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COMPARISON OF PHYLOGENIES DERIVED FROM MULTIPLE LINKAGE GROUPS: A TEST OF CHROMOSOMAL SPECIATION IN *RHOGEESSA*

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by

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Dedication

To my husband John, for giving me love, encouragement, and inspiration.

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COMPARISON OF PHYLOGENIES DERIVED FROM MULTIPLE LINKAGE GROUPS: A TEST OF CHROMOSOMAL SPECIATION IN *RHOGEESSA*

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Chromosomal rearrangements have been hypothesized to be the cause of reproductive isolation leading to speciation in diverse taxa. One model for chromosomal speciation, speciation by monobrachial centric fusions, is thought to apply to various groups of mammals, including members of the bat genus *Rhogeessa* (Chiroptera: Vespertilionidae). Specifically, this model has been proposed to account for diversification within the *R. tumida* species complex. This species group exhibits a high degree of karyotypic variation, with little to no morphological differentiation between species.

By examining phylogenetic data derived from DNA sequences of maternal, paternal and bi-parentally inherited markers, I investigate phylogenetic relationships of species within *Rhogeessa* and test expectations of the model of speciation by monobrachial centric fusions on members of the *R. tumida* complex. If chromosomal fusions caused speciation in *Rhogeessa*, I expect to see patterns of reproductive isolation

between species differing by monobrachial fusions, and therefore each chromosomal form should be a monophyletic group. My data generally follow this pattern, with the exception of potential evidence for historical hybridization between *R. tumida* (2n = 34) and *R. aeneus* (2n = 32), where none is expected under the model. There is no evidence, however, of ongoing or recent hybridization between any taxa differing karyotypically. Moreover, the speciation model predicts that all populations which contain the same set of chromosomal fusions should freely interbreed, if chromosomal rearrangements are the sole cause for reproductive isolation. My data also show an exception to this prediction based on the observation of multiple genetic lineages of karyotypically identical *R. tumida* (2n = 34). This observation indicates that chromosomal differences cannot account for genetic diversification between the different lineages of *R. tumida*. Phylogeographic analyses indicate that lineages within this species could have diverged due to differences in habitat preferences.

Overall, these data are generally consistent with speciation having occurred via reproductive isolation caused by chromosomal fusions. However, it does not appear that these rearrangements have caused complete reproductive isolation due to the evidence consistent with historical hybridization between *Rhogeessa tumida* and *R. aeneus*. The chromosomal mechanism is also not likely to be the only means by which diversification has taken place in *Rhogeessa*. Geographic factors have apparently influenced genetic divergence as well.

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Chapter 1: Introduction

Chromosomal changes are thought to play a major role in the speciation process across a wide variety of taxa (King 1993). A broad spectrum of different chromosomal changes has been hypothesized to lead to differentiation between populations, with some types of changes being more common in certain taxonomic groups than others. Recent work has verified that chromosomes can play an active role in creating reproductive isolation (Delneri et al. 2003). However, the extent of chromosomal speciation has been widely debated in the literature. Although some authors (e.g., King 1993; White 1968) believe chromosomal rearrangements are involved in the majority of speciation events, others (e.g., Coyne and Orr 2004; Futuyma and Mayer 1980) believe that chromosomal rearrangements generally become fixed only after some other event (for example, allopatry) has created an isolating barrier and the speciation process is already complete. Though not easily studied, understanding the role of chromosomal rearrangements as reproductive isolating mechanisms is critical to a complete understanding of the process of speciation.

In any given taxonomic group, even if chromosomal rearrangements are involved in creating reproductive isolation between some individuals, they are likely not the only factors involved in speciation. Though many studies have focused on either exogenous forces, such as habitat differences or historical geological events, or endogenous factors, such as chromosomal rearrangements, research evaluating the influence of both factors is rare. My dissertation examines the roles of both chromosomal rearrangements and biogeographic factors on speciation and diversification in the bat genus *Rhogeessa*.

CHROMOSOMAL MODEL

One of the most frequently observed types of chromosomal rearrangements in mammals is the centric fusion (also known as a Robertsonian fusion). In this case, two acrocentric (single-armed) chromosomes fuse at the centromere to form one larger, metacentric (bi-armed) chromosome. In the hypothesized mechanism of speciation by monobrachial centric fusions (Baker and Bickham, 1986), reproductive isolation occurs between two different populations fixed for different centric fusions involving only one common acrocentric in the fused pair (i.e., the two populations have monobrachial differences between pairs of biarmed chromosomes).

In the most simplified example of this mechanism, consider an original population containing three pairs (2n = 6, meaning there are six chromosomes in a diploid cell) of acrocentric chromosomes (Figure 1.1; modified from Baker and Bickham 1986). In population A, chromosomes 1 and 2 fuse resulting in 2n = 4 with one biarmed and one acrocentric pair. Hybrids between population A and the ancestral population are fertile because an F1 (2n = 5) will have one trivalent and one bivalent in metaphase I. The trivalent will orient on the spindle so that the biarmed chromosome goes to one pole and the two acrocentrics go to the other yielding balanced gametes. In population B, chromosomes 1 and 3 fuse (2n = 4; again, this population will form fertile hybrids with the ancestral population as described above). However, if populations A and B were to hybridize (here, A and B have monobrachial differences in their karyotypes) the hybrids would not be fertile due to the formation of a multivalent chain in metaphase I comprised of an acrocentric 2, a biarmed 1-2, a biarmed 1-3, and an acrocentric 3. Because of the inability of the chain to twist into a conformation that will ensure alternate disjunction, a high percentage of unbalanced gametes will be produced.

Based on this simplified scenario, several expectations regarding population interbreeding can be made: 1) a population with no fusions will be able to interbreed with any population containing fusions of some chromosome arms; 2) populations sharing exactly the same fusions can interbreed; 3) a population with unique fusions will be reproductively isolated from any populations having monobrachial differences from those fusions. Moreover, Baker and Bickham (1986) proposed that just a single monobrachial difference (e.g. between karyotypes of populations A and B in Figure 1.1) would be sufficient to result in complete reproductive isolation between the derived forms. These expectations of interbreeding should also influence observed phylogenetic patterns if sufficient time has elapsed since the speciation event for reciprocal monophyly to be reached. If the above expectations are met, one would expect that populations with monobrachial differences between their karyotypes should be distinct genetic lineages. Also, each different chromosomal form should be a monophyletic group because individuals sharing that karyotype should freely intebreed.

Speciation by monobrachial centric fusions is thought to have occurred in several mammalian taxa, such as *Rattus* (Baverstock et al. 1983; Baverstock et al. 1986), *Castor* (Ward et al. 1991), the *Sorex araneus* species group (Searle 1998), and the European house mouse, *Mus domesticus*. This phenomenon has been most extensively studied in *M. domesticus*, where breeding experiments have demonstrated that centric fusions result in reproductive isolation (Capanna et al. 1976; Gropp and Winking 1981; White et al. 1978). The various chromosomal races of *M. domesticus* are distributed throughout most of Europe and parts of Northern Africa and have appeared within approximately the last 10,000 years (Nachman et al. 1994). New rearrangements are still arising both in nature and in laboratory strains.

Mus domesticus provides a unique study system due to the recent appearance of karyotypic differences. Because these changes took place very recently, a lot of information about the history of these populations is available. It is well-documented that the karyotypically rearranged populations arose from a standard karyotype (the same as the *M. musculus* karyotype) of all acrocentric chromosomes (2n = 40; Corti et al. 1986; Capanna and Castiglia 2004; Nachman and Searle 1995; Hauffe et al. 2004). Many karyotypically unique populations of *M. domesticus* have been documented with contact zones identified both between populations containing monobrachial differences and between the ancestral karyotype and rearranged karyotypes.

The large number of contact zones between house mice having karyotypes differing by monobrachial fusions has allowed researchers to study their effects on reproductive isolation in natural populations. Britton-Davidian et al. (2002) performed chromosome and allozyme analyses along a contact zone between two populations in France having monobrachial differences between their karyotypes. They showed that allozyme diversity was not structured according to chromosomal variability. They also reported that at the center of the contact zone there is a high frequency of mice carrying acrocentric chromosomes. These results contradict the expectations of the model of speciation by monobrachial centric fusions. Under the model, allozyme diversity should be expected to be structured according to chromosomal differences if chromosomal changes are creating a barrier to gene flow. The discovery of acrocentrics at the center of the contact zone implies that gene flow is occurs between the populations via the acrocentric ancestral karyotypic form.

Mitochondrial DNA (mtDNA) showed individuals from either side of a contact zone between monobrachially differentiated populations in Italy to be genetically differentiated (Castiglia et al. 2002). These authors found evidence for only two

instances of hybridization between karyotypic forms through chromosomal analyses. The high level of genetic differentiation observed implies a high degree of reproductive isolation between the two populations. That different populations of *Mus* show different patterns of reproductive isolation and gene flow demonstrates that the expectations of the model of speciation by monobrachial centric fusions, while sometimes validated, may not always apply.

Microsatellite data were examined in shrews (*Sorex araneus* group) to test whether chromosomal rearrangements were the ultimate cause of reproductive isolation between monobrachially differentiated species (Basset et al 2006). Microsatellite loci were mapped to the chromosome arm on which they were located. The results showed a higher level of genetic structure in microsatellites mapped to rearranged arms compared to loci on arms in common between the species. These data demonstrate that the chromosomal rearrangements themselves affect reproductive isolation between species.

STUDY SYSTEM

Another group for which monobrachial centric fusions have been proposed as a potential cause of speciation is the bat genus *Rhogeessa* (Baker et al. 1985). *Rhogeessa* is a neotropical genus of bats in the family Vespertilionidae unusual with respect to other vespertilionids in several ways. First, there is an unusually high degree of karyotypic differentiation among taxa within the genus. Whereas most neotropical vespertilionid genera have one or two different karyotypes (Bickham 1979b), each species of *Rhogeessa* has a unique karyotype (Baker et al. 1985). Second, *Rhogeessa* has an unusual distribution pattern, where most species have relatively small, parapatric ranges. The usual pattern for bats in the neotropics is widespread, overlapping distributions (see

Nowack 1994). Third, *Rhogeessa* has a high species diversity atypical of neotropical vespertilionids.

Based on G-banding patterns (Bickham and Baker 1977), all of the different chromosomal forms (species) of *Rhogeessa* differ from each other only by centric fusions or fissions. From previous studies, it is clear that the chromosomal rearrangements of *Rhogeessa* likely occurred much longer ago than those in *Mus*. This observation, along with the very different reproductive biology of these two groups (Baker et al. 1985), makes *Rhogeessa* an excellent study system with which to contrast the results of molecular studies of *Mus domesticus*, a group currently undergoing diversification via potentially the same mechanism.

The taxonomy of the named forms of *Rhogeessa* has undergone extensive changes since the genus was first described. Prior to my study, *Rhogeessa* contained ten recognized species (Nowak 1994; Genoways and Baker 1996). Five of these species make up what I will refer to as the "*R. tumida* complex," which is the group for which the hypothesis of chromosomal speciation was proposed. The *R. tumida* complex is composed of species that were all historically classified as *Rhogeessa tumida*, but based on karyotypic differences were subsequently described as distinct species. They are morphologically indistinguishable (LaVal 1973) but vary in their karyotypes by different sets of centric fusions (Baker et al. 1985; Bickham and Baker 1977). Members of the *R. tumida* complex include: *R. tumida* (2n = 34), *R. genowaysi* (2n = 42), *R. aeneus* (2n = 32), *R. io* (2n = 30) and *R. hussoni* (2n = 52; Bickham and Baker 1977; Genoways and Baker 1996). In Chapter 1, I recognize *R. velilla* (2n = 42; identical karyotype to *R. genowaysi*) as a member of the *R. tumida* complex as well. The full list of *Rhogeessa* species names, distributions and diploid numbers is given in Table 1.1.

In previous studies, the karyotypes of all of the members of the *Rhogeessa tumida* complex (except *R. hussoni*) were banded and chromosomal arms involved in fusions were identified (Bickham and Baker 1977; Baker et al. 1985). From these chromosomal banding data it has been demonstrated that, with the exception of the 2n = 42 form, all karyotypes have monobrachial differences between them. The 2n = 42 form contains fused chromosomes, but does not have monobrachial differences to any other karyotype present in the *R. tumida* complex. Therefore, under the model of speciation by monobrachial centric fusions, it should be expected that all karyotypic forms, except possibly the 2n = 42 form, should be reproductively isolated from one another. It is important to note that banded karyotypes confirm that no hybrids between any *Rhogeessa* species have ever been collected (Bickham and Baker 1977; Baker 1984).

Prior to my study, the relationships among *Rhogeessa* species were poorly known. Baker et al. (1985) presented a phylogeny based upon their karyotypic analysis of several species; however, recent molecular work has shown that the genus they hypothesized as the closest relative of *Rhogeessa* is actually quite distant (Hoofer and Van Den Bussche 2003). Therefore, the hypothesis of relationships in Baker et al. (1985) is in doubt. Baker et al. (1985) also presented a phylogeny based on an allozyme study but were unable to resolve many of the relationships. The only molecular work to have been done on multiple *Rhogeessa* species was included in a broader study of Vespertilionidae phylogenetics (Hoofer and Van Den Bussche 2003). These authors only included two individuals from the *R. tumida* complex (*R. tumida* and *R. aeneus*), and these formed a monophyletic group within the remaining species of *Rhogeessa* sampled.

STUDY DESIGN

Whereas several authors have studied chromosomal speciation by examining patterns of gene flow between hybrid zones on the population genetic level (see above), my study examines this phenomenon in a phylogenetic framework. Such an approach will allow me to have a complete picture of the species' evolutionary history in order potentially to detect not only contemporary but historical hybridization events. Additionally, this approach will allow a better understanding of species relationships within *Rhogeessa* which are poorly understood. Previous work on morphology (LaVal 1973), chromosomes and allozymes (Baker et al. 1985) could not resolve many relationships within this group. Therefore, it is imperative to have a robust phylogeny with which to test the chromosomal model of speciation in *Rhogeessa*. A phylogenetic approach to testing the expectations of the chromosomal speciation model is the best technique when the speciation events happened in the distant past and chromosomal rearrangements are not ongoing, as in the case of *Mus*.

In Chapter 2 I examine the evolutionary history of *Rhogeessa* by studying mitochondrial DNA (mtDNA) sequences. As mentioned above, the model of speciation by monobrachial centric fusions results in several predictions about the extent of interbreeding that may occur between particular chromosomal forms. If sufficient time has elapsed since fixation of different chromosomal forms, under this model, one would expect species of *Rhogeessa* that share monobrachial homologies to be distinct genetic lineages due to the absence of gene flow. Moreover, each chromosomal form should be monophyletic due to the fact that individuals sharing the same chromosomal form should not be reproductively isolated from one another if this chromosomal model is the sole cause of speciation.

The results of the mitochondrial phylogenetic analyses form the basis for understanding the genetic relationships of *Rhogeessa* species. My results show evidence consistent with hybridization or lineage sorting between chromosomal forms (the 2n = 32 *R. aeneus* and 2n = 34 *R. tumida* karyotypes) where no interbreeding should be expected under the speciation model. However, because mtDNA is inherited maternally, it is limited in its ability to allow us to estimate the amount of gene flow that may have occurred either recently or historically between different chromosomal forms of *Rhogeessa*. Therefore, in order to test the predictions fully regarding expectations of interbreeding or reproductive isolation under the model of speciation by monobrachial centric fusions, I also examine nuclear bi-parentally inherited markers.

The mtDNA phylogeny also shows evidence for multiple lineages of the 2n = 34 karyotypic form, currently recognized as a single species, *R. tumida*. I also find evidence that a population of 2n = 42 individuals in Ecuador is karyotypically identical, yet genetically distinct from the 2n = 42 species in Chiapas Mexico, *R. genowaysi*. In this chapter I recognize the Ecuadorian population by its available name, *R. velilla*.

The mtDNA phylogeny also shows evidence for the same karyotypic form independently evolving more than once. This observation suggests that there may either be selection for the same chromosomal rearrangements or there are limited stable chromosomal arrangements available. Random processes such as drift or lineage sorting explaining this pattern seem unlikely. I intend to explore this phenomenon in more detail in future studies.

In Chapter 3, I present nuclear sequence data from a paternally inherited Y-chromosomal gene, as well as a bi-parentally inherited autosomal gene. When viewed in combination with the maternally inherited mtDNA sequence data presented in Chapter 2, these data form a robust picture of the species history of *Rhogeessa*. Each dataset shows

some evidence of potential historical hybridization and/or lineage sorting between the 2n = 34 *R. tumida* and the 2n = 32 *R. aeneus*. These two species have monobrachial differences between their karyotypes and thus are expected to be reproductively isolated from one another according to the speciation model. Additionally, they each show the 2n = 34 form to not be monophyletic, indicating that perhaps the 2n = 34 form is not a single species.

My data from all three genetic loci show phylogenetic patterns that are generally consistent with expectations of the speciation by the monobrachial centric fusions model. With the possible exception of historical hybridization between *R. tumida* and *R. aeneus*, they show reproductive isolation between chromosomal forms differing by monobrachial fusions. By looking at the history of these species in a phylogenetic framework, I can refute the possibility that there is extensive, ongoing, and recent hybridization between karyotypic forms. However, because the phylogenetic patterns expected to result from ancient hybridization and lineage sorting are similar, it is not possible to refute one hypothesis in favor of the other based on my data. Furthermore, the expectation that all individuals with the same fusions should interbreed freely is not met in the case of *R. tumida*. These observations lead me to conclude that while these data are consistent with the chromosomal speciation model, it is likely that this is not the only mechanism that has created speciation and diversification in *Rhogeessa*. Additional phylogeographic analyses conducted in this chapter indicate that genetic diversity between different 2n = 34 lineages can be explained by habitat differences. These results suggest that chromosomal and geographic factors together may have played a significant role in creating diversification in *Rhogeessa*.

My study is a novel test of the speciation by monobrachial centric fusions model. This approach has allowed me to demonstrate conclusively that in most cases the

expectations of the model are met in *Rhogeessa*. My results in combination with previous studies on European *Mus domesticus* and the *Sorex araneus* complex show that mammals as diverse as bats, mice, and shrews, despite their very different reproductive biology and behavior, have undergone the same method of reproductive isolation through chromosomal rearrangements. These different taxa have speciated on different time scales, with *Rhogeessa* being an example of historical chromosomal speciation and *Mus* showing contemporary diversification due to chromosomal changes. These studies suggest that, as hypothesized by King (1993), White (1968), and others, chromosomal rearrangements are a powerful force causing speciation in many different taxa.

Chapter 2: Evolutionary history of the genus *Rhogeessa* as revealed by mitochondrial DNA sequences

ABSTRACT

Evolutionary relationships among bats of the genus *Rhogeessa* (Chiroptera: Vespertilionidae) are poorly understood because of the morphological similarity of many of the species and the limited phylogenetic resolution using karyotypes and allozymes in previous studies. Previous karyotypic studies reported several populations that differ by Robertsonian centric fusions which led to a proposed mechanism of speciation called speciation by monobrachial centric fusions. In this chapter I present a molecular phylogenetic analysis of 90 individuals representing eight of the ten currently recognized species of *Rhogeessa* using the mitochondrial DNA gene cytochrome-*b* as well as some new karyotypic data. The molecular results are generally consistent with speciation by monobrachial centric fusions because karyotypically distinct populations typically comprise monophyletic maternal lineages. One exception was two individuals that were possible hybrids between *R. tumida* (2n = 34) and *R. aeneus* (2n = 32). Unexpectedly, I also found ostensible species level differentiation among three karyotypically identical (2n = 34) but geographically separated populations of *R. tumida*. Similarly, new karyotypic data show a population from western Ecuador to have 2n = 42 and molecular data shows it to be phylogenetically distinct from both the karyotypically identical *R. genowaysi* from Mexico and the South American *R. io* (2n = 30) to which it was previously allocated. I recognize this population by its available name, *R. velilla*. I also

found an unexpectedly close relationship between *Baeodon alleni* and *R. gracilis* and tentatively recommend these both be considered as species of *Baeodon*.

INTRODUCTION

The genus *Rhogeessa* (Chiroptera: Vespertilionidae) exhibits unusual karyotypic diversity in comparison to other New World vespertilionid bats (Bickham 1979b). Karyotypic diversity in *Rhogeessa* is characterized by species having unique sets of chromosomal fusions. Previously, these fusion events were proposed to be the cause of speciation within the *R. tumida* species group (Baker et al. 1985), a hypothesis known as speciation by monobrachial centric fusions (Baker and Bickham 1986). This speciation model states that centric fusions (a common form of chromosomal rearrangement in mammals) are not *per se* an effective isolating mechanism. However, if two populations become fixed for biarmed chromosomes that differ by having one arm in common but not the other (monobrachial differences), they will be reproductively isolated from each other because of the failure of meiosis in hybrids. In the hybrids, complex chains or rings of biarmed chromosomes differing by monobrachial centric fusions are formed in the first meiotic division. The chromosomes that comprise these multivalents fail to assort properly, which causes sterility and results in virtually instantaneous speciation. Under this model, populations differing by monobrachial rearrangements are expected to be reproductively isolated from one another, while those having no monobrachial rearrangements should be capable of interbreeding.

Currently, there are ten recognized species of *Rhogeessa* (Table 1.1), five of which belong to the *R. tumida* species complex (*R. tumida*, 2n = 34; *R. aeneus*, 2n = 32;

R. io, 2n = 30; *R. genowaysi*, 2n = 42; and *R. hussoni*, 2n = 52; Bickham and Baker 1977; Genoways and Baker 1996). Members of the *R. tumida* complex historically were considered to be conspecific because of their morphological similarities (LaVal 1973). The remaining species, all of which are morphologically distinguishable (Laval 1973), are: *R. parvula*, 2n = 44 (Bickham and Baker 1977); *R. alleni*, 2n = 30 (Volleth and Heller 1994; Volleth et al. 2006); *R. gracilis*, 2n = 30 (this study); *R. minutilla*, 2n unknown; and *R. mira*, 2n unknown. Of the ten *Rhogeessa* species, three occur in South America and the remainder in Mexico and Central America (Fig. 2.1; Table 1.1). Members of this genus exhibit unusually small, parapatric ranges, whereas most other New World vespertilionids have large, overlapping distributions. The descriptions of many *Rhogeessa* species are based on karyotypic differences rather than morphological differences. This contrasts with most vespertilionid genera which are comprised of morphologically distinct species having little or no chromosomal variability (Bickham 1979b).

Baker et al. (1985) showed that members of the *Rhogeessa tumida* complex differ in their karyotypes through a series of centric fusions (although they did not examine *R. hussoni*). *R. tumida*, *R. aeneus*, and *R. io* all have monobrachial differences from one another and therefore would be expected to be reproductively isolated from one another under the model of speciation by monobrachial centric fusions (Baker and Bickham 1986). In contrast, *R. genowaysi* has a different set of fusions relative to these species, but no monobrachial differences from them (and may be capable of interbreeding with any of those species according to the model). Based on these karyotypic observations, it should be expected that *R. tumida*, *R. aeneus* and *R. io* represent distinct genetic lineages.

Although the current taxonomic status of most *Rhogeessa* species is currently uncontroversial, the status of *R. alleni* has been viewed differently by various authors. Corbet and Hill (1991) and Duff and Larson (2004) placed it in a separate genus, *Baeodon*, whereas Honacki et al. (1982), Jones et al. (1988), Hall (1981), and LaVal (1973) considered it to be a member of *Rhogeessa*. Hoofer and Van Den Bussche (2003) placed it in the genus *Baeodon*, sister to *Rhogeessa,* based on molecular data. The karyotype of *R. alleni* was reported by Volleth and Heller (1994; see also Volleth et al. 2006). I have included *R. alleni* in this study of *Rhogeessa* for further study of its generic placement.

Very little molecular work has been done showing the degree of divergence within *Rhogeessa*. Baker et al. (1985) showed that allozymes distinguished several of the members of the *R. tumida* complex. Hoofer and Van Den Bussche (2003) included several *Rhogeessa* species (one specimen of each) in their broader study investigating phylogenetic relationships within Vespertilionidae. Included in their study were *R. aeneus* (Belize), *R. mira* (Mexico: Michoacan), *R. parvula* (Mexico: Sonora), *R. tumida* (Honduras: Valle), and *R. (Baeodon) alleni* (Mexico: Michoacan). Their analysis of mitochondrial 12S/16S rRNA genes allowed them to distinguish each *Rhogeessa* species and supported the following topology for *Rhogeessa*: (*R. alleni*, (*R. mira*, *R. parvula*), (*R. aeneus*, *R. tumida*)). This tree does not reject the hypothesis of monophyly for the *R. tumida* complex as I have defined it.

Given its unusual karyotypic diversity, the *Rhogeessa tumida* complex is an ideal system in which to investigate the relationships between chromosomal evolution and speciation. By studying this system using molecular data, I can test previous hypotheses

of speciation within the genus, investigate chromosomal evolution in the group, and determine phylogenetic relationships among species. The goals of this chapter are to examine the relationships of as many *Rhogeessa* species as possible and determine whether different karyotypic forms represent unique species and mtDNA lineages. Additionally, I examine whether the *R. tumida* complex is a monophyletic group in which chromosomal speciation may have taken place. I also present the karyotypes of *R. gracilis* and an isolated population of *Rhogeessa* from western Ecuador.

MATERIALS AND METHODS

Sampling

I sampled tissues from 90 individuals representing of eight of the ten recognized species of *Rhogeessa* (Appendix A). Sampling covered much of the geographic range of these species, including an isolated population of putative *Rhogeessa io* from western Ecuador (Fig. 2.1; labeled as *R. velilla*). Species not represented in this study are *R. minutilla* and *R. hussoni* as well as the 2n = 32 population of *R. tumida* from Nicaragua (Baker et al., 1985). Field procedures followed guidelines approved by the University of Texas, which follow recommendations of the Animal Care and Use Committee of the American Society of Mammalogists (Gannon et al. 2007). *Plecotus auritus* (2n = 32; Genbank accession number: AY665169), *Antrozous pallidus* (2n = 46; Baker and Patton 1967) and *Bauerus dubiaquercus* (2n = 44; Engstrom and Wilson 1981) were used as outgroups in the phylogenetic analysis based on the relationships among these taxa presented by Hoofer and Van Den Bussche (2003).

DNA extraction and sequencing

DNA was extracted from frozen tissue samples using a Qiagen DNeasy kit (Qiagen). The cytochrome-*b* (cyt-*b*) gene was amplified in full using the primers LGL 765 forward (GAA AAA CCA YCG TTG TWA TTC AAC T) and LGL 766 reverse (GTT TAA TTA GAA TYT YAG CTT TGG G; Bickham et al. 1995; Bickham et al. 2004). PCR was performed using 25µl reactions of the following reagents: 2.5µl 10x buffer; 2.5µl dNTP mix; 1.25µl of a 10µM solution of each primer; 0.5µl Taq DNA polymerase; 13.5-14.5µl deionized water and 1-2µl total genomic DNA. Thermal cycle conditions consisted of initial heating at 94^{o}C for 1.5 min, then 35 cycles of denaturation at 94^{o}C for 20 s, annealing at 48-50^{o}C for 30 s, and extension at 72^{o}C for 1 min, followed by an additional 7 minutes of extension at 72^{o}C.

A single band was obtained using the primers listed above. PCR products were purified using a Viogene gel extraction kit to obtain as clean a PCR product as possible. Purified products were subsequently used in standard sequencing reactions (with the same PCR primers) using Big Dye version 3.0 (Applied Biosystems, Foster City, California). Sequences were cleaned using Sephadex spin columns and samples were analyzed on an ABI3100 automated genetic analyzer (Applied Biosystems). Raw sequence data were analyzed using DNAstar software version 2 and aligned by eye using MacClade. A total of 1140bp (1088bp of which contained no missing data across all samples) was used in the phylogenetic analysis. This fragment includes only the complete cyt-*b* gene. All flanking sequences that amplified with the PCR primers were discarded prior to phylogenetic analysis.

Phylogenetic Analysis

Modeltest version 3.06 (Posada and Crandall 1998) was used to assess the appropriate model of evolution (HKY + I + Γ) for this dataset under the Akaike Information Criterion. This model was implemented in a Bayesian analysis using MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003) which generates posterior probability distributions through a Markov Chain Monte Carlo (MCMC) process. I analyzed $3x10^6$ generations of 1 cold and 3 heated Markov Chains and discarded 100,000 burn-in generations based on fluctuating likelihood scores. GARLI version 0.951 (http://www.zo.utexas.edu/faculty/antisense/garli/Garli.html) was used to generate a maximum likelihood tree, and bootstrap values were calculated using a genetic algorithm approach. No starting tree was specified in this analysis and the same model of evolution identified by Modeltest was used for both the Bayesian and GARLI analyses. Trees were visualized using TreeView version 1.6.6 (Page 1996).

Karyotypic analysis

Mitotic spreads stained with Giemsa from one specimen of *Rhogeessa gracilis* (AK11059) were prepared in the field by L. A. Ruedas and J. C. Morales. *Rhogeessa* specimens from Ecuador also were karyotyped in the field (Baker et al. 2003) by members of the 2004 Sowell Expedition from Texas Tech University. I stained karyotypes from the latter with 4,6-diamidino-2-phenylindole (DAPI) to produce banding patterns for analysis (Fig. 2.2). DAPI banding is equivalent to traditional G-banding because it stains AT regions (Sumner 1990; Ambros and Sumner 1987) and therefore karyotypes analyzed in this manner are directly comparable to those prepared by traditional G-banding methods. DAPI bands were not obtained from the *R. gracilis*

sample because of slight degradation of the karyotypes. Banded and non-differentially stained karyotypes were photographed and arranged in pairs.

RESULTS

Phylogenetic analysis

The phylogeny resulting from the cyt-*b* sequence data (Fig. 2.3) lends support to the hypothesis of monophyly of the *tumida* complex (posterior probability = 0.99). These results also show that *R. alleni* and *R. gracilis* form a clade sister to all other *Rhogeessa* species. *Rhogeessa parvula* and *R. mira* form a clade that is sister to the *R. tumida* complex. This observation is partially consistent with some of the findings of LaVal (1973) where certain morphological characters place *R. alleni* most basal followed by *R. gracilis*, with *R. mira* and *R. parvula* being closely related to one another. My genetic data, however, suggest that *R. alleni* and *R. gracilis* are more similar to one another (0.017 K2P distance) than has been hypothesized based on morphological data (LaVal 1973).

The 2n = 34 karyotypic form, *R. tumida*, previously thought to be a single broadly distributed species, occurs as four separate lineages in Fig. 3. One lineage, composed of individuals from the Pacific versant of Mexico and Central America, is sister to *R. genowaysi*. Two lineages, one comprised of individuals from the Atlantic versant of Mexico and the other from the Atlantic versant of Central America, form a polytomy with *R. aeneus*. Lastly, there are two individuals provisionally identified as *R. tumida*, one identified based on karyotype (2n = 34 confirmed from several different karyotypic

spreads) and locality and the other based on locality alone, which occur within the *R. aeneus* (2n = 32) clade. The Pacific and Atlantic *R. tumida* lineages differ by about 10% K2P distance (Table 2.1). The two Atlantic lineages differ by 2.5%, whereas *R. aeneus* differs from the two *R. tumida* individuals within that clade by about 1%.

The 2n = 42 karyotypic forms (*R. genowaysi* from Chiapas, Mexico, and the western Ecuadorian population of putative *R. io*, labeled as *R. velilla* in Fig. 2.1) appear as separate genetic lineages on the tree. The Ecuadorian form is sister to the Atlantic *tumida*/ *R. aeneus* clade, whereas *R. genowaysi* is sister to the Pacific *R. tumida* clade.

Karyotypic Analysis

The putative specimens of *Rhogeessa io* from western Ecuador possess 2n = 42 (Fig. 2.2). Differentially stained preparations using DAPI-banding allowed us to determine the arms of the biarmed chromosomes with a high degree of certainty. These bats possess the five plesiomorphic biarmed chromosomes common to the entire *R. tumida* complex (Bickham and Baker 1977): fusions of chromosomes 23/3, 22/12, 20/18, 16/17, and 21/19 (chromosomal nomenclature following Bickham 1979a and 1979b). Thus, the 2n = 42 karyotype of the Ecuadorian population of *R. io* is identical to the 2n = 42 karyotype of *R. genowaysi*, and the two species do not differ by monobrachial rearrangements with respect to the other species in the *R. tumida* complex.

The standard karyotype for *R. gracilis* had a diploid number of 2n = 30, but I was unable to obtain adequate differentially stained preparations from this specimen.

DISCUSSION

Phylogenetic relationships

My phylogenetic analysis supports the monophyly of the *Rhogeessa tumida* complex as a whole. However, it shows that populations presently considered to be *R. tumida* do not comprise a monophyletic lineage, although evidence to date shows that they all possess 2n = 34 karyotypes with the same set of chromosomal fusions. The two lineages with 2n = 42 karyotypes (*R. genowaysi* and the putative western Ecuadorian *R. io*) appear to be separate species. Within the *tumida* complex, there are three major clades: one contains *R. aeneus*, several *R. tumida* lineages with 2n = 34 karyotypes and the Ecuadorian 2n = 42 population; the second contains Pacific *R. tumida* (2n = 34) and *R. genowaysi* (2n = 42); and the third is *R. io* with 2n = 30. The first two of these three clades contains populations with identical 2n = 42 and identical 2n = 34 karyotypes. The observation that 2n = 34 forms and 2n = 42 forms do not represent monophyletic groups could be explained in two ways: 1) the karyotypes have converged on these diploid numbers; or, 2) the ancestral population to the *R. tumida* complex contained both the 2n = 34 and 2n = 42 karyotypes which have become fixed in separate mitochondrial lineages by lineage sorting (Avise 2000) or random genetic drift. A population containing 2n = 34 and 2n = 42 karyotypes would have to contain many different intermediate karyotypes as well, assuming that there is complete interbreeding between karyotypic forms. The chance that the same two karyotypes would randomly become fixed more than once is likely miniscule, making the first scenario more probable than the second.

The major unexpected result from the phylogeny is that *Rhogeessa tumida* occurs in four different clades on the tree. Under the model of speciation by monobrachial

centric fusions, one would expect all populations of 2n = 34 to be able to interbreed and thus appear as a single monophyletic lineage, which they are not based on this mtDNA dataset. In the Atlantic clade, two individuals of *R. tumida* fall within a clade of *R. aeneus*. These two *R. tumida* samples are from the provinces of Atlantida in Honduras and Izabal in Guatemala. Other individuals from these same localities fall within the Atlantic Central America *R. tumida* clade. Samples of *R. aeneus* come from Belize, the Yucatan region of Mexico, and the Petén region of Guatemala (Fig. 2.1). Three possible explanations can account for the observed relationship between *R. aeneus* and the two *R. tumida* individuals within the *R. aeneus* clade: 1) there has been incomplete lineage sorting for the cyt-*b* gene in this group; 2) there has been hybridization between *R. aeneus* and *R. tumida* in this region; or 3) *R. aeneus* is a mixture of 2n = 32 and 2n = 34 karyotypes. *Rhogeessa aeneus* and *R. tumida* differ by monobrachial rearrangements in their karyotypes (Bickham and Baker 1977), so under the hypothesis of speciation by monobrachial centric fusions one would expect them to be reproductively isolated. Hybridization seems a likely alternative in this case because of the geographic proximity of the samples and the fact that other individuals from those localities group with other *R. tumida* from Central America. If this is true, it represents the first report of hybridization among species of *Rhogeessa* sharing monobrachial differences. Nuclear bi-parentally inherited markers must be examined to test the hypothesis of hybridization between these species. Based on cyt-*b* sequence and karyotype alone, it is not absolutely certain that these individuals are hybrids. I can rule out the possibility of them being F1 hybrids because they lack a diploid number intermediate between the 2n = 32 and 2n = 34 karyotypes (the assumed two parental lineages). However, if they are hybrids from

anything greater than an F1 generation, I would most likely be unable to distinguish them karyotypically from the parental species to which they back-crossed. Therefore, nuclear sequencing must be performed in order to determine with greater certainty whether these individuals are of hybrid origin.

The third possible explanation of the relationships observed in the *Rhogeessa aeneus* clade, that *R. aeneus* is simply a population of mixed karyotypes including both 2n = 32 and 2n = 34, seems unlikely based on the fact that no intermediate karyotypes between the two forms have ever been observed. One would assume under this scenario that interbreeding between the two forms would be common if they are a single species. In that case one would expect to see intermediate karyotypes in the population.

My analysis included one individual from Darien, Panama that was most closely related to the *Rhogeessa io* samples from Venezuela and Trinidad. This specimen showed about 4.5% sequence divergence from other *R. io* individuals (K2P distance; Table 2.1). This could be sufficient divergence to indicate that there may be a distinct species in the southern part of Central America, where sampling is sparse. The relationship of *R. io* in Panama to individuals in South America should be examined in further detail with additional sampling.

I included the same individuals of *Rhogeessa alleni* used by Hoofer and Van Den Bussche (2003; TK45023) and Volleth and Heller (1994; SMF77908; also reported in Volleth et al. 2006), both of which are sister to our *R. gracilis* sample (AK11059). Within the clade of *R. gracilis/R. alleni*, samples differ from one another by only an average of 1.5% divergence (K2P distance). The *R. alleni* sample reported in Volleth and Heller (1993) and Volleth et al. (2006) is the only individual of that species for which a

karyotype has been reported. The *R. gracilis* sample (AK11059; 2n = 30) matches that karyotype as far as can be determined. This contradicts previous findings by Baker and Patton (1967), who reported a karyotype of 2n = 44 for *R. gracilis*, although later (LaVal 1973) these specimens were reported as being "almost certainly *R. parvula*". Therefore, these results are likely the first confirmed karyotype of *R. gracilis*. I compared the *R. gracilis* (AK11059) voucher to *R. alleni* not included in this study and confirmed its identification. I have been unable to locate one of the *R. alleni* vouchers and have located, but not verified, the identity of the second voucher. Because the confirmed *R. gracilis* specimen matches the karyotype of a supposed *R. alleni* specimen (and not the previously reported *R. gracilis* karyotype, although the previous karyotype was likely from a mis-identified *R. parvula*) and because it is extremely similar genetically to the *R. alleni* samples, I am still somewhat in doubt of the accuracy of the identification of the *R. alleni* samples. I am currently in the process of obtaining additional, confirmed, *R. alleni* individuals to include in future studies of *Rhogeessa*.

The phylogenetic analyses of cyt-*b* sequences cannot be used, alone, to accept or reject the chromosomal speciation hypothesis due to the limited power of mtDNA to test for gene flow between species. However, these results do show that if speciation has occurred via this mechanism in *Rhogeessa*, it is unlikely to be the only speciation mechanism at work in this group. Whereas the chromosomal speciation model predicts monophyly of the 2n = 34 chromosomal form, the analyses suggest that populations with 2n = 34 are structured more based on geography. The mountain ranges throughout the central parts of Mexico and Central America could be a potential source of genetic isolation between these clades, which is independent of karyotypic isolation. This and

other geographic speciation hypotheses can be explored if my reported phylogenetic relationships are verified with nuclear sequence data.

Taxonomy

The current precedent for the taxonomic status of *Rhogeessa alleni* comes from Hoofer and Van Den Bussche (2003) who placed this species in *Baeodon* based primarily on genetic distance from the remaining *Rhogeessa* species they examined. My study also finds *R. alleni* to be very distant from all *Rhogeessa* species except its sister taxon, *R. gracilis*. If I follow current precedent and classify *R. alleni* as a member of the genus *Baeodon* without including *R. gracilis* in that genus as well, *Rhogeessa* would be paraphyletic. There are, therefore, two options for classifying *R. alleni*: move it back into the genus *Rhogeessa* or leave it as *Baeodon alleni* and move *R. gracilis* into the genus *Baeodon* as well. I tentatively support the latter option, but strongly recommend further study on the relationship of these two species. My support for this option is based not only on their genetic distance from the remaining *Rhogeessa* species, but also on the fact that both *R. alleni* and *R. gracilis* are highly divergent morphologically from other *Rhogeessa* (LaVal 1973).

Populations currently recognized as *Rhogeessa tumida* likely comprise at least two species and possibly three. The Pacific *R. tumida* clade is genetically distinct from the Atlantic *R. tumida* clades. The Central American and Mexican Atlantic *R. tumida* clades are less distinct but might nonetheless represent different species. I tentatively support the hypothesis of three different species of *R. tumida* based on a genetic species concept (Baker and Bradley 2006). Although the genetic distance between the Atlantic

R. tumida clades and *R. aeneus* is low, I do not support a species concept based on genetic distance alone. The main criterion for supporting a genetic species concept is the lack of evidence for interbreeding between the three different lineages of *R. tumida*. I also do not currently support the hypothesis that the two Atlantic clades of *R. tumida* belong to *R. aeneus* based on the karyotypic differences between the clades. Other than the mtDNA sequences presented here, there are no morphological, karyological, or other molecular data available to justify any changes to this species at this time.

The putative *Rhogeessa io* samples from western Ecuador and *R. genowaysi* from Chiapas, Mexico share identical 2n = 42 karyotypes but are genetically distinct and geographically separated. Based on this evidence, I do not believe them to be conspecific. The Ecuadorian samples also are distinct from *R. io* (the geographically nearest *Rhogeessa* species) both genetically and karyotypically. The name *R. velilla* is available for the Ecuadorian samples. The status of *R. velilla* has changed several times since its initial description (Thomas 1903). Goodwin (1958) treated it as a subspecies of *R. parvula*, whereas LaVal (1973) considered it as a synonym of *R. tumida*. Genoways and Baker (1996), when elevating *R. io* to specific status, noted that specimens from Ecuador are morphologically more similar to *R. minutilla* than to *R. io*; however, they did not have enough data to place these specimens into either species with certainty. I am unaware of any karyotypic data existing for *R. minutilla* that may show additional similarity to specimens from Ecuador. However, because of their geographic distance from any known *R. minutilla* samples, I believe that the Ecuadorian samples should be elevated to species level as *R. velilla* and include a formal synonymy below.

Rhogeessa velilla Thomas, 1903

Rhogeessa velilla Thomas, 1903:383. Type locality "Puná, Puná Island, Gulf of Guyaquil, [Guayas Province,] Ecuador." Holotype: adult male, British Museum of Natural History number 99.8.1.5; fluid specimen.

Rhogeessa parvula velilla: Goodwin (1958:8). Name combination.

Rhogeessa (Rhogeessa) tumida: LaVal, 1973:29. Part: specimens from Puná Island, Ecuador only.

Rhogeessa io: Genoways and Baker, 1996:84. Part: specimens from Puná Island, Ecuador only.

Geographic range.—known from type locality and Guayas Province on the mainland of Ecuador.

Description.—According to Thomas (1903), R. velilla is similar to *R. io* in size, color, and proportions, except that R. velilla lacks the marked "helmet" formed by prominent sagittal and occipital crests. The baculum of *R. velilla* is similar to that of *R. minutilla* (Genoways and Baker, 1996). The karyotype of *R. velilla* is 2n = 42, differing from any species to which it was previously allocated.

Chapter 3: Molecular phylogenetics of *Rhogeessa* based on nuclear DNA sequences: A test of speciation by monobrachial centric fusions

ABSTRACT

Several members of the genus *Rhogeessa* have previously been hypothesized to have undergone speciation via chromosomal rearrangements in a model termed speciation by monobrachial centric fusions. In Chapter 2, I showed that DNA sequence data from mitochondrial cytochrome-*b* tentatively supported this hypothesis but could not explicitly test the model's expectations with regard to interbreeding between karyotypic forms. These data also showed potential evidence for hybridization or incomplete lineage sorting between *R. tumida* and *R. aeneus* as well as multiple lineages of what is currently considered to be a single species, *R. tumida*. In this chapter I present a more comprehensive test of the model of speciation by monobrachial centric fusions in *Rhogeessa*. This analysis is based on sequence data from two nuclear loci: the paternally inherited ZFY gene and the autosomal MPI gene. These results are similar to those previously found through mtDNA. The nuclear data provide results that are consistent either with incomplete lineage sorting or ancient hybridization to explain the alleles that are shared at low frequency between *R. aeneus* and *R. tumida*. From these data I can rule out the possibility of recent and ongoing hybridization between any species. I also confirm the presence of multiple genetic lineages of 2n = 34 karyotypic forms (*R. tumida*) previously observed in the analyses of mtDNA. These results are generally consistent with a model of speciation by monobrachial centric fusions in *Rhogeessa*, although this is likely not the only mechanism for speciation that has occurred in

Rhogeessa. Phylogeographic analyses indicate that habitat differences may be responsible for isolation leading to divergence between different *R. tumida* lineages.

INTRODUCTION

Bats of the genus *Rhogeessa* (Chiroptera: Vespertilionidae), with their unusual karyotypic diversity, morphological similarity, and high species diversity represent an excellent system for studying chromosomal mechanisms of speciation. Much of the interest in this genus has focused on members of the "*R. tumida* complex," a group from which the model of speciation by monobrachial centric fusions was inspired (Baker and Bickham 1986). Members of this species complex include *R. tumida* (2n = 34), *R. aeneus* (2n = 32), *R. io* (2n = 30), *R. velilla* (2n = 42), *R. genowaysi* (2n = 42) and *R. hussoni* (2n = 52; Bickham and Baker 1977; Genoways and Baker 1996). The karyotypes of *R. tumida* complex members have undergone extensive chromosomal rearrangements (all centric fusions) which, according to the hypothesized speciation model, led to reproductive isolation between populations containing different sets of fusions. All members of this group except *R. genoways*i and *R. velilla* have karyotypes that differ monobrachially (i.e., contain different fusions that have one arm but not the other in common) from other members of the group (Baker et al. 1985; Bickham and Baker 1977).

The results from Chapter 2 showed, based on mitochondrial cytochrome-*b* (cyt-*b*) sequence data, that the *R. tumida* complex was a monophyletic group in which the relationships of many species were potentially consistent with a hypothesis of speciation

by monobrachial centric fusions. A couple of exceptions were noted: 1) two individuals of 2n = 34 *R. tumida* occurred within a clade of 2n = 32 *R. aeneus*; and 2) the 2n = 34 form was not monophyletic. According to this speciation model, individuals having karyotypes that differ by monobrachial fusions should be reproductively isolated, while those having no monobrachial differences should be able to interbreed (Baker and Bickham 1986). Therefore, under the scenario of speciation by monobrachial centric fusions, one would expect monophyly of each karyotypic form and no evidence of hybridization between different karyotypic forms exhibiting monobrachial differences from one another. With respect to the observation of two *R. tumida* individuals within a clade of *R. aeneus*, I concluded that a possible explanation for the observed phylogenetic topology could be hybridization. However, I noted that mitochondrial sequence data alone is not powerful enough to conclusively demonstrate hybridization. The results could also be accounted for by incomplete lineage sorting in the mtDNA gene sequenced (Avise 2000). Therefore, nuclear markers must be examined to differentiate between these two alternative explanations.

My study has been designed to distinguish between lineage sorting and hybridization. I previously examined phylogenetic patterns from maternally inherited markers (Chapter 2), and will present here data from paternally and bi-parentally inherited markers. These three linkage groups should all have different lineage sorting periods due to their different modes of inheritance and effective population sizes (Chesser and Baker 1996; Moore 1995). Although not much is known about the behavior of *Rhogeessa*, other bats (including vespertilionids) have been shown to exhibit female philopatry and male-biased dispersal (Weyandt et al. 2005, Kerth et al. 2000; Wilkinson

1985). If one assumes the same happens in *Rhogeessa*, Y-chromosomal markers should have the shortest lineage sorting period, followed by mtDNA, and finally autosomal markers (Hoelzer 1997; Moore 1997). Therefore, Y-chromosomal markers should follow the "true" species phylogeny more closely that do the other markers. Furthermore, lineage sorting is not expected to produce congruent patterns across individual loci, other than those that are also consistent with species phylogeny.

Although they are now more widely available than in the past, Y-chromosomal and autosomal sequence data are still used relatively infrequently in combination with mtDNA to investigate species relationships (Tosi et al. 2003). The vast majority of studies that do use autosomal sequences to construct phylogenies use the consensus sequence of the two alleles (i.e., a single sequence containing ambiguous bases to represent two different alleles at once) in phylogenetic analyses. This practice can be problematic in accurate phylogenetic reconstruction, and does not clearly depict hybridization events (Bradley et al. 1993; Holloway et al. 2006). In my study, which seeks to test explicitly for hybridization and reproductive isolation, I use individual allele sequences to understand these processes better. I also selected nuclear loci that, in combination with the maternally-inherited mtDNA data already obtained (Chapter 2), will give a more complete picture of the evolutionary history of the group. Included in this study are a paternally inherited Y-chromosome gene (Zinc Finger Y; ZFY) and a bi-parentally inherited autosomal gene (manose-6-phosphate isomerase; MPI).

The autosomal locus (MPI) was previously used among other loci in an allozyme study of *Rhogeessa* species (Baker et al. 1985). All together, the allozyme data did not fully resolve the phylogenetic relationships of *Rhogeessa*, but they did succeed in

grouping members of the *R. tumida* complex as monophyletic. The MPI locus itself showed unique alleles for each karyotypic form of *Rhogeessa*, with the exception that the 2n = 34 form had one allele identical to the allele fixed in the 2n = 30 form. The only two species to have multiple alleles at the MPI locus were *R. genowaysi* (2n = 42; two alleles) and *R. tumida* (2n = 34; four alleles). Sequencing this allozyme locus should yield more characters with which to resolve the relationships between species.

The observation from Chapter 2 that the 2n = 34 form does not constitute a monophyletic group could imply that this is not a single species, as it is currently classified. LaVal (1973) studied the morphology of *Rhogeessa tumida* from throughout its range. He noted variation in several morphological characters but found no clear delineations along which to break this species up based on these differences. In fact, based on morphology, he still considered *R. io*, *R. velilla*, *R. aeneu*s and *R. genowaysi* all to be *R. tumida*. Bickham and Baker (1977) and Baker et al. (1985) studied karyotypes of the 2n = 34 form from throughout its range and consistently observed the same sets of centric fusions composing the 2n =34 karyotype. Baker et al. (1985) also studied allozyme variation in *Rhogeessa*. Based on these data they did not note any evidence to support splitting *R. tumida* into multiple species. Therefore, only DNA sequence data have supported the possibility of multiple species within the 2n =34 karyotypic form. In this chapter I intend to further investigate the possibility of multiple species within the 2n = 34 karyotypic form.

Due to their karyotypes being identical, it is unlikely that chromosomes played a role in creating diversification between the different lineages of *R. tumida* (2n = 34). The two Atlantic lineages differ by 2.5% K2P distance in cyt-*b* and the Pacific lineage differs

from the Atlantic lineages by about 10% (Chapter 2). Two major geologic events correlate to current ranges of this species and have been shown to create phylogeographic structure in other species. The older of the two is the uplift of the various mountain ranges in Mexico and Central America. Because of the deeper split between the Pacific and Atlantic lineages, and the fact that their ranges roughly correspond to either side of these mountains, this vicariance hypothesis seems reasonable. Second, a seaway may have existed at the Isthmus of Tehuantepec during the Pliocene-Pleistocene (Mulcahy et al. 2006). This dates to roughly 2.5 mya, and may better correspond to the divergence of the two Atlantic lineages. In this chapter I test whether these geologic events, as well as habitat differences, may have contributed to diversification in different lineages of *R. tumida*.

Recently, Baker and Bradley (2006) reviewed the importance of genetic data in describing species of mammals. They viewed these data in the context of the Genetic Species Concept, whereby a species is defined as "a group of genetically compatible interbreeding natural populations that is genetically isolated from other such groups" (Baker and Bradley 2006). Based on this species concept, one should be able to distinguish species using genetic data that allows for discriminating between interbreeding populations. This is critical in species groups such as the *Rhogeessa tumida* complex where there is little morphological variation and behavioral data are unknown. It is in this context that I examine additional genetic data from *Rhogeessa* in order to test hypotheses of species boundaries.

The goals of this chapter include further examination of the phylogenetic relationships among *Rhogeessa* species using nuclear DNA sequence markers.

Specifically, I test the hypothesis of relationships from mtDNA data presented in Chapter 2 and use nuclear data to examine the potential evidence for hybridization between *R. tumida* and *R. aeneus*. I also explicitly test the expectations of the speciation by monobrachial centric fusions model regarding interbreeding between karyotypic forms outlined above. Nuclear sequence data will allow me a better understanding of whether there is gene flow between karyotypic forms where none would be expected under the model. Additionally, I test alternative geographic hypotheses to explain the divergence between the different genetic lineages of *R. tumida* (2n = 34).

METHODS

Taxon Sampling

A total of 31 male *Rhogeessa* were sequenced for the ZFY dataset and 63 *Rhogeessa* for MPI (Appendix A). All samples were taken either from frozen museum tissue or tissues from animals captured in Guatemala. A map of sampling localities is shown in Fig 3.1. For the ZFY dataset, *Myotis tricolor* and *Bauerus dubiaquercus* were used as outgroups. For the MPI dataset, I compared (using BLAST; Altschul et al. 1990) a *Rhogeessa* MPI sequence against the *Myotis lucifugus* genome sequence in GenBank and used the matching sequence as an outgroup in addition to the sequence from *B. dubiaquercus*. All *Rhogeessa* species represented in Chapter 2 are included in the ZFY dataset, excluding *R. genowaysi* for which I only have a single female specimen. *R. parvula* and *R. alleni* were excluded from the MPI dataset because of my inability to amplify clean samples of this gene in these taxa.

DNA Extraction and Sequencing

DNA was extracted from frozen tissue samples using a Qiagen DNEasy Kit (Qiagen). For the ZFY gene, DNA was amplified using the primers LGL335F (5' AGACCTGATTCCAGACAGTACCA 3') and LGL331R (5' CAAATCATGCAAGGATAGAC 3'; Cathey et al. 1998). The resulting amplification was not chromosome-specific, thus resulting in the homologous region being amplified from both the X and Y chromosomes. The amplified fragment corresponded to the last intron in the ZFY cistron with some exon sequence flanking each side. These products were purified using a Viogene Gel Extraction Kit. Purified products were then amplified with the primers Las335YF (5' CCAAACAGGTGAGGGCACATA 3') and LGL331R (same as above) to obtain a Y-specific fragment. This fragment was then sequenced with the Las335YF primer.

For the MPI gene, DNA was amplified using the primers MPIEX4F (5' TGCCAACCACAAGCCAGARATGG 3') and MPIEX5R (5' GGGAGATCCGYTTCACCAACAGG 3'). The resulting amplification contained the 3' end of MPI exon 4 and the 5' end of exon 5, with an intron in between. These products were cleaned using the same methods described above. An initial sequencing reaction was performed using the same primers as in the PCR reactions. Because *Rhogeessa* are diploid for the MPI locus, this sequencing step results in a consensus of the two alleles. In the case of heterozygous individuals, polymorphic sites were identified by a double peak in the initial sequencing step, and allele-specific primers were then designed (Fig. 3.2). A list of all allele-specific primers used in this study is given in Table 3.1. Allele-specific primers were made by making the nucleotide at the 3' end of the primer specific

for one of the polymorphic bases. A second primer was made in the same way for the other base. Sequencing for each individual allele was then performed using template from the original PCR reaction (from the MPIEX4/MPIEX5 primer set) plus each allele-specific primer in separate reactions. This step resulted in separate sequences for each individual allele. Allele-specific priming was not done in the case of homozygotes or a heterozygote for only one position in the gene. In these cases, alleles could be deduced without the need for additional sequencing.

Phylogenetic Analysis

I used Modeltest version 3.06 (Posada and Crandall 1998) to determine the appropriate model of evolution for each dataset under the Akaike Information Criterion. The appropriate model (TVM for ZFY and K80 + I + Γ for MPI) was implemented in a Bayesian analysis using the MrBayes version 3.1.2 program (Ronquist and Huelsenbeck 2003) for each dataset. For MPI, 3 million generations were run and 70,000 of these were discarded as burn-in. The same number of generations was run for ZFY and 60,000 were discarded as burn-in. The models of evolution described above were also used in a maximum likelihood analysis using GARLI version 0.951 (http://www.zo.utexas.edu/faculty/antisense/garli/Garli.html). GARLI was also used to find ML bootstrap support values for clades (based on 100 replicates) for ZFY and MPI. For both datasets, all indels were discarded prior to phylogenetic analyses. With the MPI locus, each individual allele was used as an OTU in the phylogenetic analysis. Trees were visualized using TreeView version 1.6.6 (Page 1996). A total of 561 base pairs were used in phylogenetic analyses for MPI and 602 for ZFY.

Hypothesis Testing

I used parametric bootstrapping to test for monophyly of the Pacific *Rhogeessa tumida* clade in the MPI dataset (Huelsenbeck et al. 1996; Van Den Bussche et al. 1998). This was done using PAUP 4.0b10 (Swofford 2002) to obtain parsimony scores for an initial unconstrained tree, as well as a constraint tree for monophyly of all Pacific *R. tumida* individuals. Each of these heuristic searches was done using 100 addition-sequence-replicates and TBR branch-swapping. Then Mesquite version 1.12 (Maddison and Maddison 2006) was used to simulate 100 datasets under the model indicated by Modeltest for the constrained tree. These simulated datasets were used to find an expected distribution of differences in tree scores between constrained and non-constrained trees.

Phylogeographic Analysis

I implemented Analysis of Molecular Variance (AMOVA; Excoffier et al. 1992) using cyt-*b* sequences from Chapter 2 to test several alternative geographic hypotheses for diversification of multiple *R. tumida* lineages. I tested for phylogeographic structure based on: 1) mountain uplifts in Mexico and Central America; 2) historical seaway across the Isthmus of Tehuantepec; and 3) habitat differences. These analyses were performed using Arlequin version 2.001 (Schneider et al. 2000).

RESULTS

ZFY

The ZFY tree (Fig. 3.3) supports the monophyly of the *Rhogeessa tumida* complex (posterior probability = 1.00). Although I was unable to resolve many inter-

specific relationships using this slow-evolving gene, I could distinguish many of the major clades recognized in the mtDNA phylogeny (Chapter 2). Both *R. io* and *R. velilla* form distinct clades. Another clade consists of all *R. aeneus* samples, the Atlantic Mexican *R. tumida* samples and the Pacific *R. tumida* samples. All of the members of this clade share a single haplotype. Slightly different from that haplotype is the haplotype shared by all Atlantic Central American *R. tumida* individuals. As in the mtDNA phylogeny, the ZFY phylogeny shows *R. parvula* and *R. mira* as sister to the *R. tumida* complex, although they are not supported as being sister to one another as they are in the mtDNA dataset. Also similar to the mtDNA results, *R. gracilis* and *R. alleni* (both tentatively called members of the genus *Baeodon* in Chapter 2) are very closely related to one another, and distantly related to the remaining *Rhogeessa* species.

MPI

I was unable to cleanly amplify *Rhogeessa parvula* and *R. alleni* for this locus, so I have no data for this locus from those taxa. Thirty-four alleles were identified from members of the *R. tumida* complex at the MPI locus (Fig. 3.4). Several major clades are fixed for a single allele, whereas the Pacific *R. tumida* group and *R. aeneus* contain many highly variable alleles. The MPI dataset is not well resolved at the level of inter-species relationships among members of the *R. tumida* complex. However, MPI data are sufficient to confirm monophyly of most of the major clades. As in mtDNA and ZFY, the *R. tumida* complex is a monophyletic group (posterior probability = 1.00). *Rhogeessa mira* is sister to the *R. tumida* complex. *Rhogessa io* is very distinct from the other members of the *R. tumida* complex, and only two different alleles were observed in that

species. The lone unique *R. io* allele was found in the sample from Panama. Similar to the mtDNA results (Chapter 2), both *R. velilla* and the Atlantic Central American *R. tumida* both form highly supported monophyletic clades, and at the MPI locus each are fixed for a single allele. *Rhogeessa genowaysi* possesses a unique allele but is not highly supported as being different from the Pacific *R. tumida*. The Pacific *R. tumida* alleles are highly variable and are not well-supported as being monophyletic. I performed parametric bootstrapping to further test whether I can reject the hypothesis of monophyly of the Pacific *R. tumida*. The difference in parsimony scores between the unconstrained tree and the tree that was constrained for monophyly of the Pacific *R. tumida* individuals was only one. Simulated datasets were analyzed and the threshold for rejection of the monophyly hypothesis at $p<0.05$ was found to be a difference in tree score of greater than or equal to 10. Therefore, I cannot reject monophyly of the Pacific *R. tumid*a individuals based on these data. Moreover, *R. aeneus* is not monophyletic, with one allele occurring within the clade of Atlantic Mexican *R. tumida*, and two other alleles also occurring outside of the main *R. aeneus* clade, but not clustering with any other major clade. Like the Pacific *R. tumida* group, my samples of *R. aeneus* included few homozygous individuals.

Phylogeography

The results of the three AMOVAs are given in Table 3.2. The only significant result obtained was based on groups that were defined by habitat type. In this case, individuals captured in dry, semi-arid environments were significantly differentiated from those captured in humid environments. These results indicate that 86.3% of the genetic

variation in cyt-*b* sequences for these individuals is between those inhabiting dry areas and those inhabiting humid areas.

DISCUSSION

Phylogenetic Relationships

All three *Rhogeessa* DNA sequence datasets, mtDNA (Chapter 2), ZFY (Fig. 3.3) and MPI (Fig. 3.4), agree on several important issues: 1) the *R. tumida* complex is monophyletic; 2) in most cases, species that are karyotypically distinct form distinct genetic lineages; 3) there is possible evidence for hybridization or lineage sorting between *R. tumida* and *R. aeneus*; 4) what is currently known as "*R. tumida*" contains several distinct genetic lineages; 5) the two 2n = 42 species, *R. genowaysi* and *R. velilla* are genetically distinct from one another; 6) *R. alleni* and *R. gracilis* are very distantly related to all other *Rhogeessa* species.

The main differences among my analyses include the way particular relationships within the *R. tumida* complex are resolved. In most cases this is probably a lack of data in a particular dataset. For example, in the ZFY tree (Fig. 3.3), *R. aeneus*, Pacific *R. tumid*a and Atlantic Mexican *R. tumida* share one allele. This could probably be resolved better by adding more characters. This observation does, however, highlight the close relationship between Pacific and Atlantic Mexican *R. tumida* among ZFY alleles, whereas with mtDNA the Pacific *R. tumida* are very divergent from the other two lineages. Furthermore, the MPI phylogeny recognizes the Atlantic Mexican and Central American *R. tumida* clades as each being monophyletic, but fails to group the Pacific *R.*

tumida together in a clade. The parametric bootstrapping analysis shows, however, that monophyly of the Pacific clade cannot be rejected. This result is not surprising, as only one branch in the ML tree disrupts the monophyly of the Pacific clade.

Data from mtDNA (Chapter 2), ZFY, and MPI all confirm that the group currently recognized as *Rhogeessa tumida* contains multiple distinct genetic lineages. DNA sequence data distinguish three different 2n = 34 lineages: an Atlantic Mexican clade, an Atlantic Central American clade, and a Pacific clade. Cytochrome-*b* (Chapter 2) and MPI are able to distinguish all three as distinct clades and ZFY shows the Atlantic Central American form as distinct from the other two, which share a common haplotype. *R. tumida* has been studied using morphological, karyotypic, allozyme and now DNA sequence data. The morphological study of LaVal (1973) shows some variation in several morphological characters throughout the range of *R. tumida*. He did not believe there was enough difference in these characters to distinguish what are now recognized as species distinct from *R. tumida*, including *R. aeneus*, *R. io*, *R. genowaysi,* and *R. velilla*. Bickham and Baker (1977) examined banding patterns of karyotypes from individuals throughout the range of *R. tumida*. They found no differences between individuals along the Atlantic versant of Mexico/Central America or those along the Pacific versant. All 2n = 34 karyotypes were found to be composed of the same set of centric fusions. Allozyme data (Baker et al. 1985) consistently showed variability within the 2n = 34 karyotypic form at loci which were variable within the *R. tumida* complex. Although those authors did not point out geographic patterns within this variation, it could correspond to multiple species, and possibly the Pacific/Atlantic clades I have defined, within that karyotype.

Therefore, my DNA sequence data seem to be the only solid evidence at this time that these three groups of "*R. tumida*" are independently evolving lineages.

Only the ZFY gene places two different *Rhogeessa tumida* groups (the Pacific and Atlantic Mexican forms) together in the same clade. However, also in that clade are all *R. aeneu*s individuals. The low resolution within that clade is likely due to lack of variable characters for distinguishing between members of that group. No dataset I have examined shows any clear evidence of recent interbreeding between the three different lineages of "*R. tumida*."

Taxonomy

Given that there are not clear-cut morphological differences between these lineages and that they are all chromosomally identical, deciding their proper taxonomic status is difficult. I attempted to perform a detailed genetic study of whether the Pacific and Atlantic Central American forms interbreed along a potential contact zone in Guatemala. I was unable, however, to collect sufficient numbers of individuals in the contact zone to draw any firm conclusions about this point. Therefore, it is still unknown whether some degree of interbreeding is occurring between different genetic lineages of the 2n = 34 form. I do know that the Pacific clade is highly divergent from the others based on cyt-*b* and MPI and in most areas is separated geographically by mountain ranges (one known exception is in Guatemala where I have collected Pacific individuals on the Atlantic side of the mountains). Although the sampling for the Atlantic Mexican form is sparse, it seems to be separated from the Atlantic Central American form by *Rhogeessa aeneus* on the Yucatan peninsula. Thus, it is likely that the three different *R.*

tumida lineages are allopatric throughout most of their ranges. Despite this potential allopatry and the fact that they are apparently good "Genetic Species" (Baker and Bradley 2006) based on all markers sequenced, there may be morphological delineations between the species as well. I am currently conducting morphological tests prior to modifying the taxonomic status of these lineages.

The nuclear data also confirm the distant relationship of both *Rhogeessa alleni* and *R. gracilis* to the remaining *Rhogeessa* species shown in Chapter 2, where I tentatively supported placing both of these species in the genus *Baeodon*. Although I was unable to sequence MPI for *R. alleni*, results from that locus show *R. gracilis* to be very distant from the remaining *Rhogeessa*. *R. alleni* and *R. gracilis* share the same allele at the ZFY locus. These two taxa were weakly supported as the sister group to the remaining species of *Rhogeessa*. Based on these data, it is likely that placing both of these species in the genus *Baeodon* is appropriate.

Genetic Variation

A unique feature of the MPI dataset is its ability to point out the extremely variable nature of alleles present in the Pacific *Rhogeessa tumida*, compared to the other species in the *R. tumida* complex. Almost all Pacific *R. tumida* individuals are heterozygous (homozygotes are indicated by black squares in Fig. 3.4), and in many cases the two alleles within an individual are very different from one another. Many allele pairs are variable at more than two sites (in *R. aeneus*, the other clade with many heterozygotes, the alleles usually only vary at one or two sites). This stands in stark contrast to most other species in the complex, most of which are fixed for a single allele.

This observation also contrasts with the results from the cyt-*b* sequences, where within-species diversity was very similar for all three *R. tumida* lineages (Chapter 2). There are several possible explanations for this observation. Individuals from the Pacific clade span a broader geographic range than most other species in the *R. tumida* complex. This could result in more isolated populations within the species and therefore greater genetic diversity. This observation could also indicate female philopatry and greater male dispersal of Pacific *R. tumida*, although my ZFY data cannot confirm this hypothesis. This pattern is also expected in taxa from a hybrid origin (Holloway et al. 2006). I would not necessarily have noticed this variation had we not sequenced individual alleles, instead opting to perform phylogenetic analyses on the consensus sequence of two alleles. I encourage the practice of sequencing alleles when possible to uncover evolutionary processes that might otherwise go undetected.

Hybridization vs. Lineage Sorting

One similarity in all sequence datasets mentioned previously is potential evidence for hybridization or lineage sorting between *Rhogeessa tumida* and *R. aeneus*. In the cyt-*b* phylogeny, two individuals of Atlantic Central American *R. tumida* fall within the *R. aeneus* clade (Chapter 2). In the ZFY phylogeny, *R. aeneus* shares a single haplotype with all Pacific and Atlantic Mexican *R. tumida*. With MPI, one *R. aeneus* allele occurs within the Atlantic Mexican *R. tumida* clade (the other allele from that *R. aeneus* individual also clusters separately from the main *R. aeneus* clade; Fig. 3.4). For MPI and ZFY, I could sequence only one of the potential *R. tumida* hybrids from the mtDNA dataset, and it was homozygous for an MPI allele that falls within the Atlantic Central

American *R. tumida* clade. This individual also falls in the Atlantic Central American *R. tumida* clade in the ZFY tree.

I previously mentioned that a Y-chromosomal tree, due to it s shorter lineage sorting period, should follow the true species phylogeny most closely, followed by mtDNA and finally autosomal markers. Unfortunately, the ZFY marker does not provide much resolution between some members of the *R. tumida* complex. This could be due to extensive hybridization, but it is most probably a simple lack of variable characters. The cyt-*b* phylogeny presented in Chapter 2 is the most well-resolved tree and has a lineage sorting period less than that of the MPI tree presented here. The fact that the cyt-*b* tree, with its shorter lineage sorting period, does not show the same hybridization/lineage sorting pattern as the MPI tree is striking. The two different datasets show the same pattern involving different individuals from different *R. tumida* clades. The ZFY tree is in agreement with the MPI tree that no Atlantic Central American *R. tumida* are involved in lineage sorting/hybridization events with *R. aeneus*, although with ZFY we cannot rule these out between *R. aeneus* and Atlantic Mexican or Pacific *R. tumida*.

Both lineage sorting and ancient hybridization can account for the observed phylogenetic pattern. The strongest evidence for rejecting a recent hybridization hypothesis lies in comparing the cyt-*b* phylogeny to the MPI phylogeny. Incomplete lineage sorting will result in nonconcordant patterns between loci due to the fact that it is a random process and independent of the lineage sorting in different loci. My data exhibit this lack of concordance in phylogenetic patterns with respect to the relationship between *R. tumida* and *R. aeneus*. Any recent hybridization events would show the same patterns across loci. On the other hand, an ancient hybridization event can produce the

same patterns expected of lineage sorting if sufficient time has elapsed since the event. In the case of my data, the two Atlantic Central American *Rhogeessa tumida* that group with *R. aeneus* for cyt-*b* are homozygous for Atlantic Central American *R. tumida* MPI alleles. If the mitochondrial capture that produced the cyt-*b* pattern happened long ago (and divergence values in the data suggest that it did), many generations of back-crossing to the parental *R. tumida* would erase a similar pattern in MPI. The observation in the MPI tree of one *R. aeneus* allele occurring within a clade of Mexican *R. tumida* can also be explained by lineage sorting. It is evident from all of the datasets that *R. aeneus* is probably the most recently evolved species and that the Atlantic *R. tumida* are its closest sister taxa (although these relationships are not well-resolved). This is where one expects lineage sorting to be the most problematic, but also where hybridization is most likely if a complete isolating mechanism has not been established. Because the patterns expected to be produced by ancient hybridization and lineage sorting are the same, I cannot rule out either hypothesis outright. At a minimum, my data show that there is no regular, recent, or ongoing hybridization among major lineages of the *R. tumida* complex.

Chromosomal Speciation

With regard to the speciation by monobrachial centric fusions model I am testing, the nuclear sequence data reported here are able to provide stronger evidence of general support for this model than cyt-*b* sequences alone (Chapter 2). My data demonstrate that there has been no recent gene flow between species that differ from one another by monobrachial fusions. These data indicate that a scenario of speciation by monobrachial centric fusions should not be rejected, and the expectations of the model are supported.

Baker and Bickham (1986) indicated in their description of the model that the presence of monobrachial differences between karyotypes should result in instantaneous reproductive isolation and that even a single difference should be sufficient for complete reproductive isolation. My data suggest that these expectations have met in *Rhogeessa*, with the possible exception of an ancient hybridization event between *R. tumida* and *R. aeneus*. On the other hand, if monobrachial centric fusions were the only force driving reproductive isolation in *Rhogeessa*, one would expect to see evidence of gene flow between populations that do not have monobrachial differences between their karyotypes. I do not see this condition met based the presence of various lineages of *R. tumida* (2n = 34). In this case, there are three distinct genetic lineages with no evidence of gene flow among them. All three lineages share the same set of chromosomal fusions (Baker et al. 1985). All of these observations lead me to conclude that although my data are generally consistent with a hypothesis of speciation by monobrachial centric fusions, this speciation mechanism is not the only mechanism that has led to diversification within *Rhogeessa*.

Because I have repeatedly seen multiple lineages of *R. tumida* between which reproductive isolation could not have been caused by chromosomal differentiation, I tested geographic alternatives to explain these patterns. The results from the AMOVAs indicate that habitat differences may have created isolation leading to diversification between the Atlantic and Pacific lineages of *R. tumida*. Individuals from the two Atlantic clades, from Mexico and Central America, span moist or humid habitats whereas those from the Pacific clade are from dry habitats. Although my samples from Guatemala which group with the Pacific clade were captured on the Atlantic side of the Sierra Madre mountain range through Central America (all others from the Pacific clade were captured

on the Pacific coast), they were found on the semi-arid western side of the Motagua Valley. All other samples from Guatemala were collected near the coast where the habitat is much more humid. I was only able to capture one individual from "transitional" habitat of the Motagua Valley and it grouped with the Atlantic Central American clade in all of my phylogenetic analyses. The habitat in which I captured this individual is more humid than many other areas in the transition zone between habitats. The Motagua Valley may play an important role in future studies of *R. tumida* and other species that show similar divergence structured by habitat due to the dramatic habitat change over a relatively short distance.

My results of monobrachial differences creating complete reproductive isolation are similar to those in studies of shrews that also exhibit potential speciation via this mechanism. Results from hybrid zones in shrews exhibiting monobrachial differences show increased genetic structure of microsatellites on chromosomal arms involved in fusions compared to markers on arms not involved in fusions. These results show that rearrangements affect the barrier to gene flow between different karyotypic forms (Basset et al 2006). On the other hand, Britton-Davidian et al. (2002) showed that allozymes showed no structure with respect to different chromosomal forms in house mice, indicating the presence of gene flow between populations which differ by monobrachial fusions. Although some of these data, including mine, support the model of speciation by monobrachial centric fusions, some results show that the process may not be as simple as that outlined in Baker and Bickham (1986).

The presence of reciprocally monophyletic and karyotypically distinct lineages in the *R. tumida* complex in all three DNA sequence datasets is consistent with an important

role of reproductive isolation from monobrachial fusions in generating speciation events in this group. The very short branch lengths observed at the base of the *R. tumida* complex clade, and the difficulty in resolving relationships at that level in all datasets indicate that speciation and diversification at that time period was rapid. This observation is consistent with the expectations of the speciation by monobrachial centric fusions model, which states that speciation should happen virtually instantaneously with the chromosomal rearrangements (Baker and Bickham 1986). The observed phylogenetic patterns would have to correspond to rapid chromosomal change followed by rapid reproductive isolation. In fact, this expected rapid bout of chromosomal rearrangements has been observed in *Mus domesticus* by Nachman and Searle (1995) who estimated that the fixation rate of centric fusions in that species was 2.25×10^{-4} fixations per generation. If many chromosomal rearrangements were occurring during the time period corresponding to the base of the *R. tumida* complex on my tree, and the rearrangements resulted in rapid reproductive isolation, this process could lead to the phylogenetic patterns I have consistently seen in all three datasets.

The cyt-*b* phylogeny (Chapter 2) is the most well-resolved of the three datasets and shows the occurrence of two clades within the *R. tumida* complex that are both composed (at least in part) of a 2n = 34 lineage and a 2n = 42 lineage. Although not resolved at the same level, the nuclear datasets agree that there are multiple lineages of 2n = 34 and 2n = 42 karyotypes. This raises the question as to whether there may be some sort of selective advantage to possessing these particular karyotypic arrangements or whether multiple lineages which happen to have the same rearrangements have arisen by random processes. A somewhat similar observation has been made in the karyotypically

variable species *Mus domesticus*, where some chromosomes are often involved in fusions, while others have been observed rarely in fusions (Nachman and Searle 1995; Gazave et al 2003). The question of why certain chromosomes tend to pair up in fusions has been examined by other authors (Gazave et al. 2003), but explanations are rare and usually specific to certain scenarios. My findings in *Rhogeessa* of apparently parallel origins of the same karyotype suggest that there may be a limited number of stable configurations of the chromosomes. If true, this suggests caution should be applied in using karyotypic data for inferring phylogenetic relationships.

FIGURES

Figure 1.1. Simplified scenario of speciation by monobrachial centric fusions. Chromosomes on the top row represent the ancestral population. The second row contains two populations, A and B, that have different centric fusions. The third row depicts Meiosis I of hybrid offspring.

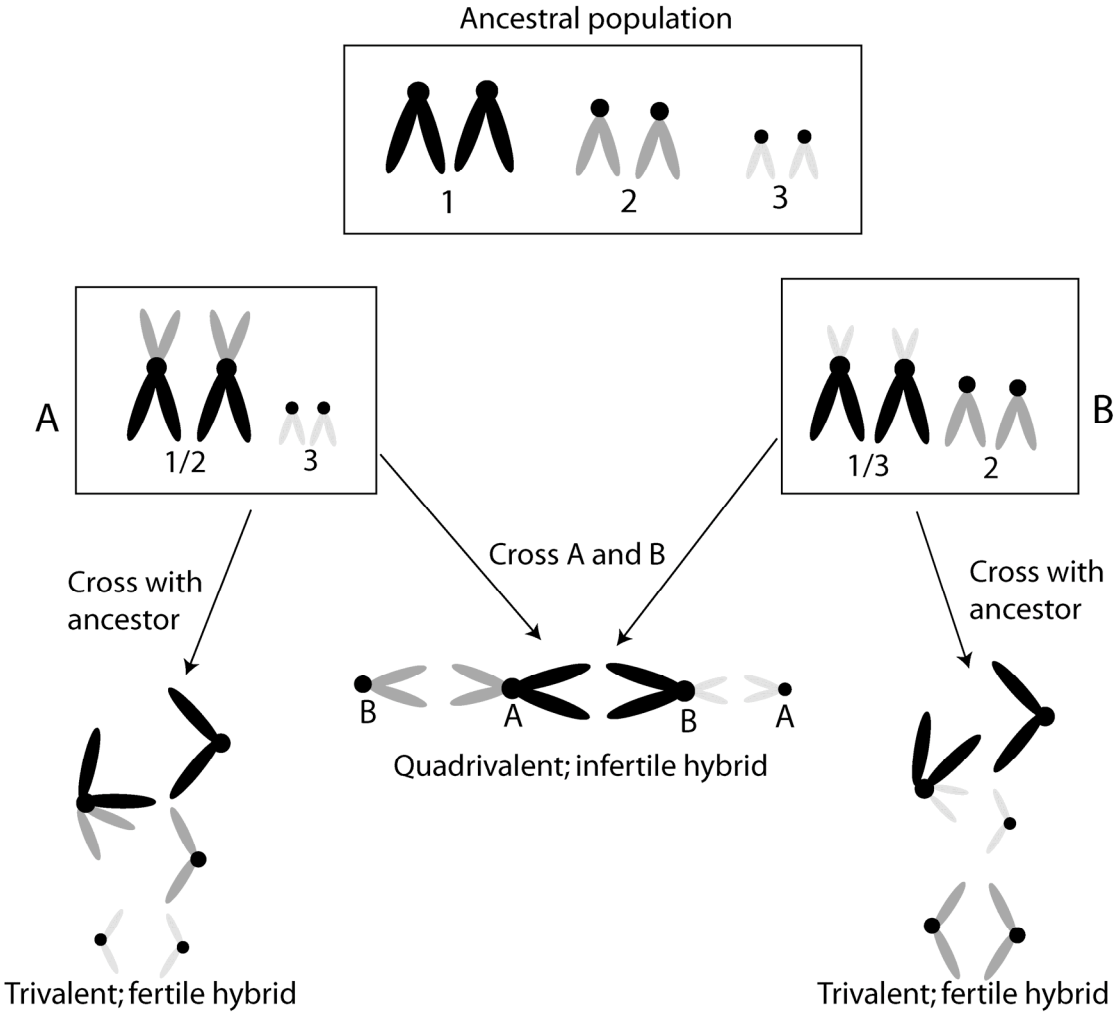


Figure 2.1. Distribution map of *Rhogeessa* samples included in Chapter 1. Collection localities in close proximity are not shown. Circled *R. tumida* localities represent sites where specimens group with the Atlantic clades; noncircled *R. tumida* localities contain individuals from the Pacific clade. The southern-most circled localities (Guatemala and Honduras) are where the two *R. tumida* which group with *R. aeneus* are from, along with *R. tumida* that group with the Central American Atlantic clade. Shading represents approximate ranges for *R. tumida*, *R. parvula*, *R. aeneus*, *R. io* and *R. velilla*. *R. genowaysi* and *R. mira* are known from only two localities near our samples indicated here. *R. gracilis* and *R. alleni* have overlapping ranges in western Mexico (*R. gracilis* from northern Jalisco to central Oaxaca; *R. alleni* from central Jalisco to central Oaxaca) and overlap the eastern part of the range limit of *R. parvula*.

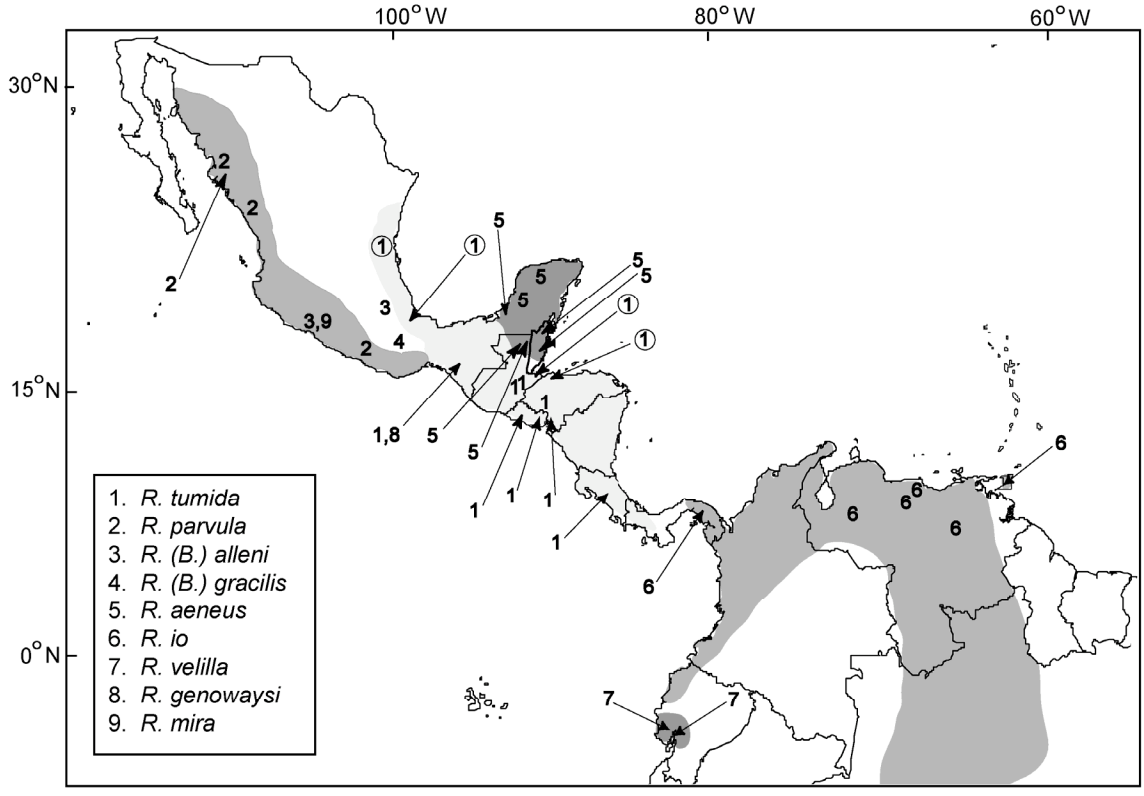


Figure 2.2. Karyotype of *R. velilla* from Ecuador (2n = 42). Numbers below biarmed chromosomes represent arms involved in the centric fusions of those chromosomes (chromosomal nomenclature following Bickham 1979a and 1979b).

Figure 2.3. Phylogeny of *Rhogeessa* based on a Bayesian analysis of cytochrome-*b* sequences. Posterior probabilities of major clades are included, followed by ML bootstrap values. An asterisk (*) indicates that the bootstrap value is <50 for that clade.

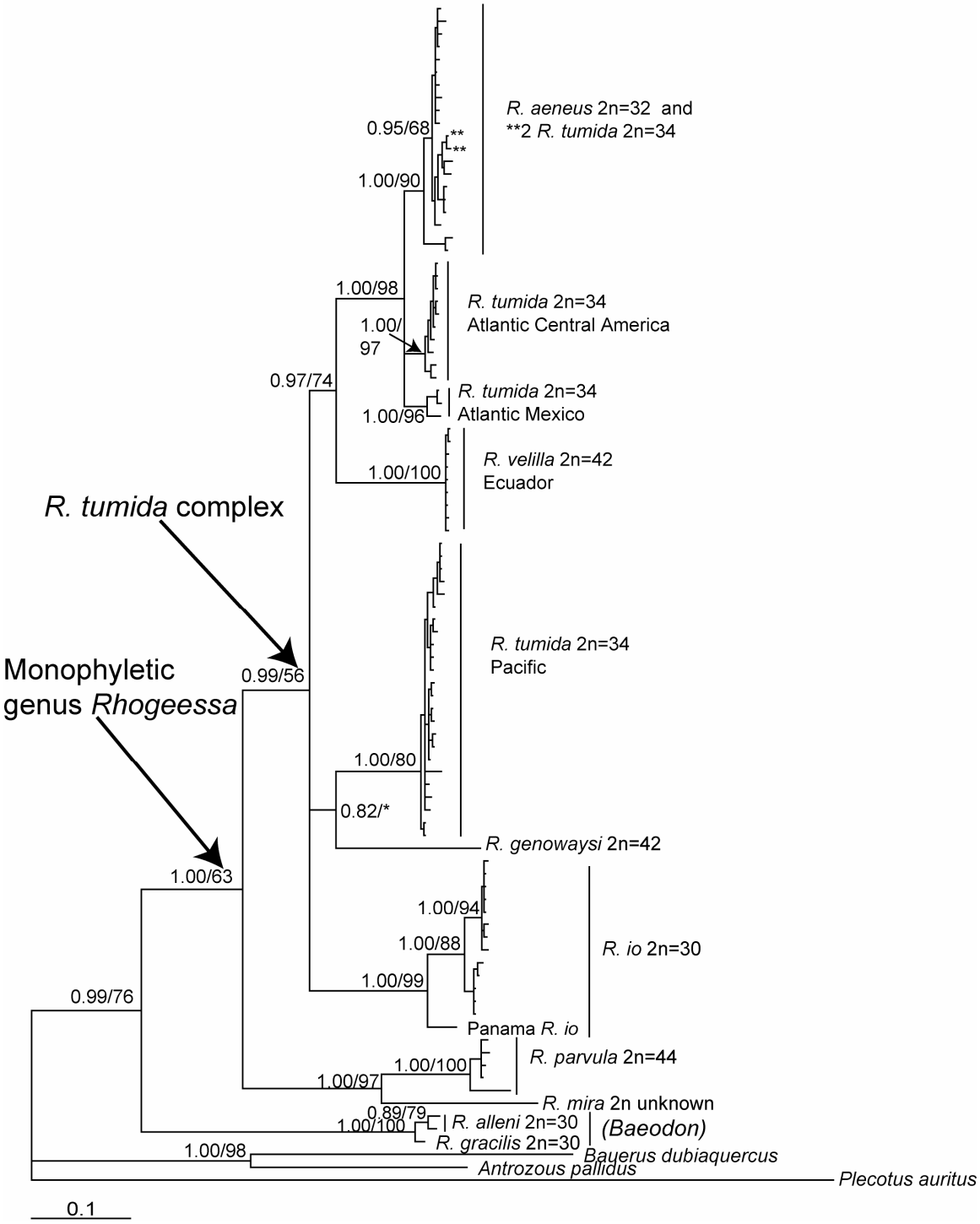


Figure 3.1. Sampling localities for ZFY and MPI. Localities in close proximity are omitted. Circled numbers represent localities only in ZFY dataset; numbers in black boxes represent samples only in MPI dataset. All other numbers represent localities in both datasets.

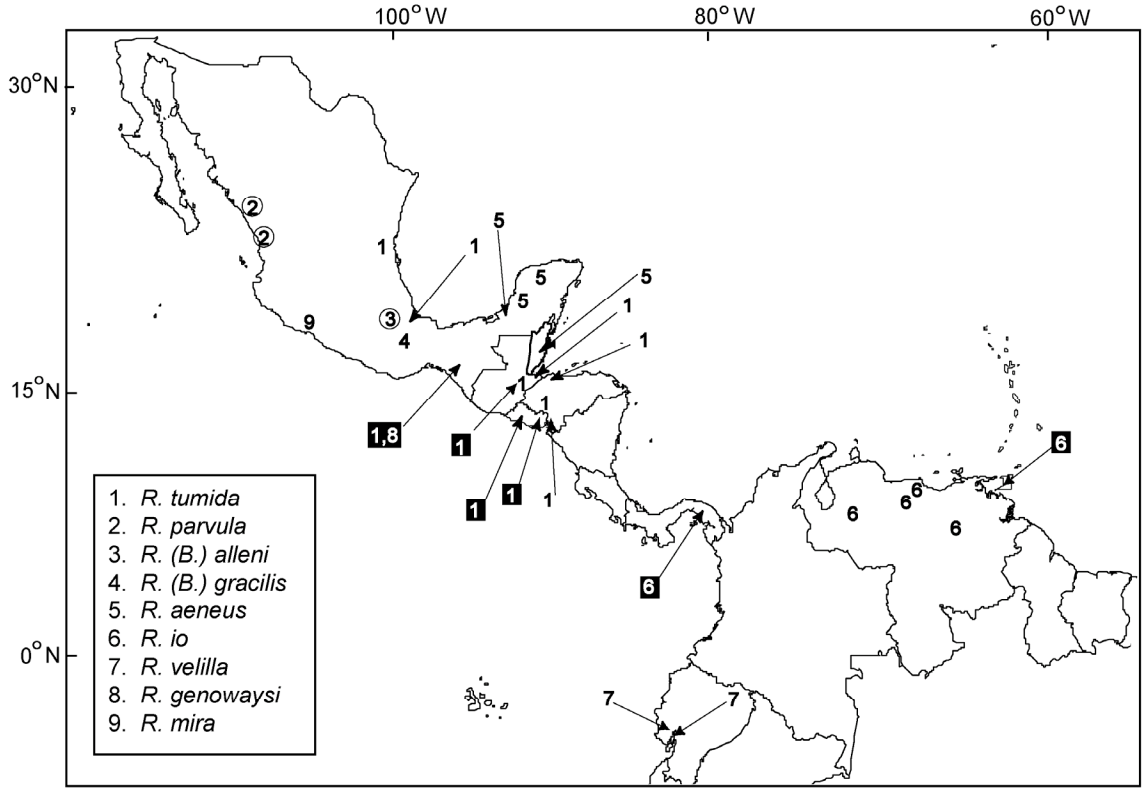


Figure 3.2. Allele-specific primer design process.

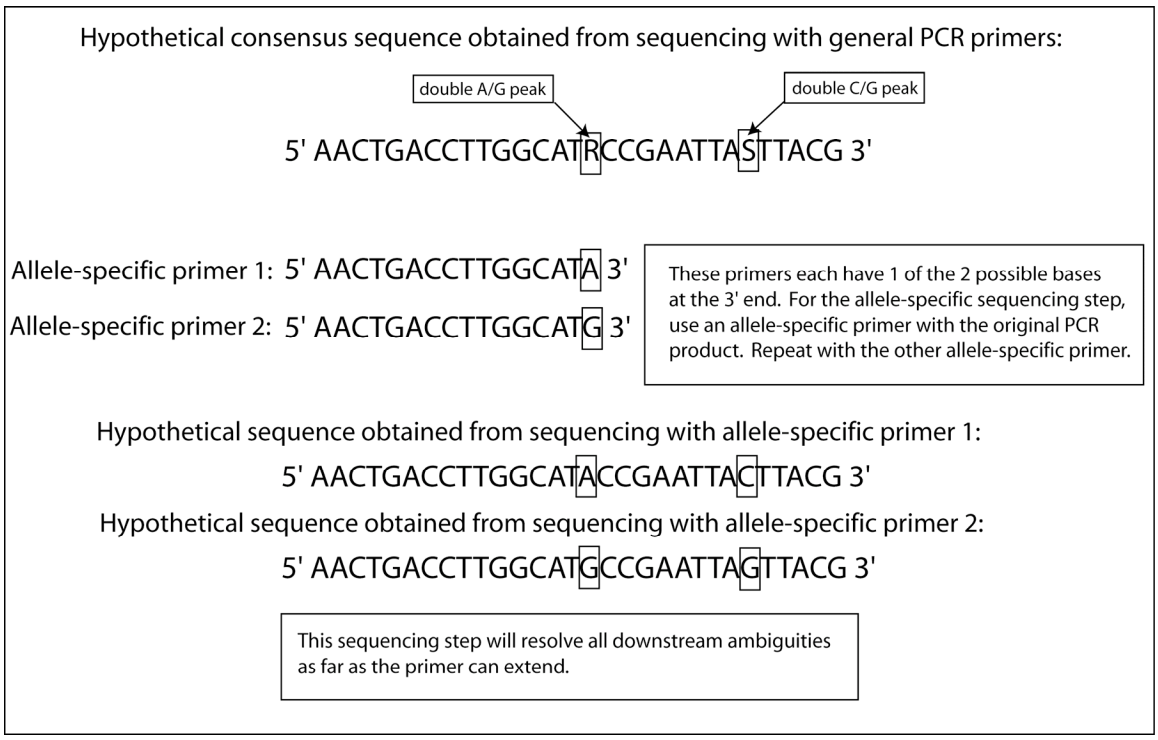


Figure 3.3. Maximum likelihood phylogeny of *Rhogeessa* based on ZFY sequences. Numbers at nodes represent ML bootstrap proportions followed by Bayesian posterior probabilities. Sample names correspond to those found in Appendix A.

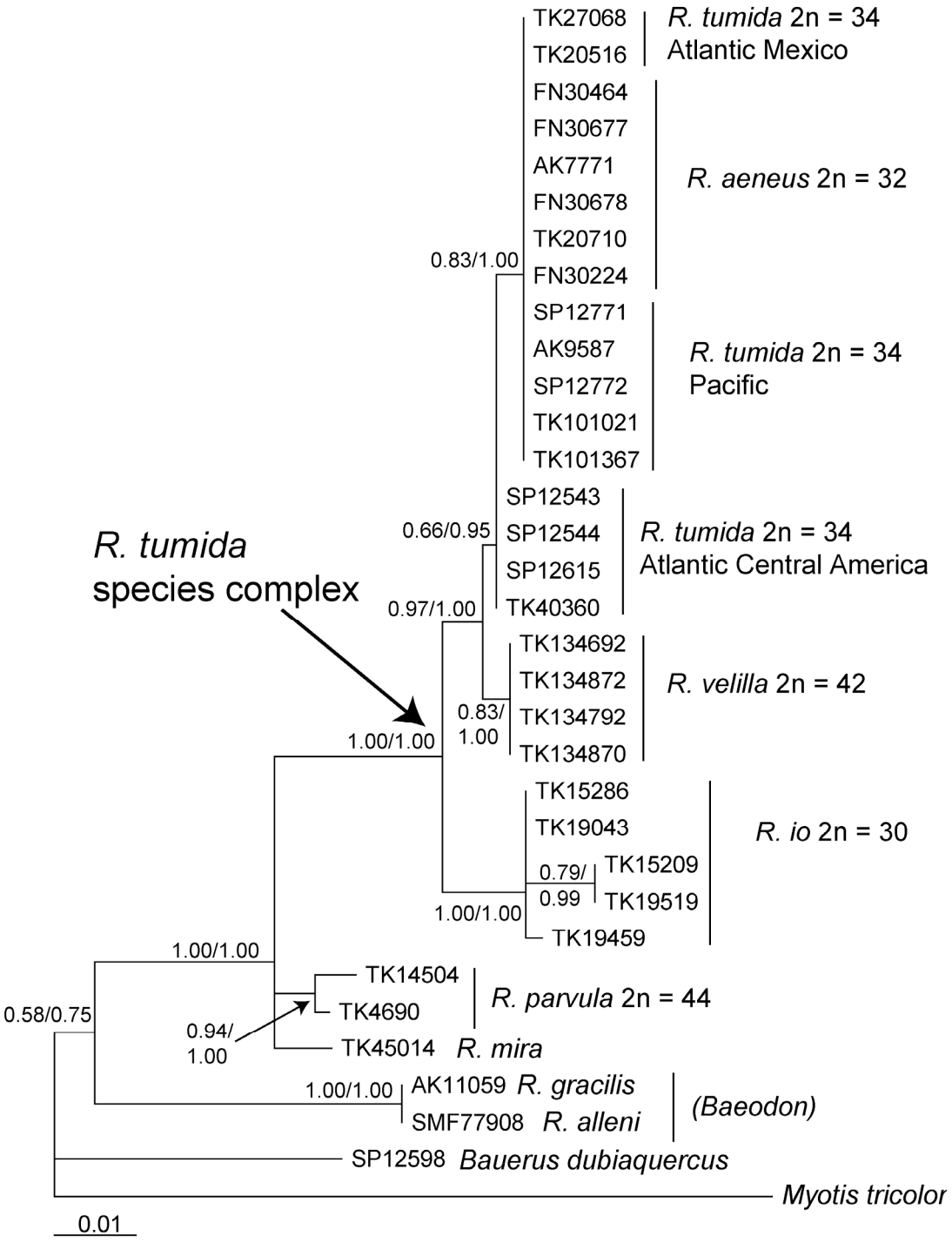
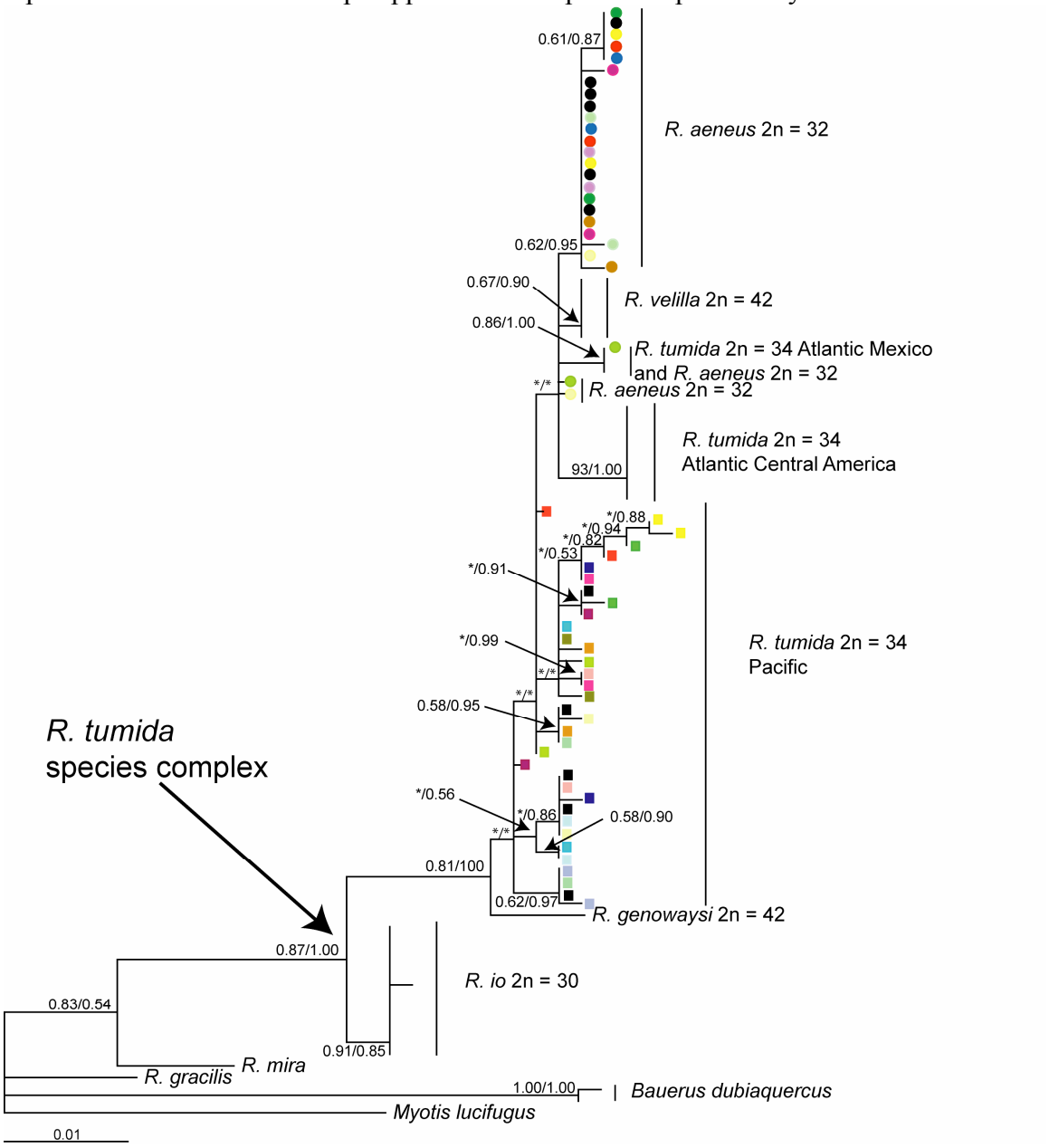


Figure 3.4. Maximum likelihood phylogeny of *Rhogeessa* based on MPI sequences. Numbers at nodes represent ML bootstrap proportions followed by Bayesian posterior probabilities. Circles represent *R. aeneus* alleles (circles of the same color represent alleles from the same individuals; a black circle represents a homozygous individual), squares represent Pacific *R. tumida* alleles (same coloring scheme as circles). Asterisks represent nodes with bootstrap support <0.50 or posterior probability <0.50.



TABLES

Table 1.1. *Rhogeessa* species. The names provided in this table reflect taxonomic status prior to my study, with the exception of *R. velilla*, which I recognize within. Species marked with an asterisk (*) belong to the *R. tumida* complex.

Species	Diploid number	Geographic range	Relevant Literature
*R. tumida**	2n = 34	Widespread from Tamaulipas, Mexico to northern Panama	Vonhoff 2000
*R. genowaysi**	2n = 42	Single locality in Chiapas, Mexico	Baker 1984; Roots and Baker 1998
*R. aeneus**	2n = 32	Yucatan region of Mexico and Belize	Audet et al. 1993
*R. io**	2n = 30	Southern Panama into South America	Baker and Genoways 1996
R. minutilla	Unknown	Northern Colombia and Venezuela	Ruedas and Bickham 1992
*R. hussoni**	2n = 52	Suriname into Brazil	Genoways and Baker 1996
R. parvula	2n = 44	Pacific coast of Mexico	LaVal 1973; Roots and Baker 2007
R. alleni	2n = 30	Mountains of western Mexico from Jalisco to Oaxaca	LaVal 1973
R. mira	Unknown	Michoacan, Mexico	Arroyo-Cabrales and Polaco 1997
R. gracilis	2n = 30	Pacific coast of Mexico from Jalisco to Oaxaca	Jones 1977
*R. velilla**	2n = 42	Southern Pacific coast of Ecuador	See Chapter 2

Table 2.1. Maximum genetic (K2P) divergence in the cytochrome-*b* gene measured within and between major clades of *Rhogeessa* and other bats examined in Chapter 1. The numbers along the diagonal represent divergence within a clade. Dashed lines indicate taxa for which only one specimen was examined.[a]

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0.014													
2	0.028	0.006												
3	0.038	0.025	0.010											
4	0.102	0.096	0.092	0										
5	0.108	0.105	0.108	0.099	0.007									
6	0.117	0.111	0.120	0.122	0.108	-								
7	0.113	0.111	0.123	0.115	0.121	0.131	0.016							
8	0.144	0.137	0.144	0.142	0.146	0.158	0.147	0.015						
9	0.157	0.156	0.155	0.160	0.158	0.161	0.016	0.108	-					
10	0.164	0.164	0.17	0.161	0.163	0.171	0.168	0.18	0.172	-				
11	0.202	0.198	0.196	0.191	0.201	0.211	0.2	0.201	0.223	0.186	-			
12	0.179	0.177	0.173	0.181	0.187	0.204	0.204	0.176	0.198	0.195	0.161	-		
13	0.167	0.169	0.173	0.17	0.166	0.176	0.168	0.187	0.181	0.017	0.186	0.197	0.011	
14	0.235	0.227	0.226	0.214	0.235	0.227	0.235	0.255	0.252	0.231	0.244	0.231	0.231	-

[a]1 = *R. aeneus* (including two *R. tumida* within that clade), 2 = *R. tumida* Atlantic Central American clade, 3 = *R. tumida* Atlantic Mexico clade, 4 = *R. velilla*, 5 = *R. tumida* Pacific clade, 6 = *R. genowaysi*, 7 = *R. io*, 8 = *R. parvula*, 9 = *R. mira*, 10 = *R. gracilis*, 11 = *Bauerus*, 12 = *Antrozous*, 13 = *Baeodon alleni*, 14 = *Plecotus autritus*

Table 3.1. Allele-specific primers used for sequencing the MPI locus.

Primer name	Primer sequence
MPI156TF	5’ GGCTAGAATACATGGGCAAT 3’
MPI156CF	5’ GGCTAGAATACATGGGCAAC 3’
MPI349GF	5’ GCCTGACTTCTTGGTTAGGG 3’
MPI349AF	5’ GCCTGACTTCTTGGTTAGGA 3’
MPI374GR	5’ GGAGCCTACAGAAGTGGGAAG 3’
MPI374TR	5’ GGAGCCTACAGAAGTGGGAAT 3’
MPI486GR	5’ TGGCTTAGGCTCTGCTTTAG 3’
MPI486AR	5’ TGGCTTAGGCTCTGCTTTAA 3’
MPI157CF	5’ GCTAGAATACATGGGCAACC 3’
MPI157GF	5’ GCTAGAATACATGGGCAACG 3’
MPI290AF	5’ TTAGTGTGCTTGCTGAGGA 3’
MPI290GF	5’ TTAGTGTGCTTGCTGAGGG 3’

Table 3.2. Results of AMOVA analyses based on cytochrome-*b* sequences in *R. tumida*. Asterisk indicates significance at $p<0.05$.

Hypothesis	Groups	Proportion of variance in haplotype diversity among groups	p-value
Mountain uplifts in Mexico and Central America as isolating mechanism	1. Individuals from Atlantic Mexican *R. tumida* clade, Atlantic Central American *R. tumida* clade, and Guatemalan samples from Atlantic side of Sierra Madres that phylogenetically group with Pacific *R. tumida* clade 2. Pacific *R. tumida* except Guatemalan samples	50.22	0.0715
Isthmus of Tehuantepec as isolating mechanism	1. Atlantic Mexican *R. tumida* (all from west side of Isthmus) 2. All other *R. tumida* (all from east side of Isthmus)	27.64	0.2216
Habitat differences as isolating mechanism	1. Atlantic Mexican and Central American *R. tumida* (represent humid environment) 2. Pacific *R. tumida* (represent semi-arid environment)	86.39	0.01069*

Appendix A

Table A1. Specimens examined. TK = Natural Science Research Laboratories, Texas Tech University; AK = Texas Cooperative Wildlife Collection, Texas A&M University; ASNHC = Angelo State Natural History Collection, Angelo State University; SGP = Sergio G. Perez collection number; FN = Royal Ontario Museum; SP = Carnegie Museum; SMF = Senckenberg-Museum, Frankfurt/Main. For the MPI locus, individuals with two sequences reported were heterozygous, those with only one sequence reported were homozygous.

Species	Museum voucher number	Locality	Cyt-*b* GenBank Accession number	ZFY GenBank Accession number	MPI GenBank Accession number(s)
Baeodon alleni	TK45023	Michoacan, Mexico	EF222375		
	SMF77908	Puebla, Mexico	EF222412	EU185125	
Rhogeessa aeneus	TK20704	Belize dist., Belize	EF222329		EU220301 EU220302
	TK20706	Belize dist., Belize	EF222361		EU220303
	TK20707	Belize dist., Belize	EF222363		EU220304 EU220305
	TK20710	Belize dist., Belize	EF222395	EU185108	EU220306 EU220307
	TK20711	Belize dist., Belize			EU220325
	TK20712	Belize dist., Belize	EF222364		EU220308 EU220309
	AK7771	Orange Walk, Belize	EF222325	EU185118	EU220310 EU220311
	FN30223	Campeche, Mexico	EF222334		EU220312 EU220313
	FN30224	Campeche, Mexico	EF222328	EU185109	EU220314
	FN30225	Campeche, Mexico	EF222327		EU220315 EU220316
	FN30226	Campeche, Mexico	EF222408		
	FN30462	Yucatan, Mexico	EF222405		EU220317 EU220318
	FN30463	Yucatan, Mexico	EF222406		EU220319 EU220320
	FN30464	Yucatan, Mexico	EF222331	EU185119	EU220321

	FN30677	Campeche, Mexico	EF222333	EU185107	EU220323 EU220324
	FN30678	Campeche, Mexico	EF222337	EU185126	
	ASNHC1414	Campeche, Mexico	EF222359		EU220326
	SGP 1030	Peten, Guatemala	EF222418		
	SGP 1140	Peten, Guatemala	EF222419		
Rhogeessa genowaysi	TK20597	Chiapas, Mexico	EF222326		EU220390
Rhogeessa gracilis	AK11059	Oaxaca, Mexico	EF222360	EU185103	EU220392
Rhogeessa io	TK15163	Guarico, Venezuela	EF222410		
	TK15164	Guarico, Venezuela	EF222384		EU220335
	TK15179	Guarico, Venezuela	EF222391		
	TK15209	Guarico, Venezuela	EF222392	EU185130	EU220336
	TK15286	Guatopo, Venezuela	EF222358	EU185124	EU220337
	TK19004	Bolivar, Venezuela	EF222393		EU220338
	TK19005	Bolivar, Venezuela	EF222394		EU220339
	TK19043	Bolivar, Venezuela	EF222347	EU185127	EU220340
	TK19450	Barinas, Venezuela	EF222404		EU220341
	TK 19458	Barinas, Venezuela	EF222348		EU220342
	TK19459	Barinas, Venezuela	EF222330	EU185110	
	TK22536	Darien, Panama	EF222369		EU220345 EU220346
	TK25079	Trinidad Nariva, Trinidad	EF222379		EU220344
	TK19519	Barinas, Venezuela	EF222407		EU220343
Rhogeessa mira	TK45014	Michoacan, Mexico	EF222336	EU185106	EU220391

Rhogeessa parvula	TK4690	Sinaloa, Mexico		EU185132	
	TK4765	Guerrero, Mexico	EF222353		
	TK14502	Sinaloa, Mexico	EF222344		
	TK14504	Sinaloa, Mexico	EF222357	EU185114	
	TK20651	Sonora, Mexico	EF222355		
	TK20653	Sonora, Mexico	EF222346		
Rhogeessa tumida	AK7136	Atlantida, Honduras	EF222370		
	AK7137	Atlantida, Honduras	EF222371		
	TK20516	Oaxaca, Mexico	EF222349	EU185115	EU220327
	TK20594	Chiapas, Mexico	EF222338		EU220356 EU220357
	TK20596	Chiapas, Mexico	EF222356		EU220358
	TK27068	Tamaulipas, Mexico	EF222345	EU185116	EU220328
	TK34866	San Salvador, El Salvador	EF222380		EU220359 EU220360
	TK34867	San Salvador, El Salvador	EF222353		
	TK34902	La Paz, El Salvador	EF222385		
	TK34980	La Paz, El Salvador	EF222390		EU220361 EU220362
	TK40186	Valle, Honduras	EF22350		EU220363 EU220364
	TK40345	Atlantida, Honduras	EF222377		EU220347
	TK40360	Atlantida, Honduras	EF222378	EU185117	
	TK101020	Valle, Honduras	EF222351		EU220365 EU220366
	TK101021	Valle, Honduras	EF222352	EU185113	EU220367 EU220368

TK101044	Valle, Honduras	EF222367		EU220369
TK101052	Valle, Honduras	EF222368		EU220370 EU220371
TK101266	Valle, Honduras	EF222409		EU220372 EU220373
TK101367	Comayagua, Honduras	EF222383	EU185128	EU220374 EU220375
TK101370	Comayagua, Honduras	EF222411		EU220389
AK1638	Tamaulipas, Mexico	EF222360		
AK7022	Gunacaste, Costa Rica	EF222335		
AK9585	Valle, Honduras	EF222326		
AK9587	Valle, Honduras	EF222372	EU185111	EU220376 EU220377
AK9613	Valle, Honduras			EU220378 EU220379
AK9615	Valle, Honduras	EF222373		
AK9617	Valle, Honduras	EF222373		
SP12543	Izabal, Guatemala	EF222396	EU185104	EU220349
SP12544	Izabal, Guatemala	EF222397	EU185112	EU220350
SP12606	Izabal, Guatemala	EF222398		EU220351
SP12615	Izabal, Guatemala	EF222399	EU185105	EU220352
SP12617	Izabal, Guatemala	EF222400		
SP12650	Izabal, Guatemala	EF222401		EU220353
SP12771	Zacapa, Guatemala	EF222402	EU185129	EU220380 EU220381
SP12772	Zacapa, Guatemala	EF222403	EU185133	EU220382 EU220383
AK 25022	El Progreso, Guatemala	EF222416		EU220384 EU220385
AK 25023	El Progreso, Guatemala	EF222413		EU220384 EU220385

	AK 25024	El Progreso, Guatemala	EF222414		EU220387 EU220388
	AK 25065	Izabal, Guatemala	EF222417		EU220354
	AK 25093	Izabal, Guatemala	EF222415		EU220355
Rhogeessa velilla	TK134692	Guayas, Ecuador	EF222341	EU185120	EU220329
	TK134693	Guayas, Ecuador	EF222342		
	TK134792	Guayas, Ecuador	EF222339	EU185121	EU220331
	TK134868	Guayas, Ecuador	EF222366		EU220333
	TK134869	Guayas, Ecuador	EF222365		EU220332
	TK134870	Guayas, Ecuador	EF222386	EU185122	EU220330
	TK134871	Guayas, Ecuador	EF222387		
	TK134872	Guayas, Ecuador	EF222388	EU185123	EU220334
	TK135175	Guayas, Ecuador	EF222389		
Antrozous pallidus	AK21090		EF222382		
Bauerus dubiaquercus	SP12598		EF222381	EU185102	EU220299 EU220300
Plecotus auritus			AY665169		
Myotis tricolor	SP13200			EU185101	

Bibliography

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. 1990. Basic local alignment search tool. J. Mol. Biol. 215:403-410.

Ambros, P. F. and A. T. Sumner. 1987. Correlation of pachytene chromomeres and metaphase bands of human chromosomes, and distinctive properties of telomeric regions. Cytogenetics and Cell Genetics 44: 223-228.

Arroyo-Cabrales, J. and O. J. Polaco 1997. *Rhogeessa mira*. Mammalian Species 550: 1-2.

Audet, D., M. D. Engstrom and M. B. Fenton. 1993. Morphology, karyology, and echolocation calls of *Rhogeessa* (Chiroptera: Vespertilionidae) from the Yucatan Peninsula. Journal of Mammalogy 74: 498-502.

Avise, J. C. 2000. Phylogeography. Cambridge: Harvard University Press.

Baker, R. J. 1984. Mammalian sympatric, cryptic species: a new species of *Rhogeessa* (Chiroptera: Vespertilionidae). Systematic Zoology 32: 178-183.

Baker, R. J. and J. L. Patton. 1967. Karyotypes and karyotypic variation of North American vespertilionid bats. Journal of Mammalogy 48: 270-286.

Baker, R. J. and J. W. Bickham 1986. Speciation by monobrachial centric fusions. Proceedings of the National Academy of Sciences 83: 8245-8248.

Baker, R. J. and R. D. Bradley. 2006. Speciation in mammals and the genetic species concept. Journal of Mammalogy 87: 643-662.

Baker, R. J., J. W. Bickham and M. L. Arnold 1985. Chromosomal evolution in *Rhogeessa* (Chiroptera: Vespertilionidae): Possible speciation by centric fusions. Evolution 39: 233-243.

Baker, R. J., M. H. Hamilton and D. A. Parish 2003. Preparations of mammalian karyotypes under field conditions. Occasional Papers, Museum of Texas Tech University 228: 1-8.

Basset, P., G. Yannic, H. Brunner, and J. Hausser. 2006. Restricted gene flow at specific parts of the shrew genome in chromosomal hybrid zones. Evolution 60: 1718-1730.

Baverstock, P. R., M. Gelder and A. Jahnke. 1983. Chromosome evolution in Australian *Rattus* – G-banding and hybrid meiosis. Genetica 60: 93-103.

Baverstock, P. R., M. Adams and C .H. S. Watts. 1986. Biochemical differentiation among karyotypic forms of Australian *Rattus*. Genetica 71: 11-22.

Bickham, J. W. 1979a. Banded karyotypes of 11 species of American bats (genus *Myotis*). Cytologia 44: 19-27.

Bickham, J. W. 1979b. Chromosomal variation and evolutionary relationships of vespertilionid bats. Journal of Mammalogy 58: 448-453.

Bickham, J. W. and R. J. Baker 1977. Implications of chromosomal variation in *Rhogeessa* (Chiroptera: Vespertilionidae). Journal of Mammalogy 58: 448-453.

Bickham, J. W., C. C. Wood and J. C. Patton. 1995. Biogeographic implications of cytochrome-b sequences and allozymes in sockeye (*Oncorhynchus nerka*). Journal of Heredity 86: 140-144.

Bickham, J. W., J. C. Patton, D. A. Schlitter, I.L. Rautenbach and R.L. Honeycutt. 2004. Molecular phylogenetics, karyotypic diversity and partition of the genus *Myotis* (Chiroptera: Vespertilionidae). Molecular Phylogenetics and Evolution 33: 333-338.

Bradley, R. D., J. J. Bull, A. D. Johnson, and D. M. Hillis. 1993. Origin of a novel allele in a mammalian hybrid zone. Proceedings of the National Academy of Sciences USA 90: 8939-8941.

Britton-Davidian, J., J. Catalan and K. Belkhir. 2002. Chromosomal and allozyme analysis of a hybrid zone between parapatric Robertsonian races of the house mouse: a case of monobrachial homology. Cytogenetic and Genome Research 96: 75-84.

Capanna, E. and R. Castiglia. 2004. Chromosomes and speciation in *Mus musculus domesticus*. Cytogenetics and Genome Research 105: 375-384.

Capanna, E., A. Gropp, H. Winking, G. Noack and M. V. Civitelli 1976. Robertsonian metacentrics in the mouse. Chromosoma 58: 341-353.

Castiglia, R., F. Annesi and E. Capanna. 2002. Contact zones between chromosomal races of *Mus musculus domesticus*. 3. Molecular and chromosomal evidence of restricted gene flow between the CD race (2n = 22) and the ACR race (2n = 24). Heredity 89: 219-224.

Cathey, J. C., J. W. Bickham and J. C. Patton. 1998. Introgressive hybridization and nonconcordant evolutionary history of maternal and paternal lineages in North American deer. Evolution 52: 1224-1229.

Chesser, R. K. and R. J. Baker. 1996. Effective sizes and dynamics of uniparentally and diparentally inherited genes. Genetics 144: 1225-1235.

Corbett, G. B. and J. E. Hill. 1991. A world list of mammalian species, 3rd edition. London: British Museum (Natural History).

Corti, M., E. Capanna and G. F. Estabrook. 1986. Microevolutionary sequences in house mouse chromosomal speciation. Systematic Zoology 35: 163-175.

Coyne, J. A. and H. A. Orr 2004. Speciation. Sunderland: Sinauer Press.

Delneri, D., I. Colson, S. Grammenoudi, I.N. Roberts, E.J. Lewis, and S.G. Oliver. 2003. Engineering evolution to study speciation in yeasts. Nature 422: 68-72.

Duff, A. and A. Larson. 2004. Mammals of the world: A checklist. New Haven and London: Yale University Press.

Engstrom, M. D. and D. E. Wilson. 1981. Systematics of *Antrozous dubiaquercus* (Chiroptera: Vespertilionidae), with comments on the status of Bauerus Van Gelder. Annals of the Carnegie Museum 50: 371-383.

Excoffier, L., P. E. Smouse and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Applications to human mitochondrial DNA restriction data. Genetics 131: 479-491.

Futuyma, D. J. and G. C. Mayer. 1980. Non-allopatric speciation in mammals. Systematic Zoology 29: 254-271.

Gannon, W. L., Sikes, R. S., and the Animal Care and Use Committee of the American Society of Mammalogists. 2007. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy 88:809-823.

Gazave, E., J. Catalan, M. G. Ramalhinho, M. L. Mathias, A. C. Nunes, D. Dumas, J. Britton_Davidian and J. –C. Auffray. 2003. The non-random occurrence of Robertsonian fusion in the house mouse. Genetical Research 81: 33-42.

Genoways, H. H. and R. J. Baker. 1996. A new species of the genus *Rhogeessa*, with comments on geographic distribution and speciation in the genus. Pp. 83-87. In: Contributions to Mammalogy: A Memorial Volume Honoring Dr. J. Knox Jones, Jr. (H.H. Genoways and R.J. Baker eds.) Museum of Texas Tech University.

Goodwin, G. G. 1958. Bats of the genus *Rhogeessa*. American Museum Novitates 1923: 1-17.

Gropp, A. and H. Winking 1981. Robertsonian translocations: Cytology, meiosis, segregation patterns and biological consequences of heterozygosity. Symposium of the Zoological Society of London 47: 141-181.

Hall, E. R. 1981. The Mammals of North America. New York: John Wiley & Sons.

Hauffe, H. C., T. Panithanarak, J. F. Dallas, J. Pialek, I. Gunduz and J. B. Searle. 2004. The tobacco mouse and its relatives: a "tail" of coat colors, chromosomes, hybridization and speciation. Cytogenetics and Genome Research 105: 395-405.

Hoelzer, G. A. 1997. Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees revisited. Evolution 51: 622-626.

Holloway, A. K., D. C. Cannatella, H. C. Gerhardt, and D. M. Hillis. 2006. Polyploids with different origins and ancestors form a single sexual polyploid species. American Naturalist 167: E88-E101.

Honacki, J. H., K. E. Kinman and J. W. Koeppl. 1982. Mammal species of the world: A taxonomic and geographic reference. Lawrence, Kansas: Allen Press.

Hoofer, S. R. and R. A. Van Den Bussche 2003. Molecular phylogenetics of the chiropteran family Vespertilionidae. Acta Chiropterologica 5(supplement): 1-63.

Huelsenbeck, J. P., D. M. Hillis and R. Nielsen. 1996. A likelihood-ratio test of monophyly. Systematic Biology 45: 546-558.

Jones, J. K., Jr. 1977. *Rhogeessa gracilis*. Mammalian Species 76: 1-2.

Jones, J. K., Jr., J. Arroyo-Cabrales, and R. D. Owen. 1988. Revised checklist of bats (Chiroptera) of Mexico and Central America. Occasional Papers of the Museum of Texas Tech University 120.

Kerth, G., F. Mayer and B. Konig. 2000. Mitochondrial DNA (mtDNA) reveals that female Bechstein's bats live in closed societies. Molecular Ecology 9: 793-800.

King, M. 1993. Species evolution: The role of chromosome change. New York: Cambridge University Press.

LaVal, R. K. 1973. Systematics of the genus *Rhogeessa* (Chiroptera: Vespertilionidae). Occasional Papers of the Museum of Natural History: The University of Kansas, Lawrence, Kansas 19: 1-47.

Maddison, W. P. and D. R. Maddison. 2006. Mesquite: a modular system for evolutionary analysis. Version 1.12 http://mesquiteproject.org

Moore, W. S. 1995. Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. Evolution 49: 718-726.

Moore, W. S. 1997. Mitochondrial-gene trees versus nuclear-gene trees, a reply to Hoelzer. Evolution 51: 627-629.

Mulcahy, D. G., B. H. Morill and J. R. Mendelson III. 2006. Historical biogeography of lowland species of toads (*Bufo*) across the Trans-Mexican Neovolcanic Belt and the Isthmus of Tehuantepec. Journal of Biogeography 33: 1889-1904.

Nachman, M. W., S. N. Boyer, J. B. Searle and C. F. Aquadro. 1994. Mitochondrial DNA variation and the evolution of Robertsonian chromosomal races of house mice, *Mus domesticus*. Genetics 136: 1105-1120.

Nachman, M. W. and J. B. Searle. 1995. Why is the house mouse karyotype so variable? Trends in Ecology and Evolution 10:397-402.

Nowak, R. M. 1994. Walker's bats of the world. Baltimore: The Johns Hopkins University Press.

Page, R. D. M. 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences 12: 357-358.

Posada, D. and K. A. Crandall. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817-818.

Ronquist, F. and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572-1574.

Roots, E. H. and R. J. Baker. 1998. *Rhogeessa genowaysi*. Mammalian Species 589: 1-3.

Roots, E. H. and R. J. Baker. 2007. *Rhogeessa parvula*. Mammalian Species 804: 1-4.

Ruedas, L. A. and J. W. Bickham. 1992. Morphological differentiation between *Rhogeessa minutilla* and *R. tumida* (Mammalia: Chiroptera: Vespertilionidae). Proceedings of the Biological Society of Washington 105: 403-409.

Searle, J. B. 1998. Selection and Robertsonian variation in nature: The case of the common shrew. Pp. 507-531 in R. G. Harrison (ed.) Hybrid Zones and the Evolutionary Process. New York: Oxford University Press.

Schneider, S., D. Roessli and L. Excoffier. 2000. Arlequin: A software for population genetics data analyses. Ver. 2.000. Genetics and Biometry Lab, Dept. of Anthropology, University of Geneva.

Sumner, A. T. 1990. Chromosome Banding. London: Unwin Hyman Ltd.

Swofford, D. L. 2002. Paup*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sunderland: Sinauer Associates, Inc..

Thomas, O. 1903. Two South American forms of *Rhogeessa*. Annals and Magazine of Natural History 7: 383.

Tosi, A. J., J. C. Morales and D. J. Melnick. 2003. Paternal, maternal and biparental molecular markers provide unique windows onto the evolutionary history of macaque monkeys. Evolution 57: 1419-1435.

Van Den Bussche, R. A., R. J. Baker, J. P. Huelsenbeck, and D. M. Hillis. 1998. Base compositional bias and phylogenetic analyses: A test of the "Flying DNA" hypothesis. Molecular Phylogenetics and Evolution 10:408-416.

Volleth, M. and K. -G. Heller. 1994. Phylogenetic relationships of vespertilionid genera (Mammalia: Chiroptera) as revealed by karyological analysis. Zeitschrift fur Zoologische Systematik und Evolutions-forschung 32: 11-34.

Volleth, M., K. -G. Heller, and J. Fahr. 2006. Phylogenetic relationships of three "Nycticeiini" genera (Vespertilionidae, Chiroptera, Mammalia) as revealed by karyological analysis. Mammalian Biology 71: 1-12.

Vonhof, M. J. 2000. *Rhogeessa tumida*. Mammalian Species 633: 1-3.

Ward, O. G., A. S. Graphodatsky, D. H. Wurster-Hill, V. R. Ermina, J. P. Park and Q. Yu. 1991. Cytogenetics of beavers: a case of speciation by monobrachial centric fusions. Genome 34: 324-328.

Weyandt, S. E., R. A. Van Den Bussche, M. J. Hamilton and D. M. Leslie, Jr. Unraveling the effects of sex and dispersal: Ozark Big-Eared bat (*Corynorhinus townsendii ingens*) conservation genetics. Journal of Mammalogy 86: 1136-1143.

White, B. J., C. Crandall, E. S. Raveche and J. H. Tjio 1978. Laboratory mice carrying three pairs of Robertsonian translocations: Establishment of a strain and analysis of meiotic segregation. Cytogenetics and Cell Genetics 21: 113-138.

White, M. J. D. 1968. Models of Speciation. Science 159: 1065-1070.

Wilkinson, G. 1985. The social organisation of the common vampire bat (*Desmodus rotundus*). II. Mating system, genetic structure and relatedness. Behavioral Ecology and Sociobiology 17: 123-134.

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