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Date July 29, 1988

MITOCHONDRIAL DNA VARIATION IN POPULATIONS OF THE MEXICAN FREE-TAILED BAT *TADARIDA BRASILIENSIS MEXICANA* (MOLLOSSIDAE)

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Mary Kathryn Gustin

August 1988

DEDICATION

I dedicate this thesis to my mother and father.

ACKNOWLEDGMENTS

A number of people have assisted me greatly in the course of research conducted for this thesis. I am particularly indebted to Dr. Gary F. McCracken for his consultation, criticism, and encouragement without which I never could have achieved the results presented herein. I am also grateful to Dr. W. Stuart Riggsby for his patience and guidance during the course of this research. My other committee members, Drs. Jeffrey MacCabe and John Kennedy have been most helpful and supportive of my efforts towards the completion of this thesis and I thank them. I wish also to express my gratitude to Gould and Billye Davis whose friendship and enthusiasm for my research has helped me enjoy the extensive field work. Thanks are also due to my family and friends without whose encouragement I would never have started this project in the first place.

ABSTRACT

Mitochondrial genotypes reflect an animal's female lineage; therefore, the distribution of different genotypes across the species range may be used to elucidate population structure. In Tadarida brasiliensis mexicana, the Mexican free-tailed bat, mitochondrial DNA phenotypes were used to address the question of whether the species range is divided into two separate migratory flyways, and whether bats from a single lineage show loyalty to particular breeding and/or maternity caves. Polymorphisms of the mitochondrial genome from 35 Mexican free-tailed bats were examined in this study. This included animals from four winter (breeding) roost sites in Mexico and animals from three summer caves (maternity) in the southwestern U. S. Composite mitochondrial phenotypes were constructed using DNA fragment patterns resulting from restriction enzyme digests. A total of ten enzymes were used, five of which yielded polymorphic fragment patterns. Results show that although several phenotypes were present in each cave, two main phenotypes were prevalent across the entire species range. These phenotypes were distributed among all caves sampled. These results support previous studies which found no significant genetic differences between caves across the species range. It was concluded that two separate migratory flyways do not exist and bats do not appear to be loyal to breeding or maternity caves.

TABLE OF CONTENTS

SECTION		PAGE
I.	INTRODUCTION	1
	The Study Animal	
II.	MATERIALS AND METHODS	9
	Sample Collection Mitochondrial DNA Isolation Fragment Patterns of Mitochondrial DNA Cloning of Mitochondrial DNA	
III.	RESULTS	25
	Clones Population Study	
IV.	DISCUSSION	35
BIBLIOGRAPHY		40
APPENDIX		45
VITA		51

LIST OF FIGURES

FIGURE		PAGE
1.	Range Map of *Tadarida brasiliensis mexicana*	5
2.	Example Gels	17
3.	Recombinant DNA Clones	26
4.	Fragment Patterns from Restriction Endonucleases	27
5.	Distribution of Composite Phenotypes	32
6.	Parsimonious Female Lineage Network	34

I. INTRODUCTION

Many barriers can limit animal dispersal to the extent that populations of conspecific animals become isolated from each other. When such isolation occurs genetic differences between populations may arise. Populations which are isolated long enough may accumulate enough genetic differences that, ultimately, speciation occurs. Barriers to dispersal can be classified into several different categories, two of which are geographic and climatic barriers. Mountain ranges and bodies of water are examples of geographic barriers which may prevent animals in populations on either side to mix freely with one another. For example, several species of Hawaiian honeycreeper (F. Drepanididae) have evolved from a common stock. Populations were founded on different islands and adapted to fill different niches. The distance between islands was large enough to prevent populations from contacting one another. Ultimately, genetic isolating mechanisms arose which prevented breeding with other populations (Amadon, 1950). Climatic barriers can include temperature gradients or differences in amount of rainfall between areas. For example, the horseshoe crab, Limulus polyphemus, occurs in coastal waters of the United States along the Atlantic Ocean and Gulf of Mexico. Two major mitochondrial DNA clonal types have been identified; one in populations north of southeastern Georgia and one in populations south of this area (Saunders, et al., 1986). Northeast Florida is a transitional area between warm temperate and tropical marine climates;

this temperature transition is a likely isolation barrier between these two clonal types.

Populations can also become isolated due to behavioral barriers. This is especially so in many migratory species where animals which may mix freely most of the year may need to return to specific sites in order to breed. Although all males and females of a species are otherwise capable of mating with one another, this behavior may limit the effective breeding population to those animals which return to the same breeding sites. For example, North Pacific salmon live as adults throughout the north Pacific Ocean during most of the year; however, they return to their natal freshwater streams in order to spawn (Hasler and Scholz, 1983).

Animals can be subject to all three types of barriers; alternatively, they may be able to overcome some limiting barriers and not others. In elucidating the population structure of migratory animals it is necessary to determine what, if any, barriers are acting to limit animal dispersal. Studies in which patterns of dispersal have been determined include the following: the horseshoe crab, Limulus polyphemus, (Saunders, et al., 1986; Selander et al., 1970); the small white-eyed songbird, Zosterops lateralis (Mayr, 1965); the grosbeaks, Pheucticus spp., (Kroodsma, 1974); North Atlantic eels, Anguilla spp. (Avise, et al., 1986); the marine catfish, Arius felis and Bagre Marinus and the toadfish, Opsanus tau and O. beta (Avise, et al., 1987).

The present study makes use of genetic differences in mitochondrial DNA to determine population structure across the range of

the migratory bat, Tadarida brasiliensis mexicana (F. Mollossidae). In the past twenty-five years many new laboratory methods have been developed which help to elucidate questions involving evolution, population structure and social behavior. Genetic analyses of population structure have been accomplished through the use of protein electrophoresis (McCracken, 1982), restriction fragment length polymorphism analysis of mitochondrial DNA (Ferris et al., 1983) and nuclear DNA (Arnason and Widegren, 1986), comparisons of sequenced portions of mitochondrial and nuclear DNA (Brown and Simpson, 1982), and immunological comparisons among different species or subspecies of animals (Neigel and Avise, 1985). The purpose of this thesis is to use restriction fragment length polymorphism analysis of mitochondrial DNA to assess the possible impact of migration patterns and site loyalties on population genetic structure of the bat, Tadarida brasiliensis mexicana.

An additional concern of population biologists is the ability to conduct genetic analyses using small tissue samples which are non-destructive to study animals. This ability is especially valuable in studies of endangered or rare species and in behavioral studies where subsequent observation of animals is desirable; however, the concept of non-destructive sampling should carry over into sampling of all animals. To this end, the work for this thesis also involves cloning a part of the T. b. mexicana mitochondrial genome. The use of these clones as probes allows analyses using very small tissue samples. Mitochondrial genome clones also will be useful in future studies involving restriction site mapping and, possibly, gene mapping.

The Study Animal

Tadarida brasiliensis mexicana, the Mexican free-tailed bat ranges throughout Mexico and north into the south- and midwestern United States to as far as southern Colorado and Kansas. To the east and west the range extends from eastern Texas and the western half of Arkansas to California. Cochrum (1969) and Barbour and Davis (1962) have subdivided the subspecies T. b. mexicana into two separate populations (Figure 1). Bats from colonies located in western Arizona and California remain resident throughout the year or show only local movements while colonies to the east are migratory. The entire population of several million individuals found East of western Arizona and West of Arkansas overwinters in Mexico. Banding studies by Cochrum (1969) revealed that the sex ratio in winter roosts is roughly 50:50, and subsequent studies (McCracken et al., 1986) corroborate this finding. Near the end of the winter, most likely in late February or early March, mating occurs. The mating system in this bat has not been studied. It also is not known whether a Mexican free-tailed bat returns to the same location year after year to mate.

After mating, Mexican free-tailed bats begin their annual migration. Only a portion of the total population migrates. Pregnant and non-pregnant females, as well as some males, migrate to the northern portions of the range; most males remain in Mexico throughout the year. Migrants apparently take several weeks to arrive at their summer roosting locations.

Banding studies suggest that genetically separate migratory flyways exist. Although several hundred thousand individual bats were

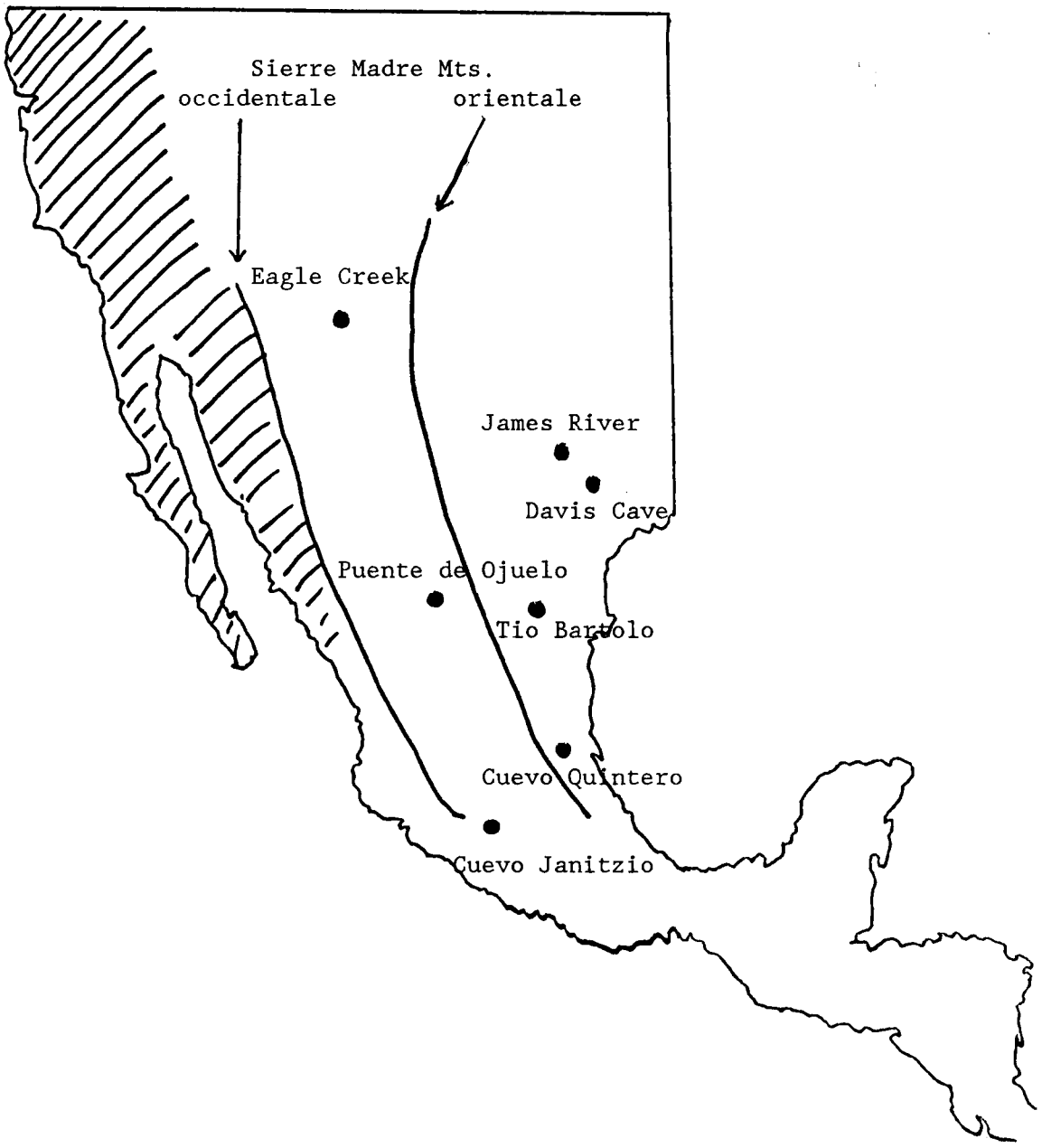


FIGURE 1. Range Map of Tadarida brasiliensis mexicana.

The range of Tadarida brasiliensis mexicana is shown above. Hatch marks indicate non-migratory populations. The Sierra Madre Occidentale and Orientale Mountains mark possible dispersal boundaries of migratory populations. Caves from which study animals were taken are indicated.

banded during the 1950's and early 1960's (Davis et al., 1962; Villa and Cochrum, 1962; and Cochrum, 1969), only a few hundred individuals were ever recaptured. Cochrum also suggested that cave loyalty may occur. Villa and Cochrum hypothesized that the Sierra Madre Orientale mountain range serves as an effective barrier to dispersal of Mexican free-tailed bats, so that bats from breeding sites east of these mountains go to summer roosting sites in Texas, Arkansas, eastern New Mexico and Oklahoma and bats from breeding sites west of these mountains go to Arizona and western New Mexico (Figure 1). Recapture of banded bats suggests that T. b. mexicana may move between roost locations on one side of the mountain range, but that they do not cross the mountain barrier (Cochrum, 1969; Villa and Cochrum, 1962; Davis et al., 1962; Constantine, 1967; Glass, 1958, 1959). The extent to which males remaining in Mexico move between roost sites is not known. Once bats arrive at the summer roosts, the population is further subdivided. Males and non-pregnant females roost in caves, under building eaves and bridges, or in other protected areas. Pregnant females form maternity colonies consisting of several million individuals in traditional cave roosts. The maternity roosts are known to be assemblages of bats from several winter roosting sites (McCracken et al., 1986).

Within these maternity roosts each female bears one pup. Births are highly synchronous with most births occurring in the later two weeks of June. Pups are weaned in about five weeks, at which time females leave the maternity caves and fly further north. All migrating Mexican free-tailed bats, including young of the year, return to Mexico during late September and October.

Recently, studies by Svoboda and coworkers (1985) and McCracken and coworkers (1986; unpublished) dispute the presence of genetically distinct flyways. These latter studies show that the entire population of Mexican free-tailed bats is genetically homogeneous among all roost sites examined throughout the range of these bats. McCracken et al. (1986; unpublished) sampled bats during both the brooding season and during the winter; roosts were sampled by Svoboda et al. only during the summer, and without regard for post-weaning movements of adult females.

In the present study, analyses of restriction fragment length polymorphisms found in mitochondrial DNA are used to determine whether Mexican free-tailed bats show loyalty (philopatry) either to winter breeding caves or to summer roosting sites. These analyses also are used to determine whether migrating Mexican free-tailed bat populations are fragmented by the Sierra Madre Orientale mountain range.

There are several characteristics of mitochondrial DNA which make its analysis advantageous for such studies (Brown, 1981, 1983; Avise and Lansman, 1983). In mammals, mitochondrial DNA is a small circular molecule between 16,000 and 17,500 base pairs in length, which can be isolated in useable quantities from relatively small tissue samples. Conversely, nuclear DNA is several orders of magnitude larger and is difficult to isolate in small, testable fragments. Studies on several species of mammals have shown that mitochondrial DNA is heterogeneous within a given species, and that interpopulation differences often are present (Avise et al., 1979; Ferris et al., 1983; Hale and Singh, 1986; Cann et al., 1987; Pumo et al., in press). Generally, all somatic

tissues of an individual have identical mitochondrial DNA, but a few cases of heteroplasmy have been documented (Shields and Wilson, 1987; Bermingham et al., 1985; Olivio et al., 1983). Another feature of mitochondrial DNA making it useful in genetic analyses is that it has a faster rate of evolution than single copy nuclear DNA. As a result, the mitochondrial DNA molecule diverges between five and ten times faster than a nuclear DNA segment of comparable length. This approximates one percent sequence divergence per million years (Brown, 1983). The characteristic of mitochondrial DNA which is most useful in the present study is that mitochondrial DNA of mammals is maternally inherited (Hauswirth and Laipis, 1982; Dawid and Blackler, 1972). Transmission genetics of mitochondrial DNA has been considered by several workers (Chapman et al., 1982; Rand and Harrison, 1986; Birky, 1978; Birky et al., 1982) and no evidence for paternal contribution to the mitochondrial DNA has been found. As a result of maternal inheritance, subdivision of populations along female lines and dispersal of females lineages may be detectable. Therefore, examination of mitochondrial DNA may reveal population structuring resulting from isolated breeding roosts or natal philopatry.

II. MATERIALS AND METHODS

Sample Collection

The Tadarida brasiliensis mexicana used in this study originally were collected for use in studies of allozyme variation. The present study made use of thirty-five animals taken from seven caves in the southwestern United States and Mexico (Figure 1). Four of the seven caves were winter roosts and presumed to be breeding caves. These are Puente de Ojuelo (PO), Cuevo Janitzio (CJ), Cuevo Tio Barolo (TB), and Cuevo Quintero (CQ). James River Cave (JR) and Davis Cave (DC) are summer maternity caves, and Eagle Creek Cave (EC) is a summer roost for males and maternity roost for some female bats. All adults and pups used in this study were euthanized by ether anaesthetic followed by exsanguination via heart puncture. Bodies were frozen in the field in liquid nitrogen and were transferred without thawing to a -80^{o} C freezer. Samples remained frozen until tissues were removed for mitochondrial isolation.

Mitochondrial DNA Isolation

Two methods were used to isolate mitochondrial DNA for analysis of population structure; both are described by Lansman et al. (1981). Isolation of mitochondrial DNA from whole animal preparation is an attempt to recover mitochondrial DNA as nearly pure as possible. A fast method for isolation of mitochondrial DNA with slight nuclear DNA contamination was used for most samples. This isolation method is ideal for population studies for several reasons: (a) small tissue samples from each animal (0.2gm) yield enriched mitochondrial DNA

suitable for fragment pattern analysis; (b) DNA from several animals can be isolated at one time; and (c) no lengthy gradient centrifugations are required, so isolation of DNA is fast.

Whole animal DNA isolation. Tissues from a single study animal were frozen in liquid nitrogen and reduced to a powder using a mortar and pestle. Ten volumes (w/v) of SEDTA (see the Appendix for all formulae) were added to the powder. One twentieth volume of 25% SDS and one fifth volume of 5 M sodium perchlorate were added to the powder suspension. An equal volume of CIP was added and the mixture was stirred for 1 hour at room temperature. Phases were separated by centrifugation at 3000g for 10 minutes. The aqueous phase was saved and an equal volume of IAC was added. Centrifugation was repeated as before, and the aqueous phase again was saved. This layer was placed under 2 volumes of ice cold 95% ethanol and allowed to remain at normal freezer temperatures (0^{o} to -10^{o} C) overnight. DNA precipitated at the ethanol/aqueous interface and was removed by winding the DNA onto a glass stirring rod. This DNA was resuspended in 10ml of 0.1 x SSC buffer while stirring overnight at 4^{o} C. Seven tenths of a milliliter of ethidium bromide stock solution (10mg/ml) was added to the DNA solution and cesium chloride was added to obtain a final density of 1.57gm/ml. Centrifugation was carried out using a Beckman Ti 50 rotor at 43,000rpm for 40 hours at 17^{o} - 21^{o} C. Tubes were removed from the rotor in the dark to prevent DNA nicking by intercalated ethidium bromide. DNA bands were located by illumination under a long wavelength ultraviolet light, and were removed separately by puncturing

the tube just below each band with a 16 gauge hypodermic needle and slowly drawing the DNA band into a syringe. Two bands were obtained in this manner: the upper band contained mostly nuclear DNA, and the lower band contained mitochondrial DNA. Intercalated ethidium bromide was removed from the DNA with water saturated sec-butanol. DNA from each band was desalted by dialysis in three six-liter changes of TE buffer washes for 4 hours, 8 hours and 12 hours, respectively.

Isolation of cytoplasmic nucleic acids. Liver or flight muscle from frozen animals was used. Liver samples weighed 0.2gm on average, and muscle samples weighed 0.1 to 0.2gm. Each sample was homogenized in 5ml of ice cold 2 x TSM buffer. One tenth milliliter of 10% Nonidet P-40 was added to each sample with vortexing; all samples then were left on ice for 5 minutes. The samples were centrifuged at 1500g for 5 minutes to pellet nuclei and large cell debris. The supernatant was added to 5ml of CIP, vigorously mixed, and allowed to incubate at 65° C for 5 minutes. Samples were centrifuged at 5400g for 10 minutes to separate phases. The aqueous layer was CIP extracted again. This aqueous layer was extracted with IAC to remove traces of phenol. The final aqueous layer was measured, and 0.1 volume of 2 M sodium acetate and 2 volumes of 95% ethanol were mixed with the aqueous phase. All samples were left at -20° C for 2 hours to allow the DNA to precipitate. Samples were centrifuged at 5400g, the supernatant was decanted, and DNA pellets were dried at room temperature. Each pellet was resuspended overnight at 4° C in 1ml of TE buffer containing

50mg/ml of RNase A. Samples were incubated for 10 minutes at 37° C to digest RNAs, then all samples were stored at -20° C until use.

Fragment Patterns of Mitochondrial DNA

Restriction endonuclease digestion of DNA. Ten type II restriction endonucleases were used in this study (Table 1). Restriction endonucleases were supplied by Bethesda Research Laboratories and New England Biolabs. Reaction conditions for the digestions were those recommended by the supplier. DNA fragments for cloning were obtained by digesting samples overnight, whereas DNA for fragment patterns of individual samples for population analysis was digested for 3 hours.

Agarose gel electrophoresis. Fragment patterns were obtained by electrophoresis of digested DNA on one or two percent agarose gels. Seakem agarose was dissolved in E buffer, the gel solution was allowed to cool to about 55° C, and the gel was cast in a vertical gel mould 140 x 150 x 4mm. Ten milliliters of two percent agarose at 55° C was cast in the mould and allowed to gel before the rest of the gel was poured in order to prevent leakage. Five microliters of loading dye (see Appendix for all formulae) was added to each sample prior to loading samples onto the gel. Each gel included lambda phage DNA digested with Hind III as a standard. For one percent gels, each gel was run at a constant voltage until the dye front reached the two percent agarose plug (approximately 2cm from the bottom of the gel apparatus). Two percent gels were allowed to run until the dye front

TABLE 1. Restriction Endonucleases.

Enzyme	Recognition sequence
Bgl II	A GATC T T CTAG A
Cla I	AT CG AT TA GC TA
EcoR I	G AATT C C TTAA G
Hind III	A AGCT T T TCGA A
Hinf I	G ANT C C TNA G
Hpa II	C CG G G GC C
Mbo I	GATC CTAG
Sma I	CCC GGG GGG CCC
Sst I	G AGCT C C TCGA G
Xba I	T CTAG A A GATC T

Type two restriction endonucleases used in the population study are listed below along with their recognition sequences. Cleavage sites are indicated.

was 5cm from the bottom of the gel apparatus. Gels were stained for 30 minutes in 500ml of water containing 25μl of ethidium bromide stock solution (10mg/ml). The DNA was visualized using a Mineralight 302nm long wave ultraviolet light transilluminator. A photographic record of each gel was made using polaroid type 57 film and a Kodak 23A Wratten gelatin filter.

Southern blots. DNA was transferred from the gel to nitrocellulose filters using the methods of Southern (1979). The gel was exposed to short wavelength ultraviolet light for 10-15 seconds in order to cause single stranded nicks in the DNA. The gel was agitated in dissociation buffer at room temperature for 30 minutes, then in neutralization buffer for 30 minutes. The gel then was placed over a reservoir of 10 x SSC on two 18 x 23cm Whatman 3MM filter paper wicks. A 14 x 16.5cm nitrocellulose filter type BA85 from Schleicher and Schuell was wetted with 10 x SSC and placed on the gel. A plastic collar was placed around the edges of the gel to prevent the nitrocellulose filter from coming in contact with the wicks. Two 14 x 16.5cm 3MM filters were placed on the nitrocellulose filter, and layers of paper towelling approximately 7cm thick were stacked on top of this. DNA was transferred from the gel to the nitrocellulose filter by blotting overnight (12-16 hours) at room temperature, then the nitrocellulose filter was rinsed in a 4 x SSC, blotted dry, and baked at 70^{o} C for 2 hours in a vacuum oven. Blots were stored at room temperature until use.

Labelling probe DNA. DNAs used as hybridization probes were radiolabelled using a Bethesda Research Laboratories nick translation kit. Each reaction mixture contained 500μg of DNA, 5μl of unlabelled deoxyribonucleotides (dATP, dGTP and dTTP), 13μl of 5' - [alpha-^{32}P]dCTP (ICN Products, Inc.), and enough water to bring the reaction mixture volume up to 45μl. Five microliters of DNase/polymerase then was added. This reaction mixture was incubated for 1.5 to 2 hours at 15^{o} C (modified from Maniatis et al., 1982). Radiolabelled probe DNA was separated from unincorporated nucleotides by column chromatography on a Sephadex G-50 column. Two types of columns (Maniatis et al., 1982) were used: spun columns with a packed bed volume of 1ml, or wet columns with a packed bed volume of about 4ml. Spun columns were equilibrated with column buffer, then spun dry at 2000g. The reaction mixture plus 50μl of column buffer was applied to the column, and the column was spun for 4 minutes at 2000g. The radiolabelled probe DNA was collected in a 1.5ml eppendorf tube during centrifugation, and unincorporated nucleotides were retained on the column. Wet columns were equilibrated with column buffer. The reaction mixture then was applied to the column and radiolabelled probe DNA was eluted with column buffer. Fractions of 500μl were collected, and those containing the radiolabelled probe DNA were pooled. Specific activity of the labelled probe DNA was determined by Cerenkov counting. Two microliters of the radiolabelled DNA was added to 10ml of water then counted. Specific activities ranged from 1 x 10^5 to 1 x 10^6 cpm/μl, assuming that all probe DNA was recovered from the column.

Hybridization to nitrocellulose blots. Radioactive probes were hybridized with DNAs on nitrocellulose blots according to the methods of Maniatis et al. (1982) as follows. Stringency conditions depended upon the specificity of the probe being used. Stringency conditions used when bovine mitochondrial DNA was used as a probe are less stringent. The more stringent parameters used with bat mitochondrial DNA probes are given in brackets. Nitrocellulose blots were prehybridized to reduce nonspecific binding of radioactive probe. Twenty milliliters of prehybridization solution was added to a plastic bag containing one nitrocellulose blot, the bag was sealed, and the blot was incubated at 42° C [65° C] for 4-24 hours. Just prior to use, radiolabelled probe was denatured by boiling for 5 minutes. Approximately 2 x 10^6 cpm of denatured probe was added to the prehybridization solution of each blot, the bag was resealed, and then sealed into another bag. Blots were hybridized at 42° C [65° C] for 16-24 hours. After hybridization, each blot was removed from its bags and washed four times for 30 minutes at 50° C [65° C]. The wash solution was 1 x SSC in 0.05% SDS. Blots were dried and wrapped in plastic wrap. Autoradiographs of each blot were made using Kodak type XAR-5 x-ray film. Blots were placed in an autoradiography folder on the outside of one of two DuPont Cronex Lightning-Plus intensifier screens, and a sheet of film was placed between the screens. Autoradiography was carried out at -80° C (Figure 2).

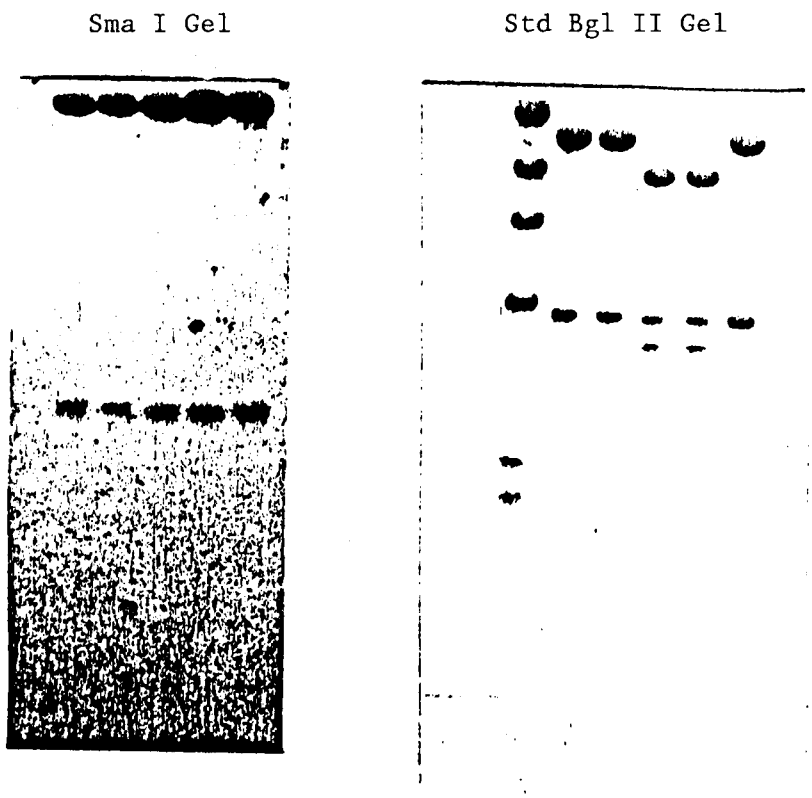


FIGURE 2. Example Gels.

Autoradiographs of restriction enzyme digest patterns are illustrated. Each column of fragments represents an individual animal. Phage λ digested with Hind III is included as a standard.

Cloning of Mitochondrial DNA

Sections of the T. b. mexicana mitochondrial genome were cloned for use as probes. These clones were radiolabelled and hybridized with DNA fragments which had been fixed onto nitrocellulose filters. These probes allowed hybridization with T. b. mexicana mitochondrial DNA to occur under stringent conditions, thus non-specific binding of probe DNA to Southern blots was reduced. Additionally, highly specific probes permitted smaller amounts of sample DNA to be used per digestion. Clones also may be of future use in restriction site and gene mapping.

TBM H1 Clone. Plasmid pUC18 was used as the vector for cloning T. b. mexicana mitochondrial DNA fragments. This vector contains one Hind III restriction site within the galactosidase gene region. T. b. mexicana mitochondrial DNA fragments obtained from a Hind III limit digest (1μg in 5μl) were mixed with Hind III-digested pUC18 (1μg in 5μl), 2μl of 10 mM ATP, 4μl of 5 x ligase reaction buffer, and 4μl of 5 x PEG. T4 DNA ligase (0.1 unit in 2μl) was added to the reaction mixture, and the mixture was incubated at 15^{o} C for 1 hour so that ligation could occur.

Escherichia coli strain TB-1 was used as the host for the pUC18/mitochondrial DNA plasmid. Cells were made competent by the calcium chloride method as described in Maniatis et al. (1982). TB-1 cells were grown in TYN medium to an absorbance reading of 0.65 at 600nm. Cell division was halted by placing the culture on ice for 10 minutes, then cells were pelletted by centrifugation at 4000g for 5

minutes at 4° C. TB-1 cells were resuspended in one half of the original volume of CaCl solution and left on ice for 15 minutes, then they were recentrifuged as before and resuspended in one fifteenth of the original volume of CaCl solution. These competent cells were left on ice for 1 hour. Twenty microliters of pUC18/insert ligated DNA was added to a 0.2ml aliquot of cells; this mixture was heat shocked at 42° C for exactly 2 minutes, then 1ml of TYN medium was added to the cell/plasmid mixture. Cells were incubated at 37° C for 1 hour, 20 minutes to allow plasmid uptake. Cells were plated onto TYN agar plates which contained ampicillin (25μg/ml) and were coated with 2% X-gal (50μl/75mm plate; 150μl/150mm plate). Cultures were incubated at 37° C for about 16 hours.

Selecting clones with mitochondrial DNA inserts. Transformants containing plasmids with inserted T. b. mexicana DNA were identified by their characteristic white color. Transformed cells containing uninterrupted plasmid DNA were blue. A 1cm streak of each white colony was made on a fresh plate using a sterile toothpick, and colonies were arranged in a grid pattern, with about 70 colonies per plate. Plates were incubated for 16-20 hours at 37° C. Replicas of each plate were made on 127mm diameter sterile nitrocellulose filters. Filters were incubated on TYN agar plates at 37° C for 4 hours to allow cells to grow. Nitrocellulose filters were prepared for autoradiography as described by Maniatis et al. (1982). Each filter was placed on Whatman 3MM paper saturated with 0.5 M NaOH for 4 minutes, then the filter was blotted dry on paper towels. This step was repeated, with the result

that all cells on the filters were lysed. Filters were neutralized by placing them on 3MM paper soaked in 1 M Tris, pH 8.0 for 4 minutes, then drying; this step was also repeated. Filters were placed on 3MM paper soaked in 1 M Tris/1.5 M NaCl for 5 minutes, and then they were blotted dry. To remove cell debris, filters were placed in 2 x SSC in 0.2% SDS and rubbed thoroughly with a Kimwipe. Cell debris was rinsed from the filters using fresh 2 x SSC. Filters were baked at 70° C for about 2 hours. Colonies which contained T. b. mexicana mitochondrial DNA inserts were identified by hybridization with a radiolabelled bovine mitochondrial DNA probe as described above. The bovine mitochondrial DNA was obtained from Dr. Deborah Smith, University of Michigan.

TBM Cl clone. Mitochondrial DNA from a single animal was used in making TBM Cl clones. The DNA was digested with Cla I (10μg/40μl) overnight. It then was electrophoresed on a 1% agarose gel as described above, except that the gel was not run to completion. A 0.5cm wide trough was cut horizontally in the gel and filled with E buffer. DNA fragments including the two mitochondrial bands were electroeluted into the trough and collected with a pipet. The DNA was concentrated by ethanol precipitation and resuspension in TE buffer. Cla I fragments were cloned in E. coli strain DH5α using plasmid pBR322 as the vector. There is a single Cla I restriction site in the promotor region of the tetracycline resistance gene of pBR322. The methods for inserting the T. b. mexicana DNA fragments into pBR322 are described below. Cla I fragments of T. b. mexicana DNA (1μg/9μl) and

Cla I digested pBR322 were added to 4μl of 5 x ligase reaction buffer, 4μl of 5 x PEG, and 2μl of 10 mM ATP, then 1μl of 1 unit/μl T4 DNA ligase was added. The reaction mixture was incubated at 15^{o} C for about 16 hours to allow ligation to occur.

Escherichia coli DH5α cells are a competent cell line available from Bethesda Research Laboratories. Competent cells were stored at -70^{o} C in 20μl aliquots until use. Cells were thawed on ice, and 1μl of ligated pBR322/insert DNA was added to the cell aliquot with gentle mixing, then cells were left on ice for an additional 30 minutes. Cells then were heat shocked at 42^{o} C for 40 seconds, then replaced on ice, and 80μl of SOC medium was added to the cells. Cells were shaken at 37^{o} C for 1 hour to allow uptake of plasmids, and then they were plated onto LB-agar plates containing 25μg/ml ampicillin. Colonies were allowed to grow at 37^{o} C overnight (12-16 hours).

Selecting clones with mitochondrial DNA inserts. Replicas of each plate were made on nitrocellulose filters. Autoradiographs were made as described above. Putative positive colonies were selected for further verification.

Minipreparation of plasmid/insert DNA. Procedures for selecting TBM C1 clones were the same as for TBM H1 clones. The selected colonies were grown in 10ml cultures of LB broth with 25μg/ml ampicillin for about 14 hours at 37^{o} C while shaking at 125rpm. Rapid isolation of plasmid DNA was accomplished using the methods of Maniatis et al. (1982). Cells were pelleted from 1.5ml of the overnight culture by centrifuging at 3000g for 1 minute, and the medium was removed. The

cell pellet was resuspended in 100μl of solution I (alkaline lysis miniprep method; see appendix). The cell suspension was allowed to stand at room temperature for 5 minutes, then 200μl of solution II was added, and the mixture was allowed to stand on ice for 5 minutes. After this time, 150μl of solution III was added and each tube was vortexed for 10 seconds then placed on ice for 5 minutes. Each tube was centrifuged as before, and the supernatant was saved. The supernatant was extracted with an equal volume of CIP, then centrifuged. Residual CIP was removed from the aqueous layer by extraction with IAC. One tenth volume of 2M Na acetate was added to the aqueous layer, followed by two volumes of ice cold 100% ethanol. The solution was cooled in a dry ice/ethanol bath for 15 minutes, and the DNA was pelletted by centrifuging at 4^{o} C for 15 minutes. The supernatant was removed and the pellets were dried, and then resuspended in 50μl of TE buffer containing 20μg/ml of DNase-free RNase A. Ten to fifteen microliters of each minipreparation was digested using either Hind III or Cla I. These digests were electrophoresed on 1% agarose gels.

Large scale isolation of recombinant DNA plasmids. Recombinant DNA plasmids containing inserts of T. b. mexicana mitochondrial DNA were isolated from E. coli chromosomal DNA using the methods of Maniatis et al. (1982). The same procedure was used for all clones. Cells were grown in 25ml of the appropriate growth medium. These cultures were shaken at 125rpm and 37^{o} C until the late log phase of growth was attained. This was determined spectrophotometrically when

the cultures gave an absorbance reading of 0.6 at 600nm. The entire culture was added to 500ml of growth medium, then shaken at 37° C until an absorbance reading of 0.4 was obtained, at which time 85mg of chloramphenicol in 2.5ml of absolute ethanol was added to each culture. Cultures were stirred overnight (about 14 hours) at 37° C and then centrifuged at 4000g for 20 minutes at 4° C. Cell pellets were resuspended in 100ml of ice cold STE buffer at pH 7.8, and centrifugation was repeated as before. Cell pellets were resuspended in 10ml of solution I (alkaline lysis procedure). This suspension remained at room temperature for 5 minutes to allow cell lysis to occur. Twenty milliliters of solution II were added to the lysed cells, and mixed thoroughly. This solution remained on ice for 10 minutes, then 15ml of solution III was added. A white flocculant material appeared in the solution; this was precipitated cell debris and proteins. The solution was cooled on ice for 20 minutes, then centrifuged at 70,000g for 20 minutes at 4° C. The supernatant was saved, and 0.6 volume of isopropanol was mixed with it. This solution remained at room temperature for 15 minutes before it was centrifuged at 12,000g for 30 minutes. The resulting DNA pellets were washed with a few drops of 70% ethanol then dried at room temperature. DNA pellets were dissolved in 8ml of TE buffer. Sample DNA, 21.4ml of TE buffer, 29.4gm of CsCl and 3ml of ethidium bromide solution (10 mg/ml) were mixed. Samples were centrifuged using a Beckman Ti60 rotor at 42,000rpm for 40 hours at 17°-21° C. The uppermost fluorescent band (plasmid band) was removed as described above. Ethidium bromide was removed by washing with 2-butanol saturated with 5 M NaCl. This DNA

was dialyzed at 4° C for 24 hours against three changes, each change using 4 liters of TE buffer. This dialysis removed the CsCl. One twentieth volume of 8 M lithium lactate, and 24μl of glycogen were added to each sample. Two volumes of ice cold 100% ethanol were added; the solution was mixed and allowed to remain at -70° C for 2 hours. DNA was pelletted by centrifugation at 12,000g for 15 minutes at 4° C. The resulting DNA pellet was allowed to dry, and was then resuspended in 1ml of TE buffer. This DNA preparation was phenol extracted to destroy any remaining DNases as described above. DNA was re-precipitated with ethanol, and the pellet was resuspended in 1ml of TE buffer. Recombinant plasmid DNA was stored at -20° C until use.

III. RESULTS

Clones

Two clones were made for use as highly specific probes. The digestion patterns of these clones as well as diagrams of vectors showing insertion sites of T. b. mexicana DNA are shown in Figure 3. The term TBM Cl represents phage vector pBR322 containing the 9.4kbp fragment from a Cla I digest of T. b. mexicana mitochondrial DNA. The term TBM Hl stands for phage vector pUC18 containing the 6.4kbp fragment from a Hind III digest of T. b. mexicana mitochondrial DNA. TBM Hl was radiolabelled by nick translation and hybridized to sample DNA to visualize fragment patterns. This was done with Hinf I and Mbo I digests. Cloned T. b. mexicana DNA has been useful in visualizing small amounts of mitochondrial DNA in fragment patterns, because less non-specific binding occurs and shorter exposure time is required than with the probe of bovine mitochondrial DNA, which was also used.

Population Study

Ten restriction endonucleases were used in this study. The approximate genome size was determined by adding together the sizes of the fragments in each different fragment pattern (Figure 4). The T. b. mexicana mitochondrial genome size is between 16.8 and 17.2 kbp in length. This is within the size range of the mitochondrial genome of higher animals (16.0 to 17.5 kbp; Brown, 1983) and is close to that found by Pumo et al. (1988) for another microchiropteran bat, Artibeus jamaisensis (16.0 kbp). Patterns from Hinf I and Mbo I digests were

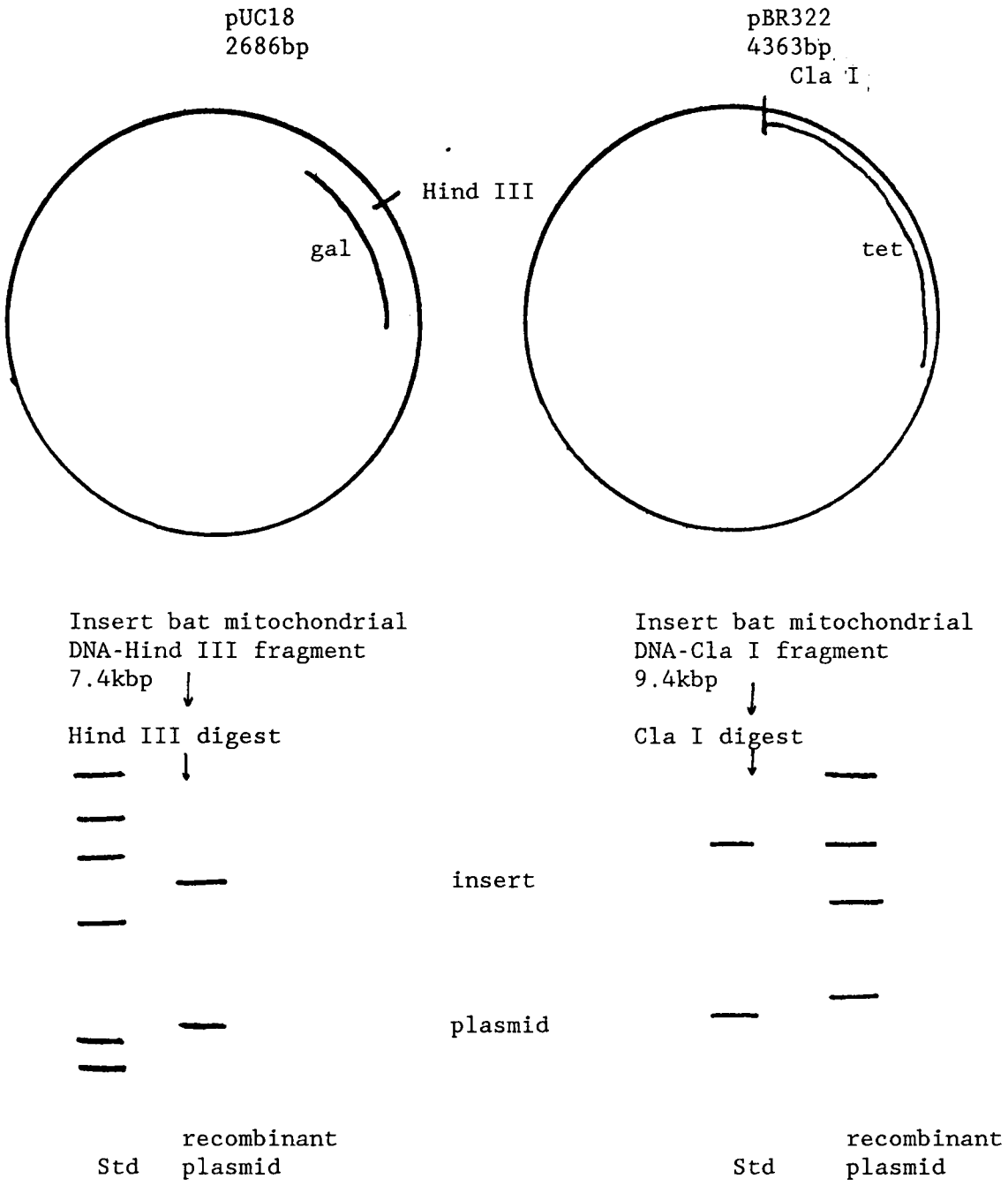


FIGURE 3. Recombinant DNA Clones.

Plasmids used to construct clones and cloning sites are given. Tadarida brasiliensis mexicana mitochondrial DNA is inserted into each plasmid. The plasmid is digested with the appropriate endonuclease to verify that the plasmid contains an insert.

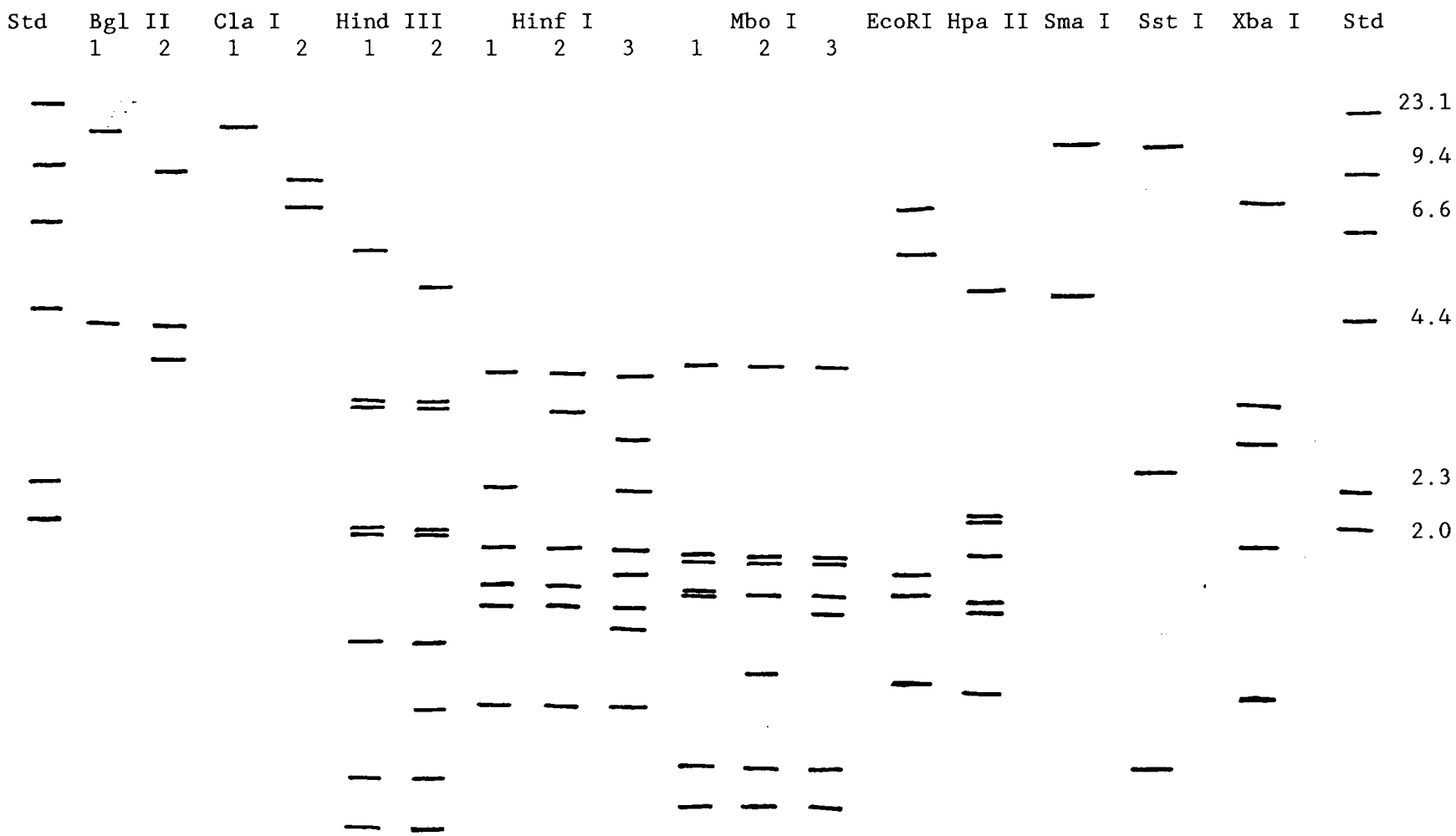


FIGURE 4. Fragment Patterns from Restriction Endonucleases.

The enzyme name and pattern numbers are listed above each pattern. Phage λ cut with Hind III is included as a standard. Mbo I and Hinf I patterns do not include all possible fragments; however, all other patterns represent the complete mitochondrial genome.

probed with TBM H1 probe. Because the TBM H1 probe includes only a portion of the mitochondrial genome, it hybridized to only a portion of the fragments in the Hinf I and Mbo I digests. As a result, not all fragments were visualized and these patterns were not used to determine the approximate genome size.

Five of the endonucleases (Eco RI, Sma I, Sst I, Xba I, and Hpa II) yielded monomorphic digestion patterns. Polymorphisms were found among fragment patterns from digests using the remaining five endonucleases (Mbo I, Hinf I, Hind III, Bgl II and Cla I). The fragment patterns are shown in Figure 4. For each polymorphic endonuclease, the most common fragment pattern is labelled '1', and the rarer patterns are labelled '2' or '3'. Hinf I patterns '1' and '2' are distributed equally across the range and each occurs in 17 animals. Endonucleases Hind III, Bgl II and Cla I each had two alternative fragment patterns, and Hinf I and Mbo I each had three alternative patterns. Table 2 gives the number of animals with each different fragment pattern. The distribution of patterns among the caves is also given.

In the case of each enzyme, changes from the '1' fragment pattern to alternative patterns can be attributed to a single mutational step. That is, alternative patterns differ from the common one by the gain or loss of a single restriction site, such that a single fragment is reduced to two smaller ones, or vice versa. The sum of the two smaller fragments is equal to the larger one. This indicates that large deletions or additions or major rearrangements have not occurred. For example, the mitochondrial genome in most of the study animals had a

TABLE 2. Distribution of Fragment Patterns.

Enzyme	Pattern #	# of animals	Caves*
Hind III	1	33	all caves
	2	2	EC, DC
Cla I	1	33	all caves
	2	2	EC, CJ
Bgl II	1	30	all caves
	2	5	JR, DC, CQ, CJ, PO
Mbo I	1	30	all caves
	2	3	EC
	3	2	EC, JR
Hinf I	1	17	all caves
	2	17	all caves
	3	1	DC

* Abbreviations for cave names are: EC, Eagle Creek Cave; DC, Davis Cave; JR, James River Cave; CQ, Cuevo Quintero; PO, Puente de Ojuelo; CJ, Cuevo Janitzio; TB, Tio Bartolo.

The fragment patterns from each polymorphic endonuclease are listed along with the number of study animals bearing each pattern and the caves in which each pattern is found.

single Cla I restriction site, so that the fragment pattern appeared as a single fragment of about 16.8 to 17.2 kbp in length. Two animals had the Cla I-2 pattern. This had one additional Cla I restriction site, so that the genome in these animals was cut into two pieces: one was 9.4 kbp in length and the other was about 7.4 kbp long.

Composite phenotypes were constructed for each animal using fragment patterns from the five polymorphic endonucleases. Nine different composite phenotypes were found among the 35 animals used in this study. Each composite phenotype is listed in Table 3 and given a letter designation. For example, phenotype B contains fragment patterns Mbo I-1, Bgl II-1, Hind III-1, Cla I-1 and Hinf I-1. Phenotype A differs from B only in that it contains fragment pattern Hinf I-2 instead of Hinf I-1; phenotype C contains the Hinf I-3 pattern. Phenotype E differs from phenotype A only in that it contains Cla I-2 instead of Cla I-1, and phenotype F differs from phenotype A only in that it contains Mbo I-2 instead of Mbo I-1. Phenotypes D, G, and H differ from phenotype B by one pattern change each; D differs in the Cla I pattern, G differs in the Bgl II pattern, and H differs in the Mbo I pattern. Phenotype K differs from H in that it contains the Hind III-2 pattern instead of the Hind III-1 pattern.

Five of the composite phenotypes were found in one animal each. Each of the remaining four composite phenotypes was found in more than one animal and more than one cave. The distribution of all phenotypes among the seven caves is illustrated in Figure 5. The composite phenotype letter is followed by the number of animals in each cave bearing that particular phenotype. Two phenotypes, B and A, represent

TABLE 3. Composite Phenotypes.

Phenotype	Mbo I	Bgl II	Hind III	Cla I	Hinf II	# of animals
A	1	1	1	1	2	15
B	1	1	1	1	1	8
C	1	1	1	1	3	1
D	1	1	1	2	1	1
E	1	1	1	2	2	1
F	2	1	1	1	2	1
G	1	2	1	1	1	5
H	3	1	1	1	1	1
K	3	1	2	1	1	2
						35

Each composite phenotype is given a letter designation. All genotypes list the fragment patterns they include for each of the five polymorphic endonucleases. The number of study animals bearing each genotype is also listed.

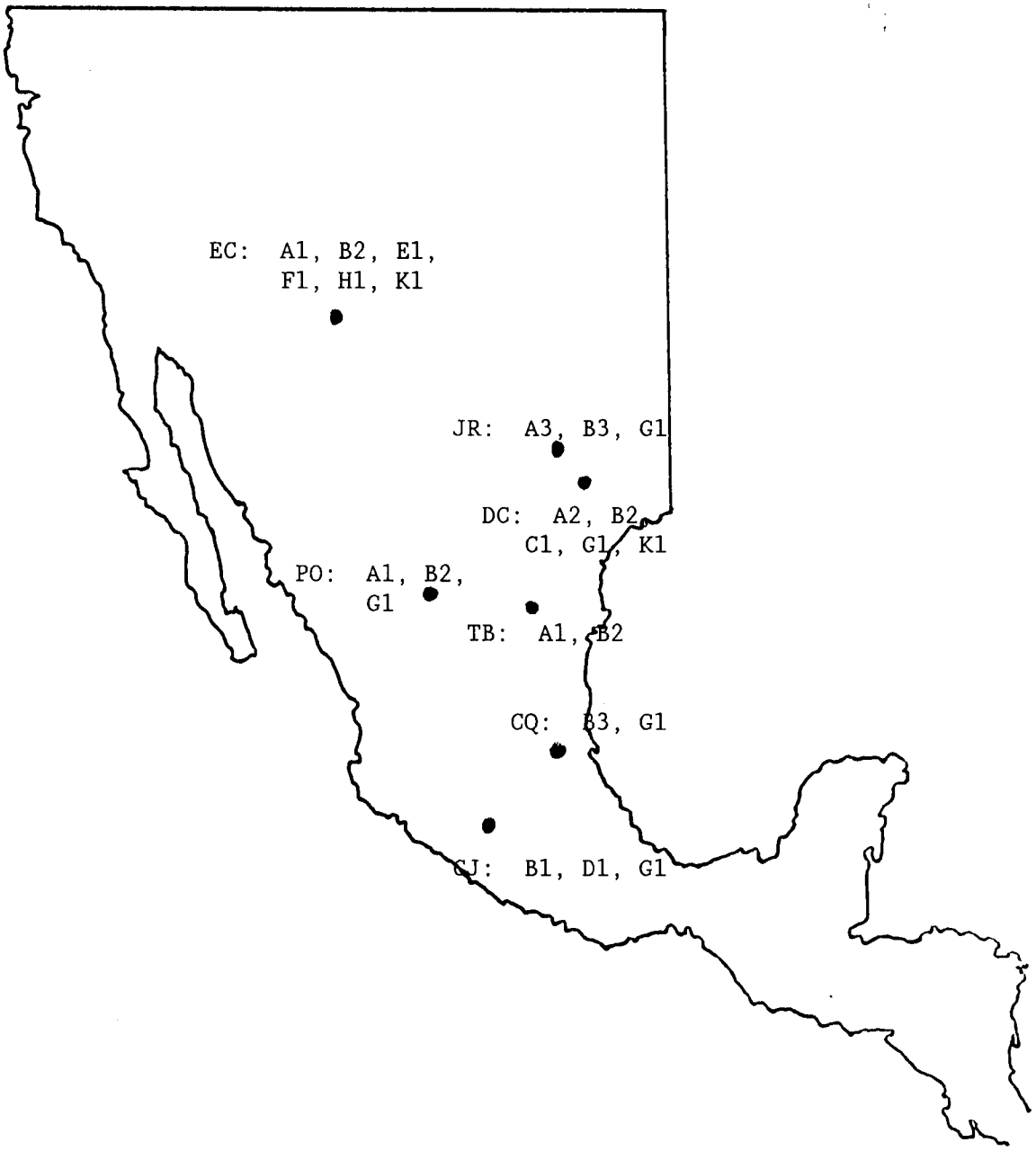


FIGURE 5. Distribution of Composite Phenotypes.

The abbreviation for each cave name is give (see text). After the cave name, the composite phenotypes found in that cave along with the number of animals bearing that genotype found in each cave is given.

66% of the sampled animals and were found in all seven caves and the five northernmost caves, respectively. Composite phenotype A was the most common, being found in 15 of the 35 study animals. Phenotype G was found in five animals, which were distributed among James River Cave, Davis Cave, Cuevo Quintero, Puente de Ojuelo and Cuevo Janitzio. Composite phenotype K was found in two animals; one was in Eagle Creek Cave and one was in Davis Cave. Each of the single-occurrence phenotypes was found as follows: phenotype C, Davis Cave; phenotype D, Cuevo Janitzio; phenotypes E, F and H, Eagle Creek Cave.

All composite phenotypes were connected into a parsimonious female lineage network (Figure 6). Both common phenotypes and their most closely related alternatives are found equally on both sides of the Sierre Madre Orientale mountains. Phenotypes A,C,D,G and H each differ from phenotype B by a single fragment pattern difference which can be attributed, in each case, to a single mutational event. Phenotypes F and E each differ from phenotype A by a single mutational event, and phenotype K differs from phenotype H in the same manner.

Upholt's (1979) equation was used to determine relatedness of composite phenotypes. For this analysis both monomorphic and polymorphic fragment patterns are used. Composite phenotypes A and C through K differ from phenotype B by less than one percent. This difference is well within the range of 0.00 to 4.0% found within single vertebrate species as reported by Brown (1981, 1983).

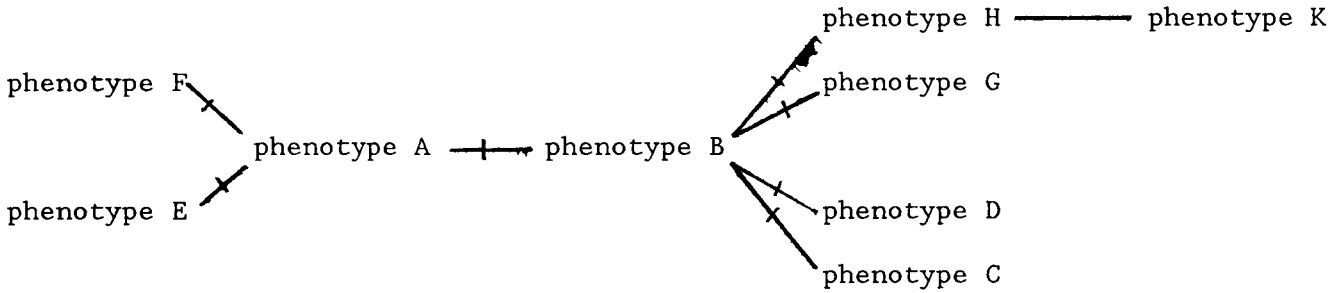


FIGURE 6. Parsimonious Female Lineage Network.

Composite phenotypes are connected by lines. Each line has hatch marks indicating the number of mutational steps separating the two connected phenotypes. In each case only one mutational step separates two connecting phenotypes. The B phenotype is separated from each other phenotype by no more than two mutational steps.

IV. DISCUSSION

Mitochondrial DNA analysis has added to the body of literature on social structure of Tadarida brasiliensis mexicana populations. Banding studies were done in the 1950's and 1960's (for a review, see Cochrum, 1969) involving many thousands of female migratory bats, but few recaptures occurred in the season in which the bats were banded, and even fewer were recaptured in subsequent seasons. Bands may have caused abnormal behavior and even death among those bats which were banded, so any patterns of movement or cave loyalty suggested by these studies would be questionable. Surveys of allozyme variation indicated that there were no significant genetic differences between populations of Mexican free-tailed bats. The most extensive survey (McCracken et al., 1986) used samples collected from breeding caves during a single breeding season and samples from summer roosting sites also taken during a single season. However, since male and female parents contribute equally to each offspring's nuclear DNA, and recombination of nuclear DNA allows each offspring the possibility of having a different genotype from either its mother or father, genetic effects of philopatry to roost sites could be masked by this recombination. Movements of bats between caves could also be sex biased, and this could mask cave loyalty of one or the other sex. For example, if males return to the same breeding cave each year but females do not, or vice versa, the gene pool of each breeding cave would be much larger than if both showed philopatry to breeding caves. Additionally, recombination of DNA would mask philopatry to maternity caves. Because mitochondrial

DNA is inherited directly from the female, and no recombination occurs, distribution of mitochondrial DNA phenotypes reflects dispersal of female lineages and can be used in concert with allozyme data to illustrate movement patterns of the migratory portion of the population as well as to indicate if philopatry to breeding or maternity caves occurs.

The social structure of T. b. mexicana caves can be examined by using both mitochondrial DNA and allozyme genetic data. Breeding caves are assemblages of male and female bats in approximately a 50:50 ratio (Villa and Cochrum, 1962). Bats in maternity caves consist of about 95 percent pregnant female bats which each give birth to a single pup while in residence. Maternity caves have been shown to contain females from several breeding caves (McCracken, in prep.). McCracken and Wilkinson (1986) have detailed methods by which allozyme genetics can be used to establish the social structure of bats. Mitochondrial DNA RFLPs are used here in concert with allozyme genetics (McCracken et al., 1986) to determine migratory patterns and philopatric associations of Mexican free-tailed bats.

The between-cave breeding cave genetic structure will be considered. If females and males were loyal to certain breeding caves, then allele frequencies as determined by allozymes would be heterogeneous across the range of T. b. mexicana. This was not the case (McCracken et al., 1986). If female bats showed philopatry, but males did not, then the allozymes would be homogeneous between caves, but mitochondrial composite phenotypes would be heterogeneous; that is, different mitochondrial phenotypes would occur in each cave. In the

situation where females are not loyal to particular breeding caves, but males are, then mitochondrial phenotypes would be homogeneous across the range and allozymes would be homogeneous between caves. If neither males nor females were philopatric to breeding caves then both genetic methods would show homogeneity between caves.

The distribution of composite phenotypes (Figure 5) supports the conclusion that two separate migratory flyways suggested by earlier banding studies do not occur in the T. b. mexicana range. These data also support the hypothesis that philopatry of Mexican free-tailed bats to breeding or maternity caves does not occur. All composite phenotypes which are found in more than one study animal are also found in more than one breeding cave and/or more than one maternity cave. The two major phenotypes (A and B) are found on both sides of the Sierra Madre Orientale mountain range. The minor phenotypes are also distributed equally on both sides of the Sierre Madre Orientale mountains. This distribution of phenotypes indicates that, as a general rule, cave philopatry does not occur. An important caveat to these conclusions is that sample sizes are so small that cave loyalty cannot be completely ruled out. Some subpopulations which were not sampled could show cave loyalty. These subpopulations could represent specific age classes of the bats or could be small populations from isolated caves. Sampling each possible genotype is not feasible, because cave populations tend to be very large (on the order of several million individuals in maternity caves and several tens of thousands in breeding caves). Although sample sizes from individual caves were not large enough to analyze these results statistically, this study clearly

suggests that behavioral, geographic and climatic barriers do not limit the movements of T. b. mexicana migratory populations.

Another important aspect of this study has been on the technical level. This involves the construction of DNA probes which can be used to visualize small amounts of DNA via hybridization. Population geneticists who have used RFLP's of mitochondrial DNA generally have sacrificed their study animals in order to obtain sufficient amounts of purified mitochondrial DNA for their studies. Tissues richest in mitochondria, such as liver and ovaries, are most often the source of mitochondrial DNA in studies on vertebrate organisms. Purification procedures require on the order of one to two grams of tissue which yield only a few micrograms of purified DNA. On the other hand, studies in which sacrifice of animals is not possible or desired could greatly benefit from information gained from mitochondrial DNA RFLP analysis. Lansman and coworkers (1981) outlined a method for isolation of mitochondrial DNA contaminated with small amounts of nuclear and cytoplasmic DNA. Hybridization conditions can be made stringent when probe DNA and sample DNA are closely related, thus reducing the possibility of nonspecific hybridization, so small amounts of mitochondrial DNA can be visualized. Clones have been used as radiolabelled probes in other studies (Kreitman and Aguade, 1986; Lansman et al., 1981).

This study successfully uses liver and biopsies of flight muscle to isolate sufficient mitochondrial DNA for analysis without sacrificing study animals. One tenth of one gram biopsies are large enough to allow for analysis by several restriction endonucleases.

There are sensitivity limits to hybridization techniques, and very small (500bp) fragments may not hybridize well. Nevertheless, the advantages gained from not having to sacrifice study animals far outweigh the disadvantages of having to use endonucleases which yield fewer but larger fragments.

Cloning the mitochondrial genome in this case would involve making several clones each of which contained only a portion of the mitochondrial genome, and there are several advantages to this. Endonucleases which have a cleavage site of four or five bases can cut the mitochondrial genome into 15 or more fragments, and patterns containing large numbers of fragments can be difficult to score accurately. Partial genome probes are useful in clarifying polymorphic fragment patterns because they visualize only a portion of the fragments in the digest. This facilitates scoring polymorphic patterns and can be helpful in mapping of cleavage sites on the DNA molecule. The cloned fragments may also be useful in studies which may involve gene mapping. Although the entire genome was not cloned for this study, efforts to accomplish this task continue. Because the main goal of this research was to use mitochondrial DNA RFLPs to assess movement patterns of Mexican free-tailed bats, cloning was a secondary goal. Clones have proven to be a useful source of probe DNA, and they have provided the methods by which sacrifice of study animals for mitochondrial genetic studies can be avoided.

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APPENDIX

APPENDIX

Composition of all solutions in the Materials and Methods section are listed here. Formulae can be found under secondary headings of sections in which they first appear.

Whole animal mitochondrial DNA isolation

SEDTA
100mM NaCl
50mM EDTA
pH 7.8-8.0

25% SDS
25g SDS
75ml water

5M Sodium chlorate
5M NaClO4 H20 in water

CIP
25 volumes distilled phenol
24 volumes chloroform
1 volume isoamyl alcohol

20 x SSC buffer stock
175.3g NaCl
88.2g sodium citrate
800.0ml water
adjust pH to 7.0 with 10N NaOH, then make up to 1 liter with water.

Ethidium bromide stock
1g ethidium bromide
100ml water
store in foil wrapped container.

Water or NaCl saturated sec-butanol
Pour equal volumes of sec-butanol and water (or 5 M NaCl) into a separatory funnel. Mix thoroughly.
Allow phases to separate, then drain off lower aqueous layer.
Add water (or 5 M NaCl) to sec-butanol layer and repeat.
Save sec-butanol layer.

TE buffer
10mM Tris adjusted to pH 7.4 with HCl
1mM EDTA

CNA isolation

2 x TSM buffer 0.06M Tris-HCl, pH 7.4
0.30M NaCl
3.00mM MgCl

10% Nonidet P-40
10ml Nonidet P-40 from Sigma Chemical Co.
90ml water

RNase A
1mg RNase A
1ml water
Store at -20 C

2M sodium acetate
272.1g sodium acetate
800ml water
Adjust to pH 5.2 with acetic acid, then bring volume to 1 liter with water.

Agarose electrophoresis

25 x E buffer
121g Tris base
28.6ml glacial acetic acid
50ml 0.5 M EDTA, pH 8.0

Loading dye
25% Ficoll
0.1% bromphenol blue
0.1% xylene cyanole FF
10mM Tris-HCl
1mM EDTA
pH to 7.8

Southern blots

Denaturation buffer
0.5M NaOH
1.5M NaCl

Neutralization buffer
1.0M Tris-HCl, pH 8.0
1.5M NaCl

Labelling probe DNA

Column buffer
0.1M NaCl
10mM Tris-HCl, pH 8.0
1mM EDTA

Hybridization to nitrocellulose filters

Prehybridization solution

10ml 20 x SSC buffer
2ml Denhardt's solution
0.1ml denatured salmon sperm DNA
0.1ml 10% SDS
7.8ml water

Denhardt's solution

5g Ficoll
5g polyvinylpyrrolidone
5g BSA (fraction V)
add water to 500mls
Sterile filter and store in -20 C freezer until use

Denatured salmon sperm DNA

Dissolve the DNA (Sigma type III) in water at a concentration of 10mg/ml. Several hours of constant stirring are necessary for dissolving the DNA. Shear the DNA by passing it through an 18 gauge hypodermic needle several times. Boil the DNA for 15 minutes, then store it in small aliquots at -20 C.

TBM H1 clone

5 x ligase reaction buffer

250mM Tris-HCl, pH 7.6
50mM MgCl
5mM dithiothreitol
store at -20 C. When ready to use, add fresh ATP to 5mM

5 x PEG

25% (w/v) polyethylene glycol 8000 in water

Calcium chloride solution

50mM CaCl
10mM Tris-HCl, pH 8.0

TYN medium

10g Bacto-tryptone
10g Bacto-yeast extract
5g NaCl
Make up to 1 liter with water. Sterilize.
For agar plates, add 20g of Bacto-agar/liter of medium.
Dissolve the agar in the medium before sterilizing.
Allow to cool before pouring plates.

Ampicillin

25mg ampicillin/1ml water
Sterile filter and store at -20 C.
Final concentration in media is 50 g/ml.

2% x-gal
100mg x-gal
5ml water

Selecting clones with mitochondrial DNA inserts

0.5 M NaOH
20g NaOH
1 liter water

1 M Tris-HCl, pH 8.0
121g Tris
900ml water
pH to 8.0
bring to 1 liter with water

1 M Tris-HCl in 1.5 M NaCl
121g Tris
g NaCl
bring to 1 liter with water

TBM Cl clone

SOC medium

LB medium
10g Bacto-tryptone
5g Bacto-yeast extract
10g NaCl
Make up to 1 liter with water. Sterilize.
For agar plates, add 20g Bacto-agar.
Dissolve the agar in the medium before sterilizing.
Allow to cool before pouring plates.

Minipreparation of plasmid/insert DNA

Alkaline lysis solution I
50 mM glucose
10 mM EDTA
25 mM Tris-HCl, pH 8.0

Alkaline lysis solution II
Prepare just prior to use.
0.2 N NaOH
1% SDS

Alkaline lysis solution III
60mls 5 M potassium acetate
11.5mls glacial acetic acid
28.5mls water

Large scale isolation of recombinant DNA plasmids

Chloramphenicol

34mg chloramphenicol/1ml 100% ethanol
Final concentration in media is 30 g/ml.

Abbreviations

C	degrees Centigrade
gm	grams
mg	milligram
g	microgram
ml	milliliter
l	microliter
mm	millimeter
cm	centimeter
nm	nanometer
M	molar
mM	millimolar
w/v	weight to volume
kbp	kilobase pairs
rpm	revolutions per minute
cpm	counts per minute
TBM H1	Clone of Tadarida brasiliensis mexicana mitochondrial DNA fragment 6.4kbp from a Hind III digest.
TBM C1	Clone of T. b. mexicana mitochondrial DNA fragment 9.4kbp from a Cla I digest.

VITA

Mary Kathryn Gustin was born in Indianapolis, Indiana on October 29, 1952. She was given the name Kitty by her mother and continues to be known by that name today. Kitty attended Indianapolis Public School #62 and Thomas Carr Howe High School, from which she graduated in June 1970. She attended DePauw University in Greencastle, Indiana for one year. From September, 1972 until May, 1974, Kitty attended Hope College in Holland, Michigan, and she graduated in May, 1974 with a Bachelor of Arts degree majoring in biology. After graduation, Kitty worked as a research technician in a number of laboratories at Indiana University, The University of Tennessee at Knoxville, and Oak Ridge National Laboratory. She began taking classes at The University of Tennessee and entered the Master's program in the Zoology Department in 1984. The Master of Science degree was awarded to her in August, 1988.

Kitty Gustin plans to continue her research career in the area of population genetics.