Genetic Diversity of Ants (Hymenoptera: Formicidae) from the Ozark-St. Francis National Forest, Arkansas, USA

by

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ABSTRACT

Limited information exists on the extent of genetic variation of ants (Hymenoptera: Formicidae) from undisturbed forest habitats in the United States. We conducted a molecular genetics study involving DNA sequencing of a portion of the mitochondrial DNA cytochrome oxidase I gene to determine the extent of genetic variation of ants from the Arkansas Ozark-St. Francis National Forest (OSFNF). Forty one samples were collected, identified to species and subjected to DNA sequencing. From these samples, five species were identified, with *Aphaenogaster texana* and *Crematogaster lineolata* being the most abundant. Genetic diversity was also the greatest for these two species. The cytochrome oxidase I marker was able to reliably distinguish all five of the species encountered. It appears that the species richness and genetic diversity of ants from the OSFNF is high, which gives support for using ants as indicators of forest health in the United States.

Keywords: mitochondrial DNA, Formicidae, Arkansas, DNA sequence.

INTRODUCTION

Maintaining a diversity of habitat types is suggested for supporting ecologically diverse ant (Hymenoptera: Formicidae) functional groups to improve forest health (Stephens & Wagner 2006). Ants have certainly been implicated in understanding agroecosystem health (Peck *et al.* 1998), so their implications in forest systems would be no different. In most instances, ants play very important roles in the process of sustaining forests. The spatial fidelity, species

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Ants are an important component of the biodiversity of forest areas of North America, and increasingly, DNA barcoding is being used for the discovery and analysis of biodiversity. We conducted a preliminary study on the utility of a DNA barcoding marker mtDNA COI for a survey of the biodiversity of ants from the OSFNF.

**MATERIALS AND METHODS**

The study was conducted at the Lake Wedington section of the Ozark-St. Francis National Forest. The Wedington section consists of 11,360 ha located in Arkansas Washington and Benton counties, that has been undisturbed since the 1930’s. Natural vegetation is generally a non-commercial growth of mixed broadleaf deciduous trees of the upland variety with post oak, Quercus stellata, black-jack oak, Q. marilandica, and black oak, Q. velutina, being the predominant upland overstory (Schalm 1973). The study used two sampling sites: Lake Wedington (36°05’38”N 94°22’28”W) and Lee Creek (35°34’48”N 94°22’03”W). The two sites were 41 km from each other. A collection permit was obtained from the USDA National Forest Service prior to insect collection. Ants were collected during 2007 from 100 m transects from underneath stumps, logs or fallen branches (> 2.5 cm diam.) using a mouth aspirator and preserved in 90% ethanol. Ants were identified to species using keys of Creighton (1950) and Warren & Rouse (1969). Voucher specimens preserved in 100% ethanol are maintained at the Arthropod Museum, Department of Entomology, University of Arkansas, Fayetteville, AR.

Alcohol-preserved specimens were allowed to dry on filter paper, and DNA was extracted from individual ant thoraces with the Puregene DNA isolation kit D-5000A (Gentra, Minneapolis, MN). Extracted DNA was resuspended in 50 µl of Tris:EDTA and stored at –20°C. Polymerase chain reaction (PCR)
of a 708 bp region of the mtDNA COI gene was conducted using the primers LCO1490-F forward: 5’-GGTCAACAAATCATAAAGATATTGG-3’ (Simon et al. 1994) and HCO2198-R reverse: 5’- TAAACTTCAGGGTGACAAAAATCA -3’ (Simon et al. 1994). The PCR reactions were conducted with 1 µl of the extracted DNA (Szalanski et al. 2000), having a profile consisting of an initial denaturation of 2 min at 94°C, followed by 40 cycles of 94°C for 45 s, 46°C for 45 s and 72°C for 60 s, and a final extension of 5 min at 72°C.

Amplified DNA from individual ants was purified and concentrated with Amicon Microcon PCR centrifugal filter devices (Millipore, Billerica, MA) according to the manufacturer’s instructions. Samples were sent to the DNA core sequencing facility at The University of Arkansas Medical, Little Rock for direct sequencing in both directions. GenBank accession numbers were FJ943556 to FJ943574 for sequences in this study. DNA sequences were aligned using BioEdit v5.89 (Hall 1999) and adjusted manually. Mitochondrial DNA haplotypes were aligned using MacClade v4 (Sinauer Associates, Sunderland, MA). Genealogical relationships among haplotypes were constructed using TCS (Clement et al. 2000).

The distance matrix option of PAUP* 4.0b10 (Swofford 2001) was used to calculate genetic distances according to the Kimura 2-parameter model of sequence evolution (Kimura 1980). Additional mitochondrial COI GenBank sequences from Crematogaster, Camponotus, Prenolepsis and Aphaenogaster ants were added to the dataset along with DNA sequences from Vespuia sp. (GenBank accession number DQ353368) which was added to act as outgroup taxon. DNA sequences were aligned using CLUSTAL W (Thompson et al. 1994). Maximum parsimony analysis on the alignments was conducted using PAUP* 4.0b10 (Swofford 2001). Gaps were treated as missing characters for all analysis. The reliability of trees was tested with a bootstrap test (Felsenstein 1985). Parsimony bootstrap analysis included 1,000 resamplings using the Branch and Bound algorithm of PAUP*.

For Bayesian analysis, the best-fitting nucleotide substitution model was chosen according to the general time reversible + gamma (GTR+G) model among 64 different models using the ModelTest v 3.7 (Posada and Crandall 1998) and PAUP* 4.0b10 (Swofford 2001) programs. Phylogenetic trees were obtained using Bayesian inference with the GTR+G model using Bayesian
Evolutionary Analysis Sampling Trees (BEAST) v1.4.2 software (Drummond and Rambaut 2003). For Bayesian inference, four Markov chains run for $10^6$ generations with a burn-in of $2 \times 10^4$ were used to reconstruct the consensus tree. Genealogical relationships among haplotypes were constructed using TCS (Clement et al. 2000).

RESULTS AND DISCUSSION

In most instances, ants play very important roles in the process of sustaining forests. The spatial fidelity, species richness, abundance and composition of ants in the OSFNF likely follows a similar pattern to other studies which suggest that ants are more pliable to changing ecological patterns within forests than many other organisms (Bennett et al. 2009). The present study was no exception. From the 41 samples, the most abundant species was Aphaenogaster texana (Emery) (37%), followed by Crematogaster lineolata (Say) (26%), Camponotus pennsylvanicus (DeGeer) (21%), Pheidole dentata Mayr (7%), and Formica fusca L. (5%). These ratios are highly influenced by the local conditions where ants were collected in the present study and should vary considerably from different locations with the OSFNF. The highest levels of genetic variation were observed in A. texana (7 distinct haplotypes) and C. lineolata (5 haplotypes) with relatively lower levels of genetic variation observed in C. pennsylvanicus (2 haplotypes) (Table 1). The presence of low genetic variation in C. pennsylvanicus from this specific site could be due,

<table>
<thead>
<tr>
<th>Species (n)</th>
<th>Location</th>
<th>Haplotypes</th>
</tr>
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<tbody>
<tr>
<td>Aphaenogaster texana (11)</td>
<td>Lake Wedington</td>
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<tr>
<td>A. texana (5)</td>
<td>Lee Creek</td>
<td>3(2), 5(1), 6(2)</td>
</tr>
<tr>
<td>Camponotus pennsylvanicus (6)</td>
<td>Lake Wedington</td>
<td>1(6)</td>
</tr>
<tr>
<td>C. pennsylvanicus (3)</td>
<td>Lee Creek</td>
<td>2(3)</td>
</tr>
<tr>
<td>Crematogaster lineolata (3)</td>
<td>Lake Wedington</td>
<td>1(1), 2(2)</td>
</tr>
<tr>
<td>C. lineolata (8)</td>
<td>Lee Creek</td>
<td>3(1), 4(5), 5(2)</td>
</tr>
<tr>
<td>Formica fusca (2)</td>
<td>Lake Wedington</td>
<td>1(1), 2(1)</td>
</tr>
<tr>
<td>Pheidole dentata (2)</td>
<td>Lee Creek</td>
<td>1(2)</td>
</tr>
<tr>
<td>P. dentata (1)</td>
<td>Lake Wedington</td>
<td>2(1)</td>
</tr>
</tbody>
</table>
Fig. 1. Bayesian phylogenetic relationship of OSFNF ant mtDNA COI sequences. Posterior bootstrap values are provided at each node, and MP bootstrap values (>75) are provided in parenthesis.
in part, to the excellent condition of the tree stands there, with few dead or decomposing trees; Alternatively, it may also be somewhat artificial based on the timing of collections which were performed during daylight hours as this species is known to be primarily nocturnal (Klotz & Reid 1992) and possesses large foraging ranges (Traniello 1977).

Of the 629 characters used for the phylogenetic analysis, a total of 266 characters (42%) were parsimony informative. The maximum parsimony analysis resulted in a tree of a length of 1373 and a confidence index value of 0.346. The Bayesian phylogenetic analysis revealed that *Camponotus pennsylvanicus* formed a sister group with *C. noveboracensis* (Fig. 1). TCS analysis (Clement et al. 2000) revealed that *A. texana* haplotype 4 was the ancestral haplotype among the seven haplotypes studied, with haplotypes 2 and 6 being the most divergent (Fig. 2). The TCS analysis of the five *C. lineolata* haplotypes revealed that haplotype 4 was ancestral and haplotype 2 was the most divergent (Fig. 3).

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**Fig. 2.** Genealogical relationships among *Aphaenogaster texana* COI haplotypes estimated by TCS (Clement *et al.* 2000). A unit branch represents one mutation and small ovals indicate genotypes that were not observed.
Maintaining a diversity of habitat types is suggested for supporting ecologically diverse ant functional groups to improve forest health (Stephens & Wagner 2006). Ants have certainly been implicated in understanding agroecosystem health (Peck et al. 1998), so their implications in forest systems would be no different. The difficulty in understanding forest health by virtue of singling out specific faunistic elements such as ants lies in the inability to easily interpret what attributes their collective activities may contribute to the overall wellness and “health” of the forest. Forests are generally considered healthy if they are irrepressible or sustainable; a healthy forest is “one that is resilient to changes” (Joseph et al. 1991); “resistant to catastrophic change and/or ability to recover after catastrophe” (Kolb et al. 1994) and has “sustained ecosystem functioning” (Wagner et al. 2000).

The genetic diversity of *A. texanna* is interesting to note since many of the ants within this genus are commonly found on the forest floor of deciduous forests, nesting under rocks and similar habitats, and yet were quite abundant at the collection locations in this study. Recent investigations of ants collected

![Genealogical relationships among *Crematogaster lineolata* COI haplotypes estimated by TCS (Clement et al. 2000). A unit branch represents one mutation and small ovals indicate genotypes that were not observed.](image-url)
from Arkansas suggest that *A. texana* can be collected many different ways (General & Thompson 2008) and is abundant throughout the state, suggesting it may be rather common in the state. The most important attribute this ant species affords forests, in terms of “health”, stems from the fact that this species and many within its genus, contribute to significant seed-dispersal (myrmecochory) mutualism. In a study of another member of the genus, *Aphaenogaster rudis*, access to seeds in an experimental forest ecosystem was noted to support the hypothesis that myrmecochory is a true mutualism (Morales & Heithaus 1998).

In similar fashion, *C. lineolata* favors somewhat sunnier sites through the forest canopy and can be found frequently tending aphids and treehoppers. The mutualism exhibited from this ant also likely contributes to improved “health” of the Ozark forest.

During the course of this there also appeared to be differences in the distribution of haplotypes of *A. texana* and *C. lineolata* between the Lake Wedington and Lee Creek sample locations. Of the seven *A. texana* haplotypes, only three were shared between each location, and for *C. lineolata* only two of the five haplotypes were shared. Bayesian phylogenetic analysis revealed that all of studied species were monophyletic (Fig. 1). As stated previously, this is most likely attributed to local conditions at each site. The resilience of a forest remains a relative unknown until exposure to catastrophic disturbance or stress. The areas evaluated for samples in this study are routinely subjected to burning and good silvicultural practices, which may partially define the degree of genetic diversity observed due to the general attraction and sustainability of said species at each location.

In conclusion we found that the mtDNA COI marker was adequate for differentiating the sampled ants species, regardless of the amount of intraspecific variation. This marker also appears to be suitable for population genetics analysis of ants from this habitat. It also appears that the species richness and genetic diversity of ants from the Ozark-St. Francis National Forest is high, which gives support for using ants as indicators of forest health in the United States in general.

**ACKNOWLEDGMENTS**

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REFERENCES


