ganglion (*1*, *2*), while in the chicken the pineal gland contains an endogenous oscillator and a photoreceptor for the *N*-acetyltransferase rhythmicity.

Menaker and his associates have shown that the free-running circadian rhythms of locomotor activity and body temperature in the house sparrow in constant darkness were abolished by pinealectomy and restored by transplantation of pineal gland (*17*). They further showed that the phase of the rhythm of locomotor activity in the recipient sparrow is determined by the phase of the rhythm of the pineal-donor sparrow. On the basis of these observations, they proposed that the avian pineal gland is an endogenous driving oscillator for the locomotor activity rhythm. Hendel and Turek have reported that melatonin controls the running activity rhythm in sparrow (*18*). These observations are consistent with the results reported here that the activity of serotonin *N*-acetyltransferase that regulates the synthesis of melatonin exhibits a circadian rhythm in isolated chicken pineal glands.

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8. Four or five pineals were placed on a nylon mesh in a Falcon plastic dish (5 cm in diameter) containing 4.5 ml of culture medium. The culture medium consisted of a Fitton-Jackson modification of BGJb medium (Gibco) supplemented with 10 percent fetal calf serum, 2 m*M* L-glutamine, ascorbic acid (0.3 mg/ml), streptomycin (100 μg/ml), and penicillin (100 U/ml). Organ culture was carried out at 40°C in an atmosphere of 95 percent O_2 and 5 percent CO_2 in a transparent incubator box placed in a CO_2 incubator. The inside of the incubator was kept dark. When indicated, a fluorescent lamp (30 W) provided light through a glass door incubator. The intensity of the illumination was 900 to 1100 lux at the level of dishes.
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Mitochondrial DNA Analyses and the Origin and Relative Age of Parthenogenetic Lizards (Genus *Cnemidophorus*)

Abstract. *Morphological, karyological, and allozyme analyses indicate that the parthenogenetic lizards* Cnemidophorus neomexicanus *and diploid* C. tesselatus *are hybrids formed, respectively, by crosses involving the bisexual species* C. tigris *and* C. inornatus, *and* C. tigris *and* C. septemvittatus. *Mitochondrial DNA, which is inherited maternally, was obtained from each of these species. Analyses of the mitochondrial DNA's and their restriction endonuclease digestion products by electron microscopy and agarose gel electrophoresis support the hybridization hypothesis by indicating that* C. tigris *(specifically the subspecies* marmoratus*) was the maternal parent species for both* C. neomexicanus *and* C. tesselatus. *Furthermore, these data imply that these two parthenogenetic species are younger than some races of* C. tigris.

Approximately 13 species of whiptail lizards (*Cnemidophorus*) are known to reproduce parthenogenetically (*1*). Ten of these species, including *C. neomexicanus* and *C. tesselatus*, occur in the southwestern United States and northern Mexico. Analyses of karyology and morphology suggested that *C. neomexicanus* and diploid *C. tesselatus* originated, respectively, by hybridization between the bisexual species *C. tigris* and *C. inornatus* (*2*), and between *C. tigris* and *C. septemvittatus* (*3*). Subsequent analyses of allozymes (*4*) added support to this hypothesis by demonstrating that both parthenogenotes are heterozygous for alleles unique to one or the other of the postulated parental spe-

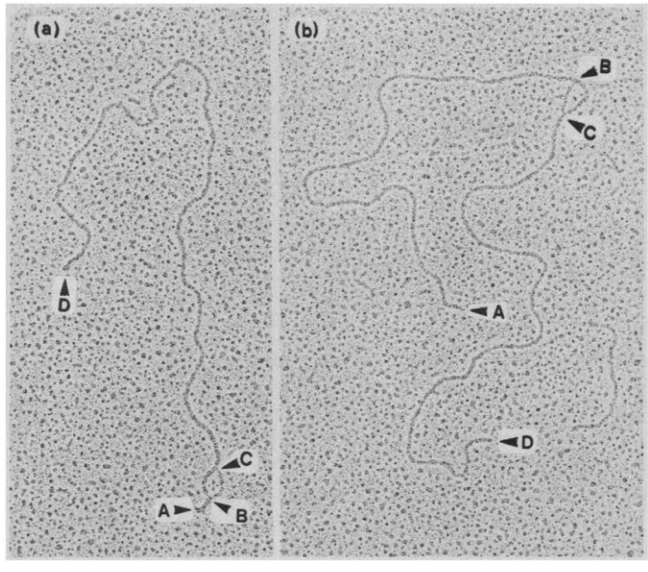

Fig. 1. Electron micrographs of fragments of mitochondrial DNA that contain the respective origins of DNA replication from (a) *Cnemidophorus inornatus* and (b) *C. tigris mundus*. The lengths AB and CD define the position of the D-loop, BC, on the fragment (*8*). During replication, D-loops expand unidirectionally, thus decreasing either AB or CD. The origin, defined as the fixed fork of the D-loop, and the direction of replication are determined by measurements of fragments that contain expanding D-loops (*8*). For the two examples above, the origin is at C, and the direction is toward A. Points A and D indicate the positions of the Eco R1 recognition sites 1 and 3 in *C. inornatus* and sites 3 and 5 in *C. t. mundus* (Fig. 3) (Table 1).

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Table 1. The recognition sites for Eco R1 and *Hind* III in mitochondrial DNA of *Cnemidophorus* species. Numbers correspond to sites mapped in Fig. 3.

Cnemidophorus species	Recognition sites	
	Eco R1	Hind III
inornatus	1,3	3,4,5,6
gularis	1,3,5	1,3,4,5,6
septemvittatus	1,2,3,4,5	1,3,4,5,6
tigris		
mundus	3,4,5	2,3,4,5,6
tigris	3,4,5	2,3,4,5,6
variolosus	2,3,5	Unknown
gracilis	3,4,5	3,4,5,7
marmoratus	3,4,5	3,4,5,6,7
neomexicanus	3,4,5	3,4,5,6,7
tesselatus	3,4,5	3,4,5,6,7

cies. Recently the role of hybridization in the origin of parthenogenetic lizards has been questioned, especially the involvement of *C. tigris* in the formation of *C. neomexicanus* (*5*).

We demonstrate here that comparative analyses of mitochondrial DNA (mtDNA), known to be maternally inherited (*6*), yield data that can be used to determine the species identity of the females that contributed the eggs that produced the first *C. neomexicanus* and *C. tesselatus*.

The mitochondrial genome in vertebrates is a closed circular duplex DNA with a unidirectional mode of replication (*7*). The position of the origin of replication (at one end of the D-loop) (Fig. 1) and the direction of replication can be determined with electron microscopy (*8*). Animal mtDNA can be cleaved by type II restriction endonucleases (*8*). When these enzymes encounter a specific DNA base sequence, they make a double-strand cleavage within the sequence (*9*). For example, the sequence recognized by the endonuclease Eco R1 is G ↓ AATTC and by the endonuclease Hind III is A ↓ AGCTT (the arrows indicate the sites of cleavage) (*9*). Thus, the circular mtDNA molecules are cleaved into fragments, the number and size of which depend directly on the number and positions of recognition sites or sequences. The size and relative order of the genome fragments can be determined by electron microscopy and by agarose gel electrophoresis, allowing the recognition sites to be mapped relative to the D-loop (*8*). For comparative purposes, homology of recognition sites between species is assumed when cleavages occur at the same (±1 percent) map position relative to the origin and direction of replication. There is evidence (*8, 10*) that the gene content and relative gene order in animal mtDNA may be highly conserved. However, the rate of mtDNA sequence evolution has been demonstrated to be at least as fast as that of the nonrepetitive portion of nuclear DNA (*6, 8, 11, 12*) and some estimates indicate an even faster rate (*8, 12*). Because of these properties, analyses of mtDNA can be especially useful in assessing relationships of closely allied species.

Mitochondrial DNA was prepared from heart, kidney, and liver tissues from individuals (*13*) of four bisexual species (*C. inornatus*, *C. gularis*, *C. tigris*, and *C. septemvittatus*) and two parthenogenetic species (*C. neomexicanus* and *C. tesselatus*) (*14*) by the procedure outlined by Brown and Vinograd (*8*). Included in the samples for *C. tigris* were individuals of the subspecies *gracilis*, *marmoratus*, *mundus*, *tigris*, and *variolosus*. To facilitate direct comparisons,

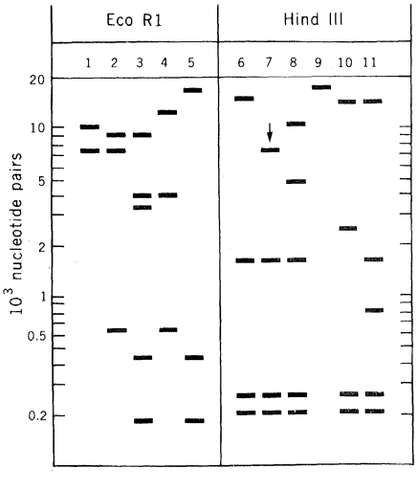

Fig. 2. Diagram of bands observed after agarose gel electrophoresis of the fragments produced by Eco R1 or Hind III digestion of mitochondrial DNA from *Cnemidophorus* species. The fragment sizes, expressed as numbers of nucleotide pairs, are based on estimates from both gel electrophoresis and electron microscopy (*15*). Lanes 1 to 5 represent the bands obtained by Eco R1 digestion of mitochondrial DNA from: lane 1, *C. inornatus*; lane 2, *C. gularis*; lane 3, *C. septemvittatus*; lane 4, *C. tigris variolosus*; lane 5, *C. tigris* (*mundus*, *tigris*, *gracilis*, *marmoratus*), *C. neomexicanus*, and *C. tesselatus*. Lanes 6 to 11 represent the bands obtained by Hind III digestion of mitochondrial DNA from: lane 6, *C. inornatus*; lane 7, *C. gularis* and *C. septemvittatus*; lane 8, *C. tigris* (*mundus* and *tigris*); lane 9, *C. tigris variolosus*; lane 10, *C. tigris gracilis*; lane 11, *C. tigris marmoratus*, *C. neomexicanus*, and *C. tesselatus* (*14*). The arrow in lane 7 indicates a band containing two fragments of essentially equal size. The Hind III band for *C. tigris variolosus* was obtained by a single determination using mitochondrial DNA from only one individual and thus is regarded as tentative.

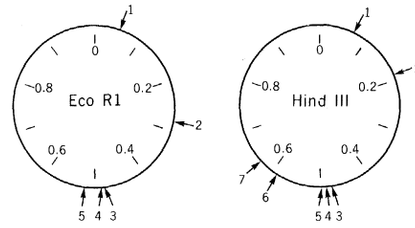

Fig. 3. Composite maps of mitochondrial DNA molecules for the *Cnemidophorus* species with the most probable sites of Eco R1 and Hind III cleavage plotted. The origin of DNA replication is at 0, replication proceeding clockwise. Numbered arrows indicate all cleavage sites revealed in the analyses. The sites present in the taxa sampled are described in the text and Table 1.

the sizes of the intact mitochondrial genomes for four species (*C. tigris mundus*, *C. inornatus*, *C. neomexicanus*, and *C. tesselatus*) were determined by contour length measurements from electron micrographs. No significant differences in size were found among the four genomes (*15*).

In contrast to the size homogeneity of the intact genomes, the sizes and numbers of mtDNA fragments produced by Eco R1 or Hind III restriction endonuclease digestion differ for most of the taxa. The sizes of the Eco R1-generated fragments (Fig. 2) differed among the samples from the four bisexual species, but the fragment sizes from *C. neomexicanus*, *C. tesselatus*, and four of the five subspecies of *C. tigris* were identical. The Hind III fragment sizes (Fig. 2) from most of the bisexual species (though not from *C. gularis* and *C. septemvittatus*) and most of the subspecies of *C. tigris* (though not from *mundus* and *tigris*) also differed. The Hind III fragment sizes from *C. neomexicanus*, *C. tesselatus*, and *C. tigris marmoratus* were identical.

Electron microscopy was used to determine the positions of the Eco R1 and Hind III cleavage sites in the mtDNA of *C. inornatus*, *C. tigris mundus*, *C. tesselatus*, and *C. neomexicanus* relative to the origin and direction of replication. Cleavage maps were prepared from these data. From the data on fragment sizes obtained by gel electrophoresis we have also been able to propose more tentative maps for the remaining taxa (Fig. 3) (Table 1). The analyses revealed a total of five Eco R1 and seven Hind III recognition sites. Both parthenogenetic species had Eco R1 sites 3, 4, and 5 and Hind III sites 3, 4, 5, 6, and 7. These conditions are shared only with *C. tigris*, the former with four of the five races and the latter with *C. t. marmoratus*. Thus,

on the basis of both data sets, *C. tigris* (but clearly not *C. inornatus* or *C. septemvittatus*) is identified as the most likely maternal parent species for both *C. neomexicanus* and *C. tesselatus*. Moreover, the Hind III data indicate that *C. t. marmoratus* was the particular geographic race that was involved in the hybridizations. This implies that the formation of both parthenogenetic species has occurred more recently than the divergence of some races of *C. tigris* (*16*).

This restriction endonuclease analysis of mtDNA of bisexual and parthenogenetic species of *Cnemidophorus* indicates a great utility for the approach. In addition to yielding data for estimating maternal parentage of parthenogenetic organisms, the focus of this study, the results underscore its potential usefulness in assessing evolutionary relationships of bisexual organisms.

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14. R. G. Zweifel [*Am. Mus. Novit.* **2235**, 1 (1965)] characterized six pattern classes (A to F) of *C. tesselatus*. It was later determined that pattern classes C to F are diploid and A and B triploid (*3*). All individuals of *C. tesselatus* used in this analysis are referable to pattern class E.
15. The genome sizes (mean number of base pairs ± standard deviation) for mtDNA's of *C. tigris mundus*, *C. inornatus*, *C. neomexicanus*, and *C. tesselatus* were, respectively, 17,600 ± 500 ($N = 19$ molecules measured), 17,400 ± 200 ($N = 20$), 17,300 ± 500 ($N = 27$), and 17,500 ± 400 ($N = 21$). Bacteriophage PM2 DNA was used as an internal size standard for contour length measurements. This DNA has 10,000 ± 250 base pairs, calculated by comparing its contour length with that of bacteriophage ϕX174 DNA (W. Brown, unpublished data). The ϕX174 genome contains ~5375 bases [F. Sanger, G. M. Air, B. G. Barrell, N. L. Brown, A. R. Coulson, J. C. Fiddes, C. A. Hutchinson III, P. M. Slocombe, M. Smith, *Nature (London)* **265**, 687 (1977)].
16. This conclusion is predicated on two assumptions: (i) *C. tigris* as a species is monophyletic and (ii) the presence of Hind III recognition sites 3, 4, 5, and 6 in four species representing two species groups indicates that they are conserved or primitive sites, at least within *C. tigris*.
17. We thank M. Bergen-Wright, R. L. Bezy, and S. Smith-Brown for assistance in the field, discussions, and criticisms; N. Hadley, N. J. Scott, A. P. Wright, and J. B. Wright for providing lizards for our study; A. Landazuri O., M. L. Cossio G., G. Quiñones L., and I. Ibarrola B. of the Direccion General de la Fauna Silvestre for facilitating our work in Mexico; J. Vinograd (deceased) and H. M. Goodman for providing laboratory facilities. Supported in part by NSF grant DEB 76-20599.
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Modified Bases Characterized in Intact DNA by Mass-Analyzed Ion Kinetic Energy Spectrometry

Abstract. *Pyrolysis of DNA into a chemical ionization source yields protonated bases and other base-containing ions. Kinetic energy spectra allow the characterization of the bases 5-methylcytosine and 1-methyladenine from underivatized salmon sperm DNA. Isomeric bases are distinguishable with this technique.*

A mass spectrometric method has been developed for the determination of the four common bases, as well as modified bases, in intact DNA. It is used to show the presence of 1-methyladenine (1-MeAde) in salmon sperm DNA. The procedure depends on kinetic energy analysis (*1*) to identify the products of collision-induced dissociation of characteristic ions in a reversed-geometry mass spectrometer (*2*) fitted with a chemical ionization (CI) ion source. This method (*3*) retains the high sensitivity of techniques (*4*) in which elemental compositions from high-resolution mass spectrometry are used to characterize the base. Our technique allows the detection of components in a mixture by providing structural information on ions which are directly formed from given molecules.

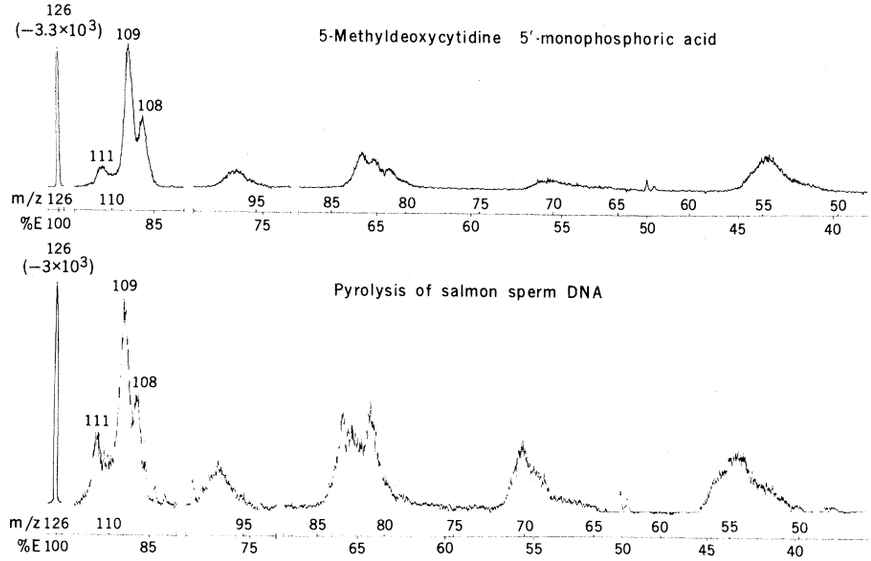

Fig. 1. A MIKE spectrum of mass 126^+ salmon sperm DNA is compared to mass 126^+ from 5-methyldeoxycytidine phosphate. The abscissa is calibrated in terms of both the mass and the kinetic energy of the fragment ions.

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