Historical Biogeography of Velvet Ants (Hymenoptera: Mutillidae) in the North American Deserts and Arid Lands

Joseph S. Wilson

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HISTORICAL BIOGEOGRAPHY OF VELVET ANTS (HYMENOPTERA: MUTILLIDAE) IN THE NORTH AMERICAN DESERTS AND ARID LANDS

by

Joseph S. Wilson

A dissertation submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Biology

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UTAH STATE UNIVERSITY
Logan, Utah

2010
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ABSTRACT

Historical Biogeography of Velvet Ants (Hymenoptera: Mutillidae) in the North American Deserts and Arid Lands

by

Joseph S. Wilson, Doctor of Philosophy

Utah State University, 2010

Major Professor: James P. Pitts
Department: Biology

Understanding the history of diversification in the North American deserts has long been a goal of biogeographers and evolutionary biologists. While it seems that a consensus is forming regarding the patterns of diversification in the Nearctic deserts in vertebrate taxa, little work has been done exploring the historical biogeography of widespread invertebrate taxa. Before a robust model of geobiotic change in the North American deserts can be proposed, it needs to be determined if the same historical events affected vertebrate and invertebrate taxa in the same way. I explored the phylogeographic patterns in four groups of widespread nocturnal velvet ants using two rDNA loci, the internal transcribed spacer regions 1 and 2 (ITS1 and ITS2). I used Bayesian phylogenetic analyses and haplotype network analyses to determine if a consistent geographic pattern exists among species and populations within each group. I also used molecular dating techniques to estimate divergence dates for each of the major
phylogenetic clades. These analyses indicate that the species-level divergences in some groups occurred in the Neogene, and likely were driven by mountain building during Miocene-Pliocene times (~5 Ma) similar to the divergences in many vertebrate taxa, while species-level divergence in other groups occurred during the Pleistocene (1.8-0.1 Ma) and were likely driven by climatic oscillations and range contractions and expansion. Several recent studies have suggested that Neogene mountain-building events were more important to the development of a diverse desert-adapted biota. My research suggests, however, that both Neogene events and Pleistocene climatic changes were influential in the development of a species-rich nocturnal velvet ant fauna.
PROFESSIONAL ABSTRACT

Historical Biogeography of Velvet Ants (Hymenoptera: Mutillidae) in the North American Deserts and Arid Lands

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Joseph S. Wilson, Doctor of Philosophy
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Department: Biology

For centuries, scientists have been intrigued by the high amount of biodiversity that is found in the deserts of North America. Recently, several studies have investigated the causes of the high diversity found in desert-dwelling mammals, reptiles, and amphibians. These studies have found that many of these organisms seem to have diversified in response to the same historical events. Little work has been done, however, on diverse desert-dwelling insect groups. In this dissertation, I investigate the patterns of genetic diversity in four groups of nocturnal wasps called velvet ants. I compare the patterns of genetic diversity to the historical events like mountain building and climate changes to determine which historical events were responsible for driving diversity in these groups of wasps. I obtained DNA from numerous velvet ant specimens and analyzed two non-coding regions of their DNA using Bayesian statistical methods implemented in several computer programs. These programs allowed me to determine if
a consistent pattern could be found across species and populations of these wasps. I used other computer programs that analyze the patterns in the DNA to estimate a date when the different species evolved. These analyses showed that some of the species-level diversification events occurred about five million years ago, during a time when many of the western mountain ranges were being formed, and other species-level evolution events occurred in the last two million years, during the most recent ice ages. Many of the recent studies investigating evolution in mammals and reptiles have suggested that most species-level evolution events occurred in response to the geologic changes that were happening about five million years ago. My research, however, shows that both the mountain-building events and the most recent ice ages influenced the evolution of velvet ants.
I would like to thank Dr. James Pitts for encouraging me to seek a PhD and for the guidance and support he provided. I would also like to thank my committee members, Dr. Carol von Dohlen, Dr. Terry Griswold, Dr. James MacMahon, and Dr. Steve Larson, for the advice and direction they provided throughout this process. I would like to thank Erik Pilgrim, Carrie Drake, Sarah Clark, Kevin Williams, Clayton Gunnell, David Tanner, and Carol von Dohlen for help with the molecular aspects of my research. I thank all those who helped me collect specimens that were used in these studies, particularly Kevin Williams, Lindsey Wilson, Erik Pilgrim, Michael Irwin, Frank Parker, and Seth Topham. I am indebted to James Pitts and Kevin Williams for the help they provided in the identification of the hundreds of specimens used here.

Funding for this research was provided in part through the AMNH Theodore Roosevelt Memorial Fund grant and the Southwestern Research Station. Additional funding was provided through the California Desert Research Fund at The Community Foundation. This research was also supported by the Utah Agricultural Experiment Station, Utah State University, Logan, Utah 84322-4810.

Lastly, I would like to thank my family for the encouragement and direction they provided to me. Particularly I would like to thank my father, Bruce J. Wilson, and my brother, Benjamin J. Wilson, for the discussions we had about my research. I am also grateful to my wife, Lindsey, and my kids, Ari and Isaac, for their patience and their willingness to take family vacations out to the desert to “catch bugs.”

Joseph S. Wilson
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Most people who visit the arid regions of North America are struck by the apparent deficiency of plant and animal life. Although these areas appear harsh and inhospitable, many taxonomic groups have diversified therein forming a unique biota. For example, the horned lizards and their relatives (Phrynosomatidae) form a diverse group of approximately 120 species in 10 genera ranging from southern Canada to western Panama, with their highest diversities in the southwestern U.S. and Mexico (Reeder and Wiens, 1996). Numerous plant taxa have also diversified in these same regions. Cacti, for example, while not wholly confined to deserts, are both abundant and diverse in North America’s arid regions (Benson, 1950). Many insect groups also exhibit high diversities in the arid regions of North America. New World bees, for instance, reach the highest diversity in the deserts of North America and Mexico (Moldenke, 1979; Michener, 2000). Another example is the wasp family Mutillidae (velvet ants), in which many arid-adapted genera have diversified extensively in western North America.

The high diversity found in the arid-adapted biota has often been attributed to Pleistocene (1.8 Ma - 0.01 Ma) climatic oscillations (Findly, 1969; Hubbard, 1973; Tanner and Banta, 1977). The effect that Pleistocene glaciation had on the unglaciated western U.S., however, remains unclear. Evidence suggests that a cool, moist environment was present across the Western deserts and arid regions throughout much of the Pleistocene (Anderson and Van Devender, 1991; Elias et al., 1992; MacKay and Elias, 1992; Jennings et al., 1993). This epoch was marked by periods of climatic cooling (glacial) with intermittent warm dry periods (inter-glacial) (Gibbard and Van
Kolfshoten, 2004). During the glacial periods, arid-adapted taxa were forced into refugia in portions of southern California, northwestern Mexico, and parts of the Chihuahuan Desert (Ayoub and Riechert, 2004; Jaeger et al., 2005; Douglas et al., 2006). Later, when the climate warmed, they re-expanded into other parts of the West. Multiple periods of isolation and subsequent expansion is thought to have catalyzed the diversification of the desert biota.

Some authors suggest that during the changing climate of the Late Quaternary (~10,000 years ago), an elevational gradient was more important in determining the distributions of some arid-adapted species than a latitudinal gradient (Hall et al., 1989; Cole, 1990; Spaulding, 1990). This suggests that many additional cryptic Pleistocene refugia may have existed at low elevations across the warm and cold deserts of western North America. While Pleistocene climate change affected distributional and population-level species dynamics, the importance of these climatic oscillations to the macroevolutionary dynamics remains debatable (Klicka and Zink, 1997).

Recently, many authors have argued against Pleistocene climatic change as the main cause of diversification, suggesting instead that late Miocene and Pliocene (~15-2 MA) geological events and subsequent aridification were the driving force behind the diversification of the arid-adapted biota (Morafka, 1977; Riddle, 1995; Orange et al., 1999). The Neogene aridification was caused by major orogenic events (i.e., the uplift of the North American Cordilleras) (Axelrod, 1979; Minckley et al., 1986; Douglas et al., 2006). These mountain building events caused changes in the circulation of moisture, prompting the aridification of western North America (Axelrod, 1979). The drying of the North American savannas promoted more arid woodlands and shrub-steppe
environments, which caused the extinction of many large mammals and was likely a factor that encouraged the diversification of many arid-adapted taxa (Webb, 1977). The uplift of the North American Cordilleras also split sympatric populations enabling independent evolution of the now isolated groups (e.g. Riddle, 1995; Orange et al., 1999). Often, studies depicting diversification in warm desert-adapted taxa find a pattern of sister species being restricted to eastern warm deserts (Chihuahuan Desert) and western warm deserts (Sonoran and Mojave deserts). This pattern has often been linked to two distinct vicariant events; the uplift of the Continental Divide (Fig. 1.1a); and the expansion of the Sea of Cortés (Fig. 1.1b), which both occurred near the Mio-Pliocene boundary (Wilson and Pitts, 2010).

The debate continues over which of these two scenarios, Pleistocene climate change or Neogene vicariance, played the central role in the diversification of arid-adapted species (Johnson and Cicero, 2004; Zink and Klicka, 2006). Likely, a combination of these two processes shaped the current desert biota (Jaeger et al., 2005; Douglas et al., 2006). Ayoub and Riechert (2004) suggest that Neogene building events may explain the diversity in some taxa, while Pleistocene climate change may have been more important for others.

Many authors have attempted to explain the patterns of diversity in the deserts of North America using molecular based historical biogeographic methods (Epps et al., 1998; Orange et al., 1999; Riddle et al., 2000; Zink et al., 2001; Jaeger et al., 2005). These studies have unearthed interesting patterns of species-level and
Figure 1.1 Map of the North American hot deserts showing two postulated vicariant events that led to diversification in desert-adapted taxa. The vicariant event marked “A” represents the uplift of the Sierra Madre Occidental, Rocky Mountains and the Colorado Plateau (Continental Divide). The vicariant event marked “B” represents the expansion of the Sea of Cortés (Bouse Embayment) that extended north to Nevada along the Colorado River drainage.

population-level diversity, yet none have been completed showing a pattern for all of the North American deserts. The majority of the studies published have focused their attention on North America’s warm deserts (Mojave, Sonoran, and Chihuahuan) because portions of these deserts maintained desert-like characteristics throughout the Pleistocene, and it is assumed that diversification was likely to occur there (Douglas et al., 2006). In order to fully understand the roles Pleistocene climate change and Neogene orogeny had
on diversification, an examination of both North America’s warm and cold deserts, as well as the surrounding arid regions, must be performed. Furthermore, the majority of the studies investigating the driving forces behind diversification of arid-adapted taxa have been done on vertebrate taxa. Additionally, few of these studies have included fossil data to date the nodes of the phylogenetic trees, resulting in estimated divergence dates with limited accuracy. Before a generalized model of historical biogeography in the Nearctic deserts can be developed, investigations into the diversification of arid-adapted arthropods must be done and compared to analyses detailing the causes of diversification in vertebrates. Among North America’s diverse arid-adapted arthropods, velvet ants (Hymenoptera: Mutillidae) are ideal candidates for historical biogeographic investigations because they are diverse and many species are widespread (Wilson and Pitts, 2008; Pitts et al., 2010). Also, because multiple velvet ant fossils are known from Dominican amber, major periods of velvet ant diversification can be dated using molecular methods calibrated with fossils.

Through this dissertation I will test and expand the current understanding of the historical biogeography of the North American arid lands biota by analyzing patterns of diversification in four velvet ant groups. First, in Chapter 2, in order to compare the causes of diversification in arid-adapted velvet ants to the proposed causes of diversification in vertebrates, I investigated the phylogeographic patterns between species of the nocturnal velvet ant genus *Dilophotopsis* and among populations of the widespread species *D. concolor* using the two internal transcribed spacer regions 1 and 2 (ITS1 and ITS2). I used Bayesian and parsimony-based analyses to estimate phylogenetic
relationships and divergence dates in order to determine if a consistent geographic pattern of diversification exists between *Dilophotopsis* and various vertebrate taxa.

In Chapter 3, I expand my analysis of the historical biogeography of velvet ants by examining the phylogeographic patterns in the *Odontophotopsis unicornis* species-group using molecular data. This species-group contains two species, *O. unicornis* and *O. erebus*, one of which is largely restricted to western deserts while the other is found primarily in eastern deserts. Although the distributions of these species seems to match the generally accepted pattern of diversification found in several vertebrate taxa, I use molecular dating techniques calibrated with fossil data to determine if late Neogene events or Pleistocene events were responsible for the diversification in this species-group.

For Chapter 4, I expanded my analysis of velvet ant diversification in the Nearctic deserts to include the Mediterranean parts of California. I analyzed the phylogeographic patterns among species and populations in the *Sphaeropthalma unicolor* species-complex, which consists of *S. unicolor*, *S. mendica*, and *S. angulifera*. While diversification in desert-adapted organisms has often been linked to Neogene mountain-building events, little work has been done investigating the causes of diversification in Mediterranean-adapted taxa that are closely related to desert-adapted taxa. I investigated the historical biogeographic patterns between the members of the *S. unicolor* species-complex, two of which are desert-adapted species and the other is Mediterranean-adapted, using the two internal transcribed spacer regions 1 and 2 (ITS1 and ITS2). I also investigated the effect Pleistocene climatic cycles had on the genetic diversity of these species using ecological niche models. This combination of molecular phylogenetic analyses and ecological niche models allowed me to form a hypothesis detailing the
causes of diversification in this group of velvet ants, and provided additional insights into the history of desert-adapted and Mediterranean-adapted species.

In Chapter 5, I explored the population-level diversification pattern in a widespread, desert-adapted velvet ant, *Sphaeropthalma arota* using molecular data. Because analyses of other taxa with similar distributions have uncovered the existence of previously unrecognized species, I investigated the possibility of *S. arota* being multiple cryptic species. Also, in order to determine if a consistent pattern of diversification exists among velvet ants, I explored the phylogeographic patterns among *S. arota* populations from across this species’ range using molecular-based phylogeographic analysis and ecological niche models.

Literature Cited


CHAPTER 2

PHYLOGEOGRAPHIC ANALYSIS OF THE NOCTURNAL VELVET ANT GENUS

*Dilophotopsis* (Hymenoptera: Mutillidae) PROVIDES INSIGHTS INTO
DIVERSIFICATION IN THE NEARCTIC DESERTS

INTRODUCTION

The diversification of the North American desert biota has been a source of speculation in the fields of evolutionary biology and biogeography for decades. The ever-growing body of work detailing evolution in the Nearctic warm deserts is beginning to show a consistent pattern, with late Neogene uplift playing a central role in diversification (e.g., Riddle & Hafner, 2006; Hafner & Riddle, in press). Often, deep genetic divergences are linked to postulated vicariant events that occurred during the late Neogene. In the hot deserts two specific events are often credited for driving diversification in desert-adapted taxa. First, the Neogene uplift of the Sierra Madre Occidental, Rocky Mountains and Colorado Plateau is thought to have fragmented an ancestral arid region leading to genetic divergences between Chihuahuan Desert populations and Sonoran Desert populations, eventually leading to speciation events (Fig. 2.1A). Second, the late Neogene extension of the Sea of Cortez, known as the Bouse Embayment, likely vicariantly isolated populations in the western Sonoran and Mojave deserts from populations in the eastern Sonoran Desert, which led to speciation (Fig. 2.1B). Phylogeographic analyses of several vertebrate taxa have revealed deep genetic

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1 This chapter has been accepted for publication and is currently in press. I have retained the copyright for this work so no copyright release was necessary. Please cite this work by using the following citation: Wilson, J. S. and J. P. Pitts. 2010. Phylogeographic analysis of the nocturnal velvet ant genus *Dilophotopsis* (Hymenoptera: Mutillidae) provides insights into diversification in the Nearctic deserts. Biological Journal of the Linnean Society. *In press.*
Figure 2.1. Map of the North American hot deserts showing two postulated vicariant events that led to diversification in desert-adapted taxa. The vicariant event marked “A” represents the uplift of the Sierra Madre Occidental, Rocky Mountains and the Colorado Plateau. The vicariant event marked “B” represents the Bouse Embayment that extended from the Sea of Cortez north to Nevada along the Colorado River drainage.

splits that have been linked to these Neogene vicariant events (e.g., Morafka, 1977; Jaeger et al., 2005; Devitt, 2006; Douglas et al., 2006). While the cold desert regions (Great Basin Desert and Colorado Plateau) have not received as much attention as the regional warm deserts, some studies suggest that the cold desert biota may also show consistent historical biogeographic patterns with Great Basin populations being genetically distinct from neighboring Colorado Plateau populations (e.g., Epps et al., 1998; Orange et al., 1999).

The majority of studies investigating the historical biogeography of the North American deserts have been completed using vertebrate taxa (e.g., Riddle, 1995; Orange
et al., 1999; Riddle et al., 2000a; 2000b; Zink et al., 2001; Jaeger et al., 2005; Riddle & Hafner, 2006; Devitt, 2006; Douglas et al., 2006). While some phylogeographic analyses have been performed with invertebrate taxa, particularly arthropods, most of these have focused on local patterns like a single desert or a transition zone between deserts rather than a region-wide analysis (e.g., Epps et al., 1998; Smith & Farrell, 2005; Crews & Hedin, 2006). Ayoub and Riechert (2004) investigated phylogeographic patterns in a widespread desert-adapted spider, but because the diversification of this species was linked to Pleistocene climatic cycles, the patterns of divergence cannot be compared to the earlier Neogene diversification events found in the vertebrate taxa.

Phylogeographic analysis using mtDNA in arthropod taxa can be difficult due to high occurrences of mitochondrial DNA transferred to the nuclear genome (NUMTs) (e.g., Andrus, 2003; Pamilo et al., 2007; Black & Bernhardt, 2009). Also, arthropods, particularly insects, are often infected with Wolbachia, a bacteria that can quickly spread throughout a population and cause changes to the dynamics of mitochondrial haplotypes (Werren, 1997). While the majority of phylogeographic analyses use mtDNA, several recent studies on invertebrates employ various nuclear genes to uncover intraspecific variation. For example, a phylogeographic study of sea urchins found that the internal transcribed spacer region 2 was more variable at the population level than the mtDNA locus COI (Iuri et al., 2007). Studies on several other taxa have also shown that the internal transcribed spacer regions 1 and 2 (ITS1 & ITS2) are variable at the population level (e.g., Rokas et al., 2001; Alvarez & Hoy, 2002; Mavárez, et al., 2002; Gómez-Zurita & Vogler, 2003; De la Rúa et al., 2007; Wilson & Pitts, 2008; 2009).
Before a generalized model of historical biogeography in the Nearctic deserts can be developed, investigations into the diversification of arid-adapted arthropods must be done and compared to analyses detailing the causes of diversification in vertebrates. One likely arthropod candidate to investigate the biogeographic history of North America’s deserts is species of the wasp family Mutillidae. Velvet ants (Hymenoptera: Mutillidae) are solitary wasps that are parasitic on other wasps and bees (Krombein, 1979). While the brightly colored diurnal genera are a well-known component of the desert insect fauna, the species-rich nocturnal groups are not well understood.

One genus of nocturnal velvet ant in particular, *Dilophotopsis*, is an ideal candidate to uncover information about diversification in the deserts through historical biogeographical analysis due to its wide range, being found in the Chihuahuan and Sonoran deserts as well as the Great Basin, Colorado Plateau, and some surrounding arid shrub-steppe lands (Wilson & Pitts, 2008). Like all velvet ants, females of *Dilophotopsis* are wingless, which limits their long distance dispersal abilities. Members of this genus do not phoretically copulate, which further limits their dispersal abilities. Also, divergence date estimates for this species can be calibrated through the Dominican amber fossil of a related genus, *Dasymutilla*.

Here we investigate the biogeographical history of the North American deserts and other arid regions through an analysis of *Dilophotopsis* to test if the same historical vicariant events drove diversification in vertebrate, as well as invertebrate taxa. This analysis adds to the current understanding of the diversification of the desert biota in two ways. Because *Dilophotopsis* is found across the west, an understanding of the history of both the warm and cold deserts can be obtained. Also, genetic divergence events can be
dated using fossil calibration points so a better understanding of causation of diversification can be gained. By comparing patterns of divergence of a wide-ranging arthropod to the patterns observed in numerous vertebrate taxa, a more complete understanding of the causes of diversification in the Nearctic deserts can be gained.

**MATERIALS AND METHODS**

**Taxon sampling**

Specimens from each of the three *Dilophotopsis* species (*D. concolor* (Cresson), *D. paron* (Cameron), and *D. stenognatha* Schuster) were collected from sites across western North America (Table 2.1) from 2002 to 2009 using black light traps and fluorescent lantern traps, and also were collected by hand using forceps. All specimens were placed directly into 95% ethanol and those used for molecular examination have been labeled as voucher specimens and deposited in the Department of Biology Insect Collection, Utah State University, Logan, UT (EMUS). Desert and arid land boundaries to discuss species distributions and historical biogeography are altered from Omernik (1987). While the Colorado Plateau is not classified as an official desert, in order to simplify the discussion of historical biogeography we will discuss it and the Great Basin Desert as both being cold deserts.

**Molecular methods**

Molecular methods including DNA extraction, PCR, and sequencing followed the protocol outlined in Pilgrim and Pitts (2006). DNA was extracted, amplified, and
sequenced from *D. concolor* individuals from 25 sites across its range. Sequences were also obtained from *D. paron* from nine sites and from *D. stenognatha* from two sites.

Because the fossil to be used for divergence date calibration is of a related genus, rather than use a genetic locus informative only at the population or species-level, a locus was needed to be able to use the same marker for the population-level analyses as well as the generic-level analyses. The two rDNA internal transcribed spacer regions (ITS1 & ITS2) have been used to discover intraspecific variation in analyses of several velvet ant species (e.g., Wilson & Pitts 2008; 2009). Both ITS1 and ITS2 have also been useful in generic-level analyses of velvet ants (Pitts *et al.*, 2010).

Sequences were analyzed with an ABI Prism 377, 3100, or 3730 Genetic Analyzer. Gel electrophoresis of each gene yielded a single band for each individual wasp and the resulting DNA was sequenced cleanly, suggesting no gene heterogeneity as seen in some other organisms (e.g., Harris & Crandall, 2000; Parkin & Butlin, 2004; Bower *et al.*, 2008). PCR products were sequenced in both directions and were combined in Sequencher 4.1 (Gene Code Corp., Ann Arbor, MI). All sequences were deposited in Genbank (Table 2.1). Genetic distances between populations in different desert regions were calculated as pairwise percentages by determining the number of differences (point mutations and insertions or deletions) divided by the number of base pairs of the longer of the two sequences.
Table 1. Descriptive information for all taxa used in the phylogenetic portion of this study.

<table>
<thead>
<tr>
<th>Species</th>
<th>Map ID (Fig. 2)</th>
<th>Voucher ID</th>
<th>Haplotype</th>
<th>Collection Location</th>
<th>ITS1 Accession #</th>
<th>ITS2 Accession #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dasymutilla heliophila</td>
<td>N/A</td>
<td>JP321</td>
<td>N/A</td>
<td>AZ: Cochise Co., 2 Mi S Wilcox</td>
<td>GU814281</td>
<td>GU814406</td>
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<tr>
<td>Dasymutilla snoworum</td>
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<td>JP443</td>
<td>N/A</td>
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<td>GU814282</td>
<td>GU814407</td>
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<tr>
<td>Dasymutilla occidentalis</td>
<td>N/A</td>
<td>Mocc1</td>
<td>N/A</td>
<td>SC: Florence Co., Florence, Pee Dee Res. &amp; Edu. Center</td>
<td>GU814283</td>
<td>GU814408</td>
</tr>
<tr>
<td>Traumatotumilla sp.</td>
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<td>JP621</td>
<td>N/A</td>
<td>Bolivia: Santa Cruz, 5km SSEBurna Vista</td>
<td>GU814284</td>
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<td>JP280</td>
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<td>EU369220</td>
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<td>JP324</td>
<td>N/A</td>
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<td>GQ223230</td>
<td>GQ814414</td>
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<tr>
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<tr>
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<td>2</td>
<td>JP528</td>
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<td>5</td>
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<td>Moj1</td>
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<td>HM189255</td>
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<td>7</td>
<td>JP848</td>
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<td>WSon</td>
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<td>EU369232</td>
</tr>
<tr>
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<td>9</td>
<td>JP83</td>
<td>Moj2</td>
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<td>GU814291</td>
<td>EU369234</td>
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<tr>
<td>Dilophotopsis stenognatha</td>
<td>10</td>
<td>JW15</td>
<td>N/A</td>
<td>AZ: Yuma Co., Yuma proving grounds</td>
<td>HM189248</td>
<td>HM189274</td>
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<tr>
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<td>11</td>
<td>JP551</td>
<td>N/A</td>
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<td>HM189274</td>
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<tr>
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<td>12</td>
<td>JP231</td>
<td>EGB2</td>
<td>UT: Cache Co., Hyrum Reservoir</td>
<td>EU369212</td>
<td>EU369227</td>
</tr>
<tr>
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<td>DQ415673</td>
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<td>14</td>
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<td>EGB1</td>
<td>NV: Elko Co., Ruby Mountains, 3.5 mi S Jiggs</td>
<td>HM189234</td>
<td>HM189260</td>
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<tr>
<td>Dilophotopsis concolor</td>
<td>15</td>
<td>JP582</td>
<td>EGB1</td>
<td>NV: White Pine Co., Steepoe Valley</td>
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<td>HM189263</td>
</tr>
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<td>Dilophotopsis concolor</td>
<td>16</td>
<td>JP580</td>
<td>EGB1</td>
<td>UT: Millard Co., 2 mi W Topaz</td>
<td>HM189235</td>
<td>HM189261</td>
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<td>Dilophotopsis concolor</td>
<td>17</td>
<td>JP583</td>
<td>EGB1</td>
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<td>EU369222</td>
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<td>18</td>
<td>JP581</td>
<td>EGB1</td>
<td>ID: Cassia Co., 1 mi SW Almo</td>
<td>HM189236</td>
<td>HM189262</td>
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<tr>
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<td>19</td>
<td>JP586</td>
<td>CP4</td>
<td>UT: San Juan Co., Valley of the gods</td>
<td>HM189246</td>
<td>HM189266</td>
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<tr>
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<td>20</td>
<td>JP587</td>
<td>CP3</td>
<td>NM: San Juan Co., 3 mi S Farmington</td>
<td>EU369211</td>
<td>EU369226</td>
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<td>21</td>
<td>ES02</td>
<td>CP1</td>
<td>UT: Garfield Co., Calf Creek, 9 mi S Boulder</td>
<td>HM189223</td>
<td>HM189249</td>
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<td>CP2</td>
<td>UT: Emery Co., Green River</td>
<td>HM189220</td>
<td>HM189225</td>
</tr>
<tr>
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<td>23</td>
<td>JP270</td>
<td>CP2</td>
<td>UT: Emery Co., Green River</td>
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<td>HM189264</td>
</tr>
<tr>
<td>Diploptilopsis concolor</td>
<td>24</td>
<td>JP584</td>
<td>CP2</td>
<td>WY: Sweetwater Co., Green River</td>
<td>HM189239</td>
<td>HM189265</td>
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<tr>
<td>Diploptilopsis concolor</td>
<td>25</td>
<td>JP502</td>
<td>WGB1</td>
<td>NV: Humboldt Co., 8 mi N Winnemucca</td>
<td>EU369214</td>
<td>EU369229</td>
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<tr>
<td>Diploptilopsis concolor</td>
<td>26</td>
<td>JP803</td>
<td>WGB1</td>
<td>NV: Lander Co., 19 mi S Battle Mountain</td>
<td>HM189245</td>
<td>HM189271</td>
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<tr>
<td>Diploptilopsis concolor</td>
<td>27</td>
<td>JP849</td>
<td>WGB1</td>
<td>ID: Owyhee Co., Bruneau Dunes State Park</td>
<td>HM189247</td>
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<tr>
<td>Diploptilopsis concolor</td>
<td>29</td>
<td>JP501</td>
<td>Eson</td>
<td>AZ: Sant Cruz Co., 5.6 mi E Lochiel</td>
<td>HM189228</td>
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<tr>
<td>Diploptilopsis concolor</td>
<td>30</td>
<td>JP1015</td>
<td>Chi2</td>
<td>AZ: Cochise Co., Wilcox Cemetery</td>
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<tr>
<td>Diploptilopsis concolor</td>
<td>31</td>
<td>JP1013</td>
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<td>AZ: Cochise Co., Cavot Rd 0.5 mi W state line</td>
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<td>HM189251</td>
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<tr>
<td>Diploptilopsis concolor</td>
<td>32</td>
<td>JP1012</td>
<td>Chi1</td>
<td>NM: Hidalgo Co., Hwy 113 11 mi S I-40</td>
<td>HM189224</td>
<td>HM189250</td>
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<tr>
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<td>33</td>
<td>JP1014</td>
<td>Chi1</td>
<td>NM: Luna Co., 14.5 mi W Demming</td>
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<td>HM189252</td>
</tr>
<tr>
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<td>JP585</td>
<td>Chi1</td>
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<td>Chi1</td>
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<td>GU841417</td>
</tr>
<tr>
<td>Diploptilopsis concolor</td>
<td>36</td>
<td>JP638</td>
<td>Chi1</td>
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<td>HM189268</td>
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<tr>
<td>Diploptilopsis concolor</td>
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<td>JP499</td>
<td>Chi1</td>
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<td>EU369223</td>
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<tr>
<td>Diploptilopsis concolor</td>
<td>38</td>
<td>JP637</td>
<td>Chi1</td>
<td>TX: Brewster Co., Big Bend Ranch State Park</td>
<td>HM189241</td>
<td>HM189267</td>
</tr>
<tr>
<td>Diploptilopsis concolor</td>
<td>39</td>
<td>JW13</td>
<td>Chi3</td>
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<td>EU369230</td>
</tr>
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<td>Diploptilopsis concolor</td>
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</tr>
<tr>
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<td>41</td>
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<td>STex</td>
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</tr>
</tbody>
</table>
**Phylogenetic and network analyses**

The two genetic loci were subjected to Bayesian analysis using MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003). Sequences were analyzed as a combined data set, with each gene partitioned separately. Appropriate models of nucleotide substitution were determined in MrModeltest version 2.3 (Nylander, 2004). Because ITS1 and ITS2 are non-coding regions, insertions and deletions (indels) can be phylogenetically informative and should be included in the analysis (Simmons & Ochoterena, 2000). While MrBayes automatically treats all gaps as missing data, we included indels in the analysis by coding them as binary characters (presence/absence of a gap) and including this as a separate data partition (Ronquist *et al*., 2005).

Bayesian analyses included four independent runs with three heated chains and one cold chain in each run. The MCMC (Markov Chain Monte Carlo) chains were set for 3,000,000 generations and sampled every 100 generations; chains were run until the average standard deviation of the split frequencies dropped below 0.01. The burn-in period for each analysis was removed after graphical determination of stationarity.

Several outgroups were included in the analysis. *Acrophotopsis eurygnatha* and *A. campylognatha* are closely related to *Dilophotopsis* (Pitts & McHugh, 2002) and were included. *Laminatilla lamellifera* was used as a more distant outgroup. In addition to the full phylogenetic analysis, a Bayesian analysis was implemented on a subset of the full data set in order to streamline the molecular dating process. Included in this analysis were *D. concolor* populations from each major clade, the same outgroups as were used in the full phylogenetic analysis, multiple *Dasymutilla* species including *D. snoworum*, *D.
occidentalis, *D. gloriosa*, and a *Traumatomutilla* species. These additional outgroups were added in order to include a fossil for calibration in the molecular dating analysis.

We constructed a parsimony-based haplotype network using the combined ITS1 and ITS2 sequences for all *Dilophotopsis* specimens using TCS version 1.21 (Clement *et al.*, 2000). The program estimated the 95% reconnection limit between haplotypes with gaps treated as a 5th character state.

**Molecular dating**

Divergence date estimates were calculated for major nodes on the tree using two methods: a penalized likelihood approach to rate smoothing using the program r8s 1.71 (Sanderson, 2003), and a Bayesian MCMC averaging approach to rate smoothing using the program BEAST v1.4.8 (Drummond & Rambaut, 2007). Because there is a disparity of fossils that can be used as calibration points in Mutillidae, two distinct dating methods were used as a way to corroborate the divergence date. While no fossils are available for *Dilophotopsis* or any of the nocturnal velvet ants, two fossils from Dominican amber, *Dasymutilla dominica* and *D. albifasciatus* (Manley & Poinar, 1991; 1999; 2003) were used to calibrate the estimated divergence dates. Based on the morphology of these fossils, they are similar to some *Dasymutilla* species as well as some *Traumatomutilla* species.

*r8s analysis.* The program r8s uses a tree description with branch lengths to estimate divergence dates. The consensus tree that resulted from the pared-down Bayesian analysis was used in the r8s analysis. The most recent common ancestor (MRCA) of the *Dasymutilla* plus *Traumatomutilla* clade was constrained to be at least 20
Ma (minage = 20) based on the placement of the fossils and the reported age of Dominican amber (Iturralde-Vinet & MacPhee, 1996). The root was fixed at 65 million years based on the estimated maximum age of Mutillidae (Grimaldi & Engle, 2005), and the penalized likelihood method with the truncated Newton algorithm was implemented to estimate rates and divergence dates.

**BEAST analysis.** The program BEAST uses the aligned sequence data to generate a tree and estimate divergence dates. The program BEAUtiv1.4.8 (Drummond & Rambaut, 2007) was used to generate the file used in BEAST with the alignment of the pared-down data set. The MRCA of the *Dasymutilla* plus *Traumatomutilla* clade was constrained to be ~ 20 Ma by giving this node a normally distributed prior with a mean age of 20 million years and a standard deviation of 1.0. The root node was limited to a mean age of 65 million years with a standard deviation of 15 million years based on the estimated age of the family (Grimaldi & Engle, 2005). A Yule process speciation prior for branching rates was implemented and the general time-reversible model with invariant sites and gamma-distributed rate variation across sites (GTR+I+Γ) was applied with base frequencies estimated during the analysis. An uncorrelated log-normal model was applied to estimate the relaxed molecular clock because this model places higher prior-density closer to the observed fossil age (Leaché & Mulcahy, 2007). The analysis was run using the default MCMC parameters.
RESULTS

Molecular and phylogenetic results

The final alignments for the non-coding genes encompassed a total of 1,551 base pairs, 521 for ITS1 and 1,030 base pairs for ITS2. In total, 54 indels were present in the ingroup alignment. The best-fit nucleotide substitution model selected for each gene was the general time-reversible model with gamma-distributed rate variation across sites (GTR+Γ). Bayesian analysis of the combined dataset produced a well-resolved consensus tree with high posterior probabilities for most of the nodes (Fig. 2.2). The Bayesian tree shows the three *Dilophotopsis* species forming distinct, deeply divergent clades, which are separated by relatively long branch lengths. Within the large clade made up of *D. concolor* populations, multiple sub-clades are present which correspond to individual deserts or arid lands (Figs 2.2, 2.3). Populations of *D. paron* show little intraspecific variation, and therefore, have no sub-clades present among populations. Because of its rarity, only two specimens of *D. stenognatha* were available for molecular analysis. Both *D. stenognatha* specimens had identical ITS1 and ITS2 sequences. Wilson and Pitts (2008) found high genetic distances between *Dilophotopsis* species. The genetic distance of *D. concolor* populations within a given desert were low (0-0.4% for ITS1 and 0-0.8% for ITS2). Genetic distances were similar for populations between desert regions (0-1.42% for ITS1 and 0.56-1.05% for ITS2: Table 2.2).
Figure 2.2. Consensus tree of Bayesian analysis of the combined ITS1 and ITS2 sequences. Numbers at each branch represent posterior probabilities. Symbols following species names correspond to symbols on the map of North American deserts and arid lands (Fig. 2.3) and mark populations forming distinct clades. The numbers within each symbol correspond to the “map location” in Table 2.1, which gives the exact collection location of each specimen.
Figure 2.3. Map of the North American deserts and arid lands showing the collection localities of *Dilophotopsis* specimens. Symbols indicate collection locality and correspond to Fig. 2.2 and Table 2.1. Arid land boundaries are as described by Omernik (1987) with some changes as in Ricketts *et al.* (1999). Specifically, the Snake River Plain and Northern Basin and Range have been combined into one region, also, the northern and southern Colorado Plateau have been combined.
Table 2.2. Genetic distances of *D. concolor* populations between desert regions. Chi= Chihuahuan Desert, CP= Colorado Plateau, EGB= Eastern Great Basin Desert, ESon= Eastern Sonoran Desert, Moj= Mojave Desert, STex= South Texas Plains, WGB=Western Great Basin Desert, and WSon= Western Sonoran Desert.

<table>
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<tr>
<th></th>
<th>EGB</th>
<th>CP</th>
<th>WGB</th>
<th>Son</th>
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<td>-</td>
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<td>0.80%</td>
<td>0.80%</td>
<td>1.41%</td>
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<td>0.84%</td>
<td>-</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.61%</td>
<td>0.81%</td>
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<td>WGB</td>
<td>0.94%</td>
<td>0.63%</td>
<td>-</td>
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<td>-</td>
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<td>0.76%</td>
<td>0.55%</td>
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<td>-</td>
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</table>

Network analyses showed results similar to the phylogenetic analyses (Fig. 2.4).

A total of 19 haplotypes were found among *Dilophotopsis* species (1 haplotype for *D. stenognatha*: 3 for *D. paron*, and 15 for *D. concolor*). The haplotypes differed greatly between species and formed three distinct networks (*D. stenognatha* not shown because the two individuals shared identical haplotypes).

**Molecular dating**

The Bayesian analysis of the pared-down dataset with additional outgroups showed the same relationships as the full phylogenetic analysis except for the cold desert clades in *D. concolor* (Fig. 2.5). While all three cold desert clades were present (western Great Basin, eastern Great Basin, and Colorado Plateau), there was no resolution among
Figure 2.4. Haplotype networks for *D. concolor* and *D. paron* overlaid on a map of the deserts of North America. Encircled populations share identical haplotypes. Each open circle along the line joining two haplotype groups represents one step. Only one haplotype was found among *D. stenognatha* populations so these were not included in the figure. Haplotypes are named according to the region in which they are found. Chi= Chihuahuan Desert, CP= Colorado Plateau, EGB= Eastern Great Basin Desert, ESon= Eastern Sonoran Desert, Moj= Mojave Desert, STex= South Texas Plains, WGB=Western Great Basin Desert, and WSon= Western Sonoran Desert. Haplotype names marked with a black rectangle represent predicted ancestral haplotypes for each species.
Figure 2.5. Phylogeny of *Dilophotopsis* species showing the estimated divergence dates for each major clade at the node of divergence. The dates estimated in the program Beast are shown first followed by the dates estimated in the program r8s (BEAST/r8s). The black circles indicate the calibration points, one being placement of the fossil at the base of the *Dasymutilla* outgroup clade, and the other limiting the age of the family.
these clades so divergence dates were only estimated for the node connecting the
eastern Great Basin clade with the western Great Basin plus Colorado Plateau clade (Fig. 2.5).

Both dating analyses suggested the divergences separating species occurred
during the period of mountain building in the late Neogene. Results from the r8s analysis
indicated that the species-level splits were between 3.8 and 3.2 Ma. Results from the
BEAST analysis suggested a slightly older dates for the species-level divergences (6.9-
4.6 Ma). BEAST also provides 95% credible intervals for each estimated date. The older
dates on the tree had a larger range of credible dates. In BEAST, the first Dilophotopsis
species to diverge, *D. paron*, was given a divergence date of 6.9 Ma (95% credibility:
10.8-4.1 Ma). The second species divergence, the split between *D. concolor* and *D.
stenognatha*, was estimated to occur at 4.6 Ma (95% credibility: 9-3.2 Ma). The
divergences between *D. concolor* populations all occurred in the Pleistocene, with the
divergence between Chihuahuan Desert clades from the rest of the populations dated to
0.7 Ma (95% credibility: 1.8-0.4 Ma). Similarly, the split between Sonoran Desert
populations and the cold desert clades was dated to 0.3 Ma (95% credibility: 1.1-0.3 Ma).
Finally, the divergence between cold desert clades was dated to 0.17 Ma (95%
credibility: 0.7-0.17 Ma). Both analyses suggested the divergences among populations of
*D. concolor* were a result of Pleistocene climatic cycles with dates ranging from 0.7 Ma
to 0.1 Ma from BEAST and from 0.5 Ma to 0.2 from r8s. Because the relationships
among *Dilophotopsis* species are not particularly well supported (0.85 posterior
probability), we will consider the relationships among species unknown and estimate the
divergence date between all species between 6.9-3.2 Ma (the range of dates given for both divergences in either dating analysis).

**DISCUSSION**

**Neogene vicariance and the diversification of Dilophotopsis**

The phylogeny of *Dilophotopsis*, along with the estimated divergence dates, clearly indicates that the species-level divergences occurred during the late Neogene, specifically near the Miocene/Pliocene boundary (Fig. 2.5). Diversification in several other arid-adapted taxa has been linked to this general time (e.g. Jaeger et al., 2005; Devitt, 2006; Douglas et al., 2006). The species of *Dilophotopsis* show a pattern similar to many vertebrate taxa, with different groups, restricted to eastern and western deserts. In *Dilophotopsis*, *D. concolor* ranges from the Chihuahuan Desert through the Colorado Plateau and the Great Basin, *D. stenognatha* is restricted to the Sonoran Desert, and *D. paron* is found only in the Peninsular Desert of Baja California, the western Sonoran, and the Mojave Desert.

Two major Neogene vicariant events have often been credited for driving diversification in arid-adapted taxa inhabiting the warm deserts, the uplift of the Continental Divide (Rocky Mountain –Sierra Madre Occidental axis), and the enlargement of the Gulf of California, often called the Bouse Sea (Morafka, 1977; Riddle & Hafner, 2006). Because there is no clear description of exactly when these geological events took place, any evolutionary events associated with dates from ~15 Ma to ~2 Ma could likely be attributed to mountain building and aridification in western North America (Wilson & Pitts, 2010).
The phylogeny of *Dilophotopsis* suggests that both the expansion of the Bouse Sea and the uplift of the Continental Divide were influential in the diversification of this genus. Based on the estimated divergence dates for the genus, and their similarities to the reported dates for the expansion of the Bouse Sea (Wilson & Pitts, 2010), the isolation of *D. paron* in the Peninsular, western Sonoran, and Mojave deserts is likely a result of this historical vicariant event. Due to the difficulty in obtaining fresh specimens from Mexico, we do not have any *D. paron* specimens in our phylogeny from Baja California so it is unclear if these populations would be nested within the western Sonoran and Mojave populations, or if they would form a distinct clade. The isolation of *D. stenognatha* in the Sonoran Desert could also be a result of the vicariance in the late Neogene. The presence of the Bouse Sea to the west, and the uplift of the Continental Divide to the east could have isolated ancestral populations, driving the evolution of *D. stenognatha*. Lastly, during the late Neogene uplift events, ancestral populations could have been isolated in the newly formed Chihuahuan Desert east of the Continental Divide, driving the evolution of *D. concolor*. The expansion of this species into the cold desert regions will be discussed in the following section.

This analysis of *Dilophotopsis* provides strong evidence that, like many arid-adapted vertebrates, species level diversification in North American desert arthropods was driven by late Neogene uplift, specifically the enlargement of the Bouse Sea and the uplift of the Continental Divide. While genetic isolation and speciation can be linked to host switches (Sorenson *et al.*, 2003), this does not seem to be the case for *Dilophotopsis* because each species is likely a generalist predator given their variation in body size (Wilson & Pitts, 2008). Furthermore, diversification of several other arid-adapted
nocturnal velvet ant species, in addition to *Dilophotopsis*, has been linked to Neogene uplift events (Pitts et al., 2010).

**Pleistocene climatic fluctuations and diversification within *D. concolor***

*Warm deserts.* Klicka and Zink (1997) suggested that, while Pleistocene climate change affected distributional and population-level species dynamics, the importance of these climatic oscillations to the macroevolutionary dynamics is debatable. Our analysis of diversification in *Dilophotopsis* supports this idea. All of the species-level divergences within *Dilophotopsis* are strongly associated with Neogene vicariant events; yet, the Pleistocene climatic cycles did play a major role in the population-level divergences within the widespread species *D. concolor* (Figs. 2.2, 2.5).

The isolation of separate populations into specific desert regions was likely a result of range reduction and expansion experienced during Pleistocene glacial and interglacial cycles. The polytomy (three-way split) at the base of the *D. concolor* clade suggests uncertainty in the relationships between the Chihuahuan Desert clade and the Southern Texas Plains clade. As in *D. paron*, obtaining fresh specimens from Mexico is difficult, but increased sampling from northern Mexico could help resolve this polytomy. Regardless of the uncertainty among the Chihuahuan Desert clade and the Southern Texas Plains clade, the most likely cause for the formation of these distinct clades is isolation in separate glacial refugia. While parts of the Chihuahuan Desert were covered with a mesic woodland during the Pleistocene (MacKay & Elias, 1992; Pendall et al., 1999; Holmgren et al., 2003), multiple desert refugia also existed (Elias et al., 1995). During the warming of the Pleistocene interglacial cycles, the Chihuahuan Desert *D.*
_concolor_ populations expanded north and west, eventually spreading to the eastern Sonoran Desert region.

The border between the Sonoran and Chihuahuan deserts is far from distinct. Ricketts _et al._ (1999) suggest the Chihuahuan Desert reaches as far west as Santa Cruz County in Arizona. Omernik (1987), on the other hand, describes the Chihuahuan Desert scarcely reaching the eastern border of Arizona, with the entire southeastern corner of Arizona designated as Madrean Archipelago due to its similarities with the Sierra Madre Mountains in Mexico. During the warming of the Pleistocene interglacials, the Chihuahuan Desert became connected to the Sonoran Desert through a relatively low elevation area in the Continental Divide often referred to as the Cochise filter-barrier (Morafka, 1977) particularly in an area in southwestern New Mexico called the Deming Plains (Hafner & Riddle, in press). The Deming Plains is a wide basin that stretches from the Arizona-New Mexico border to near the western edge of the Rio Grande near Las Cruces, New Mexico. The connection that existed between Chihuahuan and Sonoran deserts was broken during the cooling of each glacial cycle. Phylogeographic breaks have been found in multiple vertebrate taxa across the Deming Plains. For example, a shift from a Sonoran rodent fauna to a Chihuahuan rodent fauna takes place on the Deming Plains (Hafner & Riddle, in press).

The phylogeographic patterns among _D. concolor_ populations show that a genetic divergence occurred between populations in the Chihuahuan and Sonoran deserts (Figs 2.2, 2.4). We attempted to find phylogeographic evidence of the Deming Plains being the point of contact between these populations by sampling across the entire Plain and into the Madrean Archipelago region of southwestern Arizona (Fig. 2.3). Based on this
analysis, the Deming Plain is not where the genetic divergence occurred; all individuals from the Deming Plain were genetically identical, or nearly identical, to Chihuahuan Desert populations. Even individuals collected 75 km west of the Arizona/New Mexico border were genetically aligned with Chihuahuan Desert populations. Because *D. concolor* is uncommon in the Sonoran Desert, only being found in the easternmost areas bordering with the Madrean Archipelago (Wilson & Pitts, 2008), we were only able to include one individual from this area in our analysis. This individual, however, was genetically distinct from the Chihuahuan populations and may represent a Madrean Archipelago clade. Increased sampling from southeastern Arizona and northern Mexico would likely clarify the relationship between the Chihuahuan Desert clade and the Sonoran/Madrean clade. While the Deming Plain does not appear to be the point of contact between divergent *D. concolor* clades, the Cochise filter-barrier, which in its broadest sense stretches from the Baboquivari Mountains of south-central Arizona through the Deming Plains to the Rio Grande in New Mexico (Hafner & Riddle, in press) played an important role in the development of the phylogeographic patterns of this species.

**Cold deserts.** Although the processes influencing diversification in the warm deserts have been well studied, less work has been done on the historical biogeography of the cold deserts. Some studies have investigated phylogeographic patterns within the Great Basin Desert, finding a genetic split between populations on the eastern half of the desert and the western half (Epps *et al.*, 1998; Orange *et al.*, 1999). Riddle and Honeycutt (1990), through a study of grasshopper mice, found that Great Basin populations were aligned with the Columbia Plateau to the north, and that populations
from the Colorado Plateau phylogenetically grouped with the Great Plains and Wyoming Basin individuals. Other studies have suggested that the Colorado Plateau may have some similarities to the Chihuahuan Desert (e.g., Jaeger et al., 2005). Although the Colorado Plateau shares many biotic similarities with the Great Basin Desert (Ricketts et al., 1999), these areas do harbor genetically distinct populations.

As in many vertebrate taxa, the phylogeographic patterns of *D. concolor* populations inhabiting the cold deserts were influenced by the cyclic nature of the Pleistocene climate changes. There are three distinct cold desert lineages in *D. concolor*, one in the eastern Great Basin, one in the western Great Basin, and one in the Colorado Plateau. The formation of these lineages is likely the result of recent range expansion events from glacial refugia. An ecological niche model based on a different species of nocturnal velvet ant found in the cold deserts suggests that glacial age refugia could have existed in the northwestern Mojave Desert in the low valleys around Death Valley, in the northeastern Mojave Desert along the Virgin River in northwestern Arizona and southwestern Utah, and in several canyon bottoms in the Colorado Plateau (Wilson unpub data). It is likely that these same areas also provided glacial refugia for many other cold desert-adapted species. Expansion from possible Mojave refugia into the low-elevation western Great Basin (the Lahontan Basin) and the low-elevation eastern Great Basin (the Bonneville Basin) could have resulted in isolated groups separated by the relatively higher areas in the central Great Basin. In *D. concolor*, the phylogeographic patterns suggest that the eastern Great Basin populations are more closely related to the Colorado Plateau populations than they are to the western Great Basin populations. Because southwestern Utah is a unique area where the Colorado Plateau, the Great Basin
Desert, and the Mojave Desert meet, range expansion from the eastern Mojave refugium north into the Bonneville Basin and northeast into the Colorado Plateau could have caused the formation of two separate populations on either side of the Wasatch Mountains. The increased structuring of sub-clades within the Colorado Plateau clade compared to either Great Basin clade could be the result of isolation into several refugia in the canyon bottoms and low elevation valleys in the Colorado Plateau during the late Pleistocene, which may have led to genetic isolation during the glacial cycles. An in-depth phylogeographic analysis, with increased sampling of these populations, is needed to fully investigate the effect of Pleistocene glacial cycles on *D. concolor* populations isolated to a specific desert region.

Recent range expansion from the deserts to other arid regions is apparent from haplotypes matching desert populations being found in other regions. For example, a specimen collected near the Bruneau Sand Dunes in the Snake River Plain of southwestern Idaho phylogenetically matches the western Great Basin populations. Another specimen collected in southern Idaho, near City of Rocks National Preserve, has identical ITS1 and ITS2 haplotypes to individuals from the eastern Great Basin clade. A specimen from the Wyoming shrub-steppe, near Green River Wyoming, is genetically identical to several individuals from the Colorado Plateau indicating recent range expansion into this northern region. Lastly, an individual collected near Chimney Rock National Historic Site, Nebraska, shares ITS1 and ITS2 haplotypes with individuals from the Chihuahuan desert in Texas, indicating recent range expansion.
Conclusions: Understanding the diversification in the Nearctic desert biota

As more data become available, it is becoming clear that a majority of the species-level diversity within the arid lands of North America is associated with Neogene mountain building events rather than Pleistocene climatic oscillations. For example, studies of multiple newts and salamanders (Tan & Wake, 1995; Shaffer et al., 2004), toads (Jaeger et al., 2005), several species of snake (Devitt, 2006; Douglas et al., 2006), birds (Klicka & Zink, 1997), and mice (Riddle, 1995), as well as plants (Moore & Jansen, 2006), suggest that major diversification in these lineages occurred well before the Pleistocene, and was likely driven by drying which was caused by mountain uplift. While it is clear that the Pleistocene climatic shift affected the distributions and population structure of numerous species, there is little evidence that these climatic cycles played much of a role in speciation within arid-adapted taxa.

The phyogeography of Dilophotopsis supports the postulation that Neogene mountain building was the major cause of diversification among arid-adapted species. Pleistocene climatic changes, while not contributing to the species-level diversity in this group, did have a major effect on the distributions and population-level diversity within D. concolor. This represents one of the first analyses of a widespread invertebrate taxon in the arid lands of North America and it suggests that additional analyses using invertebrates will add to the understanding of the historical biogeography of the region.
REFERENCES


Riddle BR. 1995. Molecular biogeography in the pocket mice (*Perognathus* and *Chaetodipus*) and grasshopper mice (*Onychomys*): the late Cenozoic development of a North American aridlands rodent guild. *Journal of Mammalogy* 76: 283-301.


CHAPTER 3

PLEISTOCENE DIVERSIFICATION OF THE *ODONTOPHOTOPSIS UNICORNIS*
SPECIES-GROUP (HYMENOPTERA: MUTILLIDAE)

Phylogeographic analyses use the genetic population structure within widespread species, and among closely related species to understand their geographic distributions and gain insights into the geobiotic history of a region (Avise 2000). Many recent studies have suggested that a majority of the species-level diversification in the arid-adapted North American biota was driven by Neogene vicariant events (i.e., mountain-building events) rather than by Pleistocene climatic oscillations (Riddle 1995, Orange et al. 1999, Douglas et al. 2006). It is clear, however, that the Pleistocene climate change had a large effect on the distribution and population-level divergence within species, but the importance of these climatic oscillations to the macroevolutionary dynamics is debatable (Klicka and Zink 1997).

Velvet ants (Hymenoptera: Mutillidae) are an often unnoticed yet common element of North America’s desert environments. All velvet ants are solitary parasitic wasps that parasitize other aculeate Hymenoptera, including bees (Apoidea) (Krombein 1979, Nonveiller 1990, Brothers 1995). While the colorful, diurnal species are most frequently encountered, a rich and abundant fauna of nocturnal velvet ants also exists. With over 205 known nocturnal species in nine genera found throughout western North

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2 This chapter has been published in *Annals of the Entomological Society of America*. Permission has been given for the reproduction of this work here (Appendix A). Please use the following citation when referring to this work: Wilson, J. S., and J. P. Pitts. 2010. Pleistocene Diversification of the *Odontophotopsis unicornis* Species-Group (Hymenoptera: Mutillidae). *Annals of the Entomological Society of America*. 103: 555-565.
America, members of this family are ideal subjects for revealing phylogeographic patterns in the deserts and arid regions (Pitts et al. 2010).

The *Odontophotopsis unicornis* species-group currently contains only the species *O. erebus* (Melander) and *O. unicornis* Schuster (Pitts 2007). The species-group is easy to recognize and is distinctive among the North American nocturnal mutillid fauna due, in part, to unique clypeal and mandibular morphology (Pitts 2007). The distinction between the species in this group, which is based on differences in the length of the clypeal tubercle and on very slight differences in the genitalia, can be occasionally difficult to discern. Because of the morphological similarities within the species-group, Pitts (2007) suggested that future molecular studies may show that these two species represent one highly variable species.

Extreme sexual dimorphism occurs in mutillids, with the result that many species and even genera are known only from a single sex (Brothers 1995). Sex associations for the nocturnal velvet ants are further compounded by the great morphological similarity of the species, and because, although males are easily collected with light traps, females are rarely caught. Molecular techniques are now available to make sex associations using species-specific genetic loci (Pilgrim and Pitts 2006, Pitts et al. 2007, Pilgrim et al. 2008). Historically, the species in the *Odontophotopsis unicornis* species-group were known only from males. The female of *O. erebus* was only recently described (Pitts et al. 2007), while the female of *O. unicornis* remains unknown. Discovering the female of *O. unicornis* can add to our understanding of the species-group by making available morphological characters of the female, which may better define the species boundaries within the group.
The biogeographical pattern of the *O. unicornis* species-group is also interesting. One member, *O. erebus*, is wide ranging from western Kansas, Nebraska, Oklahoma, and Texas west to Arizona, Nevada, New Mexico, and Utah, and south into northern Mexico. This species, however, is absent from the Mojave and western Sonoran deserts. The other member, *O. unicornis*, is found in the Sonoran and Mojave deserts of Arizona, Nevada, and California into northern Mexico. The ranges of these two species overlap broadly in the eastern Sonoran Desert over most of southern Arizona. This overlap leads one to further question the distinctness of these two species.

The purposes of this study are to 1) determine the validity of the species in the *O. unicornis* species-group using both molecular and morphological data, 2) uncover any phylogeographic patterns within this species-group and associate the genetic divergences with historical geological or climatological events, using molecular dating techniques calibrated with fossil data, and, determine if the there is good evidence for discrete species, 3) describe and associate the female of *O. unicornis*.

**Materials and Methods**

**Trapping Methods.** During the summers of 2005–2008, field studies were conducted throughout the southwestern U.S. to collect fresh specimens of both sexes of nocturnal velvet ants. Collections were made of male and female nocturnal mutillids at 60 field sites across the southwestern U.S. Specimens were collected using black light traps, fluorescent lantern traps, and by hand. Those collected with light traps were captured in soapy water and were transferred into 95% ethanol, while all hand-collected specimens were placed directly into 95% ethanol. All specimens were identified to the species level
except for some female specimens that were sorted to morphospecies because they had not yet been associated with males. Samples were collected from various sites across the range of each species in the *O. unicornis* species-group (Fig. 3.1). A total of 20 *O. erebus* specimens (19 males and 1 female) were sampled, as well as four male *O. unicornis* specimens. Also, two unknown female specimens that were morphologically similar to the female of *O. erebus* were included. All specimens were examined for both morphological and molecular characters.

**Molecular Methods.** Molecular techniques including, DNA extraction, PCR, and sequencing were preformed following the protocol described by Pilgrim and Pitts (2006). The following primers were used to amplify the ITS1 and ITS2 regions of the nuclear genome. The primers 5′-GATTACGTCCCTGCCCCTTG-3′ (forward-18S) and 5′-CGATGATCAAGTGTCCTGCA-3′ (reverse-5.8S) (both from Pilgrim et al. 2002) were used for the ITS1 locus and 5′-GGCTCGTGGAAATCGATGAAGA-3′ (forward 5.8S) (modified from Weekers et al. 2001) and 5′-GCTTATTAATATGCTTAAATTCAGCGG-3′ (Weekers et al. 2001) were used for ITS2. PCR took place in a 20 µl volume with conditions of 3 mM MgCl2, 200 pM dNTPs, 2 units of *Taq* polymerase, 1 mM of each primer, and standard PCR buffer concentration. For each PCR, approximately 20 ng of template DNA was added to the reaction. The PCR program included an initial step of 94°C for 150 sec, followed by 35 cycles of 94°C for 30 sec, 52°C (ITS1) or 56°C (ITS2) for 60 sec, and 72°C for 60 sec, with a final step of 72°C for 10 min. Amplified products were visualized on agarose gels
stained with ethidium bromide. Successful PCR products were cleaned using isopropanol purification.

The two internal transcribed spacers (ITS1 and ITS2) were sequenced for representatives of each available species and sex, sequences were aligned, and females were associated with males based on identical or nearly identical DNA sequences for those loci (i.e., very small genetic distances). The methods proposed by Pilgrim and Pitts (2006) were followed for performing sex associations. ITS1 and ITS2 were sequenced.
for at least one female of each morphospecies and several male specimens of each
described species. PCR was used to amplify the ITS1 and ITS2 regions of the nuclear
genome. Gel electrophoresis of each gene yielded a single band for each individual wasp
and the resulting DNA was sequenced cleanly, suggesting no gene heterogeneity as seen
in some other organisms (Harris and Crandall 2000, Parkin and Butlin 2004, Bower et al.
2008). PCR products were sequenced in both directions and sequence contigs assembled
using Sequencher 4.0 (Gene Code Corp., Ann Arbor, MI). DNA sequences were aligned
using Clustal W (Thompson et al. 1994) and intraspecific and interspecific genetic
distances were calculated from these alignments. Genetic distances between species were
calculated as pairwise percentages by determining the number of differences (point
mutations and insertions or deletions) divided by the number of base pairs of the longer
of the two sequences. ITS1 and ITS2 sequences were deposited in GenBank (Accession
Nos. HM030444-HM030491; Table 3.1).

**Phylogenetic and Haplotype Network Methods.** The two genetic loci were
subjected to Bayesian analysis using MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003).
Sequences were analyzed as a combined data set, with each gene partitioned separately
with all parameters unlinked across loci. Appropriate models of nucleotide substitution
were determined in MrModeltest version 2.3 (Nylander 2004). Bayesian analyses
included four independent runs with three heated chains and one cold chain in each run.
The MCMC chains were set for 3,000,000 generations and sampled every 100
Table 3.1. Descriptive information for all taxa used in the phylogenetic portion of this study.

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<tr>
<th>Species</th>
<th>Species ID # (Figs. 3.1, 3.2, 3.4)</th>
<th>Voucher ID</th>
<th>Sex</th>
<th>Collection Location</th>
<th>ITS1 Accession #</th>
<th>ITS2 Accession #</th>
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<td><em>Dasymutilla occidentalis</em></td>
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generations; chains were run until the average standard deviation of the split frequencies dropped below 0.01. The burn-in period for each analysis was removed after graphical determination of stationarity. Several outgroups were included in the analysis. *Odontophotopsis rubiventris* (Schuster) and *O. armata* Schuster are closely related to the *O. unicornis* species-group (Pitts et al. 2010) and were included. *Odontophotopsis delodonta* Viereck was used as a more distant outgroup. In addition to the full phylogenetic analysis, a Bayesian analysis was implemented on a subset of the full data set in order to streamline the molecular dating process. Included in this analysis were two *O. erebus* populations, two *O. unicornis* populations, the same outgroups as were used in the full phylogenetic analysis, and multiple *Dasymutilla* species including *D. snoworum* (Cockerell), *D. occidentalis* (L.), *D. gloriosa* (Saussure), and a *Traumatomutilla* species. These additional outgroups were added in order to include a fossil for calibration in the molecular dating analysis.

A parsimony-based haplotype network was constructed using the combined ITS1 and ITS2 sequences for all *Dilophotopsis* specimens using TCS version 1.21 (Clement et al. 2000). The program estimated the 95% reconnection limit between haplotypes with gaps treated as missing data.

**Molecular Dating Methods.** Divergence date estimates were calculated for major nodes on the tree using two methods: a penalized likelihood approach to rate smoothing using the program r8s 1.71 (Sanderson 2003), and a Bayesian MCMC averaging approach to rate smoothing using the program BEAST v1.4.8 (Drummond and Rambaut 2007). Because there is a disparity of fossils that can be used as calibration points in Mutillidae, two distinct dating methods were used as a way to corroborate the
divergence date. While no fossils are available for *Odontophotopsis* or any of the nocturnal velvet ants, two fossils from Dominican amber, *Dasymutilla dominica* Manley and Poinar and *D. albifasciatus* Manley and Poinar (Manley and Poinar 1991, 1999, 2003) were used to calibrate the estimated divergence dates. Based on the morphology of these fossils, they appear to be most closely related to the basal members of the genus *Dasymutilla* (Williams, unpublished data).

*r8s analysis.* The program *r8s* uses a tree description with branch lengths to estimate divergence dates. The consensus tree that resulted from the paired-down Bayesian analysis was used in the *r8s* analysis. The most recent common ancestor (MRCA) of the *Dasymutilla* plus *Traumatomutilla* clade was constrained to be at least 20 Ma (minage = 20) based on the placement of the fossils and the reported age of Dominican amber (Iturralde-Vinet and MacPhee 1996). The root was fixed at 65 million years based on the estimated maximum age of Mutillidae (Grimaldi and Engel 2005), and the penalized likelihood method with the truncated Newton algorithm was implemented to estimate rates and divergence dates.

*BEAST analysis.* The program *BEAST* uses the aligned sequence data to generate a tree and estimate divergence dates. The program BEAUtv1.4.8 (Drummond and Rambaut 2007) was used to generate the file used in *BEAST* with the alignment of the paired-down data set. The MRCA of the *Dasymutilla* plus *Traumatomutilla* clade was constrained to be \( \sim 20 \) Ma by giving this node a normally distributed prior with a mean age of 20 million years and a standard deviation of 1.0. The root node was limited to a mean age of 65 million years with a standard deviation of 15 million years based on the estimated age of the family (Grimaldi and Engle 2005). A Yule process speciation prior
for branching rates was implemented and the general time-reversible model with invariant sites and gamma-distributed rate variation across sites (GTR+I+Γ) was applied with base frequencies estimated during the analysis. An uncorrelated log-normal model was applied to estimate the relaxed molecular clock because this model places higher prior-density closer to the observed fossil age (Leaché and Mulcahy 2007). The analysis was run using the default MCMC parameters.

**Taxonomic Methods and Terminology.** The following acronyms are for institutions or collections housing the material discussed in the current study: Department of Entomology, Academy of Natural Sciences, Philadelphia, Pennsylvania, USA (ANSP); Department of Entomology and Entomological Museum, Department of Biology, Utah State University, Logan, Utah, USA (EMUS); National Museum of Natural History, Smithsonian, Washington D.C., USA (NMNH).

Based on Ferguson (1967), we adopt the following notation for punctures in the order of decreasing coarseness: reticulate, coarse, moderate, small, fine and micropunctate. Micropunctate refers to punctures that are extremely shallow and do not have vertical walls or sharp margins. Small refers to punctures that do have slight vertical walls and are separated by at least 5X their diameter. We use the term “Brachyplumose setae” for setae with barbs that are less than, or equal to, the diameter of the shaft at the attachment of the barb. The term “plumose setae” is used for setae that have longer barbs. The term “tibial spurs” is used instead of “calcaria.” The acronyms T2, T3, etc., denote the second, third, etc., metasomal tergites, respectively. Similarly, S2, S3, etc., signifies the second, third, etc., metasomal sternites, respectively.
**Results**

**Molecular Results.** Genetic distances were low among populations of a single species. For *O. unicornis*, all ITS1 sequences were identical and for ITS2 the distances were from 0.0-0.2%. For *O. erebus*, distances were similarly low, with 0.0-0.4% for ITS1 and 0.0-0.1% for ITS2. Interspecific distances, however, were higher, with a distance of 1.2% for ITS1 and 2.0% for ITS2. Genetic distances of the unknown females, which resembled the female of *O. erebus*, to the males of *O. unicornis* were low (0% for ITS1 and 0.2% for ITS2).

**Phylogenetic, Haplotype Network and Dating Results.** The best-fit nucleotide substitution model selected for each gene was the general time-reversible model (GTR) (Lanave et al. 1984). Bayesian analysis of the combined ITS1 and ITS2 data set resulted in a well-supported tree with posterior probabilities of 1.0 for most nodes (Fig. 3.2). The topology showed two distinct clades in the *O. unicornis* species-group, one made up of *O. unicornis* populations and the other consisting of *O. erebus* populations (Fig. 3.2). There was no resolution within the *O. unicornis* clade but one sub-clade was present in the *O. erebus* clade, which was made up of populations from the Chihuahuan Desert. The analysis on the paired-down data set to be used in the molecular dating analysis resulted in a tree depicting the same relationships among ingroup taxa as the consensus tree from full analysis (Fig. 3.3).
Figure 3.2. Consensus tree of Bayesian analysis of the combined ITS1 and ITS2 sequences. Numbers at each node represent posterior probabilities. Symbols following species names correspond to symbols on the map of the distributions of the species in the *O. unicornis* species-group (Fig. 3.1). The numbers within each symbol correspond to the “Species ID #” in Table 3.1 which gives the exact collection location of each specimen. Estimated divergence dates are given for the node connecting *O. unicornis* and *O. erebus*. 
Both molecular dating analyses resulted in similar date estimates for the divergence between *O. unicornis* and *O. erebus*. The analysis using the program r8s suggested that these species diverged at about 1.2 Ma and the analysis using the program BEAST suggested a divergence date of 0.77 Ma (95% credibility dates from ~9.5-0.5 Ma). The haplotype network analysis resulted in two haplotypes, one representing *O. unicornis* populations and one representing *O. erebus* populations (Fig. 3.4). Similar to the phylogeny, there is little genetic structuring in the haplotype networks.

**Morphological Results.** Careful examination of numerous specimens of both species in the *O. unicornis* species-group revealed consistent morphological differences between the males of *O. erebus* and *O. unicornis*. Study of the clypeal tubercle has
revealed that this structure on *O. erebus* is an extension of the base of the clypeus (Figs. 3.5-3.7), while the tubercle of *O. unicornis* is an extension of both the clypeus and the frons (Figs. 3.5 and 3.6). The genitalia are not informative for separating these two species (Figs. 3.9-3.12).

Based on the above molecular and morphological data, we are describing the female of *O. unicornis*. Also, we provide diagnoses of the *O. unicornis* species-group, and for each of the species in this group.

**Odontophotopsis unicornis species-group**

**Diagnosis of male.** This species-group is easily characterized by the unique mandibles (Fig. 3.8), which are bidentate apically with a weak ventral excision, a weak to moderate ventrobasal angulation or tooth, and dorsal and ventral margins that are sharply and strongly carinate appearing somewhat lamellate (Figs. 3.5-3.8). This species-group also has a pair of small, triangulate, mesosternal spines, is nocturnal having testaceous to
Figures 3.5-3.12. *Odontophotopsis unicornis*: Fig. 3.5. head, frontal view; Fig. 3.6. head, lateral view; Fig. 3.8. mandible; Fig. 3.9. genitalia, lateral view; Fig. 3.10. penal valve. *Odontophotopsis erebus*: Fig. 3.7. head, lateral view; Fig. 3.11. genitalia, lateral view; Fig. 3.12. penal valve (Figs. 3.5, 3.6, 3.8-3.12 from Pitts 2007; Fig. 3.7 from Pitts et al. 2007).

Stramineous integumental coloration and large ocelli, and has dense fringes of dense plumose setae located on the apices of the metasomal segments. Additional characters can be found in Pitts (2007).
**Diagnosis of female.** The female of this species-group can be diagnosed by the following unique combination of characters: the first metasomal segment is petiolate with the second; the sides of the propodeum are punctate; the pygidium is laterally defined by carinae with weak longitudinally striate sculpturing; the mandibles have a distinct basal angulate tooth on ventral margin; and the coloration and setal pattern, specifically the presence of the various colors of decumbent setae and the density of plumose setae composing the metasomal fringes, is characteristic.

**Remarks.** The females of *O. erebus* and *O. unicornis* are similar to *O. succinea* Viereck in that they both have distinctly margined pygidium both laterally and apically. They differ from both *O. succinea* and *O. melicausa* (Blake) by the lack of transverse sinuate carinae on the mesosomal dorsum and by the lack of a large basal tooth on the ventral margin of the mandible. These two females are also quite similar to *Sphaeropthalma diomeda* (Fox) and *S. halcyone* (Fox), and it is possible that the latter two species actually belong in *Odontophotopsis*. Although these latter two species have well-developed basal teeth on the ventral margin of the mandible, they may be confused with *O. erebus* due to similarities in coloration, but the ventral mandibular tooth in *O. erebus* is not as well developed. Pitts et al. (2007) suggested informally that *S. diomeda* and *O. erebus* may be synonymous given that they differ only in coloration of the metasomal setal fringes. After studying several more female specimens of *O. erebus*, this is no longer believed to be the case given differences in mandibular morphology, as well as head shape and pygidial sculpturing.
Odontophotopsis erebus (Melander)


**Diagnosis of male.** This species can be distinguished from *O. unicornis* by the clypeus being concave and having a tuberculate process at median proximal margin that is not longer than wide (Fig. 3.7). Also, the dorsal carina of the mandible is present on the distal third, the anterior margin of the clypeus is indistinctly emarginated, the ocellar area is concolorous with the head, and the cuspis is not narrowed medially having stout setae throughout (Figs. 3.9-3.12).

**Diagnosis of female.** The female of *O. erebus* can be separated from the female of *O. unicornis* by the legs being concolorous with the body, and the decumbent setae on the dorsum of the mesosoma and second tergite of the metasoma being orangish brown to brown (Fig. 3.13).

![Figure 3.13. Habitus of the female of *Odontophotopsis erebus.*](image-url)
**Distribution.** Widely distributed from western Kansas, Nebraska, Oklahoma, and Texas west to Arizona, Nevada, New Mexico, and Utah and south into northern Mexico. Absent from the Mojave, Great Basin, and western Sonoran deserts (Krombein 1979, Pitts 2007).

**Remarks.** This female of this species is fully described in Pitts et al. (2007) and the male of this species is discussed at length in Pitts (2007).

*Odontophotopsis unicornis* Schuster


**Diagnosis of male.** In this species, the clypeus is also concave with tuberculate process at median proximal margin, but the process is narrowly linguiform, is produced downward over clypeus, is prominent and is much longer than wide (Fig. 3.6). The anterior margin of the clypeus is distinctly emarginate and turned outward, the ocellar area usually is concolorous with the head, but sometimes slightly infuscated, the cuspis is slightly narrowed medially having an apex with stout setae, medially having thinner setae, and an inner margin with circular area of dense short setae (Figs. 3.9-3.12).

**Diagnosis of female.** The female of *O. unicornis* can be separated from the female of *O. erebus* by the legs being light yellow and not concolorous with the body, and the decumbent setae on the dorsum of the mesosoma and second tergite of the metasoma being bright orange (Fig. 3.14).
Description of female. Coloration and setal pattern. Body reddish brown to brown; legs light yellow. Mandibular apex black. Flagellum light yellow to dark yellow. Decumbent setae dense, concealing sculpture of head and mesosomal dorsum; setae distinctly plumose especially at base of setal stalk. Head with dense decumbent white to pale golden plumose setae and erect white to pale golden brachyplumose setae. Genal region less densely pubescent. Anterior margin of pronotum, pleurae, and vertical face and lateral faces of propodeum with erect white brachyplumose setae. Dorsum of mesosoma with dense decumbent bright orange plumose setae and sparser erect brachyplumose setae; color changes to white laterally and on dorsal face of propodeum. T1 covered with erect white brachyplumose setae. T2 with decumbent orange plumose setae and erect brachyplumose setae. T1-T5 and S2-S5 with fringe of dense white fluffy plumose setae. Fringe of T2-T4 obscures proceeding disk. Legs with white brachyplumose setae.
Head. Head rounded posteriorly, not as wide as mesosoma, moderately punctate. Eye slightly ovate, distance from posterior mandibular articulation ~2.5X visible length of pedicel. Clypeus protruding anteriorly, posteromedially produced into low triangular tubercle. Antennal scrobes lacking dorsal carina. Antennal tubercle with multiple carinae running parallel to apical margin. Flagellomere I ~1.2X length of pedicel. Flagellomere II ~1.2X and FIII ~1.4X length of pedicel. Flagellomeres II-X produced apically on ventral side; appearing crenulate. Mandible bidentate apically. Ventral mandibular margin with basal angulation; excision as wide as 0.2X basal width of mandible. Genal carina absent.

Mesosoma. Mesosoma obpyriform, slightly longer than broad; broadest medially. Mesosoma densely confluently punctate on dorsum; punctures becoming larger posteriorly. Propleuron completely, mesopleuron medially running vertically, and extreme ventral region of propodeal side punctate. Humeral angle dentate. Epaulet prominent. Scutellar scale and transverse sinuate carina absent. Mesosternum with low transverse tubercle present medially just anterior to mesocoxa. Metasternum tridentate, median tooth ~4X as long as lateral teeth. Mid and hind tibiae with two rows of spines on outer margin and each with pair of apical tibial spurs.

Metasoma. Segment 1 subpetiolate with segment 2. Tergite 1 with small sparse punctures. Tergite 2 with dense moderate punctures anteriorly; punctures becoming more widely spaced posteriorly (interstitial distance ≥ puncture width). Tergite 2 with felt line; ~0.33X length of tergite. Tergite 6 with distinct pygidial area defined laterally and apically by thickened up-turned margin; surface weakly longitudinally striate throughout.
Sternite 2 with slight anteromedian tumid region. Sternite 2-S5 with punctation similar to tergites.

*Length.* ~ 6-9 mm.

**Distribution.** The Sonoran and Mojave Deserts of Arizona, Nevada, California into northern Mexico (Pitts 2007).

**Remarks.** It is somewhat difficult to differentiate the species of the *O. unicornis* species-group based on females. This is not surprising given the difficulty of separating the females of other related taxa (Pitts et al. 2004, Pitts 2006). The two species basically differ only in setal and leg coloration, as well as that *O. unicornis* has slightly denser punctation on T2. The two species overlap greatly in range in southern Arizona, and in this area locality data are not a good indicator for identifying the females. The males of these species, on the other hand, are usually not difficult to distinguish and differ mainly in the shape and position of the tubercle on the clypeus (Figs. 3.1-3.3).

**Discussion**

Pitts (2007) stated that the distinction between the genitalia and clypeal tubercles of the males of the two species in the *O. unicornis* species-group can be occasionally difficult to discern and future molecular data may show that these species represent one highly variable species. The present morphological and molecular analysis of the *O. unicornis* species-group, however, clearly indicates that the two species in this group are distinct (Fig. 3.2). While the molecular distances between the species in this group are slightly lower than has been found in other mutillids (Wilson and Pitts 2008, 2009), the
phylogenetic and haplotype analyses, together with the morphological characters, support the individuality of *O. unicornis* and *O. erebus*.

Several genetic analyses of mutillid wasps have shown that conspecifics often have identical or nearly identical ITS1 and ITS2 sequences (Pilgrim and Pitts 2006, Pitts et al. 2007, Pilgrim et al. 2008). The genetic distances between the unknown females that were included in the analysis and *O. unicornis* were comparable to distances found in other sex-association studies and suggest that these females are *O. unicornis*.

The geographic distributions of these two species (Fig. 3.1) show similar patterns to other North American desert taxa, including other mutillid wasps, with one species being restricted to the eastern deserts (Chihuahuan and Great Basin deserts and the Colorado Plateau) and the other species restricted to the western deserts (Mojave and Sonoran deserts) (Morafka 1977, Riddle 1995, Wilson and Pitts 2008). An east/west split, like that seen in the *O. unicornis* species-group, has been observed in several other desert-adapted taxa and has often been associated with Neogene mountain building (Morafka 1977, Jaeger et al. 2005, Devitt 2006, Douglas et al. 2006). Morafka (1977) first attempted to explain the phenomenon of sister species being restricted to eastern and western deserts by describing a hypothetical ancient desert region called Mojavia, which extended from the modern Mojave Desert east through the Sonoran and Chihuahuan deserts. This vast desert region was subsequently split into eastern and western deserts by the uplift of the Continental Divide (made up of the Rocky Mountains and the Sierra Madre Mountains), which occurred in the late Neogene, from about 15-2 Ma (Morafka 1977). While this scenario seems to explain the phyllogeographic patterns in many animals, including other mutillid wasps (Jaeger et al. 2005, Devitt 2006, Douglas et al.
2006, Wilson and Pitts 2008), the estimated divergence date associated with the development of the *O. unicornis* species-group suggests that the evolution of these species was driven by more recent events.

Based on the proposed location of Pleistocene desert refugia, the pattern of sister species inhabiting eastern and western deserts could be attributed to effects of isolation in eastern and western refugia. Evidence of Pleistocene refugia for desert taxa exists in the Chihuahuan Desert (Elias et al. 1992) and the Sonoran Desert (Van Devender 1990, Van Devender et al. 1990, Hunter et al. 2001). If a species was widespread in the deserts during Pleistocene interglacials, it could have been forced into refugia in both the east and west during the onset of a glacial cycle. This isolation could have led to the same pattern of species distribution that has often been associated with Neogene mountain building. Divergence dates are necessary to be able to distinguish between Neogene and Pleistocene diversification.

Both divergence date estimations for the *O. unicornis* species-group suggest that the diversification within this group occurred during the mid to late Pleistocene (Fig. 3.2). Pleistocene age diversification has been found in population-level analyses (e.g. Ayoub and Riechert 2004), yet the effect Pleistocene climate change had on species-level divergence has been questioned due to the lack of evidence (Klicka and Zink 1997). Divergence date calculations, such as these, can be affected by inadequate sampling; if taxa are considered to be sister species erroneously, the calculated divergence dates can be mistaken as younger than actual. Our results, however, of young divergence dates placed in the Pleistocene are not the artifact of an incorrect assumption of the monophyly of the *O. unicornis* species-group. *Odontophotopsis erebus* and *O. unicornis* are
undoubtedly sister taxa. This conclusion is based on the degree of morphological similarity between these two species and also is based on a comparison of sequences of these two species with ~75% of *Odontophotopsis* species and more complete phylogenetic analyses (e.g., Pitts et al. 2010; unpublished data). As such, this analysis, along with other recent studies (e.g., Pitts et al. 2010), provides strong evidence of species-level diversification being driven by Pleistocene climatic oscillations.

Unlike the phylogeographic patterns observed in other mutillid wasps (Wilson and Pitts 2008, 2009), there is little or no phylogenetic structure within either species in the *O. unicornis* species-group (Fig. 3.2). This lack of genetic structuring could be a result of extensive gene flow between distant populations, or it could be a result of relatively recent range expansion. While the current study did not attempt to measure gene flow between populations, it is unlikely that the lack of variation in ITS1 and ITS2 among the populations of *O. unicornis* and *O. erebus* is due to wide-ranging gene flow. Population-level analyses of other mutillid wasps have shown genetic structuring using both ITS1 and ITS2, so it is doubtful that the *O. unicornis* species-group species differ drastically in behavior that would cause extensive gene flow. The results of the molecular dating analyses suggest, however, that *O. unicornis* and *O. erebus* evolved recently in the mid to late Pleistocene and this recent origin could explain the lack of phylogenetic structure among distant populations. Because each species presumably did not evolve until the mid Pleistocene, there has not been sufficient time for remote populations to develop phylogenetically informative mutations in ITS1 or ITS2. Future fine-scale analyses, like microsatellite analyses, may uncover population-level genetic structure among populations in the *O. unicornis* species-group.
While Neogene geologic events have often been cited as the driving force behind the majority of species-level divergences in desert-adapted species, this study shows that not all taxa showing an east/west pattern of genetic divergence were influenced by the same historical events. Because both Neogene events and Pleistocene events can lead to similar patterns of genetic divergence, it is imperative that divergence dates are estimated so more accurate historical biogeographic hypotheses can be formed.

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CHAPTER 4


INTRODUCTION

Historical biogeographical tools are increasingly being used to gain insights into the evolutionary history of organisms, which often adds to the understanding of the geobiotic history of an area. Western North America has received a great deal of attention from biogeographers because the diverse geologic and climatic history of this region has led to numerous barriers to gene flow and high levels of diversity in some groups. Several studies have focused on uncovering the history of the hot deserts of North America (e.g., Epps et al., 1998; Orange et al., 1999; Marshall & Liebherr, 2000; Riddle et al., 2000a; Riddle et al., 2000b; Zink et al., 2001; Jaeger et al., 2005; Riddle & Hafner, 2006). Many others have focused on the diverse landscape of California’s Central Valley and surrounding Mediterranean areas (e.g., Moritz et al., 1992; Tan & Wake, 1995; Rodriguez-Robles et al., 1999; Shaffer et al., 2004).

Today, the Central Valley of California has been converted almost entirely to agricultural lands, but, in its pristine state, three main plant communities were found in the valley, namely freshwater marsh, riparian woodland, and the most common, dry savanna-grassland (Schoenherr, 1992; Ricketts et al., 1999). As one of California’s arid regions, the Central Valley, especially the southern end of the valley, shares many characteristics with the desert lands to the south and east (Schoenherr, 1992). These
similarities may be due, in part, to the geobiotic history of these areas. Several million years ago, the climate of California’s Mediterranean areas and deserts were nearly equivalent. Before a rain shadow was formed as California’s mountains uplifted, the desert areas resembled Mediterranean grasslands with patches of woodland and scattered playa lakes, similar to the climate of the coastal areas (Merriam, 1919; Tidwell & Nambudiri, 1989; Park & Downing, 2001). This historical similarity between California’s Mediterranean and desert areas may provide insights into the development of the diverse arid-adapted biota that exists in these areas.

Several arid-adapted taxa live in California’s Mediterranean areas, and some are endemic or near-endemic to these areas. For example, many species of rodents, reptiles, birds, insects, and plants are found only in the Central Valley (Ricketts et al., 1999). Many of these endemic taxa have close relatives inhabiting the nearby deserts. Recently, a phylogeographic analysis has shown the relationship between some of the arid-adapted taxa within the Central Valley and their relatives in the deserts. Mulcahy (2008), in a study of nightsnakes (Hypsiglena torquata), suggested that populations from the Central Valley and the coastal areas form a clade that is sister to a clade made up of Sonoran, Mojave and Great Basin desert populations.

Because no divergence dates have been proposed for these speciation events, the underlying causes, whether they were mountain uplift during the late Neogene or more recent climatic changes during the Pleistocene, have not been confirmed. Although the discussion continues questioning whether the majority of diversification in arid-adapted taxa was caused by Neogene mountain uplift or Pleistocene climate change, the growing body of evidence suggests that much of the diversification in desert-dwelling species was
driven by mountain uplift rather than Pleistocene climatic oscillations (e.g., Orange et al., 1999; Jaeger et al., 2005; Riddle & Hafner, 2006). It can be difficult, however, to determine if mountain uplift was the main factor in the diversification of western organisms due, in large part, to the wide range of dates given for these uplift events (Wilson & Pitts, 2010). The majority of accounts, both palaeobiological and geological, place the uplift of the western mountain ranges between 15 Ma and 2 Ma, so any divergence events linked to dates within this range could be associated with mountain uplift, and divergences occurring more recently than 2 Ma would likely be associated with the cooling and warming cycles present throughout the Pleistocene (Wilson & Pitts, 2010).

In an analysis of the species boundaries in the nocturnal velvet ant *Sphaerophalma unicolor* (Cresson) (Hymenoptera: Mutillidae), Wilson and Pitts (2009) found a similar relationship to those described by Mulcahy (2008). *Sphaerophalma unicolor* is largely restricted to the California’s Central Valley and coastal areas, while its sister species, *S. mendica* (Blake), is found in the Sonoran, Mojave and Great Basin deserts (Wilson & Pitts, 2009). Also, *S. mendica* has two distinct colour forms, a light-coloured form and a dark-coloured form, which seem to be geographically isolated (Wilson & Pitts, 2009). While the divergence date for *S. unicolor* and *S. mendica* is unknown, estimated divergence dates for related *Sphaerophalma* species suggest that the split between *S. unicolor* and *S. mendica* may have occurred in the Pleistocene rather than during Neogene mountain uplift (Pitts et al., 2010).

In addition to genetic tools, ecological niche models (ENMs) are increasingly being used to understand the evolutionary history of numerous taxa (e.g., Buckley et al.,
Historical biogeographical analyses can provide valuable information regarding the evolutionary history of an area, especially when vicariant events play a major role in diversification. However, when the driving forces behind diversification are more convoluted, comprising a mixture of extinction, migration, and vicariance, understanding the changes in a species’ distribution over time can add to the understanding of the history of diversification. ENMs use the recorded occurrence data for a species combined with bioclimatic variables for these areas to predict the possible distribution for the species and to estimate what climatic variables influence the distribution of a species (Peterson, 2001; Soberon & Peterson, 2005). Recently, multiple datasets have become available that model historical climates, so ENMs can be generated to predict the distribution of a species during the Last Glacial Maximum (LGM ~20,000 years BP: Braconnot et al., 2007) and during the last interglacial (LIG; ~120,000 - 140,000 years BP) (Otto-Bliesner et al., 2006). A combination of molecular data, estimated divergence dates, and ENMs based on Pleistocene climates can provide several lines of evidence regarding the historical biogeography of a region.

Here we investigate the relationships between populations of nocturnal velvet ants in the *S. unicolor* species-complex (*S. unicolor*, *S. mendica*, and *S. angulifera* Schuster) in order to gain an understanding of the events that led to the diversification of Mediterranean-adapted organisms and related desert-adapted organisms. We use phylogenetic techniques to determine relationships between species and among populations within each species. Molecular dating techniques, calibrated with fossils from Dominican Amber, are used to estimate divergence dates for speciation events as well as population level divergences. Also, we develop ENMs for *S. unicolor* and *S.
*mendica* using current climate data and Pleistocene climate reconstructions from the LGM and LIG to understand how historical ecological processes affected the distribution of each species, which is key to understanding the patterns of genetic diversification.

**MATERIALS AND METHODS**

**Study system**

Members of the *S. unicolor* species-complex (Fig. 4.1), like all velvet ants (Hymenoptera: Mutillidae), are solitary wasps that are parasitic on other wasps and bees as larvae or prepupae (Krombein, 1979), while feeding on nectar as adults (Wilson *et al.*, 2010). The long-distance dispersal abilities of the species in the *S. unicolor* species-complex are limited because as in all velvet ants, the females are wingless and these species do not copulate phoretically. Little is known about the natural history of most nocturnal velvet ant species, including those in the *S. unicolor* species-complex. No data are available for *S. angulifera* likely due to its rarity. Until recently, *S. unicolor* and *S. mendica* were considered the same species (Wilson & Pitts, 2009), so all available descriptions regarding natural history must be applied to both species. Both *S. unicolor* and *S. mendica* are generalist predators, parasitizing a wide variety of ground nesting bees and wasps (Ferguson, 1962). Based on the variety of habitats each of these species has been collected in, ranging from sand dunes to shrub-steppe grasslands to dry mountain meadows, both species seem to be generalists in terms of habitat requirements. Little is known about the activity patterns of these wasps. While *S. unicolor* and *S. mendica* are both wide-ranging species, they are not consistently collected throughout the year or during any particular part of the collecting season. This seeming stochastic nature
Figure 4.1. Female and Male specimens of the nocturnal velvet ants *S. unicolor* and *S. mendica*.

of their activity may be due to a combination of unknown abiotic and biotic factors. Regardless of the factors causing the apparent indiscriminate activity patterns of these species, these patterns make obtaining large numbers of fresh specimens for molecular analysis problematic.

**Taxon sampling**

Specimens from three species of the *S. unicolor* species-complex (*S. unicolor*, *S. mendica*, and *S. angulifera*) were collected from sites across western North America (Fig.
from 2002 to 2009 using black-light traps, fluorescent lantern traps, and by hand using forceps. All specimens were placed directly into 95% ethanol and those used for molecular examination have been labelled as voucher specimens and deposited in the Department of Biology Insect Collection, Utah State University, Logan, UT (EMUS).

**Molecular methods**

An attempt was made to sample from various parts of the range of each species (Fig. 4.2, Table 4.1). DNA extraction and amplification of the two rDNA internal transcribed spacer regions (ITS1 & ITS2) followed the protocols outlined by Pilgrim and Pitts (2006). Sequences were analyzed with an ABI Prism 377, 3100, or 3730 Genetic Analyzer. Gel electrophoresis of each gene yielded a single band for each individual wasp and the resulting DNA was sequenced cleanly, suggesting no gene heterogeneity as seen in some other organisms (e.g., Harris & Crandall, 2000; Parkin & Butlin, 2004; Bower *et al.*, 2008). PCR products were sequenced in both directions and sequence contigs assembled using Sequencher 4.0 (Gene Code Corp., Ann Arbor, MI). DNA sequences were aligned using Clustal W (Thompson *et al.*, 1994). ITS1 and ITS2 sequences will be deposited in GenBank upon publication in a peer-reviewed journal.

**Phylogenetic and haplotype network analysis**

The two genetic loci were subjected to Bayesian analysis using MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003). Sequences were analyzed as a combined data set, with each gene partitioned separately with all parameters unlinked across loci. Appropriate models of nucleotide substitution were determined in MrModeltest version 2.3 (Nylander,
Figure 4.2. Map of the North American deserts and arid lands showing the collection localities of *S. unicolor* species-complex specimens. Symbols with numbers correspond to Fig. 4.3 and Table 4.1. Squares represent *S. unicolor* specimens, circles represent *S. mendica* specimens with dark circles marking the localities of specimens with dark colouration and light circles representing those specimens with light colouration. Stars mark the collection localities of *S. angulifera*. Arid land boundaries are as described by Omernik (1987) with some modifications, specifically, the northern and southern Colorado Plateau have been combined.

2004). Bayesian analyses included four chains were set for 3,000,000 generations and sampled every 100 generations. Because independent runs with three heated chains and one cold chain in each run. The MCMC samples taken this close together might be autocorrelated, and therefore give a biased estimate of the posterior distribution, we also ran an analysis with the MCMC chains set for 50,000,000 generations and sampled every 1000 generations. A 10% burn-in was removed after graphical determination of
Table 4.1. Descriptive information for all taxa used in the phylogenetic portion of this study including voucher ID number, and collection locality.

<table>
<thead>
<tr>
<th>Species</th>
<th>Map ID (Fig. 4.2)</th>
<th>Voucher ID</th>
<th>Collection Location</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Dasymutilla heliophila</em></td>
<td>N/A</td>
<td>JP321</td>
<td>AZ: Cochise Co., 2 Mi S Willcox</td>
</tr>
<tr>
<td><em>Dasymutilla snoworum</em></td>
<td>N/A</td>
<td>JP443</td>
<td>TX: Hidalgo Co., Bensten- Rio Grande Valley State Park</td>
</tr>
<tr>
<td><em>Dasymutilla occidentalis</em></td>
<td>N/A</td>
<td>Moccf1</td>
<td>SC: Florence Co., Florence, Pee Dee Res. &amp; Edu. Center</td>
</tr>
<tr>
<td><em>Traumatomutilla sp.</em></td>
<td>N/A</td>
<td>JP621</td>
<td>Bolivia: Santa Cruz, 5km SSE Burna Vista</td>
</tr>
<tr>
<td><em>Laminatilla lamelliferia</em></td>
<td>N/A</td>
<td>JP280</td>
<td>AZ: Cochise Co., Indian Bread Rocks, Happy Camp Rd</td>
</tr>
<tr>
<td><em>Pseudomethoca contumax</em></td>
<td>N/A</td>
<td>JP233</td>
<td>USA: UT: Cache Co., Hyrum Reservoir</td>
</tr>
<tr>
<td><em>Odontophotopsis tenuiptera</em></td>
<td>N/A</td>
<td>JP569</td>
<td>AZ: Cochise Co., Paradise Rd, 3 mi W Portal</td>
</tr>
<tr>
<td><em>Sphaeropthalma pinalea</em></td>
<td>N/A</td>
<td>JP761</td>
<td>AZ: Cochise Co., Carr Canyon, Huachuca Mtns</td>
</tr>
<tr>
<td><em>S. triangularis</em></td>
<td>N/A</td>
<td>JP111</td>
<td>AZ: Cochise Co., San Pedro River Conservation Area</td>
</tr>
<tr>
<td><em>S. triangularis</em></td>
<td>N/A</td>
<td>JP108</td>
<td>AZ: Cochise Co., San Pedro River Conservation Area</td>
</tr>
<tr>
<td><em>S. angulifera</em></td>
<td>1</td>
<td>JP276</td>
<td>CA: San Bernardino Co., 5 mi S Barstow</td>
</tr>
<tr>
<td><em>S. angulifera</em></td>
<td>2</td>
<td>JW04</td>
<td>UT: Washington Co., West of Bloomington</td>
</tr>
<tr>
<td><em>S. angulifera</em></td>
<td>3</td>
<td>JP934</td>
<td>UT: Washington Co., Beaver Dam Slope</td>
</tr>
<tr>
<td><em>S. mendica</em></td>
<td>4</td>
<td>JP802</td>
<td>ID: Owyhee Co., nr Bruneau Dunes State Park</td>
</tr>
<tr>
<td><em>S. mendica</em></td>
<td>5</td>
<td>JP1073</td>
<td>NV: Pershing Co., Rye Patch Dam, 21 mi N Lovelock</td>
</tr>
<tr>
<td><em>S. mendica</em></td>
<td>6</td>
<td>JP626</td>
<td>NM: San Juan Co., 3 mi S Farmington</td>
</tr>
<tr>
<td><em>S. mendica</em></td>
<td>7</td>
<td>JP625</td>
<td>UT: San Juan Co., Valley of the gods</td>
</tr>
<tr>
<td><em>S. mendica</em></td>
<td>8</td>
<td>JW03</td>
<td>UT: Garfield Co., Alvey Wash, 5 km S Escalante</td>
</tr>
<tr>
<td><em>S. mendica</em></td>
<td>9</td>
<td>JP556</td>
<td>UT: Garfield Co., Alvey Wash, 5 km S Escalante</td>
</tr>
<tr>
<td><em>S. mendica</em></td>
<td>10</td>
<td>KW08</td>
<td>CA: Riverside Co., Corn Springs, 5 mi S Desert Center</td>
</tr>
<tr>
<td><em>S. mendica</em></td>
<td>11</td>
<td>JW12</td>
<td>UT: Garfield Co., Alvey Wash, 7 km S Escalante</td>
</tr>
<tr>
<td><em>S. mendica</em></td>
<td>12</td>
<td>JP345</td>
<td>UT: Garfield Co., Calf Creek, 10 km S Boulder</td>
</tr>
<tr>
<td><em>S. mendica</em></td>
<td>13</td>
<td>JP933</td>
<td>UT: Washington Co., Beaver Dam Slope</td>
</tr>
</tbody>
</table>
Species | Map ID (Fig. 4.2) | Voucher ID | Collection Location
--- | --- | --- | ---
*S. mendica* | 14 | JP555 | NV: Nye Co., Pahrump
*S. unicolor* | 15 | JW02 | NV: Washoe Co., Washoe Lake State Park
*S. unicolor* | 16 | JP97 | CA: Riverside Co., Bautista Canyon
*S. unicolor* | 17 | JP850 | OR: Lake Co., Bullard Canyon, 2 mi E lakeview
*S. unicolor* | 18 | JP712 | CA: Soloano Co., Suisun City, Rush ranch
*S. unicolor* | 19 | JP641 | CA: Soloano Co., Suisun City, Rush ranch
*S. unicolor* | 20 | JP558 | CA: Solano Co., Stebbins Cold Canyon Reservoir
*S. unicolor* | 21 | JP557 | CA: Kern Co., 10 mi W sw Mckittrick
*S. unicolor* | 22 | JP553 | CA: Yuba Co., Shad Pad
*S. unicolor* | 23 | JP102 | CA: Riverside Co., Bautista Canyon
*S. unicolor* | 24 | JP552 | CA: Los Angeles Co., San Dimas experimental forest
*S. unicolor* | 25 | JP100 | CA: Riverside Co., Bautista Canyon

Stationarity. Convergence and burn-in were assessed using Tracer v1.4.1 (Rambaut and Drummond, 2007). Several outgroups were included in the analysis. *Sphaeropthalma pinalea* Schuster and *S. triangularis* (Blake) are closely related to the *S. unicolor* species-complex (Schuster, 1958; Pitts et al., 2010) and were included. *Laminatilla lamellifera* Schuster and *Odontophotopsis tenuiptera* Schuster were used as more distant outgroups (Pitts et al., 2010).

In addition to the full phylogenetic analysis, a Bayesian analysis was implemented on a subset of *S. unicolor* species-complex samples in order to streamline the molecular dating process by focusing only on the major clades. The full dataset was pared down to include a single *S. unicolor* specimen, two *S. mendica* specimens (one representing the dark morph and one representing the light morph), and a single *S. angulifera* specimen. This analysis also included one specimen of each of the outgroup species including *D.*
snoworum Cockerell, *D. occidentalis* (Linnaeus), *D. gloriosa* (Saussure), a *Traumatomutilla* species, and *Pseudomethoca contumax*. These additional outgroups were added in order to include a fossil for calibration in the molecular dating analysis.

Parsimony-based haplotype networks were constructed using the combined ITS1 and ITS2 sequences for all specimens in the *S. unicolor* species-complex using TCS version 1.21 (Clement *et al.* 2000). The reconnection limit was set to 95% between haplotypes with gaps treated as missing data.

**Molecular dating analyses**

Divergence date estimates were calculated for major nodes on the tree using two methods: a penalized likelihood approach to rate smoothing using the program r8s 1.71 (Sanderson, 2002), and a Bayesian MCMC averaging approach to rate smoothing using the program BEAST v1.4.8 (Drummond & Rambaut, 2007). Because there are a limited number of fossils that can be used as calibration points in Mutillidae, two distinct dating methods were used as a way to corroborate the stability of the estimated divergence dates. While no fossils are available for *Sphaeropthalma* or any of the nocturnal velvet ants, two fossils from Dominican amber, *Dasymutilla dominica* and *D. albifasciatus* (Manley & Poinar, 1991; 1999; 2003) were used to calibrate the estimated divergence dates. The dating of Dominican amber remains controversial, with the youngest proposed age of 15-20 million years (Ma) based on Foraminifera (Iturralde-Vinent and MacPhee, 1996) and the oldest of 30-45 Ma based on coccoliths (Schlee, 1990). Because previous analyses that used velvet ant fossils in Dominican amber to estimate divergence dates imply that constraining the fossil to an age of 20 million years results in the most
reasonable divergence date estimates (Pitts et al., 2010), we used this date in both the r8s and the BEAST analyses. Based on the morphology of the available fossils, both species appear to be most closely related to the basal members of the genus Dasymutilla (K.A. Williams, unpublished data).

*r8s analysis*

The program r8s uses a tree description with branch lengths to estimate divergence dates. The consensus tree that resulted from the pared-down Bayesian analysis was used in the r8s analysis. The most recent common ancestor (MRCA) of the Dasymutilla plus Traumatomutilla clade was constrained to be at least 20 Ma (minage = 20) based on the placement of the fossils and the generally accepted age of Dominican amber (Iturralde-Vinet & MacPhee, 1996). The root was fixed at 65 million years based on the estimated maximum age of Mutillidae (Grimaldi & Engle, 2005), and the penalized-likelihood method with the truncated Newton algorithm was implemented to estimate rates and divergence dates. To estimate the effects phylogenetic uncertainty had on age estimations, 1000 trees from the stationary sample of Bayesian trees were independently dated using a PL analysis in r8s (Antonelli, 2009; Pitts et al., 2010). The mean age and the upper and lower 95% bounds were computed for each node using the software TreeAnnotator v.1.4.8 (Rambaut and Drummond, 2007).

*BEAST analysis*

The program BEAST uses the aligned sequence data to generate a tree and estimate divergence dates. The program BEAUtiv1.4.8 (Drummond & Rambaut, 2007) was used to generate the file used in BEAST with the alignment of the pared-down data
set. The MRCA of the *Dasymutilla* plus *Traumatomutilla* clade was constrained to be 20 million years old with a standard deviation of 5 million years to account for some of the variability within the range of reported ages for Dominican amber (Iturralde-Vinent and MacPhee, 1996). The root node was limited to a mean age of 65 million years with a standard deviation of 15 million years based on the estimated age of the family (Grimaldi & Engle, 2005). Because in the pared-down data set single terminals represent individual species, a Yule process speciation prior for branching rates was implemented and the general time-reversible model with invariant sites and gamma-distributed rate variation across sites (GTR+I+Γ) was applied with base frequencies estimated during the analysis. An uncorrelated lognormal model was applied to estimate the relaxed molecular clock because this model places higher prior-density closer to the observed fossil age (Leaché & Mulcahy, 2007). The analysis was run using the default MCMC parameters with the MCMC chains being set for 10,000,000 generations and sampled every 1000 generations. Convergence and burn-in were assessed using Tracer v1.4.1 (Rambaut and Drummond, 2007).

**Ecological niche models**

Ecological niche models were developed for *S. unicolor* and *S. mendica* in order to gain insights into the genetic diversity among populations of each of these species. For the ENMs, we used occurrence data based on the examination of over 1,000 specimens from 12 entomological museums from across the U.S. (see Wilson & Pitts, 2009). Several specimens did not have geographic coordinates associated with them. For these localities, coordinates were estimated using the collection description from the label and
the software Google Earth 5.0 (http://earth.google.com). Sixty-six localities were used to generate the ENM for *S. unicolor*, and 54 localities were used in the ENM for *S. mendica*.

Models were first developed for each species based on current climatic conditions, and then these models were projected onto LGM climate surfaces and LIG climate surfaces. Current climate data consisted of 19 bioclimatic variables from the Worldclim data set (version 1.4), which were the product of global land area interpolation of climate point data from 1950-2000 at a spatial resolution of 30 arc-seconds (available at http://www.worldclim.org). LGM climate data were based on models developed through the Paleoclimate Modelling Intercomparison Project (PMIP2) at a resolution of 2.5 arcmin (Braconnot *et al*., 2007; available at http://www.worldclim.org). LIG climate data were based on models from Otto-Bliesner *et al.* (2008) at a resolution of 30 arc-seconds (available at http://www.worldclim.org). All climate variables were clipped to western North America using ArcGIS 9.2 (ESRI, Inc., Redlands, California).

Ecological niche models were generated using Maxent 3.3.1 (http://www.cs.princeton.edu/~schapire/maxent/), with the default parameters following Phillips *et al.* (2006). The predictive ability of the model based on current climate data was evaluated using the area under the receiver operating characteristic curve (AUC) with 80% of the data used in training and 20% retained as test points.
RESULTS

Molecular and phylogenetic results

The final alignments encompassed 506 base pairs for ITS1 and 949 base pairs for ITS2. While some insertions and deletion polymorphisms (indels) were present in the alignment, both intergenic regions aligned cleanly. The best-fit nucleotide substitution model selected for each gene was the general time-reversible model with invariant sites (GTR+I). Both Bayesian analyses of the combined dataset, the one that was run for 3,000,000 generations and the one that was run for 50,000,000 generations, resulted in consensus trees with identical topologies and posterior probabilities. Posterior probabilities were high for most of the nodes (Fig. 4.3). The Bayesian tree shows the three *Sphaeropthalma* species forming distinct, well-supported clades. Within the *S. unicolor* clade, there is little resolution among individual populations (Fig. 4.3). Within the *S. mendica* clade, however, there is some genetic structuring of sub-clades that seems to be associated with desert/ecosystem type (Figs. 4.2, 4.3). These sub-clades also appear to be associated, in part, with the two colour forms of *S. mendica* (Figs. 4.2, 4.3). While only three populations of *S. angulifera* were sampled, the analysis revealed a deep split between the population from Barstow and the populations from southwestern Utah (Figs. 4.2, 4.3). The analysis on the pared-down data set for the molecular dating analysis resulted in a tree depicting the same relationships among ingroup taxa as the consensus tree from the full analysis (Fig. 4.4).
Figure 4.3. Consensus tree of the Bayesian analysis using the combined ITS1 and ITS2 sequences. Numbers above each node represent posterior probabilities. Symbols following the species names correspond to symbols on the map of North American deserts and arid lands (Fig. 4.2). The numbers within each symbol correspond to the “map location” in Table 4.1, which gives the collection location of each specimen.
Figure 4.4. Chronogram of the paired-down data set. Black circles represent the calibration points that were used in the molecular dating analyses. Numbers at each node are mean estimated divergence dates presented as r8s/BEAST. Posterior probabilities are given at the right of each node; posterior probabilities of 1.0 are represented with asterisks. The 95% credibility of estimated divergence dates are represented with grey bars at each node. Estimates from r8s are represented with a dark bar and estimates from BEAST are represented with a light bar.

Haplotype network analysis

Haplotype network analysis resulted in three unique haplotypes among *S. unicolor* populations, 10 unique haplotypes among *S. mendica* populations and three haplotypes in the three *S. angulifera* populations sampled. Haplotype networks of *S. unicolor* and *S. mendica* are shown overlaid on a map of the deserts of western North America (Fig. 4.5). The three *S. angulifera* populations did not form a network due to
the large genetic distance between individuals, so *S. angulifera* is not included in Figure 4.5. The network of *S. mendica* haplotypes shows a grouping of light-coloured populations and a grouping of dark-coloured populations (Fig. 4.5).

**Molecular dating**

Both molecular dating analyses resulted in similar divergence date estimates (Fig. 4.4). Both analyses suggested the divergences separating *S. angulifera* from the other
two species occurred in the Pliocene (r8s 2.3 Ma, 95% credibility 1.7-3.4 Ma; BEAST 2.8 Ma, 95% credibility 2.5-10.7 Ma). The divergence between *S. unicolor* and *S. mendica*, however, was associated with dates in the early Pleistocene (r8s 1.7 Ma, 95% credibility 1.1-2.6 Ma; BEAST 1.7 Ma, 95% credibility 1.4-7.8 Ma). The separation of major lineages in *S. mendica*, particularly the separation of the light and dark colour forms was associated with the middle Pleistocene (r8s 0.7 Ma, 95% credibility 0.2-1.3 Ma; BEAST 1.0 Ma, 95% credibility 0.4-3.4 Ma).

**Ecological niche modelling**

Ecological Niche Models for each species were better than random (*S. unicolor*: training AUC = 0.993; test AUC = 0.989; *S. mendica*: training AUC = 0.979; test AUC = 0.935). The relative contributions of the 19 bioclimatic variables to the ENM differed for each species. For *S. unicolor*, the top three bioclimatic variables contributing to the ENM were precipitation of warmest quarter (82.2 %), precipitation of the coldest quarter (8.0 %), and precipitation seasonality (4.8 %). The top three bioclimatic variables contributing to the ENM of *S. mendica* were precipitation of wettest month (66.3 %), mean temperature of coldest quarter (11.1 %), and precipitation of driest month (5.3 %).

The ENM predictions for *S. unicolor* show a predicted distribution throughout the Central Valley of California and the coastal areas, with some suitable habitat in isolated mountain valleys including some areas east of the Sierra Nevadas (Fig. 4.6). The ENM predictions for *S. mendica* show a wide range of suitable habitats from valleys in the southern Great Basin Desert, the Colorado Plateau, into the Mojave and western Sonoran deserts (Fig. 4.6).
Figure 4.6. Ecological niche models for each species in the *S. unicolor* species-complex based on current climatic conditions in the first row, Last Glacial Maximum (~21,000 years BP) climatic conditions in the second row, and the Last Interglacial (~120,000-140,000 years BP) in the last row. The areas shaded blue represent the most suitable habitat for each species with the darkest blue being the most suitable.

The projection of these models onto Pleistocene climate surfaces suggested each species experienced dramatic range contractions during the glacial cycles (Fig. 4.6). While the desert-adapted species, *S. mendica*, experienced range expansions during the
interglacials, the Mediterranean-adapted species, *S. unicolor*, was forced into more isolated refugia in the interglacials than during the glacial cycles, with predicted suitable habitat only occurring around the San Francisco Bay and portions of the western Sacramento Valley. Because these models are based on 30 arc-second climate grids (~1 km), it is possible that additional microrefugia existed during the interglacial cycles but remain undetected because of the coarse resolution of the available climate surfaces.

Because fossil evidence indicates earlier glacial and interglacial cycles caused similar environmental changes to those experienced during the most recent cycles (e.g., Litwin *et al.*, 1999; Woolfenden, 2003), in the following discussion we will assume that the range expansions and contractions predicted in the ENMs occurred in a similar fashion in the earlier Pleistocene cycles.

**DISCUSSION**

**Diversification driven by Neogene uplift**

Much of western North America resembled a dry Mediterranean-like savanna before the late Neogene mountain-building events (Webb, 1977; Wilson & Pitts, 2010). As the mountains rose, many of the Nearctic deserts formed in the growing rain shadow (Wilson & Pitts, 2010), leaving the areas on the windward side of the mountains a dry, Mediterranean-like environment (Minnich, 2007). The drying out of the deserts during this period seems to have been influential in the diversification of many arid-adapted groups (e.g., velvet ants: Pitts *et al.*, 2010; rattle snakes: Douglas *et al.*, 2006; spiny lizards: Leaché & Mulcahy, 2007). Because the historical Mediterranean-like climate of western North America was similar to the climate parts of California have maintained for
millions of years (Minnich, 2007), the Mediterranean parts of California may represent a refugium from the desertification experienced in the late Neogene. Thus, in groups with desert-adapted species sister to Mediterranean-adapted species, the major divergence events could likely be associated with mountain building and desert formation events that occurred between 15-2 Ma (Wilson & Pitts, 2010). Presumably as the California mountains uplifted, many taxa would have been vicariantly isolated on either side of the rising mountains, with the populations on the windward side remaining in a Mediterranean climate and the populations on the leeward side evolving in the newly formed arid climate.

While our analysis shows that not all of the divergence events in the *S. unicolor* species-complex were associated with Neogene mountain building, these events were influential in the split of *S. angulifera* from the ancestor of *S. unicolor* and *S. mendica*. Late Neogene uplift could have separated populations of the ancestor of the *S. unicolor* species-complex, leaving some populations in the developing deserts where strong selection pressure would cause the evolution of a more arid-adapted species, *S. angulifera*. This supposition is supported by the phylogenetic placement of *S. angulifera*, the estimated divergence dates (Figs. 4.3, 4.4), and the fact that *S. angulifera* is found only in the Mojave and western Sonoran deserts (Wilson & Pitts, 2009).

Because of its rarity, only three populations of *S. angulifera* were included in the analysis. More extensive sampling of this species from across its range would provide data for future phylogeographic analyses may reveal that the deep divergences between the populations we sampled actually represent species-level differences.
Diversification driven by Pleistocene climatic cycling

While the divergence of *S. angulifera*, and many other velvet ant species (Pitts *et al.*, 2010) from their sister lineages is associated with Neogene uplift, our analysis of the *S. unicolor* species-complex suggests that Pleistocene influences were responsible for the pattern of sister species showing isolation in desert and Mediterranean regions. Although the estimated divergence dates place this speciation event in the early Pleistocene, the confidence intervals show that this divergence could have occurred anywhere from 1.1-7.8 million years ago (Fig. 4.4). Because previous analyses involving this species-group have suggested Pleistocene-age diversification (Pitts *et al.*, 2010), and because of the morphological similarity and close genetic distance between *S. unicolor* and *S. mendica*, we feel that the estimation of an early Pleistocene origin of these species is accurate.

A Pleistocene divergence between Mediterranean-adapted species and arid-adapted species is not necessarily a novel suggestion. Lee *et al.* (2003) proposed that the split between North America’s two magpie species (*Pica hudsonia* and *P. nuttalli*), one of which is restricted to the Mediterranean parts of California while the other is widespread throughout the west, occurred in the middle Pleistocene. This divergence is not entirely comparable to many of the other relationships showing a Mediterranean and desert relationship, because the ancestor of these magpie species only arrived in North America from Asia during the Pleistocene (Lee *et al.*, 2003), long after the climatic connection between the deserts and the coastal areas existed.

The early Pleistocene split between the Mediterranean-adapted *S. unicolor* and the arid-adapted *S. mendica* was likely primarily driven by climatic changes and subsequent
range contractions and expansions. We propose a scenario in which the ancestor of *S. unicolor* and *S. mendica* was a Mediterranean-adapted species inhabiting the areas on the windward side of the mountains following its split with *S. angulifera*, which was caused by the late Neogene uplift. This ancestor could have been widespread throughout the Mediterranean areas of the Central Valley and the coastal areas in California. The onset of global cooling that began in the Pleistocene likely caused range contractions for the Mediterranean-adapted ancestral species. Fossil evidence points to the existence of several glacial refugia for Mediterranean-adapted species along the coast and in parts of the Central Valley (Johnson, 1977; Coltrain *et al.*, 2004). In the early Pleistocene, while the suitable habitat for Mediterranean-adapted taxa was being restricted into isolated refugia, intense selection pressure was placed on those populations inhabiting the portions of the range that were cooling, possibly driving the evolution of a more cold-tolerant species (*S. mendica*). Those populations that were able to survive in the glacial refugia as Mediterranean-adapted species became *S. unicolor*. Although this hypothesis is not directly testable with our data, the opposite scenario, with the ancestor of *S. unicolor* and *S. mendica* being desert-adapted and *S. unicolor* evolving into a Mediterranean-adapted species from a desert-adapted ancestor is unlikely given the palaeoecological data.

During the early Pleistocene glacial cycles, the newly evolved, cold tolerant *S. mendica* could have migrated along the foothills of the Central Valley over the transverse range at the south end of the valley into the ice-age deserts. Fossil evidence from the southern Sierra Nevada foothills indicates that a more xeric plant community, adapted to cooler temperatures, was present in areas that today house Oak-chaparral plant
assemblages (Cole, 1983). Many features of the glacial-age western Sierra Nevada biota resembled the modern-day eastern slopes of the Sierras (Cole, 1983), which provide suitable habitat for *S. mendica* (Fig. 4.6). The ENM of *S. mendica* implies that several desert areas to the south and east of the Mediterranean parts of California could have been refugia during the glacial cycles (Fig. 4.6). Fossil evidence supports the idea that *S. mendica* could have thrived in the glacial-age Mojave Desert because the area housed widespread xeric woodlands (Koehler *et al.*, 2005), similar to those found in the Great Basin and Colorado Plateau today where *S. mendica* is widespread.

While *S. unicolor* and *S. mendica* both have relatively large ranges spanning a variety of microhabitats, there is a big difference in the amount of intraspecific genetic variation found in each of these species. The desert-adapted species *S. mendica* is genetically diverse, with 10 unique haplotypes forming several distinct clades (Fig. 4.3). *Sphaeropthalma unicolor*, on the other hand, exhibited much less genetic diversity, with only three distinct haplotypes and little phylogenetic structuring (Fig. 4.3). This difference in genetic diversity between these two closely related species is likely due to the availability of Pleistocene refugia. The number of Pleistocene refugia utilized by each species can be estimated using phylogenetic patterns along with the ENMs.

Although the ENM of *S. mendica* suggests that the most suitable habitat during the LGM was in the mountains of northern Mexico, the divergence of major *S. mendica* clades dating to approximately 1 Ma suggests that this species was isolated into at least three different glacial refugia. Possible locations of these glacial refugia are revealed in the ENM of *S. mendica* during the LGM (Fig. 4.6). Suitable habitat existed during the glacial cycles in the western Sonoran Desert, the low valleys of the Northern Mojave
Desert, and the low elevations in the northern Colorado Plateau in the Uintah Basin (Fig. 4.6). While suitable habitat is also predicted in the Lahontan Basin in northwestern Nevada, we do not consider this area a likely refugium because a freshwater lake covered this area during the LGM (Wilson & Pitts, 2010). Major *S. mendica* lineages, regardless of the lack of resolution among these lineages, appear to be correlated to these glacial refugia.

*Sphaerophalma unicolor* also experienced drastic range contractions during the Pleistocene glacial cycles. While the ENM for this species suggests several areas along the coast and in the western side of the Central Valley maintained suitable conditions throughout the glacial cycles, there is little phylogeographic structuring among populations, which would be expected from isolation in separate refugia (Figs. 4.3, 4.5). While this lack of phylogeographic structure could be a result of limited sampling, we propose that it is due to dramatic range contraction during the Pleistocene and recent radiation from a limited number of Pleistocene refugia. The ENM of *S. unicolor* insinuates that its range greatly contracted during the LIG, with only limited areas around the San Francisco Bay and portions of the western Sacramento Valley having probabilities of suitable conditions over 70%. Although the ENM does not indicate that suitable habitat existed along the coast, because these models do not take into account maritime effects, we suggest that suitable Mediterranean-like habitats likely also existed in some areas along the California coast. Additionally, undetected microrefugia may have existed elsewhere in the Central Valley and Coastal mountains, which could have provided habitat for Mediterranean adapted species during the Pleistocene. The lack of genetic variation among *S. unicolor* populations, however, suggests that microrefugia
likely did not support isolated populations during this time. The predicted range contractions *S. unicolor* experienced in the glacial and interglacial cycles, as well as the lack of genetic variation among distant populations, indicate that Pleistocene climatic cycles restricted the suitable Mediterranean habitat to a limited number of refugia, likely along the coast, which resulted in a genetic bottleneck prior to Holocene range expansion.

**Conclusions**

Our analysis of the *S. unicolor* species-complex suggests that the diversification of arid-adapted Mediterranean species and desert-adapted species is likely a result of Pleistocene climatic fluctuations rather than Neogene mountain uplift. These fluctuations drove the evolution of cold-tolerant and arid-adapted species and allowed dispersal to the surrounding deserts. While this model of evolution seems to fit the species in the *S. unicolor* species-complex, much more work should be done on the other Mediterranean-adapted taxa to determine if similarities exist in the diversification of these other species. Although the effect that Pleistocene climatic cycles had on population-level diversification is well established, their effect on species-level divergences is debatable (Klicka & Zink, 1997). The diversification of the *S. unicolor* species-complex provides strong evidence that the climatic cycling experienced throughout the Pleistocene did, in fact, drive species-level diversification in at least some taxa.

**REFERENCES**


CHAPTER 5

PHYLOGEOGRAPHY AND ECOLOGICAL NICHE MODELS OF

SPHAEROPTHALMA AROTA (HYMENOPTERA: MUTILLIDAE) REVEAL A
CRYPTIC SPECIES-COMPLEX AND UNIQUE BIOGEOGRAPHIC HISTORY

1. Introduction

Increasing availability of DNA sequences over the past 20 years has led to an exponential increase in studies documenting cryptic species, which are groups of closely related species that are morphologically indistinguishable (Bickford et al., 2007). In addition to providing important information that can be helpful to conservationists (e.g., Bowen et al., 1993; Ravaoarimanana et al., 2004), uncovering cryptic species can advance our understanding of the processes that led to diversification. While cryptic species have been described in a multitude of organisms, new species complexes are expected to exist even in some well-studied groups, particularly among arthropods (Bickford et al., 2007). Several of the cryptic arthropod species that have been discovered were the result of phylogenetic analyses of widespread species (e.g., Hendrixson and Bond, 2005; Williams et al., 2006; Kuhlmann et al., 2007). Furthermore, phylogenetic analyses of widespread taxa are useful for investigating the historical biogeography of North America’s arid lands (Wilson and Pitts, 2008).

Among North America’s diverse arid-adapted arthropods, velvet ants (Hymenoptera: Mutillidae) are ideal candidates for historical biogeographic

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3 This chapter has been submitted for publication in Molecular Phylogenetics and Evolution and was coauthored by J.P. Pitts, S. L. Clark and K. A. Williams. Permission has been granted by the required coauthors for this research to be included in my dissertation (Appendix B).
investigations (Wilson and Pitts, 2008; Pitts et al., 2010). Additionally, several velvet ant species are widespread and may be composed of multiple cryptic species. While various molecular analyses have been performed with North American velvet ants, no cryptic species-complexes have been discovered. In fact, phylogenetic analyses of the brightly colored diurnal genus *Dasymutilla*, revealed gross over-splitting rather than the existence of cryptic species (e.g., Pilgrim et al., 2008, 2009). In one instance 17 named species were found to be a single species (Pilgrim et al., 2009). The over-splitting of this genus is likely due to highly variable color forms that are present in a single species (Pilgrim et al., 2009). Unlike the colorful diurnal velvet ants, the species-rich nocturnal genera have not received as much taxonomic attention. Studies investigating some widespread nocturnal velvet ant genera did result in the discovery of previously unrecognized species (e.g., Wilson and Pitts, 2008, 2009; Pitts et al., 2009). These are not cryptic, however, and can be diagnosed solely using morphological differences.

As the body of work describing the driving forces behind velvet ant diversification in the Nearctic deserts grows, a common pattern is beginning to form. The majority of species-level diversification events are linked to late Neogene mountain building (Wilson and Pitts, 2008; Pitts et al., 2010; Wilson and Pitts 2010a). Several analyses of arid-adapted species have found a geographic pattern with sister species being separated into eastern and western deserts. For example, many studies have found a pattern with one species being restricted to the Chihuahuan Desert and its sister species being restricted to the Sonoran and Mojave deserts (e.g., Morafka, 1977; Jaeger et al., 2005; Devitt, 2006; Douglas et al., 2006; Pitts et al., 2010; Wilson and Pitts 2010a). Additional investigations into patterns of diversification in widespread species will add to
our understanding of the driving forces behind the development of a diverse arid lands biota.

*Sphaeropthalma arota* is a widespread nocturnal velvet ant ranging from Texas to California. Because phylogenetic analyses of other velvet ant species with similar ranges uncovered the existence of previously unrecognized species (Wilson and Pitts, 2008), analysis of *S. arota* may also uncover the existence of currently unrecognized taxa. Additionally, *S. arota* has had six different names in the past, based largely on color variation and differences in the tuberculate condition of the posterior margin of the clypeus. Ferguson (1967) synonymized five species known only from male specimens into *S. helicaon* after a study of the type specimens. Recently, the males of *S. helicaon* were associated with the taxon known from female specimens only, *S. arota* (Pitts et al., 2009). Because *S. arota* is found in all of North America’s hot deserts, as well as many neighboring arid regions, phylogeographic analysis of this species can provide insights into the development of the desert biota. Because of wide distribution and morphological variation, closer examination of this species, including tests of species limits, is warranted (e.g., Wilson and Pitts, 2008).

Here we report molecular evidence that suggests *S. arota* is actually multiple species. We compare the historical biogeographic patterns of divergence among these species to patterns in other desert-adapted organisms. We develop ecological niche models (ENMs) for each species to determine their potential distribution so possible barriers to gene flow can be identified. Molecular dating techniques calibrated with fossils from Dominican amber are used to estimate dates for major diversification events so these events can be linked to past geologic and climatic events. Also, we investigate
the morphological characters that were historically associated with the synonyms of
*S. arota* to determine if they can be used to differentiate the species discovered through molecular analyses.

2. Materials and Methods

2.1 Taxon sampling

*Sphaeropthalma arota* specimens were collected from sites across western North America (Fig. 5.1, Table 5.1) from 2002 to 2009 using black-light traps, fluorescent

**Figure 5.1.** Map of western North America showing the collection localities of *S. arota* specimens. Numbers correspond to Fig. 2 and localities of sampled populations listed in Table 5.1.
Table 5.1. Descriptive information for all taxa used in the phylogenetic portion of this study.

<table>
<thead>
<tr>
<th>Species</th>
<th>Map ID (Fig. 5.2)</th>
<th>Voucher ID</th>
<th>Lineage</th>
<th>Collection Location</th>
</tr>
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<tr>
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<td>JP321</td>
<td>N/A</td>
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<td>JP443</td>
<td>N/A</td>
<td>TX: Hidalgo Co., Bensten- Rio Grande Valley State Park</td>
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<td>N/A</td>
<td>Moccfl</td>
<td>N/A</td>
<td>SC: Florence Co., Florence, Pee Dee Res. &amp; Edu. Center</td>
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<td>JP621</td>
<td>N/A</td>
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<td>Lineage</td>
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<td>Sphaeropthalma arota</td>
<td>48</td>
<td>JP1215</td>
<td>C</td>
<td>NM: Otero Co., 5 mi E La Luz</td>
</tr>
<tr>
<td>Sphaeropthalma arota</td>
<td>49</td>
<td>JP1213</td>
<td>C</td>
<td>NM: Luna Co., Rock Hound SP</td>
</tr>
<tr>
<td>Sphaeropthalma arota</td>
<td>50</td>
<td>Jp971</td>
<td>C</td>
<td>NM: Hidalgo Co., Granite Gap 9.5 mi S Steins</td>
</tr>
<tr>
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<td>51</td>
<td>JP518</td>
<td>C</td>
<td>NM: Hidalgo Co., Granite Gap 9.5 mi S Steins</td>
</tr>
<tr>
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<td>52</td>
<td>JP948</td>
<td>C</td>
<td>TX: Brewster Co., Big Bend Ranch State Park</td>
</tr>
<tr>
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<td>53</td>
<td>Jp947</td>
<td>C</td>
<td>TX: Brewster Co., Big Bend Ranch State Park</td>
</tr>
<tr>
<td>Sphaeropthalma arota</td>
<td>54</td>
<td>JP1202</td>
<td>D</td>
<td>CA: Kern Co., 22 mi S Maricopa</td>
</tr>
<tr>
<td>Sphaeropthalma arota</td>
<td>55</td>
<td>JP961</td>
<td>D</td>
<td>CA: San Diego Co., 4 mi E Campo</td>
</tr>
<tr>
<td>Sphaeropthalma arota</td>
<td>56</td>
<td>JP1065</td>
<td>D</td>
<td>CA: San Diego Co., 1 km W Boulivard</td>
</tr>
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</table>
lantern traps, and by hand using forceps. An attempt was made to sample from various parts of the range of *S. arota*. Due to the difficulty in obtaining specimens from Mexico, however, few samples were obtained from this area. All specimens were placed directly into 95% ethanol; those used for molecular examination have been labeled as voucher specimens and deposited in the Department of Biology Insect Collection, Utah State University, Logan, UT (EMUS).

2.2 Molecular methods

DNA extraction and amplification of the two rDNA internal transcribed spacer regions (ITS1 & ITS2) followed the protocols outlined by Pilgrim and Pitts (2006). Sequences were analyzed with an ABI Prism 3730 Genetic Analyzer. Gel electrophoresis of each gene yielded a single band for each individual wasp and the resulting DNA was sequenced cleanly, suggesting no gene heterogeneity as seen in some other organisms (e.g., Harris and Crandall, 2000; Parkin and Butlin, 2004; Bower et al., 2008). PCR products were sequenced in both directions and sequence contigs were assembled using Sequencher 4.0 (Gene Code Corp., Ann Arbor, MI). DNA sequences were aligned using
Clustal W (Thompson et al., 1994) and alignments were visually inspected and corrected in MacClade 4.07 (Maddison and Maddison, 2005). ITS1 and ITS2 sequences will be deposited in GenBank upon publication in a peer-reviewed journal. Genetic distances between major clades were calculated as pairwise percentages by determining the number of differences (point mutations and insertions or deletions) divided by the number of base pairs of the longer of the two sequences.

2.3 Phylogenetic analysis

The two genetic loci were subjected to Bayesian analysis using MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003). Sequences were analyzed as a combined data set, with each gene partitioned separately with all parameters unlinked across loci. Appropriate models of nucleotide substitution were determined in MrModeltest version 2.3 (Nylander, 2004). Bayesian analyses included four independent runs with three heated chains and one cold chain in each run. The MCMC chains were set for 3,000,000 generations and sampled every 100 generations; chains were run until the average standard deviation of the split frequencies dropped below 0.01. The burn-in period for each analysis was removed after graphical determination of stationarity. Convergence and burn-in were assessed using Tracer v1.4.1 (Rambaut and Drummond, 2007). Several outgroups were included in the analysis, including *Sphaeropthalma coaequalis* and *S. marpesia*, which are closely related to *S. arota* (Pitts et al., 2010). In addition to the full phylogenetic analysis, a Bayesian analysis was implemented on a subset of *S. arota* samples in order to streamline the molecular dating process by focusing only on the major clades. Multiple populations from each major clade were included in this analysis.
along with the same outgroups as were used in the full analysis. Also, multiple
Dasymutilla species were added including D. snoworum, D. occidentalis, D. heliophila,
and a Traumatomutilla species. These additional outgroups were added in order to
include a fossil calibration point in the molecular dating analysis.

2.4 Molecular dating analyses

Divergence date estimates were calculated for major nodes on the tree using two
methods: a penalized likelihood approach to rate smoothing using the program r8s 1.71
(Sanderson, 2002), and a Bayesian MCMC averaging approach to rate smoothing using
the program BEAST v1.4.8 (Drummond and Rambaut, 2007). Because there is a
disparity of fossils in the family Mutillidae to be used as calibration points, two distinct
dating methods were used as a way to corroborate the divergence dates. While no fossils
are available for Sphaeropthalma or any of the nocturnal velvet ants, two fossils from
Dominican amber, Dasymutilla dominica and D. albifasciatus (Manley and Poinar, 1991,
1999, 2003) were used to calibrate the estimated divergence dates. Based on the
morphology of these fossils, they appear to be most closely related to the basal members
of the genus Dasymutilla (Pitts et al., 2010).

2.5 r8s analysis

The program r8s uses a tree description with branch lengths to estimate
divergence dates. The consensus tree that resulted from the pared-down Bayesian
analysis was used in the r8s analysis. The most recent common ancestor (MRCA) of the
Dasymutilla plus Traumatomutilla clade was constrained to be at least 20 Ma (minage =
20) based on the placement of the fossils and the reported age of Dominican amber
(Iturralde-Vinet and MacPhee, 1996). The root was fixed at 65 million years based on
the estimated maximum age of Mutillidae (Grimaldi and Engle, 2005), and the penalized-
likelihood method with the truncated Newton algorithm was implemented to estimate
rates and divergence dates.

2.6 BEAST analysis

The program BEAST uses the aligned sequence data to generate a tree and
estimate divergence dates. The program BEAUtiv1.4.8 (Drummond and Rambaut, 2007)
was used to generate the file used in BEAST with the alignment of the pared-down data
set. The MRCA of the Dasymutilla plus Traumatomutilla clade was constrained to be
~20 Ma by giving this node a normally distributed prior with a mean age of 20 million
years and a standard deviation of 5.0. The root node was limited to a mean age of 65
million years with a standard deviation of 15 million years based on the estimated age of
the family (Grimaldi and Engle, 2005). A Yule process speciation prior for branching
rates was implemented and the general time-reversible model with invariant sites and
gamma-distributed rate variation across sites (GTR+I+Γ) was applied with base
frequencies estimated during the analysis. An uncorrelated lognormal model was applied
to estimate the relaxed molecular clock because this model places higher prior-density
closer to the observed fossil age (Leaché and Mulcahy, 2007). The analysis was run using
the default MCMC parameters with the MCMC chains being set for 10,000,000
generations and sampled every 1000 generations. Convergence and burn-in were assessed
using Tracer v1.4.1 (Rambaut and Drummond, 2007).
2.7 Ecological niche models

Ecological niche models were developed for each major *S. arota* clade. For the ENMs, we used occurrence data based only on the specimens that were included in the phylogenetic analysis. Several specimens did not have geographic coordinates associated with them. For these localities, coordinates were estimated using the collection description from the label and the software Google Earth 5.0 (http://earth.google.com).

Models were developed using current climatic conditions based on 19 bioclimatic variables from the Worldclim data set (version 1.4), which were the product of global land area interpolation of climate point data from 1950-2000 at a spatial resolution of 30 arc-seconds (Hijmans et al., 2005; available at http://www.worldclim.org). These included BIO1 = annual mean temperature; BIO2 = mean diurnal range in temperature; BIO3 = isothermality; BIO4 = temperature seasonality; BIO5 = maximum temperature of the warmest month; BIO6 = minimum temperature of the coldest month; BIO7 = temperature annual range; BIO8 = mean temperature of the wettest quarter of the year; BIO9 = mean temperature of the driest quarter of the year; BIO10 = mean temperature of the warmest quarter of the year; BIO11 = mean temperature of the coldest quarter of the year; BIO12 = annual precipitation; BIO13 = precipitation of the wettest month; BIO14 = precipitation of the driest month; BIO15 = precipitation of seasonality (coefficient of variation); BIO16 = precipitation of the wettest quarter of the year; BIO17 = precipitation of the driest quarter of the year; BIO18 = precipitation of warmest quarter of the year; BIO19 = precipitation of coldest quarter of the year. All climate variables were clipped to North America using ArcGIS 9.2 (ESRI, Inc., Redlands, California). Ecological niche models were generated using Maxent 3.3.1
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In order to determine if the major lineages of *S. arota* inhabit significantly different environmental niches we performed an analysis of similarity test (ANOSIM) using the program Community Analysis Package (CAP: version 4.1.3.384, Pisces Conservation Ltd., Lymington, UK), which compares the niches of the four major lineages (A-D) based on the 19 bioclimatic variables. Also, we compared the values of four bioclimatic variables between sister lineages to understand the specific ecological constraints that are important to determining the range of specimens from each major lineage. Because our sample sizes of each lineage are small, we used a non-parametric test, the Mann-Whitney U test, to determine if each distinct lineage inhabited a different niche. The variables used in these tests were BIO5 (maximum temperature of the warmest month), BIO6 (minimum temperature of the coldest month), BIO18 (precipitation of warmest quarter of the year), and BIO19 (precipitation of coldest quarter of the year) because these variables differ between distinct desert regions and have been used to define boundaries of specific deserts (MacMahon and Wagner, 1985).

3. Results

3.1 Molecular and phylogenetic analyses

The final alignments encompassed 518 base pairs for ITS1 and 967 base pairs for ITS2. The best-fit nucleotide substitution model selected for each gene was the general time-reversible model with invariant sites and gamma-distributed rate variation across
Figure 5.2. Phylogenetic relationships of *Sphaerophalma arota* based on the 95% majority rules consensus tree of the Bayesian analysis using the combined ITS1 and ITS2 sequences. Numbers and asterisks above each node represent posterior probabilities with asterisks representing probabilities of 1.0. Numbers on each *S. arota* terminal correspond to collection localities (Fig. 5.1, Table 5.1). Letters marking each major clade (A-D) distinguish the four cryptic species in the *S. arota* species-complex.
sites (GTR+I+Γ). Bayesian analysis of the combined molecular data produced a well-supported phylogeny (Fig. 5.2). This tree clearly shows that \textit{S. arota} can be split into four distinct, deeply divergent lineages (Fig. 5.2: A-D). Lineage A is composed of specimens from the western Sonoran Desert of southern California. Lineage B is made up of specimens from southeastern Arizona and Sonora, Mexico. Lineage C is composed of specimens from Texas to California. Lineage D is made up of specimens from various localities in southern California. Although no morphological characters were found that could be used to separate these lineages, genetic distances were high between each lineage (Table 5.2). Intralineage genetic distances are low within lineage A-C (0-0.65%). Genetic distances are higher within lineage D (0.7-1.3%). Although the four lineages are genetically distinct, analysis of the morphological characters historically used in the descriptions of the \textit{S. arota} synonyms revealed no consistent morphological differences between distinct clades.

\textbf{Table 5.2.} Genetic distances of major \textit{S. arota} and a closely related species, \textit{S. coaequalis}. Major lineages (A-D) correspond to Fig. 5.2. ITS1 distances are displayed above the diagonal and ITS2 distances are below the diagonal.

<table>
<thead>
<tr>
<th></th>
<th>\textit{S. arota} Lineage A</th>
<th>\textit{S. arota} Lineage B</th>
<th>\textit{S. arota} Lineage C</th>
<th>\textit{S. arota} Lineage D</th>
<th>\textit{S. coaequalis}</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{S. arota} Lineage A</td>
<td>-</td>
<td>5.8-6.0%</td>
<td>4.6-4.7%</td>
<td>5.1-5.7%</td>
<td>7.20%</td>
</tr>
<tr>
<td>\textit{S. arota} Lineage B</td>
<td>4.9-5.3%</td>
<td>-</td>
<td>2.8-3.3%</td>
<td>4.0-4.3%</td>
<td>6.6-6.8%</td>
</tr>
<tr>
<td>\textit{S. arota} Lineage C</td>
<td>6.1-6.5%</td>
<td>6.1-6.9%</td>
<td>-</td>
<td>1.6-1.8%</td>
<td>6%</td>
</tr>
<tr>
<td>\textit{S. arota} Lineage D</td>
<td>5.9-6.1%</td>
<td>6.1-6.6%</td>
<td>2.2-3.1%</td>
<td>-</td>
<td>6.4-6.8%</td>
</tr>
<tr>
<td>\textit{S. coaequalis}</td>
<td>7.9-8.1%</td>
<td>7.8-8.2%</td>
<td>7.9-8.2%</td>
<td>6.8-7.1%</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 5.3. Consensus tree of the Bayesian analysis of the pared-down data set. The open circle represents the fossil calibration point based on two fossils from Dominican amber. The black circle represents the constraint placed on the root node corresponding to the estimated age of the family Mutillidae. Numbers at each node are mean estimated divergence dates presented as r8s/BEAST.

3.2 Molecular dating

The Bayesian analysis of the pared-down data set with additional outgroups showed the same relationships as the full phylogenetic analysis (Fig. 5.3). Both dating
analyses suggested the divergences separating the major *S. arota* clades occurred during the period of mountain building in the late Neogene. Results from the r8s analysis indicated that the split between clade A and B occurred at about 4.1 Ma and the split between clade C and D occurred at about 3.2 Ma. The r8s analysis shows that the diversification of this cryptic species-complex began around 5.6 Ma. The BEAST analysis suggested older dates. The split between clade A and B occurred at 7.2 Ma (95% credibility: 2.7-9.5 Ma) and the split between clade C and D occurred at 5.8 Ma (95% credibility: 2.2-8.1 Ma). The origin of this species-complex was estimated to be at 9.3 Ma (95% credibility: 4.4-13.4 Ma).

### 3.3 Ecological niche modeling

The four lineages in *S. arota* cannot be distinguished based on morphology alone, therefore, only those specimens that were analyzed molecularly could reliably be assigned to a group (A-D). Because of this, only the specimens used in the phylogenetic analysis could be used to generate ENMs. Five collection localities were used to develop the ENM for lineage A, 19 localities for lineage B, 24 localities for lineage C, and eight localities for lineage D.

Because these models are based on limited numbers of specimens, we report only the predicted range supported by >70% probability of suitable habitat. The ENM predictions for lineage A suggested that this group is largely restricted to the low elevation areas in the western Sonoran Desert (Colorado Desert) (Fig. 5.4). The ENM predictions for lineage B show that this group is primarily found in the mountainous regions in southeastern Arizona (Madrean Archipelago) and parts of Sonora Mexico (Fig.
The ENM for lineage C indicates a broad distribution spanning from the Mojave Desert to parts of the Chihuahuan Desert (Fig. 5.4). The ENM for lineage D suggest that this group is largely restricted to the Mediterranean areas of California and Baja California, Mexico (Fig. 5.4).

**Figure 5.4.** Ecological niche models for each species in the *S. arota* species-complex based on current climatic conditions. Only areas with an estimated probability of suitable habitat above 70% are marked for each species. Circles marked with a letter (A-D) represent locations used to develop the model for each species. Letters correspond to major clades on the phylogenetic tree (Fig. 5.2).
The relative contributions of the 19 bioclimatic variables to the ENM differed for each species. The top three bioclimatic variables contributing to the ENM for lineage A were BIO5 (maximum temperature of the warmest month), BIO14 (precipitation of the driest month), and BIO9 (mean temperature of the driest quarter). The top three bioclimatic variables contributing to the ENM for lineage B were BIO14 (precipitation of the driest month), BIO3 (isothermality), and BIO8 (mean temperature of the wettest quarter). The top three bioclimatic variables contributing to the ENM for lineage C were BIO12 (annual precipitation), BIO14 (precipitation of the driest month), and BIO1 (annual mean temperature). The top three bioclimatic variables contributing to the ENM for lineage D were BIO14 (precipitation of the driest month), BIO9 (mean temperature of the driest quarter), and BIO18 (precipitation of the warmest quarter).

The analysis of similarity (ANOSIM) showed that the ecological niches inhabited by each lineage are significantly different (p=0.001). The Mann-Whitney U test of BIO5 indicated that the sister lineages A and B and the sister lineages C and D are found in areas with significantly different maximum temperatures (Fig. 5.5: A/B: U_{0.05} (2), 10, 18 = 0; p<0.001. C/D: U_{0.05} (2), 8, 24 = 142; p=0.05). The Mann-Whitney U test of BIO6 showed that sister lineages A and B inhabited areas with significantly different minimum temperatures (Fig. 5.5: U_{0.05} (2), 10, 18 = 42; 0.05>p>0.02), but the difference in minimum temperature between sister lineages C and D was not significant (Fig. 5.5: U_{0.05} (2), 8, 24 = 51; 0.01>p>0.05). The Mann-Whitney U test of BIO18 clearly showed that sister lineages live in areas with significantly different amounts of precipitation during the warmest quarter of the year (Fig. 5.6: A/B: U_{0.05} (2), 10, 18 = 180; p<0.001; C/D: U_{0.05} (2), 8, 24 = 167; 0.002>p>0.001). Also, the Mann-Whitney U test of BIO19 indicated that that sister
lineages live in areas with significantly different amounts of precipitation during the coldest quarter of the year (Fig. 5.6: A/B: U_{0.05} (2), 10, 18 = 168; p<0.001; C/D: U_{0.05} (2), 8, 24 = 29; 0.005<p<0.002).

Figure 5.5. Graphs comparing the summer and winter precipitation values for individuals of each species in the S. arota species-complex with comparisons made between sister species.
**Figure 5.6.** Graphs comparing the minimum and maximum temperature values for individuals of each species in the *S. arota* species-complex with comparisons made between sister species.

4. Discussion

4.1 Cryptic species

The genetic distances between the four major lineages are comparable to interspecific genetic distances reported from analyses of other velvet ants (Pilgrim and Pitts, 2006; Wilson and Pitts, 2008, 2009). Furthermore, phylogenetic reconstructions support the distinctness of each lineage. The genetic distances and the phylogenetic reconstructions suggest that *S. arota* is four distinct species (Table 5.2, Fig. 5.2). While
the genetic distances among populations of lineage D are slightly higher than those found in the other lineages, we feel that this lineage represents a distinct species given its geographic range and the high posterior probability support for its monophyly. Because of the genetic and phylogenetic evidence for the presence of four distinct species in the \textit{S. arota} species-complex, we will hereafter discuss each lineage as a distinct species (species A-D).

Several morphological characters have been proposed to diagnose the synonyms of \textit{S. arota} (Ferguson, 1967). These proposed characters consisted of differences in leg coloration and the state of a longitudinal carina on both the clypeus and the scutellum. Examination of these characters revealed no consistent differences between the four \textit{S. arota} species. In addition, we examined genitalic characters and potential differences between the marginal cell and the length of the stigma. These additional morphological investigations also revealed no differences between species. Because no notable morphological differences could be found separating these species, the \textit{S. arota} species-complex represents the first cryptic species-complex described in the family Mutillidae.

While the species in the \textit{S. arota} species-complex cannot be distinguished morphologically, three of the four species can be tentatively associated with historical names based on the distribution of the type specimens. Species A is only known from two areas in the western Sonoran Desert, Deep Canyon and Corn Springs, and likely represents an undescribed species. Schuster (1958) described \textit{S. helicaon diegueno} from San Carlos, Arizona; this subspecies was later synonymized with \textit{S. helicaon} by Ferguson (1967). Based on distributional data, \textit{S. h. diegueno} is likely a member of our species B suggesting that this subspecies is actually a valid species, not a synonym, in the \textit{S. arota}
species-complex. Species C is distributed from Texas to California but is largely absent from the Sonoran Desert. Because species C is the only species in the *S. arota* species-complex that is found in Nevada, it can be associated with *S. helicaon*, which was originally described based on a specimen collected in Nevada. Species D is largely restricted to the mesic parts of southern California and is often found in mountainous areas or areas with surface water. Thus, species D can be associated with *S. arota*, which was described from a specimen from San Diego, California.

Even though we can tentatively associate the four cryptic species in the *S. arota* species-complex to named species, we do not suggest that taxonomists attempt to assign members of this species-complex to these historic names. Rather, because of the difficulty in determining the geographic delimitations of each species, we suggest that the members of this group be identified as the *S. arota* species-complex.

4.2 Biogeography

Although the four species in the *S. arota* species-complex are genetically distinct, there is not an obvious biogeographic pattern among these species. For example, species A, C, and D all are found in southern California within 80 km of each other. Even though members of this species-complex are often collected relatively close to each other, the ANOSIM results indicate that each of the four *S. arota* species is associated with a distinct ecological niche based on the 19 Worldclim bioclimatic variables. Additionally, the ENMs indicate that little overlap exists in the suitable habitat for each species.

The results of our examination of the four bioclimatic variables that are important to desert-adapted species (minimum and maximum temperature and rainfall) can help
identify the specific ecological niches for each species. For example, species A is adapted to hotter and drier climates than its sister species, species B (Figs 5.5, 5.6). The ENM for species A indicates that this species is most likely to be found in the western Sonoran Desert and along the lower Colorado River (Fig. 5.4), which includes some of the warmest and driest parts of North America (MacMahon and Wagner, 1985). The ENM of species B, on the other hand, shows that this species occurs primarily in the mountainous southeastern corner of Arizona and into parts of the Sierra Madre Occidental Mountains of Mexico (Fig. 5.4).

The distinctness of the niches of species C and D are not as clear as the niches of species A and B. While there are significant differences in the average maximum and minimum rainfall and maximum temperature values between species C and D, there is a large amount of overlap in the individual values (Figs 5.5, 5.6). In general, species D seems to be adapted to more mesic areas with cooler summer temperatures, warmer winter temperatures, and high winter precipitation. This assertion of a mesic-adapted species D is supported by its ENM, which shows suitable habitat throughout the Nearctic Mediterranean regions. Species C, however, is found across the North American deserts and appears to be adapted to areas with high summer temperatures and cooler winter temperatures compared to species D (Fig. 5.5). The ENM of Species C predicts suitable habitat in isolated parts of the Chihuahuan, Sonoran, and Mojave Deserts (Fig. 5.4).

Although there is a clear difference in rainfall and temperature preferences between each species, selected individuals are outliers, not grouping with the other members of their species. Some of the differences in temperature and rainfall values of these outlying individuals can be explained by the elevation or latitude from which they
were collected. For example, the four specimens of species B found in Sonora Mexico have much higher minimum temperature estimates than do the other representatives of this species (Fig. 5.5). The habitat where these four specimens were collected, however, is consistent with habitats inhabited by other members of this species. Also, one specimen of species D has drastically different values for the maximum temperature and the winter precipitation (Figs 5.5, 5.6). This individual was collected in Afton Canyon, an area in the Mojave Desert that has a significantly different microhabitat than nearby areas due to the continual presence of water. While this location is in the middle of the Mojave Desert, it houses some bee species normally found in more mesic regions (T. Griswold, pers. comm.). Even though there is variation in some of the bioclimatic variables experienced by these species, this variation is generally explainable by examining the microhabitat and other factors from each collection location.

4.3 Historical biogeography

While the growing body of work detailing evolution in the Nearctic warm deserts is beginning to show a consistent pattern, with late Neogene uplift playing a central role in diversification (e.g., Riddle and Hafner, 2006; Hafner and Riddle, in press), little work has been done examining patterns of diversification in widespread arthropods (Wilson and Pitts, 2010a). The pattern of diversification that is emerging from multiple studies of arid-adapted species suggests that the uplift of the continental divide in the late Neogene divided an ancestral desert region and led to a pattern seen in many organisms, with sister species restricted to eastern (Chihuahuan) and western (Mojave and Sonoran) deserts (e.g., Morafka, 1977; Jaeger et al., 2005; Devitt, 2006; Douglas et al., 2006; Wilson and
Pitts, 2010a). The historical biogeography of the *S. arota* species-complex does not fit in this common evolutionary pattern. The ENMs of each of the species in the *S. arota* species-complex show little overlap between the ranges of the four species, suggesting that vicariant events may have played a major role in speciation. While some of the divergences in this group can be linked to historical events that drove diversification in other taxa, the current model of an east/west pattern of diversification in desert-adapted species does not easily explain all of the evolutionary events in this group.

The sister relationship between species A and species B does seem to be associated with known vicariant events. While both of these species are associated with the Sonoran Desert and neighboring areas (Madrean Archipelago), species A is largely confined to areas west of the Colorado River and species B is primarily found in the mountainous regions of the Sonoran Desert and the Madrean Archipelago. A low elevation desert area called the Lower Colorado Subunit of the Sonoran Desert separates these two species. The divergence between species A and B was dated to the Miocene-Pliocene times (~ 4 Ma by r8s and ~ 7 Ma by BEAST). From about 4-8 Ma much of the Lower Colorado Desert was covered by water, a formation known as the Bouse Embayment, which stretched from the Sea of Cortez as far north as southern Nevada (Wilson and Pitts, 2010b). This extension of the Sea of Cortez vicariantly isolated populations into the eastern and western Sonoran Desert and drove speciation in several taxa (Jaeger et al., 2005; Devitt, 2006; Wilson and Pitts, 2010a). It is likely that the split between species A and Species B was also driven by the presence of the Bouse Embayment. At approximately the same time many of the Nearctic mountain ranges were experiencing episodes of uplift (Wilson and Pitts, 2010b), which may be responsible for
the evolution of the montane-adapted species B. These late Neogene uplift events also caused the formation of a rain shadow in the low elevation areas of southern California which may have driven the evolution of the desert-adapted species A.

The sister relationship between species C and D may also be associated with late Neogene uplift events based on the estimated divergence dates (r8s: 3.2 Ma, BEAST: 5.8 Ma). Before a rain shadow was formed as California’s mountains uplifted, many parts of the modern deserts resembled Mediterranean grasslands with patches of woodland and scattered playa lakes, similar to the climate of the coastal areas (Merriam, 1919; Tidwell and Nambudiri, 1989; Park and Downing, 2001). Because the historical Mediterranean-like climate of western North America was similar to the climate California has maintained for millions of years (Minnich, 2007), the Mediterranean parts of California may represent a refugium from the desertification experienced in the late Neogene. Thus, in groups with desert-adapted species sister to Mediterranean-adapted species, the major divergence events could likely be associated with mountain building and desert formation events that occurred between 2-15 Ma (Wilson and Pitts, 2010b). The relationship between species C and D shows such a pattern, with the Mediterranean-adapted species D being sister to the desert-adapted species C and is likely a result of the uplift of the California mountain ranges and the subsequent aridification of the deserts. Although several of the specimens of species D were collected on the eastern side of the mountains, which is generally more desert-like than the western slopes, the majority of these collections were made in areas with perennial water sources which effectively create more Mediterranean-like microhabitats.
Within species C, four well-supported clades are associated with distinct desert regions. Specifically, we see a large Chihuahuan Desert clade, a Colorado Plateau clade, a Mojave Desert clade, and a Sonoran Desert clade. Due to the lack of sequence divergence between populations, the relationship between the distinct desert clades remains unclear. Because of their genetic similarity and the estimated divergence dates (Fig. 5.3: r8s 0.8 Ma; BEAST 1.6 Ma), it is likely that the formation of these clades is a result of isolation in separate Pleistocene refugia, as has been reported from analyses of other velvet ants (Wilson and Pitts, 2010a).

While several recent analyses have pointed to the uplift of the continental divide as a major driving force in the evolution of desert-adapted taxa (e.g., Morafka, 1977; Jaeger et al., 2005; Devitt, 2006; Douglas et al., 2006; Wilson and Pitts, 2010a), our analysis of evolution in the S. arota species-complex suggests that other late Neogene events influenced the diversification of this group. Rather than a clear east/west split among species in our phylogeny, we find three of the four species restricted to the western deserts with only some populations from the widespread species C living in the eastern deserts. Although the uplift of the continental divide did not drive diversification in this species-complex, the uplift of California’s southern mountains and the expansion of the Bouse Embayment did drive evolution in these wasps.

4.4 Conclusions

This phylogeography of S. arota reveals four deeply divergent lineages. Genetic distances indicate that these four lineages represent four distinct species. No morphological characters were found that can be used to identify these species.
Therefore, *S. arota* represents the first known cryptic species complex described in Mutillidae. The analyses of the bioclimatic variables influencing the distributions of each species provide evidence for the distinctiveness of the ecological niche of each species. The ENMs show that little overlap exists between the probable distributions of each species. Divergences between sister species are linked to late Neogene events. Both the uplift of the southern California mountain ranges and the presence of the Bouse Embayment were influential in the diversification of the *S. arota* species-complex. This biogeographic history differs from patterns of diversification in other desert-adapted organisms in that the uplift of the continental divide, which also occurred in the late Neogene, does not seem to have influenced the diversification of *S. arota*.

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CHAPTER 6
SUMMARY AND CONCLUSIONS

While the evolution of desert-adapted vertebrates has often been linked to Neogene vicariant events (e.g., Morafka, 1977; Jaeger et al., 2005; Devitt, 2006; Douglas et al., 2006), it is unclear if these same events drove diversification in arid-adapted insects as well. In this dissertation, I examined the patterns of diversification in four nocturnal velvet ant groups (Hymenoptera: Mutillidae) in order to determine if a consistent pattern of diversification exists between desert-adapted vertebrates and invertebrates. Because similar patterns of diversification may be linked to different historical events, I used molecular dating techniques to estimate the age of major divergences. While the phylogeographic patterns in some velvet ant groups show similar geographic structuring and seem to be associated with the same historical events that led to diversification in several vertebrate groups, not all diversification events among velvet ants can be linked to the same historical events.

In Chapter 2, I analyzed phylogeographic patterns of the widespread velvet ant genus *Dilophotopsis*. The analyses indicated that the species-level divergences in *Dilophotopsis* occurred in the Neogene, and were likely driven by mountain building during the Miocene-Pliocene boundary (~5 Ma). Furthermore, the different *Dilophotopsis* species show similar biogeographic patterns to those found in many vertebrate taxa (e.g., Morafka, 1977; Jaeger et al., 2005; Devitt, 2006; Douglas et al., 2006). The population-level divergences within *Dilophotopsis* species occurred during the Pleistocene (1.8-0.1 Ma). This study showed that similar patterns of diversification exist in vertebrate and
invertebrate taxa, specifically, that species-level divergences are linked to Neogene events and that population-level divergences are linked to Pleistocene events. This study also supports the model of evolution that suggests Neogene uplift of the western mountains isolated populations into eastern and western deserts and drove diversification in arid-adapted groups.

In Chapter 3, I performed a phylogeographic analysis of another widespread velvet ant species-group, the *Odontophotopsis unicornis* species-group. This species-group has a similar range to *Dilophotopsis* and shows a similar biogeographic pattern with sister species being restricted to eastern and western deserts. The analysis of the *O. unicornis* species-group resulted in a well-supported phylogenetic tree that reinforces the notion that *O. unicornis* and *O. erebus* are distinct species. Unlike the analysis of *Dilophotopsis*, little or no phylogenetic structuring was found among populations of either species. The molecular dating analysis suggested that these species evolved in the middle Pleistocene (~1 Ma). The lack of phylogeographic structuring in each of the species of the *O. unicornis* species-group is likely due to the recent origin of these species. This analysis represented one of the few instances of Pleistocene age species-level divergences in desert-adapted taxa.

The development of a diverse desert-adapted velvet ant biota cannot be thoroughly investigated without looking into the causes of diversification between desert-adapted species and closely related Mediterranean-adapted species. In Chapter 4, I investigated the causes of diversification in the *Sphaeropthalma unicolor* species-complex, a group of nocturnal velvet ants with desert-adapted species and Mediterranean-adapted species. This phylogenetic reconstruction showed that each of the three species
in the *S. unicolor* species-complex form a distinct, well-supported clade with little population-level structuring within the Mediterranean-adapted *S. unicolor* and more structuring among desert-adapted *S. mendica* populations. Molecular dating analyses suggested that the split between *S. angulifera* and the other two species occurred in the late Neogene and the split between *S. unicolor* and *S. mendica* occurred in the early Pleistocene. While both late Neogene and early Pleistocene events were influential in the diversification of the *S. unicolor* species-complex, Pleistocene climatic fluctuations seem to be responsible for the split between the Mediterranean-adapted species and the desert-adapted species. Although the majority of species-level diversification events in arid-adapted taxa seem to be driven by Neogene vicariant events, Pleistocene climatic changes apparently did drive species-level diversification in some velvet ant groups.

In Chapter 5, I investigated the phylogeographic patterns among populations of the wide-ranging velvet ant *Sphaeropthalma arota*. This analysis indicated that *S. arota* can be split into four deeply divergent lineages that likely represent distinct species. No morphological characters were found that can be used to identify these four species, making the *S. arota* species-complex the first documented cryptic species-complex in Mutillidae. Divergence date estimates indicated that major diversification events in the *S. arota* species-complex can be linked to late Neogene mountain building and aridification events, specifically the uplift of the mountain ranges in southern California and the expansion of the Bouse Sea. The history of diversification of the *S. arota* species-complex differs from the history of several other arid-adapted species, because the uplift of the continental divide seems to not have influenced diversification in this group.
Although the growing body of literature detailing diversification in desert-adapted taxa suggests that species-level divergence events are associated with Neogene mountain-building events, this dissertation shows that a mixture of Neogene and Pleistocene events were responsible for the diversification of velvet ants. Often, researchers assume that diversification in taxa with similar biogeographic patterns (i.e., sister species being restricted to eastern and western deserts) was driven by the same historical events. My work shows that this is not necessarily the case. Both *Dilophotopsis* species and species in the *Odontophotopsis unicornis* species-group show a similar biogeographic pattern, yet, diversification in these groups is linked to different historical events.

A recent analysis of velvet ant evolution by Pitts *et al.* (2010) also suggests that diversification in this group was driven by both Neogene and Pleistocene events. While a majority of analyses done on vertebrate taxa show that Neogene events are more important to species-level diversification than are Pleistocene events, as more work is done, researchers may find that, as in velvet ants, the diversification of the desert biota was driven by both Neogene and Pleistocene events.

**Literature Cited**


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APPENDICES
Appendix A. Copyright Release Letter
August 1, 2010

Joseph S. Wilson
Utah State University
5305 Old Main Hill
Logan, UT 84322
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Annals of the Entomological Society of America
Journal Address

Dear Dr. Kahan:

I am preparing my dissertation in the Biology Department at Utah State University. I hope to complete my degree in the fall of 2010.

An article, Pleistocene Diversification of the *Odontophotopsis unicornis* Species-Group (Hymenoptera: Mutillidae), of which I am first author, and which appeared in your journal, Vol. 103(4) 555-565, reports an essential part of my dissertation research. I would like permission to reprint it as a chapter in my dissertation. Reprinting the chapter may necessitate some revision. Please note that USU sends dissertations to Bell & Howell Dissertation Services to be made available for reproduction.

I will include an acknowledgment to the article on the first page of the chapter. Copyright and permission information will be included in a special appendix. If you would like a different acknowledgment, please so indicate.

Please indicate your approval of this request by signing in the space provided, and attach any other form necessary to confirm permission.

If you have any questions, please call me at the number above or send me an e-mail message at the above address. Thank you for your assistance.

Joseph S. Wilson

I hereby give permission to Joseph Wilson to reprint the requested article in his dissertation, with the following acknowledgment:

(This chapter was published in *Annals of the Entomological Society of America* in July of 2010 and is reprinted here with permission. Please cite information from this chapter by using the following reference:


Signed ________________________________

Date    August 2, 2010
Appendix B. Coauthor Permission Letters
2 August 2010

Joseph Wilson has my permission to include the following paper, which was submitted for publication, of which I was a co-author, in his doctoral dissertation.

Wilson, J. S., Clark, S. L., Williams, K. A. and Pitts, J. P. “Phylogeography and ecological niche models of *Sphaeropthalma arota* (Hymenoptera: Mutilidae) reveal a cryptic species-complex and unique biogeographic history”.

Sarah L. Clark
2 August 2010

Joseph Wilson has my permission to include the following paper, which was submitted for publication, of which I was a co-author, in his doctoral dissertation.

Wilson, J. S., Clark, S. L., Williams, K. A. and Pitts, J. P. “Phylogeography and ecological niche models of Sphaerophaarma arota (Hymenoptera: Mutillidae) reveal a cryptic species-complex and unique biogeographic history”.

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## Education

**PhD, Biology**, Utah State University, Department of Biology  
Dissertation Title: "Historical Biogeography of velvet ants (Hymenoptera: Mutillidae) in the North American Deserts and Arid Lands". Advisor: James P. Pitts.

**B.S., Biology**, Utah State University, Department of Biology (May 2005) GPA: 3.37  
Utah Valley University (2004-2005) Major: Biology GPA: 3.34  
Southern Utah University (Fall 2002) Major: Biology GPA: 3.90

## Publications

**Peer-reviewed Publications**

**Wilson, J.S., S.L. Clark, K.A. Williams and J.P. Pitts**  


**Pitts, J.P., J.S. Wilson, K.A. Williams, and N.F. Boehme. 2010.** Nocturnal velvet ant males (Hymenoptera: Mutillidae) of Deep Canyon, California including four new species and a fifth new species from Owens Lake Valley, California. Zootaxa. 2553: 1-34.


Research Reports


**Funding**

2009  Theodore Roosevelt Memorial Grant from the American Museum of Natural History. Biodiversity and endemism in velvet ants (Hymenoptera: Mutillidae) of the Madrean Sky Islands. $1,000.

2007  Research Grant from the California Desert Research Fund at The Community Foundation of Riverside and San Bernardino Counties. Endemism and diversity in velvet ants (Hymenoptera: Mutillidae) of the Southern California deserts. $3,194.

2006  Theodore Roosevelt Memorial Grant from the American Museum of Natural History. Phylogeography of deserticolous nocturnal Mutillidae (Hymenoptera). $1,130.

**Teaching Experience**

2009-2010  **Teaching Assistant Coordinator**, Biology 1610/1620, Utah State University. I trained teaching assistants how to perform the experiments in the weekly biology lab and advised them regarding appropriate teaching techniques. I also assisted in writing tests and quizzes and aided in the development of laboratory curriculum.

2009-2010  **Honors Teaching Assistant**, Biology 1610/1620 Honors Laboratory, Utah State University. In addition to standard TA duties, I designed and led activities that encouraged problem solving and critical-thinking skills.

2006-2010  **Student Mentor**, Utah State University, Logan, Utah. Supervisor: Dr. James Pitts. I mentored five undergraduate students who were conducting molecular biology research on multiple arthropod groups. Two of these students presented their research at regional entomology conferences.

2006-2010  **Assistant Laboratory Coordinator**, Biology 1610/1620 Laboratory, Utah State University. I set up the weekly biology lab to be used by 500-700 students. I ordered supplies and instructed teaching assistants on proper use of laboratory equipment.

2005-2010  **Teaching Assistant**, Biology 1610/1620 Laboratory, Utah State University. For three sections of 30 students, I lectured for 20-30 min. and supervised the weekly three-hour lab. Lab topics ranged from the cell cycle and Mendelian genetics to fungi and plant diversity in Biology 1610 and from phylogenetics and evolution to animal behavior and diversity in Biology 1620.

2009  **Guest Lecturer**, Biology 1620, Utah State University. I gave a series of lectures on animal diversity, behavior, and conservation to the general biology class (500+ students).
2008 **Invited Lecturer**, Entomology Club, Utah State University. I presented a 15 min. lecture to the USU Entomology Club on the evolution of nocturnal velvet ants in western North America.

2008 **Wildlife Specialist**, Utah Envirothon, Moab, Utah. I taught 19 teams of high school students about bee diversity and conservation in the desert southwest, with particular focus on land-management issues.

2007 **Biologist**, Teton Science School BioBlitz, Jackson, Wyoming. I instructed students, teachers, and community members (over 350 total participants) on arthropod identification and the importance of arthropods to native ecosystems in conjunction with a visit from E.O. Wilson.

2007 **Guest Entomologist**, Adams Elementary School, Logan, Utah. I taught students and parents about the science of entomology and the importance of insects.

2007 **Wildlife Specialist**, Utah Envirothon, Salt Lake City, Utah. I taught 16 teams of high school students about adaptation and evolution, focusing principally on the adaptations of shorebirds.

### Presentations

#### Seminars

Wilson, J.S. and J.P. Pitts. 2010. How did California’s geologic history affect velvet ants (Hymenoptera: Mutillidae)? Presented at the Pacific Branch Entomological Society of America meeting.


#### Poster Presentations


Research Experience

2005-2010 PhD candidate, Utah State University, Logan, Utah. Supervisor: Dr. James Pitts. Duties: Collected molecular data from multiple arid-adapted arthropod species in order to examine patterns of diversification in the Nearctic deserts.

2007-2010 Insect Systematics and Evolution Laboratory manager, Utah State University, Logan, Utah. Supervisor: Dr. James Pitts. Duties: instructed undergraduate and graduate students in the proper use of molecular biology tools. Techniques included DNA extraction, PCR, gel electrophoresis, DNA purification, and sequencing reactions.

2005 Biological Aid, Bioekistic Biological Consulting, St. George, Utah. Supervisor: Marshall Topham. Duties: Surveyed public lands for sensitive reptiles, amphibians, and birds prior to proposed utility work.


2003-2005 Biological Technician, USDA-ARS Bee Biology and Systematics Laboratory, Logan, Utah. Supervisor: Dr. Terry Griswold. Duties: Investigated the diversity and biogeographical patterns of native bees from multiple arid environments in Utah.


Awards and Honors

2010 First Place in Student Oral Competition at the Entomological Society of America Annual Meeting (Pacific Branch), held in Boise, Idaho on April 11-13.

2010 Third Place in Lecture Session at the 2010 Intermountain Graduate Research Symposium, held in Logan, Utah on March 31 2010.

2008 Second Place in Student Oral Competition for President’s Prize in Revisions and Evolution at the Entomological Society of America National Meetings, held in Reno, Nevada on November 16-19.

Professional Society Memberships

Entomological Society of America
International Society of Hymenopterists
Southwestern Association of Naturalists

Served as a reviewer for the Botanical Journal of the Linnean Society.