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Genetics

Tandem duplications in animal mitochondrial DNAs: Variation in incidence and gene content among lizards

(cleavage maps/transfer hybridization/D loop/gene rearrangement)

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ABSTRACT **Size, location, gene content, and incidence were determined for 10 lizard mitochondrial DNA duplications. These range from 0.8 to 8.0 kilobases (kb) and account for essentially all of the observed size variation (17–25 kb). Cleavage-site mapping and transfer-hybridization experiments indicate that each duplication is tandem and direct, includes at least one protein or rRNA gene, and is adjacent to or includes the D loop-containing control region. Duplication boundaries are nonrandomly distributed, and most appear to align with tRNA genes, suggesting that these may play a role in the duplication process. Duplications are infrequent and usually restricted to particular individuals or populations. They appear to be ephemeral; in no case is the same duplication shared by mitochondrial DNAs from closely related species. Mitochondrial DNA duplications occur significantly more often in triploid than diploid lizards and at similar frequencies in hybrids and nonhybrids.**

Although well known for its rapid rate of sequence evolution (1, 2), animal mtDNA also has several highly conserved features, including its genetic content and small size. Virtually all animal mtDNAs studied contain genes for two rRNAs, 22 tRNAs, and the same 13 proteins and have a control region with sequences regulating DNA replication and transcription (3, 4). Their small size [16.0–18.0 kilobases (kb); refs. 4 and 5] is partly a consequence of simple genetic organization: there are no introns, and intergenic spacers are absent or very small (3, 4).

Size differences in the control region, ranging from a few base pairs (bp) to 1 or 2 kb, are common among closely related animal mtDNAs (4, 6). These differences can be due to variation in the number of nucleotides in homopolymer tracts, to variation in the copy number of short tandem repeats, and to the duplication or deletion of unique sequences (4, 6).

Recently, mtDNAs that are 5–10 kb larger than normal have been observed in some lizards (ref. 7; unpublished data), snakes (L. D. Densmore and F. Rose, personal communication), newts (8), frogs (9), fish (M. Hall, personal communication), and nematodes (10). The sequence additions have been characterized for mtDNAs from some lizards (7) and newts (8) and have been shown to be tandem duplications that include protein genes and/or rRNA genes as well as the control region. Similar duplications caused the mtDNA size increases in at least some of the other taxa (M. Hall, personal communication; B. Hyman, personal communication), indicating that the *de novo* duplication of mtDNA coding sequences is a widespread phenomenon.

We have characterized physical, genetic, and evolutionary properties of 10 duplications in mtDNAs from seven species of *Cnemidophorus*. Cleavage-site mapping and transfer-hybridization experiments were used to determine the size, boundaries, and genetic content of each duplication. We also have compared the incidence of mtDNA duplication in diploid vs. triploid and in hybrid vs. nonhybrid nuclear backgrounds.

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MATERIALS AND METHODS

Purification, restriction endonuclease digestion, end-labeling, autoradiography, transfer-hybridization, nick-translation, and cleavage-site mapping of *Cnemidophorus* mtDNA have been described (7, 11–13). All digests were analyzed electrophoretically with both agarose (0.6–2.0%) and polyacrylamide (3.5–6.0%) gels. Radioactive probes were prepared from standard-length *Cnemidophorus* mtDNA, from cloned rRNA sequences of gorilla mtDNA (1.4-kb fragment; ref. 14), and from sequences of the genes for cytochrome *b*, glutamine tRNA, and NADH dehydrogenase subunit 6 (ND6) (Fig. 1*A*). The latter were isolated from digests of cloned *Mus* mtDNA provided by D. Clayton. In sequential hybridizations of the same filter with these probes, the preceding probe was completely removed as described (7).

RESULTS

Physical Characteristics. Size variation was examined in mtDNAs from 306 individuals of seven *Cnemidophorus* species by electrophoretic analysis of restriction endonuclease digests. Of the 306 samples, 263 had standard-size (S) mtDNAs (i.e., between 17.0 and 17.8 kb). Minor size differences (up to a few hundred base pairs) were frequent and appeared to stem from copy-number variation of a 64-bp tandem repeat that occurs in the control region (13). Forty-three samples from seven species had larger (L) mtDNAs (i.e., 18.0–25.0 kb, Table 1). When compared with the S mtDNAs of conspecific individuals, the L mtDNAs correspond to eight different increments, ranging from 0.8 to 8.0 kb in size (Table 1).

Representatives of each L mtDNA were compared to the corresponding S mtDNA by electrophoretic analysis of restriction endonuclease digests (Table 1; see also Fig. 3). Depending on the endonuclease used, the differences between the L and S digests fell into one of three basic types: (*i*) a size increment, α, of one fragment of L (type I); (*ii*) one additional fragment of size α in L (type II); and (*iii*) two or more additional fragments in L that sum to α, of which one differs in size from any S fragment (type III). These three types of difference are most easily explained by a direct tandem duplication of a sequence (size α), in which there is either no cleavage site (type I), one cleavage site (type II), or more than one cleavage site (type III) for a given restriction endonuclease.

Abbreviations: ND1 to ND6, NADH dehydrogenase subunits; S, standard size; L, larger.

Table 1. Size, restriction digest classification, and frequency of 10 mtDNA duplications in seven *Cnemidophorus* species

		cozumela					*uniparens*			
	*exsanguis**	*c. maslini*	*c. cozumela*	*gularis*	*septem-vittatus*	*opatae*†	*I*	*II*	*III*	*inornatus*
L genome, kb	22.2	22.2	25.0	22.1	21.1	21.9	24.0	18.6	18.0	19.1
Duplication, kb	4.8	5.0	8.0	4.8	3.8	4.8	6.8	1.5	0.9	1.1
Frequency	7/25	2/2	11/11	1/21	1/9	1/3	1/32	13/32	1/32	3/29
Restriction enzyme										
Ava I (a)	I	I		I	I					I
*Bam*HI (b)	II 4.8	II 5.0	II 6.6, 1.4‡			I	I			I
Bcl I (c)	I	II 5.0	II 8.0	I	I	III (3.4/3.0) *1.4*	III 3.2, 1.6, *2.0*	I	I	I
*Eco*RI (e)	I	I	I	II 4.8	II 4.0	I	I	II 1.5	II 0.9	I
*Eco*RV (v)	I	I	I	II 4.8	I					I
*Eco*0109 (d)	III 0.8, 3.7, *0.5*	III 0.8, 3.7, *0.5*	III 1.6, 4.1, *2.3*§		III 0.3, 2.1, *1.3*	III (3.6/3.2) 0.3, *1.0*	III 3.3, 0.8, 0.3, *2.4*	II 1.6	II 0.9	II 1.1
*Hin*dIII (h)	I	I	I	II 4.9	II 3.8					I
Nci I (n)	II 4.8			I	I	II 4.8	II 6.8	I	I	I
Nco I (o)		III 2.2, *2.9*	III 4.6, *3.3*		II 3.7	II (4.7/4.3)	II 7.0			II 1.1
Nhe I (g)	No site	III 0.1, *4.8*	III 0.1, *7.8*	III 0.1, *4.7*	II 3.6	II 4.8	I§	I	I	I
Pvu II (p)	II 4.8	II 4.9	II 8.0	I	I	I§	III 0.9, *6.0*			I
Sal I (l)	I	I	I	I	I					I
Spe I (i)		III 0.3, *4.7*	III 0.3, *7.7*	No site	I	III 0.3 (*4.5/4.1*)	III 0.3, *6.6*	I	I	I
Sst II (s)	III 1.7, *3.2*	III 1.7, *3.4*	III 1.7, *6.1*	II 4.7	II 3.8	III 1.7 (*3.1/2.7*)	III 1.7, *5.1*	I	I	I
Xba I (x)	I	I	I	I	No site		I			I

Each restriction enzyme digest is classified according to the types (I, II, and III) described in the text. The sizes of the additional fragments from types II and III digests (in kb) are given where appropriate. For type III digests, the additional fragment that does not comigrate with S fragments is in italics. For the *C. opatae* mtDNA, the heteroplasmic additional fragments are given in parentheses.

*Two other *exsanguis* mtDNA samples had duplications of 6.8 kb and >4.5 kb, but these were not adequately characterized for inclusion here.

†The duplication was heteroplasmic in the *opatae* sample (see text).

‡Secondary modification by a site gain within one copy of the duplicated sequence.

§Secondary modification by a site loss within one copy of the duplicated sequence.

The cleavage-site information was used to map the approximate position of each putative duplication in relation to the cleavage map for its corresponding S genome. This required analysis with 7–15 restriction endonucleases (Table 1). For example, S and L mtDNAs from *C. gularis* were compared by digestion with 12 restriction endonucleases (Table 1). One enzyme, *Spe* I, did not cleave this mtDNA, 6 produced digests in which one L fragment was 4.8 kb larger (type I), 4 produced digests in which L had an additional fragment of 4.8 kb (type II), and 1 produced digests in which L had two unusual fragments of 0.1 and 4.7 kb (type III). These data show that the duplication includes one cleavage site for each of *Eco*RI, *Eco*RV, *Hin*dIII, and *Sst* II and two cleavage sites for *Nhe* I. In relation to the cleavage map for S mtDNAs from *C. gularis*, the duplication includes an *Sst* II site at one end and an *Eco*RV site at the other (Fig. 1).

The locations of the different duplications were compared by aligning the corresponding S cleavage maps (Fig. 1). Three of the 10 duplications (those in *C. c. maslini, C. exsanguis*, and *C. opatae* mtDNAs) have approximately the same size and location. The duplication boundaries were mapped to regions of 0.1–0.6 kb (Fig. 1*A*). These boundaries are nonrandomly distributed. Seven duplications have boundaries within 100 bp of a particular *Eco*0109 cleavage site (Fig. 1*B*). Clustering of boundaries is also evident in other regions (e.g., the left end of the 4.8-kb duplications from unrelated mtDNAs of *C. opatae, C. c. maslini*, and *C. exsanguis*; Fig. 1*A*). However, there are at least 12 different boundaries among the 10 duplications.

In six cases, one copy of a duplicate sequence has been modified by base substitution or length mutation resulting in deviations from the three expected types of L digests. For example, *Bam*HI digestion of *C. c. cozumela* L mtDNA should produce an 8.0-kb type II fragment; instead all samples had two different fragments, of 6.6 and 1.4 kb. Double-digestion experiments showed that an additional *Bam*HI site is present in one copy. Cleavage-site alterations within one copy were also evident in the three cases where the number of unusual fragments was one less than predicted (Table 1). A 150-bp deletion was observed in one copy of the 4.8-kb duplicate sequence from an individual of *C. exsanguis* (7). Additional complexity arises when the duplications span a region of short tandem repeats that are themselves subject to variation in copy number. This is exemplified by the *C. opatae* L mtDNA (Fig. 2). In this sample, heteroplasmy for type I modifications showed that both L and S genomes were present. Further, the L genomes were of two types (L_1 and L_2) that differed in the length of one of the tandem repeat regions (Fig. 2).

Gene Content. The mtDNA gene order, including the arrangement of tRNA genes, is identical in all vertebrates studied (4). The cleavage-site maps of *Cnemidophorus* mtDNAs and, thus, the duplications can be aligned with the genetic map for the mtDNA of vertebrates by using two highly conserved *Sst* II sites that occur in the rRNA sequences (15) and the D-loop structure (11). Of the 10 duplications, 7 include the entire control region and one or more of the rRNA genes, and 5 span the gene for cytochrome *b* (Fig. 1*A*). The maximum extensions of the duplications are represented by the 6.8-kb duplication in *C. uniparens*, which includes at least part of the ND2 gene sequence, and those from *C. c. cozumela* and *C. gularis*, which include ND6 and at least part of ND5 genes. The most consistent feature of the genetic content of the duplications is the presence of sequences in or adjacent to the control region (Fig. 1*A*).

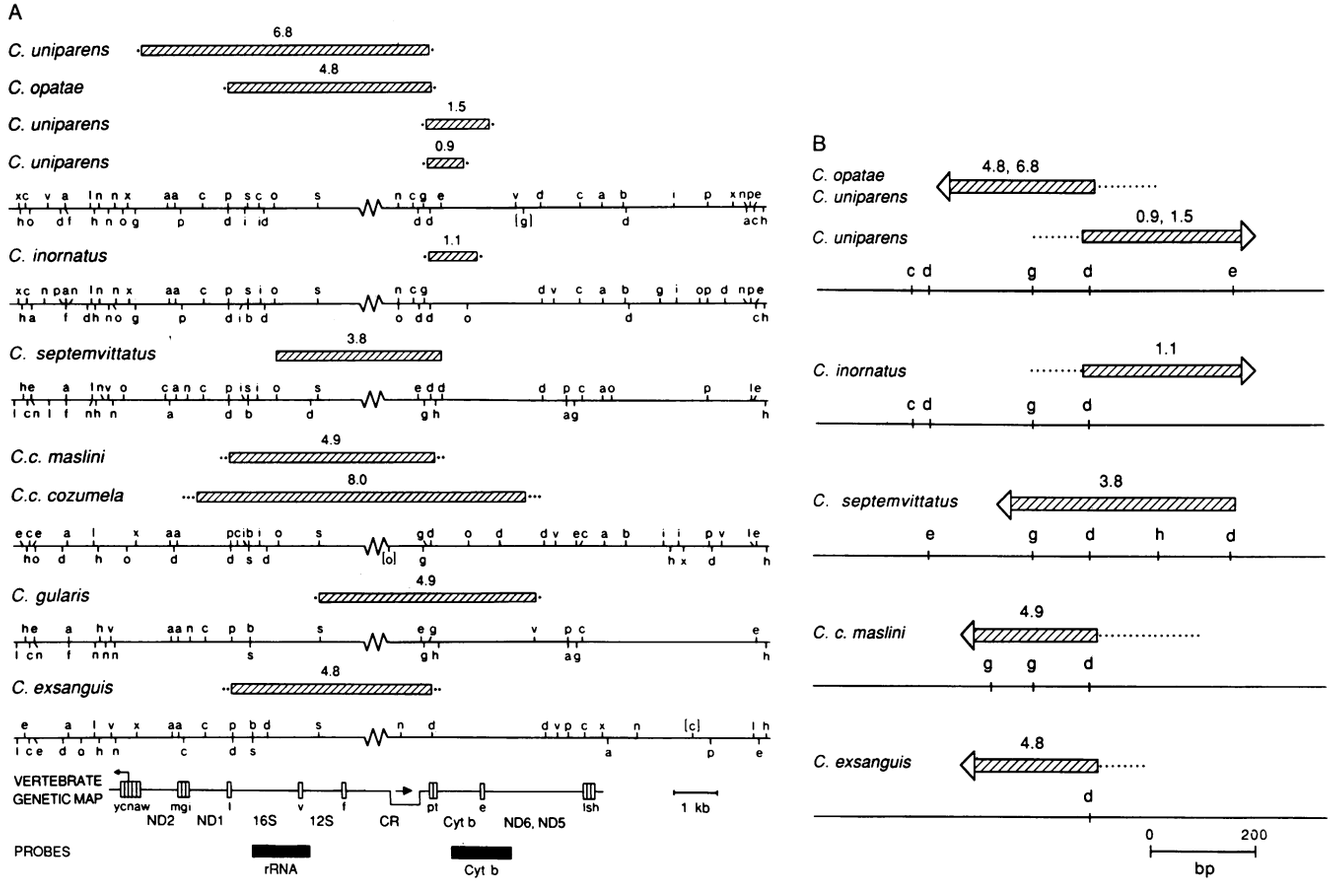

FIG. 1. Characterizations of 10 mtDNA duplications. (*A*) Location and size of the duplications in relation to their corresponding S mtDNA cleavage maps. Saw-tooth sections indicate regions of small tandem repeats. Bars indicate the regions definitely included within duplications; dots indicate the maximum possible extensions. Numbers over the bars indicate the duplication sizes (in kb). The approximate alignment with part of the vertebrate mtDNA genetic map is shown. The rRNA and "Cyt *b*" probes are located at the bottom. ND1, ND2, ND5, and ND6, subunits of NADH dehydrogenase gene; 12S and 16S, small and large rRNAs; Cyt *b*, cytochrome *b*; CR, control region. Cleavage site abbreviations are explained in Table 1. The single-letter code on the vertebrate genetic map represents tRNA genes (see ref. 4). (*B*) Detail of duplication boundaries from the region of the cytochrome *b* gene to the control region junction. Symbols and abbreviations are as in *A*.

The above inferences about the genetic content of the duplications were tested by transfer-hybridization experiments. Representative L mtDNAs were digested with restriction endonucleases that produced type II or III fragment patterns, electrophoresed, transferred to a nylon membrane, and sequentially hybridized with radioactive probes specific for S mtDNA, mitochondrial rRNA genes, and the cytochrome *b*–ND6 gene region. As expected, all duplicated sequences hybridized with S mtDNA (e.g., see Fig. 3). Those from *C. uniparens* (6.8 kb only), *C. opatae*, *C. septemvittatus*, *C. c. cozumela*, *C. c. maslini*, and *C. exsanguis* hybridized to the rRNA gene probe (e.g., see Fig. 3), and those from *C. uniparens* (1.5 and 0.9 kb only), *C. inornatus*, *C. c. cozumela*, and *C. gularis* hybridized to the cytochrome *b*–ND6 probe (e.g., see Fig. 3).

Although approximate, the alignment of the mtDNAs with the genetic map suggests that most of the duplication boundaries are at or near tRNA genes. The region in which seven of the boundaries occur (Fig. 1*B*) appears to correspond to the proline and threonine tRNA genes. Similarly, the positions of the left ends of the *C. opatae*, *C. c. maslini*, and *C. exsanguis* duplications appear to be at a leucine tRNA gene (Fig. 1*A*). Within the limits of cleavage site mapping, 12 of the 20 boundaries appear to be located at tRNA genes, and a further 3 are near tRNA genes; the remaining 5 appear to be located well within structural genes (Fig. 1*A*).

Evolutionary Properties. The mtDNA duplications were generally present at low frequency (Table 1), and each was restricted to a single lizard within a population or to a single population within a species. All *C. c. maslini* and *C. c. cozumela* mtDNAs examined have duplications, but only one population of each was surveyed. In no case do closely related species share a particular mtDNA duplication. This is particularly notable for mtDNAs from *C. inornatus*, *C. opatae*, and *C. uniparens*, since the last two species acquired their mtDNAs from the first (L. D. Densmore, C.M., J. W. Wright, and W.M.B., unpublished data). Although some individuals from each of these species have duplications, there are different boundaries in each, and the *C. inornatus* mtDNAs that are most closely related to those from *C. opatae* and *C. uniparens* lack duplications altogether. Conversely, 4.8-kb duplications with similar boundaries have evolved independently in distantly related mtDNAs of *C. c. maslini*, *C. exsanguis*, and *C. opatae*.

The genus *Cnemidophorus* is unusual in that approximately one-third of its 45 species are parthenogenetically reproducing interspecific hybrids that are diploid or triploid (reviewed in refs. 16 and 17). The effect of nuclear background on the incidence of mtDNA duplications can be assessed by comparing mtDNAs from diploid and triploid hybrid-parthenogens to those from the bisexual (nonhybrid) species that provided their mtDNA. mtDNA duplications occur in all three types of lizard: diploid nonhybrids, diploid hybrids, and triploid hybrids (Table 2). Several of the frequencies in Table 2 are biased by the presence of multiple individuals bearing the same mtDNA duplication. When

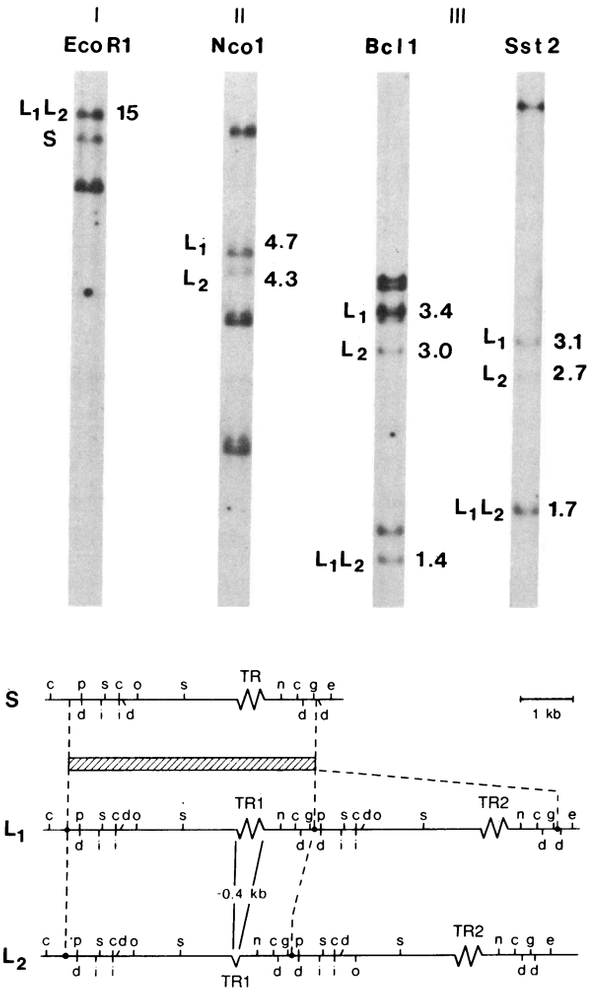

FIG. 2. Cleavage analysis of mtDNA from the heteroplasmic *C. opatae* sample. (*Upper*) Agarose gel electrophoresis of types I, II, and III restriction endonuclease digests (defined in text). The presence of both S and L genomes is demonstrated by the *Eco*RI digest. The presence of two L genomes, L_1 and L_2, that differ by 400 bp can be seen in the *Nco* I, *Bcl* I, and *Sst* II digests. (*Lower*) Cleavage maps showing the length changes inferred among S, L_1, and L_2. L_1 is derived from S by a 4.8-kb duplication. L_2 can be derived from L_1 by the loss of 400 bp from one of the two sets of tandem repeats (TR1).

these redundancies are removed (Table 2), the incidence of mtDNA duplications does not differ significantly between hybrids and nonhybrids [log-likelihood ratio (G_{adj}) = 2.80; $0.1 > P > 0.05$] but is significantly higher in triploids than in diploids (G_{adj} = 6.91; $0.01 > P > 0.005$).

DISCUSSION

The above evidence extends our previous observation (7) that L mtDNAs arise from S mtDNAs by direct tandem duplications of sequences that include structural genes. That the additional DNA is derived from mtDNA is shown by its hybridization to probes made from S mtDNA. That it results from a direct, tandem duplication is shown by the three types of fragment patterns produced by restriction endonucleases. That the duplications include structural genes is confirmed by transfer-hybridization experiments using probes for specific genes.

There is evidence for divergence between duplicated sequences within the same molecule. These can differ from one another by the gain/loss of cleavage sites (base substitution) or by length mutations. The latter may be due to sequence deletions (7) or to differences in the copy number of short, tandemly repeated sequences. Preliminary cleavage-site comparisons of mtDNA duplications that are geographically widespread (in *Cnemidophorus sexlineatus* and *Heteronotia binoei*; C.M., D. Vyas, and W.M.B., unpublished data) indicate that the amount of divergence due to cleavage-site changes or deletions can be considerable. In at least one case, the L* mtDNA of *C. exsanguis* (7), a deletion in one copy of the duplicated sequence may have disrupted the functions of one set of rRNA(s).

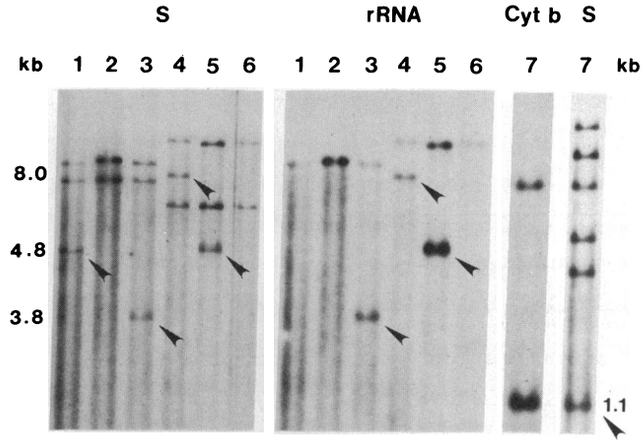

FIG. 3. Comparisons of L and S fragments after hybridization with radioactive probes. Lane digests: 1–3, *Eco*RI; 4–6, *Pvu* II; 7, *Eco*0109. Lane fragments: 1, *C. gularis* (L, 4.8 kb); 2, *C. gularis* (S); 3, *C. septemvittatus* (L, 3.8 kb); 4, *C. c. cozumela* (L, 8.0 kb); 5, *C. c. maslini* (L, 4.8 kb); 6, *C. angusticeps* (S) and *C. inornatus* (L, 1.1 kb). Arrows indicate additional fragments. S, rRNA, and Cyt *b* refer to radioactive probes of the entire S mtDNA and the regions shown in Fig. 1*A*. The observed hybridizations confirm the sequence content of the duplications predicted by Fig. 1*A*.

Duplications appear to be slightly more common in triploid than in diploid lizards (Table 2). Notably, mtDNA duplications in *Heteronotia binoei* also appear to be restricted to the triploid hybrid-parthenogenetic form. However, the majority of triploid *Cnemidophorus* lack mtDNA duplications, and some duplications are present in animals that are not triploid. The evidence from *Cnemidophorus* does not support the suggestion (8) that large DNA insertions are more frequent in hybrids.

Table 2. Comparisons of duplication incidence in mtDNAs from hybrid vs. nonhybrid and diploid vs. triploid nuclear backgrounds

	Nuclear background			
		Hybrid		
mtDNA	Non-hybrid	Diploid	Triploid	Total
C. inornatus	3/29 (1)	0/3	16/51 (4)	16/54 (4)
C. tigris	0/38	0/75	0/9	0/84
C. costatus	0/16	—	9/25 (2)	9/25 (2)
C. angusticeps	0/2	13/24 (2)	—	13/24 (2)
C. gularis	2/30 (2)	0/4	—	0/4
Total	5/115	13/106	25/85	38/191
Duplication incidence	3/113	2/95	6/66	8/161

The mtDNAs from parthenogens are listed according to the maternal bisexual species from which they are derived. The proportions cited are the number of individuals with L mtDNAs divided by the total number of individuals in the sample. The numbers in parentheses indicate the number of distinct duplications in each category. The duplication incidence was calculated by removing redundant individuals having the same duplication.

The restricted geographic and/or phylogenetic distributions of the mtDNA duplications in *Cnemidophorus*, *Heteronotia*, and other species (*Triturus*, ref. 8; *Culaea*, M. Hall, personal communication) suggest that they are relatively short-lived on an evolutionary time scale. This could result from a purely mechanistic bias, if one copy of the duplication were frequently and efficiently excised, or from a selective bias, if duplications disrupted mtDNA metabolism. Large molecules may be less efficiently replicated; a similar suggestion was made to account for a slight bias against larger mtDNAs in crickets and flies (18–20). Such intracellular competition requires the presence of L and S genomes (heteroplasmy), which appears to be rare (1/43 *Cnemidophorus*). If this mechanism is responsible for the loss of L genomes during evolution, then the selection against them must be strong. Further studies are needed both on the long-term inheritance and on the metabolic capabilities of the L genomes.

In comparison with animal mtDNAs, duplications are common among mtDNAs from plants, fungi, and protists, and in chloroplast DNAs of land plants (5, 21, 22). The *Cnemidophorus* mtDNA duplications appear to be ephemeral, whereas land-plant chloroplast DNA duplications are long-lived (23). Duplications in *Cnemidophorus* mtDNA also differ from those of most other mtDNAs and chloroplast DNAs (*i*) in being direct rather than inverted and (*ii*) in the rarity of recombination-associated events such as high-frequency excision/isomerization and the rapid homogenization of the duplicated sequences (see Table 3 in ref. 22). These observations accord with the apparent lack of intermolecular recombination mechanisms in animal mtDNA (reviewed in refs. 6 and 24). The *Cnemidophorus* mtDNA duplications are most similar to those of *Euglena* chloroplast DNA (22) in being direct and tandem and in appearing to lack the means to homogenize duplicate sequences.

The large mtDNA duplications in *Cnemidophorus* and other organisms could arise through replication slippage (25) as suggested for smaller tandem repeats and homopolymer tracts in mtDNAs (reviewed in refs. 4 and 6). This process would be facilitated by the presence of dispersed short repeats (26, 27). Cleavage-site mapping suggests that 15 of the 20 duplication boundaries are at or near tRNA genes. Perhaps portions of some tRNA genes are similar enough for them to function as dispersed repeats. Alternatively, their secondary structure may be important. All of the duplicated sequences lie within the region that is single-stranded for a lengthy period during the highly asymmetric replication of mtDNA (28). The tRNA genes might fold while single-stranded to provide secondary structures that somehow act as signals for the duplication process. The proposed alignment of duplication boundaries with tRNA genes is testable by sequence analysis, which may also provide additional information about duplication mechanisms.

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1. Brown, W. M., George, M., Jr., & Wilson, A. C. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1967–1971.
2. Vawter, L. & Brown, W. M. (1986) *Science* **234,** 194–196.
3. Attardi, G. (1985) *Int. Rev. Cytol.* **93,** 93–145.
4. Brown, W. M. (1985) in *Molecular Evolutionary Genetics*, ed. MacIntyre, R. J. (Plenum, New York), pp. 95–130.
5. Sederoff, R. R. (1984) *Adv. Genet.* **22,** 1–108.
6. Moritz, C., Dowling, T. E. & Brown, W. M. (1987) *Annu. Rev. Ecol. Syst.* **18,** 269–292.
7. Moritz, C. & Brown, W. M. (1986) *Science* **233,** 1425–1427.
8. Wallis, G. P. (1987) *Heredity* **58,** 229–238.
9. Kessler, L. G. & Avise, J. C. (1985) *Mol. Biol. Evol.* **2,** 109–125.
10. Powers, T. O., Platzer, E. G. & Hyman, B. C. (1986) *Curr. Genet.* **11,** 71–77.
11. Brown, W. M. & Wright, J. W. (1979) *Science* **203,** 1247–1249.
12. Wright, J., Spolsky, C. & Brown, W. M. (1983) *Herpetologica* **39,** 410–416.
13. Densmore, L. D., Wright, J. W. & Brown, W. M. (1985) *Genetics* **110,** 689–707.
14. Hixson, J. E. & Brown, W. M. (1986) *Mol. Biol. Evol.* **3,** 1–18.
15. Carr, S. M., Brothers, A. J. & Wilson, A. C. (1987) *Evolution* **41,** 176–190.
16. Wright, J. W. (1978) *Science* **201,** 1152–1154.
17. Darevsky, I. S., Kupriyanova, L. A. & Uzzell, T. (1985) in *Biology of the Reptilia*, eds. Gans, C. & Billett, F. (Wiley, New York), Vol. 15B, pp. 412–526.
18. Solignac, M., Genermont, J., Monnerot, M. & Mounolou, J.-C. (1984) *Mol. Gen. Genet.* **197,** 183–188.
19. Hale, L. R. & Singh, R. S. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 8813–8817.
20. Rand, D. M. & Harrison, R. G. (1986) *Genetics* **114,** 955–970.
21. Clark-Walker, G. D. (1985) in *The Evolution of Genome Size*, ed. Cavalier-Smith, T. (Wiley, New York), pp. 277–297.
22. Palmer, J. D. (1985) *Annu. Rev. Genet.* **19,** 325–354.
23. Palmer, J. D. & Stein, D. B. (1986) *Curr. Genet.* **10,** 823–833.
24. Brown, W. M. (1983) in *Evolution of Genes and Proteins*, eds. Nei, M. & Koehn, R. K. (Sinauer, Sunderland, MA), pp. 62–88.
25. Streisinger, G., Okada, Y., Emrich, J., Newton, J., Tsugita, A., Terzaghi, E. & Inouye, M. (1966) *Cold Spring Harbor Symp. Quant. Biol.* **31,** 77–84.
26. Efstratiadis, A., Posakony, J. W., Maniatis, T., Lawn, R. M., O'Connell, C., Spritz, R. A., DeRiel, J. K., Forget, B. G., Weissman, S. M., Slightom, J. L., Blechl, A. E., Smithies, O., Baralle, F. E., Shoulders, C. C. & Proudfoot, N. J. (1980) *Cell* **21,** 653–668.
27. Albertini, A. M., Hofer, M., Calos, M. P. & Miller, J. H. (1982) *Cell* **29,** 319–328.
28. Clayton, D. A. (1982) *Cell* **28,** 693–705.