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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13. Voucher specimens were prepared from all individuals used in the analyses ($N = 32$) and are deposited in the Natural History Museum of Los Angeles County (LACM). Abbreviated locality data and catalog numbers are presented here: more complete data are available on request. *Cnemidophorus gularis*, Nuevo Leon: near Mina (121627) and near Villa de Garcia (121628); *C. inornatus*, Chihuahua: near Samalayuca (122408), and New Mexico: near Hatch (121635-36); *C. neomexicanus*, New Mexico: near Albuquerque (122405, 127357-59), near Socorro (127354-56), and near Hatch (121621); *C. septemvittatus*, Chihuahua: near Rio Florida (121629-30, 122407); *C. tesselatus*, Chihuahua: near Meoqui (121623), and New Mexico: near Hatch (121622) and near Santa Rosa (127362); *C. tigris gracilis*, Arizona: near Tempe (127367-73); *C. t. marmoratus*, Chihuahua: near Meoqui (121624); *C. t. mundus*, California: San Benito County (127364); *C. t. tigris*, Nevada: Mineral County (127365-66); and *C. t. variolosus*, Nuevo Leon: near Villa de Garcia (121626).
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*.
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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.

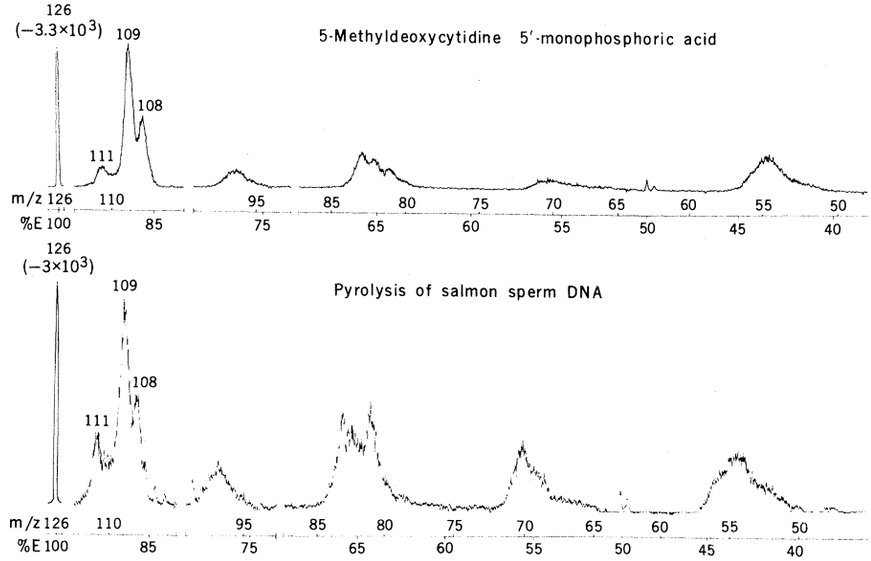

Fig. 1. A MIKE spectrum of mass 126^+ salmon sperm DNA is compared to mass 126^+ from 5-methyldeoxycytidine phosphate. The abscissa is calibrated in terms of both the mass and the kinetic energy of the fragment ions.

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The mass-analyzed ion kinetic energy (MIKE) spectrum (Fig. 1) of the ion m/z 126^+ from intact, underivatized salmon sperm DNA exemplifies the power of the technique by establishing the presence of 5-methylcytosine (5-MeCyt), which is present as less than 2 percent of the total base content (*5*).

The procedure involves three levels of simultaneous separation and analysis. The first is slow pyrolysis of the intact lyophilized submilligram DNA sample on a standard heated direct insertion probe in the ionization chamber of a chemical ionization source (*6*). The temperature (175° to 250°C) of the pyrolysis determines the composition of the ion beam and can be optimized to enhance a specific component relative to its abundance in the mixture. The second step is mass analysis by a magnetic sector, which selects the ions characteristic of the component being studied and directs them into a collision region where they undergo induced fragmentations. The kinetic energies of the fragment ions are then analyzed by an electric sector to give the resulting MIKE spectrum, which is characteristic of the structure of the mass-analyzed ion. The MIKE spectrum is solely dependent on the composition of the mass-analyzed ion beam. A beam free from interfering ions gives a reproducible energy spectrum that is independent of ion source conditions. This procedure can be thought of as insertion of a structurally diagnostic probe between ion mass analysis and detection.

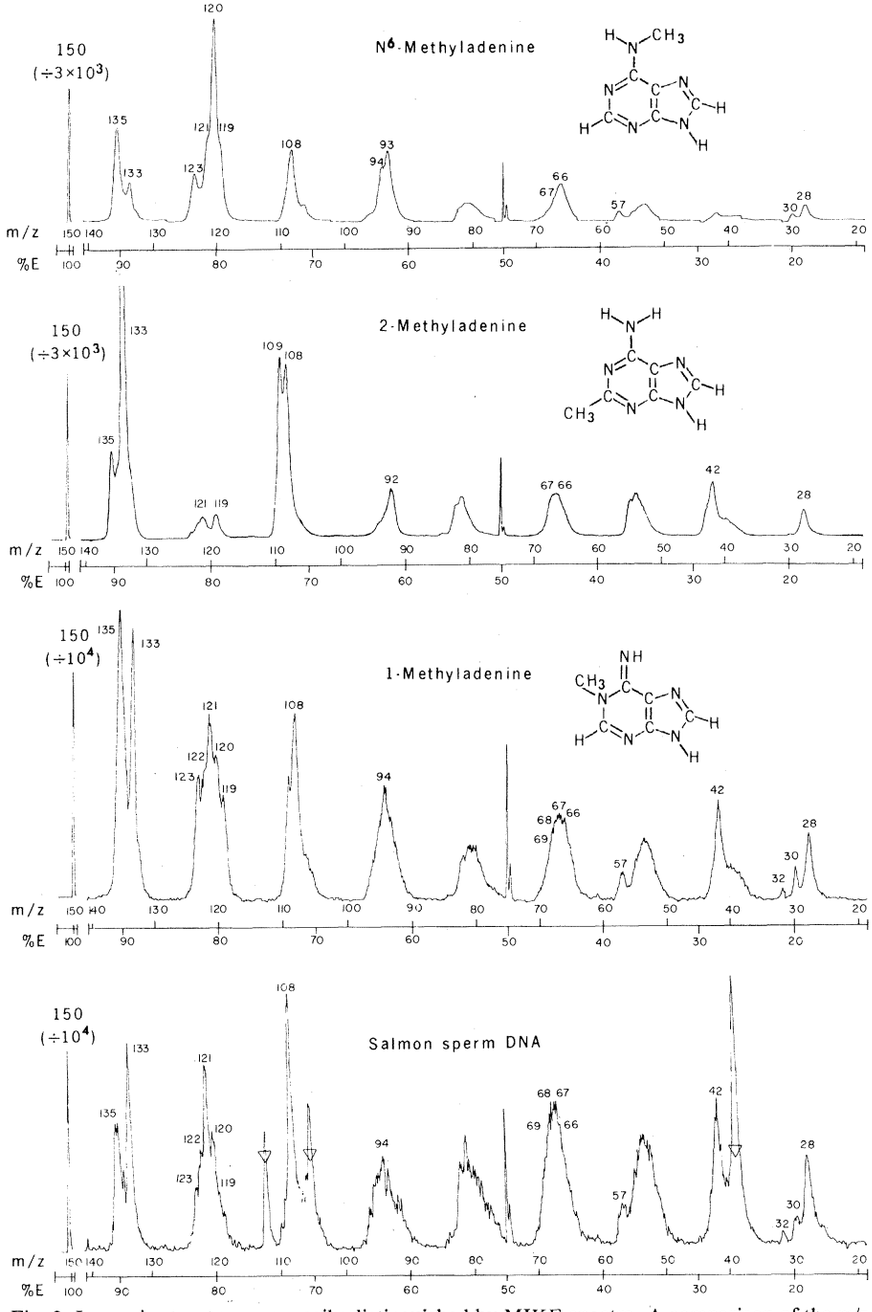

Fig. 2. Isomeric structures are easily distinguished by MIKE spectra. A comparison of the m/z 150 from salmon sperm DNA with three isomeric methyladenines identifies the modified residue in the native DNA as 1-methyladenine. Fragments from interfering ions identified in lower temperature scans are indicated by triangles.

When ions corresponding in mass to protonated bases are subjected to analysis by the MIKE spectrum technique, comparison of the resulting spectrum with that of the authentic compound can be used to characterize the ion. The MIKE spectrum structural assignment goes much further than simply identifying the mass of the ion, as is shown by the detection of the rare deoxynucleoside 5-methyldeoxycytidine (5-MedCyd) in salmon sperm DNA (Calbiochem) (Fig. 1). The most abundant ion in the mass spectrum of the authentic modified nucleoside monophosphate (Sigma) is 126^+. In DNA, however, 126^+ cannot be assumed to originate from 5-MedCyd because of the complexity of the sample. Conclusive proof of the ion's origin is seen by comparison of the energy spectrum from the authentic nucleotide's 126^+ peak with that from the underivatized salmon sperm DNA. In contrast, a determination by high-resolution mass spectrometry of the elemental composition of 126^+ as $C_5H_8N_3O$ would provide a less secure assignment.

Isomeric bases can be distinguished by MIKE spectra even in experiments where complex samples are studied (*7*). Figure 2 compares the MIKE spectra of the protonated forms N^6-methyladenine (N^6-MeAde), 1-MeAde (P-L Biochemicals), and 2-methyladenine (2-MeAde) (Cyclochemical Corp.). The isomers are readily distinguishable in these pyrolysis-independent spectra. When complex mixtures, rather than pure compounds, are sampled, the mass-analyzed primary beam, here m/z 150, may contain extraneous ions. This does not preclude the analysis as illustrated by experiments in which lyophilized salmon sperm DNA is slowly heated and energy spectra are taken as a function of time and temperature. The relative abundance of interfering ions is dependent on the composition of the mass-analyzed beam, which varies with time and temperature. Initial spectra are assigned to the complex between protonated cytosine plus a 38-amu fragment (*8*). At higher temperatures a spectrum due chiefly

to methyladenine is observed (Fig. 2) (*9*). Our major finding is that the methyladenine sampled in the pyrolyzate is clearly 1-MeAde (*10*). Crude attempts at quantification by comparing MIKE peak heights indicated that 1-MeAde was less than 1 percent adenine. Treatment of both native and denatured salmon sperm DNA with ribonuclease A does not eliminate or noticeably attenutate the signal from 1-MeAde. This lessens the possibility that an RNA impurity is serving as the source of this modified base. Also, RNA should give N^6-MeAde as well (*11*). This is not observed.

In conclusion, a powerful new method is presented for the identification of modified nucleosides in intact DNA. Used in conjunction, CI and MIKE spectra provide the maximum advantages for pyrolysis studies on intact DNA. The MIKE spectrum of a single characteristic ion (for example, base $+H^+$) is used to supply structural information on ions formed from the pyrolyzate and to indicate the presence of particular nucleosides in the native DNA. Isomeric bases can be distinguished, and other ions having the same mass does not invalidate the procedure. The characterization is more secure than earlier procedures based on recognition of sets of characteristic ions or gas chromatography retention times (*12*). The detection of 1-methyldeoxyadenosine is noteworthy since it has not been detected in DNA previously (*13*).

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9. This spectrum shows many characteristic differences from that of N^6- and 2-MeAde and in each instance matches that of 1-MeAde. This base is stable under the conditions of the experiment making isomerization during pyrolysis an unlikely possibility. In an extraction and chromatographic study, Lis *et al.* [A. W. Lis, R. K. McLaughlin, D. I. McLaughlin, *Physiol. Chem. Phys.* **6**, 527 (1974)] isolated N^6-MeAde from salmon sperm. It is possible [P. Brooks and P. D. Lawley, *J. Chem. Soc. (London)* (1960), p. 530.] that 1-MeAde isomerized under the conditions of the work-up to N^6-MeAde.
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Premature Senescence in Cultured Skin Fibroblasts from Subjects with Cystic Fibrosis

Abstract. *Cultured skin fibroblasts from subjects with cystic fibrosis exhibited normal population doubling times in early passages. After about 13 cumulative population doublings, cystic fibrosis lines doubled more slowly than controls and ceased doubling after about 19 weekly passages. Control lines continued doubling for 27 passages. The premature senescence noted in cells from subjects with cystic fibrosis reconciles controversial observations of cell doubling reported in the literature. Data presented here demonstrate that experiments with cystic fibrosis cells in late passage may generate misleading results since differences from control lines may be ascribed to generalized senile changes rather than to specific results of the cystic fibrosis genotype.*

Cystic fibrosis (CF) is a lethal exocrinopathy (*1*) and is transmitted within families as an autosomal recessive condition. The primary gene product responsible for CF is not known. Although fibroblasts are not exocrine cells they are regarded typically as secretory cells. Because of their accessibility and retention of the donor's genetic properties over many generations in vitro, skin fibroblasts are a good model system for CF investigations. Reports from this and other laboratories suggest that skin fibroblasts in culture from subjects with CF do manifest abnormalities (*2*). As with much of the CF literature, however, frequent controversy and failure to confirm reported findings with these cells have occurred. A particular area of disagreement concerns cell population kinetics. Several laboratories reported that skin fibroblasts in culture from CF subjects divide more slowly than those from control subjects (*3*). Other investigations, including our own, failed to detect any differences in population doubling time (PDT) between lines from controls and from subjects with CF (*4*, *5*).

In experiments that we report here the PDT was dramatically increased in monolayers derived from CF subjects in comparison with control lines matched for sex, age, and passage number. In an attempt to determine the basis for these divergent conclusions we analyzed weekly mean population doublings (MPD) in terms of passage number in lines that had been maintained routinely. We found that in early passages (until approximately seven weekly subcultures had been made) no difference between CF and control lines occurred. With further passages, however, cell lines derived from subjects with CF doubled more slowly than matched control lines. Additional experiments confirmed that the replicative life-span (cumulative population doublings) and the number of subcultures before the cessation of cell renewal is significantly less in cells derived from subjects with CF. That is, in comparison with controls, cells derived from patients with CF manifest premature senescence. We propose that the subculture passage number or cell population generation at which PDT is determined is a crucial factor when CF cells are compared with controls. These findings appear to reconcile the opposing points of view concerning PDT in CF fibroblast monolayers and have major implications for interpretation of metabolic data derived from CF fibroblasts.

Skin fibroblast cultures were obtained, maintained, frozen, and thawed as described (*5*). Cell lines from eight subjects with CF (five males and three females; mean age 11 years) and five control subjects (two males and three females; mean age 13.2 years) at passages 9 and 10 were

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