+	+	+
0.063	+	+	+
0.056	+	+	+
0.035	+	+	+
N:	78	3	5

[a] Site loss: 1.29 kb + 0.98 kb → 2.28 kb.
[b] Site loss: 1.25 kb + 0.209 kb → 1.46 kb.
[c] Variable fragment (CV fragment; see text and Densmore et al. [1985]).

Three pairs of fragments (1.33 kb, 0.411 kb, and 0.247 kb) comigrated. All mtDNAs except that from Brewster County were St. The Brewster County mtDNA differed from St by two *Rsa* I site changes, one an apparent site gain (fragments of 1.27 kb + 0.52 kb = 1.77 kb fragment of St) and the other an apparent site loss (fragment of 0.97 kb = 0.495-kb + 0.463-kb fragments in St).

For those *C. tesselatus* mtDNAs analyzed with *Mbo* I, *Msp* I, *Rsa* I, and *Taq* I, 51 of 57 mtDNAs were St in all digests (Table 5). Of the site changes detected in *C. tesselatus*, two occurred in all F mtDNAs, one in F′, and one in E′ (Table 5). Fourteen of the *C. t. marmoratus* mtDNAs were St for all four enzyme digests, with the majority of the restriction-site variation limited to the Brewster County mtDNA (Table 5).

A total of twelve restriction-site polymorphisms were found among the approximately 118 sites surveyed. From these data, we calculated two estimates of mtDNA sequence variability: 1) the average number of nucleotide substitutions per individual mtDNA (π; Nei and Li, 1979) and 2) the mean number of nucleotide substitutions per site between mtDNA genotypes (δ; Nei and Tajima, 1983). The π for all 96 individuals with a *C. t. marmoratus* mitochondrial genome was 0.08%; for all *C. tesselatus*, it was 0.06%; and for all *C. t. marmoratus* it was 0.10%. The nine mtDNA genotypes identified by the 12 site changes differed by an average of 0.012 substitutions per site (Table 6).

The mtDNAs were clustered according to the sequence divergence estimates using UPGMA (Sneath and Sokal, 1973), and a phenogram was constructed (Fig. 3). Phylogenetic relationships were assessed by treating the restriction-site differences as binary characters (Table 7) and subjecting them to the Wagner parsimony algorithms in PAUP. The topology of the tree (Fig. 4) represents a consensus of 945 equally parsimonious trees (length = 14, consistency index = 0.93). Because almost all the differences between the mtDNA genotypes involved single site changes, neither analysis was capable of adequately resolving relationships among most *C. tesselatus*, *C. neomexicanus*, and two of the *C. t. marmoratus* (El Paso Co. and Hidalgo Co.). The *Rsa* I site change shared by the E′ *C. tesselatus* and most of the *C. neomexicanus* mtDNAs further complicated these attempts. Either E′ has independently gained this parallel *Rsa* I site or it has independently gained and lost an *Msp* I site. While the homoplasy is explained by either hypothesis, a gain-loss has a slightly higher likelihood than parallel independent gains (Templeton, 1983; Li, 1986). In summary, only the Brewster

TABLE 4. *Taq* I fragment sizes (in kb) of mtDNAs from *Cnemidophorus tesselatus*, by pattern class (A–F), and from *C. neomexicanus* and *C. tigris marmoratus*, by locality (LU = Luna Co., NM [*C. neomexicanus*]; AL = Albuquerque, NM [*C. neomexicanus*]; BR = Brewster Co., TX [*C. tigris marmoratus*]; EP = El Paso Co., TX [*C. t. marmoratus*]; HD = Hidalgo Co., NM [*C. t. marmoratus*]) (see Appendix). A "+" indicates the presence of one fragment, and "++" indicates the presence of two fragments of equal size. A "−" indicates the absence of a fragment that is present in St (see text). An asterisk (*) indicates the presence of the 35-bp size variation in one or more of the mtDNAs (DV fragment of Densmore et al. [1985]). *N* is the number of mtDNAs examined.

Fragment	Samples	
	A–F, LU, AL EP, HD	BR
1.86–2.25[c]	+	+
1.77	+	−[a]
1.70	+	+
1.33	++	++
1.27		+[a]
0.99	+	+
0.97		+[b]
0.77	+	+
0.71	+*	+
0.64	+	+
0.542	+	+
0.528	+	+
0.518	+	++[a]
0.495	+	−[b]
0.463	+	−[b]
0.443	+	+
0.411	++	++
0.382	+	+
0.285	+	+
0.247	++	++
0.190	+	+
0.177	+	+
0.131	+	+
0.125	+	+
0.098	+	+
0.074	+	+
0.052	+	+
N:	80	1

[a] Site gain: 1.77 kb → 1.27 kb + 0.518 kb.
[b] Site loss: 0.495 kb + 0.463 kb → 0.97 kb.
[c] Variable fragment (see text and Densmore et al. [1985]).

TABLE 5. Cleavage-site polymorphism in mtDNAs from *Cnemidophorus tesselatus*, by pattern class (A–F) and from *C. neomexicanus* and *C. tigris marmoratus*, by locality (LU = Luna Co., NM [LU1 and LU2 are two individuals]; AL = Albuquerque, NM; BR = Brewster Co., TX; EP = El Paso Co., TX; HD = Hidalgo Co., NM). Site polymorphism coding: St = Standard pattern; a–f refer to footnotes in whichever of Tables 1–4 correspond to the enzyme (e.g., a(1) refers to footnote a in Table 1); NA indicates that the analysis was not performed.

Species	Class/ locality	mtDNA numbers	Restriction enzyme			
			Mbo I	*Msp* I	*Rsa* I	*Taq* I
C. tesselatus	A	1–8	St	St	St	St
	B	9–10	St	St	St	St
	C	11–16	St	St	St	St
		17	St	St	NA	NA
		18–24	St	St	St	St
		25–27	St	NA	NA	NA
		28–30	St	St	St	St
	D	31–36	St	St	St	St
		37	St	NA	NA	NA
		38–42	St	St	St	St
	E′	43	St	St	a(3)	NA
	E	44–45	St	NA	NA	NA
		46–47	St	St	St	St
		48	St	NA	St	NA
		49–52	St	St	St	St
		53	St	NA	St	St
		54–59	St	St	St	St
		60	St	St	NA	St
		61	St	St	St	St
		62	St	NA	NA	NA
		63	St	St	NA	St
		64–67	St	NA	NA	NA
		68–70	a(1)	St	b(3)	St
	F′	71	a(1), b(1)	St	b(3)	St
	F	72	a(1)	St	b(3)	St
C. neomexicanus	AL	1	c(1)	a(2)	a(3)	St
	LU1	2	St	a(2)	St	St
	LU2	3	St	a(2)	a(3)	St
C. tigris marmoratus	BR	1	d(1), e(1)	a(2)	St	a(4), b(4)
	EP	2–7	f(1)	St	St	St
	HD	8–21	St	St	St	St

County *C. t. marmoratus* mtDNA (distinguished by five site differences) was consistently separated from that of all other taxa by both phenetic and phylogenetic analyses. Among the six *C. tesselatus* pattern classes, only the mtDNA of pattern-class F (which includes F′) was distinct.

DISCUSSION

The Ancestry of the Unisexuals and the Question of Reciprocity

These results rigorously identify *C. tigris marmoratus* as the source of the mtDNA found in all *C. tesselatus* and *C. neomexicanus* and, thus, as the maternal parent species in the hybridizations that led to their formation. The involvement of *C. septemvittatus* and *C. sexlineatus* as the paternal parent species in hybridizations that led, respectively, to the diploid and triploid *C. tesselatus* pattern classes follows from this; their involvement in the formation of *C. tesselatus* was previously demonstrated by skin-grafting, karyotypic, morphological, and allozyme studies (Maslin, 1967; Wright and Lowe, 1967; Neaves, 1969; Parker and Selander, 1976; Parker, 1979; Dessauer and Cole, 1984, 1986, 1989). We found no *C. septemvittatus* or *C. sexlineatus* mtDNA in any *C. tesselatus*. Although this observation had been made for three class-E *tesselatus*

by Brown and Wright (1979), it was possible that reciprocal hybridization, in which either *C. septemvittatus* or *C. tigris marmoratus* was the maternal parent species, might have been responsible for part of the dorsal-pattern diversity present in the *C. tesselatus* complex. The mtDNA data clearly exclude this possibility.

C. neomexicanus is also a unisexual with a *marmoratus*-like mtDNA and, thus, must also have had *C. t. marmoratus* as its maternal parent species (Brown and Wright, 1979). Mitochondrial DNAs sampled across the entire geographic range of *C. inornatus* (the paternal parent of *C. neomexicanus*) are distinct from each other and very different from *C. neomexicanus* mtDNA (data not shown; see Brown and Wright, 1979; Densmore et al., 1989). An analysis of mtDNA variation in *C. inornatus* and documentation of *C. inornatus* as the mtDNA source in yet another complex of parthenogenetic *Cnemidophorus* is presented in an accompanying paper (Densmore et al., 1989).

These data do not allow us to determine the specific populations of *C. tigris marmoratus* that are most likely to have contributed the mtDNA to *C. tesselatus* or to *C. neomexicanus*. Only the *C. t. marmoratus* mtDNA from Brewster County differs from St by more than a single restriction site (Tables 1–4), and no *C. t. marmoratus* mtDNA is identical to St. The *C. t. marmoratus* mtDNAs from Hidalgo County differ from all *C. tesselatus* mtDNAs by having an additional *Eco*R V site (Fig. 4; also see Densmore et al. [1985]); similarly, the cleavage patterns of *C. t. marmoratus* from El Paso County differ from St by the presence of an additional *Mbo* I site (Tables 1, 5; Fig. 4).

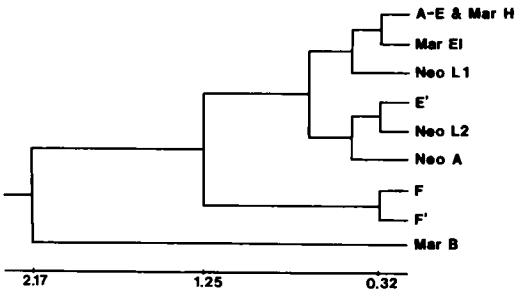

FIG. 3. UPGMA dendrogram of *Cnemidophorus tesselatus*, *C. neomexicanus*, and *C. tigris marmoratus* mtDNA distances. Distance estimates are from Table 6. Abbreviations: A–F (including E' and F') = the *C. tesselatus* pattern classes; Neo L1 and Neo L2 = individual *C. neomexicanus* from Luna Co., NM; Neo A = *C. neomexicanus* from Albuquerque, NM; Mar B, Mar El, and Mar H = *C. tigris marmoratus* from Brewster Co., TX, El Paso Co., TX, and Hidalgo Co., NM, respectively.

Relative Age of the C. tesselatus *Complex*

Mitochondrial-DNA sequence variation in the *C. tesselatus* complex is extremely low. Among individuals of the six pattern

TABLE 6. Percentage sequence divergences among nine mtDNA cleavage types found in *Cnemidophorus tesselatus*, *C. neomexicanus*, and *C. tigris marmoratus*. The divergence estimates are based on aggregate comparisons of the *Mbo* I, *Msp* I, *Rsa* I, and *Taq* I digests (Tables 1–4). The number of cleavage sites in each comparison appears (in bold type) on the diagonal. The divergence estimates and their standard errors appear below and above the diagonal, respectively. The cleavage types are grouped by pattern class (A–F) for *C. tesselatus* and by locality (LU = Luna Co., NM [LU1 and LU2 are two individuals]; AL = Albuquerque, NM; BR = Brewster Co., TX; EP = El Paso Co., TX; HD = Hidalgo Co., NM) for *C. t. marmoratus* and *C. neomexicanus*. The UPGMA-derived dendrogram (Sneath and Sokal, 1973) for these estimates is shown in Figure 3.

mtDNA sample	mtDNA sample								
	A–E, HD	E'	F	F'	LU1	LU2	AL	EP	BR
A–E, HD	**118**	0.19	0.26	0.33	0.19	0.27	0.33	0.18	0.44
E'	0.32	**117**	0.33	0.39	0.27	0.19	0.27	0.26	0.49
F	0.64	0.97	**118**	0.19	0.37	0.42	0.47	0.24	0.53
F'	0.97	1.31	0.32	**117**	0.39	0.44	0.49	0.35	0.58
LU1	0.32	0.64	1.19	1.31	**116**	0.19	0.27	0.26	0.46
LU2	0.64	0.32	1.53	1.65	0.32	**117**	0.19	0.33	0.51
AL	0.98	0.65	1.88	2.00	0.65	0.32	**115**	0.39	0.59
EP	0.32	0.64	0.53	1.07	0.64	0.97	1.31	**119**	0.49
BR	1.65	2.00	2.34	2.71	1.77	2.13	2.73	1.98	**115**

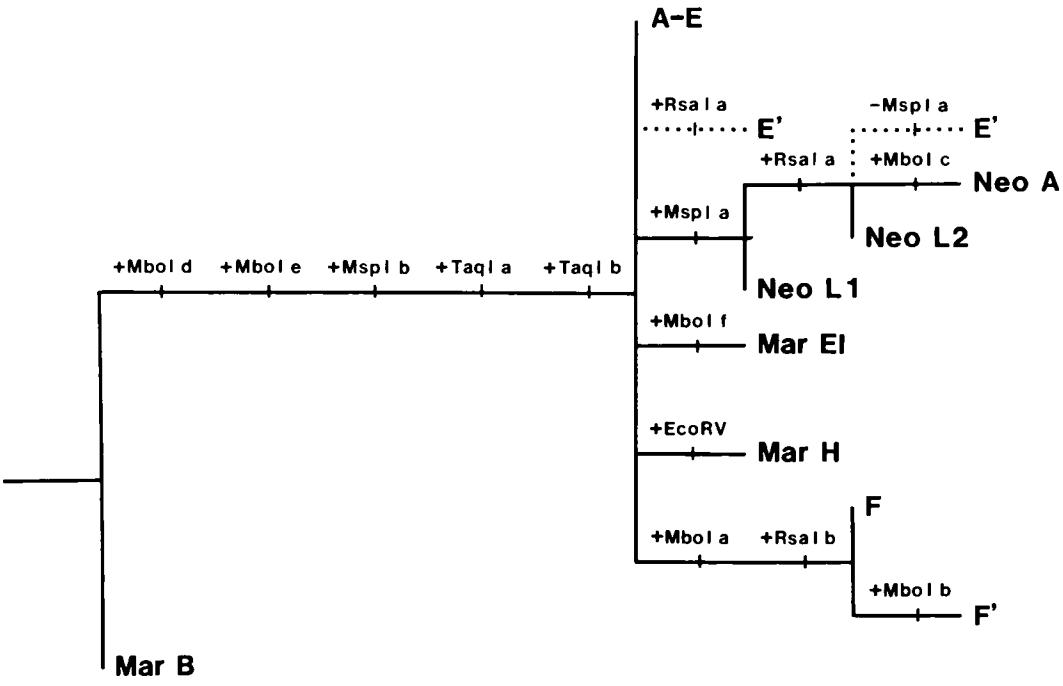

FIG. 4. The relationships of *Cnemidophorus tesselatus, C. neomexicanus,* and *C. tigris marmoratus* mtDNAs inferred by parsimony analysis (PAUP), using the data in Table 7. Abbreviations: A–F (including E' and F') = the *C. tesselatus* pattern classes; Neo L1 and Neo L2 = individual *C. neomexicanus* from Luna Co., NM; Neo A = *C. neomexicanus* from Albuquerque, NM; Mar B, Mar El, and Mar H = *C. tigris marmoratus* from Brewster Co., TX, El Paso Co., TX, and Hidalgo Co., NM, respectively. The tree was rooted using the most divergent *C. t. marmoratus* mtDNA (Mar B) as the outgroup. Character changes from this root (i.e., site gains or losses) are indicated by + or −. Dotted lines indicate uncertainty due to possible homoplasy in an *Rsa* I site in *C. tesselatus* E' and two *C. neomexicanus* (see Tables 1–5 and text).

classes, the π value (0.06%) is the lowest reported for a natural population and is 6–70 times lower than those reported for humans (0.4%; Brown and Goodman, 1979; Brown, 1980; Cann et al., 1984, 1987), great apes (0.6–5.0%; Ferris et al., 1981), *Peromyscus polionotus* and *P. maniculatus* (1.0% and 2.0%, respectively; Avise et al., 1979*b*), and *Geomys pinetis* (2.0%; Avise et al., 1979*a*). Because of the rapid rate of nucleotide substitution in vertebrate mtDNA (see Brown, 1985), the near absence of such variation (Tables 5, 6; Figs. 3, 4) strongly supports Parker and Selander's (1976) suggestion that the *C. tesselatus* complex may be of very recent origin. While the absolute age cannot be determined from these data, the large mtDNA sequence divergence between *C. t. marmoratus* (and thus *C. tesselatus* and *C. neomexicanus*) and the other subspecies of *C. tigris* (Figs. 1, 2) suggests that the formation of both *C. tesselatus* and *C. neomexicanus* postdated the *C. tigris* radiation (Brown and Wright, 1979).

Biochemical Estimates of the Number of Hybridization Events

The low level of allozymic diversity among *C. tesselatus* pattern-classes is consistent with the hypothesis that most diploids could have arisen from a very small number of hybridizations (Parker and Selander, 1976). Although they were able to distinguish some 12 distinct diploid genotypes (Parker, 1979), Parker and Selander (1976) concluded that most did not result from multiple hybridizations and that the remainder provided only equivocal support for the multiple-hybridization hypothesis. They determined that three of the genotypes were probably due to post formational mutation events, because the unique alleles were

TABLE 7. Cleavage-site variation in *Cnemidophorus* mtDNA, expressed as binary characters (1 = present, 0 = absent). The informative cleavage sites are as characterized in Tables 1–5 (a–f refer to footnotes in corresponding tables) and (for *Eco*R V) in the text. Mitochondrial-DNA sources are grouped by pattern class (A–F) for *C. tesselatus* and by locality (LU = Luna Co., NM [LU1 and LU2 are two individuals]; AL = Albuquerque, NM; BR = Brewster Co., TX; EP = El Paso Co., TX; HD = Hidalgo Co., NM). The most parsimonious (consensus) tree derived using these characters is shown in Figure 4.

	Cleavage site												
	Mbo I						*Msp* I		*Rsa* I		*Taq* I		*Eco*R V
mtDNA source	a	b	c	d	e	f	a	b	a	b	a	b	a
C. tesselatus, A–E	0	0	0	1	1	0	0	1	0	0	1	1	0
C. tesselatus, E′	0	0	0	1	1	0	0	1	1	0	1	1	0
C. tesselatus, F	1	0	0	1	1	0	0	1	0	1	1	1	0
C. tesselatus, F′	1	1	0	1	1	0	0	1	0	1	1	1	0
C. neomexicanus, LU 1	0	0	0	1	1	0	1	1	0	0	1	1	0
C. neomexicanus, LU2	0	0	0	1	1	0	1	1	1	0	1	1	0
C. neomexicanus, AL	0	0	1	1	1	0	1	1	1	0	1	1	0
C. tigris marmoratus, HD	0	0	0	1	1	0	0	1	0	0	1	1	1
C. t. marmoratus, EP	0	0	0	1	1	1	0	1	0	0	1	1	0
C. t. marmoratus, BR	0	0	0	0	0	0	0	0	0	0	0	0	0

present in single, widely disjunct populations, and they concluded that five others were probably the result of local recombination events. The remaining four genotypes presumably originated from independent hybridizations. This deduction was based on the presence of multiple phosphoglucose isomerase (*Pgi*) and leucyl-alanine peptidase (*Pep*) alleles shared by a few *C. tesselatus* diploids and some *C. septemvittatus*. While independent hybridizations *could* have been responsible for the distribution of the alleles observed in *C. tesselatus*, Parker and Selander (1976) noted that electromorphs of apparently identical migration may be "allelically heterogeneous" (King and Ohta, 1975) and that the current geographic distribution of *C. septemvittatus* with these alleles is widely separated from any *C. tesselatus* that share them. Similarly, the low allozymic diversity found in *C. neomexicanus* is consistent with the hypothesis that, as in the case of *C. tesselatus*, few hybridizations were involved in its formation (Parker and Selander, 1984; Cole et al., 1988).

The mtDNA data offer no evidence for more than three independent hybridizations leading to the four diploid pattern-classes of *C. tesselatus*. Only four restriction-site differences from St were found among all *C. tesselatus* mtDNAs. Three of these were limited to pattern-class F, of which two were shared by all five class-F individuals (Tables 1, 3). It is reasonable to regard the unshared difference as a mutation that arose in one of the F lineages after the formation of *C. tesselatus*, but this seems less likely for two differences shared by all class-F lineages. We regard a separate hybrid origin for pattern-class F as more likely. The fourth difference, a site loss, was noted in only one *C. tesselatus* individual (E′; Table 3). However, two *C. neomexicanus* mtDNAs also lacked this site (Table 3). It is possible, therefore, that the E′ mtDNA reflects a third hybridization event (Fig. 4). However, extensive human-mtDNA comparisons indicate that convergent site losses can occur even when sequence variability is low (Brown, 1980; Cann et al., 1984, 1987). Given this, it is not unreasonable to assume that the site loss in E′ arose postformationally. Thus, it is possible that as few as one hybridization event produced pattern classes C, D, and E, that a second produced class F, and that classes A and B derive from the same event that produced C, D and E, with their triploid condition being due to subsequent hybridization(s).

Overall, no clear correlation can be drawn between the mtDNA and allozyme data sets regarding the absolute number of different hybridizations that produced the diploid pattern-classes of *C. tesselatus*. We analyzed the critical protein loci (*Pgi* and *Pep*) in 20 individual *C. tesselatus* of pattern-classes C and E from the same localities that Parker

and Selander (1976) sampled, using their electrophoretic protocols (kindly provided by E. D. Parker). Although we were able to resolve the same set of alleles that they reported at one of the two localities, the mtDNA restriction analyses revealed no site differences from St. However, the alleles in question are shared by *C. tesselatus* and *C. septemvittatus,* but not by *C. t. marmoratus.* Because mtDNA is a marker for maternal lineages only, it cannot be used to evaluate the hypothesis of multiple origins involving different paternal parents that is suggested by the nuclearly encoded enzyme markers. For this, studies employing either skin grafting or anlysis of nuclear DNA (e.g., restriction-fragment length polymorphisms) ought to be more informative.

The triploid pattern-classes A and B were produced by hybridization of *C. tesselatus* (presumably one or more class-C or class-E lizards) with *C. sexlineatus* (Wright and Lowe, 1967). The suggestion that some class-C individuals may also be triploid (Parker and Selander, 1976; Parker, 1979) is supported by indirect evidence from analysis of mtDNA-size distribution and variation (Densmore et al., 1985). Because these putative triploids occur sympatrically with pattern-class D (see Zweifel, 1965), it is likely that they arose by hybridization between class-D *C. tesselatus* and *C. sexlineatus.* If these independent inferences are correct, this hybridization was almost certainly distinct from the one(s) that produced pattern-classes A and B.

Parthenogenesis in Cnemidophorus

All present data are consistent with the hypothesis that interspecific hybridization can lead directly to the formation of parthenogenetic lineages (see Dessauer and Cole [1989] and Moritz et al. [1989*a*] for recent reviews). Because the ranges of many *Cnemidophorus* overlap, many such hybridizations are possible. The lack of parthenogenetic taxa corresponding to most of these overlaps suggests that hybridization events leading to parthenogenesis are rare, either because hybridization itself is rare or because the production of a hybrid that is also genetically capable of parthenogenetic reproduction is rare. In support of the former interpretation, we know of no authenticated cases of naturally occurring interspecific hybrids between relevant *Cnemidophorus* species except for the unisexuals themselves. In support of the latter, attempts to produce unisexuals by hybridizing bisexual *Cnemidophorus* under laboratory conditions have been unsuccessful, even when the parent species for naturally occurring unisexuals were used. However, it is difficult to assess the effects of the artificial laboratory environment on mating and egg viability in these reconstitution experiments (C. J. Cole, pers. comm., unpubl.).

Regardless of mechanism, the absence of reciprocity and the extremely low levels of nucleotide divergence among *C. tesselatus* and *C. neomexicanus* mtDNAs are consistent with the hypothesis that unisexual-generating hybridizations are rare. Both the range of nucleotide diversity and the number of different genotypes were greater among mtDNAs from *C. t. marmoratus* than among *C. tesselatus* or *C. neomexicanus* (Tables 1–4). Results in other complexes of unisexual *Cnemidophorus* are concordant with this hypothesis (Densmore et al., 1989; Moritz et al., 1989*b*).

Among parthenogenetic *Cnemidophorus,* mtDNA homogeneity appears to be the rule. Other parthenogenetic complexes of *Cnemidophorus* repeat the pattern of having a single mtDNA type present in several morphologically and allozymically distinct, geographically widespread forms (Densmore et al., 1989; Moritz et al., 1989*b*).

Before the genetic mechanisms that give rise to and maintain parthenogenesis can be understood, new genetic data about interspecific hybridization are needed. These can be obtained by field studies of contact zones and by experimental breeding and hybridization studies. The identification of the underlying genetic and molecular mechanisms that affect the viability of interspecific hybrids will significantly increase our understanding of the process of parthenogenesis, both in *Cnemidophorus* and in other unisexual taxa.

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APPENDIX

All *Cnemidophorus* used in these analyses have been deposited as voucher specimens in the herpetological collection of the Natural History Museum of Los Angeles County (LACM). Abbreviated locality data and LACM catalog numbers are presented in the table below; more complete specimen data may be requested from J.W.W.

Taxon	Collection localities	*N*	Voucher specimens
C. neomexicanus	Albuquerque, Bernalillo Co., NM	1	122405
	NE edge of Deming, along RR tracks, Luna Co., NM	2	134347, 134356
C. septemvittatus	Along Río Florida at Ciudad Jimenez, Chihuahua, Mexico	3	121629–121630, 122407
	15.8 mi (by Hwy. 57) NW of Santa Cruz, Coahuila, Mexico	1	130629
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. tesselatus (pattern-class A)	Fremont Co. sanitary landfill, 3 mi N of Florence on Hwy. 61, Fremont Co., CO	8	131869–131876
C. tesselatus (pattern-class B)	Red Top Ranch, ca. 30 mi ENE of Walsenburg, Pueblo Co., CO	2	131877–131878
C. tesselatus (pattern-class C; 17 diploids and 3 [presumed] triploids	Higbee, Otero Co., CO	3	134804–134805, 134807
	Conchas Lake State Park, San Miguel Co., NM	17	128344, 128347–128358, 128360, 134251, 134255–134256
C. tesselatus (pattern-class D)	2 mi S of Higbee on Hwy. 109, Otero Co., CO	3	128338–128340
	3.5 mi S of Higbee, Otero Co., CO	2	128341–128342
	Higbee, Otero Co., CO	3	134803, 134806, 134808
	Conchas Lake State Park, San Miguel Co., NM	4	128345–128346, 128359, 134257
C. tesselatus (pattern-class E)	0.3 mi N of Engle, Sierra Co., NM	4	131886, 131888, 134231–134232
	Ash Canyon, 8.7 mi WSW of Engle and 1.25 mi SE of Elephant Butte, Sierra Co., NM	4	134227–134230
	1 mi S of Engle, Sierra Co., NM	16	134233–134237, 134339–134342, 134344–134350
	Franklin Mountains, E of El Paso, El Paso Co., TX	1	128362
C. tesselatus (pattern-class F)	San Antonio Canyon, Chinati Mountains, Presidio Co., TX	3	128282–128284
	Pinto Canyon, Chinati Mountains, Presidio Co., TX	2	128285, 128361
C. tigris gracilis	Cochise Co., AZ	1	134675
	Tempe, Maricopa Co., AZ	7	127367–127373
C. t. marmoratus	1.6 mi NE of Steins, Hidalgo Co., NM	14	134259–134271, 134273
	Black Gap Wildlife Management Area, Brewster Co., TX	1	130269
	NE of El Paso, El Paso Co., TX	6	130263–130268
C. t. variolosus	11.3 mi (by Hwy. 57) NW of Santa Cruz, Coahuila, Mexico	2	130261–130262
	15.8 mi (by Hwy. 57) NW of Santa Cruz, 12 mi (by Hwy. 57) NNW of La Gamuza, Coahuila, Mexico	2	130259–130260
	1 mi S, 1 mi W of Villa de Garcia, Nuevo Leon, Mexico	1	121626