The Dissertation Committee for Scott Evan Solomon Certifies that this is the approved version of the following dissertation:

BIOGEOGRAPHY AND EVOLUTION OF WIDESPREAD LEAFCUTTING ANTS, *ATTA* SPP. (FORMICIDAE, ATTINI)

Committee:

Ulrich G. Mueller, Supervisor

Lawrence E. Gilbert

Mark A. Kirkpatrick

C. Randal Linder

Kenneth R. Young

BIOGEOGRAPHY AND EVOLUTION OF WIDESPREAD LEAFCUTTING ANTS, *ATTA* SPP. (FORMICIDAE, ATTINI)

by

Scott Evan Solomon, B.S.

Dissertation

Presented to the Faculty of the Graduate School of
The University of Texas at Austin
in Partial Fulfillment
of the Requirements
for the Degree of

Doctor of Philosophy

The University of Texas at Austin
August, 2007

Dedication

To my parents, Ira and Susan, for showing me the world

Acknowledgements

This project, and my studies in general, would never have been completed without the help of a great many people. First, my parents, who taught me that “C’s” are never acceptable. They also showed me the art of travel from a very young age, and probably never anticipated the effect it would have on me.

I have had many great teachers and mentors throughout the years, who showed me the wonder that exists in nature and taught me how to understand and appreciate it. My first advisor, Martin Wikelski, had a profound influence on my career as both a field and laboratory scientist, and showed me how to do both while still having fun. He also introduced me to Ulrich Mueller, to whom I am indebted for taking me on as a graduate student and supporting me over the years, and for giving me enough freedom to pursue any inclination, scientific or otherwise. Thank you also to Patrick Abbot, who showed me (and many of my fellow students) how to think about the big picture, and who was instrumental both in formulating the idea for this project and in securing funding for it.

Many thanks to all of my labmates, especially A. Mikheyev and C. Rabeling, who have provided support and camaraderie throughout the years, and who have become not only my trusted colleagues but also my good friends. Thank you also to the many other current and former students in the Department who have helped immensely with my

endless questions and consultations, especially J. Brown, S. Hedtke, S. Ron, B. Wee, and D. Zwickl.

There are countless people to thank for assisting me in the field and for otherwise helping obtain specimens, and there is never enough space to thank all of them. This project would simply not have been possible without their help. In particular, I would like to acknowledge Mauricio Bacci, Jr., who arranged all of the logistics for fieldwork in Brazil, and oversaw much of the subsequent laboratory analyses for the material collected there. I look forward to our ongoing collaborations.

I have been fortunate to work with many talented and enthusiastic undergraduate research assistants, who aided greatly in the organization of collections and especially in molecular analyses. In particular, S. Haferkamp, H. Luong, S. Narasimhan, and D. Seval made significant contributions to this project. Our laboratory technicians, M. Cooper and J. Scott have also been instrumental in keeping the lab running smoothly.

I would like to thank the following funding sources for their contributions: the National Science Foundation (DDIG: DEB 0407772; IGERT program in computational phylogenetics at UT), the Organization for Tropical Studies, the Smithsonian Tropical Research Institute, the Amazon Conservation Association, and The University of Texas at Austin Graduate Program in Ecology, Evolution, and Behavior.

Finally, thank you to my wife, Catharina, for her enduring support and understanding, and for reminding me always about the things that are most important.

BIOGEOGRAPHY AND EVOLUTION OF WIDESPREAD LEAFCUTTING ANTS, *ATTA* SPP. (FORMICIDAE, ATTINI)

Publication No._____________

Scott Evan Solomon, Ph.D.
The University of Texas at Austin, 2007

Supervisor: Ulrich G. Mueller

Leafcutter ants (*Atta* spp.) are Neotropical herbivores that play important ecological roles, but are also notorious pests, causing millions of dollars in annual damage to agriculture across their range. Three species, *A. cephalotes*, *A. sexdens*, and *A. laevigata*, stand out as the most widespread and economically important. Two studies were conducted using these three species to better understand their biogeography and evolutionary history.

First, using a maximum entropy niche-based modeling approach, locality information for these three species was used to (1) accurately describe the current geographic range of each species, (2) determine what factors limit their respective ranges, and (3) identify areas where each species is capable of becoming established. By comparing the model's predictions with published records and targeted surveys, a more accurate picture of the current ranges of each species was obtained. Areas in which a

species does not currently occur, but that are predicted to be suitable, may reveal the ecological factors limiting the spread of these species. Such areas may also represent potential sites for invasion by these ants, with potentially devastating results.

Second, these species were used to test the leading biogeographic hypotheses on the origins of high Amazonian diversity, an issue that remains unresolved despite much research. The hypotheses are the riverine barrier, Pleistocene refugia, and marine incursion hypotheses, each of which has been tested almost exclusively on vertebrates. A comparative, molecular phylogeographic approach was combined for the first time with paleodistribution modeling for the last glacial maximum to test these hypotheses on an insect. All analyses rejected the predictions of the riverine barrier hypothesis for each species. Tests of gene tree topology could not reject the refugia hypothesis for *A. sexdens*, while population-genetic and historical demography analyses failed to reject both the refugia and marine incursion hypotheses for all three species. However, coalescent-based estimates of population divergences for each species suggest that current population structure formed recently, suggesting that Miocene marine incursions have not promoted diversification in these species. Therefore, of the hypotheses examined, only the Pleistocene refugia hypothesis can explain the current population structure of Amazonian leafcutter ants.

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

The Amazon Basin harbors perhaps the greatest diversity of terrestrial life on earth (Wilson 1999, Gentry 1988, Wilson 1987, Lewinsohn, Freitas and Prado 2005). Why Amazonia, and the tropics in general, contain more species than temperate regions remains unclear (Moritz et al. 2000, Bush 1994). This question has typically been approached from either an ecological or an evolutionary perspective. From an ecological perspective, a number of hypotheses have been put forth to address the proximate causes of tropical species diversity, or how such diversity is maintained. On the other hand, the ultimate causes of high tropical diversity are addressed by evolutionary hypotheses that compare either the origin of tropical species (speciation), the loss of tropical species (extinction), or the relative rates of each.

The overall goal of this thesis is to test the evolutionary hypotheses on the origins of Amazonian species diversity. Most such hypotheses are concerned with how allopatry is achieved in Amazonia, since allopatric speciation is currently believed to be the predominant mode by which most species arise (Coyne and Orr 2004). The leading hypotheses on allopatric speciation in Amazonia are the riverine barrier hypothesis, the marine incursion hypothesis, and the Pleistocene refugia hypothesis (Haffer 1997, Bush

1994, Moritz et al. 2000), and are described in detail in Chapter 3. These hypotheses, although meant to be general enough to apply to all Amazonian species, have only been tested in a rigorous way using vertebrate model systems (Moritz et al. 2000). Since vertebrates comprise a relatively small proportion of tropical diversity (Wilson 1999), the general applicability of these findings to other Amazonian taxa is unclear.

The following chapters use leafcutter ants of the genus Atta as a model system for testing the previously mentioned hypotheses. These insects are an appropriate model system for such an analysis for several reasons. First, they are ubiquitous and abundant across the Amazon Basin, as well as in adjacent areas (Weber 1972, Gonçalves 1960, Gonçalves 1967). Second, they are easy to collect due to their large nest sizes and status as agricultural pests, which combine to make them well known to local residents (Hölldobler and Wilson 1990, Cherrett 1986a). Third, leafcutter ants are ecologically important as major herbivores and critical participants in nutrient cycling as they bring organic matter into their subterranean nests to cultivate their mutualistic fungi (Garrettson et al. 1998, Moutinho, Nepstad and Davidson 2003, Sternberg et al. 2007).

Three species of Atta are present in the Amazon Basin: *A. cephalotes*, *A. sexdens*, and *A. laevigata* (Gonçalves 1967, Kempf 1972, Weber 1972). The second chapter of this thesis examines the biogeography of these three species. Previous studies have focused either on restricted geographic regions (Bonetto 1959, Pollard 1982, Gonçalves 1967, Gonçalves 1951), individual countries (Daguerre 1945, Gonçalves 1960, Mariconi 1970, Fowler 1983, Mackay and Mackay 1986, Gustavo, Brener and Ruggiero 1994), or have only crudely outlined the entire range of selected species (Kempf 1972, Weber 1972).

Chapter 2 provides the most comprehensive estimate to date of the current geographic ranges of these species by combining known collecting localities and reports from the literature. Further, the potential range of each species is estimated using a maximum entropy (maxent), niche-based modeling approach (Phillips 2004, Phillips, Anderson and Schapire 2006). The relative importance of different abiotic variables for predicting whether a species occurs in a given locality are compared to determine what factors are most important for limiting the ranges of these species. Finally, the estimated potential range of each species is compared to an estimate of its current actual range, using absence data from detailed surveys. Areas in which the species does not currently occur but that are predicted to be suitable are interpreted as either, (1) areas in which the species has not had an opportunity to become established because it has not arrived there, or (2) areas in which a species has failed to become established due to some external factors. Chapter two concludes with a discussion of possible biotic and other ecological factors (e.g. competition, predation, diseases, etc.) that may explain the discrepancies between estimates of each species' current range and its potential range.

Chapter 3 uses a comparative, molecular phylogeographic approach to test the leading hypotheses on allopatric diversification in Amazonian leafcutter ant species. Phylogeography bridges the gap between population genetics and phylogenetics, and thereby provides insight into processes that occur at the interface between processes that occur within and between species (Avise et al. 1987, Avise 2000). This study is the first to use such an approach to explicitly test the hypotheses mentioned above on an Amazonian insect.

The predictions of each hypothesis (described in Table 3.1) were tested using multiple population-genetic and phylogenetic tools. Samples of each species were obtained from populations spanning much of the geographic range of each species. A section of mitochondrial DNA (mtDNA) was sequenced for a single individual from each colony. The sequences obtained varied in length from 635 to 701 base pairs, and span parts of the cytochrome oxidase I (COI) and tRNA-leucine (tRNAleu) genes, as well as the entire intergenic spacer region between these genes.

To test the predictions of the Pleistocene refugia hypothesis, paleodistribution analyses were performed to predict where each species was capable of existing during the last glacial maximum (LGM). Using the maxent models for current climatic conditions described in chapter two, the potential range of each species at the LGM was estimated by projecting the model onto a reconstruction of climatic conditions at the LGM. This approach provides an independent, a priori prediction of where each species was capable of occurring during the LGM, thereby avoiding many of the potential problems and biases often associated with tests of the refugia hypothesis (see Chapter 3). This study is the first to combine paleodistribution modeling with molecular analyses in an Amazonian species.

The cumulative results of all tests of the hypotheses in question suggest that, of the hypotheses examined, only the Pleistocene refugia hypothesis adequately explains the population structure currently present in these species of leafcutter ants. Although these results do not eliminate the possibility that other hypotheses (e.g. ecological gradients) may have been important in the formation of these and other species, they do provide renewed support for the role of recent climate change in the structuring of extant Amazonian species. Species-level phylogenetic analyses could provide additional support for the role of recent climate change in the formation of these ant species, or may suggest that other factors, including those examined here, have been important in past speciation events; such analyses are currently underway (Bacci et al. in prep.). In addition, future studies that combine paleodistribution modeling with molecular phylogeographic analyses, especially on invertebrates, will determine how general the conclusions of this study are to diversification processes in other Amazonian species.

Chapter 2: Estimating the potential geographic ranges of widespread leafcutter ants (*Atta* spp.) using a maximum entropy modeling approach

INTRODUCTION

Leafcutter ants (Formicidae: Attini) are among the most conspicuous, ecologically successful, and economically important of all insects in the Neotropics (Hölldobler and Wilson 1990; Cherrett 1986; Weber 1972). In particular, species in the genus *Atta*, while playing important roles in ecosystem function (Sternberg et al. 2007; Brener and Silva 1995; Garrettson et al. 1998; Moutinho, Nepstad, and Davidson 2003), also have devastating effects on human agriculture (Varon et al. 2007; Hernandez et al. 1999; Robinson and Fowler 1982; Cherrett 1986, 1986, 1986) due in part to their larger mature colony sizes and greater degree of polymorphism in comparison to their sister leafcutter genus *Acromyrmex*.

The species of *Atta* that are most destructive as agricultural pests, incoincidentally are those with the widest geographic distributions and broadest dietary breadths (Cherrett 1986; Hölldobler and Wilson 1990; Weber 1972; Cherrett and Peregrine 1976; Cherrett 1986). In this regard, two species, *A. cephalotes* and *A. sexdens* stand out as having the largest known ranges (Weber 1972; Hölldobler and Wilson 1990; Kempf 1972), the latter of which can be divided into several recognized subspecies (Borgmeier 1959) that have distinct evolutionary histories (Bacci et al. in prep). A third species, *A. laevigata*, has a somewhat smaller distribution (Weber 1972; Kempf 1972) but nevertheless is responsible for substantial economic impacts where it occurs (Hernandez et al. 1999; Jaffe 1986).

Previous biogeographic studies of leafcutter ants have focused either on restricted geographic regions (Bonetto 1959; Pollard 1982; Gonçalves 1967, 1951), individual countries (Daguerre 1945; Gonçalves 1960; Mariconi 1970; Fowler 1983; Mackay and Mackay 1986; Gustavo, Brener, and Ruggiero 1994) or have only crudely outlined the entire range of selected species (Kempf 1972; Weber 1972). No detailed reports exist on species' current geographic boundaries, their potential geographic ranges, or the factors that limit the spread of these species beyond their current extent.

Identifying the factors limiting the spread of these species is important not only for understanding their ecology, but also to predict where these species might be capable of spreading in the future. Although leafcutter ants are not generally considered invasive, some *Acromymyrmex* populations have been introduced and become established on Caribbean islands (Mikheyev, Mueller, and Abbot 2006; Pollard 1982; Cherrett and Peregrine 1976; Cherrett 1968), and ecologists and conservationists have long feared the devastation that could occur should something similar occur with *Atta* (Hölldobler and Wilson 1990).

The goals of this study were (1) to accurately describe the current geographic range of each of the three previously mentioned *Atta* species, (2) to estimate which factors are most important for limiting their respective ranges, and (3) to identify where each species is likely to be capable of becoming established. The recent application of the maximum entropy approach to niche-based species distribution modeling (Phillips, Anderson, and Schapire 2006) provides a powerful tool for accomplishing these goals.

MATERIALS AND METHODS

Estimates of the potential geographic range of each species were made using Maxent verson 2.3 (Phillips, Anderson, and Schapire 2006). Maxent uses presence-only species occurrence records (i.e. latitudes & longitudes of known species sightings) and environmental data (i.e. GIS layers) as input. In general, the Maxent approach seeks to estimate an unknown ("target") distribution using incomplete information about the target distribution and a given set of constraints. For modeling species potential geographical ranges, the occurrence data are considered to be the incomplete sample of a larger, unknown geographical distribution, and the environmental data are used as constraints (Dudik, Phillips, and Schapire 2004; Phillips, Anderson, and Schapire 2006). A recent comparison of methods for niche-based modeling of species potential ranges identified Maxent as among the best approach currently available in terms of predictive performance (Elith et al. 2006).

Localities used as known presence records for each species of leafcutter ant (Appendix A) came primarily from observations of nests or foraging workers by the author. Additional localities were obtained from collaborators (U. Mueller, A. Himler, N. Gerardo, C. Currie, A. Little, A. Mikheyev, S. Villamarin). Geographic coordinates for each locality were obtained using a handheld GPS unit (Garmin eTrex). Museum specimens, although abundant for many species of *Atta*, were generally not used in these analyses because (1) they often do not contain detailed geographic coordinates indicating where the collection was made, and (2) older collections may not accurately reflect where a species occurs today, especially for species sensitive to human-induced habitat alteration (Anderson 2003; Vasconcelos and Cherrett 1995).

Twenty bioclimatic layers (Table 2.1) for the entire New World were obtained from the WorldClim dataset (http://www.worldclim.org; version 1.4), each with a resolution of approximately 10 km. The methods used to generate these layers are described in Hijmans et al. (Hijmans et al. 2005). The “auto features” option was selected in Maxent for all analyses. In addition, the following settings were used for the full training runs for each species: 500 maximum iterations, a convergence threshold of 1.0E-5, “minimize memory use,” and a regularization multiplier equal to 1.0. A jackknife test was used to determine the effect that each environmental layer had on the overall performance of the full training model

Two approaches were used to determine whether the predictions generated by Maxent were better than random predictions. First, the area under the receiver Operating Characteristic curve (AUC), a commonly used measurement for comparison of model performance (Elith et al. 2006), was calculated for each species. The AUC varies from 0 to 1, with greater scores indicating better discrimination ability; an AUC greater than 0.5 indicates that the model discriminates better than random (Elith et al. 2006).

Second, a separate analysis was conducted by randomly splitting the localities into two sets: training and testing. The training set (75% of localities for *A. cephalotes*, 90% for *A. laevigata* and *A. sexdens*) was used to build the model while the testing set was used to test the predictive ability of that model. The number of localities used for testing versus training was dependent on how many sites were available for each species. To test the predictive ability of the model, Maxent's cumulative prediction was converted to a binary (i.e. presence vs. absence) prediction. Ten different thresholds were used for this conversion, and the extrinsic omission rate (the fraction of test localities that are outside the area in which the species is predicted to occur) was tested against the null hypothesis that it is no better than a random prediction (of equal area) using a one-tailed binomial test (Phillips, Anderson, and Schapire 2006). The same settings were used as for the full training runs, except for the number of test samples (25% for *A. cephalotes*, 10% for *A. laevigata* and *A. sexdens*).

RESULTS

95 unique presence records were obtained for *Atta cephalotes*, 51 for *A. sexdens*, and 41 for *A. laevigata* (Appendix A; Figure 2.1, left panel). The potential geographic ranges for each species estimated by the full training runs are shown in Figure 2.1 (right panel). The area under the receiver operating characteristic curve (AUC) was 0.996, 0.983, and 0.986 for *A. cephalotes*, *A. laevigata*, and *A. sexdens*, respectively. Furthermore, out of the ten different thresholds used to obtain a binary (i.e. presence/absence) prediction, all ten were significantly better than random models for all three species (Table 2.2).

The results of jackknife tests to determine the relative importance of each environmental variable for each species are shown in Figure 2.2. For *A. cephalotes*, the most important single variable was annual precipitation and the least important was altitude. The most important variable for *A. sexdens* was temperature seasonality, while the least important was precipitation seasonality. For *A. laevigata*, the most important single variable was isothermality (ratio of mean diurnal temperature range to annual temperature range), while the least important was precipitation of driest month. Thus for *A. cephalotes*, precipitation seems most important while *A. sexdens* and *A. laevigata* are more influenced by temperature. However, removing any single variable had a negligible effect on each model's predictive ability.

DISCUSSION

The Maxent approach (Dudik, Phillips, and Schapire 2004; Phillips 2004; Phillips, Anderson, and Schapire 2006) is a powerful new tool for estimating the potential geographic range of a species for which presence-only data are available. Using a subset of localities for which each leafcutter ant species is known to currently occur, Maxent accurately predicted the locations of other known sites. Using all of the known localities, full models were produced with AUC values ranging between 0.983 and 0.996. Given that AUC values greater than 0.75 are considered to contain "a useful amount of discrimination," (Elith et al. 2006, p.137), and that the average AUC for South American plant species in a recent comparison of available methods (Elith et al. 2006) was 0.78, the models generated in the current study appear to be comparatively good.

The data used to model the conditions in which each species is most likely to occur were abiotic variables limited to various measures of precipitation, temperature, and elevation. Future analyses that include additional environmental data could determine whether other variables might also be relevant for these species. Of particular interest are variables related to human habitation and disturbance, such as land-use and landcover, as these species can be affected by such anthropogenic factors (Vasconcelos and Cherrett 1995; Vasconcelos et al. 2006).

Although the abiotic factors used in this analysis performed well in terms of their ability to predict where each species currently exists, any niche-based approach will almost always produce an overestimate of the actual, current geographic range of a species (Phillips, Anderson, and Schapire 2006). This is because the model output is an estimate of the species' fundamental niche and not its realized niche (Hutchinson 1957). The realized niche may be constrained by intrinsic and extrinsic biotic factors, such as interspecific competition, predation, dispersal ability, and the presence or absence of important parasites, mutualists, or diseases (but see Araujo and Guisan 2006).

Absence data, although often difficult to obtain and potentially unreliable due to the fact that proving the absence of anything is virtually impossible (Graham et al. 2004; Anderson 2003), leafcutter ants of the genus *Atta* provide a possible exception. Since mature Atta nests are conspicuous landscape features (Moreira et al. 2004; Hölldobler and Wilson 1990; Weber 1972) and the ants themselves are such notorious pests with large foraging territories (Cherrett 1986a, 1986b), it is unlikely that the presence of these insects could go unnoticed by local human residents. Therefore, surveys using interviews with locals, combined with active searches by trained experts, are likely to produce an accurate determination of whether leafcutters are present or absent from a particular region. Identifying which exact species is/are present (e.g. *A. sexdens* vs. *A. cephalotes*), however, requires finding a representative specimen, despite the ubiquity of common names for each genus and, occasionally, each species (Weber 1972). Nevertheless, the use of local knowledge can be a powerful tool for surveying leafcutter ant populations. By combining the estimated distribution maps with absence data obtained from published literature and surveys near the edge of each species' geographic range, the factors limiting the spread of each species beyond their current limits may be discernable.

Individual species accounts

Atta cephalotes

A. cephalotes is known to occur from Southern Mexico through Central America and across the Amazon Basin, with an apparently disjunct population in the Atlantic Coastal Forests of Brazil (Gonçalves 1951, 1960, 1967; Mariconi 1970; Weber 1972; Hölldobler and Wilson 1990; Correa et al. 2005; Kempf 1972) (Figure 2.1A). Throughout this region, it is considered a major pest on crops such as citrus, coffee and cocoa, as well as ornamental plants such as roses and other flowers (Cherrett 1986; Cherrett and Peregrine 1976).

The abiotic factors most important for limiting the range of this species, according to the full Maxent model, were primarily related to precipitation (Figure 2.2A). This is in agreement with observations by other authors that *A. cephalotes* is primarily a wet forest species (Weber 1959; Gonçalves 1960, 1967), and it can generally be found throughout most humid, lowland forests of Central and South America. Although it is often more abundant in secondary growth and other marginally disturbed habitats (Farji-Brener 2001), it is rarely found far from forest cover of some sort (S. Solomon, pers. obs.). Surveys conducted in January 2003 in southern Mexico (Chiapas, Oaxaca, Tabasco, and Veracruz) suggest that the northwestern limit of this species' range is near the Sierra de los Tuxtlas (Solomon, pers. obs.). This is where mean annual precipitation and, as a result, the probability of occurrence as predicted by Maxent, both decline precipitously (Figure 2.1B).

The southern range limit predicted by Maxent, however, is less in agreement with survey data. *A. cephalotes* is not known to occur in the Brazilian states of Mato Grosso do Sul, Goias, or São Paulo despite the high probability of its occurrence in parts of these states as predicted by Maxent (Figure 2.1B). A survey in February-March 2004 in Mato Grosso near the town of Alta Floresta suggested that the southern edge of this species' distribution in this region is roughly concordant with the limits of standing forest. Incorporating data on current landcover may therefore fine-tune the predictive model for this species.

The Maxent models predicted that a disjunct population of *A. cephalotes* should occur along the Atlantic Coast of Brazil, roughly between the cities of Rio de Janiero in the south and Natal in the north. Indeed, the species has been recorded from much of this region (Gonçalves 1951; Correa et al. 2005; Delabie et al. 1997) and is not known from the intervening cerrado and caatinga habitats. The historical cause of its isolation from the rest of the species' range has been the subject of speculation (Gonçalves 1951, 1960, 1967), including an anecdotal report by Weber (Weber 1972) that it was accidentally introduced in the 1930s.

The Maxent models also predicted that *A. cephalotes* should occur with high probability on most Caribbean islands, including the Greater and Lesser Antilles, as well as on the Galápagos Islands in the Pacific (Figure 2.1B). With the exception of Trinidad and the Bocas del Toro archipelago of western Panama, where *A. cephalotes* is well known and abundant, this species is not known to occur on any other Caribbean islands. It should also be noted that *A. cephalotes* is reported in the literature to occur on Cocos Island, Costa Rica (Kempf 1972; Hogue and Miller 1981), an isolated volcanic island in the tropical Eastern Pacific Ocean, roughly halfway between the Galápagos Islands and mainland Central America. However, recent surveys determined that this species does not currently occur on this island, and most likely never has (Solomon and Mikheyev 2005).

Atta sexdens

A. sexdens ranges from Costa Rica to northern Argentina (Kempf 1972; Weber 1972). This species has traditionally been divided into three subspecies (*A. s. sexdens*, *A. s. piriventris*, and *A. s. rubropilosa*) (Borgmeier 1959), and recent molecular phylogenetic analyses suggest that these subspecies correspond to evolutionarily distinct taxa (Bacci et al. in prep). The present study considered only the subspecies *A. sexdens* sexdens, which has the widest distribution of the sexdens group. *A. sexdens sexdens* is a serious pest everywhere it occurs, and has been shown to readily colonize human-altered habitats such as parks and agricultural fields (Zanuncio et al. 2002; Delabie et al. 1997; Weber 1972, 1959).

According to the full Maxent model, the abiotic factors most important for limiting the range of *A. sexdens* were those related to temperature (Figure 2.2B). However, *A. sexdens* appears to be more versatile with respect to microclimate than A. cephalotes, as it can be found nesting in closed-canopy forest or in open grassland, and is often associated with disturbed habitats (Weber 1959; Solomon, pers. obs.). The Maxent model predicted that the probability of this species occurring north of Nicaragua is relatively low, and this seems to be consistent with survey data (Solomon, pers. obs.). The species apparently does not occur in western Ecuador (Solomon, pers. obs.) where it is predicted to occur with high relative probability. The actual southern edge of *A. sexdens*' range is not well known, due in part to the unresolved taxonomy of this species group. *A. sexdens* was predicted to occur with some likelihood on islands in the Lesser Antilles, including Trinidad, and also on Hispaniola and Jamaica; this species is not known to occur on any of these islands.

Atta laevigata

A. laevigata, although more geographically restricted than the previous species, ranges across much of the eastern Amazon Basin, across the cerrados of central and southern Brazil, and north into the llanos of Venezuela and part of Colombia (Figure 2.1E) (Kempf 1972; Gonçalves 1960, 1967; Mackay and Mackay 1986). This species, which is more common in open habitat than closed forest (Gonçalves 1960, 1967) is a serious pest on pine plantations (Jaffe 1986) as well as cocoa, manioc, and some fruit trees (Gonçalves 1967) It is commonly seen nesting along roads, which serve as corridors for expanding its range into areas that might otherwise be inaccessible (Vasconcelos et al. 2006).

According to the Maxent analyses, temperature variability limits the distribution of *A. laevigata* (Figure 2.2C). However, the predicted range for this species disagrees with survey data to a greater extent than for the previous two species. *A. laevigata* is not known to occur west of the Andes, including Central America, despite the high relative probability of the Maxent model for parts of Panama, Nicaragua, Honduras, and western Ecuador (Figure 2.1F). Likewise, surveys in southeast Peru in 2004 did not find this species (Solomon pers. obs.). It was also predicted to occur on many Caribbean islands (Figure 2.1F) but is not known to occur on any, including Trinidad (Kempf 1972; Cherrett 1968; Pollard 1982).

Factors limiting the spread of leafcutting ants

The three leafcutter ant species examined in this study do not currently occupy all suitable habitats, according to the Maxent models. There are a number of possible reasons why this might be so. First, they do not occur on many Caribbean or Pacific islands. The only exceptions are that *A. cephalotes* occurs on the Caribbean islands of Trinidad and Tobago as well as on the Bocas del Toro islands of Panama. However, these continental islands have been connected to mainland South America and Central America during recent periods of lower sea levels (Carr-Brown 1972), so their presence there does not necessarily indicate having dispersed across the sea. The absence of *A. cephalotes* from most Caribbean islands is almost certainly due to limitations of the dispersal ability of mated queens. Although their precise dispersal ability is not known, estimates range from 10 to a maximum of 50 km in *A. texana* (Moser 1967).

The factors that have limited the spread of these species across the continental regions of South and Central America are less clear. Some physical barriers to dispersal may play a role, especially in the case of *A. laevigata*, which does not occur west of the Andes (Mackay and Mackay 1986; Kempf 1972). However, the Andes do not seem to have prevented the spread of *A. cephalotes* or *A. sexdens*, both of which occur on either side, and their present distributions were probably achieved after the rise of the northern Andes in the Miocene (Hoorn et al. 1995). Furthermore, suitable habitat appears to exist for all three species through several corridors in the Colombian Andes as well as for *A. cephalotes* and *A. laevigata* in northern Peru (Figure 2.1: B, D, F). *Atta* can generally be found up to 1,500 meters in elevation (Solomon, pers. obs.), and some species have been reported as high as 2,000 meters (Weber 1972; Mackay and Mackay 1986), making these corridors potentially suitable.

Predation by army ants has been suggested as a determinant in structuring tropical ant communities (Kaspari and O'Donnell 2003). Indeed, the army ant *Nomamyrmex esenbeckii* is perhaps the only well documented predator of mature colonies of Atta (Sanchez-Pena and Mueller 2002; Swartz 1998; Powell and Clark 2004). Powell and Clark's (Powell and Clark 2004) study of predation by *N. esenbeckii* on *A. cephalotes* and *A. columbica* in Panama hinted that these predators do not distinguish between *Atta* species as potential prey; anecdotes and reports in the literature of attacks on other *Atta* as well as *Acromyrmex* species support this conclusion (Borgmeier 1955). However, studies on the differential effects of army ant predation on leafcutter ant demography are necessary before predation by army ants can be shown to influence the geographic ranges of *Atta* species.

Since leafcutter ants are generalist herbivores (Weber 1972), it seems unlikely that the availability of foraging material would limit the spread of these species. Although they display preferences for particular substrates, most species are capable of utilizing an enormous breadth of organic material to cultivate their fungal gardens (Rockwood 1976; Gonçalves 1960, 1967). Unlike some leafcutter ant species that specialize on monocots or dicots, the three species discussed here are capable of harvesting both kinds of vegetation (Cherrett 1986), and in laboratory settings they accept a wide variety of material including many items that they would not naturally encounter.

Parasitoid flies of the family Phoridae are thought to play an important role in structuring ant communities by mediating the outcome of interspecific competition (Feener 2000; LeBrun and Feener 2002; Mehdiabadi, Kawazoe, and Gilbert 2004; Philpott 2005). Phorid parasitoids are known to attack each of the *Atta* species discussed here (Orr 1992; Braganca and Medeiros 2006; Tonhasca, Braganca, and Erthal 2001; Erthal and Tonhasca 2000; Disney 1996), and some evidence suggests that the presence of phorids disrupts foraging behavior in *Atta* (Orr 1992; Braganca, Tonhasca, and Della Lucia 1998). Individual phorid species are known to parasitize multiple *Atta* species (Disney 1996), indicating a general lack of host specificity. This suggests that the presence or absence of phorids probably does not limit the range of the *Atta* species in question since potential parasites exist beyond their current distribution. However, the role that phorid parasitoids play, directly or indirectly, in the outcome of competition between *Atta* species deserves further investigation.

Since leafcutter ants are obligately dependent upon their fungal cultivars for food, it seems conceivable that the distribution of any particular leafcutter ant species might, in part, be a function of the abiotic or biotic limitations of its fungal cultivar. However, recent molecular work suggests that all leafcutter ant species (*Acromyrmex* and *Atta*) cultivate the same species/strain of cultivar across their entire geographic range and that, in any one region, the leafcutter ant species appear to exchange fungal cultivars with some frequency (Mikheyev, Mueller, and Abbot 2006; Bruschi et al. in prep). Therefore, given the fact the other leafcutter species exist beyond the current distributional limits to each of the *Atta* species investigated in this study, it is unlikely that the fungal cultivar plays a role in limiting these species' geographic ranges.

Likewise, the pathogenic fungi and mutualistic actinomycete bacteria that are thought to play important roles in the ecological and evolutionary dynamics of these ants do not appear to be species-specific (Cafaro and Currie 2005) and therefore are unlikely to limit the geographic ranges of the species in question for the same reason mentioned above.

Although evidence from research on habitat selection, foraging patterns, and territoriality of sympatric species of *Atta* (Rockwood 1973; Weber 1959; Delabie et al. 1997; Rao 2000) hint that interspecific competition may occur, surprisingly few direct studies exist on this subject. More work is needed in this area to determine whether competition from other *Atta* or *Acromyrmex* species might play roles in delineating the boundaries of leafcutter ant species.

CONCLUSIONS

This study is the first to combine known occurrence data with predictive niche-based distribution modeling to describe the potential geographic range of leafcutter ants. Since the model's output represents an estimation of a species' fundamental niche, not its realized niche (Hutchinson 1957), this approach necessarily overpredicts the actual current range of a given species (Elith et al. 2006). However, by using published records and targeted surveys, including interviews with local inhabitants, absence data can be used to obtain a more accurate picture of the current range of these species (Graham and Hijmans 2006). Areas in which a species does not currently occur, but that are predicted to be suitable, represent potential sites for further investigation into what ecological factors have prevented the spread of the species thus far. Such areas, especially some Caribbean islands (Cherrett 1968; Pollard 1982), may be prone to future establishment of these species and care should thus be taken to avoid accidental introductions.

Name	Variable
BIO1	Annual Mean Temperature
BIO2	Mean Diurnal Range (Mean of monthly (max temp - min temp))
BIO3	Isothermality ((BIO2/BIO7)*100)
BIO4	Temperature Seasonality (standard deviation *100)
BIO5	Max Temperature of Warmest Month
BIO6	Min Temperature of Coldest Month
BIO7	Temperature Annual Range (BIO5-BIO6)
BIO8	Mean Temperature of Wettest Quarter
BIO9	Mean Temperature of Driest Quarter
BIO10	Mean Temperature of Warmest Quarter
BIO11	Mean Temperature of Coldest Quarter
BIO12	Annual Precipitation
BIO13	Precipitation of Wettest Month
BIO14	Precipitation of Driest Month
BIO15	Precipitation Seasonality (Coefficient of Variation)
BIO16	Precipitation of Wettest Quarter
BIO17	Precipitation of Driest Quarter
BIO18	Precipitation of Warmest Quarter
BIO19	Precipitation of Coldest Quarter
Altitude	Elevation above sea level

Table 2.1: Environmental layers used in predictive modeling of leafcutter ant species (from http://www.worldclim.org/bioclim.htm). The methods used to generate these layers are described in Hijmans et al. (2005).

Cumulative threshold	Threshold description	Fractional predicted area	Training omission rate	Test omission rate	*P*-value
A. cephalotes					
1	Fixed cumulative value	0.232	0	0.022	8.94E-33
5	Fixed cumulative value	0.152	0	0.044	4.23E-51
10	Fixed cumulative value	0.114	0	0.044	0.00E+00
11.906	Minimum training presence	0.104	0	0.044	0.00E+00
69.158	10 percentile training presence	0.008	0.096	0.156	0.00E+00
48.324	Equal training sensitivity and specificity	0.022	0.022	0.089	0.00E+00
54.815	Minimum training sensitivity plus specificity	0.016	0.022	0.111	0.00E+00
21.753	Equal test sensitivity and specificity	0.067	0.022	0.067	0.00E+00
47.237	Minimize test sensitivity plus specificity	0.023	0.022	0.067	0.00E+00
2.009	Balance training omission, predicted area and threshold value	0.198	0	0.022	8.77E-40
A. sexdens					
1	Fixed cumulative value	0.208	0.017	0.167	1.91E-03
5	Fixed cumulative value	0.144	0.033	0.167	3.31E-04
10	Fixed cumulative value	0.109	0.033	0.167	8.55E-05
0.876	Minimum training presence	0.212	0	0.167	2.10E-03
56.136	10 percentile training presence	0.018	0.083	0.167	1.25E-08
22.16	Equal training sensitivity and specificity	0.067	0.067	0.167	7.48E-06
56.136	Minimum training sensitivity plus specificity	0.018	0.083	0.167	1.25E-08
3.034	Equal test sensitivity and specificity	0.167	0.033	0.167	6.65E-04
56.136	Minimize test sensitivity plus specificity	0.018	0.083	0.167	1.25E-08
0.876	Balance training omission, predicted area and threshold value	0.212	0	0.167	2.10E-03

Cumulative threshold	Threshold description	Fractional predicted area	Training omission rate	Test omission rate	P-value
A. laevigata					
1	Fixed cumulative value	0.277	0	0	1.26E-04
5	Fixed cumulative value	0.188	0.014	0	8.46E-06
10	Fixed cumulative value	0.143	0.014	0	1.21E-06
2.532	Minimum training presence	0.229	0	0	3.27E-05
22.508	10 percentile training presence	0.082	0.085	0	2.56E-08
21.887	Equal training sensitivity and specificity	0.085	0.085	0	3.08E-08
20.834	Minimum training sensitivity plus specificity	0.088	0.056	0	4.15E-08
44.532	Equal test sensitivity and specificity	0.032	0.169	0	3.59E-11
44.532	Minimize test sensitivity plus specificity	0.032	0.169	0	3.59E-11
2.209	Balance training omission, predicted area and threshold value	0.236	0	0	4.13E-05

Table 2.2: Results of threshold–dependent evaluation of model performance. Each threshold was used to obtain a binary (presence vs. absence) prediction for each species. Fractional predicted area = proportion of total area predicted to contain the species; Omission rate = rate of failure to predict a species' occurrence where it is known to occur. *P*-values indicate the probability for each threshold that the model's predictions are better than random using a one-tailed binomial test.

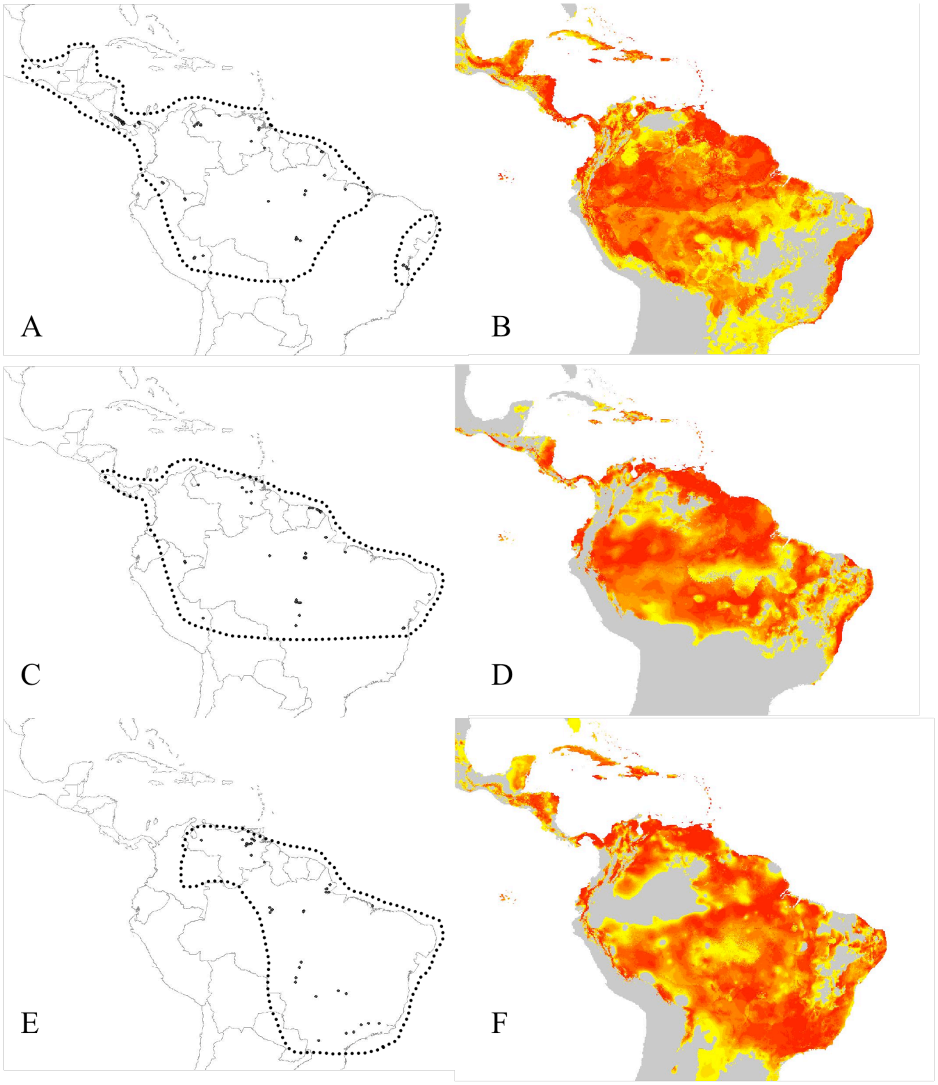

Figure 2.1: Niche-based modeling of the geographic ranges of three *Atta* species (A–B, *Atta cephalotes*; C–D, *Atta sexdens*; E–F, *Atta laevigata*). Left panel: the localities used as training data for the model for each species are indicated with a dot. The approximate current range of this species, based on published distribution maps (Gonçalves, 1960; Weber, 1972) is outlined with a dotted line for each species. Right panel: The corresponding continuous predictions of the current geographic range for each species, with warmer colors indicating a higher probability of occurrence.

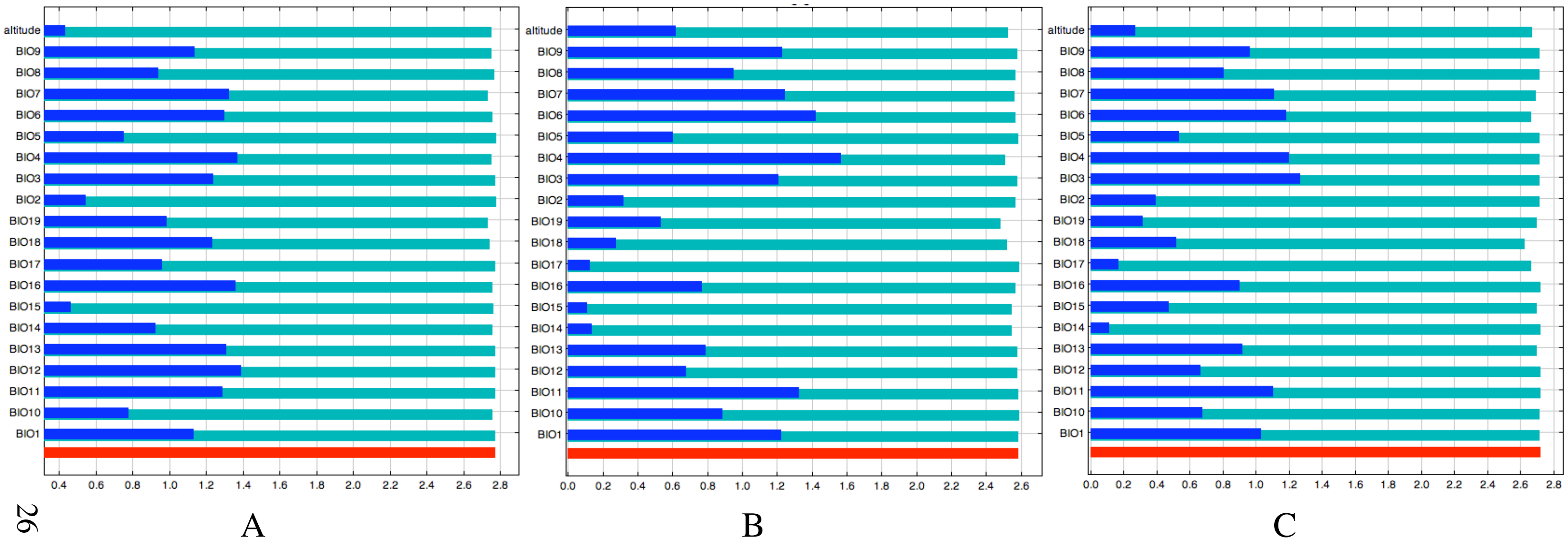

Figure 2.2: Results of jackknife tests of variable importance for each species. The environmental variables used to build the model are shown on the y-axis, and the relative contribution of each variable to model gain is shown on the x-axis. Dark blue bars show the contribution each variable makes to model gain when only that variable is used. Light blue bars show the model gain when that variable is removed. The red bar on the bottom of each graph shows the total model gain when all variables are used.

Chapter 3: Comparative molecular phylogeography and paleodistribution modeling of leafcutter ants (Atta spp.) sort hypotheses on the origins of Amazonian species diversity

INTRODUCTION

Tropical regions around the world are well known for the rich diversity of life they harbor. Yet, the reasons why the tropics contain more species than temperate and arctic regions remain unclear (Rohde 1992; Hillebrand 2004; Wright et al. 2006; Weir and Schluter 2007). Research into this area, which is fundamental to the fields of biogeography, ecology, and conservation biology, has generally been divided between the mechanisms that generate tropical diversity (Moritz et al. 2000; Leigh et al. 2004; Jablonski et al. 2006; Weir and Schluter 2007) versus those that maintain it (Pianka 1966; Phillips et al. 1994).

The Amazon Basin has been of particular interest in this matter, as it harbors perhaps the world's greatest terrestrial biodiversity (Wilson 1987; Gentry 1988; Wilson 1999; Lewinsohn et al. 2005). As is true for the study of speciation in general (Coyne and Orr 2004), much of the focus has been on the biogeography of processes generating diversity in the Amazon Basin (Moritz et al. 2000). Many hypotheses on the biogeography of Amazonian speciation have been suggested (Bush 1994; Haffer 1997). Perhaps the earliest can be traced to observations by Alfred Russell Wallace, who noted that rivers often separate related species of birds and primates (Wallace 1852). This idea was later shaped into the riverine barrier hypothesis, which suggests that tropical rivers, which are wide and numerous, may serve as barriers to gene flow for terrestrial organisms, thus promoting allopatric divergence of populations restricted to either side (Patton et al. 1994; Peres et al. 1996; Gascon et al. 2000; Matocq et al. 2000; Moritz et al. 2000; Patton et al. 2000).

The riverine barrier hypothesis has received mixed support. On the one hand, major Amazonian rivers do seem to restrict dispersal of passerine birds (Hayes and Sewlal 2004), small primates (Hershkovitz 1977), lizards (Avila-Pires 1995; Pellegrino et al. 2005), and Riodinid butterflies (Hall and Harvey 2002). However, extensive molecular and morphological work on small mammals and frogs along the Juruá River, a major tributary of the Amazon, has failed to detect a significant river barrier effect (Patton et al. 1994; Gascon et al. 2000; Patton et al. 2000).

A second hypothesis on the evolutionary origin of Amazonian species stems from evidence that elevated sea levels, most recently during the early Miocene (15–23 mya), flooded much of the Amazon basin in salty or brackish water (Rasanen et al. 1995; Vonhof et al. 2003; Hovikoski et al. 2005; Hovikoski et al. 2007; Latrubesse et al. 2007). This would have restricted all terrestrial organisms inhabiting the Amazon region to become isolated in areas of higher elevation, namely near the Andes to the west, the Guiana Shield to the north, and the Brazilian Shield to the south (Figure 2.1). The result, as for the refugia model, would be the subsequent divergence of these populations. Support for this marine incursion hypothesis has been found in woodcreepers (Aleixo 2004) and freshwater fish (Lovejoy et al. 2006).

A third hypothesis, like the riverine barrier model, was also inspired by distributions of Amazonian birds. Haffer (Haffer 1969) noted that avian species richness in South America was unevenly distributed, with certain regions containing substantially more species than others. Furthermore, these regions tended to receive more abundant rainfall than the rest of Amazonia. He suggested that historical climate changes, specifically during glacial maxima, may have restricted the distribution of wet forests in Amazonia. The species that inhabit these forests would likewise have become more restricted, resulting in the possibility for allopatric speciation. Haffer (Haffer 1969) proposed the presence of several Pleistocene forest refugia along the periphery of the Amazon Basin, as well as in the Chocó region west of the Andes. The Pleistocene refugia hypothesis has subsequently been expanded to include some refugia outside Amazonia (Haffer and Prance 2001).

The Pleistocene refugia hypothesis has generated a substantial amount of research on Amazonian speciation (Bush 1994; Haffer 1997), as well as tropical speciation in general (Moritz et al. 2000). Although some studies (Brown et al. 1974; Brown 1982; Mayr and Ohara 1986; Fjeldsa 1994; Brower 1996) have found support for its predictions (Table 2.1), most have not (Smith et al. 2001; Aleixo 2004; Dick et al. 2004; Cheviron et al. 2005; Aleixo 2006). Furthermore, the refugia hypothesis has been criticized because (1) geological and paleoclimatic data do not generally support the conclusion that wet forests were highly fragmented during the Pleistocene (Colinvaux et al. 1996; Colinvaux et al. 2000; Colinvaux and De Oliveira 2001; Colinvaux et al. 2001), (2) the locations and size of forest refugia, if they did exist, would be different for each species because of different environmental tolerances (Bush 1994; Moritz et al. 2000), (3) some areas that have been proposed as refugia because they appear to contain greater species diversity can be explained as artifacts of sampling biases (Nelson et al. 1990), (4) the ages of many extant Amazonian species pre-date the Pleistocene, suggesting they were generated by earlier mechanisms (Bush 1994; Moritz et al. 2000; Wilf et al. 2003).

Two recent developments have allowed new insights into the predictions made by these hypotheses (see Table 3.1). First, advances in molecular techniques have not only increased the amount of data available for analysis, they also permit a more quantitative evaluation of species and population histories, which are essential for testing competing hypotheses on tropical diversification (Moritz et al. 2000). Although molecular reconstructions of the biogeography of past speciation events seems promising, the dynamic nature of species' geographic ranges makes these inferences somewhat tenuous (Losos and Glor 2003). An alternative approach is to examine the current population structure of widespread species. Such phylogeographic analyses can provide insight into the processes responsible for generating allopatry by giving not only a snapshot of the current population structure, but also a window into the past through the reconstruction of gene trees and historical demography (Avise et al. 1987; Moritz et al. 2000; Knowles 2004).

The second recent development combines new reconstructions of paleoclimates with a flurry of novel techniques for modeling species distributions under current as well as past (or future) climate conditions. These paleodistribution analyses have provided a means of independently assessing the extent to which past climate has influenced species' geographic ranges (Hugall et al. 2002; Elith et al. 2006; Ruegg et al. 2006), thereby avoiding many of the pitfalls associated with assumptions about the presence and location of putative forest refugia.

As Hugall et al. (Hugall et al. 2002) demonstrated for snails in the Australian Wet Tropics, these two approaches (molecular phylogeography and paleodistribution reconstruction) can be used in a complimentary fashion to test the hypotheses that have long generated fierce debate among students of tropical biology. However, paleoclimate data for the Amazon basin are not nearly as complete as for the Australian Wet Tropics (Bermingham et al. 2005), so such an approach has not yet been utilized for Amazonian species. Furthermore, the few studies that have used a molecular phylogeographic approach to test these supposedly universal hypotheses have primarily focused on vertebrate taxa (Patton et al. 1994; Peres et al. 1996; Lougheed et al. 1999; Patton et al. 2000; Smith et al. 2001; Aleixo 2004), which represent only a small proportion of the total diversity of the Amazonian region (Wilson 1987; Erwin 1991; Wilson 1999; Lewinsohn et al. 2005).

In this study, we used three co-distributed species of leafcutter ants in the genus *Atta* to test these biogeographic hypotheses on Amazonian diversification using a combination of comparative molecular phylogeography and paleodistribution modeling. Leafcutter ants are widespread throughout the Neotropics (Weber 1972; Hölldobler and Wilson 1990). They are generalist herbivores, cutting fresh vegetation as a food source for their mutualistic fungal gardens (Mueller et al. 1998). In fact, leafcutter ants are considered to be the dominant herbivores of the Neotropics (Hölldobler and Wilson

1990), and play a key ecological role in nutrient cycling as they bring organic material deep into their subterranean nests (Garrettson et al. 1998; Moutinho et al. 2003).

Three leafcutter ant species, *A. cephalotes*, *A. sexdens*, and *A. laevigata*, are ideal for testing the hypotheses in question because (1) they co-occur throughout much of the Amazon Basin, as well as in adjacent areas (Gonçalves 1967; Weber 1972), (2) the three species display a range of environmental tolerances (Weber 1959; Gonçalves 1960), permitting an evaluation of how historical climatic changes have differentially influenced each, and (3) they can be easily collected due to their enormous colony sizes (Hölldobler and Wilson 1990; Moreira et al. 2004) and infamous status as agricultural and garden pests (Cherrett and Peregrine 1976).

We used these three species as independent tests of the predictions of each hypothesis (summarized in Table 3.1). Furthermore, we hypothesized that, since these species have similar distributions, dispersal abilities, and life histories (Weber 1959; Gonçalves 1960, 1967; Weber 1972; Cherrett 1986b, c, a), the riverine barrier hypothesis and marine incursion hypothesis should both apply equally to all three. However, because of the ecological differences between the species, particularly in terms of their sensitivity to aridity (Chapter 2.), we hypothesized that each species would respond differently to historical climate change since the Pleistocene. Specifically, we predicted that species that are more restricted to wet forest habitats should track the distribution of wet forests more closely than species with greater environmental flexibility. As a result, these wet forest specialists were predicted to show a stronger response to wet forest fragmentation at the LGM, if such fragmentation occurred. To test these predictions, we used a rigorous statistical framework consisting of gene tree reconstructions, population genetic analyses, historical demographic analyses, and paleodistribution modeling.

MATERIALS AND METHODS

Collection of samples and molecular analyses

The 33 sampling locations (Figure 3.1, Appendix B) were chosen to allow testing of the hypotheses in question and to maximize coverage within each species' geographic range. Individual worker ants were collected at nests or along foraging trails and preserved in 95% ethanol during transport to The University of Texas at Austin (for samples collected outside Brazil) or the Universidade Estadual Paulista, Rio Claro, Brazil (for samples collected in Brazil), where they were stored at 4°C. The location of all samples was recorded using a handheld GPS unit (Garmin eTrex).

Total genomic DNA was extracted from one individual per colony using either the DNeasy Blood and Tissue Kit (QIAGEN) or the AccuPrep Genomic DNA Extraction Kit (Bioneer, Inc.). Several sets of mtDNA primers (Table 3.2) were used to amplify two sections of the cytochrome oxidase I (COI) gene, as well as an intergenic spacer, and a portion of the tRNA-leucine gene. PCR reactions contained 1 μl each of genomic DNA (approximately 10 ng), 1X reaction buffer, dNTPs, and MgCl2, 0.04 μl of Taq polymerase, and 5.96 μl of water for a total reaction volume of 10 μl. Average PCR conditions were as follows, with slight modifications depending on the annealing temperatures of individual primer pairs: Initial denaturation at 95°C for 3 minutes was followed by 35 cycles of 95°C for 5 seconds, and an annealing temperature that increased by 0.5°C for each successive round of amplification, beginning at 45°C, for 20 seconds each round, with a final elongation step of 68°C for 15 seconds. PCR products were analyzed by running 3 μl of the product on a 1.5% agarose gel and subsequently visualized with ethidium bromide staining. For samples that successfully amplified, the remaining 7 μl of PCR product were purified by polyethylene glycol (PEG) precipitation, using a 1:1 PCR product/20% PEG mixture which was incubated for 15 min at 37°C followed by a 10-min centrifugation at 2,688 X g and two washes with 80% ethanol.

Cycle sequencing reactions were performed for both forward and reverse sequences using the ABI BigDye Terminator Kit (version 3.1). Sephadex column purification was used to clean the cycle-sequencing product, which was then analyzed on a PRISM 3100 genetic analyzer (Applied Biosystems). Forward and reverse sequences were assembled into individual contigs using SeqMan II v.5.05 (DNASTAR) and alignments between sequneces were created initially using Clustal X (Thompson et al. 1999) and then adjusted manually in MacClade v. 4.06 (Maddison and Maddison 2000).

Paleodistribution modeling

Estimates of the potential geographic range of each species during the last glacial maximum (LGM), approximately 21 kya, were made using MaxEnt version 2.3 (Phillips et al. 2006). The MaxEnt approach estimates a species' potential geographic distribution using known occurrence localities and environmental data for those localities (Dudik et al. 2004; Phillips 2004; Phillips et al. 2006). Niche-based models of the current distribution of each species using 20 environmental layers (Table 2.1) were projected onto an estimate of the same environmental layers during the LGM (see Chapter 2 for details on how the models were created; see Ruegg et al. 2006 for a description of how the layers for the LGM were generated). A binary (presence vs. absence) prediction for the LGM was necessary for hypothesis tests (see below). To obtain a binary prediction, threshold values were chosen that minimized the commission (false positive) rate for current conditions, based on absence data obtained from recent surveys (see Chapter 2: Discussion). The cumulative probability thresholds chosen for *A. cephalotes*, *A. sexdens*, and *A. laevigata* were 1, 5, and 5, respectively. The results of the paleodistribution models were used in subsequent analyses to provide a priori population groupings for all tests of the refugia hypothesis in the following way: areas that were predicted to provide contiguous blocks of suitable habitat during the LGM (using the binary prediction) were grouped together as a single population (Figure 3.2: C, F, I); areas that were predicted not to be suitable were ignored for the purposes of hypothesis testing (see below).

Gene tree topology tests

Each hypothesis makes a specific prediction about the genealogical relationships between populations across the geographic range of each species (see Table 2.1). Specifically, given enough time, isolated populations that have diverged evolutionarily are expected to become reciprocally monophyletic (Avise 2000; Moritz et al. 2000). The relationships predicted by a strict interpretation of each hypothesis, assuming complete lineage sorting, were converted into backbone constraint topologies as follows. For the riverine barrier hypothesis, populations occurring on either bank (i.e. north and south) of the Amazon River should be reciprocally monophyletic. For the marine incursion hypothesis, populations near the eastern base of the Andes, on the Brazilian Shield, and on the Guyana Shield should be reciprocally monophyletic. For the refugia hypothesis, populations that were predicted by the paleodistribution models to persist during the last glacial maximum (Figure 3.2, middle rows) should be reciprocally monophyletic.

To determine whether these predictions were met, mitochondrial DNA gene trees were estimated, using unique haplotypes, with maximum likelihood and Bayesian inference techniques. Maximum likelihood searches were performed with a beta version of GARLI (Zwickl 2006) that allows backbone constraints (version 0.952 Beta), with default settings and parameters estimated according to the model of evolution selected using the Akaike Information Criterion (AIC) as implemented in ModelTest (Posada and Crandall 1998). The best tree consistent with the constraint topology for each hypothesis was then found using identical settings. In order to assess whether the null hypothesis represented by the constraint trees could be rejected, the difference between the log-likelihood values of the best constrained and best unconstrained trees was used as a test statistic, with statistical significance assessed through simulation (parametric bootstrap or SOWH test (Hillis et al. 1996; Goldman et al. 2000)). One hundred simulated datasets were generated using Seq-gen (Rambaut and Grassly 1997), with parameters estimated by PAUP* (Swofford 2002) from the best constrained tree under each constraint.

Constrained and unconstrained searches were performed in GARLI on the simulated data using identical settings as for the empirical data. The distribution of differences between constrained and unconstrained searches on the simulated data was used to assess the significance of the test statistic; the *p* value was equal to the number of simulated datasets (out of 100 replicates) with a difference in log-likelihood scores between constrained and unconstrained searches greater than the empirical difference. The null hypothesis (i.e. constraint topology) was rejected when *p* values were less than 0.05.

Bayesian searches were conducted in MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003). Four separate runs were conducted, each with four incrementally heated chains and uninformative, default priors; convergence and optimal burn-in were assessed as described in (Brown and Lemmon 2007) using the program MrConverge (Lemmon, in prep). After discarding burn-in, the posterior samples of tree topologies for each run were combined in PAUP*; the combined posterior sample was then filtered with the constraint tree for each hypothesis. The proportion of trees retained by the filter was the Bayesian posterior probability of that hypothesis.

Population Genetic Structure

To determine whether populations are structured as predicted by each of the hypotheses in question (Table 3.1), two types of population-genetic analyses were performed, using all ingroup haplotypes for each. Analysis of molecular variance (AMOVA) was used, as implemented in Arlequin 3.11 (Excoffier et al. 2005), to calculate the percentage of variance explained by *a priori* population groupings in a hierarchical framework (Excoffier et al. 1992). The population structure was defined for each species/hypothesis, as for the constraint trees in phylogenetic analyses. Tamura and Nei distances with an alpha shape parameter were used to compute the pairwise distance matrix for all AMOVA calculations, as this is the most complex model of sequence evolution currently available in Arlequin (Excoffier et al. 2005). Transitions and transversions were given equal weight, while deletions (i.e. gaps) were ignored. Statistical significance of variance components was assessed using the permutation procedures described in the Arlequin user's manual (http://cmpg.unibe.ch/software/arlequin3/arlequin31.pdf).

To further test for the presence of barriers to gene flow, as predicted for each hypothesis, simple and partial Mantel tests (Mantel 1967; Smouse et al. 1986) were conducted on the following matrices. First, the pairwise maximum likelihood genetic distance between individuals (as defined *a priori* for each species/hypothesis) was calculated in PAUP, using the model of sequence evolution selected by the AIC in ModelTest (Posada and Crandall 1998). Second, the pairwise geographic distance (in kilometers) was calculated using the program Range (http://earthquake.usgs.gov/research/software/#Range). Third, the presence or absence of a potential barrier between two individuals was coded as a binary character and converted to a pairwise barrier matrix. If the straight-line distance between two individuals crossed the barrier of interest (e.g. the Amazon River in the case of the riverine barrier hypothesis), then the barrier was coded as present; if not, the barrier was coded as absent.

For each hypothesis/species, simple Mantel tests assessed the correlation between pairwise genetic distance matrices and the pairwise barrier matrix. Furthermore, isolation by distance was tested for by a simple Mantel test of the pairwise genetic distance and pairwise geographic distance. If both of the above tests were statistically significant, a partial Mantel test was conducted to determine whether the genetic distance between individuals was correlated with the presence of a potential barrier when the effects of geographic distance are removed. All Mantel tests were conducted with the program zt (Bonnet and Van de Peer 2002) and used 10,000 permutations to assess statistical significance.

Demographic Analyses

Two types of analyses were performed using Arlequin 3.11 (Excoffier et al. 2005) to test the predictions of both the refugia and marine incursion hypotheses that populations restricted to an isolated region should show signs of population bottlenecks and subsequent population expansion (Table 3.1). Tajima's *D* statistic (Tajima 1989) which is expected to be negative for populations that have experienced recent population growth (Rogers 1995), was calculated for each *a priori* population for each hypothesis. Significance was tested, as described in the Arlequin manual (http://cmpg.unibe.ch/ software/arlequin3/arlequin31.pdf) by simulating random samples under a model of population equilibrium, where the p value is equal to the number of simulated values less than or equal to the observed value of *D*.

Second, pairwise nucleotide mismatch distributions were calculated for each population. A population that is at equilibrium is expected to have a multi-model mismatch distribution due to the stochastic shape of its gene tree, whereas populations that have experienced recent growth should have a unimodal mismatch distribution resulting from a star-like gene tree (Slatkin and Hudson 1991; Rogers and Harpending 1992). A model of stepwise population expansion was estimated using a generalized least-square approach (Schneider and Excoffier 1999), and its validity was tested as follows: The sum of squared deviations (SSD) between the observed and the simulated (i.e. expected) mismatch distributions was used as a test statistic; 1000 bootstrap simulations of the data were performed, and the SSD was calculated for each; the null hypothesis of population expansion was rejected when fewer than 5% of the simulated SSD values were greater than the observed SSD. To further test whether the observed mismatch distributions deviated from the null expectations characteristic of an expanding population, Harpending's Raggedness Index (Harpending 1994) was calculated. This index has greater values for distributions that are multimodal, as expected for stationary (i.e. non-expanding) populations. Significance for Harpending's Raggedness Index was assessed through bootstrap simulation as described for the SSD.

Coalescent dating of population divergence

The refugia and marine incursion hypotheses make similar predictions about how populations should be structured (see Table 3.1). However, these two hypotheses make predictions on vastly different temporal scales. On the one hand, the refugia model predicts that current population structure formed since the last glacial maximum (LGM), approximately 21,000 years ago. In contrast, the population structure predicted by the marine incursion hypothesis should date to the Miocene, approximately 5-23 million years ago.

To discriminate between these alternative scenarios, a coalescent dating approach was used. The results of the phylogenetic analyses for each species were used to determine where the most basal split between all sampled populations. The approximate date of this split, in years before present (ybp), was estimated as follows. The isolation-with-migration model developed by Nielsen and Wakeley (Nielsen and Wakeley 2001) was implemented in the program MDIV (http://www.binf.ku.dk/~rasmus/webpage/mdiv.html), following the suggestions made by Nielsen and Wakeley (Nielsen and Wakeley 2001) and Carstens et al. (Carstens et al. 2005). This model simultaneously approximates the divergence time (*T*) between two populations that share a common ancestor, the migration rate (*M*) between these populations, and a measure of genetic diversity (θ) in a Bayesian framework using a Markov chain Monte Carlo method. The model assumes equal effective population sizes in both populations and that these populations are not exchanging migrants with any other populations (Nielsen and Wakeley 2001).

Preliminary analyses on each population pair were conducted using the default values of $M_{\max}=10$, $T_{\max}=5$, and the suggested default value for $\theta_{\max}$. Based on the results of these preliminary analyses, priors for $T_{\max}$ of 1, 5, and 20 were used for *A. cephalotes*, *A. sexdens*, and *A. laevigata*, respectively. The full searches used 2 million MCMC chains, of which 500,000 were discarded as burnin. All searches used the HKY model of sequence evolution and uninformative priors. The estimates for T were converted into time in years since divergence (T_{div}) using the equations, $T = T_{\mathrm{div}} /2Ne$, and, $\theta = 4Ne\mu$, where Ne is the effective population size, and μ is the mutation rate in substitutions per sequence per generation. Since the mutation rate for the gene used in this study is not known for *Atta*, an approximate value of 1.5% per million years was used, as this was the average value for COI in a recent survey across the arthropods (Quek et al. 2004). To determine how sensitive the results were to the mutation rate, a range of values was used, corresponding to the minimum and maximum mutation rates observed for this gene in arthropods (Quek et al. 2004, Table 2.4). To complete the conversion into units of time in years, a generation time of 4 years was used, based on life history data from Autuori (Autuori 1947) and observations by U. Mueller (pers. comm.). The refugia hypothesis was rejected if the upper 95% confidence interval (CI) of Tdiv was less than 21,000 ybp, as this would indicate that any population structure present in the species was generated after the last glacial maximum. Likewise, the marine incursion hypothesis was rejected if the upper 95% CI of Tdiv was less than 5 million ybp, as this would suggest diversification after the Miocene, when marine incursions are thought to have been present in South America (Rasanen et al. 1995; Hovikoski et al. 2005; Lovejoy et al. 2006; Hovikoski et al. 2007; Latrubesse et al. 2007).

RESULTS

Summary of data collected

Samples for molecular analysis were obtained from 118 *Atta cephalotes* colonies, 44 *Atta sexdens* colonies, and 30 *Atta laevigata* colonies, spanning the known geographic range of each species (see Appendix B). Two disjunct sections of mitochondrial DNA, encompassing part of the Cytochrome Oxidase I (COI) and tRNA-Leucine (tRNAleu) genes, as well as the entire intergenic spacer between COI and tRNAleu were sequenced for all samples. The sequences were concatenated into a single alignment that varied in length from 635 basepairs in *A. cephalotes* to 701 basepairs in *A. sexdens* and *A. laevigata*. Several nuclear pseudogenes were accidentally amplified and sequenced for *A. cephalotes* (see Martins et al. in prep for details) and were not used in subsequent analyses; all sequences included in the final alignments for each species appeared to be functional, mitochondrial loci, as no premature stop codons or frameshift mutations were detected. Additional sequences for outgroup taxa (*Atta columbica*, *Atta mexicana*, and *Atta texana*), used for phylogenetic analyses of *A. cephalotes* were obtained from specimens available in the Mueller Lab at The University of Texas at Austin.

Paleodistribution modeling

Maps comparing the predicted geographic range of each species under current conditions and during the last glacial maximum (LGM), approximately 21 kya, are shown in Figure 3.2. For *A. cephalotes*, the predicted LGM range spanned most of the Amazon

Basin, with a contiguous population throughout the Guiana Shield (Figure 3.2B-C). This range is somewhat reduced from the estimated current distribution of the species (Figure 3.2A.). Other areas with high probability of occurrence during the LGM include the Atlantic Coastal Forests of Brazil, lower Central America and the Chocó region of South America west of the Andes, and upper Central America into central Mexico. For *A. sexdens*, the paleodistribution model predicts a more fragmented distribution during the LGM (Figure 3.2E-F). The largest block of inhabitable range is in the southwestern Amazon Basin, from approximately just west of Manaus to the southwestern edge of the Peruvian Andes. Other blocks of inhabitable areas during the LGM for *A. sexdens* include the Guiana Shield; the Atlantic Coastal Forests of Brazil; an area south of the mouth of the Amazon River, roughly between Belem and São Luis; northwestern Colombia/eastern Panama; and Nicaragua. For *A. laevigata*, the model predicted the presence of a large area of unsuitable habitat spanning much of the Amazon Basin (Figure 3.2H-I). The remaining populations occur to the north and south of the Amazon Basin, and are themselves somewhat fragmented.

Gene tree reconstruction

For each species, both maximum likelihood (ML) and Bayesian searches yielded gene trees with similar topologies (Figures 3.3–3.5). The gene tree for *A. cephalotes* (Figure 3.3) is relatively unresolved, with nested sets of paraphyletic populations. The most basal individuals are from populations in the western Amazon lowlands and the eastern foothills of the Andes, and are paraphyletic with respect to all other populations of this species. The most derived populations are in the Atlantic Coastal Forest of Brazil, which are disjunct from the remainder of the species' range (see Chapter 2).

In contrast, the gene tree for *A. sexdens* (Figure 3.4) is well resolved, with a basal split corresponding to a roughly north-south division between populations, each of which is further subdivided into additional clades. The northern clade consists of (1) an eastern clade, encompassing populations from the Atlantic Coastal Forest in Bahia, Brazil as well as from Belem, just south of the mouth of the Amazon River; and (2) a western Amazonian clade, containing individuals from eastern Peru and the southern edge of the Amazon Basin in northern Mato Grosso, Brazil. The southern clade is divided into (1) a lower Amazon River clade, consisting of populations along both sides of the lower Amazon River from Manaus to near its mouth in Amapá, Brazil, as well as populations from French Guyana to the north; and (2) a Venezuelan clade, containing populations that range from the eastern foothills of the Venezuelan Andes, across the Llanos to the lower Orinoco River plain.

The gene tree for *Atta laevigata* is also somewhat unresolved (Figure 3.5). The most basal populations are from the Guiana Shield, and are paraphyletic with respect to all other populations. The most derived populations of this species occur along the lower Amazon River.

The results of parametric bootstrap and Bayesian hypothesis tests gave similar results in all cases (Table 3.3). The parametric bootstrap analyses suggest that, with one exception, the best gene trees consistent with the topologies predicted by each of the three hypotheses are significantly less likely than the best overall trees. Therefore, the strict predictions of these hypotheses regarding population monophyly (see Table 3.1) were rejected. The exception is the topology predicted by the refugia hypothesis for *Atta sexdens*, which could not be rejected by the parametric bootstrap analysis. Likewise, in the Bayesian hypothesis tests, the constraint topology for the refugia hypothesis as applied to *A. sexdens* was the only case in which any trees were retained by the filter, indicating strong support for that topology (Bpp = 0.843). The topology tests therefore consistently rejected the strict predictions of each hypothesis in all cases except for the refugia hypothesis for *A. sexdens*, which could not be rejected.

Population Genetic Analyses

The results of analysis of molecular variance (AMOVA) analyses are shown in Table 3.4. For each hypothesis tested, the AMOVA results should reveal a significant amount of genetic variance explained by differences among groups assuming the predictions of a given hypothesis were true. This is the case only for the refugia hypothesis as applied to *Atta cephalotes*. In most cases, the majority of the genetic variance occurred among populations within a region. It should be noted that negative percentages can occur in AMOVA analyses because they are computed based on covariance components, which occasionally take on negative values when the true value is zero; since all percentages must sum to 100, the presence of a negative percentage can cause other percentages to be greater than 100. All negative percentages and percentages greater than 100 should be interpreted as not significantly different from zero and 100, respectively (discussed in Arlequin FAQ section at http://lgb.unige.ch/arlequin/software/2.000/doc/faq/faqlist.htm).

No correlation was found for any of the three species between the pairwise genetic distance between individuals and the presence of the Amazon River between them (Table 3.5), suggesting that the Amazon River does not serve as a barrier between populations on either bank. In contrast, a significant correlation between genetic distance and the barrier of interest was found for both the refugia and marine incursion hypotheses for all three species, even when the effects of isolation by distance were factored out (note that for *A. laevigata*, the predictions of the marine incursion and refugia models are identical).

Two types of analyses were performed to test the predictions of both the refugia and marine incursion hypotheses that populations restricted to an isolated region should show signs of population bottlenecks and subsequent population expansion (Table 3.6). For the mismatch distribution, two measures of goodness-of-fit were used to determine whether the null hypothesis of population expansion could be rejected: the Sum of Squared Deviations (SSD) test and Harpending's Raggedness Index. The Raggedness Index was unable to reject the null hypothesis of population expansion in all instances for which there were sufficient data. In contrast, the SSD index rejected the sudden expansion model in 7 out of the 13 instances for which there was sufficient data. There three instances in which all three measures are consistent with a recent population expansion: the Atlantic Coast Forest population in *A. cephalotes* (marine incursion and refugia hypotheses), the Brazilian Shield population in *A. sexdens* (marine incursion hypothesis), and the Guiana Shield population in *A. laevigata* (marine incursion and refugia hypotheses).

Coalescent dating

The distributions of T_{div}, the date of the oldest measurable split between extant populations for each species are shown in Figure 3.7. The mode, upper, and lower 95% confidence intervals of T_{div} for a range of mutation rates and generation times are given in Table 2.7. For all three species, the oldest dates of population divergence are too recent to be consistent with the marine incursion hypothesis, since all extant population structure seems to have formed after the Miocene. In fact, the confidence intervals for the dates of population divisions for all three species fall within the Pleistocene (Figure 3.7). Therefore, the results of the coalescent dating analysis could not reject the Pleistocene refugia hypothesis for any of the three species.

DISCUSSION

This study is the first to use a comparative, molecular phylogeographic approach to explicitly test the leading theories on Amazonian speciation in an insect. The combined results of gene tree topology tests and population-genetic analyses (summarized in Table 3.8) strongly rejected all predictions of the riverine barrier model for all three species of leafcutter ants. This result is perhaps not too surprising given the dispersal ability of *Atta* reproductives (males and queens), which fly from their natal nests on nuptial flights and then search for a site in which to establish their new colony (Moser 1967). Although the exact dispersal abilities of *Atta* species are not known, the maximum range for a mated queen is thought to be no more than 50 km (Moser 1967). Nevertheless, the Amazon River is known to pose a barrier to other flying animals, such as butterflies and birds (Hall and Harvey 2002; Hayes and Sewlal 2004), so it does not seem unreasonable that winged ants may not be able to cross it. Based on the results of the present study however, it appears that gene flow regularly occurs across the Amazon River in the three species examined. Although the potential barrier effects of other major rivers in the Amazon Basin were not tested in this analysis, the lack of a significant effect of the lower Amazon River suggests that smaller rivers are unlikely to structure populations of leafcutter ants.

This study is also the first to combine paleodistribution models with molecular data to test the Pleistocene refugia hypothesis in South America. Paleodistribution modeling of species ranges during the LGM circumvents one of the major criticisms of the refugia hypothesis, namely that the location and size of putative forest refugia are likely to be different for every species considered (Bush 1994; Moritz et al. 2000). The results of paleodistribution models in the current study strengthen this argument, since each species is predicted to have responded differently to environmental conditions at the LGM (Figure 3.2). Interestingly, the paleoclimate model used in this study predicts that conditions supporting wet forest persisted throughout much of the Amazon Basin during the LGM, as is suggested by an increasing amount of fossil pollen and other geological data (Colinvaux et al. 2000; Colinvaux et al. 2001). However, this reconstruction of

Pleistocene climate conditions contradicts claims by proponents of the refugia model that wet forest only existed along the margins of the Amazon Basin during the LGM (Haffer 1969; Simpson and Haffer 1978; Haffer 1997; Haffer and Prance 2001). As a result of the paleoclimate model's predictions, the wet forest species, A. cephalotes, is predicted to have been the least fragmented of the three species examined during the LGM (Figure 3.2, middle rows). Instead, *A. sexdens* and *A. laevigata* were predicted to have more dissected ranges, with *A. laevigata* being divided into roughly two populations, one to the north of the Amazon Basin and one to the south (Figure 3.2H-I).

Some support was found for predictions of both the refugia and marine incursion hypotheses (Table 3.8). However, these hypotheses make very similar predictions in many cases (especially for *A. laevigata*), so differentiating between them can be difficult. Topology tests to determine whether a priori population groupings predicted by each hypothesis are reciprocally monophyletic were not significant for the marine incursion hypothesis for any species (Table 3.3). However, both parametric bootstrap and Bayesian hypothesis tests were consistent with the population structure predicted by the refugia hypothesis, as determined from paleodistribution modeling, for *Atta sexdens*, but not for *A. cephalotes* or *A. laevigata* (Table 3.3). Reciprocal monophyly is expected to eventually occur for populations that do not exchange migrants. However, incomplete lineage sorting is expected to also produce paraphyletic and polyphyletic gene trees as populations are split by barriers to gene flow (Avise 2000, chapter 4). Thus, failure to detect reciprocal monophyly does not necessarily indicate that populations are not diverging, especially if the suspected barrier promoting divergence appeared recently.

In fact, the gene trees for *A. cephalotes* and *A. laevigata* are consistent with more conservative predictions made by the marine incursion and refugia hypotheses about the shapes of gene trees. For instance, the lack of differentiation seen in the gene tree for A. cephalotes is consistent with the low levels of population subdivision predicted by the paleodistribution models for the refugia hypothesis. Furthermore, the marine incursion hypothesis predicts that the most ancestral populations should be in areas unlikely to have been inundated by rising sea levels (Nores 1999; Aleixo 2004). This prediction holds true for *A. cephalotes*, where the most basal populations are those in the Western Amazon, including several populations near the base of the Andes, and some from the Brazilian Shield. Likewise, for *A. laevigata*, the most basal populations are those in the Guiana Shield, which would not have experienced flooding (Rasanen et al. 1995; Nores 1999; Hovikoski et al. 2005; Hovikoski et al. 2007). Since the paleodistribution models for *A. laevigata* predicted that it would have been restricted to occur only to the north (along the Guiana Shield) and to the south (on the Brazilian Shield) of the Amazon Basin at the LGM, this finding is also consistent with the refugia hypothesis for this species.

Population genetic tests of the marine incursion and refugia hypotheses provided mixed results. Analysis of molecular variance (AMOVA) tests suggest that the groupings predicted by the refugia hypothesis for *A. cephalotes* explained a significant percent of the variance in this species, but not for *A. sexdens* or *A. laevigata* (Table 3.4). The groupings predicted by the marine incursion hypothesis did not explain a significant percentage of the variation in any of the three species. In contrast, for both the marine incursion and refugia hypotheses, the presence of the barrier of interest was significantly correlated with genetic distance between individuals for all three species once the effects of geographic distance were removed using partial Mantel tests (Table 3.5).

Evidence for population bottlenecks and subsequent expansion was found for several populations, as predicted by both the refugia and marine incursion models. Specifically, results of mismatch distributions and Tajima's D test were both consistent with recent population growth in the Atlantic Coast Forest population of *A. cephalotes*, the Brazilian Shield population of *A. sexdens*, and the Guiana Shield population of *A. laevigata* (Table 3.6).

The results discussed so far suggest some support for both the refugia and marine incursion hypotheses (Table 3.8). However, these hypotheses make predictions on vastly different temporal scales. The Pleistocene refugia hypothesis predicts that population structure should date to the Pleistocene (approximately 10,000–1.8 million ybp), while the marine incursion hypothesis suggests that populations were last subdivided during the Miocene (5 million–23 million ybp). To distinguish between these alternative scenarios, we used the mtDNA gene trees as a guide to divide all samples into two populations by looking for the best supported, basal split in each tree. The Bayesian posterior probability of the time since divergence of these two populations was then estimated using an MCMC search. The results of these coalescent analyses suggest that, for each species, all extant population structure developed subsequent to the Miocene (Figure 3.7, Table 3.7). These results seem to rule out the possibility of Miocene marine incursions as the primary factor structuring genetic variation in extant populations of leafcutter ants. Although our method for grouping populations may not reflect the actual population history, it is somewhat less arbitrary than any other means of a priori population assignment. Future analyses using additional genetic loci or incorporating information on the phylogenetic relationships between closely related species would allow for corroboration of these results.

Combining all of the results of the paleodistribution models with the molecular phylogeographic analyses for all three species (Table 3.8), the available data are therefore most consistent with the predictions of the Pleistocene refugia hypothesis. However, these results do not support the traditional formulation of this hypothesis, namely that small isolated pockets of wet forest at the periphery of the Amazon Basin are responsible for generating extant species diversity. Our study did find that climate changes at the LGM caused some species to have more fragmented ranges and therefore more extant population structure than others. However, the species that currently are the most structured are not those most restricted to wet forests as we predicted. Instead, *A. sexdens* and *A. laevigata*, which have greater environmental flexibility than *A. cephalotes*, seem to have been more affected by Pleistocene climate change. These results demonstrate how ecological differences, even between congeneric species, can determine the influence that historical climate change has had on genetic population structure.

Care must be taken when interpreting the results of an analysis that rely on only a single, mitochondrial marker, since the reconstructed gene trees may not match the actual species trees (Shaw 2002; Gomez-Zurita and Vogler 2003). Further analyses using additional genetic loci are needed in order to definitively determine whether the gene trees reconstructed by the present study match the actual species history (Avise 2000). Estimates of dates for population divergences are also known to be susceptible to error when only a single locus is used (Edwards and Beerli 2000; Arbogast et al. 2002); however, estimates obtained using the isolation-with-migration model are typically older than the actual date of divergence (Carstens and Knowles 2007), reinforcing our conclusion that the population structures observed in the current study are of recent origin. Phylogenetic analyses of the entire genus could reveal whether the biogeography and timing of speciation in *Atta* is also consistent with the refugia hypothesis, and would permit testing of other hypotheses that rely less on biogeography, such as the ecological gradient hypothesis (Moritz et al. 2000); such analyses are currently underway (Bacci et al. in prep).

CONCLUSIONS

The results of this study provide renewed support for the idea that historical climate change, as recently as the Pleistocene, may be important for structuring populations of Amazonian species (Bush 1994; Moritz et al. 2000; Colinvaux et al. 2001). However, the role that climate change has played needs to be further evaluated across a diversity of taxa. More research on insects and other invertebrates, which comprise the majority of tropical species diversity (Wilson 1999), are especially needed to make a truly general statement about what mechanisms are responsible for generating high Amazonian species diversity. New approaches that permit a combination of paleodistribution modeling and comparative, molecular phylogeography provide promising tools to accomplish this goal.

Predictions	Riverine barrier	Pleistocene refugia	Marine incursion	Methods
Reciprocal monophyly of populations:	on north and south of Amazon River	in different refugia	in Eastern base of Andes, Brazilian Shield, and/or Guiana Shield	Parametric bootstrap, Bayesian hypothesis tests
Basal populations:	N/A	in refugia	in Eastern base of Andes, Brazilian Shield, and/or Guiana Shield	ML and Bayesian gene tree reconstruction
Derived populations:	N/A	outside refugia	in Amazonian lowlands	ML and Bayesian gene tree reconstruction
Barrier to gene flow:	Amazon River	areas between refugia	Amazonian lowlands	AMOVA, Mantel tests
Population history includes:	N/A	population expansion	population expansion	Mismatch distributions, Tajima's D
Population structure formed:	continually	during LGM (21 kya)	during Miocene (5-23 mya)	MDIV

Table 3.1: Summary of the predictions of each hypothesis and the methods used to test them.

Primer Name	Forward/ Reverse	Primer Sequence (5'-3')	Reference
Jerry	F	CAACATTTATTTTGATTTTTTGG	Simon et al., 1994
George I	F	ATACCTCGACGTTATTCAGA	Wetterer et al., 1998
AntF	F	ATTCATTCTTATCTTGAAATATTATTTC	Martins et al. in prep.
Ben	R	GCTACTACATAATAKGTATCATG	T. Schultz pers. comm.
AntR	R	TTCATAAGTTCAGTATCATTGGTG	Martins et al. in prep.
SESR1EXT1	R	ATTATTAAGTCGTATGTAGGGGA	This study
ASMtRNAleuR1	R	CAATGCACTATTCTGCCATATTAAA	This study

Table 3.2: Mitochondrial DNA primers used for amplification and sequencing of ants in the present study.

Constraint Name	Parametric Bootstrap Tests -ln L constrained	-ln L uncon-strained	Difference	*p*	Bayesian Tests # consistent trees	# trees in posterior sample	Bpp
Atta cephalotes							
Amazon River	2263.5432	2195.4377	68.1055	<0.001	0	223872	0
Marine Incursion	2266.8397	2195.4377	71.4019	<0.001	0	223872	0
Refugia	2270.3737	2195.4377	74.936	<0.001	0	223872	0
Atta sexdens							
Amazon River	2159.8489	2096.8147	63.0342	<0.001	0	11832	0
Marine Incursion	2104.9211	2096.8147	8.10643	<0.001	0	11832	0
Refugia	2096.8836	2096.8147	0.0689	0.15	9975	11832	0.843
Atta laevigata							
Amazon River	1965.9731	1933.9096	32.0635	<0.001	0	7812	0
Refugia/Marine*	1974.7753	1933.9096	40.8657	<0.001	0	7812	0

Table 3.3: Results of gene tree topology tests. For the parametric bootstrap analyses, *p* values less than 0.05 indicate rejection of the null hypothesis (i.e. the constraint tree). Bpp is the Bayesian posterior probability of a given constraint topology.

* The predictions of the Pleistocene refugia and marine incursion hypotheses are identical for *A. laevigata*

Species	Hypothesis	N	Among regions	Among populations within regions	Within populations
A. cephalotes	Amazon River	20	-7.81	54.41*	53.40*
	Marine Incursion	47	30.24	48.96*	20.79*
	Refugia	82	40.19*	33.40*	26.41*
A. sexdens	Amazon	17	-48.91	119.87*	29.04*
	Marine Incursion	24	20.3	74.13*	5.57*
	Refugia	30	39.42	56.06*	4.52*
A. laevigata	Amazon	13	-98.56	186.84*	11.72*
	Marine Incursion/ Refugia	18	59.24	-0.36	41.12*

Table 3.4: Results of Analyses of Molecular Variance (AMOVA). For each hypothesis, population structure was defined as predicted by each hypothesis (see text). The percentage of variance explained by each hierarchical grouping is shown, with an asterix indicating statistical significance as assessed by permutation. Negative percentages and percentages greater than 100 should be interpreted as not significantly different than zero and 100, respectively. The "among regions" grouping is the grouping of interest for the purposes of hypothesis testing in this study.

Species	Hypothesis	Gen Dist x Barrier		Gen Dist x Geog Dist		Partial	
		r	*p*	*r*	*p*	*r*	*p*
A. cephalotes	Riverine	0.113	0.12714	0.729	0.0001	N/A	N/A
	Marine	0.265	0.00002	0.665	0.00001	-0.149	0.00003
	Refugia	0.364	0.00001	0.498	0.00001	0.076	0.00589
A. sexdens	Riverine	0.119	0.07756	0.801	0.00001	N/A	N/A
	Marine	0.561	0.00014	0.785	0.00001	-0.396	0.00138
	Refugia	0.593	0.00001	0.574	0.00001	0.251	0.00009
A. laevigata	Riverine	0.024157	0.355466	0.707757	0.00128	N/A	N/A
	Marine/ Refugia	0.580717	0.00977	0.383795	0.02435	0.472043	0.0073

Table 3.5: Results of simple and partial Mantel tests of matrix correlation. For each hypothesis, the correlation between corrected, pairwise genetic distance between individuals and the presence or absence of the barrier of interest was tested using a simple Mantel test (Gen Dist x Barrier). The correlation between genetic and geographic distances (Gen Dist x Geog Dist) was assessed to test for isolation by distance. If a significant correlation was found between both matrix comparisons, a partial Mantel test was conducted on all three matrices to determine whether the presence of the barrier of interest was significantly correlated with genetic distance once the effects of geographic distance are factored out (Partial). All tests used 10,000 permutations to assess statistical significance.

			Mismatch Distribution				Tajima's *D*	
Species	Hypothesis	Population	SSD	*p*	Raggedness	*p*	*D*	*p*
A. cephalotes	Marine Incursion	Andes	0.00854517	0.428	0.08645395	0.315	-1.1544	0.142
		Guiana Shield	0.03722933	0.231	0.0538843	0.592	0.25014	0.639
		Brazilian Shield	0.04740862	0.092	0.02787182	0.589	0.79628	0.827
	Refugia	Atlantic Coast	0.0200829	0.299	0.08930211	0.3	-1.65893	0.033
		Greater Amazonia	0.02135842	0.679	0.02513078	0.547	-0.59127	0.309
		S Central America	0.24169597	0.004	0.01579238	1	-1.85767	0.007
		N Central America	0.08286126	0.309	0.14	0.845	1.21852	0.864
A. sexdens	Marine Incursion	Brazilian Shield	0.20368588	0.137	0.47	0.191	-1.21852	0.026
		Guiana Shield	0.36626913	0	0.21440472	0.98	-0.13367	0.483
		Andes	N/A	N/A	N/A	N/A	N/A	N/A
	Refugia	Bahia	0.4999998	0	0.75	0.966	-0.78012	0.198
		Western Amazon	0.32561711	0	0.59027778	0.931	-1.03227	0.222
		Guianas	0.31271602	0.003	0.25859645	0.972	0.0198	0.577
A. laevigata	Marine Incursion/ Refugia	Guiana Shield	0.01959799	0.181	0.10577614	0.212	-2.31554	0
		Brazilian Shield	N/A	N/A	N/A	N/A	0	1

Table 3.6: Results of pairwise nucleotide mismatch distribution and Tajima's (1989) *D* tests for historical population expansion for populations defined *a priori* for each hypothesis. See text for details.

Species	μ	L	95_{Low}	T_{div}	95_{High}
A. cephalotes	1%	4	25,394	43,701	92,913
	1.5%	**4**	**16,929**	**29,134**	**61,942**
	2%	4	12,697	21,850	46,457
	1%	6	16,929	29,134	61,942
	1.5%	6	11,286	19,423	41,295
	2%	6	8,465	14,567	30,971
A. sexdens	1%	4	178,317	329,886	816,690
	1.5%	**4**	**118,878**	**219,924**	**544,460**
	2%	4	89,158	164,943	408,345
	1%	6	118,878	219,924	544,460
	1.5%	6	79,252	146,616	362,974
	2%	6	59,439	109,962	272,230
A. laevigata	1%	4	191,771	927,476	1,652,720
	1.5%	**4**	**127,848**	**618,317**	**1,101,813**
	2%	4	95,886	463,738	826,360
	1%	6	127,848	618,317	1,101,813
	1.5%	6	85,232	412,211	734,542
	2%	6	63,924	309,159	550,907

Table 3.7: Estimates of population divergence times, 95% confidence intervals (T_{div}, 95_{Low} and 95_{High}, respectively, in years before present) used for hypothesis testing are given for a range of mutation rates (μ, in percent sequence divergence per million years) and generation times (L, in years). The values used for discussion are shown in bold.

Species	Prediction	Test	Riverine barrier	Pleistocene refugia	Marine incursion
A. cephalotes	Reciprocal monophyly of relevant populations	Parametric bootstrap	–	–	–
		Bayesian hypothesis tests	–	–	–
	Relevant basal and derived populations	ML and Bayesian trees	N/A	+	–
	Evidence for predicted barrier to gene flow	AMOVA	–	+	–
		Mantel Tests	–	+	+
	History of population expansions	Mismatch Distributions	N/A	+	+
		Tajima's *D*	N/A	+	–
	Appropriate age of oldest population division	MDIV	N/A	+	–
A. sexdens	Reciprocal monophyly of relevant populations	Parametric bootstrap	–	+	–
		Bayesian hypothesis tests	–	+	–
	Relevant basal and derived populations	ML and Bayesian trees	N/A	N/A*	N/A*
	Evidence for predicted barrier to gene flow	AMOVA	–	–	–
		Mantel Tests	–	+	+
	History of population expansions	Mismatch Distributions	N/A	+	+
		Tajima's *D*	N/A	–	+
	Appropriate date for oldest population division	MDIV	N/A	+	–
*A. laevigata***	Reciprocal monophyly of relevant populations	Parametric bootstrap	–	–	–
		Bayesian hypothesis tests	–	–	–
	Relevant basal and derived populations	ML and Bayesian trees	N/A	+	+
	Evidence for predicted barrier to gene flow	AMOVA	–	–	–
		Mantel Tests	–	+	+
	History of population expansions	Mismatch Distributions	N/A	+	+
		Tajima's *D*	N/A	+	+
	Appropriate date for oldest population division	MDIV	N/A	+	+

Table 3.8: Overview of results. A "+" indicates that the test could not reject the predictions of a hypothesis for a given species; A "–" indicates rejection of the predictions; "N/A" means the prediction does not apply to that hypothesis/species.

* The gene tree for *A. sexdens* could not resolve which populations were basal or derived

** The predictions for the Pleistocene refugia and marine incursion models are identical for *A. laevigata*

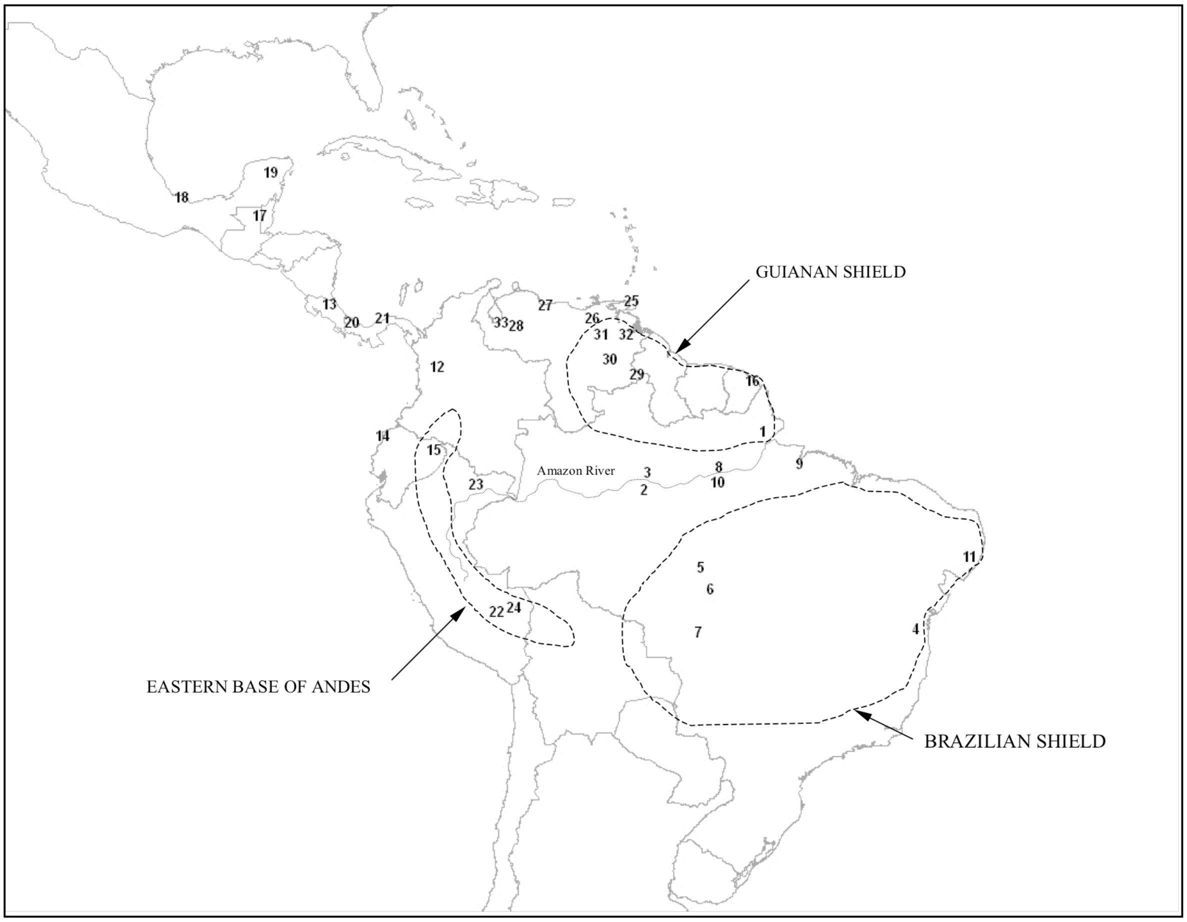

Figure 3.1: Map of collection areas used in this study, listed by number (some areas contain multiple collection sites, which for simplicity are not shown here. See Appendix B for a list of all collection sites). Dotted lines depict the approximate boundaries of areas not flooded by Miocene marine incursions (adapted from Aleixo 2004).

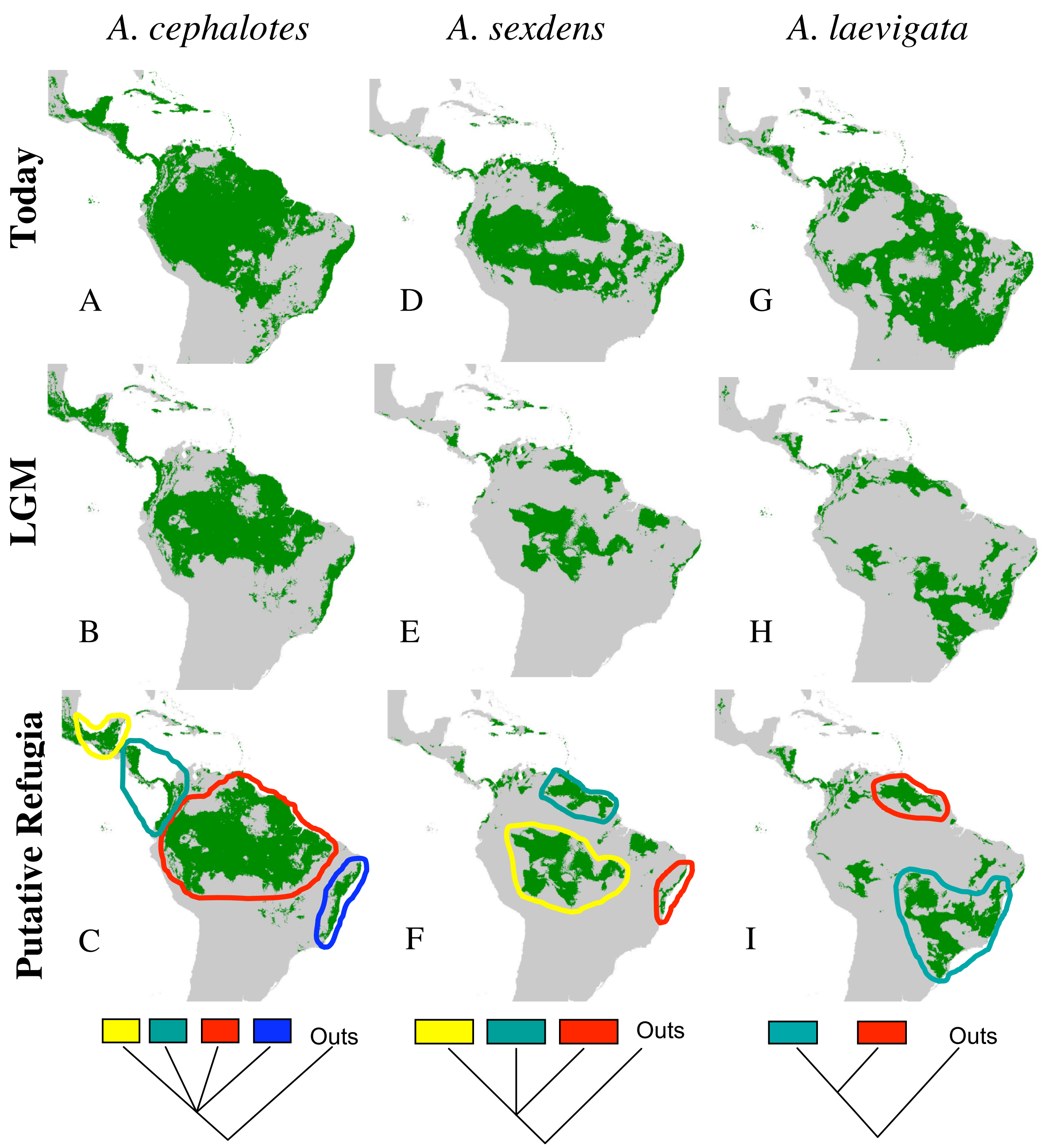

Figure 3.2: Results of maxent binary distribution models for the three species under current conditions (top row) and at the last glacial maximum (bottom rows). Areas shaded in green are predicted to be suitable for a given species. A–C: *A. cephalotes*, D–F: *A. sexdens*, G–I: *A. laevigata*. The results of paleo-distribution models were used to determine a priori population groupings (colored outlines) to test the refugia model; the corresponding constraint trees used in gene tree topology tests are shown below each column.

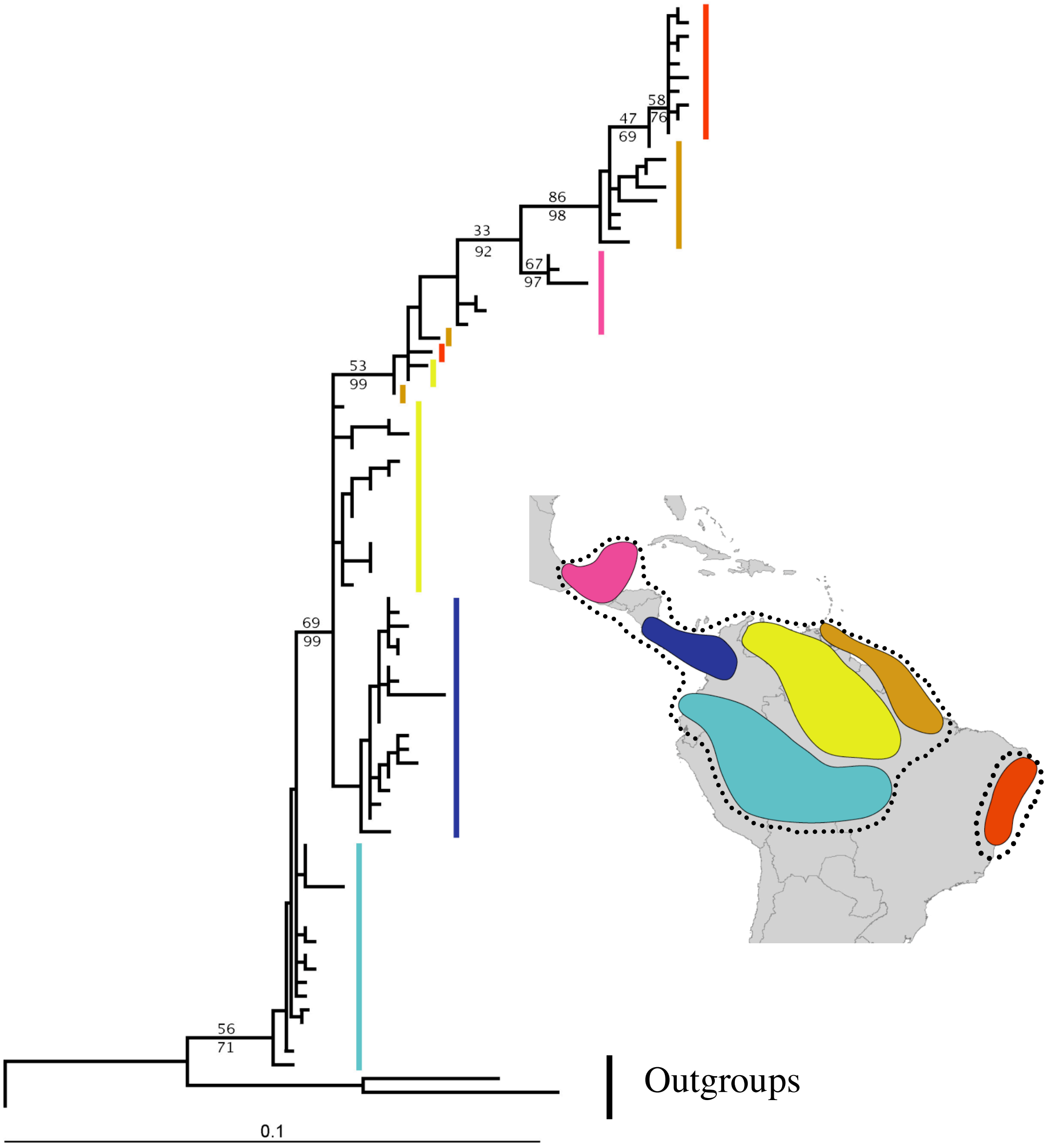

Figure 3.3: Maximum likelihood gene tree for *Atta cephalotes*. Support values are 100 ML Bootstrap (top) and Bayesian posterior probabilities (bottom). Outgroup sequences used for rooting were from *A. columbica*, *A. texana*, and *A. mexicana*. Colored lines correspond to regions shown on map. Dotted lines indicate the approximate range of the species.

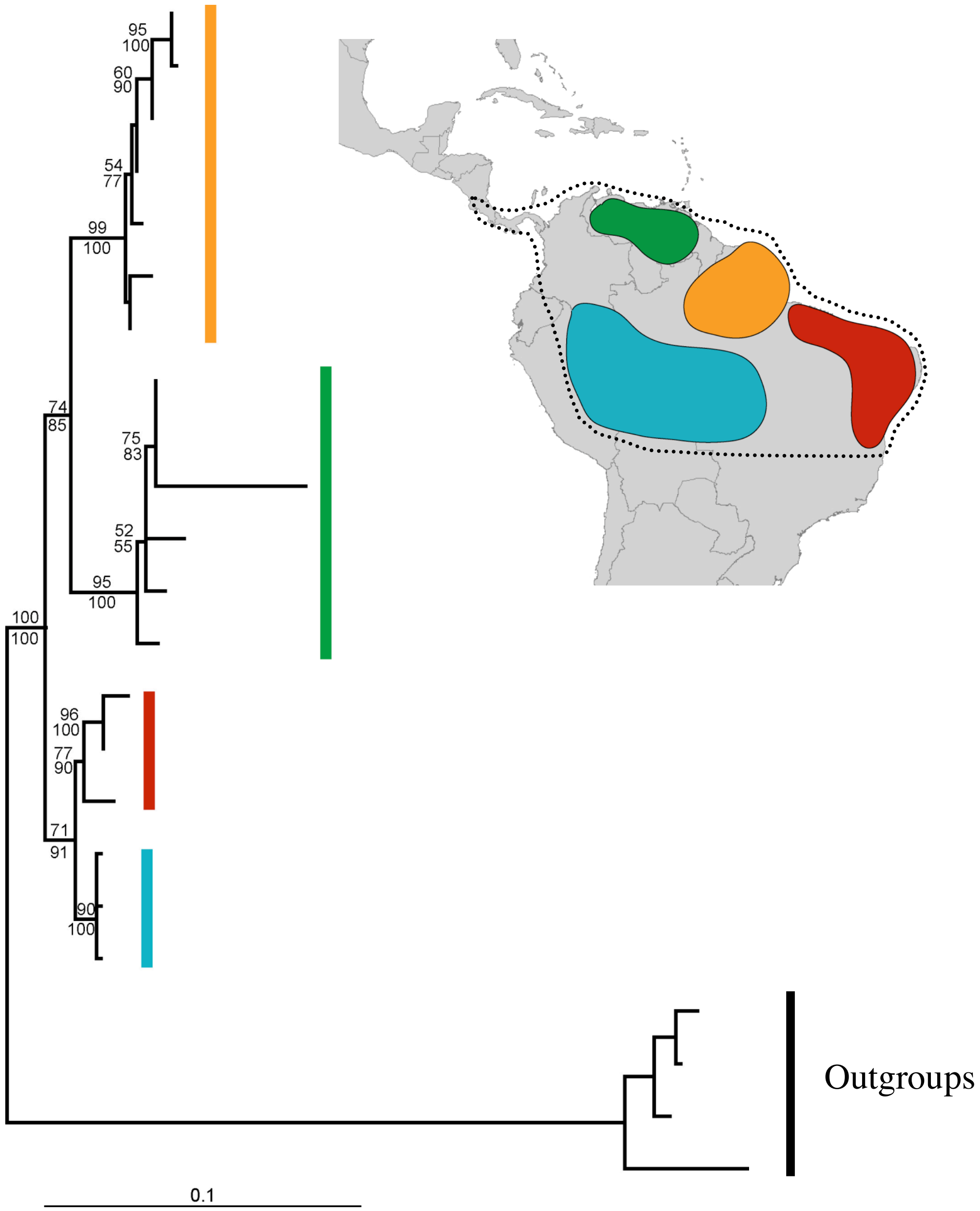

Figure 3.4: Maximum likelihood gene tree for *Atta sexdens*. Support values are 100 ML Bootstrap (top) and Bayesian posterior probabilities (bottom). Outgroup sequences used for rooting were from *A. laevigata*. Colored lines correspond to regions shown on map. A dotted line indicates the approximate range of the species.

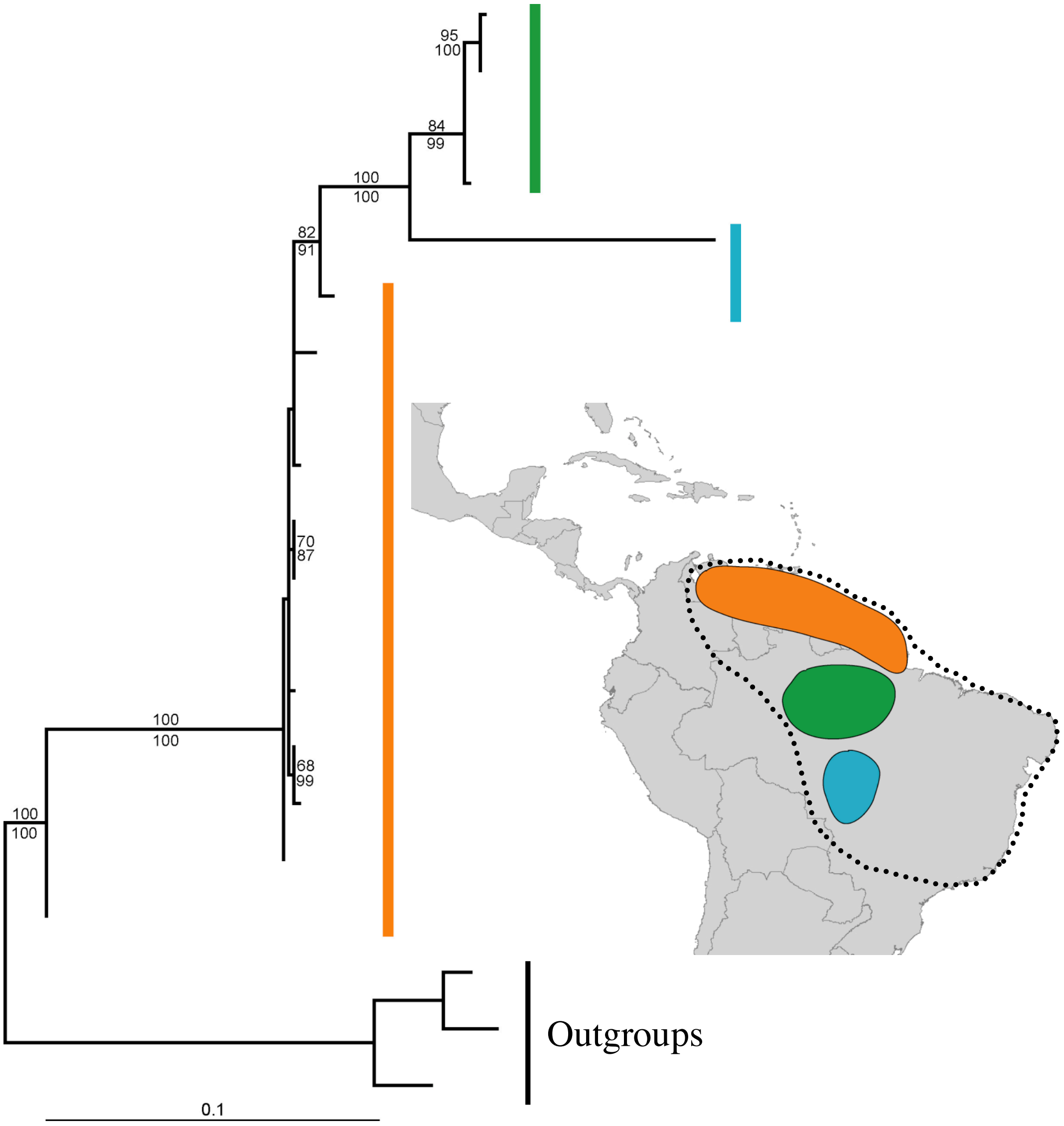

Figure 3.5: Maximum likelihood gene tree for *Atta laevigata*. Support values are 100 ML bootstrap replicates (top) and Bayesian posterior probabilities (bottom). Outgroup sequences used for rooting were from *A. sexdens*. Colored lines correspond to regions shown on map. A dotted line indicates the approximate range of the species.

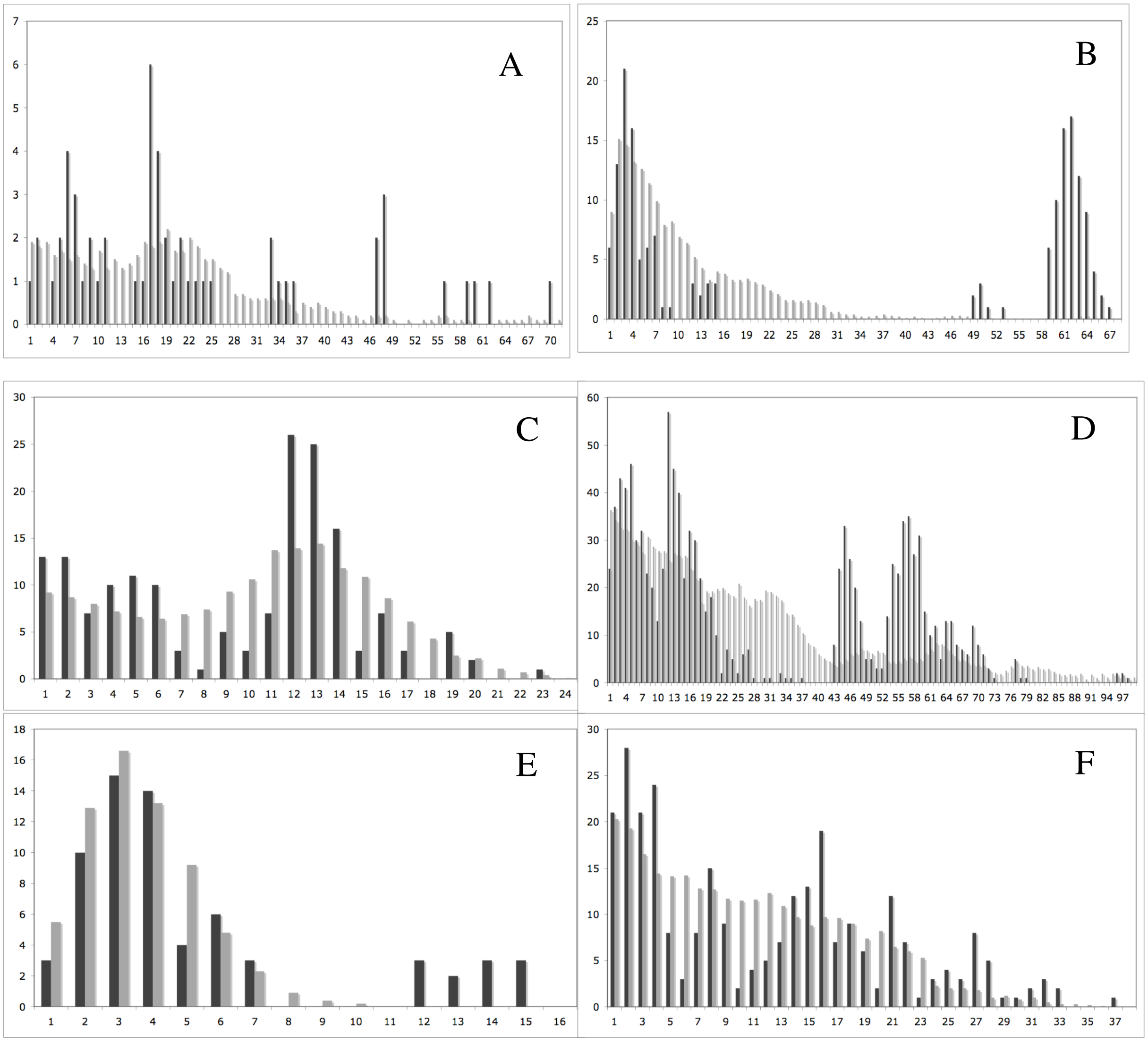

Figure 3.6: Pairwise nucleotide mismatch distributions for population groupings of each species defined a priori by the marine incursion and refugia hypotheses. Dark gray bars show the frequency of observed numbers of pairwise nucleotide mismatches within a population; light gray bars show expected pairwise mismatches under the sudden expansion model (see text). A–C, *Atta cephalotes* populations as defined under the marine incursion hypothesis: (A) Guiana Shield, (B) Brazilian Shield, (C) Eastern base of Andes; D–G, *Atta cephalotes* populations as defined under the refugia hypothesis: (D) Greater Amazonia/Guiana Shield, (E) Brazilian Atlantic Coastal Forests, (F) lower Central America/Chocó, (continued on next page)

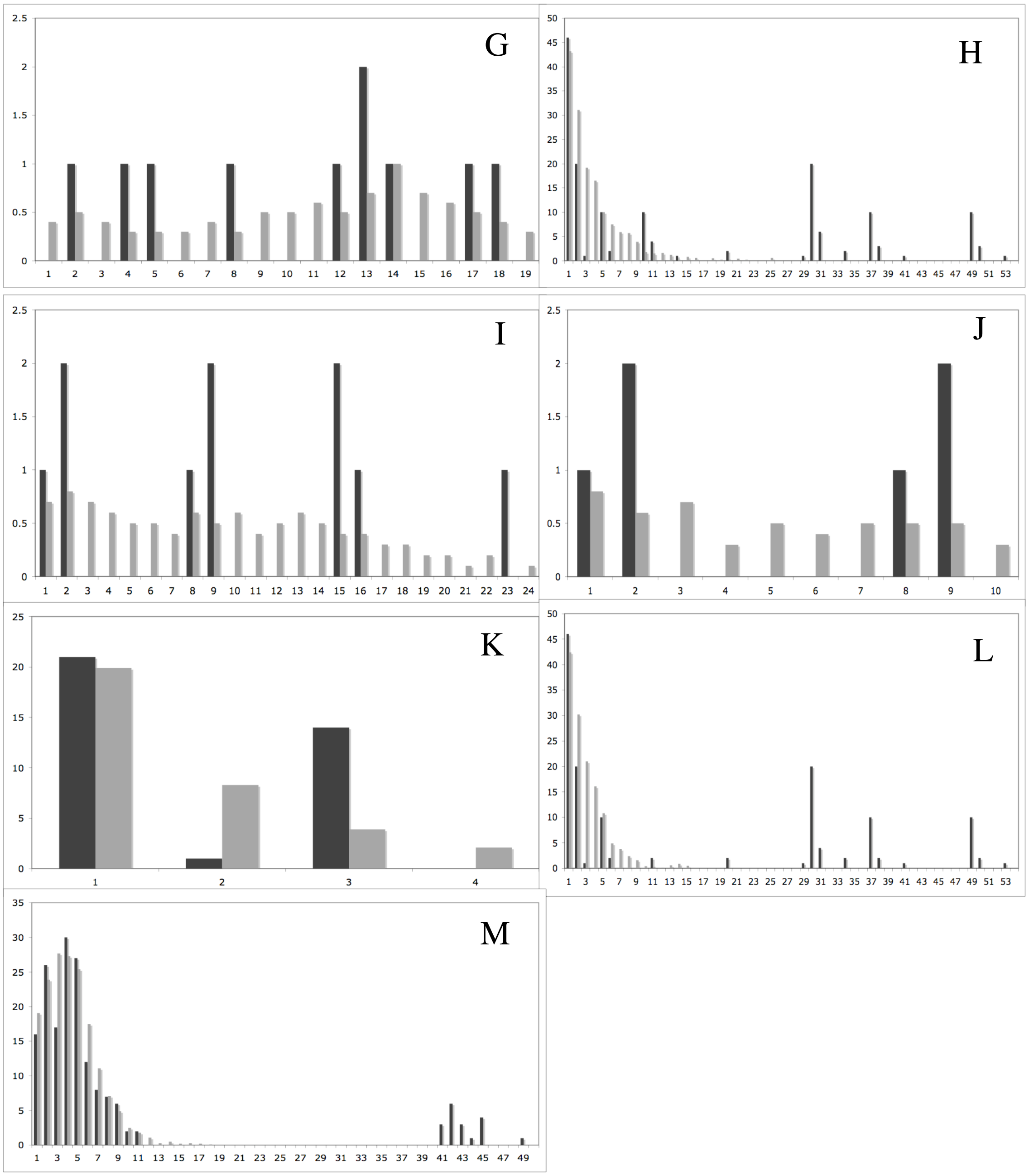

Figure 3.6, continued: *A. cephalotes* refugia populations: (G) upper Central America & Mexico; H–I, *A. sexdens* populations as defined by the marine incursion hypothesis: (H) Guiana Shield, (I) Brazilian Shield; J–L, *A. sexdens* populations as defined by the refugia hypothesis: (J) Bahia, (K) Western Amazonia, (L) Guiana Shield; M, *A. laevigata* populations as defined by the refugia & marine incursion hypotheses: (M) Guiana Shield.

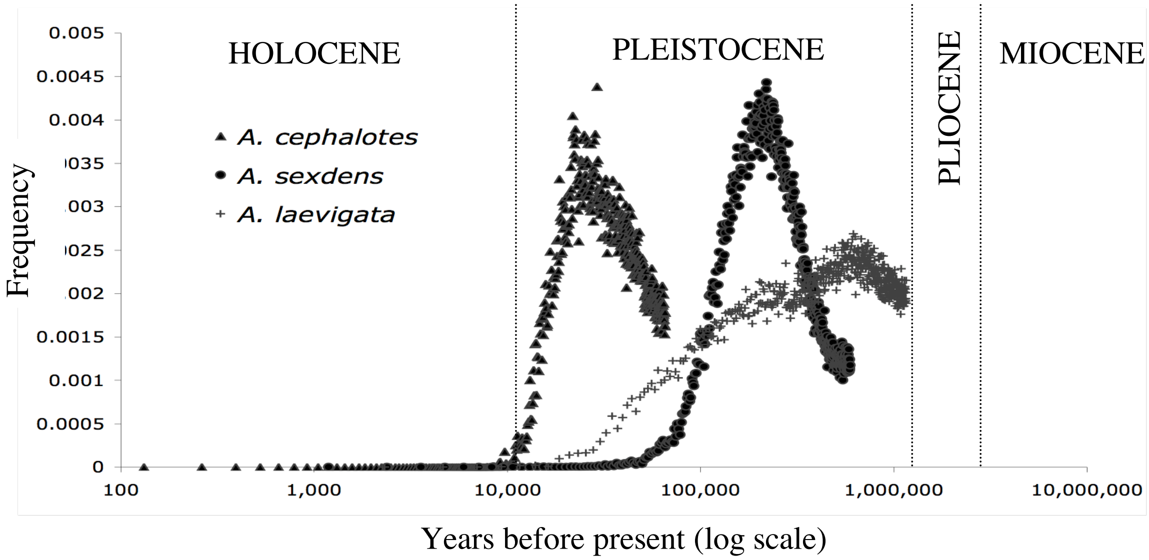

Figure 3.7: Posterior distributions of T_{div}, the time since the oldest population division for each species, assuming a mutation rate of 1.5% per million years and a generation time of 4 years. The mode and 95% confidence limits are given in Table 2.7. The approximate boundaries of geological epochs (names in uppercase) are shown with dotted lines.

Appendices

APPENDIX A: Localities used as training points for predictive niche-based modeling. Localities are defined as a site in which a species was directly observed or collected, either at a nest or along a foraging trail. Latitude and longitude were recorded using a handheld GPS receiver.

Species	Site	Country	State/Prov.	Latitude	Longitude
A. cephalotes	Macapa 1	BRAZIL	Amapa	0.60082	-51.75435
A. cephalotes	Macapa 2	BRAZIL	Amapa	0.60029	-51.75318
A. cephalotes	Macapa 3	BRAZIL	Amapa	0.61917	-51.70161
A. cephalotes	Macapa 4	BRAZIL	Amapa	0.62059	-51.69178
A. cephalotes	Carreiro da Varzea	BRAZIL	Amazonas	-3.65538	-60.26173
A. cephalotes	CEPLAC	BRAZIL	Bahia	-14.75536111	-39.23255556
A. cephalotes	Farm	BRAZIL	Bahia	-14.20027778	-39.81586111
A. cephalotes	Fazenda Cascata	BRAZIL	Bahia	-14.41355556	-39.33088889
A. cephalotes	Ipiau	BRAZIL	Bahia	-14.09619444	-39.78102778
A. cephalotes	Ubaitaba	BRAZIL	Bahia	-14.83975	-39.02688889
A. cephalotes	Alta Floresta 1	BRAZIL	Mato Grosso	-9.86249	-56.07603
A. cephalotes	Alta Floresta 2	BRAZIL	Mato Grosso	-9.59214	-56.02422
A. cephalotes	Alta Floresta 3	BRAZIL	Mato Grosso	-9.65121	-56.01799
A. cephalotes	Alta Floresta 4	BRAZIL	Mato Grosso	-9.55657	-55.997
A. cephalotes	Alta Floresta 5	BRAZIL	Mato Grosso	-9.53129	-55.99617
A. cephalotes	Alta Floresta 6	BRAZIL	Mato Grosso	-9.75642	-55.86272
A. cephalotes	Alta Floresta 7	BRAZIL	Mato Grosso	-10.05337	-55.43224
A. cephalotes	Alenquer 1	BRAZIL	Para	-1.90357	-54.64113
A. cephalotes	Alenquer 2	BRAZIL	Para	-1.92631	-54.63676
A. cephalotes	Alenquer 3	BRAZIL	Para	-1.91676	-54.62662
A. cephalotes	Belem	BRAZIL	Para	-1.68755	-48.54977
A. cephalotes	Santerem	BRAZIL	Para	-2.55767	-54.72733
A. cephalotes	Frei Caneca	BRAZIL	Pernambuco	-8.720383333	-35.84425
A. cephalotes	La Selva	COSTA RICA	Heredia	10.4299	-84.0097
A. cephalotes	Banano del Sur	COSTA RICA	Limon	9.874966667	-83.009
A. cephalotes	Cahuita 1	COSTA RICA	Limon	9.736033333	-82.83843333
A. cephalotes	Cahuita 2	COSTA RICA	Limon	9.735816667	-82.83786667
A. cephalotes	Cahuita 3	COSTA RICA	Limon	9.7343	-82.82808333
A. cephalotes	Cahuita 4	COSTA RICA	Limon	9.73845	-82.82213333
A. cephalotes	Matina-Limón	COSTA RICA	Limon	10.02683333	-83.24896667
A. cephalotes	Moín	COSTA RICA	Limon	9.992483333	-83.12618333
A. cephalotes	Pacuarito	COSTA RICA	Limon	10.10153333	-83.46098333
A. cephalotes	Puerto Viejo de Talamanca 1	COSTA RICA	Limon	9.652666667	-82.75198333
A. cephalotes	Puerto Viejo de Talamanca 2	COSTA RICA	Limon	9.638683333	-82.6934
A. cephalotes	Puerto Viejo de Talamanca 3	COSTA RICA	Limon	9.6396	-82.69281667

Species	Site	Country	State/Prov.	Latitude	Longitude
A. cephalotes	Puerto Viejo de Talamanca 4	COSTA RICA	Limon	9.640666667	-82.6928
A. cephalotes	Puerto Viejo de Talamanca 5	COSTA RICA	Limon	9.64005	-82.69256667
A. cephalotes	Tres Rosales	COSTA RICA	Limon	10.50718333	-84.0309
A. cephalotes	Valle de la Estrella	COSTA RICA	Limon	9.743916667	-82.93256667
A. cephalotes	Westfalia 1	COSTA RICA	Limon	9.935983333	-83.00621667
A. cephalotes	Westfalia 2	COSTA RICA	Limon	9.9356	-83.00588333
A. cephalotes	La Selva	ECUADOR	Sucumbios	-0.497516667	-76.37471667
A. cephalotes	Tiputini	ECUADOR	Orellana	-0.638250	-76.14931667
A. cephalotes	Amazon Nature Lodge 1	FRENCH GUYANA	N/A	4.5518	-52.21196667
A. cephalotes	Amazon Nature Lodge 2	FRENCH GUYANA	N/A	4.559766667	-52.2068
A. cephalotes	Amazon Nature Lodge 3	FRENCH GUYANA	N/A	4.557833333	-52.2039
A. cephalotes	Amazon Nature Lodge 4	FRENCH GUYANA	N/A	4.55705	-52.17618333
A. cephalotes	Kaw Boat Landing	FRENCH GUYANA	N/A	4.498266667	-52.05238333
A. cephalotes	Rt. N1 Kourou-St.Laurent	FRENCH GUYANA	N/A	5.266383333	-52.91666667
A. cephalotes	Palenque1	MEXICO	Chiapas	17.49168333	-92.02481667
A. cephalotes	Palenque2	MEXICO	Chiapas	17.49153333	-92.02375
A. cephalotes	Palenque3	MEXICO	Chiapas	17.49	-92.02366667
A. cephalotes	Palenque4	MEXICO	Chiapas	17.49005	-92.02361667
A. cephalotes	Palenque5	MEXICO	Chiapas	17.51018333	-91.98591667
A. cephalotes	Temascal	MEXICO	Oaxaca	18.23121667	-96.4189
A. cephalotes	Sierra de los Tuxtlas	MEXICO	Veracruz	18.48846667	-95.06698333
A. cephalotes	Almirante-Changuinola 1	PANAMA	Bocas del Toro	9.33111	-82.46096
A. cephalotes	Almirante-Changuinola 2	PANAMA	Bocas del Toro	9.29537	-82.42819
A. cephalotes	Isla Bastimentos	PANAMA	Bocas del Toro	9.34018	-82.17691
A. cephalotes	Isla Colón	PANAMA	Bocas del Toro	9.39198	-82.23982
A. cephalotes	Isla Cristobal	PANAMA	Bocas del Toro	9.29051	-82.26089
A. cephalotes	Isla Popa	PANAMA	Bocas del Toro	9.20105	-82.13069
A. cephalotes	Punta Peña-Almirante 1	PANAMA	Bocas del Toro	9.14857	-82.32436
A. cephalotes	Punta Peña-Almirante 2	PANAMA	Bocas del Toro	9.02712	-82.30523
A. cephalotes	Punta Peña-Almirante 3	PANAMA	Bocas del Toro	9.04552	-82.30249
A. cephalotes	Punta Peña-Almirante 4	PANAMA	Bocas del Toro	9.0982	-82.2895
A. cephalotes	Punta Peña-Almirante 5	PANAMA	Bocas del Toro	8.99978	-82.26275
A. cephalotes	Coclesito	PANAMA	Cocle	8.68655	-80.4557

Species	Site	Country	State/Prov.	Latitude	Longitude
A. cephalotes	Fort Sherman	PANAMA	Colon	9.32605	-79.95731
A. cephalotes	Fort Sherman	PANAMA	Colon	9.33388	-79.79851
A. cephalotes	BCI	PANAMA	Panama	9.16425	-79.84784
A. cephalotes	Pipeline Road	PANAMA	Panama	9.118769	-79.7097
A. cephalotes	Huancaria	PERU	Cusco	-12.90274	-71.42362
A. cephalotes	Pillcopata 1	PERU	Cusco	-13.03925	-71.50857
A. cephalotes	Pillcopata 2	PERU	Cusco	-13.03933	-71.50725
A. cephalotes	Pillcopata 3	PERU	Cusco	-13.02342	-71.49075
A. cephalotes	Pillcopata 4	PERU	Cusco	-13.02591	-71.48086
A. cephalotes	Pillcopata 5	PERU	Cusco	-13.02226	-71.46418
A. cephalotes	ACTS	PERU	Loreto	-3.2489	-72.90908
A. cephalotes	Explorama Lodge	PERU	Loreto	-3.44336	-72.84978
A. cephalotes	CICRA	PERU	Madre de Dios	-12.56895	-70.1002
A. cephalotes	Salvación	PERU	Madre de Dios	-12.82841	-71.36393
A. cephalotes	Rancho Grande Biological Station	VENEZUELA	Aragua	10.34846	-67.68436
A. cephalotes	Calderas 1	VENEZUELA	Barinas	8.83354	-70.4947
A. cephalotes	Calderas 2	VENEZUELA	Barinas	8.84314	-70.49066
A. cephalotes	Calderas 3	VENEZUELA	Barinas	8.85996	-70.47611
A. cephalotes	Calderas 4	VENEZUELA	Barinas	8.89603	-70.44917
A. cephalotes	Calderas 5	VENEZUELA	Barinas	8.91012	-70.44672
A. cephalotes	Kukenan Camp	VENEZUELA	Bolívar	5.10819	-60.82989
A. cephalotes	Canaima	VENEZUELA	Bolívar	6.2465	-62.85368
A. cephalotes	Campamento Rio Grande 1	VENEZUELA	Delta Amacuro	8.13954	-61.68963
A. cephalotes	Campamento Rio Grande 2	VENEZUELA	Delta Amacuro	8.4224	-61.67191
A. cephalotes	Monte Aventino	VENEZUELA	Merida	9.00528	-71.08352
A. cephalotes	La Palmita	VENEZUELA	Merida	8.73499	-71.44686
A. cephalotes	Monte Carmelo	VENEZUELA	Trujillo	9.25677	-70.85779
A. sexdens	Macapa 1	BRAZIL	Amapa	0.67436	-51.53008
A. sexdens	Macapa 2	BRAZIL	Amapa	0.6772	-51.50669
A. sexdens	Manaus	BRAZIL	Amazonas	-2.31131	-60.02489
A. sexdens	Barra do Rocha	BRAZIL	Bahia	-14.18722222	-39.65983333
A. sexdens	Ipiau	BRAZIL	Bahia	-14.09619444	-39.78102778
A. sexdens	roadside	BRAZIL	Bahia	-14.20027778	-39.81586111
A. sexdens	Alta Floresta 1	BRAZIL	Mato Grosso	-10.06242	-55.59001
A. sexdens	Alta Floresta 2	BRAZIL	Mato Grosso	-10.05337	-55.43224
A. sexdens	Alta Floresta 3	BRAZIL	Mato Grosso	-9.89753	-56.09404
A. sexdens	Alta Floresta 4	BRAZIL	Mato Grosso	-9.895055556	-55.90791667
A. sexdens	Alta Floresta 5	BRAZIL	Mato Grosso	-9.86249	-56.07603
A. sexdens	Alta Floresta 6	BRAZIL	Mato Grosso	-9.5746	-56.01333
A. sexdens	Novo Motum	BRAZIL	Mato Grosso	-13.74634	-56.05287
A. sexdens	Sinop	BRAZIL	Mato Grosso	-12.08227778	-55.51755556
A. sexdens	Alenquer 1	BRAZIL	Para	-1.92631	-54.63676
A. sexdens	Alenquer 2	BRAZIL	Para	-1.91676	-54.62662
A. sexdens	Alenquer 3	BRAZIL	Para	-1.90357	-54.64113
A. sexdens	Belem 1	BRAZIL	Para	-1.68755	-48.54977
A. sexdens	Belem 2	BRAZIL	Para	-1.416666	-48.417555
A. sexdens	Santerem 1	BRAZIL	Para	-2.66756	-54.6574
A. sexdens	Santerem 2	BRAZIL	Para	-2.64342	-54.78015

Species	Site	Country	State/Prov.	Latitude	Longitude
A. sexdens	Santerem 3	BRAZIL	Para	-2.55767	-54.72733
A. sexdens	Frei Caneca	BRAZIL	Pernambuco	-8.720383333	-35.84425
A. sexdens	Cayenne Airport	FRENCH GUYANA	N/A	4.823033333	-52.3643
A. sexdens	Km 194 Route N1	FRENCH GUYANA	N/A	5.470383333	-53.57206667
A. sexdens	Mont Rorora	FRENCH GUYANA	N/A	4.886016667	-52.26111667
A. sexdens	Montjoly 1	FRENCH GUYANA	N/A	4.885116667	-52.26281667
A. sexdens	Montjoly 2	FRENCH GUYANA	N/A	4.911966667	-52.27306667
A. sexdens	Montjoly 3	FRENCH GUYANA	N/A	4.912566667	-52.27195
A. sexdens	Montjoly 4	FRENCH GUYANA	N/A	4.92245	-52.28185
A. sexdens	Montjoly 5	FRENCH GUYANA	N/A	4.935983333	-52.28626667
A. sexdens	Montjoly 6	FRENCH GUYANA	N/A	4.944683333	-52.31465
A. sexdens	Mt. Pariacabo, Kourou	FRENCH GUYANA	N/A	5.159433333	-52.67355
A. sexdens	Rt N1 Kourou-St.Laurent 1	FRENCH GUYANA	N/A	5.239266667	-52.90688333
A. sexdens	Rt N1 Kourou-St.Laurent 2	FRENCH GUYANA	N/A	5.266383333	-52.91666667
A. sexdens	Rt N1 Kourou-St.Laurent 3	FRENCH GUYANA	N/A	5.480766667	-53.5649
A. sexdens	St. Laurent	FRENCH GUYANA	N/A	5.48925	-54.00153333
A. sexdens	Tonate-Kourou Road	FRENCH GUYANA	N/A	5.0709	-52.5439
A. sexdens	Gamboa	PANAMA	Panama	9.369722	-79.931944
A. sexdens	ACTS	PERU	Loreto	-3.2489	-72.90908
A. sexdens	Explorama Lodge	PERU	Loreto	-3.44336	-72.84978
A. sexdens	CICRA	PERU	Madre de Dios	-12.56895	-70.1002
A. sexdens	El Tigre 1	VENEZUELA	Anzoátegui	8.82739	-64.14829
A. sexdens	El Tigre 2	VENEZUELA	Anzoátegui	8.85397	-64.204
A. sexdens	UCV Campus	VENEZUELA	Aragua	10.27306	-67.61294
A. sexdens	Canaima	VENEZUELA	Bolívar	6.2465	-62.85368
A. sexdens	Cd. Bolivar 1	VENEZUELA	Bolívar	8.13762	-63.54602
A. sexdens	Cd. Bolivar 2	VENEZUELA	Bolívar	8.14594	-63.55125
A. sexdens	Cd. Bolivar-Cd. Guayana	VENEZUELA	Bolívar	8.21699	-62.85557
A. sexdens	Mte Carmelo 1	VENEZUELA	Trujillo	9.28387	-70.86011
A. sexdens	Mte Carmelo 2	VENEZUELA	Trujillo	9.28387	-70.86011
A. laevigata	Ferreira Gomes	BRAZIL	Amapa	0.73981	-51.33325
A. laevigata	Macapa 1	BRAZIL	Amapa	0.16766	-51.12546
A. laevigata	Macapa 2	BRAZIL	Amapa	0.17151	-51.51285
A. laevigata	Macapa 3	BRAZIL	Amapa	0.6772	-51.50669
A. laevigata	Tartarugalzinho	BRAZIL	Amapa	1.43379	-50.89864
A. laevigata	Manaus 1	BRAZIL	Amazonas	-3.085277	-60.01
A. laevigata	Manaus 2	BRAZIL	Amazonas	-2.69491	-59.73894
A. laevigata	Manaus 3	BRAZIL	Amazonas	-2.61851	-59.61978
A. laevigata	Manaus 4	BRAZIL	Amazonas	-2.31131	-60.02489
A. laevigata	Brasilia	BRAZIL	Dto. Federal	-16.366666	-48.466666
A. laevigata	Serranopolis	BRAZIL	Goias	-15.873611	-49.606388
A. laevigata	São Luis	BRAZIL	Maranhão	-2.9	-44.45
A. laevigata	Araguainha	BRAZIL	Mato Grosso	-16.84	-53.01
A. laevigata	Itauba	BRAZIL	Mato Grosso	-11.21058	-55.30494
A. laevigata	Posto Gil	BRAZIL	Mato Grosso	-14.47869444	-56.16755556

Species	Site	Country	State/Prov.	Latitude	Longitude
A. laevigata	roadside	BRAZIL	Mato Grosso	-13.75377778	-56.05347222
A. laevigata	Sinop 1	BRAZIL	Mato Grosso	-12.09205	-55.51761
A. laevigata	Sinop 2	BRAZIL	Mato Grosso	-12.08227778	-55.51755556
A. laevigata	Jardim	BRAZIL	Minas Gerais	-21.503333	-46.209166
A. laevigata	Lavras	BRAZIL	Minas Gerais	-21.26	-45.03
A. laevigata	Uberlandia	BRAZIL	Minas Gerais	19	-48.31
A. laevigata	Viçosa	BRAZIL	Minas Gerais	-21.266666	-43.483333
A. laevigata	Santerem	BRAZIL	Para	-2.93924	-54.9285
A. laevigata	Santerem	BRAZIL	Para	-2.82138	-54.90031
A. laevigata	Farol	BRAZIL	Parana	-24.11	-52.62
A. laevigata	Botucatu	BRAZIL	São Paulo	-22.9	-48.46
A. laevigata	Santa Barbara D'Oeste	BRAZIL	São Paulo	-22.7	-47.291388
A. laevigata	El Tigre	VENEZUELA	Anzoátegui	8.82739	-64.14829
A. laevigata	Soledad	VENEZUELA	Anzoátegui	8.22513	-63.50855
A. laevigata	Barinitas	VENEZUELA	Barinas	8.77066	-70.42052
A. laevigata	Kukenan Camp	VENEZUELA	Bolívar	5.10819	-60.82989
A. laevigata	Canaima	VENEZUELA	Bolívar	6.2465	-62.85368
A. laevigata	Cd. Bolivar	VENEZUELA	Bolívar	7.88762	-63.59712
A. laevigata	Cd. Bolivar-Cd. Guayana 1	VENEZUELA	Bolívar	8.09461	-63.20366
A. laevigata	Cd. Bolivar-Cd. Guayana 2	VENEZUELA	Bolívar	8.21699	-62.85557
A. laevigata	Cd. Guayana 1	VENEZUELA	Bolívar	8.22086	-62.85172
A. laevigata	Cd. Guayana 2	VENEZUELA	Bolívar	8.23616	-62.83675
A. laevigata	Cd. Guayana 3	VENEZUELA	Bolívar	8.25965	-62.81322
A. laevigata	Temblador 1	VENEZUELA	Monagas	8.73389	-62.38002
A. laevigata	Temblador 2	VENEZUELA	Monagas	8.97284	-62.75017
A. laevigata	Temblador 3	VENEZUELA	Monagas	9.00554	-62.66763

APPENDIX B: List of all specimens used for molecular analyses and their collection locality information. Site numbers refer to areas in Figure 2.1. Latitude and longitude were recorded using a handheld GPS receiver.

Species	ColonyID	Latitude	Longitude	Country	State/ Province	Site Name	Site #
Atta cephalotes	650	6.468333	-75.035555	COLOMBIA	Antioquia	San Roque	12
Atta cephalotes	651	6.469166	-75.034722	COLOMBIA	Antioquia	San Roque	12
Atta cephalotes	688	6.534722	-75.884444	COLOMBIA	Antioquia	Santafé de Antioquia	12
Atta cephalotes	692	6.533333	-75.883333	COLOMBIA	Antioquia	Santafé de Antioquia	12
Atta cephalotes	693	6.535277	-75.883888	COLOMBIA	Antioquia	Santafé de Antioquia	12
Atta cephalotes	697	5.667222	-75.850833	COLOMBIA	Antioquia	Andes	12
Atta cephalotes	699	5.97	-75.72	COLOMBIA	Antioquia	Venecia	12
Atta cephalotes	AGH03061101	-0.497516667	-76.37471667	ECUADOR	Sucumbios	La Selva	15
Atta cephalotes	AGH030612-01	-0.497516667	-76.37471667	ECUADOR	Sucumbios	La Selva	15
Atta cephalotes	AL030612-02	-0.497516667	-76.37471667	ECUADOR	Sucumbios	La Selva	15
Atta cephalotes	AL030614-02	-0.638250	-76.14931667	ECUADOR	Orellana	Tiputini	15
Atta cephalotes	ATMCAVE	17.216666	-88.833333	BELIZE	Cayo	ATM Cave	17
Atta cephalotes	CINCIZOO	10.683611	-61.283333	TRINIDAD	N/A	?	25
Atta cephalotes	E909	-14.201777	-39.82375	BRAZIL	Bahia	Aurelino Leal	4
Atta cephalotes	E958	-9.55657	-55.997	BRAZIL	Mato Grosso	Alta Floresta	5
Atta cephalotes	KM-GU	5.3	-59.9	GUYANA	Potaro-Siparuni	?	29
Atta cephalotes	NMG030609-04	-0.497516667	-76.37471667	ECUADOR	Sucumbios	La Selva	15
Atta cephalotes	NMG030611-01	-0.497516667	-76.37471667	ECUADOR	Sucumbios	La Selva	15
Atta cephalotes	RIH-ESMEC	0.403333	-79.973333	ECUADOR	Esmeraldes	Finca Tenorio-La Tola	14
Atta cephalotes	SES020106-01	9.652666667	-82.75198333	COSTA RICA	Limon	Puerto Viejo de Talamanca	20
Atta cephalotes	SES020107-01	9.638683333	-82.6934	COSTA RICA	Limon	Puerto Viejo de Talamanca	20

Species	ColonyID	Latitude	Longitude	Country	State/ Province	Site Name	Site #
Atta cephalotes	SES020107-02	9.6396	-82.69281667	COSTA RICA	Limon	Puerto Viejo de Talamanca	20
Atta cephalotes	SES020107-05	9.736033333	-82.83843333	COSTA RICA	Limon	Cahuita	20
Atta cephalotes	SES020107-07	9.73845	-82.82213333	COSTA RICA	Limon	Cahuita	20
Atta cephalotes	SES020108-03	9.874966667	-83.009	COSTA RICA	Limon	Banano del Sur	20
Atta cephalotes	SES020109-01	9.992483333	-83.12618333	COSTA RICA	Limon	Moín	20
Atta cephalotes	SES020109-02	10.02683333	-83.24896667	COSTA RICA	Limon	Matina-Limón	20
Atta cephalotes	SES020110-01	10.50718333	-84.0309	COSTA RICA	Limon	Tres Rosales	20
Atta cephalotes	SES020113-01	10.4299	-84.0097	COSTA RICA	Heredia	La Selva Arboretum	13
Atta cephalotes	SES020113-04	10.42926667	-84.00995	COSTA RICA	Heredia	La Selva Arboretum	13
Atta cephalotes	SES020113-06	10.42951667	-84.0106	COSTA RICA	Heredia	La Selva Arboretum	13
Atta cephalotes	SES020113-07	10.42966667	-84.01073333	COSTA RICA	Heredia	La Selva Arboretum	13
Atta cephalotes	SES020113-10	10.43008333	-84.01118333	COSTA RICA	Heredia	La Selva Arboretum	13
Atta cephalotes	SES020524-14	9.32605	-79.95731	PANAMA	Colon	Fort Sherman	21
Atta cephalotes	SES020531-04	8.99978	-82.26275	PANAMA	Bocas del Toro	Punta Peña-Almirante	20
Atta cephalotes	SES020602-05	9.02712	-82.30523	PANAMA	Bocas del Toro	Punta Peña-Almirante	20
Atta cephalotes	SES020603-02	9.29537	-82.42819	PANAMA	Bocas del Toro	Almirante-Changuinola	20
Atta cephalotes	SES020604-02	9.0982	-82.2895	PANAMA	Bocas del Toro	Punta Peña-Almirante	20
Atta cephalotes	SES020606-04	9.34018	-82.17691	PANAMA	Bocas del Toro	Isla Bastimentos	20
Atta cephalotes	SES020609-01	9.20105	-82.13069	PANAMA	Bocas del Toro	Isla Popa	20
Atta cephalotes	SES020609-02	9.20105	-82.13069	PANAMA	Bocas del Toro	Isla Popa	20
Atta cephalotes	SES020609-03	9.20105	-82.13069	PANAMA	Bocas del Toro	Isla Popa	20
Atta cephalotes	SES030112-02	18.48846667	-95.06698333	MEXICO	Veracruz	Sierra de los Tuxtlas	18
Atta cephalotes	SES030112-04	18.48846667	-95.06698333	MEXICO	Veracruz	Sierra de los Tuxtlas	18
Atta cephalotes	SES040120-04	-8.720383333	-35.84425	BRAZIL	Pernambuco	Frei Caneca	11
Atta cephalotes	SES040120-01	-8.720383333	-35.84425	BRAZIL	Pernambuco	Frei Caneca	11

Species	ColonyID	Latitude	Longitude	Country	State/ Province	Site Name	Site #
Atta cephalotes	SES040121-03	-8.720383333	-35.84425	BRAZIL	Pernambuco	Frei Caneca	11
Atta cephalotes	SES040123-01	-14.75536111	-39.23255556	BRAZIL	Bahia	CEPLAC	4
Atta cephalotes	SES040123-03	-14.75536111	-39.23255556	BRAZIL	Bahia	CEPLAC	4
Atta cephalotes	SES040123-06	-14.75536111	-39.23255556	BRAZIL	Bahia	CEPLAC	4
Atta cephalotes	SES040123-07	-14.75536111	-39.23255556	BRAZIL	Bahia	CEPLAC	4
Atta cephalotes	SES040124-01	-14.83975	-39.02688889	BRAZIL	Bahia	Ubaitaba	4
Atta cephalotes	SES040124-02	-14.83975	-39.02688889	BRAZIL	Bahia	Ubaitaba	4
Atta cephalotes	SES040124-03	-14.83975	-39.02688889	BRAZIL	Bahia	Ubaitaba	4
Atta cephalotes	SES040125-01	-14.41355556	-39.33088889	BRAZIL	Bahia	Fazenda de Cascata	4
Atta cephalotes	SES040125-02	-14.41355556	-39.33088889	BRAZIL	Bahia	Fazenda de Cascata	4
Atta cephalotes	SES040129-03	-9.75642	-55.86272	BRAZIL	Mato Grosso	Alta Floresta	5
Atta cephalotes	SES040129-06	-9.75642	-55.86272	BRAZIL	Mato Grosso	Alta Floresta	5
Atta cephalotes	SES040131-04	-10.05337	-55.43224	BRAZIL	Mato Grosso	Alta Floresta	5
Atta cephalotes	SES040131-06	-10.05337	-55.43224	BRAZIL	Mato Grosso	Alta Floresta	5
Atta cephalotes	SES040131-12	-9.86249	-56.07603	BRAZIL	Mato Grosso	Alta Floresta	5
Atta cephalotes	SES040131-13	-9.86249	-56.07603	BRAZIL	Mato Grosso	Alta Floresta	5
Atta cephalotes	SES040204-03	-1.68755	-48.54977	BRAZIL	Para	Belem	9
Atta cephalotes	SES040208-06	0.62059	-51.69178	BRAZIL	Amapa	Macapa	1
Atta cephalotes	SES040208-10	0.60082	-51.75435	BRAZIL	Amapa	Macapa	1
Atta cephalotes	SES040214-03	-1.90357	-54.64113	BRAZIL	Para	Alenquer	8
Atta cephalotes	SES040215-03	-1.92631	-54.63676	BRAZIL	Para	Alenquer	8
Atta cephalotes	SES040220-01	-3.65538	-60.26173	BRAZIL	Amazonas	Carreiro da Varzea	2
Atta cephalotes	SES040305-01	5.10819	-60.82989	VENEZUELA	Bolivar	Kukenan Camp	29
Atta cephalotes	SES040528-01	-12.90274	-71.42362	PERU	Cusco	Pillcopata	22
Atta cephalotes	SES040528-06	-12.90274	-71.42362	PERU	Cusco	Pillcopata	22

Species	ColonyID	Latitude	Longitude	Country	State/ Province	Site Name	Site #
Atta cephalotes	SES040528-08	-12.90274	-71.42362	PERU	Cusco	Pillcopata	22
Atta cephalotes	SES040530-03	-12.82841	-71.36393	PERU	Madre de Dios	Salvación	22
Atta cephalotes	SES040605-01	-12.56895	-70.1002	PERU	Madre de Dios	CICRA	24
Atta cephalotes	SES040605-03	-12.56895	-70.1002	PERU	Madre de Dios	CICRA	24
Atta cephalotes	SES040605-06	-12.56895	-70.1002	PERU	Madre de Dios	CICRA	24
Atta cephalotes	SES040605-07	-12.56895	-70.1002	PERU	Madre de Dios	CICRA	24
Atta cephalotes	SES040606-01	-12.56895	-70.1002	PERU	Madre de Dios	CICRA	24
Atta cephalotes	SES040607-01	-12.56895	-70.1002	PERU	Madre de Dios	CICRA	24
Atta cephalotes	SES040609-01	-12.56895	-70.1002	PERU	Madre de Dios	CICRA	24
Atta cephalotes	SES040609-05	-12.56895	-70.1002	PERU	Madre de Dios	CICRA	24
Atta cephalotes	SES040613-02	-3.44336	-72.84978	PERU	Loreto	Explorama Lodge	15
Atta cephalotes	SES040615-03	-3.44336	-72.84978	PERU	Loreto	Explorama Lodge	15
Atta cephalotes	SES040615-05	-3.44336	-72.84978	PERU	Loreto	Explorama Lodge	15
Atta cephalotes	SES040616-02	-3.2489	-72.90908	PERU	Loreto	ACTS	15
Atta cephalotes	SES040616-03	-3.2489	-72.90908	PERU	Loreto	ACTS	15
Atta cephalotes	SES040617-02	-3.2489	-72.90908	PERU	Loreto	ACTS	15
Atta cephalotes	SES040617-03	-3.2489	-72.90908	PERU	Loreto	ACTS	15
Atta cephalotes	SES050730-04	10.34846	-67.68436	VENEZUELA	Aragua	Rancho Grande Biological Station	27
Atta cephalotes	SES050801-04	10.34846	-67.68436	VENEZUELA	Aragua	Parque Nacional Henri Pittier	27
Atta cephalotes	SES050801-05	10.34846	-67.68436	VENEZUELA	Aragua	Parque Nacional Henri Pittier	27
Atta cephalotes	SES050801-06	10.34846	-67.68436	VENEZUELA	Aragua	Parque Nacional Henri Pittier	27
Atta cephalotes	SES050803-01	9.25677	-70.85779	VENEZUELA	Trujillo	Monte Carmelo	33
Atta cephalotes	SES050803-02	9.25677	-70.85779	VENEZUELA	Trujillo	Monte Carmelo	33

Species	ColonyID	Latitude	Longitude	Country	State/ Province	Site Name	Site #
Atta cephalotes	SES050803-03	9.00528	-71.08352	VENEZUELA	Merida	Monte Aventino	33
Atta cephalotes	SES050803-04	9.00528	-71.08352	VENEZUELA	Merida	Monte Aventino	33
Atta cephalotes	SES050803-05	9.00528	-71.08352	VENEZUELA	Merida	Monte Aventino	33
Atta cephalotes	SES050803-06	9.00528	-71.08352	VENEZUELA	Merida	Monte Aventino	33
Atta cephalotes	SES050804-02	8.73499	-71.44686	VENEZUELA	Merida	Parque Recreacional La Palmita	33
Atta cephalotes	SES050804-03	8.73499	-71.44686	VENEZUELA	Merida	La Palmita	33
Atta cephalotes	SES050804-04	8.73499	-71.44686	VENEZUELA	Merida	La Palmita	33
Atta cephalotes	SES050807-11	8.84314	-70.49066	VENEZUELA	Barinas	Calderas	28
Atta cephalotes	SES050817-02	8.13954	-61.68963	VENEZUELA	Delta Amacuro	Campamento Rio Grande	32
Atta cephalotes	SES050817-03	8.13954	-61.68963	VENEZUELA	Delta Amacuro	Campamento Rio Grande	32
Atta cephalotes	SSP0304XX-XX	20.4	-88.36	MEXICO	Yucatan	Chichén Itzá	19
Atta cephalotes	SV030618-09	-0.638250	-76.14931667	ECUADOR	Orellana	Tiputini	15
Atta cephalotes	TIKAL	17.13	-89.24	GUATEMALA	Petén	Tikal	17
Atta cephalotes	UGM050719-03	4.559766667	-52.2068	FRENCH GUYANA	N/A	Amazon Nature Lodge	16
Atta cephalotes	UGM050727-01	4.5518	-52.21196667	FRENCH GUYANA	N/A	Amazon Nature Lodge	16
Atta cephalotes	UGM050727-02	4.557833333	-52.2039	FRENCH GUYANA	N/A	Amazon Nature Lodge	16
Atta cephalotes	UGM050727-03	4.498266667	-52.05238333	FRENCH GUYANA	N/A	Kaw Boat Landing	16
Atta cephalotes	UGM050727-04	4.55705	-52.17618333	FRENCH GUYANA	N/A	Amazon Nature Lodge	16
Atta cephalotes	UGM950108-05	10.683611	-61.283333	TRINIDAD	N/A	Simla	25
Atta cephalotes	UGM950109-04	10.683611	-61.283333	TRINIDAD	N/A	Simla	25
Atta cephalotes	UGM950111-02	10.683611	-61.283333	TRINIDAD	N/A	Simla	25
Atta cephalotes	UGM950111-03	10.683611	-61.283333	TRINIDAD	N/A	Simla	25
Atta cephalotes	UGM950113-07	10.683611	-61.283333	TRINIDAD	N/A	Simla	25
Atta cephalotes	UGM950114-10	10.683611	-61.283333	TRINIDAD	N/A	Simla	25

Species	ColonyID	Latitude	Longitude	Country	State/ Province	Site Name	Site #
Atta sexdens	CR060807-01	-1.453502778	-48.47670833	Brazil	Para	Belem	9
Atta sexdens	CR060817-05	-2.48382	-54.95919	Brazil	Para	Alter do Chao	10
Atta sexdens	SES040124-07	-14.18722222	-39.65983333	BRAZIL	Bahia	Roadside	4
Atta sexdens	SES040124-09	-14.09619444	-39.78102778	BRAZIL	Bahia	Ipiau	4
Atta sexdens	SES040125-05	-14.20027778	-39.81586111	BRAZIL	Bahia	Roadside	4
Atta sexdens	SES040125-06	-14.20027778	-39.81586111	BRAZIL	Bahia	Farm	4
Atta sexdens	SES040131-11	-10.06242	-55.59001	BRAZIL	Mato Grosso	Alta Floresta	5
Atta sexdens	SES040208-02	0.6772	-51.50669	BRAZIL	Amapa	Macapa	1
Atta sexdens	SES040212-06	-2.64342	-54.78015	BRAZIL	Para	Santerem	10
Atta sexdens	SES040213-03	-2.55767	-54.72733	BRAZIL	Para	Santerem	10
Atta sexdens	SES040215-07	-1.92631	-54.63676	BRAZIL	Para	Alenquer	8
Atta sexdens	SES040215-08	-1.92631	-54.63676	BRAZIL	Para	Alenquer	8
Atta sexdens	SES040215-10	-1.92631	-54.63676	BRAZIL	Para	Alenquer	8
Atta sexdens	SES040216-03	-2.58981	-54.57096	BRAZIL	Para	Santerem	10
Atta sexdens	SES040221-01	-2.31131	-60.02489	BRAZIL	Amazonas	Manaus	3
Atta sexdens	SES040606-03	-12.56895	-70.1002	PERU	Madre de Dios	CICRA	24
Atta sexdens	SES040613-01	-3.44336	-72.84978	PERU	Loreto	Explorama Lodge	23
Atta sexdens	SES040614-01	-3.44336	-72.84978	PERU	Loreto	Explorama Lodge	23
Atta sexdens	SES040614-02	-3.44336	-72.84978	PERU	Loreto	Explorama Lodge	23
Atta sexdens	SES040614-03	-3.44336	-72.84978	PERU	Loreto	Explorama Lodge	23
Atta sexdens	SES040615-01	-3.44336	-72.84978	PERU	Loreto	Explorama Lodge	23
Atta sexdens	SES040618-01	-3.2489	-72.90908	PERU	Loreto	ACTS	23
Atta sexdens	SES040619-03	-3.2489	-72.90908	PERU	Loreto	ACTS	23
Atta sexdens	SES050728-02	10.27306	67.61294	VENEZUELA	Aragua	UCV Campus	27
Atta sexdens	SES050728-08	10.27306	67.61294	VENEZUELA	Aragua	UCV Campus	27
Atta sexdens	SES050802-01	9.28387	-70.86011	VENEZUELA	Trujillo	Monte Carmelo	33
Atta sexdens	SES050802-02	9.28387	-70.86011	VENEZUELA	Trujillo	Monte Carmelo	33
Atta sexdens	SES050814-01	8.85397	-64.204	VENEZUELA	Anzoátegui	El Tigre	26
Atta sexdens	SES050814-06	8.82739	-64.14829	VENEZUELA	Anzoátegui	El Tigre	26
Atta sexdens	SES050815-01	8.14594	-63.55125	VENEZUELA	Bolívar	Ciudad Bolivar	31
Atta sexdens	SES050824-01	6.2465	-62.85368	VENEZUELA	Bolívar	Canaima	30
Atta sexdens	UGM050721-02	4.92245	-52.28185	FRENCH GUYANA	N/A	Montjoly	16
Atta sexdens	UGM050721-05	4.886016667	-52.26111667	FRENCH GUYANA	N/A	Mont Rorora	16
Atta sexdens	UGM050723-09	4.885116667	-52.26281667	FRENCH GUYANA	N/A	Montjoly	16
Atta sexdens	UGM050724-01	4.823033333	-52.3643	FRENCH GUYANA	N/A	Cayenne Airport	16
Atta sexdens	UGM050724-10	5.159433333	-52.67355	FRENCH GUYANA	N/A	Mt. Pariacabo, Kourou	16
Atta sexdens	UGM050725-01	5.239266667	-52.90688333	FRENCH GUYANA	N/A	Rt N1 Kourou-St.Laurent	16

Species	ColonyID	Latitude	Longitude	Country	State/ Province	Site Name	Site #
Atta sexdens	UGM050725-02	5.239266667	-52.90688333	FRENCH GUYANA	N/A	Rt N1 Kourou-St.Laurent	16
Atta sexdens	UGM050725-03	5.239266667	-52.90688333	FRENCH GUYANA	N/A	Rt N1 Kourou-St.Laurent	16
Atta sexdens	UGM050725-05	5.266383333	-52.91666667	FRENCH GUYANA	N/A	Rt N1 Kourou-St.Laurent	16
Atta sexdens	UGM050726-10	5.48925	-54.00153333	FRENCH GUYANA	N/A	St. Laurent	16
Atta sexdens	UGM050726-11	5.48925	-54.00153333	FRENCH GUYANA	N/A	St. Laurent	16
Atta sexdens	UGM050726-13	5.488083333	-53.98501667	FRENCH GUYANA	N/A	St. Laurent	16
Atta sexdens	UGM050726-15	5.470383333	-53.57206667	FRENCH GUYANA	N/A	Km 194 Route N1	16
Atta laevigata	CR060816-01	-2.48382	-54.95919	BRAZIL	Para	Alter do Chao	10
Atta laevigata	CR060817-02	-2.48382	-54.95919	BRAZIL	Para	Alter do Chao	10
Atta laevigata	CR060819-04	-2.53024	-54.94993	BRAZIL	Para	Alter do Chao	10
Atta laevigata	CR060820-09	-2.93728	-54.98359	BRAZIL	Para	Floresta National do Tapajos	10
Atta laevigata	CR060903-07	-2.8907	-59.96969	BRAZIL	Amazonas	EMBRAPA Amazonia Ocidental	3
Atta laevigata	CR060904-25	-2.89824	-59.9903	BRAZIL	Amazonas	EMBRAPA Amazonia Ocidental	3
Atta laevigata	SES040127-01	-14.47869444	-56.16755556	BRAZIL	Mato Grosso	Posto Gil	7
Atta laevigata	SES040201-02	-11.21058	-55.30494	BRAZIL	Mato Grosso	Itauba	6
Atta laevigata	SES040207-01	1.43379	-50.89864	BRAZIL	Amapa	Tartarugal-zinho	1
Atta laevigata	SES040207-03	0.73981	-51.33325	BRAZIL	Amapa	Ferreira Gomes	1
Atta laevigata	SES040207-04	0.73981	-51.33325	BRAZIL	Amapa	Ferreira Gomes	1
Atta laevigata	SES040209-07	0.16766	-51.12546	BRAZIL	Amapa	Macapa	1
Atta laevigata	SES040218-02	-2.69491	-59.73894	BRAZIL	Amazonas	Manaus	3
Atta laevigata	SES040218-04	-2.69491	-59.73894	BRAZIL	Amazonas	Manaus	3
Atta laevigata	SES040221-02	-2.31131	-60.02489	BRAZIL	Amazonas	Manaus	3
Atta laevigata	SES050807-16	8.77066	-70.42052	VENEZUELA	Barinas	Barinitas	28

Species	ColonyID	Latitude	Longitude	Country	State/ Province	Site Name	Site #
Atta laevigata	SES050807-17	8.77066	-70.42052	VENEZUELA	Barinas	Barinitas	28
Atta laevigata	SES050807-18	8.77066	-70.42052	VENEZUELA	Barinas	Barinitas	28
Atta laevigata	SES050814-02	8.82739	-64.14829	VENEZUELA	Anzoátegui	El Tigre	26
Atta laevigata	SES050814-08	8.22513	-63.50855	VENEZUELA	Anzoátegui	Soledad	31
Atta laevigata	SES050814-10	8.22513	-63.50855	VENEZUELA	Anzoátegui	Soledad	31
Atta laevigata	SES050814-11	8.22513	-63.50855	VENEZUELA	Anzoátegui	Soledad	31
Atta laevigata	SES050814-13	8.22513	-63.50855	VENEZUELA	Anzoátegui	Soledad	31
Atta laevigata	SES050816-01	8.09461	-63.20366	VENEZUELA	Bolívar	Cd. Bolivar-Cd. Guayana	31
Atta laevigata	SES050816-04	8.25965	-62.81322	VENEZUELA	Bolívar	Cd. Guayana	31
Atta laevigata	SES050816-05	8.25965	-62.81322	VENEZUELA	Bolívar	Cd. Guayana	31
Atta laevigata	SES050816-06	8.25965	-62.81322	VENEZUELA	Bolívar	Cd. Guayana	31
Atta laevigata	SES050818-06	8.73389	-62.38002	VENEZUELA	Monagas	Temblador	31
Atta laevigata	SES050818-08	8.73389	-62.38002	VENEZUELA	Monagas	Temblador	31
Atta laevigata	SES050819-02	8.97284	-62.75017	VENEZUELA	Monagas	Temblador	31

References

Aleixo, A. 2004. Historical diversification of a Terra-firme forest bird superspecies: A phylogeographic perspective on the role of different hypotheses of Amazonian diversification. Evolution 58:1303-1317.

Aleixo, A. 2006. Historical diversification of floodplain forest specialist species in the Amazon: a case study with two species of the avian genus Xiphorhynchus (Aves : Dendrocolaptidae). Biological Journal of the Linnean Society 89:383-395.

Anderson, R. P. 2003. Real vs. artefactual absences in species distributions: tests for Oryzomys albigularis (Rodentia : Muridae) in Venezuela. Journal of Biogeography 30:591-605.

Araujo, M. B., and A. Guisan. 2006. Five (or so) challenges for species distribution modelling. Journal of Biogeography 33:1677-1688.

Arbogast, B. S., S. V. Edwards, J. Wakeley, P. Beerli, and J. B. Slowinski. 2002. Estimating divergence times from molecular data on phylogenetic and population genetic timescales. Annual Review of Ecology and Systematics 33:707-740.

Autuori, M. 1947. Contribuição para o conhecimento da Saúva (Atta spp.) IV. O saúveiro depois da primeira revoada (Atta sexdens rubropilosa Forel, 1908). Arquivos do Instituto de Biologia de São Paulo 187:39–70.

Avila-Pires, T. C. S. 1995. Lizards of Brazilian Amazonia (Reptilia: Squamata). Zoologische Verhandelingen 299:1–706.

Avise, J. C., J. Arnold, R. M. Ball, E. Bermingham, T. Lamb, J. E. Neigel, C. A. Reeb & N. C. Saunders (1987) Intraspecific phylogeography - the mitochondrial-DNA bridge between population-genetics and systematics. Annual Review of Ecology and Systematics, 18, 489-522.

Avise, J. C. 2000. Phylogeography: the history and formation of species. Harvard University Press, Cambridge, MA.

Bacci, M., A. C. O. Silva-Pinhati, S. E. Solomon, V. G. Martins, A. O. R. Carvalho, U. G. Mueller, and L. G. E. Vieira. In prep. Phylogeny of the ants in the genus Atta (Hymenoptera: Formicidae) based on mitochondrial and nuclear DNA sequeneces.

Bermingham, E., C. W. Dick, and C. Moritz, eds. 2005. Tropical rainforests: past, present, and future. The University of Chicago Press, Chicago, IL.

Bonnet, E., and Y. Van de Peer. 2002. zt: a software tool for simple and partial Mantel tests. Journal of Statistical software 7:1–12.

Bonetto, A. A. 1959. Las hormigas "cortadoras" de la Provincia de Santa Fe (Géneros Atta y Acromyrmex). Pp. 1–79. Ministerio de Agricultura y Ganadería, Santa Fe, Argentina.

Borgmeier, T. 1955. Die Wanderameisen der Neotropischen Region. Studia Entomologica 3:1–720.

Borgmeier, T. 1959. Revision der Gattung Atta Fabricius (Hymenoptera, Formicidae). Studia Entomologica 22:321–390.

Braganca, M. A. L., and Z. C. S. Medeiros. 2006. Occurrence and biological characteristics of parasitoid phorids (Diptera : Phoridae) of the leaf-cutting ant Atta laevigata (Smith) (Hymenoptera : Formicidae) in Porto Nacional, TO, Brazil. Neotropical Entomology 35:408-411.

Braganca, M. A. L., A. Tonhasca, and T. M. C. Della Lucia. 1998. Reduction in the foraging activity of the leaf-cutting ant Atta sexdens caused by the phorid Neodohrniphora sp. Entomologia Experimentalis Et Applicata 89:305-311.

Brener, A. G. F., and J. F. Silva. 1995. Leaf-cutting ants and forest groves in a tropical parkland savanna of Venezuela - facilitated succession. Journal of Tropical Ecology 11:651-669.

Brower, A. V. Z. 1996. Parallel race formation and the evolution of mimicry in Heliconius butterflies: A phylogenetic hypothesis from mitochondrial DNA sequences. Evolution 50:195-221.

Brown, J. M., and A. R. Lemmon. 2007. The importance of data partitioning and the utility of Bayes factors in Bayesian phylogenetics. Systematic Biology in press.

Brown, K. S. 1982. Paleoecology and regional patterns of evolution in Neotropical forest butterflies. Pp. 255–308. Biological diversification in the tropics. Columbia University Press, New York.

Brown, K. S., F. R. S. Sheppard, and J. R. G. Turner. 1974. Quaternary refugia in tropical America: evidence from race formation in Heliconius butterflies. Proceedings of the Royal Society of London, Series B 187:369–378.

Bruschi, S., J. Scott, S. E. Solomon, A. S. Mikheyev, P. Abbot, T. R. Schultz, S. Rehener, J. J. Boomsma, M. Bacci, U. Mueller, and et. al. in prep. Biogeography and evolution of the fungi cultivated by leafcutter ants (Formicidae: Attini).

Bush, M. B. 1994. Amazonian speciation - a necessarily complex model. Journal of Biogeography 21:5-17.

Cafaro, M. J., and C. R. Currie. 2005. Phylogenetic analysis of mutualistic filamentous bacteria associated with fungus-growing ants. Canadian Journal of Microbiology 51:441-446.

Carr-Brown, B. 1972. The Holocene/Pleistocene contact in the offshore area east of Galeota Point, Trinidad, West Indies. Memorias de la VI Conferencia Geológia del Caribe:381–397.

Carstens, B. C., S. J. Brunsfeld, J. R. Demboski, J. M. Good, and J. Sullivan. 2005. Investigating the evolutionary history of the Pacific Northwest mesic forest ecosystem: Hypothesis testing within a comparative phylogeographic framework. Evolution 59:1639-1652.

Carstens, B. C., and L. L. Knowles. 2007. Shifting distributions and speciation: species divergence during rapid climate change. Molecular Ecology 16:619-627.

Cherrett, J. M. 1968. Some aspects of the distribution of pest species of leaf-cutting ants in the Caribbean. Proceedings of the American Society for Horticultural Science (Tropical Region) 12:295–310.

Cherrett, J. M. 1986a. History of the leaf cutting ant problem. Pp. 10-17. Lofgren, C. S. and R. K. Vander Meer.

Cherrett, J. M. 1986b. The biology pest status and control of leaf-cutting ants. Pp. 1-38. Russell, G. E.

Cherrett, J. M. 1986c. The economic importance and control of leaf-cutting ants. Pp. 165–192. Economic impact and control of social insects. Praeger, New York.

Cherrett, J. M., and D. J. Peregrine. 1976. A review of the status of leaf-cutting ants and their control. Proceedings of the Association of Applied Biologists 84:124–128.

Cheviron, Z. A., S. J. Hackett, and A. P. Capparella. 2005. Complex evolutionary history of a Neotropical lowland forest bird (Lepidothrix coronata) and its implications for historical hypotheses of the origin of Neotropical avian diversity. Molecular Phylogenetics and Evolution 36:338-357.

Colinvaux, P. A., and P. E. De Oliveira. 2001. Amazon plant diversity and climate through the Cenozoic. Palaeogeography Palaeoclimatology Palaeoecology 166:51-63.

Colinvaux, P. A., P. E. De Oliveira, and M. B. Bush. 2000. Amazonian and Neotropical plant communities on glacial time-scales: The failure of the aridity and refuge hypotheses. Quaternary Science Reviews 19:141-169.

Colinvaux, P. A., P. E. DeOliveira, J. E. Moreno, M. C. Miller, and M. B. Bush. 1996. A long pollen record from lowland Amazonia: Forest and cooling in glacial times. Science 274:85-88.

Colinvaux, P. A., G. Irion, M. E. Rasanen, M. B. Bush, and J. de Mello. 2001. A paradigm to be discarded: Geological and paleoecological data falsify the HAFFER & PRANCE refuge hypothesis of Amazonian speciation. Amazoniana-Limnologia Et Oecologia Regionalis Systemae Fluminis Amazonas 16:609-646.

Correa, M. M., A. G. D. Bieber, R. Wirth, and I. R. Leal. 2005. Occurrence of Atta cephalotes (L.) (Hymenoptera : Formicidae) in Alagoas, Northeastern Brazil. Neotropical Entomology 34:695-698.

Coyne, J. A., and H. A. Orr. 2004. Speciation. Sinauer Associates, Inc., Sunderland, MA.

Daguerre, J. B. 1945. Hormigas del género Atta F. de la Argentina. Revista de la Sociedad Argentina de Entomología 12:438–460.

Delabie, J., I. C. do Nascimento, E. Fonseca, R. B. Sgrilo, P. A. O. Soares, A. B. Casimiro, and M. Furst. 1997. Biogeografia das formigas cortadeiras (Hymenoptera; Formicidae; Myrmicinae; Attini), de importância econômica no leste da Bahia e nas regiões periferias dos estados vizinhos. Agrotrópica 9:49–58.

Dick, C. W., D. W. Roubik, K. F. Gruber, and E. Bermingham. 2004. Long-distance gene flow and cross-Andean dispersal of lowland rainforest bees (Apidae : Euglossini) revealed by comparative mitochondrial DNA phylogeography. Molecular Ecology 13:3775-3785.

Disney, R. H. L. 1996. A key to Neodohrniphora (Diptera: Phoridae), parasites of leaf-cutter ants (Hymenoptera: Formicidae). Journal of Natural History 30:1377–1389.

Dudik, M., S. J. Phillips, and R. E. Schapire. 2004. Performance guarantees for regularized maximum entropy density estimation. Pp. 472-486. Learning Theory, Proceedings. Springer-Verlag Berlin, Berlin.

Edwards, S. V., and P. Beerli. 2000. Perspective: Gene divergence, population divergence, and the variance in coalescence time in phylogeographic studies. Evolution 54:1839-1854.

Elith, J., C. H. Graham, R. P. Anderson, M. Dudik, S. Ferrier, A. Guisan, R. J. Hijmans, F. Huettmann, J. R. Leathwick, A. Lehmann, J. Li, L. G. Lohmann, B. A. Loiselle, G. Manion, C. Moritz, M. Nakamura, Y. Nakazawa, J. M. Overton, A. T. Peterson, S. J. Phillips, K. Richardson, R. Scachetti-Pereira, R. E. Schapire, J. Soberon, S. Williams, M. S. Wisz, and N. E. Zimmermann. 2006. Novel methods improve prediction of species' distributions from occurrence data. Ecography 29:129-151.

Erthal, M., and A. Tonhasca. 2000. Biology and oviposition behavior of the phorid Apocephalus attophilus and the response of its host, the leaf-cutting ant Atta laevigata. Entomologia Experimentalis Et Applicata 95:71-75.

Erwin, T. L. 1991. How many species are there - revisited. Conservation Biology 5:330-333.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 14:47–50.

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

Farji-Brener, A. G. 2001. Why are leaf-cutting ants more common in early secondary forests than in old-growth tropical forests? An evaluation of the palatable forage hypothesis. Oikos 92:169-177.

Feener, D. J. 2000. Is the assembly of ant communities mediated by parasitoids? Oikos 90:79-88.

Fjeldsa, J. 1994. Geographical patterns for relict and young species of birds in Africa and South America and implications for conservation priorities. Biodiversity and Conservation 3:207-226.

Fowler, H. G. 1983. Distribution patterns of Paraguayan leaf-cutting ants (Atta and Acromyrmex) (Formicidae: Attini). Studies on Neotropical Fauna and Environment 18:121–138.

Garrettson, M., J. F. Stetzel, B. S. Halpern, D. J. Hearn, B. T. Lucey, and M. J. McKone. 1998. Diversity and abundance of understorey plants on active and abandoned nests of leaf-cutting ants (Atta cephalotes) in a Costa Rican rain forest. Journal of Tropical Ecology 14:17-26.

Gascon, C., J. R. Malcolm, J. L. Patton, M. N. F. da Silva, J. P. Bogart, S. C. Lougheed, C. A. Peres, S. Neckel, and P. T. Boag. 2000. Riverine barriers and the geographic distribution of Amazonian species. Proceedings of the National Academy of Sciences of the United States of America 97:13672-13677.

Gentry, A. H. 1988. Tree species richness of upper Amazonian forests. Proceedings of the National Academy of Sciences of the United States of America 85:156-159.

Goldman, N., J. P. Anderson, and A. G. Rodrigo. 2000. Likelihood-based tests of topologies in phylogenetics. Systematic Biology 49:652-670.

Gomez-Zurita, J., and A. P. Vogler. 2003. Incongruent nuclear and mitochondrial phylogeographic patterns in the Timarcha goettingensis species complex (Coleoptera, Chrysomelidae). Journal of Evolutionary Biology 16:833-843.

Gonçalves, C. R. 1951. Saúvas do nordeste do Brasil. Boletim Fitossanitário 5 (Zoologia):1–43.

Gonçalves, C. R. 1960. Distribuição, biologia e ecologia das saúvas. Divulgação Agronómica 12:2–10.

Gonçalves, C. R. 1967. As formigas cortadeiras da Amazônia, dos generos "Atta" Fabr. e "Acromyrmex" Mayr (Hym., Formicidae). Atas do Simpósio sôbre a Biota Amazônica 5 (Zoologia):181–202.

Graham, C. H., S. Ferrier, F. Huettman, C. Moritz, and A. T. Peterson. 2004. New developments in museum-based informatics and applications in biodiversity analysis. Trends in Ecology & Evolution 19:497-503.

Graham, C. H., and R. J. Hijmans. 2006. A comparison of methods for mapping species ranges and species richness. Global Ecology and Biogeography 15:578-587.

Gustavo, A., F. Brener, and A. Ruggiero. 1994. Leaf-cutting ants (Atta and Acromyrmex) inhabiting Argentina - patterns in species richness and geographical range sizes. Journal of Biogeography 21:391-399.

Haffer, J. 1969. Speciation in Amazonian forest birds. Science 165:131–137.

Haffer, J. 1997. Alternative models of vertebrate speciation in Amazonia: An overview. Biodiversity and Conservation 6:451-476.

Haffer, J., and G. T. Prance. 2001. Climatic forcing of evolution in Amazonia during the Cenozoic: On the refuge theory of biotic differentiation. Amazoniana-Limnologia Et Oecologia Regionalis Systemae Fluminis Amazonas 16:579-605.

Hall, J. P. W., and D. J. Harvey. 2002. The phylogeography of Amazonia revisited: New evidence from riodinid butterflies. Evolution 56:1489-1497.

Harpending, H. C. 1994. signature of ancient population-growth in a low-resolution mitochondrial-DNA mismatch distribution. Human Biology 66:591-600.

Hayes, F. E., and J. A. N. Sewlal. 2004. The Amazon River as a dispersal barrier to passerine birds: effects of river width, habitat and taxonomy. Journal of Biogeography 31:1809-1818.

Hernandez, J. V., C. Ramos, M. Borjas, and K. Jaffe. 1999. Growth of Atta laevigata (Hymenoptera : Formicidae) nests in pine plantations. Florida Entomologist 82:97-103.

Hershkovitz, P. 1977. Living New World monkeys (Platyrrhini) with an introduction to primates. University of Chicago Press, Chicago, IL.

Hijmans, R. J., S. E. Cameron, J. L. Parra, P. G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25:1965-1978.

Hillebrand, H. 2004. On the generality of the latitudinal diversity gradient. American Naturalist 163:192-211.

Hillis, D. M., B. K. Mable, and C. Moritz. 1996. Applications of molecular systematics: the state of the field and a look to the future. Pp. 515–543. Molecular Systematics. Sinauer Associates, Inc., Sunderland, MA.

Hogue, C. L., and S. E. Miller. 1981. Entomofauna of Cocos Island, Costa Rica. The Smithsonian Institution, Washington, DC.

Hölldobler, B., and E. O. Wilson. 1990. The ants. Harvard University Press, Cambridge, MA.

Hoorn, C., J. Guerrero, G. A. Sarmiento, and M. A. Lorente. 1995. Andean tectonics as a cause for changing drainage patterns in Miocene northern South America. Geology 23:237-240.

Hovikoski, J., M. Rasanen, M. Gingras, S. Lopez, L. Romero, A. Ranzi, and J. Melo. 2007. Palaeogeographical implications of the Miocene Quendeque Formation (Bolivia) and tidally-influenced strata in southwestern Amazonia. Palaeogeography Palaeoclimatology Palaeoecology 243:23-41.

Hovikoski, J., M. Rasanen, M. Gingras, M. Roddaz, S. Brusset, W. Hermoza, and L. R. Pittman. 2005. Miocene semidiurnal tidal rhythmites in Madre de dios, Peru. Geology 33:177-180.

Hugall, A., C. Moritz, A. Moussalli, and J. Stanisic. 2002. Reconciling paleodistribution models and comparative phylogeography in the Wet Tropics rainforest land snail Gnarosophia bellendenkerensis (Brazier 1875). Proceedings of the National Academy of Sciences of the United States of America 99:6112-6117.

Hutchinson, G. E. 1957. Concluding remarks. Pp. 145–159. Cold Spring Harbor Symposia on Quantitative Biology.

Jablonski, D., K. Roy, and J. W. Valentine. 2006. Out of the tropics: Evolutionary dynamics of the latitudinal diversity gradient. Science 314:102-106.

Jaffe, K. 1986. Control of Atta and Acromyrmex spp. in pine tree plantations in the Venezuelan llanos. Pp. 409–416. Fire ants and leaf-cutting ants: biology and management. Westview Press, Boulder.

Kaspari, M., and S. O'Donnell. 2003. High rates of army ant raids in the Neotropics and implications for ant colony and community structure. Evolutionary Ecology Research 5:933-939.

Kempf, W. W. 1972. Catálogo Abreviado das Formigas da Região Neotropical (Hymenoptera: Formicidae). Studia Entomologica 15:3–344.

Knowles, L. L. 2004. The burgeoning field of statistical phylogeography. Journal of Evolutionary Biology 17:1-10.

Latrubesse, E. M., S. A. F. da Silva, M. Cozzuol, and M. L. Absy. 2007. Late Miocene continental sedimentation in southwestern Amazonia and its regional significance:

Biotic and geological evidence. Journal of South American Earth Sciences 23:61-80.

LeBrun, E. G., and D. H. Feener. 2002. Linked indirect effects in ant-phorid interactions: impacts on ant assemblage structure. Oecologia 133:599-607.

Leigh, E. G., P. Davidar, C. W. Dick, J. P. Puyravaud, J. Terborgh, H. ter Steege, and S. J. Wright. 2004. Why do some tropical forests have so many species of trees? Biotropica 36:447-473.

Lewinsohn, T. M., A. V. L. Freitas, and P. I. Prado. 2005. Conservation of terrestrial invertebrates and their habitats in Brazil. Conservation Biology 19:640-645.

Losos, J. B., and R. E. Glor. 2003. Phylogenetic comparative methods and the geography of speciation. Trends in Ecology & Evolution 18:220-227.

Lougheed, S. C., C. Gascon, D. A. Jones, J. P. Bogart, and P. T. Boag. 1999. Ridges and rivers: a test of competing hypotheses of Amazonian diversification using a dart-poison frog (Epipedobates femoralis). Proceedings of the Royal Society of London Series B-Biological Sciences 266:1829-1835.

Lovejoy, N. R., J. S. Albert, and W. G. R. Crampton. 2006. Miocene marine incursions and marine/freshwater transitions: Evidence from Neotropical fishes. Journal of South American Earth Sciences 21:5-13.

Mackay, W., and E. Mackay. 1986. Las hormigas de Colombia: arrieras del género Atta (Hymenoptera: Formicidae). Revista Colombiana de Entomología 12:23–30.

Maddison, D. R., and W. P. Maddison. 2000. MacClade 4: analysis of phylogeny and character evolution. Sinauer Associates, Inc., Sunderland, MA.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research 27:209–220.

Mariconi, F. A. M. 1970. As Saúvas. Agronômica Ceres, São Paulo.

Matocq, M. D., J. L. Patton, and M. N. F. da Silva. 2000. Population genetic structure of two ecologically distinct Amazonian spiny rats: Separating history and current ecology. Evolution 54:1423-1432.

Mayr, E., and R. J. Ohara. 1986. The biogeographic evidence supporting the Pleistocene forest refuge hypothesis. Evolution 40:55-67.

Mehdiabadi, N. J., E. A. Kawazoe, and L. E. Gilbert. 2004. Phorid fly parasitoids of invasive fire ants indirectly improve the competitive ability of a native ant. Ecological Entomology 29:621-627.

Mikheyev, A. S., U. G. Mueller, and P. Abbot. 2006. Cryptic sex and many-to-one colevolution in the fungus-growing ant symbiosis. Proceedings of the National Academy of Sciences of the United States of America 103:10702-10706.

Moreira, A. A., L. C. Forti, A. P. P. Andrade, M. A. C. Boaretto, and J. F. S. Lopes. 2004. Nest architecture of Atta laevigata (F. Smith, 1858) (Hymenoptera : Formicidae). Studies on Neotropical Fauna and Environment 39:109-116.

Moritz, C., J. L. Patton, C. J. Schneider, and T. B. Smith. 2000. Diversification of rainforest faunas: An integrated molecular approach. Annual Review of Ecology and Systematics 31:533-563.

Moser, J. C. 1967. Mating activities of Atta texana (Hymenoptera, Formicidae). Insectes Sociaux 14:295–312.

Moutinho, P., D. C. Nepstad, and E. A. Davidson. 2003. Influence of leaf-cutting ant nests on secondary forest growth and soil properties in Amazonia. Ecology 84:1265-1276.

Mueller, U. G., S. A. Rehner, and T. R. Schultz. 1998. The evolution of agriculture in ants. Science 281:2034-2038.

Nelson, B. W., C. A. C. Ferreira, M. F. Dasilva, and M. L. Kawasaki. 1990. Endemism centers, refugia and botanical collection density in Brazilian Amazonia. Nature 345:714-716.

Nielsen, R., and J. Wakeley. 2001. Distinguishing migration from isolation: A Markov chain Monte Carlo approach. Genetics 158:885-896.

Nores, M. 1999. An alternative hypothesis for the origin of Amazonian bird diversity. Journal of Biogeography 26:475-485.

Orr, M. R. 1992. Parasitic flies (Diptera, Phoridae) influence foraging rhythms and caste division-of-labor in the leaf-cutter ant, Atta cephalotes (Hymenoptera, Formicidae). Behavioral Ecology and Sociobiology 30:395-402.

Patton, J. L., M. N. F. Da Silva, and J. R. Malcolm. 2000. Mammals of the Rio Juruá and the evolutionary and ecological diversification of Amazonia. Bulletin of the American Museum of Natural History:1-306.

Patton, J. L., M. N. F. Dasilva, and J. R. Malcolm. 1994. Gene genealogy and differentiation among arboreal spiny rats (Rodentia, Echimyidae) of the Amazon basin - a test of the riverine barrier hypothesis. Evolution 48:1314-1323.

Pellegrino, K. C. M., M. I. Rodrigues, A. N. Waite, M. Morando, Y. Y. Yassuda, and J. W. Sites. 2005. Phylogeography and species limits in the Gymnodactylus darwinii complex (Gekkonidae, Squamata): genetic structure coincides with river systems in the Brazilian Atlantic Forest. Biological Journal of the Linnean Society 85:13-26.

Peres, C. A., J. L. Patton, and M. N. F. daSilva. 1996. Riverine barriers and gene flow in Amazonian saddle-back tamarins. Folia Primatologica 67:113-124.

Phillips, O. L., P. Hall, A. H. Gentry, S. A. Sawyer, and R. Vasquez. 1994. Dynamics and species richness of tropical rain-forests. Proceedings of the National Academy of Sciences of the United States of America 91:2805-2809.

Phillips, S. J. 2004. A maximum entropy approach to species distribution modeling. Proceedings of the Twenty-first Century International Conference on Machine Learning.

Phillips, S. J., R. P. Anderson, and R. E. Schapire. 2006. Maximum entropy modeling of species geographic distributions. Ecological Modelling 190:231-259.

Philpott, S. M. 2005. Trait-mediated effects of parasitic phorid flies (Diptera : Phoridae) on ant (Hymenoptera : Formicidae) competition and resource access in coffee agro-ecosystems. Environmental Entomology 34:1089-1094.

Pianka, E. R. 1966. Latitudinal Gradients in Species Diversity: A Review of Concepts. The American Naturalist 100:33–46.

Pollard, G. V. 1982. A review of the distribution, economic importance and control of leaf-cutting ants in the Caribbean region with an analysis of current control programmes. Pp. 43–61. Urgent plant pest and disease problems in the Caribbean. IICA, Port of Spain.

Posada, D., and K. A. Crandall. 1998. Model selection and model averaging in phylogenetics: advantages of the AIC and Bayesian approaches over likelihood ratio tests. Bioinformatics 14:817–818.

Powell, S., and E. Clark. 2004. Combat between large derived societies: A subterranean army ant established as a predator of mature leaf-cutting ant colonies. Insectes Sociaux 51:342-351.

Quek, S. P., S. J. Davies, T. Itino, and N. E. Pierce. 2004. Codiversification in an ant-plant mutualism: Stem texture and the evolution of host use in Crematogaster (Formicidae : Myrmicinae) inhabitants of Macaranga (Euphorbiaceae). Evolution 58:554-570.

Rambaut, A., and N. C. Grassly. 1997. Seq-Gen: An application for the Monte Carlo simulation of DNA sequence evolution along phylogenetic frees. Computer Applications in the Biosciences 13:235-238.

Rao, M. 2000. Variation in leaf-cutter ant (Atta sp.) densities in forest isolates: the potential role of predation. Journal of Tropical Ecology 16:209-225.

Rasanen, M. E., A. M. Linna, J. C. R. Santos, and F. R. Negri. 1995. Late Miocene tidal deposits in the Amazonian foreland basin. Science 269:386–390.

Robinson, S. W., and H. G. Fowler. 1982. Foraging and pest potential of Paraguayan grass-cutting ants (Atta and Acromyrmex) to the cattle industry. Zeitschrift Fur Angewandte Entomologie-Journal of Applied Entomology 93:42-54.

Rockwood, L. 1973. Distribution, density, and dispersion of two species of Atta (Hymenoptera: Formicidae) in Guanacaste Province, Costa Rica. Journal of Animal Ecology 42:803–817.

Rockwood, L. L. 1976. Plant selection and foraging patterns in 2 species of leaf-cutting ants (Atta). Ecology 57:48-61.

Rogers, A. R. 1995. Genetic evidence for a Pleistocene population explosion. Evolution 49:608-615.

Rogers, A. R., and H. Harpending. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Molecular Biology and Evolution 9:552-569.

Rohde, K. 1992. Latitudinal gradients in species diversity - the search for the primary cause. Oikos 65:514-527.

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

Ruegg, K. C., R. J. Hijmans, and C. Moritz. 2006. Climate change and the origin of migratory pathways in the Swainson's thrush, Catharus ustulatus. Journal of Biogeography 33:1172-1182.

Sanchez-Pena, S. R., and U. G. Mueller. 2002. A nocturnal raid of Nomamyrmex army ants' on Atta leaf-cutting ants (Hymenoptera : Formicidae) in Tamaulipas, Mexico. Southwestern Entomologist 27:221-223.

Schneider, S., and L. Excoffier. 1999. Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates very among sites: Application to human mitochondrial DNA. Genetics 152:1079-1089.

Shaw, K. L. 2002. Conflict between nuclear and mitochondrial DNA phylogenies of a recent species radiation: What mtDNA reveals and conceals about modes of speciation in Hawaiian crickets. Proceedings of the National Academy of Sciences of the United States of America 99:16122-16127.

Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu, and P. Flook. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain-reaction primers. Annals of the Entomological Society of America 87:651-701.

Simpson, B. B., and J. Haffer. 1978. Speciation patterns in Amazonian forest biota. Annual Review of Ecology and Systematics 9:497-518.

Slatkin, M., and R. R. Hudson. 1991. Pairwise comparisons of mitochondrial-DNA sequences in stable and exponentially growing populations. Genetics 129:555-562.

Smith, T. B., C. J. Schneider, and K. Holder. 2001. Refugial isolation versus ecological gradients. Genetica 112:383-398.

Smouse, P. E., J. C. Long, and R. R. Sokal. 1986. Multiple-regression and correlation extensions of the Mantel test of matrix correspondence. Systematic Zoology 35:627-632.

Solomon, S. E., and A. S. Mikheyev. 2005. The ant (Hymenoptera : Formicidae) fauna of Cocos Island, Costa Rica. Florida Entomologist 88:415-423.

Sternberg, L. D., M. C. Pinzon, M. Z. Moreira, P. Moutinho, E. I. Rojas, and E. A. Herre. 2007. Plants use macronutrients accumulated in leaf-cutting ant nests. Proceedings of the Royal Society B-Biological Sciences 274:315-321.

Swartz, M. B. 1998. Predation on an Atta cephalotes colony by an army ant, Nomamyrmex esenbeckii. Biotropica 30:682-684.

Swofford, D. L. 2002. PAUP*: Phylogenetic Analysis Using Parsimony (and Other Methods) 4.0 Beta. Sinauer Associates, Inc., Sunderland, MA.

Tajima, F. 1989. The effect of change in population-size on DNA polymorphism. Genetics 123:597-601.

Thompson, J. D., F. Plewniak, and O. Poch. 1999. A comprehensive comparison of multiple sequence alignment programs. Nucleic Acids Research 27:2682-2690.

Tonhasca, A., M. A. L. Braganca, and M. Erthal. 2001. Parasitism and biology of Myrmosicarius grandicornis (Diptera, Phoridae) in relationship to its host, the leaf-cutting ant Atta sexdens (Hymenoptera, Formicidae). Insectes Sociaux 48:154-158.

Varon, E. H., S. D. Eigenbrode, N. A. Bosque-Perez, and L. Hilje. 2007. Effect of farm diversity on harvesting of coffee leaves by the leaf-cutting ant Atta cephalotes. Agricultural and Forest Entomology 9:47-55.

Vasconcelos, H. L., and J. M. Cherrett. 1995. Changes in leaf-cutting ant populations (Formicidae, Attini) after the clearing of mature forest in Brazilian Amazonia. Studies on Neotropical Fauna and Environment 30:107-113.

Vasconcelos, H. L., E. H. M. Vieira-Neto, F. M. Mundim, and E. M. Bruna. 2006. Roads alter the colonization dynamics of a keystone herbivore in Neotropical savannas. Biotropica 38:661-665.

Vonhof, H. B., F. P. Wesselingh, R. J. G. Kaandorp, G. R. Davies, J. E. van Hinte, J. Guerrero, M. Rasanen, L. Romero-Pittman, and A. Ranzi. 2003. Paleogeography of Miocene Western Amazonia: Isotopic composition of molluscan shells constrains the influence of marine incursions. Geological Society of America Bulletin 115:983-993.

Wallace, A. R. 1852. On the monkeys of the Amazon. Proceedings of the Zoological Society of London 20:107–110.

Weber, N. A. 1959. Ecological relations of three Atta species in Panama. Ecology 50:141–147.

Weber, N. A. 1972. Gardening Ants, The Attines. The American Philosophical Society, Philadelphia, PA.

Weir, J. T., and D. Schluter. 2007. The latitudinal gradient in recent speciation and extinction rates of birds and mammals. Science 315:1574-1576.

Wetterer, J. K., T. R. Schultz, and R. Meier. 1998. Phylogeny of fungus-growing ants (Tribe Attini) based on mtDNA sequence and morphology. Molecular Phylogenetics and Evolution 9:42-47.

Wilf, P., N. R. Cuneo, K. R. Johnson, J. F. Hicks, S. L. Wing, and J. D. Obradovich. 2003. High plant diversity in Eocene South America: Evidence from Patagonia. Science 300:122-125.

Wilson, E. O. 1987. The arboreal ant fauna of Peruvian Amazon forests - a 1st assessment. Biotropica 19:245-251.

Wilson, E. O. 1999. The Diversity of Life. W. W. Norton and Company, New York.

Wright, S., J. Keeling, and L. Gillman. 2006. The road from Santa Rosalia: A faster tempo of evolution in tropical climates. Proceedings of the National Academy of Sciences of the United States of America 103:7718-7722.

Zanuncio, J. C., E. T. Lopes, R. Zanetti, D. Pratissoli, and L. Couto. 2002. Spatial distribution of nests of the leaf cutting ant Atta sexdens rubropilosa (Hymenoptera : Formicidae) in plantations of Eucalyptus urophylla in Brazil. Sociobiology 39:231-242.

Zwickl, D. J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. The University of Texas at Austin.

Vita

Scott E. Solomon was born June 21, 1979 in Tucson, Arizona to Ira and Susan Solomon. Scott was raised primarily in Champaign, Illinois, though his family traveled frequently and he also lived briefly in Coronado, California and Franklin Lakes, New Jersey. He graduated from University High School in Urbana, Illinois in May 1996 and entered the University of Illinois at Urbana-Champaign in August of that year. Scott studied biology and Spanish, and spent a semester abroad at the Universidad San Francisco de Quito in Ecuador, where he got his first taste of tropical biology. He returned to Ecuador the following year as a field assistant for Martin Wikelski in the Galápagos Islands, his first experience conducting research in the field. After receiving his B.S. in Cell and Structural Biology in May 2000, Scott entered graduate school at The University of Texas at Austin in August 2000. Scott attended the Organization for Tropical Studies' course on tropical ecology in the summer of 2001, where he solidified his interest in tropical biology and biogeography. He has since conducted fieldwork throughout Central and South America, as well as on Cocos Island (Costa Rica) and Fiji.

Permanent address: 2918 RR 620N #183, Austin, TX 78734

This dissertation was typed by the author.