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I am submitting herewith a dissertation written by Amy Louise Russell entitled "Population Genetics and Effective Population Size of the Brazilian Free-Tailed Bat, *Tadarida brasiliensis* (Chiroptera: Molossidae)." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Ecology and Evolutionary Biology.

Gary F. McCracken
Major Professor

Christine Boake
Department Head

We have read this dissertation and recommend its acceptance:

Christine Boake

Daniel Simberloff

Nancy B. Simmons

Randall L. Small

Accepted for the Council:

Anne Mayhew
Vice Provost and Dean of Graduate
Studies

(Original signatures are on file with official student records.)

POPULATION GENETICS AND EFFECTIVE POPULATION SIZE OF THE
BRAZILIAN FREE-TAILED BAT, *TADARIDA BRASILIENSIS*
(CHIROPTERA: MOLOSSIDAE)

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Degree
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Amy Louise Russell

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DEDICATION

This dissertation is dedicated to my husband, John Daniel Morelli, for his emotional support and intellectual contributions, to my parents, James and Marsha Russell, for their continual support and encouragement of my education, and to the rest of my family, especially Lois Boak and Clifford Morelli.

“And hast thou slain the Jabberwock?
Come to my arms my beamish boy!
O frabjous day! Callooh! Callay!
He chortled in his joy.”
Lewis Carroll
“Through the Looking Glass”

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ABSTRACT

The purpose of this study was to examine patterns of genetic structuring and diversity at multiple spatial and taxonomic scales within the Brazilian free-tailed bat, *Tadarida brasiliensis* (Chiroptera: Molossidae). All analyses were completed using DNA sequence data from a non-coding, hypervariable region of the mitochondrial genome.

I explored the impact of variation in migratory behaviors on genetic structuring and gene flow in the Mexican free-tailed bat, *T. b. mexicana*. These analyses revealed that substantial gene flow exists among groups differentiated by both migratory tendency and direction, and suggest that migratory behavior in these populations is a plastic response to environmental conditions.

I investigated patterns of molecular diversity and genetic structuring within and among four subspecies of *T. brasiliensis* that are distributed throughout mainland North, Central, and South America. I found significant differentiation in populations from South America from those in North and Central America, suggesting that these regions may represent distinct species. Analyses of South American populations reveal a genetic division that is consistent with a model of regional isolation of populations caused by the Andes Mountains acting as a barrier to gene flow. Mitochondrial sequence data do not support the differentiation of the three recognized subspecies (*T. b. cynocephala*, *T. b.*

mexicana, and *T. b. intermedia*) in North and Central America at the subspecific level; I have made taxonomic recommendations that reflect these data.

Having determined that colonies within and among *T. b. mexicana* and *T. b. intermedia* are linked by high levels of gene flow, I used several statistical techniques to estimate the effective size of the combined subspecies as a single population, and to determine whether that population is consistent with a model of population growth, stasis, or decline. A maximum likelihood estimate of the effective size of this population ($N_e = 28.4$ million females) is the largest effective population size yet estimated for any mammal, and estimated measures of genetic diversity are among the highest for any organism. Multiple analyses of these parameters support a model of growth for this population.

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I. INTRODUCTION

This dissertation contains five parts, including this introduction. Parts 2, 3, and 4 are written as manuscripts for publication and are formatted for the target journals. Part 2 will be submitted to *Molecular Ecology*, part 3 will be submitted to *Evolution*, and part 4 will be submitted to *Nature*. In part 5 I summarize the dissertation and identify areas of possible research proceeding from this work. Preliminary analyses of a portion of the data included in this dissertation were published as a chapter in a symposium proceedings entitled “Functional and Evolutionary Ecology of Bats” (Russell and McCracken in press).

In part 2 I examine genetic structuring within North American populations of *Tadarida brasiliensis mexicana*, an extremely abundant migratory subspecies that occurs throughout Mexico and much of the southern and western United States. This subspecies has been subdivided into groups that are characterized by different migratory behaviors and that have been hypothesized as being genetically distinct (Cockrum 1969). Using mitochondrial DNA (mtDNA) sequence data, I test whether differences in migratory behavior among regional groups of *T. b. mexicana* have resulted in the genetic differentiation of those groups.

In part 3 I examine genetic structuring among four recognized subspecies, *T. b. cynocephala*, *T. b. mexicana*, *T. b. intermedia*, and *T. b. brasiliensis*, that occur in North, Central, and South America. These subspecies are defined by differences in behavior, morphology, and/or geographical range. However, previous data from allozymes (McCracken and Gassel 1997) and morphology (Schmidley 1991) suggest that gene flow occurs between *T. b. cynocephala* and

T. b. mexicana, at least at the interface of their ranges. I use phylogenetic and population genetic analyses of mitochondrial DNA (mtDNA) sequence data to investigate genetic structure between populations from North and South America, as well as to analyze patterns of genetic structure among populations of the putative subspecies within each continent.

In part 4 I use mtDNA sequence data to estimate the effective population size of *T. b. mexicana* and to determine whether the genetic data support models of population growth, decline, or stability over time.

In part 5 I summarize the conclusions from the previous parts, and propose areas for further study.

II. GENETIC VARIATION AND MIGRATION IN THE MEXICAN FREE-TAILED
BAT (*TADARIDA BRASILIENSIS MEXICANA*)

1. Abstract

The Mexican free-tailed bat, *Tadarida brasiliensis mexicana*, exhibits variation in migratory behavior over its range. Some populations undergo seasonal long-distance migrations to warmer climates in winter, whereas others are resident through the winter in more northern regions and hibernate. Variation is also exhibited among populations in their migratory routes, and long-term banding studies document that populations are largely faithful to a single migratory route. These observations have led to the prediction that behaviorally defined migratory groups make up structured gene pools. Tests of this prediction using allozyme markers have documented high levels of gene flow among migratory groups, with no evidence for the structuring of gene pools. I use phylogenetic and population genetic analyses of mitochondrial DNA sequence data to examine genetic structuring among three of the four previously defined migratory groups. I find no evidence supporting the maintenance of separate gene pools within this subspecies, and conclude that sufficient gene flow occurs among colonies to genetically homogenize the entire subspecies. These analyses imply that the entire *T. b. mexicana* subspecies evolves as a single, very large population.

2. Introduction

Differences in migratory behavior can potentially separate migratory from non-migratory populations during the mating season, and be an effective factor promoting reproductive isolation. Additionally, differences in the date of departure or the use of different migratory routes may separate populations during the mating season. Separate populations of numerous wide-ranging species are known to exhibit differences in migratory behaviors that include migratory tendency, direction, and timing (Gall *et al.* 1992; Buerkle 1999; Wirth and Bernatchez 2001; Hatase *et al.* 2002; Kimura *et al.* 2002; Dallimer *et al.* 2003).

Studies using presumably neutral genetic loci have found that variation in migratory behavior is often associated with genetic differentiation. Bird species that breed in different locations due to differences in migratory behavior typically have genetically structured populations (Buerkle 1999; Kimura *et al.* 2002). The same pattern has been found for aquatic animals that return to their natal areas to breed, such as loggerhead turtles (*Caretta caretta*; Hatase *et al.* 2002) and Chinook salmon (*Oncorhynchus tshawytscha*; Gall *et al.* 1992). Recent analyses of the migratory European eel (*Anguilla anguilla*) have found evidence of significant genetic structure in a species previously thought to be panmictic (Daemen *et al.* 2001; Wirth and Bernatchez 2001; Maes and Volckaert 2002). Conversely, some studies have found no association of genetic structuring with differences in migratory behavior. Dallimer *et al.* (2003), using microsatellites and morphological characters, found no genetic differentiation between groups of red-

billed quelea (*Quelea quelea*) that breed in the same time and place in Africa, but afterwards migrate in different directions.

Studies of genetic structuring among populations of a number of migratory and non-migratory bat species (reviewed in Burland and Worthington Wilmer 2001), typically demonstrate a lack of differentiation among migratory populations (McCracken *et al.* 1994; Webb and Tidemann 1996; Wilkinson and Fleming 1996), but there are exceptions. The migratory species *Nyctalus noctula* (Chiroptera: Vespertilionidae) exhibits significant genetic isolation-by-distance over a broad geographic scale (Petit *et al.* 1999). For migratory *Myotis myotis* (Chiroptera: Vespertilionidae), the Gibraltar Strait has been shown to be an effective barrier to gene flow (Castella *et al.* 2000). In contrast, significant differentiation among populations is typical for non-migratory species of bats (Worthington Wilmer *et al.* 1994; Burland *et al.* 1999; Rossiter *et al.* 2000). Migration versus seasonal occupancy of the same area is often cited as an explanation for the observed genetic pattern; however, few studies have examined genetic structuring in a species that demonstrates intraspecific variation in migratory behavior (Svoboda *et al.* 1985; McCracken *et al.* 1994; McCracken and Gassel 1997).

The Brazilian free-tailed bat, *Tadarida brasiliensis* (Chiroptera: Molossidae), is one of the most abundant bat species in the Western Hemisphere, occurring in colonies numbering up to tens of millions of individuals (McCracken *et al.* 1994). It is found throughout Central and South America and in North America from the Atlantic to the Pacific coast, north to 40 °N latitude (Hall

1981). Within North America, the subspecies *T. b. mexicana* is located in the southwest to the west coast of the United States and in Mexico (Figure 2.1). Colonies in the southwestern United States are migratory, with bats overwintering in central Mexico (Villa-R. 1956; Villa-R. and Cockrum 1962) and dispersing in February and March. Migration appears to be female-biased with many males moving shorter distances and remaining in Mexico. Mating occurs during February and March in transient roosts located in northern Mexico and in Texas (Villa-R. and Cockrum 1962; McCracken and Wilkinson 2000; A. Keely pers. com.). In June and July pregnant females congregate in maternity colonies in caves located as far north as southern Kansas (Glass 1958; Davis *et al.* 1962; Kunz *et al.* 1980). After giving birth, the females and young remain in maternity colonies until the young are weaned. The bats then disperse rapidly, forming smaller colonies in buildings, caves, and mines from August to early October. Beginning as early as September, the bats return to Mexico.

The *mexicana* subspecies was the subject of long-term banding studies during the 1950s and 1960s in which over 430,000 bats were banded throughout much of the southwestern United States and Mexico. Approximately 1.5% of these banded bats were recovered (reviewed in Cockrum 1969; Glass 1982; McCracken *et al.* 1994; Russell and McCracken in press). From band recapture records, Cockrum (1969) described four geographically and behaviorally distinct migratory groups (Figure 2.1), characterized as follows: Group A, resident populations in California and southern Oregon that do not migrate, but undergo

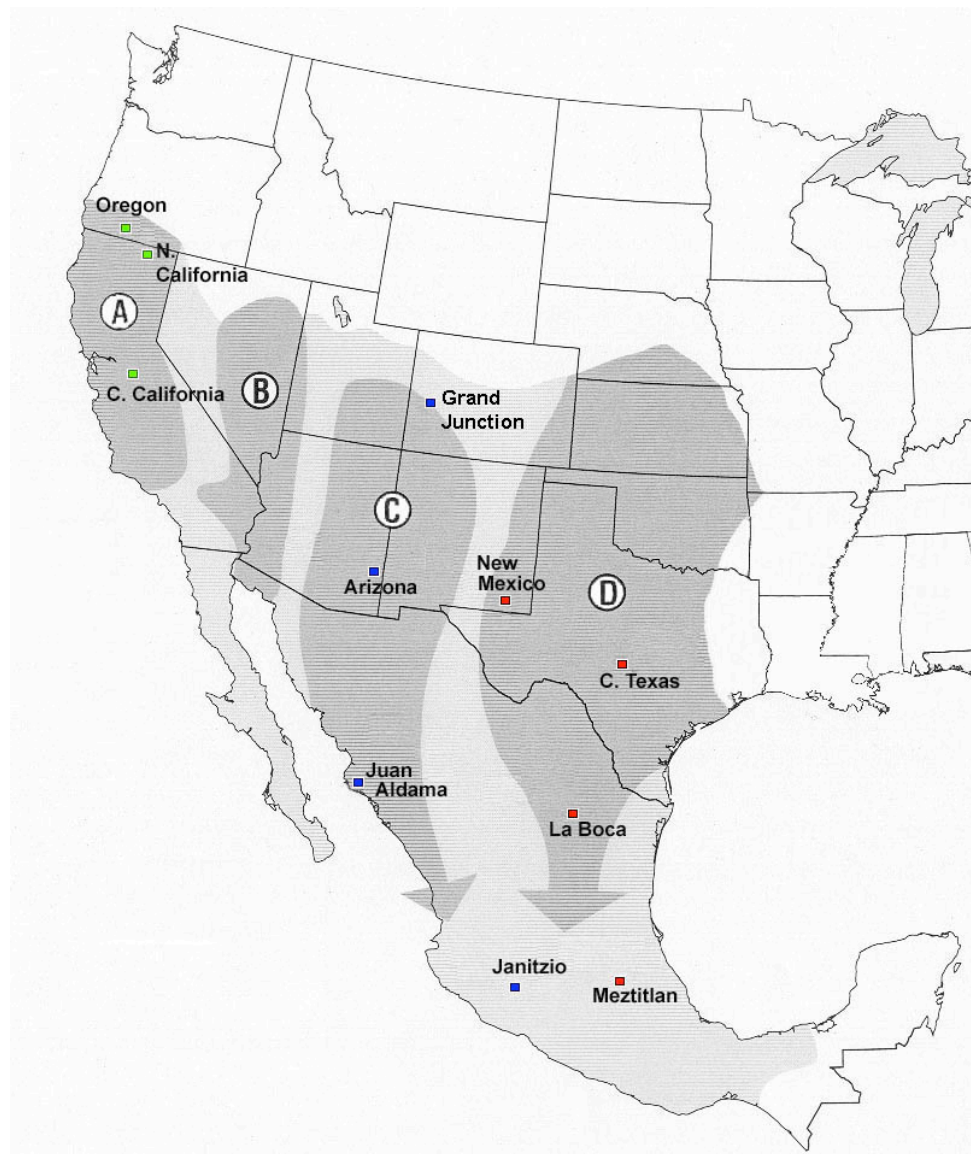


Figure 2.1. Range of *T. b. mexicana* with ranges of the putative migratory groups given as shaded areas (from Cockrum 1969). Sampled populations are indicated, with the designated migratory group indicated by color. Samples from the non-migratory group A are shown in green, the western long-distance migratory group C in blue, and the eastern long-distance migratory group D in red.

only local seasonal movements and hibernate in winter; Group B, located in western Arizona, southern Nevada, and southeastern California, migrates relatively short distances, perhaps into Baja California or into the low interior valleys of southern California; Group C, located in central and eastern Arizona and western New Mexico, migrates long distances through Sonora and Sinaloa and utilizes a flyway on the western side of the Sierra Madre Mountains; Group D, located in central and eastern New Mexico, western Kansas, Oklahoma, and Texas, migrates long distances along a flyway on the eastern side of the Sierra Madre.

This single subspecies, therefore, demonstrates variation in migratory tendency (migratory versus non-migratory) and migratory direction (eastern versus western migratory corridors). Tremendous amounts of banding and observational data directly measure migratory tendency and movement patterns throughout the range. Thus, these bats present an excellent opportunity to examine the influence of migratory behavior on population genetic structure. Here I evaluate patterns of genetic diversity using mitochondrial DNA (mtDNA) sequence data. Although nuclear microsatellite and nuclear sequence data are not available for this species, I use these mitochondrial data in combination with existing nuclear allozyme data (Svoboda *et al.* 1985; McCracken *et al.* 1994; McCracken and Gassel 1997) to explore the impact of variation in migratory behavior on genetic structuring of populations.

3. Materials and Methods

Sample Collection

Samples representing three of the four migratory groups described by Cockrum, including the two long-distance migratory groups and the western non-migratory group, were provided by colleagues. Samples from colonies belonging to the short-distance migratory group B were not available. Bats were captured using 4'x4' harp traps or mist nets erected at the entrance of each colony, or with hand nets within roosts. The bats were sexed, and placed in cloth bags prior to tissue sampling. Two 3-mm wing biopsy punches, one from each wing, were taken from each bat for DNA analysis (Worthington Wilmer and Barratt 1996). The bats were then released on the same night at the place of capture. The tissue samples were stored on ice in either NaCl-saturated 20% DMSO or silica gel desiccant in the field and at -80°C upon return to the lab. Tissue samples were obtained from a total of 94 bats from eleven locations throughout the subspecies' range, with an average sample size of 8.5 bats per population (Table 2.1; Figure 2.1).

DNA Isolation and Amplification

I isolated total genomic DNA using a standard phenol-chloroform-isoamyl alcohol (25:24:1), ethanol precipitation method (modified from Sambrook *et al.* 1989) or using a DNeasy® DNA isolation kit (Qiagen), and stored in 1/10 Tris-EDTA (TE). I then quantified the isolates using a Hoefer DyNA Quant® 200

Table 2.1. Sampling information. Colonies are listed from east to west by increasing longitude. The sample size indicates the number of individuals that were sequenced from each colony. Samples were donated by colleagues as indicated.

Colony Name	Location	Coordinates	Sample Size	Collected By
Meztitlan	El Salitre, Meztitlan, Hidalgo, Mexico	20:07N, 98:44W	9	R. Medellín
CTexas	James River Cave, Mason Co., TX, USA	30:44:57N, 99:13:56W	10	G. McCracken
LaBoca	Cueva La Boca, Nuevo Leon, Mexico	25:41N, 100:15W	12	R. Medellín
Janitzio	Isla de Janitzio, Michoacán, Mexico	19:42N, 101:07W	11	R. Medellín
NewMexico	Carlsbad Caverns National Park, Eddy Co., NM, USA	32:10:32N, 104:22:34W	10	L. McWilliams
Colorado	Grand Junction, Mesa Co., CO, USA	39:04N, 108:33W	4	K. Navo
JAldama	Ejido Juan Aldama, Sinaloa, Mexico	25:45N, 108:57W	12	R. Medellín
Arizona	Eagle Creek Cave, Greenlee Co., AZ, USA	33:03:11N, 109:19:46W	8	G. McCracken
CCalifornia	Merced Co., CA, USA	37:28:11N, 120:29:56W	10	E. Pierson

Table 2.1. Continued.

Colony Name	Location	Coordinates	Sample Size	Collected By
NCalifornia	Lava Beds National Monument, Siskiyou Co., CA, USA	41:53:18N, 121:22:16W	3	S. and C. Cross
Oregon	Jackson Co., OR, USA	42:11:25N, 122:41:58W	5	E. Arnett

fluorometer (Amersham Pharmacia), and diluted them to a standard concentration of 10 ng/ μ l for the polymerase chain reaction (PCR). I amplified all samples using primers F(mt): 5'-GTTGCTGGTTTCACGGAGGTAG-3', and P(mt): 5'-TCCTACCATCAGCACCCAAAGC-3' (Wilkinson and Chapman 1991) for the control region (D-loop) of the mitochondrial genome, located near the tRNA^{pro} gene. These primers yielded a product approximately 500 bp long. To obtain adequate amounts of DNA for sequencing reactions, I performed amplifications in eight 12 μ L reaction volumes, each containing 1.04 mM MgCl₂, 0.1 mM dNTPs, 1 Unit Taq DNA polymerase (Promega), 1.2 μ l Promega 10X buffer, 10 ng genomic DNA, and 14 pmol of each primer. The amplification involved an initial denaturation at 94 °C for two minutes followed by 30 cycles at 94 °C for one minute, 55 °C for 1.5 minutes, and 72 °C for two minutes. I combined the PCR products of the eight reactions and purified the target fragment using gel band excision (MinElute® kit, Qiagen).

DNA Sequencing

I sequenced the gel-purified DNA fragment using the Prism® BigDye™ terminator cycle sequencing kit version 2 (Applied Biosystems) in a 10 μ L reaction containing 3 μ L of ready reaction mix, 7 pmol of primer, and 50-100 ng of purified PCR product. The sequencing reaction consisted of 25 cycles at 96 °C for 10 seconds, 50 °C for 5 seconds, and 60 °C for 4 minutes, with temperatures changing at 1 °C per second. I cleaned the sequencing reactions of

unincorporated nucleotides using Centri-Sep columns (Princeton Separations) according to the manufacturer's instructions, and analyzed the reaction on an ABI 3100 automated sequencer. I sequenced twenty arbitrarily chosen individuals from both directions to quantify the average rate of sequencing error. Because this rate of error was low ($\sim 2.1 \times 10^{-3}$ per nucleotide), I sequenced most individuals using only the P(mt) primer. All sequences were deposited with GenBank.

The control region of the mitochondrial genome is characterized in most bat species by variable numbers of tandemly repeated 81-bp sequence units (Wilkinson *et al.* 1997). In *T. brasiliensis*, I found that most individuals possess three of these repeat units, although I observed length variation representing up to six repeats. I detected sixteen individuals representing seven locations that possessed more than three repeat units. These individuals were found in all of the three sampled migratory groups, and there was no detectable geographical bias to their distribution (data not shown). In this study, I included only individuals possessing three repeat units to avoid complications in sequence alignment.

Sequence Analysis

I aligned all sequences using the default settings in CLUSTALW (Thompson *et al.* 1994). The alignment was then edited by eye and cropped to a common length of 474 bp. Sequence variation included twelve insertion/deletions (indels), each one bp in length, making alignment unambiguous. In all analyses, indels were treated as a fifth character, rather than as missing data.

Analyses of DNA sequence data are made more accurate and efficient by specifying an appropriate model of evolution. These models detail parameters of evolutionary change such as nucleotide frequencies and the rates of change between pairs of nucleotides; individual models make simplifying assumptions about some or all of these parameters. I conducted a likelihood analysis using MODELTEST version 3.06 (Posada and Crandall 1998) to determine which of 56 models of evolution best fit the data. This analysis begins by assessing the likelihood of the data under the most simplified Jukes-Cantor (1969) model, a model that assumes equal nucleotide frequencies and equal rates of change between all pairs of nucleotides. Simplifying assumptions of the Jukes-Cantor model are then individually replaced by direct estimates of different parameters, and the likelihood of the data is assessed under these new models of evolution. The model that best describes the data is identified using likelihood ratio tests. Subsequent phylogenetic and population genetic analyses were carried out using the model assumptions and parameter values specified.

I used ARLEQUIN version 2.000 (Schneider *et al.* 2000) to calculate descriptive parameters of molecular diversity such as haplotype diversity (h) and nucleotide diversity (π) for each colony, and identified haplotypes shared among colonies. Haplotype diversity reflects the number of different haplotypes in the data set, and is calculated according to Nei (1987):

$$\hat{h} = \frac{n}{n-1} \left(1 - \sum_{i=1}^k p_i^2 \right)$$

(1)

where n = the number of haplotype copies in the sample, k = the number of haplotypes, and p_i = the sample frequency of the i -th haplotype. Nucleotide diversity reflects the evolutionary distance among the sampled haplotypes, and is calculated using the method of Tajima (1983):

$$D = \frac{1}{k(k-1)} \sum_{i=1}^k \sum_{j=i+1}^k p_i p_j d_{ij} \quad (2)$$

where k = the number of haplotypes; p_i = the frequency of haplotype i , and d_{ij} is the minimum number of mutations having occurred since the divergence of haplotypes i and j .

Phylogenetic Analyses

I employed a phylogenetic approach to determine whether variation in migratory behavior as described in the literature results in cladistic differentiation of separate gene pools. Due to the large number of haplotypes, the data set was not amenable to a traditional maximum likelihood analysis. Therefore, I used a Bayesian likelihood analysis to look for the phylogenetic signal among the migratory groups (MRBAYES version 3.0B4; Huelsenbeck and Ronquist 2001).

As implemented in MRBAYES, this analysis uses a Metropolis-coupled Markov chain Monte Carlo, or (MC)³, approach with a Metropolis-Hastings sampling algorithm (Huelsenbeck *et al.* 2002). In this approach, a single Markov chain begins with a random tree, T , with topology τ and branch lengths λ . Next, a new tree designated T' is proposed. This new tree is accepted as the next state

of the chain with the probability described by Metropolis *et al.* (1953) and Hastings (1970) and based upon the observed data X :

$$R = \min \left[1, \frac{f(X | \theta')}{f(X | \theta)} \times \frac{f(\theta)}{f(\theta')} \times \frac{f(\theta | X \theta')}{f(\theta | X \theta)} \right] \quad (3)$$

where $f(X | \theta')/f(X | \theta)$ is the likelihood ratio, $f(\theta)/f(\theta')$ is the prior ratio, and $f(\theta | X \theta')/f(\theta | X \theta)$ is the proposal ratio. Because searches using a single Markov chain may be more prone to convergence upon locally optimal trees, multiple chains may be run in parallel to allow a more thorough search of treespace. In these Metropolis-coupled Markov chains, a single “cold” chain samples from the posterior distribution of interest, $\Pr[\theta | X]$. Other “heated” chains sample from distributions obtained by raising the cold distribution by β_i , where $\beta_i = 1/(1 + iT)$ and T is a specified “temperature” parameter. At regular intervals, two chains are randomly picked and an attempt is made to change their states using a normal Metropolis-Hastings step as described above. Thus, the effect of heating is to enable heated chains to traverse valleys between isolated hilltops in the likelihood surface more easily and to provide the cold chain with more probable alternatives of new states. In this way, the cold chain is mixed more thoroughly by being exposed to isolated hilltops that would not otherwise have been explored due to the intervening valleys of highly improbable intermediate states.

I ran four Markov chains for 500,000 generations each with sampling every 100 generations, resulting in a sample of 5000 trees. The chains were heated using the temperature scaling factor $T = 0.2$. Mutation rates were

assumed to follow a gamma distribution with six substitution categories. I discarded the first 1000 trees, and constructed a 50% majority consensus phylogram from the remaining 4000 trees using PAUP* (Swofford 1998).

I used a cladistic analysis of gene flow to quantify the genetic structure shown in the tree (Slatkin and Maddison 1989). This analysis assigns character states (in this case, migratory group designations) to every branch in a tree in such a way as to minimize the number of changes between states on the tree, and then counts the number of gene flow events between migratory groups as the number of character state changes. This same procedure is then carried out with 1000 randomly joining trees to create a null distribution of gene flow events. The presence of genetic structure among populations is determined by comparing the number of inferred gene flow events in the observed tree with this null distribution.

In many intraspecific analyses, a hierarchical tree format may be inappropriate for representing relationships among haplotypes because the period of time over which the data have evolved is so short that ancestral and descendant haplotypes exist concurrently or because reticulate relationships may exist among haplotypes (Posada and Crandall 2001). In such cases, a haplotype network can better depict relationships among the sampled haplotypes and can portray uncertainties about those relationships using loops to indicate possible recombination, homoplasy, or reverse mutations. I constructed a minimum spanning network using ARLEQUIN version 2.000.

Population Genetic Analyses

Because barriers to gene flow that have developed recently may not be detectable in a phylogenetic analysis, population genetic analyses combining information on haplotype frequency, genetic distance, and geographic distribution can be more instructive concerning shorter-term processes (Neigel 2002). I calculated pairwise F_{ST} values between colonies to identify pairs of colonies that were genetically distinct using the method of Slatkin (1991):

$$F_{ST} = \frac{f_0 - f_1}{1 - f_1} \quad (4)$$

where f_0 is the probability of identity by descent of two different haplotypes drawn from the same population and f_1 is the probability of identity by descent of two haplotypes drawn from two different populations. I also conducted a Mantel test to test for a correlation between genetic distance (F_{ST}) and geographic distance. Geographic coordinates for each colony were determined using the Astro-dienst website (<http://www.astro.com/cgi/aq.cgi?lang=e>), and the geographic distance between each pair of colonies was calculated as the direct aerial distance using the How Far Is It? website (<http://www.indo.com/distance/>). Colonies having the same migratory behavior were pooled into single populations for analyses of molecular variance (AMOVA; Excoffier *et al.* 1992) to detect evidence of genetic structuring among migratory groups. Using the software package ARLEQUIN, I tested for significant structuring between non-migratory and migratory types, and among Cockrum's three migratory groups. The resulting measure of Φ_{ST} is the

correlation of random haplotypes within populations relative to random pairs drawn from the whole subspecies. Significance was determined by comparing the observed variance measures with null distributions obtained from 16,000 random permutations of the data. I transformed pairwise F_{ST} values between behaviorally distinct populations into estimates of $N_e m$, the average number of migrants per generation using an equation from Wright (1951), modified for mitochondrial data (Birky *et al* 1983):

$$F_{ST} = \frac{1}{(2N_e m + 1)}.$$
(5)

4. Results

Molecular Diversity

The D-loop of *T. b. mexicana* is characterized by very high levels of diversity (Table 2.2). I identified 86 different haplotypes (91.5%) in the sample of 94 individuals. In contrast to this high overall haplotype diversity ($h = 0.998$), I found a relatively low sequence diversity (π per site = 0.045), indicating that the many haplotypes I detected were closely related to one another. Seventy-eight of the 86 different haplotypes occurred as singletons, and the remaining eight were each found in only two individuals. Of the eight haplotypes that were shared by two individuals, five (62.5%) were in individuals in the same migratory group, of

Table 2.2. Molecular diversity indices for all colonies; n = number of individuals sequenced; n_h = number of different haplotypes; h = haplotype diversity; π = nucleotide diversity. The overall number of haplotypes is less than the sum of n_h for all individual colonies due to the presence of haplotypes shared between colonies.

Colony	Migratory Group	n	n_h	h	π
CTexas	D	10	10	1.000	0.035 ± 0.019
NewMexico	D	10	10	1.000	0.051 ± 0.028
Meztitlan	D	9	9	1.000	0.046 ± 0.025
LaBoca	D	12	11	0.985	0.051 ± 0.027
Colorado	C	4	4	1.000	0.047 ± 0.032
Arizona	C	8	8	1.000	0.051 ± 0.029
JuanAldama	C	12	12	1.000	0.040 ± 0.021
Janitzio	C	11	11	1.000	0.056 ± 0.030
CCalifornia	A	10	10	1.000	0.039 ± 0.021
NCalifornia	A	3	3	1.000	0.056 ± 0.043
Oregon	A	5	4	0.900	0.033 ± 0.021
overall		94	86	0.998	0.045 ± 0.022

which two were in the same colony. The three haplotypes present in multiple migratory groups were shared between the two long-distance migratory groups C and D. No haplotypes were shared between individuals in migratory versus non-migratory groups.

Evolution of this section of the mtDNA molecule conforms to the predictions of the Tamura-Nei model of evolution (Tamura and Nei 1993), which was first derived to describe the evolution of the control region in primate mtDNA. This model specifies unequal base frequencies and a higher rate of transition than transversion mutations with different rates of transitions between purines than between pyrimidines. In my data, I found that adenine constituted nearly 50% of the nucleotides in an average sequence ($\pi_A = 0.448$, $\pi_C = 0.189$, $\pi_G = 0.086$, $\pi_T = 0.278$). The observed transition:transversion rate was 7.68, and I observed over 1.5 times as many transitions between pyrimidines ($R_{C-T} = 72.215$) as between purines ($R_{A-G} = 42.768$). Nearly a third of the sites were invariant ($I = 0.327$), and the region was characterized by a gamma-distributed mutation rate with the shape parameter $\alpha = 0.5503$.

Phylogenetic Analyses

Bayesian likelihood analysis using *T. b. brasiliensis* as an outgroup revealed no evidence of long-term segregation of gene pools among migratory groups. A strict consensus summary of the 4000 trees resulting from the analysis is completely comb-shaped and reveals no structure among the data (not shown). More cladistic structure was found in a 50% majority rule consensus

tree, but this structure does not correspond with any clustering of groups correlating with their migratory behavior (Figure 2.2). I attempted to quantify the level of structuring in the genealogy using Slatkin and Maddison's (1989) cladistic analysis of gene flow. The many multifurcations in the genealogy and the resulting ambiguities in the character state of many internal branches biased the number of gene flow events downwards relative to the completely bifurcating random trees. The highly unresolved structure in the observed genealogy may indicate a starlike phylogeny resulting from recent population growth (Part 4); in any case, it yields no clear signal for these analyses.

I constructed a minimum spanning network to depict the relationships among haplotypes (Figure 2.3). This network also shows no clear pattern of structure among behaviorally differentiated groups. Most sequential haplotypes in the network were separated by multiple mutation steps (mean = 6.1 steps, range 1 to 21). Although this lack of intermediate haplotypes is not unexpected in a species with very large population sizes, it does preclude a statistically rigorous analysis of genetic structuring in the network, such as a nested cladistic analysis (Templeton 1998).

Population Genetic Analyses

F_{ST} values between all pairs of colonies were low with only one pair of colonies (La Boca which is migratory, and Oregon which is non-migratory) showing an F_{ST} significantly greater than zero (Table 2.3). Except for these and La Boca and central California ($F_{ST} = 0.047$, $P = 0.086$), all other pairwise F_{ST}

Figure 2.2. A 50% consensus tree from a Bayesian likelihood analysis. Only trees sampled after the burn-in period were used to construct the consensus. Support indices are the percent of trees having the given partition, and are equivalent to the posterior probabilities from the Bayesian analysis. Migratory group designations were traced onto the tree for a cladistic analysis of gene flow (Slatkin and Maddison 1989), and are indicated by branch color, following the same color code as in Figure 2.1. Internal branches are assigned to migratory groups following a parsimony criterion; branches in gray could not be unambiguously assigned to a single group. The Argentina1 haplotype, shown in orange, was used as an outgroup for comparison.

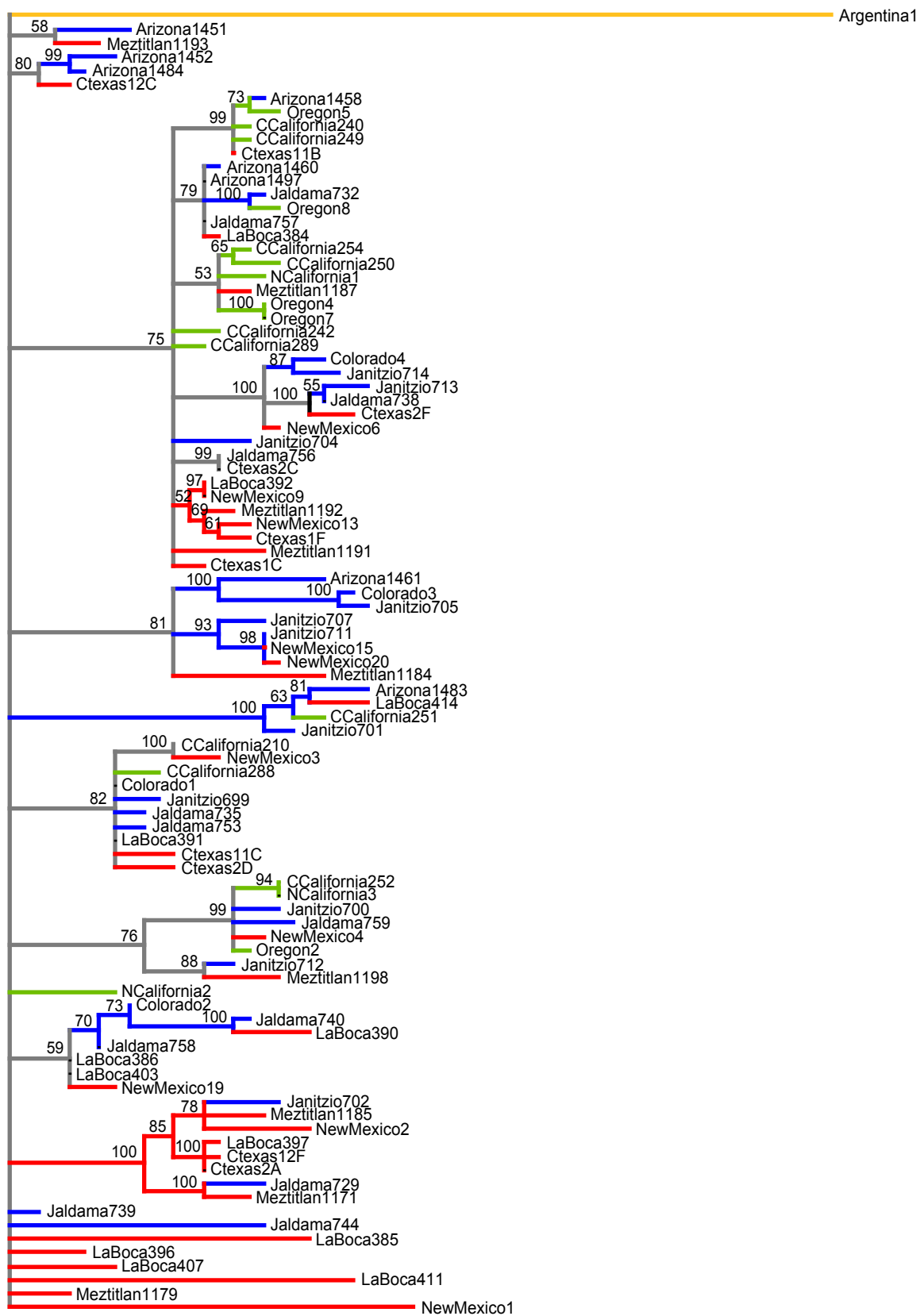


Figure 2.3. Minimum spanning network of all haplotypes. Migratory groups are indicated by tone; white is group A, gray is group C, and black is group D. Smaller circles represent unique haplotypes, and larger circles represent haplotypes found in two individuals. Haplotypes having two tones were found in two migratory groups. The number of mutations at each step of the network is indicated by hatch marks.

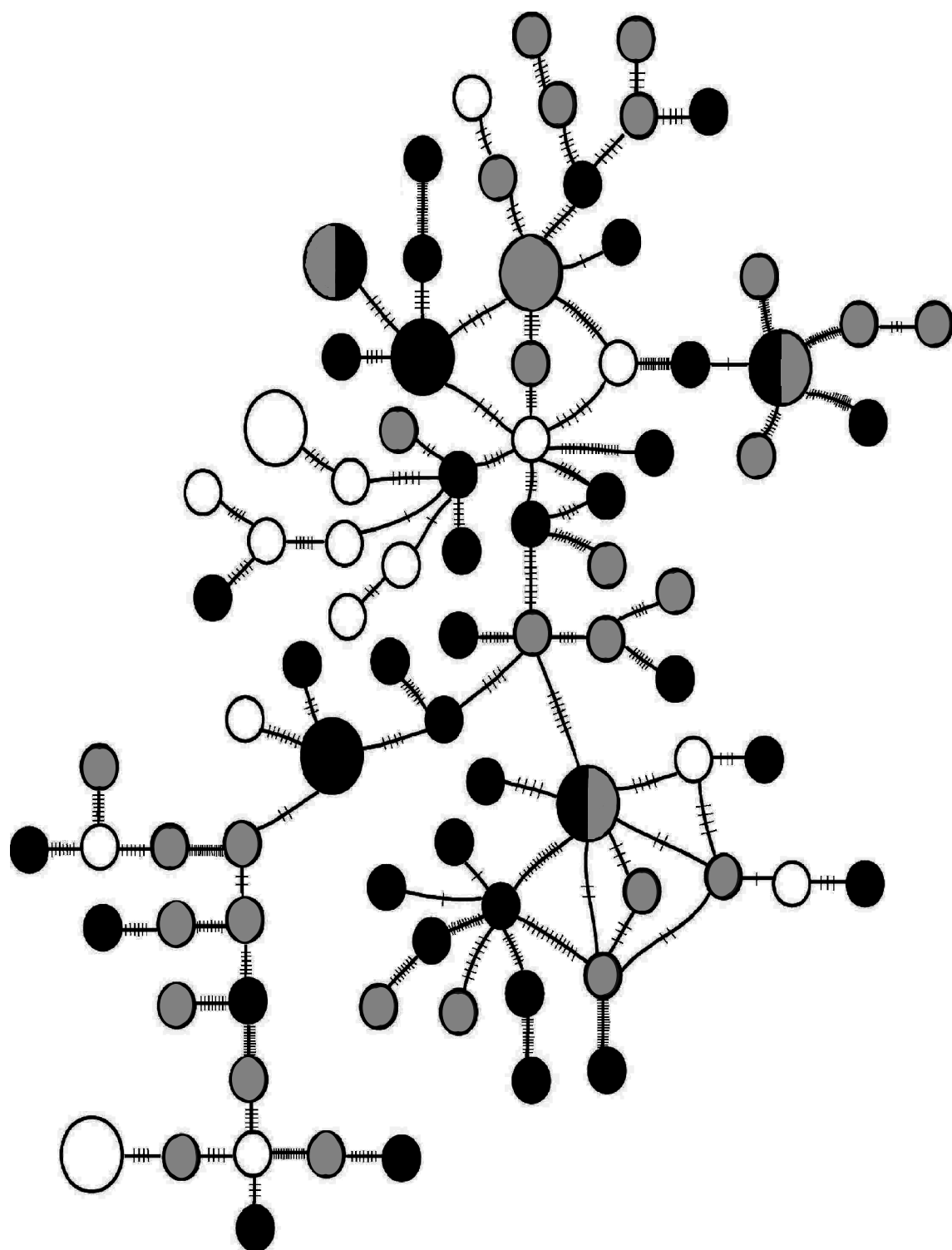


Table 2.3. Pairwise F_{ST} between individual colonies. * $P < 0.05$. Significant P values are given above the diagonal.

	CTexas	NMexico	Colorado	Arizona	CCalif.	NCalif.	Oregon	JAldama	Janitzio	LaBoca
CTexas	-									
NMexico	0.012	-								
Colorado	-0.024	-0.060	-							
Arizona	0.001	-0.008	-0.042	-						
CCalifornia	-0.006	0.010	0.009	-0.004	-					
NCalifornia	-0.037	-0.125	-0.124	-0.119	-0.045	-				
Oregon	0.069	0.026	0.084	0.077	-0.012	-0.053	-			0.021
Jaldama	-0.036	0.011	-0.052	-0.011	-0.009	-0.102	0.064	-		
Janitzio	0.005	-0.052	-0.089	-0.003	0.004	-0.117	0.028	-0.004	-	
LaBoca	0.020	0.014	-0.061	-0.045	0.047	-0.101	0.128*	-0.005	0.017	-
Meztitlan	-0.025	-0.028	-0.057	-0.019	0.015	-0.137	0.027	-0.028	-0.031	0.001

measures were nonsignificant at $P > 0.10$. An analysis of molecular variance (AMOVA) of all colonies pooled into their respective migratory groups revealed no evidence of genetic structuring among groups with nearly all genetic variation in the data set explained by variation among individuals within colonies ($\Phi_{ST} = 0.004$, $P = 0.290$; Table 2.4). An AMOVA analysis of differentiation between the non-migratory and the pooled migratory groups showed that approximately 2% of the total variance was explained by differences between migratory and non-migratory groups, and about 98% of all genetic variance was present among colonies within their respective group ($\Phi_{ST} = 0.021$, $P = 0.054$; Table 2.4). The weak signal of genetic structuring between migratory and non-migratory groups is due in large part to the genetic differentiation between the migratory colony at Cueva La Boca in eastern Mexico and the non-migratory colonies in California and Oregon. An AMOVA analysis with the La Boca sample removed reveals the impact of that single colony on genetic structuring in the entire data set ($\Phi_{ST} = 0.015$, $P = 0.111$). Whether Cueva La Boca is of any special significance in the life history of *T. b. mexicana* is not certain, but it is notable that this is the only colony in my sample to share a haplotype with the southeastern subspecies *T. b. cynocephala* (Part 3). A Mantel test revealed no correlation between genetic distance (F_{ST}) and geographic distance ($r = -0.017$, $P = 0.536$; Figure 2.4).

The average numbers of migrants per generation among migratory groups and between migratory and non-migratory populations were estimated using pairwise Φ_{ST} measures as in equation 5 (Table 2.5). These pairwise analyses reveal no genetic distinction between the migratory groups C and D ($\Phi_{ST} =$

Table 2.4. AMOVA analyses of genetic structuring between the non-migratory and the pooled migratory populations and among the three groups. *P* values indicate the probability of obtaining a lower F_{ST} statistic by chance in 16,000 random permutations of the data.

Hierarchical level	% variation	F_{ST} statistic	<i>P</i> value
<i>Between migratory and non-migratory populations</i>			
Among pops	2.14	$F_{ST} = 0.021$	0.054
Within pops	97.86		
<i>Among all groups</i>			
Among groups	0.37	$F_{ST} = 0.004$	0.290
Within groups	99.63		

Figure 2.4. Mantel test of the correlation between genetic distance (F_{ST}) and geographic distance. The regression line and its equation are shown.

Table 2.5. Pairwise F_{ST} measures between migratory and non-migratory populations and between migratory groups. P values are the significance of the F_{ST} measures. Estimates of migration ($N_e m$) are derived from F_{ST} values using equation 5 in the text.

	F_{ST}	P value	$N_e m$
Migratory vs. non-migratory	0.021	0.054	23.31
A-C	0.018	0.128	27.28
A-D	0.024	0.065	20.33
C-D	-0.011	0.950	undef.

-0.011), and indicate that the groups are of the same population. Gene flow between migratory and non-migratory groups ($N_e m = 20.33$ and 27.28) and between non-migratory and pooled migratory populations ($N_e m = 23.31$) is estimated at levels that are sufficient to homogenize the gene pools (Wright 1931).

5. Discussion

Variation in migratory behavior that spatially separates populations during mating can serve as a mechanism for reproductive isolation. *Tadarida brasiliensis mexicana* consists of four groups that have been considered distinct because of migratory behaviors, however, my mtDNA sequence data do not indicate genetic structure either among previously described migratory groups or between migratory and non-migratory populations. Neither phylogenetic nor population genetic analyses provide evidence that migratory behavior is associated with the genetic differentiation of migratory groups or migratory versus non-migratory populations, but rather, they indicate that significant gene flow occurs between these groups. These results suggest that any differences in migratory tendency and direction revealed by the recovery of banded bats may be due to behavioral plasticity.

My estimates of F_{ST} for *T. b. mexicana* ($F_{ST} = 0.004$ to 0.021 ; Table 2.4) indicate a lack of genetic structure that is similar to that found in other widely dispersing species. Buerkle (1999) found no evidence of genetic structuring

among five geographically dispersed populations of a migratory subspecies of prairie warblers, *Dendroica discolor discolor* (pairwise $\Delta_{ST} = -0.014$ to 0.091). Eastern populations of the monarch butterfly, *Danaus plexippus*, overwinter in large colonies in Mexico and migrate north over several generations during the summer. Allozyme analyses revealed that summer populations are not genetically structured (average $F_{ST} = 0.009$), and the authors concluded that eastern monarch butterflies consist of a single panmictic population (Eanes and Koehn 1978). In the red-winged blackbird, *Agelaius phoeniceus*, allozyme analyses revealed that populations representing five named subspecies from across the United States are not genetically structured (average $F_{ST} = 0.043$) (Gavin *et al.* 1991). While indices of genetic structuring from allozyme data are not directly comparable to those from mitochondrial data due to differences in mode of inheritance, rate of evolution, and the effective size of the population of reference, data from one marker may be transformed using an equation given by Crochet (2000):

$$F_{ST(mitochondrial)} = \frac{4F_{ST(nuclear)}}{1 + 3F_{ST(nuclear)}}. \quad (6)$$

This equation yields a transformed measure of genetic structure of $F_{ST(mtDNA)} = 0.035$ for eastern populations of *D. plexippus* and $F_{ST(mtDNA)} = 0.152$ for populations of *A. phoeniceus*.

Comparison of mtDNA and Allozyme Data

My results are consistent with those of previous studies that used allozyme data to investigate genetic differentiation among migratory groups of *T. b. mexicana* (Svoboda *et al.* 1985; McCracken *et al.* 1994; McCracken and Gassel 1997). Using data from six polymorphic loci to examine genetic structuring among *T. b. mexicana* colonies located in migratory groups C and D, Svoboda *et al.* (1985) concluded that the sampled colonies were slightly differentiated (overall $F_{ST} = 0.052$; $P < 0.005$), although pairwise measures of genetic identity were very high (Nei's (1978) $I = 0.972$ to 1.000). McCracken *et al.* (1994) also investigated the pattern of genetic structuring between the two long-distance migratory groups C and D, employing separate analyses of winter colonies in Mexico and summer colonies in the U.S., as well as analyzing males and females separately. In all of these analyses McCracken *et al.* (1994) found no evidence of significant genetic structuring, with an average $F_{ST} = 0.008$ over 15 variable loci among all sampled colonies in the United States. McCracken *et al.* (1994) also pointed out that the high F_{ST} of Svoboda *et al.* (1985) was due to one of the six loci (PGM-2) biasing the overall estimate of F_{ST} , and that this locus significantly deviated from Hardy-Weinberg equilibrium in all populations. McCracken and Gassel (1997) examined allozymes for evidence of genetic structuring between the non-migratory group A and the eastern long-distance migratory group D. They found no significant differences in allele frequencies among colonies, and no significant genetic differentiation of the colonies ($F_{ST} = 0.014$, $P > 0.05$).

To compare indices of genetic structuring using allozymes and mitochondrial markers, data from one set of markers must first be transformed to account for the different modes of inheritance (Crochet 2000). Because mitochondrial DNA is inherited solely through the mother while nuclear markers are inherited from both parents, the size of the population of reference for mtDNA is one-quarter that for nuclear markers and therefore undergoes lineage sorting at a much faster rate. Using a formula adapted from Crochet (2000):

$$F_{ST(nuclear)} = \frac{F_{ST(mitochondrial)}}{4 \times 3F_{ST(mitochondrial)}}, \quad (7)$$

I transformed F_{ST} for three sampling subgroups from the mitochondrial data into nuclear analogs (Table 2.6). The transformed indices of genetic structuring from mitochondrial data are up to one order of magnitude less than genetic structuring indices from nuclear allozyme data. This discrepancy may be due to the gender-specific migratory behavior in this species. Because females migrate longer distances than males, maternally inherited mtDNA haplotypes are more exposed to potential gene flow. Separate analyses of allozyme data of males from winter colonies in Mexico ($F_{ST} = 0.016$) and migratory females from summer colonies in the U.S. ($F_{ST} = 0.008$) reveal a greater degree of genetic structure among the non-migratory males (McCracken *et al.* 1994). It is worth emphasizing, however, that neither mitochondrial nor allozyme data show significant differentiation of populations at any spatial scale.

Table 2.6. Comparison of genetic structuring from nuclear allozyme and mitochondrial sequence data. Indices of genetic structure for mtDNA data are given as F_{ST} values that were transformed to allow for comparison with nuclear data. In data from McCracken *et al.* (1994) genetic structure among migratory colonies in the USA was estimated from samples of females collected during the summer. Genetic structure among migratory colonies in Mexico was estimated from samples of males collected during the winter. Genetic structure among migratory and non-migratory colonies in McCracken and Gassel (1997) was analyzed using data from both males and females.

Locus	F_{ST}	P	Reference
<i>Among migratory colonies (USA)</i>			
Allozymes (15 loci)	0.008	ns	McCracken <i>et al.</i> (1994)
D-loop (mtDNA)	0.002	ns	this study
<i>Among migratory colonies (Mexico)</i>			
Allozymes (15 loci)	0.021	ns	McCracken <i>et al.</i> (1994)
D-loop (mtDNA)	-0.006	ns	this study
<i>Among migratory and non-migratory colonies (USA)</i>			
Allozymes (10 loci)	0.014	ns	McCracken and Gassel (1997)
D-loop (mtDNA)	-0.006	ns	this study

Alternative Explanations of mtDNA Patterns

Neigel and Avise (1986) showed that isolated populations take $2N$ to $3N$ generations to reach reciprocal monophyly (*i.e.*, distinct groups that do not share any haplotypes with other such groups). Large populations are consequently expected to retain ancestral polymorphisms for a significant period of time following reproductive isolation. Thus, for populations as large as those of *T. b. mexicana* ($N_e = 2.84 \times 10^7$; Part 4), the retention of ancestral polymorphisms can bias estimates of gene flow upwards, even for populations that are reproductively isolated (Neigel 2002). Therefore, while the presence of reciprocal monophyly is powerful evidence for the evolutionary independence of lineages, taxa may be evolving independently even in the absence of monophyly.

To determine whether the estimated high levels of gene flow in *T. b. mexicana* reflect reality or are merely the artifact of retained ancestral polymorphism, I turned to direct estimates of gene flow from banding records. Over a span of nearly thirty years, records were published of 31 bats that switched migratory groups (Mohr 1952; Villa-R. and Cockrum 1962; Mumford 1963; Constantine 1967; Barbour and Davis 1969; Glass 1982; Svoboda *et al.* 1985). In studies at Carlsbad Caverns in western New Mexico, Constantine (1967) documented recoveries of 64 banded bats in places other than where they were banded. Of these, 13 recoveries (20.3%) involved individuals that moved between the eastern and western migratory groups (groups C and D). Notably, the longest documented movement was from Carlsbad Caverns, New Mexico, in the eastern migratory group D to Las Garrochas Cave, Jalisco, in the

western migratory group C (Villa-R. and Cockrum 1962). Movement has also been noted between other migratory groups, although much less frequently, such as one individual banded in western Nevada in group B that was recovered in western Kansas in group D (Svoboda *et al.* 1985). Rather than a lack of gene flow between these areas, the infrequency of documented movements between non-migratory and migratory populations may reflect the fact that almost all banding studies on *T. b. mexicana* have involved only migratory populations C and D in the southwestern United States and Mexico. The presence of large colonies in locations between migratory groups, such as Ojuela Cave in the Sierra Madre Occidental (McCracken *et al.* 1994) and Orient mine in the San Luis Valley of Colorado (Svoboda *et al.* 1985), also suggests that population subgroups are not as distinct as indicated by Cockrum (1969), and that opportunities exist for gene flow between these putatively distinct migratory groups of *T. b. mexicana*.

The lack of genetic differentiation among migratory groups in my data could potentially be explained by the use of an inappropriate genetic marker. If the mutation rate at the mtDNA D-loop were too high, then any phylogeographic signal would be lost due to saturation. I do not believe this to be the case for two reasons. First, use of the same sequence region to examine genetic structure among multiple subspecies of *T. brasiliensis* has shown clear patterns (Part 3). Second, my results are consistent with allozyme studies of the same migratory groups, which also did not find any genetic structuring (McCracken *et al.* 1994; McCracken and Gassel 1997). If the lack of structure in the mtDNA D-loop were

due to signal saturation, I might still expect the genetic structure to manifest itself in the more slowly evolving nuclear allozymes (Crochet 2000). Therefore, I conclude that the lack of phylogeographic signal in the D-loop sequences reflects the absence of genetic structuring among *T. b. mexicana* migratory groups and not the artifact of signal saturation in the data.

Migratory Behavior

The existence of behaviorally distinct populations of *T. b. mexicana* is amply documented in the literature. Colonies of tens to thousands of bats are routinely found throughout the year in California, Oregon, Nevada, and Utah (Benson 1947; Jewett 1955; Kruttsch 1955; Ruffner *et al.* 1979; Perkins *et al.* 1990), while colonies throughout Texas, Oklahoma, New Mexico, Arizona, and Mexico are up to several orders of magnitude larger and undergo seasonal migrations (Villa-R. 1956; Glass 1959, 1982; Villa-R. and Cockrum 1962; Cockrum 1969). Additionally, the majority of banding data record individuals being banded and recaptured within the same migratory corridor, suggesting that the bats maintain distinct migratory routes. However, all efforts to detect genetic differentiation corresponding with these behaviorally distinct groups, using mitochondrial and nuclear loci, have failed to detect such structure. Therefore, in *T. b. mexicana*, differences in migratory tendency and direction have not led to gene pool differentiation.

Migratory behavior in *T. b. mexicana* appears to be a plastic response to differential environmental cues. This has been demonstrated in northern

goshawks (*Accipiter gentilis atricapilus*), where Kennedy and Ward (2003) used experimental food supplementation to examine the effect of extra food on dispersal behavior in juveniles, and found that birds with access to abundant food did not disperse during the length of the study, whereas those without food supplements were not found in their natal areas after becoming independent. Migrating *T. b. mexicana* are known to follow large migrating populations of corn earworm moths, *Helicoverpa zea* (McCracken 1996). The large movements of moths are an environmental cue that is not present in regions where the bats are non-migratory, and may present an environmental stimulus that encourages migratory behavior. Alternatively, migratory behaviors, including the tendency and direction of migration, may be learned. Some behaviors documented in *T. b. mexicana* circumstantially support learning. In a long-term study of the colony at Carlsbad Caverns, New Mexico, Constantine (1967) noted that in all movements the older adults preceded younger adults who “followed [their elders’] example” (p. 25). In a comprehensive review of distribution records for this species, Genoways *et al.* (2000) proposed a mechanism by which individuals dispersing from crowded maternity colonies in the late summer discover potential new roosting sites that are presumably remembered and relocated in following summers. For this long-lived species, the adaptability conferred by the ability to learn may well be advantageous. The differential migratory behaviors of *T. b. mexicana* present an interesting system for the potential study of learned behaviors in Chiroptera.

III. GENETIC STRUCTURING AND SUBSPECIES STATUS OF CONTINENTAL
POPULATIONS OF THE BRAZILIAN FREE-TAILED BAT
(*TADARIDA BRASILIENSIS*)

1. Abstract

Tadarida brasiliensis is a highly vagile bat species that is migratory through part of its range. Recognized subspecies in the United States and Mexico are characterized by differences in behavior and in morphology, whereas subspecies in South and Central America are defined by differences in their geographical ranges. I used DNA sequence data from the D-loop of the mitochondrial genome to analyze genetic structuring among putative subspecies from mainland North, Central, and South America. Both phylogenetic and population genetic analyses reveal significant structuring between combined North and Central American versus South American populations, with sequence divergences between these populations comparable to those between other species of mammals. Populations within South America, nominally all of the same subspecies, are significantly structured with the Andes Mountains appearing to act as a barrier to gene flow. Conversely, populations within North and Central America are no more structured than is typical of populations of the same subspecies of other mammals.

2. Introduction

Different populations of migratory species may live in sympatry during one season while being geographically separated in another. Populations of migratory birds that are recognized as different subspecies often share the same

habitat during the winter, but move to separate geographical regions during the breeding season (Kimura *et al.* 2002). Similarly, races of salmon are segregated into different river systems during reproduction (Nielsen *et al.* 1999). In these examples, mating and the production of offspring occur in the same location following seasonal migration. Populations, defined as breeding units, are thus determined by geographic locality.

Conversely, in migratory bat species, mating may be separated from parturition by several weeks and thousands of miles (Koopman 1983). The determination of sympatry in bat populations is further complicated by the nocturnal habits of these animals, and because mating in natural populations is rarely observed. Although mating in natural populations of bats may be difficult to observe owing to the nature of the animals, its signal is carried in the bats' genomes. Here I use genetic data to interpret patterns of population structure in a migratory bat.

The Brazilian free-tailed bat, *Tadarida brasiliensis*, is one of the most abundant bat species in the Western Hemisphere, occurring in colonies numbering up to tens of millions of individuals (Wilkins 1989; Part 4). It is found throughout Central and South America and in North America from the Atlantic to the Pacific coast, north to 40 °N latitude (Hall 1981; see Figure 3.1). Bats in the following geographical areas have been named as subspecies based on distributional and behavioral differences, as well as slight, but not diagnostic, morphological differences (Schwartz 1955; Wilkins 1989). Within North America,



Figure 3.1. Range of *T. brasiliensis*. Populations sampled in this study are indicated by dots, with their subspecific designation indicated by color. Ranges of individual subspecies are numbered as follows: (1) *T. b. cynocephala* with colonies in blue, (2) *T. b. mexicana* with colonies in red, (3) *T. b. intermedia* with colonies in green, and (4) *T. b. brasiliensis* with colonies in orange.

two subspecies of *T. brasiliensis* are currently described: *T. b. cynocephala* LeConte (1831) in the southeastern United States, and *T. b. mexicana* Saussure (1860) in the southwestern United States and in Mexico. *T. b. intermedia* Shamel (1931) is described for Central America from southern Mexico to Costa Rica. A fourth subspecies, *T. b. brasiliensis* Geoffroy St. Hilaire (1824), is thought to occupy the entire species range in South America, and has been recorded as far north as the Heredia Province of Costa Rica.

Most studies of behavior have concerned the two North American subspecies, *T. b. cynocephala* and *T. b. mexicana*, and taxonomic recognition of these subspecies is based largely on behavioral differences in migration, hibernation, and roosting habits (Carter 1962; Barbour and Davis 1969). Conversely, relatively little such information is available for populations of the other mainland subspecies, and taxonomic recognition of *T. b. intermedia* and *T. b. brasiliensis* is based largely on geographic distribution. The *brasiliensis* subspecies is known to migrate in part of its range (Marques 1991), but population subdivision has not been investigated in South America.

The *mexicana* subspecies roosts in caves and man-made structures in colonies of up to several million individuals and generally migrates to winter roosts in Mexico. Mating occurs in February and March in transitional roosts in northern Mexico and southern Texas during the northward migration (Villa-R. and Cockrum 1962; McCracken and Wilkinson 2000). The vast majority of migrating *T. b. mexicana* are pregnant females (Villa-R. 1956; Davis *et al.* 1962; Cockrum 1969) with large numbers of adult males remaining in Mexico. Males that migrate

occupy smaller colonies separate from the large maternity colonies. After the young become independent, the bats disperse from the maternity caves, often moving further north and roosting in smaller groups before the southward migration begins in August and September (Glass 1982).

The *cynocephala* subspecies roosts primarily in tree hollows and man-made structures in colonies that rarely exceed several thousand individuals (Bain 1981). Mating occurs in early spring, although direct observations of mating in natural populations have not been verified. The peak of parturition occurs two to four weeks earlier in *T. b. cynocephala* than in *T. b. mexicana* (Sherman 1937; Kruttsch 1955). Differences between *T. b. cynocephala* and *T. b. mexicana* are most evident in winter, when *T. b. cynocephala* undergoes only local seasonal movements and spends the winter months in torpor, interrupted by periodic feeding activity during warmer periods (Sherman 1937; LaVal 1973). Movements of individuals among colonies may occur in winter during these warmer periods, as evidenced by significant fluctuations in population size (Kiser and Glover 1997). While *T. b. cynocephala* maintain some activity throughout the year, there is no evidence that they undertake long-distance migration similar to that of *T. b. mexicana*.

Measurements of the skull and forearm have been used to distinguish *T. b. mexicana*, *T. b. cynocephala*, and *T. b. brasiliensis* (Carter 1962; Owen *et al.* 1990). The mean values differ between subspecies, but the ranges of these morphological characters overlap broadly (Table 3.1). Due to this overlap, it has

Table 3.1. Morphological measurements of characters used to define three subspecies of *T. brasiliensis*. Measurements are given in mm.

Skull Length				
Subspecies	Sex	Sample Size	Mean $\pm$ s.d.	Range
<i>cynocephala</i>	male	128	17.48 $\pm$ 0.307	16.7 – 18.4
	female	152	17.16 $\pm$ 0.306	16.1 – 18.2
<i>mexicana</i>	male	173	16.94 $\pm$ 0.308	16.2 – 17.7
	female	164	16.72 $\pm$ 0.274	16.1 – 17.4
<i>brasiliensis</i>	both	41	17.28 $\pm$ 0.400	16.1 – 17.8
Zygomatic Breadth				
Subspecies	Sex	Sample Size	Mean $\pm$ s.d.	Range
<i>cynocephala</i>	male	123	10.27 $\pm$ 0.211	9.6 – 10.8
	female	144	10.10 $\pm$ 0.189	9.6 – 10.6
<i>mexicana</i>	male	162	9.78 $\pm$ 0.186	9.3 – 10.4
	female	104	9.70 $\pm$ 0.159	9.3 – 10.2
<i>brasiliensis</i>	both	32	9.95 $\pm$ 0.282	9.1 – 10.5

Data from Owen *et al.* (1990).

been argued that these characters differentiate the taxa poorly (Schwartz 1955; McCracken and Gassel 1997; Russell and McCracken in press), and most arguments for the separation of the taxa have relied more on behavioral and geographical differences than on morphology.

Although these differences in behavior and, to a lesser extent, morphology seem to differentiate *T. b. cynocephala* and *T. b. mexicana*, many studies have demonstrated considerable plasticity in these same traits. Western populations of *T. b. mexicana* roost in buildings and rock crevices in relatively small populations of several thousand individuals and remain in the United States to hibernate through the winter (Grinnell 1918; Jewett 1955; Kruttsch 1955; Perkins *et al.* 1990). Furthermore, putatively migratory populations of *T. b. mexicana* have been found during the winter in southeastern Texas and colonies have recently been reported in the winter as far west as Austin (B. French, pers. comm.). These populations may represent opportunities for interbreeding between these two recognized subspecies (Spenrath and LaVal 1974; Bennett 2000; B. French pers. comm.). Specimens that are intermediate in cranial measurements between *T. b. cynocephala* and *T. b. mexicana* have been described from a population in southeastern Texas, and the intermediate individuals have been attributed to possible interbreeding between the two subspecies (Schmidley *et al.* 1977).

The taxonomic status of these forms has been in contention for many years. Excepting *T. b. intermedia* (Shamel 1931), the taxa were originally described as separate species (Geoffroy St. Hilaire 1824; LeConte 1831;

Saussure 1860). H. Allen (1893) synonymized *Nyctinomus* (= *Tadarida*) *mexicana* and *N. cynocephala* with *N. brasiliensis*, without differentiation at the subspecific level. G. Allen (1908) used morphological data to justify a recommendation that *N. brasiliensis* and *N. mexicana* be synonymized, and that *N. cynocephala* be subordinated to a subspecies of *N. brasiliensis*. These recommendations, however, were disregarded, and the taxa continued to be treated as separate species until Schwartz (1955) recommended that all species in the *brasiliensis* group be subordinated to subspecies of *Tadarida brasiliensis*. Carter (1962) later argued that behavioral, distributional, and morphological differences among *T. b. cynocephala*, *T. b. mexicana*, and *T. b. brasiliensis* warrant their recognition as separate species. Carter never directly addressed the status of *T. b. intermedia*, although he included colonies from southern Mexico and Guatemala in his sampling of *T. b. mexicana*.

Molecular data also have been employed to address these issues. Along with behavioral and morphological data, Owen *et al.* (1990) considered data from a single allozyme locus, concluding that the total evidence supported the differentiation of *T. b. cynocephala* and *T. b. mexicana* at the specific level. This conclusion was not supported by McCracken and Gassel (1997), who found that the two subspecies differed only in allele frequencies, with no fixed differences at twenty-two allozyme loci. They estimated an average genetic similarity between *T. b. cynocephala* and *T. b. mexicana* of $I = 0.978$ (range 0.962 to 0.994) for Nei's (1978) genetic identity statistic, comparable to estimates typically found between populations of the same subspecies (Avice 1974). They also found that

a colony in Arkansas was intermediate in allele frequencies and shared rare alleles with both subspecies, suggesting that the colony represented an area of interbreeding.

Here I use mitochondrial DNA (mtDNA) sequence data in an effort to clarify patterns of genetic structuring among these subspecies of *T. brasiliensis* at multiple geographical scales. At the intercontinental scale I compare South American with North and Central American populations. Within each continent I examine patterns of structure among colonies of *T. b. brasiliensis* and among North and Central American subspecies. Additionally, I analyze genetic structure within the non-migratory North American subspecies *T. b. cynocephala*. I use these data to contribute a perspective from maternal lineages regarding gene pool structure and the longstanding taxonomic issues in *T. brasiliensis*.

3. Materials and Methods

Sample Collection

Tissue samples were obtained from a total of 220 bats from 25 locations in North, Central, and South America, with an average sample size of 8.8 bats per population (Table 3.2; Figure 3.1). I collected the samples from the Louisiana colony; all other samples were provided by colleagues. The bats were captured in 4'x4' harp traps or mist nets erected at the entrance of each colony, or using hand nets within roosts. The bats were sexed and stored temporarily in cotton bags prior to tissue sampling. Two 3-mm wing biopsy punches, one from each

Table 3.2. Sampling information. Within each subspecies, locations are listed from east to west by increasing longitude. The sample size (n) indicates the number of individuals that were sequenced from each colony.

Subspecies	Sample Name	n	Location	Geographical Coordinates
<i>T. b. cynocephala</i>	NCarolinaA	9	Mecklenberg County, North Carolina, USA	35:46N 78:38W
<i>T. b. cynocephala</i>	NCarolinaB	7	Wake County, North Carolina, USA	35:14N 80:51W
<i>T. b. cynocephala</i>	SCarolina	9	Columbia, South Carolina, USA	34:02:21N 80:53:11W
<i>T. b. cynocephala</i>	EFlorida	10	Sanford, Florida, USA	28:47:21N 81:16:33W
<i>T. b. cynocephala</i>	WFlorida	10	Ponce de Leon, Florida, USA	30:43:19N 85:56:13W
<i>T. b. cynocephala</i>	Louisiana	10	Baton Rouge, Louisiana, USA	30:26:56N 91:07:34W
<i>T. b. cynocephala</i>	Arkansas	9	Conway, Arkansas, USA	35:05:20N 92:27:11W
<i>T. b. cynocephala</i>	ETexas	10	Huntsville, Texas, USA	30:42:43N 95:32:59W
<i>T. b. mexicana</i>	Meztitlan	9	El Salitre Cave, Hidalgo, Mexico	20:07N 98:44W
<i>T. b. mexicana</i>	CTexas	10	James River Cave, Texas, USA	30:44:57N 99:13:56W
<i>T. b. mexicana</i>	LaBoca	12	Cueva La Boca, Nuevo Leon, Mexico	25:41N 100:15W
<i>T. b. mexicana</i>	Janitzio	11	Isla de Janitzio, Michoacán, Mexico	19:42N 101:07W

Table 3.2. Continued.

Subspecies	Sample Name	<i>n</i>	Location	Geographical Coordinates
<i>T. b. mexicana</i>	NewMexico	10	Carlsbad Caverns Nat'l Park, New Mexico, USA	32:10:32N 104:22:34W
<i>T. b. mexicana</i>	Colorado	4	Grand Junction, Colorado, USA	39:04N 108:33W
<i>T. b. mexicana</i>	JuanAldama	12	Ejido Juan Aldama, Sinaloa, Mexico	25:45N 108:57W
<i>T. b. mexicana</i>	Arizona	8	Eagle Creek Cave, Arizona, USA	33:03:11N 109:19:46W
<i>T. b. mexicana</i>	CCalifornia	10	Merced County, California, USA	37:28N 120:30W
<i>T. b. mexicana</i>	NCalifornia	3	Lava Beds Nat'l Monument, California, USA	41:53:18N 121:22:16W
<i>T. b. mexicana</i>	Oregon	5	Jackson County, Oregon, USA	42:11:25N 122:41:58W
<i>T. b. intermedia</i>	Guatemala	4	El Rancho, Guatemala	14:21N 91:00W
<i>T. b. intermedia</i>	Chiapas	11	San Francisco Cave, Chiapas, Mexico	16:15N 92:08W
<i>T. b. brasiliensis</i>	Brazil	10	Porto Alegre, Brazil	30:01:59S 51:13:48W
<i>T. b. brasiliensis</i>	Argentina	10	Rosario, Argentina	32:57S 60:40W
<i>T. b. brasiliensis</i>	Santiago	7	Santiago, Chile	33:26S 70:40W
<i>T. b. brasiliensis</i>	Concepción	10	Concepción, Chile	36:46S 73:03W

wing, were taken from each bat for DNA analysis according to the method of Worthington Wilmer and Barratt (1996). The bats were then released at the site of capture. Wing biopsy punches were stored in either NaCl-saturated 20% DMSO or silica gel desiccant in the field, and at -80°C in the lab.

DNA Isolation and Amplification

I isolated total genomic DNA using a standard phenol-chloroform-isoamyl alcohol (25:24:1), ethanol precipitation method (modified from Sambrook *et al.* 1989) or using a DNeasy® DNA isolation kit (Qiagen), and stored in 1/10 Tris-EDTA (TE). I then quantified the isolates using a Hoefer DyNA Quant® 200 fluorometer (Amersham Pharmacia), and diluted them to a standard concentration of 10 ng/ μl for the polymerase chain reaction (PCR).

PCR was carried out using primers F(mt): 5'-GTTGCTGGTTTCACGGA-GGTAG-3', and P(mt): 5'-TCCTACCATCAGCACCCAAAGC-3' (Wilkinson and Chapman 1991) for the control region (D-loop) of the mitochondrial DNA molecule, located near the tRNA^{pro} gene. These primers yielded a product approximately 500 bp long. To obtain sufficient PCR product for sequencing, I carried out PCR amplifications in eight 12- μL reaction volumes, each containing 1.04 mM MgCl₂, 0.1 mM dNTPs, 1 Unit *Taq* DNA polymerase, 1.2 μl Promega 10X buffer, 10 ng genomic DNA, and 14 pmol of each primer. The amplification involved an initial denaturation at 94°C for two minutes followed by 30 cycles at 94°C for one minute, 55°C for 1.5 minutes, and 72°C for two minutes. I then

combined the eight reactions into a common sample, and purified the PCR product using gel band excision (MinElute® kit, Qiagen).

DNA Sequencing

I performed sequencing reactions using the PRISM® BigDye™ Terminator Cycle Sequencing Ready Reaction kit version 2 (Applied Biosystems). Each 10 µL sequencing reaction contained 50-100 ng of PCR product, 7 pmol of primer, and 3 µL of the BigDye ready reaction mix. The sequencing temperature profile consisted of 25 cycles at 96 °C for 10 seconds, 50 °C for 5 seconds, and 60 °C for 4 minutes, with temperature changes between each step occurring at a speed of 1 °C per second. Sequencing fragments were cleaned of unincorporated nucleotides using Centri-Sep columns (Princeton Separations), and analyzed on an ABI 3100 automated sequencer (Applied Biosystems). I sequenced twenty arbitrarily chosen individuals from both directions to estimate the rate of sequencing error. Because this rate was low ($\sim 2.1 \times 10^{-3}$ per sequence), I sequenced most individuals using only the P(mt) primer. All sequences were deposited in the GenBank database.

The D-loop of the mitochondrial genome is characterized in many bat species by variable numbers of tandemly repeated 81-bp sequence units (Wilkinson *et al.* 1997). In *T. brasiliensis*, I found that most individuals possess three of these repeat units, while others possessed up to six repeats. I detected 41 individuals representing twenty-two locations that possessed more than three

repeat units. These individuals were found in *T. b. cynocephala* ($n = 22$, from thirteen sites, six of which were not included in this study), *T. b. mexicana* ($n = 16$, from seven sites), and *T. b. intermedia* ($n = 3$, from two sites). Within North and Central America, there was no detectable geographical bias to their distribution (data not shown). No size variation was detected in the South American subspecies *T. b. brasiliensis*. To avoid problems with sequence alignment, I included in these analyses only individuals that were characterized by three repeats.

Sequence Analyses

Sequences were aligned using the default settings in CLUSTALW (Thompson *et al.* 1994), and edited by eye. The alignment resulted in a sequence length of 477 bp. Sequence variation included five insertion/deletions (indels), of which the longest was five bp. In all analyses, indels were treated as a fifth character, rather than as missing data.

Phylogenetic and population genetic analyses are made more accurate and efficient when carried out under an appropriate model of molecular evolution. The model may specify such parameters as nucleotide frequency, substitution rates, proportion of invariant sites, and α , the shape of the gamma distribution for variable mutation rates. I used the likelihood analysis implemented in MODELTEST version 3.06 (Posada and Crandall 1998) to quantify these evolutionary parameters and to determine which of 56 models of evolution best described the data. This analysis uses likelihood ratio tests of increasingly more

complex models to identify the model that best describes the data. Subsequent phylogenetic and population genetic analyses were carried out using the model assumptions and parameter values specified.

I used ARLEQUIN version 2.000 (Schneider *et al.* 2000) to calculate descriptive parameters of molecular diversity such as haplotype diversity (h) and nucleotide diversity (π) for each colony, and identified haplotypes shared among colonies. Haplotype diversity reflects the number of different haplotypes in the data set, and is calculated using the method of Nei (1987):

$$\hat{h} = \frac{n}{n-1} \left(1 - \sum_{i=1}^k p_i^2 \right) \quad (1)$$

where n = the number of haplotype copies in the sample, k = the number of haplotypes, and p_i = the sample frequency of the i -th haplotype. Nucleotide diversity reflects the evolutionary distance among the sampled haplotypes, and is calculated according to Tajima (1983):

$$\pi = \sum_{i=1}^k \sum_{j<i}^k p_i p_j d_{ij} \quad (2)$$

where k = the number of haplotypes; p_i = the frequency of haplotype i , and d_{ij} is an estimate of the number of mutations having occurred since the divergence of haplotypes i and j .

Phylogenetic Analyses

I used a phylogenetic approach to determine whether the subspecies consist of cladistically differentiated gene pools. I used a Bayesian likelihood analysis, as implemented in MRBAYES version 3.0B4 (Huelsenbeck and Ronquist 2001). This analysis uses a Metropolis-coupled Markov chain Monte Carlo, or (MC)³, approach with a Metropolis-Hastings sampling algorithm (Huelsenbeck *et al.* 2002). In this approach, a single Markov chain begins with a random tree, θ , with topology θ and branch lengths θ . Next, a new tree designated θ' is proposed. This new tree is accepted as the next state of the chain with the probability described by Metropolis *et al.* (1953) and Hastings (1970) and based upon the observed data X :

$$R = \min \left[1, \frac{f(X|\theta')}{f(X|\theta)} \times \frac{f(\theta)}{f(\theta')} \times \frac{f(\theta|\theta')}{f(\theta'\theta)} \right] \quad (3)$$

where $f(X|\theta')/f(X|\theta)$ is the likelihood ratio, $f(\theta)/f(\theta')$ is the prior ratio, and $f(\theta|\theta')/f(\theta'\theta)$ is the proposal ratio. Because searches using a single Markov chain may be more prone to convergence upon locally optimal trees, multiple chains may be run in parallel to allow a more thorough search of treespace. In these Metropolis-coupled Markov chains, a single “cold” chain samples from the posterior distribution of interest, $\Pr[\theta|X]$. Other “heated” chains sample from distributions obtained by raising the cold distribution by β_i , where $\beta_i = 1/(1 + iT)$ and T is a specified “temperature” parameter. At regular intervals, two chains are randomly picked and an attempt is made to change their states using a normal

Metropolis-Hastings step as described above. Thus, the effect of heating is to enable heated chains to traverse valleys between isolated hilltops in the likelihood surface more easily and to provide the cold chain with more probable alternatives of new states. In this way, the cold chain is mixed more thoroughly by being exposed to isolated hilltops that would not otherwise have been explored due to the intervening valleys of highly improbable intermediate states.

I ran four Markov chains of 1,000,000 generations each with sampling every 100 generations. The chains were heated using the temperature scaling factor $T = 0.2$. The MRBAYES program allows for the calculation of only one, two, or six substitution types; because my data were characterized by three substitution types, I specified the general time-reversible model with six substitution types. A proportion of the sites remained invariable, while the other sites mutated at one of four rates following a categorical gamma distribution. This analysis resulted in a sample of 10,000 trees which were rooted using homologous sequences from two individuals of *Chaerephon pumilus* (Chiroptera: Molossidae) as an outgroup. Because the Markov chains begin from random trees, some number of trees having low likelihood scores will be sampled before the chains converge upon the state with the highest sampled likelihood. Using a log likelihood plot as a guide, I discarded the first 8500 sampled trees as a burn-in, and constructed a 50% majority rule consensus tree from the remaining 1500 trees using PAUP* (Swofford 1998).

Population Genetic Analyses

I used analyses of molecular variance (AMOVA; Excoffier *et al.* 1992) as implemented in ARLEQUIN to test for genetic structuring at each geographical level. This analysis apportions total genetic variance into hierarchical categories and determines significance by comparing observed genetic structure with null distributions obtained from 16,000 random permutations of the data. Where I found significant genetic structuring, I further explored the causes of that structure by calculating pairwise sequence divergence or pairwise F_{ST} among the taxa using the method of Slatkin (1991):

$$F_{ST} = \frac{f_0 - f_1}{1 - f_1} \quad (4)$$

where f_0 is the probability of identity by descent of two different haplotypes drawn from the same population and f_1 is the probability of identity by descent of two haplotypes drawn from two different populations. I used Mantel tests to investigate hypotheses of genetic isolation-by-distance, testing for a correlation between genetic distance (F_{ST}) and geographic distance. Geographic coordinates for each colony were determined using the Astro-dienst website (<http://www.astro.com/cgi/aq.cgi?lang=e>), and the geographic distance between each pair of colonies was calculated as the direct aerial distance using the How Far Is It? website (<http://www.indo.com/distance/>).

I calculated asymmetrical rates of migration among subspecies using a (MC)³ likelihood method as implemented in MIGRATE version 1.6.9 (Beerli and

Felsenstein 1999). In this analysis, the likelihood of the mutation-scaled and subpopulation-specific migration and population size parameters, $\mathcal{M}_i (= m/\mu)$ and $\mu_i (= 2N_e\mu)$, is estimated using a likelihood formula from Kuhner *et al.* (1995):

$$L(P) = \sum_G \Pr(D | G) \Pr(G | P) \quad (5)$$

where the likelihood of the population parameters are based on the joint probabilities of the data given a genealogy and of the genealogy given a set of parameter values, summed over all sampled genealogies. For each genealogy, the probability of the genealogy given a set of specified parameter values is estimated as the probability that a migration event happens at the beginning of a coalescent interval multiplied by the probability that nothing happens during that interval, summed over all such coalescent intervals, or:

$$\Pr(G | P) = \prod_{i=1}^2 \left(\left[\frac{2}{\mu_i} \right]^{v_i} M_i^{w_i} \right) \prod_{j=1}^T p_j \quad (6)$$

where p_j , the probability that nothing happens during the coalescent interval, is:

$$p_j = \exp\left(-u_j \prod_{i=1}^2 \left(k_{ji} M_i + \frac{k_{ji}(k_{ji} - 1)}{\mu_i} \right)\right), \quad (7)$$

v_i is the total number of coalescences in subpopulation i , w_i is the sum of the number of migration events to subpopulation i over all time intervals T , u_j is the length of time interval j , and k_{ji} is the number of lineages in subpopulation i during

time interval j (Beerli and Felsenstein 1999). The genealogy is then accepted or rejected based on the probability of the data, given the genealogy. When reiterated many times, this process results in a posterior distribution of trees, from which the $\mathcal{M}_i$ and $\square_i$ parameters may be estimated by maximizing the likelihood ratio:

$$\frac{L(P)}{L(P_0)} = \frac{1}{n} \prod_i^n \frac{\Pr(G_i | P)}{\Pr(G_i | P_0)} \quad (8)$$

where n is the number of sampled genealogies and P_0 represents the initial parameter values (Beerli and Felsenstein 1999). As with the phylogenetic analysis using MRBAYES, a Metropolis-Hastings importance sampling approach is used to bias the search of treespace towards the most probable genealogies, making the analysis feasible for large data sets. I executed this analysis using four Markov chains simultaneously with adaptive heating at starting temperatures of 1, 4, 7, and 10. Within each chain, I ran ten short searches of 100,000 steps each and three long searches of 1,000,000 steps each, with sampling of chains at every 200 steps. Start parameters of $\mathcal{M}$ and $\square$ were generated from calculations of F_{ST} , and $\square$ was kept constant throughout the analysis.

4. Results

Molecular Diversity

The mitochondrial D-loop is extremely diverse in *T. brasiliensis*. I found 191 different haplotypes in the sample of 220 individuals, yielding an overall haplotype diversity (h) of 0.998 (Table 3.3). Individual subspecies were similarly diverse, with haplotype diversity ranging from 0.976 for *T. b. brasiliensis* to 1.0 for *T. b. intermedia*. Nucleotide diversity (π) was low for the subspecies ($\pi = 0.042$ to 0.057) indicating that the large number of haplotypes present in the sample all diverged relatively recently.

The total data set is best described by the Tamura-Nei model of evolution (Tamura and Nei 1993) with the proportion of sites being invariable (I) = 0.318 and a gamma-distributed mutation rate (shape parameter $\alpha = 0.650$). This model was originally described for primate mitochondrial control region sequence data, and specifies different substitution rates for transition mutations between purines ($R_{A-G} = 34.009$), transitions between pyrimidines ($R_{C-T} = 56.856$), and transversions ($R_{TV} = 1.000$). I found a heavy bias in the nucleotide composition towards adenine ($\pi_A = 0.432$, $\pi_C = 0.194$, $\pi_G = 0.113$, $\pi_T = 0.262$).

Genetic Structuring Between Continental Populations

Both phylogenetic analyses and population genetic analyses support the distinction of South American *T. b. brasiliensis* from the North and Central American subspecies. In a Bayesian likelihood analysis, a deep separation of

Table 3.3. Estimates of molecular diversity within each subspecies; n = number of individuals sequenced; n_h = number of different haplotypes; h = haplotype diversity; π = nucleotide diversity.

	n	n_h	h	π
<i>T. b. cynocephala</i>	74	64	0.996	0.042 ± 0.021
<i>T. b. mexicana</i>	94	86	0.998	0.045 ± 0.022
<i>T. b. intermedia</i>	15	15	1.000	0.042 ± 0.022
North and Central America	183	163	0.999	0.047 ± 0.023
<i>T. b. brasiliensis</i>	37	28	0.976	0.057 ± 0.028
overall	220	191	0.998	0.085 ± 0.041

South American from North and Central American subspecies was supported in 100% of the genealogies (Figure 3.2). In addition to single nucleotide polymorphisms, *T. b. brasiliensis* differed from North and Central American subspecies in having five indels up to five bp in length. Although these indels certainly contributed to the structure evident in the phylogeny, that structure did not disappear nor did it lose its significance when the indels were removed from the sequences (analysis not shown).

Significant structure between subspecies from different continents was also detected using population genetic analyses. In an AMOVA analysis among all four subspecies, over half ($\Phi_{CT} = 0.565$; $P < 0.01$) of the total variance in the data is due to genetic structuring among subspecies (Table 3.4). Using nested AMOVA analyses in which each subspecies in turn was excluded from the analysis, I found that genetic structuring at the subspecific level dropped precipitously only when *T. b. brasiliensis* was excluded from the analysis ($\Phi_{CT} = 0.093$; $P < 0.01$). The genetic differences between South versus North and Central American subspecies also are clearly evident in analyses of average sequence divergences within and among subspecies (Table 3.5). While the North and Central American subspecies differ from each other by less than 1% when corrected for variation within subspecies, the three subspecies each differed from *T. b. brasiliensis* by at least 13%. The Monte Carlo Markov chain likelihood method to estimate asymmetrical migration rates among subspecies (Beerli and Felsenstein 1999) yielded point estimates and upper confidence limits of migration rates between *T. b. brasiliensis* and the other sampled subspecies

Figure 3.2. An 50% consensus tree from a Bayesian likelihood analysis. Only trees sampled after the burn-in period were used to construct the consensus. Support indices are the percent of trees having the given partition, and are equivalent to the posterior probabilities from the Bayesian analysis. Subspecies designations were traced onto the tree and are indicated by branch color, following the same color code as in Figure 3.1. Internal branches are assigned to subspecies following a parsimony criterion; branches in gray could not be unambiguously assigned to a single subspecies. The tree was rooted using homologous sequences from *Chaerephon pumilus*, represented by the black branches. Portions of the tree denoted as A and B are shown in more detail in Figures 3.3 and 3.5, respectively.

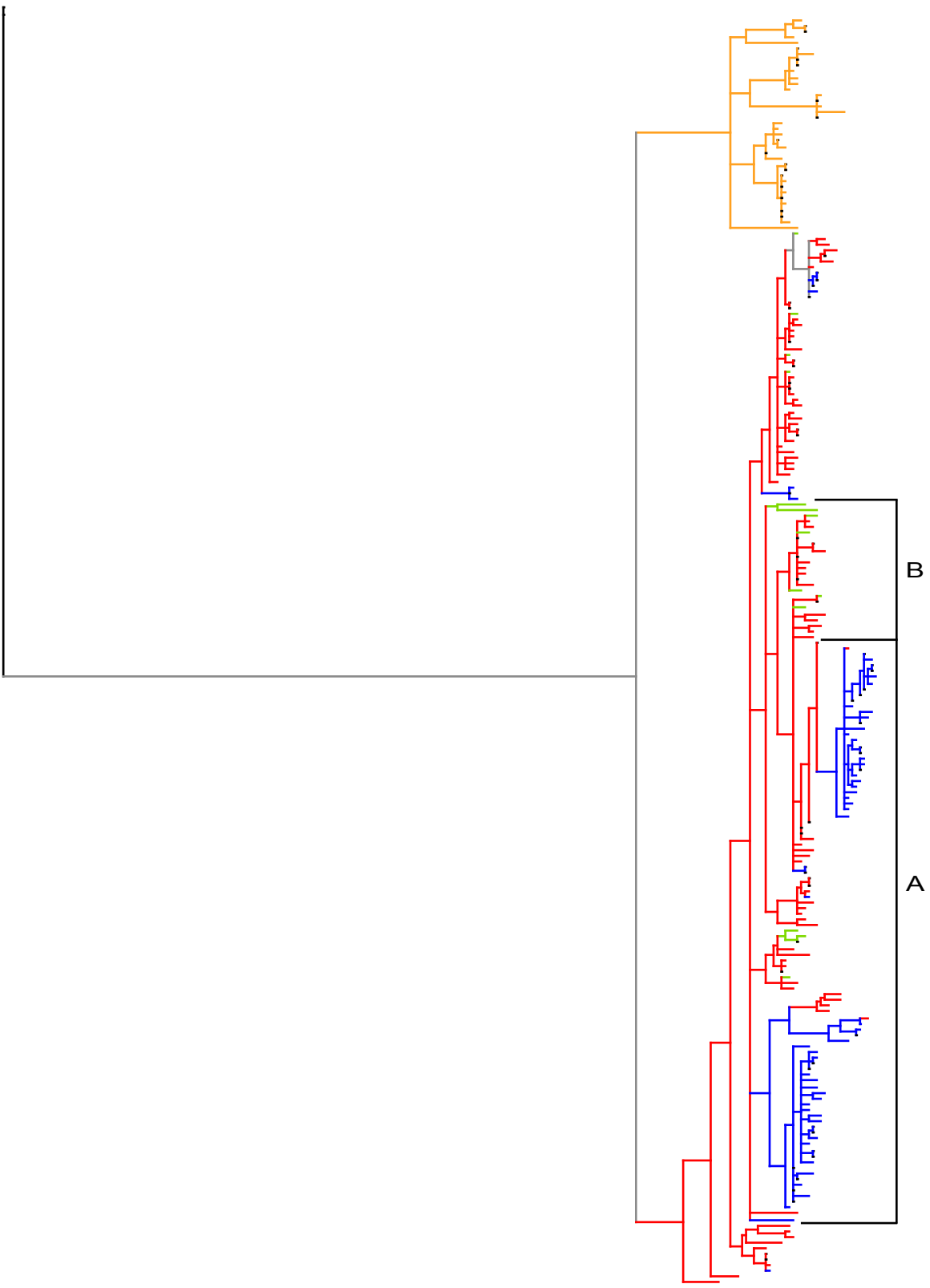


Table 3.4. Nested analyses of molecular variance (AMOVA). For each analysis, either all of the samples were included or one of the four subspecies was excluded from the analysis. P values for Φ_{CT} (among subspecies) and Φ_{SC} (among colonies, within subspecies) represent the probability of obtaining a higher Φ statistic by chance alone, determined by 16,000 permutations of the data. P values for Φ_{ST} (within colonies) represent the probability of obtaining a lower Φ statistic by chance alone, determined by 16,000 permutations of the data. * $P < 0.01$.

	All samples		Exclude <i>T. b. cynocephala</i>		Exclude <i>T. b. mexicana</i>		Exclude <i>T. b. intermedia</i>		Exclude <i>T. b. brasiliensis</i>	
	% variation	Φ -stat	% variation	Φ -stat	% variation	Φ -stat	% variation	Φ -stat	% variation	Φ -stat
Among subsp	56.52	0.565*	66.72	0.667*	68.18	0.682*	58.49	0.585*	9.34	0.093*
Among colony, within subsp	3.55	0.082*	3.00	0.090*	4.79	0.151*	3.41	0.082*	2.13	0.024
Within colony	39.94	0.601*	30.28	0.697*	27.02	0.730*	38.10	0.619*	88.52	0.115*

Table 3.5. Average sequence divergences within and between subspecies, calculated by the method of Tamura and Nei (1993). Diagonal elements are divergences within subspecies. Measures of uncorrected sequence divergence are above the diagonal; divergences corrected for variation within subspecies are below the diagonal. Significant differences between subspecies are indicated by an asterisk.

	<i>T. b.</i> <i>cynocephala</i>	<i>T. b.</i> <i>mexicana</i>	<i>T. b.</i> <i>intermedia</i>	<i>T. b.</i> <i>brasiliensis</i>
<i>T. b.</i> <i>cynocephala</i>	0.042	0.049 $P < 0.0001^*$	0.048 $P < 0.0001^*$	0.187 $P < 0.0001^*$
<i>T. b.</i> <i>mexicana</i>	0.005 $P < 0.0001^*$	0.045	0.043 $P = 0.634$	0.181 $P < 0.0001^*$
<i>T. b.</i> <i>intermedia</i>	0.006 $P = 0.0004^*$	0.0003 $P = 0.239$	0.042	0.186 $P < 0.0001^*$
<i>T. b.</i> <i>brasiliensis</i>	0.137 $P < 0.0001^*$	0.130 $P < 0.0001^*$	0.137 $P < 0.0001^*$	0.057

of less than one in all instances (Table 3.6). Wright (1931) showed that populations isolated by levels of gene flow less than one individual per generation are able to diverge through random genetic drift.

Genetic Structure Within South America

Due to the significant differentiation of South from North and Central American subspecies, I suspected that molecular evolutionary parameters estimated for the entire data set might not accurately describe either group separately. Therefore, I conducted a likelihood analysis of each continental population separately to determine which model of evolution best describes each smaller data set. The South American subspecies *T. b. brasiliensis* conformed best to the HKY85 (Hasegawa *et al.* 1985) model, with a gamma-distributed mutation rate ($\alpha = 0.251$). This model is similar to the Tamura-Nei model described above except that only two mutation categories are specified, those being transitions and transversions (Ti/Tv ratio = 11.681). The data set was characterized by unequal base frequencies, with adenine again being the most prevalent ($\pi_A = 0.431$, $\pi_C = 0.189$, $\pi_G = 0.097$, $\pi_T = 0.283$).

Population genetic analyses revealed significant structure within South America. In an AMOVA analysis, I found that nearly a third of the total variance within *T. b. brasiliensis* is due to differences among colonies ($\pi_{ST} = 0.315$; $P < 0.001$; Table 3.7). Although a Mantel test found no significant association between genetic and geographical distances ($r = 0.646$; $P = 0.122$), pairwise measures of F_{ST} among colonies do reveal a geographic pattern in the genetic

Table 3.6. Maximum likelihood estimates of $N_e m$ among subspecies with 90% confidence intervals given in parentheses. Directional pairwise rates of migration are given as migration from the taxon named in the row into the taxon named in the column.

	<i>T. b.</i> <i>brasiliensis</i>	<i>T. b.</i> <i>intermedia</i>	<i>T. b.</i> <i>mexicana</i>	<i>T. b.</i> <i>cynocephala</i>
<i>T. b.</i> <i>brasiliensis</i>	-	4.85×10^{-12} (4.2×10^{-12} , 0.25)	0.182 (0.02, 0.66)	2.43×10^{-12} (2.1×10^{-12} , 0.25)
<i>T. b.</i> <i>intermedia</i>	1.93×10^{-15} (1.7×10^{-15} , 0.39)	-	5.58 (3.95, 7.56)	1.02 (0.43, 1.99)
<i>T. b.</i> <i>mexicana</i>	9.38×10^{-13} (8.2×10^{-13} , 0.38)	14.56 (11.41, 18.18)	-	3.15 (1.82, 5.12)
<i>T. b.</i> <i>cynocephala</i>	0.288 (0.07, 0.77)	0.72 (0.31, 1.39)	1.01 (0.51, 1.78)	-

Table 3.7. AMOVA analyses of each subspecies. This analysis was not done on *T. b. intermedia* because it was sampled from only two colonies.

	<i>T. b.</i> <i>cynocephala</i>	<i>T. b.</i> <i>mexicana</i>	<i>T. b.</i> <i>brasiliensis</i>
Among colonies	6.34%	-0.24%	31.48%
Within colonies	93.66%	100.24%	68.52%
Φ_{ST}	0.063 $P = 0.021$	-0.002 $P = 0.551$	0.315 $P < 0.001$

structure (Table 3.8). The largest genetic differences are found between colonies separated by the Andes mountain range ($F_{ST} = 0.336$ to 0.405 ; $P < 0.01$).

Although some significant genetic structuring on the eastern side of the Andes also is indicated by this analysis ($F_{ST} = 0.206$; $P = 0.010$), conclusions are limited by the small number of colonies sampled.

Genetic Structure Within North and Central America

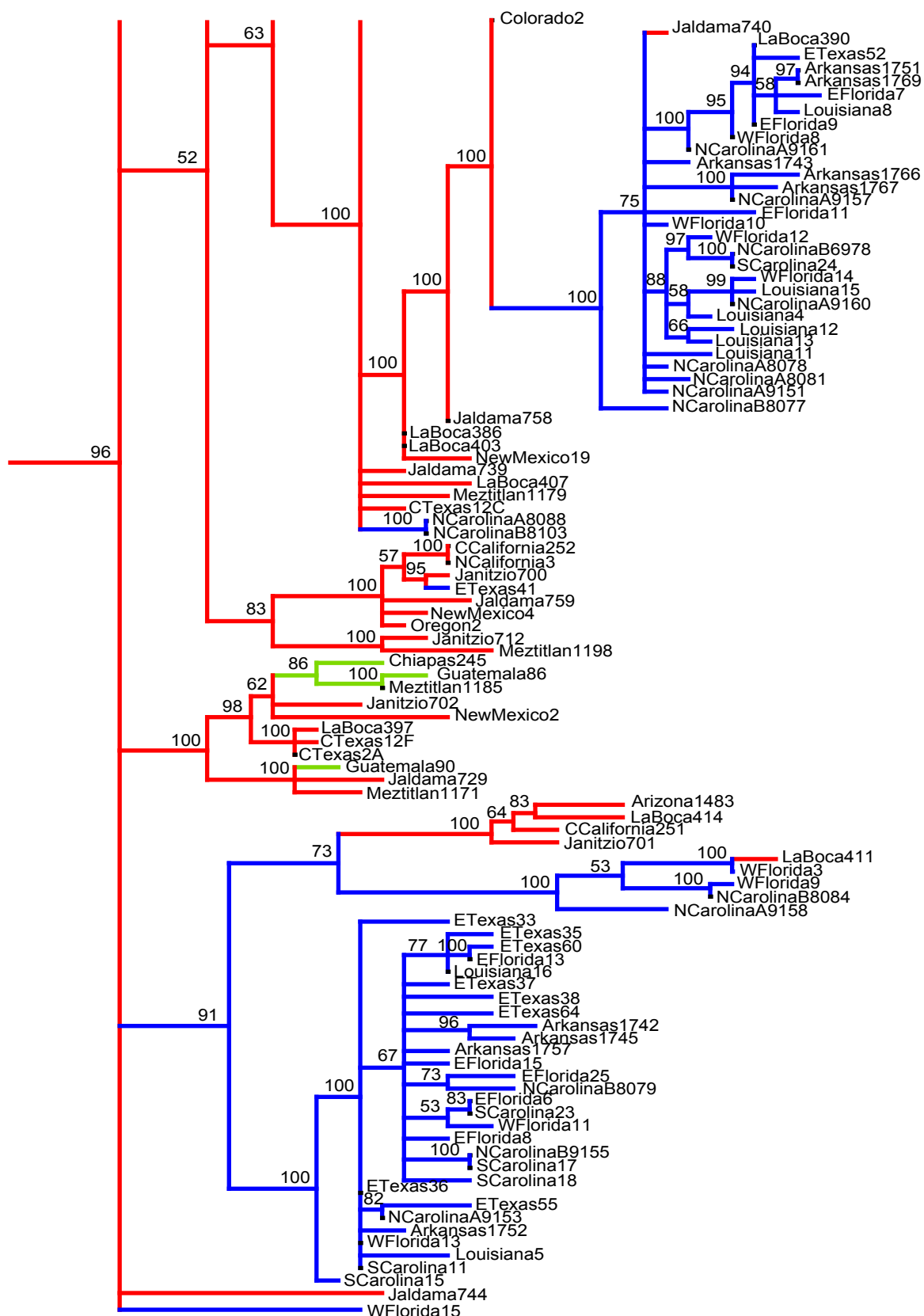
Using a likelihood analysis, I found that the North and Central American data set best conformed to a Tamura-Nei model of molecular evolution, with almost a third of the sites being invariable ($I = 0.327$) and other sites following a gamma-distributed mutation rate ($\alpha = 0.517$). This model specifies unequal base frequencies ($\pi_A = 0.437$, $\pi_C = 0.201$, $\pi_G = 0.102$, $\pi_T = 0.261$), and different mutation rates for transitions between purines ($R_{A-G} = 53.807$), transitions between pyrimidines ($R_{C-T} = 83.416$), and all transversions ($R_{TV} = 1.000$). These parameters were used in all analyses of genetic structuring within North and Central American populations.

Mitochondrial sequence data do not support differentiation of the three taxa, *T. b. cynocephala*, *T. b. mexicana*, and *T. b. intermedia*, at the subspecific level. A phylogenetic analysis of the data did not reveal separation of these subspecies similar to that between the combined North and Central American versus South American populations (Figure 3.2). Although individuals from the putative range of *T. b. cynocephala* were divided into two well-supported clades, there is no apparent geographical basis to that division (Figure 3.3). Additionally,

Table 3.8. Pairwise F_{ST} among *T. b. brasiliensis* colonies. P values for significant F_{ST} measures are given above the diagonal. * $P \leq 0.01$. Colonies in Argentina and Brazil are located east of the Andes Mountains, and colonies in Concepción and Santiago are located in Chile west of the Andes.

	Argentina	Brazil	Concepción	Santiago
Argentina	-	0.010	0.001	0.002
Brazil	0.206*	-	< 0.001	0.003
Concepción	0.405*	0.369*	-	
Santiago	0.401*	0.336*	-0.100	-

Figure 3.3. Portion denoted as “A” in the 50% consensus tree from a Bayesian likelihood analysis from Figure 3.2. This part of the tree contains most samples identified as *T. b. cynocephala*. Sampling locations are indicated.



these clades are nested among lineages from *T. b. mexicana* and *T. b. intermedia*, with each *cynocephala* clade as closely related to *mexicana* lineages as it is to the other *cynocephala* clade (Figure 3.2). Haplotypes of putative *T. b. cynocephala* from North Carolina, South Carolina, Florida, Louisiana, and eastern Texas also are interspersed among lineages that were sampled from the putative range of *T. b. mexicana* (Figure 3.2, locations not shown).

Although I found evidence for significant gene flow among all of the North and Central American subspecies, population genetic analyses indicate some structuring of lineages among the taxa. An AMOVA analysis of the data revealed significant structuring among subspecies over the entire continent ($\Phi_{CT} = 0.093$; $P < 0.01$; Table 3.9). Nested AMOVA analyses revealed that the genetic structure at the subspecific level became non-significant only when *T. b. cynocephala* was excluded from the analysis ($\Phi_{CT} = 0.007$; $P = 0.213$), indicating that *T. b. cynocephala* is significantly different from *T. b. mexicana* and *T. b. intermedia*. However, the corrected sequence divergences between *T. b. cynocephala* and *T. b. mexicana* and between *T. b. cynocephala* and *T. b. intermedia* were less than one percent (0.5% and 0.6%, respectively; Table 3.5). A Mantel test of the correlation between geographic and genetic distances among all North and Central American populations sampled was not significant ($r = 0.178$; $P = 0.061$; Figure 3.4), but does suggest the influence of isolation-by-distance. Additionally, maximum likelihood estimates of $N_e m$ revealed significant levels of migration among *T. b. cynocephala*, *T. b. mexicana*, and *T. b. intermedia* (Table 3.6). All but one estimate of $N_e m$ between *T. b. cynocephala* and another North or Central

Table 3.9. Nested AMOVA analyses of North and Central American populations. P values for Φ_{CT} (among subspecies) and Φ_{SC} (among colonies, within subspecies) represent the probability of obtaining a higher Φ statistic by chance alone, determined by 16,000 permutations of the data. P values for Φ_{ST} (within colonies) represent the probability of obtaining a lower Φ statistic by chance alone, determined by 16,000 permutations of the data. * $P < 0.01$

	All North and Central America		Exclude <i>T. b.</i> <i>cynocephala</i>		Exclude <i>T. b.</i> <i>mexicana</i>		Exclude <i>T. b.</i> <i>intermedia</i>	
	% variation	Φ -stat	% variation	Φ -stat	% variation	Φ -stat	% variation	Φ -stat
Among subsp	9.34	0.093*	0.69	0.007	11.50	0.115*	10.58	0.106*
Among colonies, within subsp	2.13	0.024	-0.42	-0.004	5.44	0.061*	2.02	0.023
Within colonies	88.52	0.115*	99.73	0.003	83.06	0.169*	87.39	0.126*

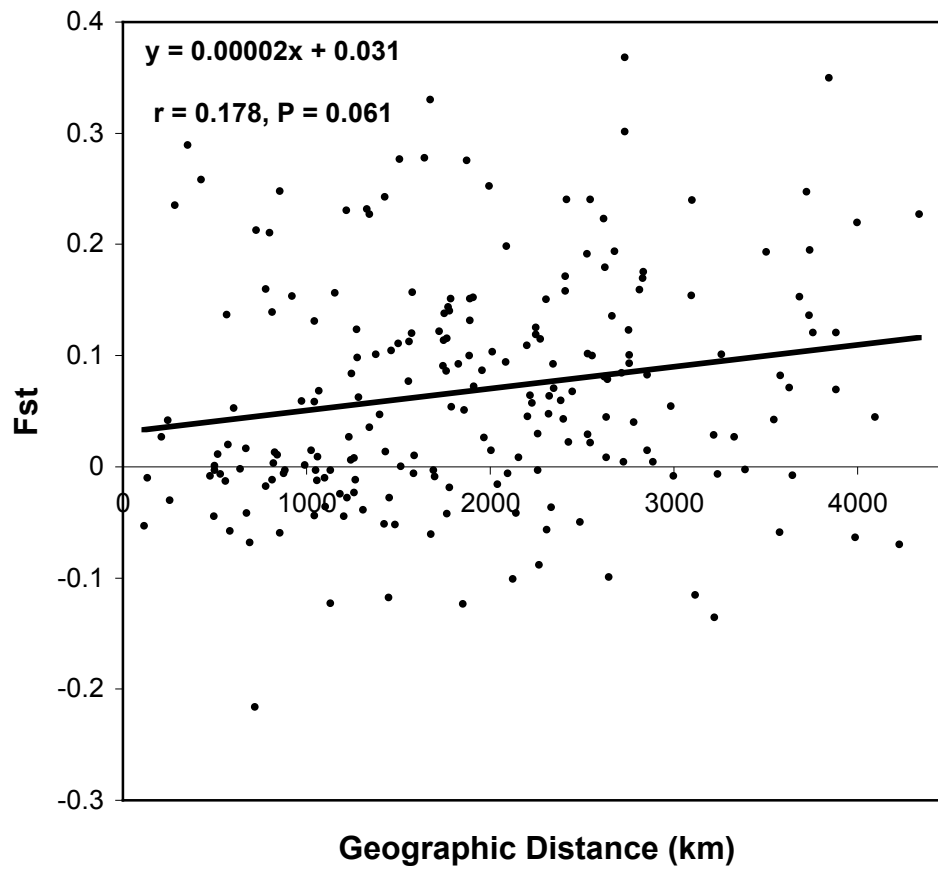
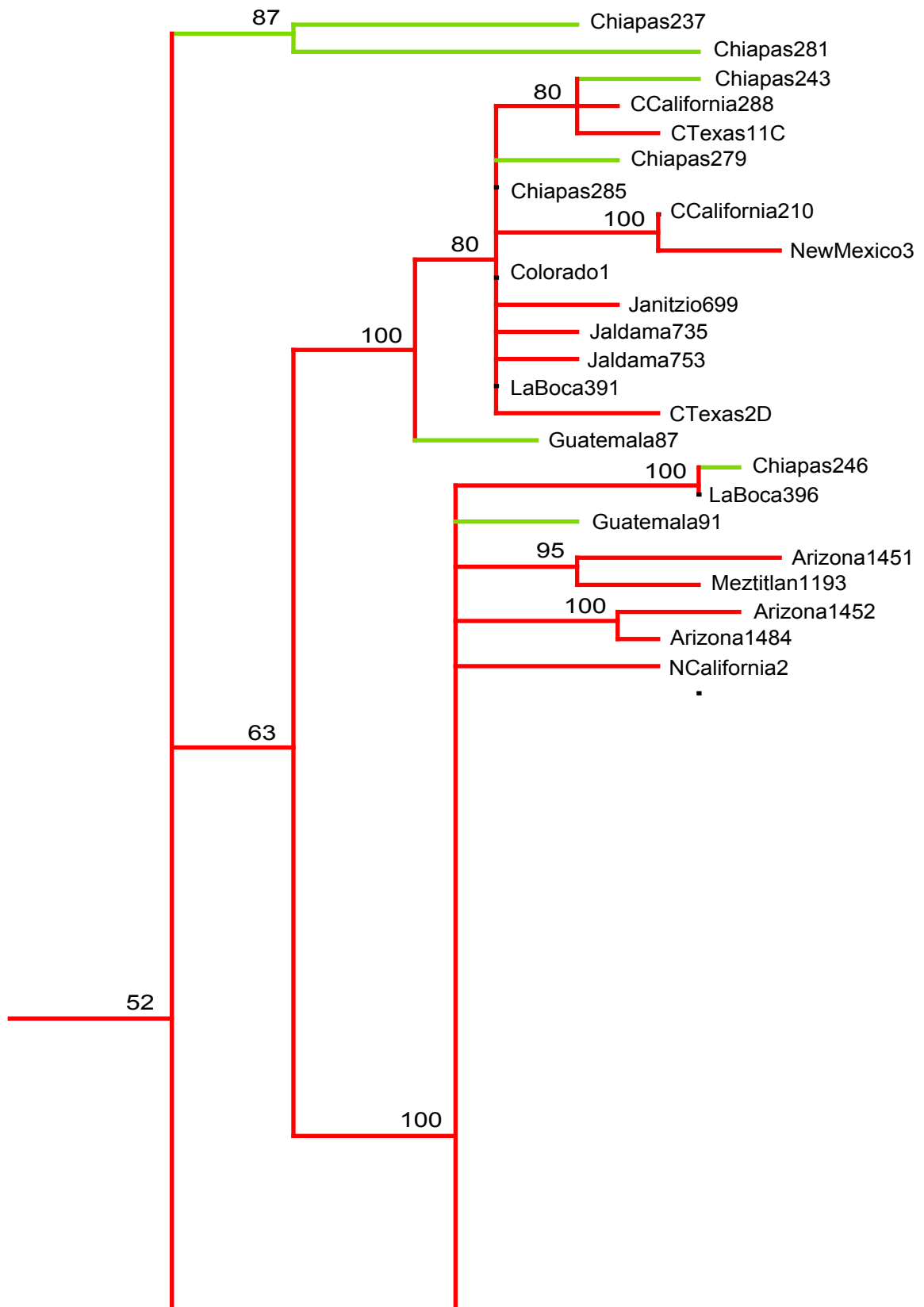


Figure 3.4. Relationship between genetic distance (F_{ST}) and geographic distance (in km) for all North and Central American colonies. The equation of the regression line is given in the figure.

American subspecies (from *T. b. cynocephala* into *T. b. intermedia*) were greater than one individual per generation. Wright (1931) showed that migration rates of this magnitude are sufficient to prevent divergence of gene pools through genetic drift. Migration from *T. b. mexicana* into *T. b. cynocephala* was estimated at 3.15 individuals per generation, and was significantly greater than in the opposite direction ($N_e m = 1.01$). This difference in directional migration rates between non-migratory *T. b. cynocephala* and adjoining migratory populations of *T. b. mexicana* may reflect differences in the subspecies' tendencies for long-distance movements. Asymmetrical migration rates were also estimated between *T. b. mexicana* and *T. b. intermedia*, with estimates from *T. b. mexicana* into *T. b. intermedia* ($N_e m = 14.56$) significantly greater than in the opposite direction ($N_e m = 5.58$). The difference in estimated directional migration rates between *T. b. mexicana* and *T. b. intermedia* may be due to my small sample size ($n = 15$) for the latter subspecies.

I found no evidence for genetic structuring between *T. b. mexicana* and *T. b. intermedia* in either the phylogenetic or population genetic analyses. In a phylogenetic analysis, there was no clustering of *T. b. intermedia* lineages separately from *T. b. mexicana* (Figure 3.5). Population genetic analyses of structure between these subspecies revealed no differentiation of gene pools. In an AMOVA analysis I found that less than one percent of the total variance in the data set was due to differentiation of the subspecies ($\Phi_{CT} = 0.007$; $P = 0.213$; Table 3.9). The subspecies diverged at an average of only 0.03% of nucleotides, less than the average sequence divergence within either subspecies (Table 3.5).

Figure 3.5. Portion denoted as “B” in the 50% consensus tree from a Bayesian likelihood analysis from Figure 3.2. This part of the tree contains most samples identified as *T. b. intermedia*. Sampling locations are indicated.



Maximum likelihood estimates of migration rates between the subspecies were higher from *T. b. mexicana* to *T. b. intermedia* ($N_m = 14.56$) than in the opposite direction ($N_m = 5.58$), but both estimates were much higher than one (Table 3.6).

Genetic Structuring Within T. b. cynocephala

I expected *a priori* that *T. b. cynocephala*, being a non-migratory subspecies, would show significant genetic structuring over a regional scale. Phylogenetic analyses reveal the clustering of most *T. b. cynocephala* haplotypes into two groups (Figures 3.2, 3.3). However, these groups do not correspond to geographic location. I turned to population genetic analyses to further explore genetic patterns in the data. An AMOVA analysis revealed significant genetic structuring among colonies ($\Phi_{ST} = 0.063$; $P = 0.021$; Table 3.7). However, genetic distances between colonies were not correlated with geographic distances, as a Mantel test was non-significant (Figure 3.6). I used an analysis of pairwise F_{ST} to determine if the genetic structure in the data could be explained by differentiation among certain colonies (Table 3.10). This analysis revealed that four of seven significant F_{ST} measures included the colony in eastern Texas. The impact of the eastern Texas colony on the genetic structure within *T. b. cynocephala* was made apparent in an AMOVA analysis excluding that colony from the data set ($\Phi_{ST} = 0.026$; $P = 0.174$).

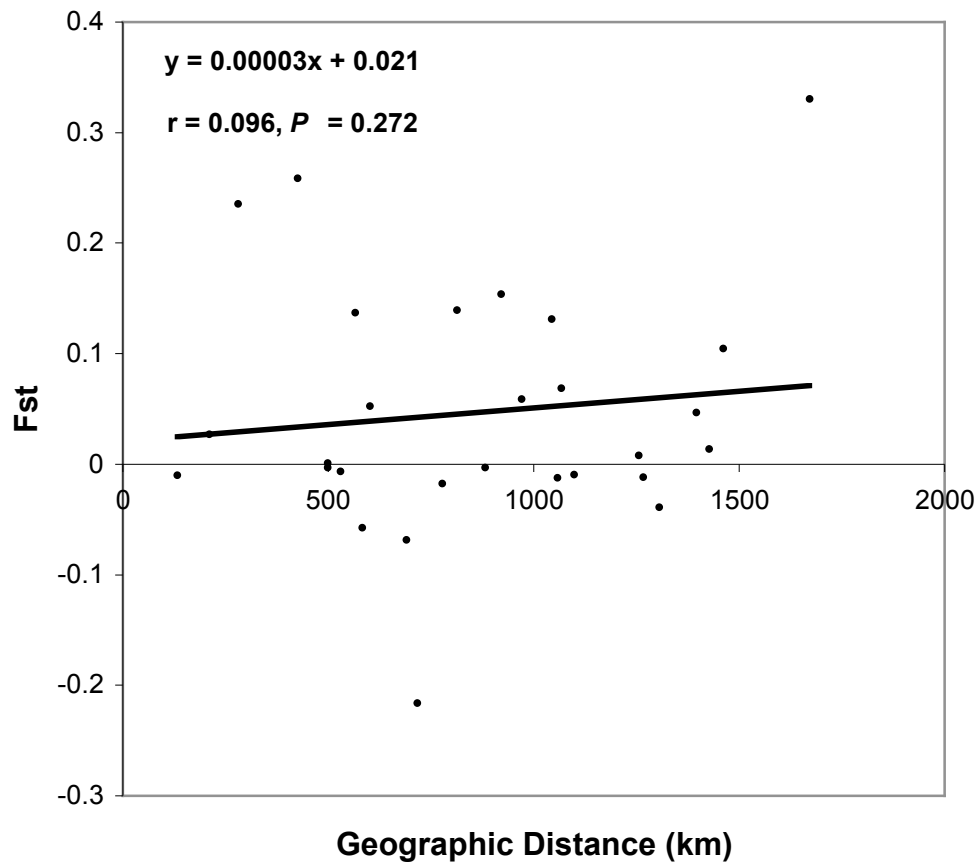


Figure 3.6. Relationship between genetic distance (F_{ST}) and geographic distance (in km) for all colonies in the putative subspecies *T. b. cynocephala*. The equation of the regression line is given.

Table 3.10. Pairwise F_{ST} among *T. b. cynocephala* colonies. P values for significant F_{ST} measures are given above the diagonal. * $P < 0.05$; ** $P < 0.01$.

	ETexas	Arkansas	EFlorida	WFlorida	Louisiana	NCarolinaA	NCarolinaB	SCarolina
ETexas	-			0.015	0.005	0.003	0.048	
Arkansas	0.138	-						
EFlorida	0.048	-0.012	-			0.042		
WFlorida	0.155*	-0.017	0.001	-				
Louisiana	0.261**	-0.007	0.060	-0.002	-			0.048
NCarolinaA	0.332**	0.008	0.140*	-0.002	-0.039	-		0.010
NCarolinaB	0.105*	-0.013	-0.021	-0.069	-0.009	0.027	-	
SCarolina	0.013	0.069	-0.058	0.053	0.132*	0.237**	-0.009	-

5. Discussion

Genetic Structuring Between Continental Populations

Earlier authors have indicated the possibility of gene flow between populations of *T. brasiliensis* in North, Central, and South America. G. Allen (1908) recommended synonymizing *Nyctinomus* (= *Tadarida*) *brasiliensis* and *N. mexicana* after finding no morphological differences between populations from Buenos Aires, Argentina, and Texas, USA. Schmidley (1983) indicated that *T. b. mexicana* migrates south into Central America, and perhaps as far as South America.

I demonstrate significant differences between *T. b. brasiliensis* and the North and Central American subspecies using mtDNA sequence data. This division is supported by both phylogenetic and population genetic analyses. The phylogenetic analysis using Bayesian likelihood criteria supports segregation of *T. b. brasiliensis* from North and Central American populations with a posterior probability of 1.0 (Figure 3.2). AMOVA analyses (Table 3.4) and estimates of sequence divergence of approximately 13% (Table 3.5) reveal significant differentiation between populations on the two continents. In other mammals, comparable estimates of D-loop sequence divergence range from 10.3% - 11.3% between *Leptonycteris curasoae* and *L. nivalis*, two recognized and geographically isolated species of migratory bats (Wilkinson and Fleming 1996), and 10.2% - 18.8% between species of European rabbits in the genus *Lepus* (Pierpaoli *et al.* 1999). It is also noteworthy that, whereas each of the three North

and Central American subspecies possessed individuals having more than three repeat units in the HV1 region of the D-loop, no individuals of *T. b. brasiliensis* were ever detected with more than three repeats.

The level of genetic differentiation between *T. b. brasiliensis* and the combined North and Central American population of *T. b. cynocephala*, *T. b. mexicana*, and *T. b. intermedia* suggests that these gene pools in North and Central America versus South America may deserve recognition at the species level. However, due to the absence of samples from northern South America and most of Central America, the data are not informative as to whether the differentiation of gene pools is due to genetic isolation-by-distance or to the existence of the respective populations as separate species. Supporting evidence from nuclear loci and from analyses of populations in northern South America is required before the species question can be resolved. In the event that such a revision is warranted, I point out the precedence of *Tadarida cynocephala* LeConte (1831) over *T. mexicana* Saussure (1860) or *T. intermedia* Shamel (1931) as a species name for the North and Central American populations.

Genetic Structure Within South America

Population genetic analyses revealed significant genetic structuring within South America, with over 30% of the variance in the data due to differences among colonies (Table 3.7). This genetic structuring is also reflected in the nucleotide diversity (π = 5.7%) of *T. b. brasiliensis* (Table 3.3). This statistic

varied from 4.2% to 4.5% within North and Central American subspecies, and was no larger than 4.7% in all of North and Central America, indicating that the South American populations are more differentiated than are different recognized subspecies in North and Central America. Calculations of pairwise F_{ST} between colonies reveal that the most differentiated pairs of colonies are those separated by the Andes mountain range, indicating that the mountains act as a barrier to gene flow between populations (Table 3.8). The average sequence divergence between colonies separated by the Andes was 3.1%, while the sequence divergence between colonies on the same side of the mountain range varied from 0.3% to 1.8%. For context, estimates of D-loop sequence divergence between migratory populations of humpback whales (*Megaptera novaeangliae*) range from 0 – 0.282% (Baker *et al.* 1990), and from 0 – 6.1% for highly structured populations of the ghost bat (*Macroderma gigas*) that are not given subspecific rank (Worthington Wilmer *et al.* 1994). Our analyses support the existence of significant genetic structure among South American populations of *T. brasiliensis*. Further analyses including populations from northern South America are needed.

Genetic Structuring Within North and Central America

I found no support in the mtDNA sequence data for the differentiation of lineages within North and Central America at the subspecific level. Populations of *T. b. intermedia* were not genetically distinguishable from *T. b. mexicana* in any analysis. *T. b. intermedia* lineages were dispersed among *T. b. mexicana*

lineages within the phylogeny and formed no distinct cluster (Figure 3.5). Sequences from these two subspecies diverged at only 0.03% (Table 3.5), and estimated migration rates between them were sufficient to homogenize the populations (Table 3.6). Carter (1962) categorized populations of *T. b. intermedia* from Chiapas, Mexico, and Guatemala as *T. b. mexicana* based on morphological criteria, and found no differences among colonies considered to be *T. b. mexicana*. My analyses of mtDNA variation support Carter's implication that *T. b. intermedia* be synonymized with *T. b. mexicana*. My recommendation is limited to populations of *T. b. intermedia* from southern Mexico and Guatemala, pending analyses of other populations from Central America.

The low level of genetic differentiation between *T. b. cynocephala* and *T. b. mexicana* in my mtDNA sequence data is consistent with an overall lack of differentiation among populations of the purported subspecies seen in behavioral, morphological, and nuclear allozyme data. A clear definition of subspecies based on migratory behavior is complicated by the presence of non-migratory populations in the range of *T. b. mexicana*, a subspecies characterized as being migratory (Benson 1947; Part 2). The possibility of gene flow between *T. b. cynocephala* and *T. b. mexicana* has been shown from banding studies. It is also supported by observations of the presence of a year-round *T. b. mexicana* colony in College Station, Texas, only 137 km from resident colonies of *T. b. cynocephala*, and reports of an adult male identified as *T. b. mexicana* that was banded in western Oklahoma and recovered in Louisiana (Spenrath and LaVal 1974; Glass 1982). Morphological distinctions between the taxa are blurred by

the documentation of individuals of intermediate size (Schmidley 1991) and by the overlap in the ranges of measurements that characterize the putative subspecies (Schwartz 1955). Populations located close to the interface of the subspecies' ranges have been characterized in allozyme studies as being intermediate in allele frequencies and possessing rare alleles from both eastern and western populations (McCracken and Gassel 1997). The mtDNA sequence divergences of no more than 1.9% among colonies throughout North America are comparable to the sequence divergence between weakly differentiated clades within subspecies of the migratory bat *Leptonycteris curasoae yerbabuenae* (2.1%; Wilkinson and Fleming 1996).

Thus, all evidence indicates that North American populations of *T. brasiliensis* maintain substantial gene flow despite differences in migratory behavior. I recommend that *T. b. cynocephala* and *T. b. mexicana* be synonymized as the same subspecies, with the name *T. b. cynocephala* LeConte (1831) having precedence over *T. b. mexicana* Saussure (1860).

Genetic Structuring Within T. b. cynocephala

Populations of *T. b. cynocephala* are characterized as resident colonies undergoing only local movements (Barbour and Davis 1969; Wilkins 1989). This description leads to an *a priori* expectation of significant genetic structure among colonies. I found a significant level of cladistic structure within the subspecies, but this was not correlated with any geographic structuring among colonies. Pairwise F_{ST} analyses revealed that the genetic structure in the data was due

significantly to differentiation of the colony in Huntsville, Texas. This colony has been described as containing both *T. b. mexicana* and *T. b. cynocephala* (Bennett 2000), and is located 74 km from College Station, Texas, where a colony of *T. b. mexicana* is present throughout the year (Spenrath and LaVal 1974; Schmidley 1991). Thus, it is likely that our sample from eastern Texas contains haplotypes from individuals that are currently placed into two subspecies designations. An AMOVA analysis of putative colonies of *T. b. cynocephala*, excluding the colony from eastern Texas, reveals no genetic structure among the remaining colonies. Therefore, I find that a lack of migratory behavior in this species is not associated with strong genetic structuring among colonies.

6. Conclusions

Koopman (1983) elegantly articulated that a problem exists in bat systematics, because the temporal and spatial separations of mating and offspring production make it difficult to define populations, subspecies, and even species. The difficulty of observing mating in these animals has often led to definitions of populations based on the location of maternity sites. In cases for which direct records of gene flow are scarce, population admixture may be inferred indirectly from genetic analyses. I have demonstrated the utility of genetic data for inferring patterns of gene flow among animals that are difficult to monitor directly. Although mating has seldom been documented in *T. brasiliensis*,

I have provided evidence for substantial gene flow between taxa that were previously thought to be largely isolated.

IV. EXTREMELY LARGE EFFECTIVE POPULATION SIZE IN A MIGRATORY BAT SPECIES

1. Abstract

The effective size (N_e) of a population determines the distribution of coalescence times in a genealogy, the expected rate of loss of genetic variation, and the expected time to divergence of populations through genetic drift. Populations with a small effective size can suffer inbreeding depression in the short-term (Nieminen *et al.* 2001) and a loss of evolutionary potential and flexibility in the long-term (Falk and Holsinger 1991). Here I demonstrate in a migratory bat genetic diversity estimated from mitochondrial DNA (mtDNA) sequence data that is among the largest of any previously reported, regardless of gene or organism. My estimate of N_e for this species is the largest yet reported for a mammal, and is comparable to estimates for species that are parasites and commensals of humans (Vilá *et al.* 1999; Walton *et al.* 2000; Hughes and Verra 2001; Mes 2003).

2. Introduction

A theoretical concept, effective population size (N_e) is defined as the size of a randomly mating gene pool that experiences the same effect from genetic drift as the actual population (Wright 1931). Demographic variables such as sex ratio and family size may cause the effective size of a population to deviate from the census size (N). Variation in family size, fluctuations in population size over time, and unequal sex ratios will decrease N_e relative to N . N_e can be directly

estimated from these demographic data (Lande and Barrowclough 1987; Ardren and Kapuscinski 2003) or, alternatively, it can be indirectly estimated from DNA sequence diversity. Estimates of N_e from DNA sequence data are derived from estimates of genetic diversity, π ($= 2N_e\mu$, for haplotype data). These estimates of N_e are strongly dependent on the mutation rate (μ), but provided that an estimate of μ is available, N_e can easily be derived from π . Several methods for estimating π have been derived (Watterson 1975; Tajima 1983; Kuhner *et al.* 1998), and are reviewed in detail elsewhere (Crandall *et al.* 1999). Generally, the methods fall into two broad categories: summary statistical analyses and genealogical analyses. Tajima's (1983) estimate of π_π , obtained from nucleotide diversity (π), and Watterson's (1975) estimate of π_s , obtained from the number of segregating sites (S), are estimates based on descriptive statistics. Felsenstein (1992) has shown that these summary statistical methods are inefficient and do not make use of much of the information inherent in DNA sequences. Genealogically based methods make full use of both direct sequence variation and inferred coalescent relationships. For example, Kuhner *et al.* (1998) use sequence data to construct genealogies and then determine the most likely value of π_{ML} , given the maximum-likelihood genealogy.

Fluctuations in population size leave a signature in DNA sequence data. This signature may be detected using tests of neutrality, comparisons of estimates of π , or maximum likelihood-based estimates of population growth. Neutrality tests such as Fu's (1997) F_S are designed to detect evidence of

selection under a model of demographic equilibrium. Because this test is based on an assumption of a stable population size, the F_S statistic may yield spurious results for a selectively neutral locus if the population being sampled is not at demographic equilibrium. Fu's F_S is especially sensitive to demographic expansion, which has been shown to result in large negative values of F_S (Fu 1997). When population size changes drastically, it has been shown that π_θ is more sensitive to the size of the original population whereas π_s is more sensitive to the size of the current population (Tajima 1989a). Tajima's D (1989b) compares these two estimates of π and, assuming selective neutrality, can be used to make inferences regarding the demographic history of the population. Additionally, the rate of population growth can itself be estimated from DNA sequence data using maximum likelihood analyses (Kuhner *et al.* 1998).

The Mexican free-tailed bat, *Tadarida brasiliensis mexicana*, is one of the most abundant bats in the Western Hemisphere. It is distributed throughout the southwestern and western United States and Mexico, where it roosts in colonies of several million individuals (Figure 4.1; Davis *et al.* 1962). The bats are so numerous that their emergences are detected regularly on Doppler radar (McCracken 1996), and radar studies show that individuals can fly up to 50 km in a single night (Williams *et al.* 1973). These bats are seasonally migratory throughout most of their range, but populations in California and Oregon hibernate during the winter (Davis *et al.* 1962). Banding studies in the 1950s and 1960s showed that individuals could travel at least 1500 km annually (Constantine 1967; Cockrum 1969).



Figure 4.1. Ranges of *T. b. mexicana* and *T. b. intermedia* (Hall 1981). Sampled populations are indicated by black squares. Putative *T. b. intermedia* colonies include Chiapas and Guatemala, all others are *T. b. mexicana*.

Although previous authors (Cockrum 1969) have suggested that geographic populations of these bats are distinguished by their migratory behavior and may be genetically isolated, analyses of mtDNA sequence data reveal no significant structuring among populations throughout North and Central America (Parts 2, 3), a result that is consistent with earlier allozyme studies (McCracken *et al.* 1994). Numerous recoveries of banded individuals also demonstrate high levels of movement among colonies (Constantine 1967; Svoboda *et al.* 1985). Thus, the total evidence indicates that colonies of *T. b. mexicana* are connected by high levels of gene flow and consist of a single, extremely large population. Here I use several approaches to estimate the effective size of this population and to assess if the trajectory of the population is increasing, decreasing, or demographically stable.

3. Materials and Methods

Sample Collection

Samples representing thirteen colonies distributed throughout the range of *T. b. mexicana* and the northern range of the Central American subspecies *T. b. intermedia* were collected by colleagues (Table 4.1). Bats were captured using 4'x4' harp traps or mist nets erected at the entrance of each colony, or using hand nets within roosts. The bats were sexed and stored temporarily in cotton bags prior to tissue sampling. Two 3-mm tissue biopsies, one from each

Table 4.1. Sampling information. Locations are listed from east to west by increasing longitude. The sample size indicates the number of individuals that were sequenced from each colony.

Subspecies	Sample Name	Sample Size	Location	Geographical Coordinates
<i>T. b. intermedia</i>	Guatemala	4	El Rancho, Guatemala	14:21N 91:00W
<i>T. b. intermedia</i>	Chiapas	11	San Francisco Cave, Chiapas, Mexico	16:15N 92:08W
<i>T. b. mexicana</i>	Meztitlan	9	El Salitre Cave, Hidalgo, Mexico	20:07N 98:44W
<i>T. b. mexicana</i>	CTexas	10	James River Cave, Texas, USA	30:44:57N 99:13:56W
<i>T. b. mexicana</i>	LaBoca	12	Cueva La Boca, Nuevo Leon, Mexico	25:41N 100:15W
<i>T. b. mexicana</i>	Janitzio	11	Isla de Janitzio, Michoacán, Mexico	19:42N 101:07W
<i>T. b. mexicana</i>	NewMexico	10	Carlsbad Caverns Nat'l Park, New Mexico, USA	32:10:32N 104:22:34W
<i>T. b. mexicana</i>	Colorado	4	Grand Junction, Colorado, USA	39:04N 108:33W
<i>T. b. mexicana</i>	JuanAldama	12	Ejido Juan Aldama, Sinaloa, Mexico	25:45N 108:57W
<i>T. b. mexicana</i>	Arizona	8	Eagle Creek Cave, Arizona, USA	33:03:11N 109:19:46W
<i>T. b. mexicana</i>	CCalifornia	10	Merced County, California. USA	37:28N 120:30W
<i>T. b. mexicana</i>	NCalifornia	3	Lava Beds Nat'l Monument, California, USA	41:53:18N 121:22:16W
<i>T. b. mexicana</i>	Oregon	5	Jackson County, Oregon, USA	42:11:25N 122:41:58W

wing, were taken from each bat for DNA analysis according to the method of Worthington Wilmer and Barratt (1996). The bats were then released at the site of capture. Wing biopsies were stored in either NaCl-saturated 20% DMSO or silica gel desiccant in the field and at -80°C upon return to the lab. Tissue samples were obtained from a total of 109 bats, with an average sample size of 8.4 bats per site.

DNA Isolation and Amplification

I isolated total genomic DNA using a standard phenol-chloroform-isoamyl alcohol (25:24:1), ethanol precipitation method (modified from Sambrook *et al.* 1989) or using a DNeasy® DNA isolation kit (Qiagen), and stored in 1/10 TE. I then quantified the isolates using a Hoefer DyNA Quant® 200 fluorometer (Amersham Pharmacia), and diluted each to a standard concentration of 10 ng/ μL for the polymerase chain reaction (PCR). I amplified all samples using primers F(mt): 5'-GTTGCTGGTTTCACGGAGGTAG-3', and P(mt): 5'-TCCTACCATCAG-CACCCAAAGC-3' (Wilkinson and Chapman 1991) for the control region (D-loop) of the mitochondrial DNA molecule, located near the tRNA^{Pro} gene. These primers yielded a product approximately 500 bp long. To obtain adequate amounts of PCR product for DNA sequencing, I carried out amplifications in eight 12- μL reaction volumes, each containing 1.04 mM MgCl_2 , 0.1 mM dNTPs, 1 Unit *Taq* DNA polymerase, 1.2 μL Promega 10X buffer, 10 ng genomic DNA, and 14 pmol of each primer. The amplification involved an initial denaturation at 94°C

for two minutes followed by 30 cycles at 94 °C for one minute, 55 °C for 1.5 minutes, and 72 °C for two minutes. I combined the eight reactions into a common sample, and purified the target fragment using gel band excision (MinElute® kit, Qiagen).

DNA Sequencing

I performed sequencing reactions using the PRISM® BigDye™ Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems) in a 10 µL reaction containing 50-100 ng of PCR product, 7 pmol of primer, and 3 µL of the BigDye ready reaction mix. The sequencing temperature profile consisted of 25 cycles at 96 °C for 10 seconds, 50 °C for 5 seconds, and 60 °C for 4 minutes, with temperature changes between each step occurring at a speed of 1 °C per second. I cleaned the sequencing reactions of unincorporated nucleotides using Centri-Sep columns (Princeton Separations) according to the manufacturer's instructions, and analyzed the reaction on an ABI 3100 automated sequencer. I sequenced twenty arbitrarily chosen individuals from both directions to estimate the rate of sequencing error. Because this rate was low ($\sim 2.1 \times 10^{-3}$ per sequence), I sequenced most individuals using only the P(mt) primer. All sequences were deposited in the GenBank database (accession numbers X-Y).

The D-loop of the mitochondrial genome is characterized in many bat species by variable numbers of tandemly repeated 81-bp sequence units (Wilkinson *et al.* 1997). In *T. brasiliensis*, I found that most individuals possessed

three of these repeat units, while others possessed up to six repeats. I detected nineteen individuals representing nine locations that possessed more than three repeat units. These individuals were found in both *T. b. mexicana* ($n = 16$, from seven sites) and *T. b. intermedia* ($n = 3$, from two sites), and there was no detectable geographical bias to their distribution (data not shown). To avoid problems with sequence alignment, I included in these analyses only individuals that were characterized by three repeats.

Sequence Analysis

I aligned all sequences using the default settings in CLUSTALW (Thompson *et al.* 1994). The alignment was then edited by eye and cropped to a common length of 477 bp. Sequence variation included five indels, of which the longest was one bp. In all analyses, indels were treated as a fifth character, rather than as missing data.

Using a likelihood analysis (Posada and Crandall 1998), I determined that the data best fit the Tamura-Nei model of evolution (Tamura and Nei 1993) with a proportion of sites being invariable ($I = 0.3266$) and a gamma-distributed mutation rate with the shape parameter $\alpha = 0.5503$. This model specifies different substitution rates for transition mutations between purines ($R_{A-G} = 42.768$), transitions between pyrimidines ($R_{C-T} = 72.215$), and transversions ($R_{TV} = 1.000$). I found a heavy bias in the nucleotide composition towards adenine ($\pi_A = 0.432$, $\pi_C = 0.198$, $\pi_G = 0.104$, $\pi_T = 0.265$). All subsequent analyses were conducted using the assumptions and parameters of that model.

Estimation of Population Genetic Parameters

Previous analyses revealed no evidence of genetic structuring among colonies of *T. b. mexicana* ($\Phi_{ST} = -0.002$; $P = 0.551$; Part 2) or between *T. b. mexicana* and *T. b. intermedia* (average sequence divergence = 0.03%; $P = 0.239$; Part 3). Thus, I estimated all population parameters under the assumption that all of the sampled colonies constitute a single population.

I used ARLEQUIN version 2.000 (Schneider *et al.* 2000) to obtain estimates of $\Phi_{\square}$, Φ_s , Fu's F_S , and Tajima's D . The estimate of $\Phi_{\square}$ is based on the mean number of pairwise differences ($\square$) and is calculated as (Tajima 1983):

$$Exp(\square) = \Phi_{\square}. \quad (1)$$

The estimate of Φ_s is based on the number of segregating sites (S) and is calculated as (Watterson 1975):

$$\Phi_s = \frac{S}{a_1} \quad (2)$$

where

$$a_1 = \sum_{i=1}^{n-1} \frac{1}{i} \quad (3)$$

is a correction for the sample size n . Fu's (1997) test of selective neutrality evaluates the probability of observing a random neutral sample with a number of

alleles less than or equal to the observed value, given the observed number of pairwise differences. Fu (1997) called this probability S_{π}

$$S_{\pi} = \Pr(K \geq k_{obs} \mid \pi = \pi_{\pi}) \quad (4)$$

and defined his F_S statistic as:

$$F_S = \ln\left(\frac{S_{\pi}}{1 - S_{\pi}}\right) \quad (5)$$

Tajima's (1989b) D statistic is calculated as:

$$D = \frac{\pi_{\pi} - \pi_s}{\sqrt{\text{Var}(\pi_{\pi} - \pi_s)}}. \quad (6)$$

This statistic was derived as a test for selective neutrality, but has been shown to indicate past changes in population size, when selective neutrality can be assumed (Tajima 1989a). The significance of Fu's F_S and Tajima's D were determined by comparison with 16,000 random samples generated under an hypothesis of selective neutrality and demographic equilibrium, and represent the proportion of random F_S or D values less than or equal to the observed.

I used FLUCTUATE version 1.5 (Kuhner *et al.* 1998) to obtain estimates of π_{ML} and the exponential growth rate of the population, g , measured in units of $(1/\pi)$ *generations. The strategy of this analysis is to first sample genealogies based on their posterior probabilities given the data and trial values of the parameters π and g . These sampled genealogies are then used to evaluate the

relative likelihood of other parameter values (Kuhner *et al.* 1998). In the first phase of the analysis, the probability of a given tree is estimated as:

$$\Pr(G | \mu, g) = \prod_i \frac{e^{gt_s} e^{\frac{k(k-1)}{2g} (e^{gt_s} - e^{gt_e})}}{\prod} \quad (7)$$

where k is the number of lineages during coalescent interval i , t_s is the time at the tipward end of the interval, and t_e is the time at the rootward end of the interval (Kuhner *et al.* 1998). Each genealogy is then accepted or rejected based on the probability of the data given the genealogy. After many reiterations, this process results in a set of genealogies from which the joint maximum likelihood estimates of μ and g may be estimated as the maximum of:

$$\frac{L(\mu, g)}{L(\mu_0, g_0)} = \frac{1}{n} \prod_G \frac{\Pr(G | \mu, g)}{\Pr(G | \mu_0, g_0)}. \quad (8)$$

I implemented this analysis using four differentially heated Markov chains simultaneously to allow a wider search of treespace. I used a UPGMA distance tree as the initial genealogy and Watterson's estimate of μ_s for μ_0 . From this starting point I ran ten short searches of 10,000 steps each with sampling of trees every 200 steps, followed by two long searches of 100,000 steps with sampling every 500 steps. The entire analysis was run three times from different random seeds. These results were not significantly different from each other and,

in the interest of being conservative, I have reported the smallest estimated value of θ_{ML} .

4. Results

Estimates of Effective Population Size

Using the summary statistical methods of Tajima (1983) and Watterson (1975) I estimated $\theta_{\pi} = 0.045 \pm 0.022$ per site from nucleotide diversity and $\theta_S = 0.067 \pm 0.017$ per site from segregating sites (Table 4.2). Assuming a mutation rate of $\mu = 1.88 \times 10^{-8}$ per site per year (Pesole *et al.* 1999), these estimates of θ translate to effective population sizes of $1.2 \times 10^6 \pm 0.59 \times 10^6$ and $1.8 \times 10^6 \pm 0.45 \times 10^6$ females, respectively. Using the genealogically based method of Kuhner *et al.* (1998), I estimated genetic diversity from a maximum-likelihood analysis as $\theta_{ML} = 1.068 \pm 0.08$, indicating an effective population size of $2.84 \times 10^7 \pm 0.21 \times 10^7$ females.

Variation in Effective Population Size

For *T. b. mexicana*, I calculated $F_S = -13.13$ ($P < 0.001$) for the *T. b. mexicana* population, consistent with a model of population growth. This population also was characterized by a significantly negative Tajima's (1989b) D ($= -0.28$; $P < 0.001$), indicating that the mean number of nucleotide differences (π) and the number of segregating sites (S) for this population are consistent with a model of population growth. This demographic model is also supported by

Table 4.2. Genetic diversity (π), effective population size (N_e), and exponential growth rate (g) estimates for *T. b. mexicana*

π	N_e	g	Analytical Method
0.045 $\pm$ 0.022	1.2 x 10 ⁶ $\pm$ 0.59 x 10 ⁶	-	From nucleotide diversity (π_π ; Tajima 1983)
0.067 $\pm$ 0.017	1.8 x 10 ⁶ $\pm$ 0.45 x 10 ⁶	-	From segregating sites (π_s ; Watterson 1975)
1.068 $\pm$ 0.08	2.84 x 10 ⁷ $\pm$ 0.21 x 10 ⁷	128.79 $\pm$ 7.87	Maximum likelihood (π_{ML} ; Kuhner <i>et al.</i> 1998)

comparisons of θ_{ML} with both $\theta_{\square}$ and θ_s . While the magnitude of the difference between the coalescent-based θ_{ML} and the summary statistic-based $\theta_{\square}$ and θ_s may be due, in part, to the known upward bias for θ_{ML} , especially when using data from a single locus (Kuhner *et al.* 1998), θ_{ML} is expected to be greater than $\theta_{\square}$ and θ_s in growing populations (Matocq 2002). Using a maximum likelihood analysis (Kuhner *et al.* 1998), the exponential growth rate of the population (g) is estimated from the mutation rate as 128.79 ± 7.87 . This estimate differs significantly from zero, and implies that the population fits a model of exponential growth over time. Thus, tests of selective neutrality, comparisons of θ estimates, and direct estimates of the growth rate using maximum likelihood are consistent with a model of demographic growth, and reject models of demographic decline or equilibrium.

5. Discussion

These estimates of θ_{ML} and $\theta_{\square}$ for *T. b. mexicana* are among the highest estimates (per site) that I have found in the literature, regardless of organism or gene (Table 4.3). My estimate of $\theta_{\square}$ is similar to that for coyotes ($\theta_{\square} = 0.046$; Vilá *et al.* 1999), although estimates of $N_{e(\square)}$ for the two species vary due to differences in generation time. Additionally, $\theta_{\square}$ and N_e for *T. b. mexicana* greatly exceeds that for human populations ($\theta_{\square} = 0.002$, $N_e = 5.3 \times 10^4$; Clark *et al.*

Table 4.3. Estimates of $\theta_{\square}$ and N_e for assorted taxa. Estimates for *H. sapiens* and *P. falciparum* are roughly four times larger than mtDNA-derived estimates because they are derived from nuclear loci.

Organism	Species Name	Locus	θ_{ML}	$N_{e(ML)}$	$\theta_{\square}$	$N_{e(\square)}$	Citation
Human	<i>Homo sapiens</i>	Lipoprotein lipase (nDNA)	-	-	0.002 ± 0.001	$5.3 \times 10^4 \pm 2.66 \times 10^4$	Clark <i>et al.</i> 1998
American eel	<i>Anguilla rostrata</i>	RFLP (mtDNA)	-	-	0.0011	5.5×10^4	Awise <i>et al.</i> 1986
Ghost bat	<i>Macroderma gigas</i>	Control region (D-loop)	-	-	0.022	5.9×10^5	Worthington Wilmer <i>et al.</i> 1999
Parasitic nematode	<i>Mazamastongylus ocoidei</i>	ND4 (mtDNA)	2.23 ± 0.32	$2.23 \times 10^6 \pm 0.32 \times 10^6$	-	-	Mes 2003
Parasitic nematode	<i>Teladorsagia circumtineta</i>	ND4 (mtDNA)	7.09 ± 0.87	$7.09 \times 10^6 \pm 0.87 \times 10^6$	-	-	Mes 2003
Malaria parasite	<i>Plasmodium falciparum</i>	Protein-coding loci (nDNA)	0.0505 ± 0.0212	$1.05 \times 10^7 \pm 0.44 \times 10^7$	-	-	Hughes and Verra 2001
Coyote	<i>Canis latrans</i>	Control region 1	0.373 ± 0.103	$3.73 \times 10^6 \pm 1.03 \times 10^6$	0.046 ± 0.025	$4.6 \times 10^5 \pm 2.5 \times 10^5$	Vilá <i>et al.</i> 1999
Mosquito	<i>Anopheles dirus</i>	Cytochrome oxidase 1	0.757 ± 0.38	$5.0 \times 10^8 \pm 2.5 \times 10^8$	0.0064 ± 0.0002	$4.2 \times 10^6 \pm 0.13 \times 10^6$	Walton <i>et al.</i> 2000

Table 4.3. Continued.

Organism	Species Name	Locus	π_{ML}	$N_{e(ML)}$	π_D	$N_{e(D)}$	Citation
Mexican free-tailed bat	<i>T. b. mexicana</i>	Control region (D-loop)	1.068 ± 0.08	$2.84 \times 10^7 \pm 0.21 \times 10^7$	0.045 ± 0.022	$1.2 \times 10^6 \pm 0.59 \times 10^6$	this study

1998) as well as migratory animals with high gene flow such as American eels *Anguilla rostrata* ($\theta_{\square} = 0.0011$, $N_e = 5.5 \times 10^4$; Avise *et al.* 1986). Comparable estimates for other bat species range from $\theta_{\square} = 0.013$ ($N_e = 3.5 \times 10^5$) for migratory *Nyctalus noctula* (Petit *et al.* 1999) and $\theta_{\square} = 0.016$ ($N_e = 4.3 \times 10^5$) for migratory *Leptonycteris curasoae* (Wilkinson and Fleming 1996) to $\theta_{\square} = 0.021$ ($N_e = 5.6 \times 10^5$) for migratory *Myotis myotis* (Ruedi and Castella 2003) and $\theta_{\square} = 0.022$ ($N_e = 5.9 \times 10^5$) for a large population of *Macroderma gigas* (Worthington Wilmer *et al.* 1999). Maximum likelihood estimates of θ and N_e for *T. brasiliensis* exceed those for parasitic protists ($\theta_{ML} = 0.0505$, $N_e = 1.05 \times 10^7$; Hughes and Verra 2001) and coyotes ($\theta_{ML} = 0.373$, $N_e = 3.73 \times 10^6$; Vilá *et al.* 1999). I have found in the literature only two species with estimates of θ_{ML} exceeding that for *T. b. mexicana*; they are nematode parasites of livestock, *Mazamastrongylus ocoidei* ($\theta_{ML} = 2.23 \pm 0.32$) and *Teladorsagia circumtincta* ($\theta_{ML} = 7.09 \pm 0.87$) (Mes 2003). Despite the high estimates of genetic diversity, maximum likelihood estimates of N_e for these nematodes are less than that for *T. b. mexicana*, possibly due to differences in mutation rates. Although θ_{ML} for *Anopheles dirus* ($= 0.757$) is less than that estimated for *T. b. mexicana*, its maximum likelihood estimate of N_e exceeds that for *T. b. mexicana*, possibly due to its shorter generation time or to differences in mutation rates. Thus, I find that *T. b. mexicana* has the highest effective population size measured for any mammal, with $N_e = 28.4$ million females as estimated by θ_{ML} and $N_e = 1.2$ million females as estimated by $\theta_{\square}$. The effective population size of these bats is more similar to

those of human-associated parasite or commensal species than it is to most other mammals (Vilá *et al.* 1999; Walton *et al.* 2000; Hughes and Verra 2001; Mes 2003).

The genetic data also indicate that the effective size of this population has recently increased (Table 4.2). Other lines of evidence indicate that *T. b. mexicana* may have recently experienced a population expansion beyond what is detectable in these genetic data. Individual summer maternity colonies that number in the tens of millions are sustained by enormous populations of insect crop pest species, including the corn earworm (*Helicoverpa zea*), the tobacco budworm (*Heliothis virescens*), and the beet armyworm (*Spodoptera exigua*) (Lee and McCracken 2002). The northward migration of *T. b. mexicana*, many of which are pregnant females (Villa-R. and Cockrum 1962), coincides with the migration of these pest insects (McCracken 1996), and the bats exploit this high-energy food source during the especially energy-demanding period of pregnancy and lactation (Lee and McCracken 2002). The development and expansion of agriculture in Mexico and the southwestern United States, especially the development of mechanized large-scale agriculture in the recent past, may have promoted growth of the *T. b. mexicana* population through increased resource availability in the form of crop pest species. Additionally, northward geographic expansion of these bats has been documented over the last century, and may reflect a response to changing climatic conditions (Genoways *et al.* 2000).

Despite the large census and effective population sizes that characterize this species, and the genetic signal indicating recent population growth,

significant declines have been documented at several colonies (Cockrum 1970; Altenbach *et al.* 1979; McCracken 1986; Wahl 1993; McCracken in press). These declines have been variously attributed to human alteration of cave environments or deliberate vandalism. Due in part to its low rate of reproduction, long lifespan, and habit of forming very large aggregations in relatively few places, *T. brasiliensis* is classified by the International Union for the Conservation of Nature (IUCN) as near threatened (IUCN 2003), making it, probably, the most numerous species of conservation concern.

V. CONCLUSIONS AND DIRECTIONS FOR FUTURE RESEARCH

1. Conclusions

Part II

- Mitochondrial DNA sequence data show no genetic structure among groups of *T. b. mexicana* that follow different migratory routes.
- Migratory and non-migratory populations of *T. b. mexicana* are not genetically differentiated as revealed by mtDNA sequence data.
- The conclusions of this study indicate that substantial gene flow exists between migratory groups and are consistent with results of previous allozyme studies and inferences from recoveries of banded bats.
- The lack of genetic structure among behaviorally distinct groups suggests that migratory behavior in these populations is a plastic response to environmental conditions.

Part III

- Significant genetic structure exists between populations of *T. brasiliensis* in South America and the other recognized subspecies in North and Central America. The level of divergence between these populations is comparable to that between species of other mammals. Populations may represent different species.
- Populations within South America are significantly differentiated, with the Andes Mountains acting as a barrier to gene flow.

- Mitochondrial sequence data do not support the differentiation of the three recognized subspecies (*T. b. cynocephala*, *T. b. mexicana*, and *T. b. intermedia*) in North and Central America at the subspecific level.
- Populations of putative *T. b. intermedia* from Chiapas, Mexico, and Guatemala are completely subsumed within the phylogeny of *T. b. mexicana* and should be synonymized with *T. b. mexicana*.
- Within my sampling, populations recognized as *T. b. cynocephala* consist of two major clades. However, *T. b. cynocephala* clades are not geographically structured and both clades are more closely related to clades of *T. b. mexicana* than to each other. I recommend synonymizing *T. b. mexicana* and *T. b. cynocephala* as a single subspecies, *T. b. cynocephala*.
- The lack of long-distance seasonal migration in non-migratory colonies of *T. b. cynocephala* is not associated with strong genetic structuring among colonies.

Part IV

- The lack of genetic structure among colonies of putative *T. b. mexicana* and *T. b. intermedia* indicates that these colonies form a single, large population.
- A maximum likelihood analysis of the effective size of this population at $N_e = 28.4$ million females is the largest effective population size yet estimated for any mammal. Estimated measures of genetic diversity are among the highest for any organism.

- Multiple analyses of the data support a model of demographic expansion for this population of *T. brasiliensis*.

2. Directions for Future Research

The results of this dissertation reveal many opportunities for further study of genetic structuring in a highly vagile, migratory species. In part 3, I found differentiation between populations of *T. brasiliensis* from North and Central America versus South America that is comparable to the sequence divergence found between species of other mammals. Due to the absence of samples from northern South America and most of Central America, the data are not informative as to whether the observed differentiation of gene pools is due to the existence of these populations as separate species or to genetic isolation-by-distance. Data from this unsampled region are needed to test whether the North and South American populations are linked by indirect gene flow through populations in intermediate regions. Additionally, analyses of populations within South America indicate the presence of significant genetic structuring. Populations of *T. b. brasiliensis* may exist as separate gene pools and perhaps separate taxa. More complete sampling of South American populations is needed.

Conclusions from mitochondrial data apply only to females. This limitation is of particular importance when males and females differ in their behavior, as has been documented for migratory populations of *T. b. mexicana* (Villa-R.

1956). Studies of genetic structure in males versus females are needed using nuclear DNA, especially Y-chromosome markers, which would serve to delineate the population genetic effects of gender-biased dispersal.

The existence of distinctive mtDNA clades of *T. b. cynocephala* that exist without geographic structure raise questions regarding the phylogeographic history of these bats that demand further study. These clades may be the consequence of separate introductions to North America from South America. *Tadarida* may have expanded its range northward through Central America, or through the Antilles, or in both directions. Depending on the direction and timing of its range expansion, there may be zones of secondary contact in the Antilles, in Central America, or elsewhere (Owen *et al.* 1990). Five additional subspecies of *T. brasiliensis* are endemic to islands in the Caribbean. By combining data from this study with additional sampling from Antillean subspecies, these hypotheses concerning patterns and timing of geographic radiation might be tested.

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VITA

Amy Louise Russell was born on March 9, 1973, in Lancaster, Ohio. She attended East and Chief Tarhe Elementary Schools, General Sherman Junior High School, and Stanbery Freshman School in Lancaster, and graduated from Lancaster High School in 1990. She graduated in 1994 from Bowling Green State University in Bowling Green, Ohio, with a B.S. in Biology and Environmental Science. In 1997, Amy received a M.S. in Biology from Bowling Green State University, completing a thesis entitled “The genetics and evolution of the *mariner* transposable DNA element in natural populations of *Drosophila simulans*”.

Amy is currently pursuing a Ph.D. in Ecology and Evolutionary Biology at the University of Tennessee in Knoxville. She will begin a Gaylord Donnelley Environmental Fellowship for postdoctoral studies at Yale University in 2003.