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RESEARCH ARTICLE

Phylogeography of the imperiled Comanche Harvester Ant (*Pogonomyrmex comanche*)

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Abstract

One of the few imperiled ant species in North America is the Comanche Harvester Ant, *Pogonomyrmex comanche*. Despite its status, little is known about its natural history throughout its range in the western Gulf Coastal Plain of North America. This study presents a regional phylogeographic analysis of *P. comanche* across sites in its natural range as a first step to learning more about this species. By using COI genotyping, we discovered that the center of genetic diversity is found in central Texas, which is typical for many species that found refugia in the southern North America during Pleistocene glaciations. Although diversity was slightly lower in northern populations, there was no evidence of recent population expansion into northern latitudes. Rather, some deviations from neutrality were consistent with population contraction in the northern regions (Arkansas, Oklahoma). The high diversity and relative rarity of identical sequences among samples were also consistent with dispersal limitation. The exact mechanisms driving its decline are currently unknown, but a combination of dispersal limitation and habitat loss seem likely causes.

Keywords Dispersal limitation · Gulf Coastal Plain · 'Lost Pines' of Central Texas · mtDNA · Population contraction · Texas

Introduction

Ants are among the most ecologically successful group of animals; they occur nearly everywhere on the planet and are exceptionally abundant in mid to low latitudes (Moreau and Bell 2013; Schultheiss et al. 2022). Their success is partially attributed to colony life, so that once established, colonies may achieve numerical dominance, as well as their interactions with other organisms (Davidson et al. 2003; Parker and Kronauer 2021; Hill et al. 2022; Costa-Silva et al. 2023). Despite their overall success and ecological importance, more than 150 species are thought to be endangered (Talavera et al. 2015; Balzani et al. 2022; IUCN 2024).

Southeastern North America is considered a global hotspot of biodiversity due to the degree of endemism and overall species richness found in this region (Soltis et al. 2006; Sorrie and Weakley 2006; Noss et al. 2015). The region extends along the Gulf of Mexico from Texas to Florida and northward along the Atlantic Ocean. Although much information exists on the phylogeography of plants and vertebrates in this region, comparatively little is known about insects and particularly the ants that are found in this large region. Most of the southern USA escaped the brunt of Pleistocene glaciations and many northern ant species found refugia associated with more stable climates in Florida, Texas or northern Mexico before expanding northward during the Holocene (Soltis et al. 2006). Consequently, the genetic variation of many species may exhibit a pattern typical of expanding populations with most variation being found in ancestral (southern) regions and comparatively less found in regions more recently occupied (Avise 2000; Al-Rabab'ah and Williams 2004; Seal et al. 2015; Harrison and Noss 2017).

Pogonomyrmex is a genus of harvester ants found in mostly arid regions in North and South America (MacMahon et al. 2000; Johnson 2001) This genus has unique external nest forms that aid in their identification in the field. *Pogonomyrmex* nests can be identified by the presence of

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a flattened "disk" covered with small pebbles or charcoal, among other objects or a crater of sand with a central entrance (Smith and Tschinkel 2005; Smith and Tschinkel 2007). The internal nest structure consists of downward branching tunnels and chambers that house the queen, the worker ants, the brood and the collected seeds (Tschinkel 2004). *Pogonomyrmex* ants are presumably ecologically important as they collect vast amounts of seeds and frequently dig deep nests (MacMahon et al. 2000; Tschinkel 2015; Tschinkel and Kwapich 2016). In the southeastern North America, three species of *Pogonomyrmex* occur. Two (*P. barbatus* and *P. comanche*) are found west of the Mississippi River, whereas *P. badius* has an eastern distribution (Johnson 2000). While *P. barbatus* and *P. badius* have been subjects of many studies that range from population genetics to behavior and ecological impacts (Smith and Tschinkel 2006; Smith et al. 2011; Mott et al. 2015; Tschinkel and Kwapich 2016), very little is known about *P. comanche* beyond their presence on regional species lists and anecdotal observations (Strandtmann 1942; Johnson 2000; Dash and Hooper-Bùi 2008; Warriner 2011).

Pogonomyrmex comanche is predominantly distributed in the prairie or savannas overlaying sandy soils of Arkansas, Louisiana, Kansas, Oklahoma and Texas (Johnson 2000), in particular the Post Oak Savannah Ecoregion of the western Gulf Coastal Plain (Diggs et al. 2006). It thus occupies a habitat similar to *P. badius*, except that it is found to the west of the Mississippi River (Fig. 1). This species is associated with the *P. californicus* complex (Johnson et al. 2013; Johnson and Moreau 2016; Smith 2023). One of the unique features of the biology of *P. comanche* is that it is, along with the rarely observed *P. bigbendensis* (Francke and Merickel 1982), among one of the few ant species listed at being at risk of extinction by the states of Louisiana and Texas. For example, Louisiana and Texas both list *P. comanche* as globally imperiled/vulnerable (G2/G3 and regionally imperiled (S2) (TPWD 2017; LDWF 2020) (Fig. 2).

The goal of this study was to identify regional patterns of genetic diversity in *P. comanche* across a North–South gradient of its distribution, a region from central Texas to Oklahoma and Arkansas. We found that southern populations were genetically more diverse than those found in the North and there may be evidence of population contraction in northern regions.

Fig. 1 Photographs of *Pogonomyrmex comanche* nest and in its native habitat in Bastrop, County, Texas

Materials and methods

Specimen collection

Colonies were sampled in three regions. We sampled a 'South' region that corresponded to several localities in the central part of Texas. The 'Central region was characterized by several localities near Tyler, Texas, and was broadly between the Trinity River and the Red River, a major river in the southern USA. We defined the 'North' region as localities north of the Red River and consisted of a site in Norman, Oklahoma, and two sites in southern Arkansas (Miller County Sandhills Natural Area and Arkansas Oak Natural Area) (Table 1).

DNA extraction and mitochondrial DNA analysis

From each colony sampled in the field, a single individual worker was selected for DNA extraction. DNA was extracted from whole individual workers using a QIAamp DNA Micro Kit (QIAGEN), and a 779-bp sequence was obtained from the COI-tRNA Leucine-COII region of mitochondrial DNA

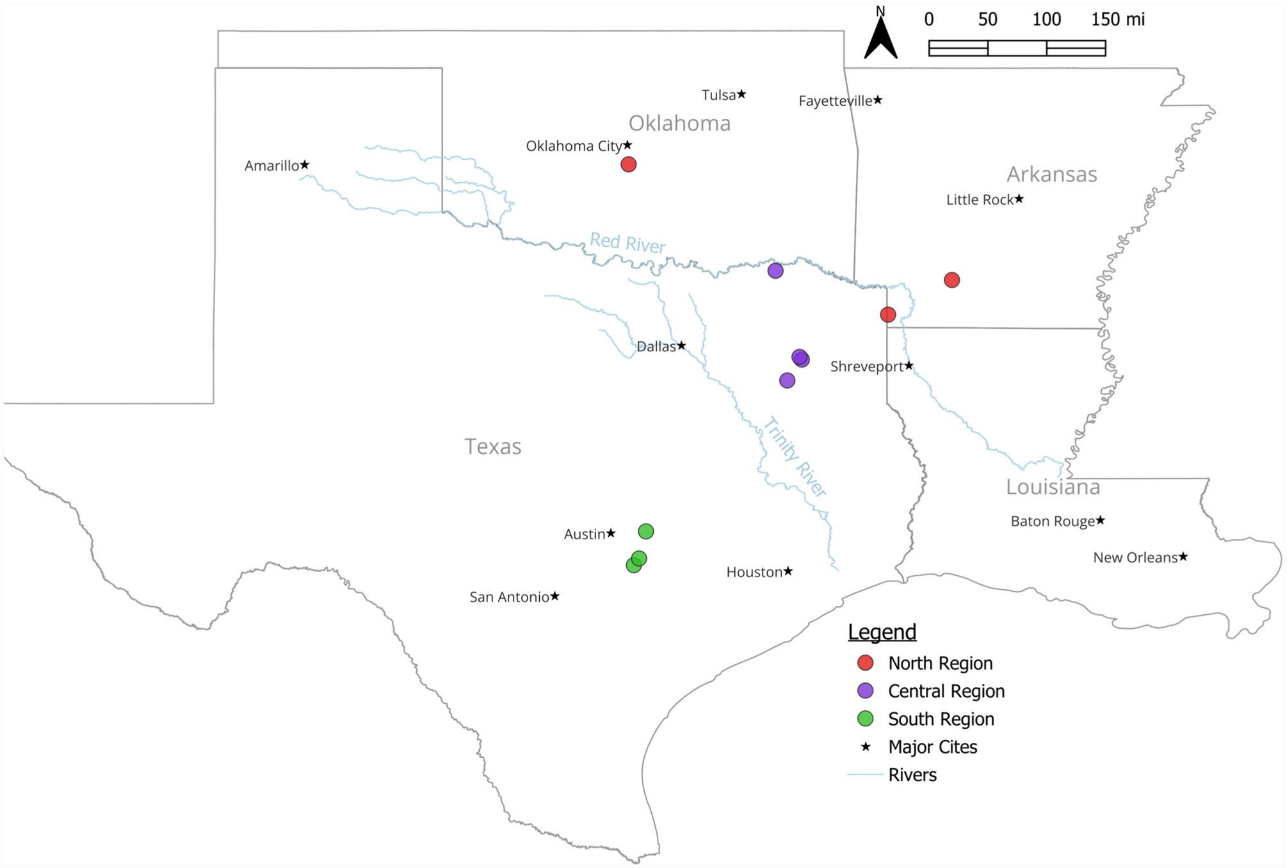

Fig. 2 Localities of *Pogonomyrmex comanche* sampling sites in this study. Colors of sampling sites represent designated regions. Major rivers and cities and state names are noted for reference

Table 1 Sample sizes of the collection localities and the associated GPS coordinates

Region		County	State	Colonies	Latitude	Longitude
South	Caldwell County	Bastrop	Texas	1	29.83865	– 97.4332
South	Camp Swift	Bastrop	Texas	17	30.29194	– 97.271
South	Red Rock	Bastrop	Texas	1	29.9285	– 97.3636
Central	Jarvis Christian College	Wood	Texas	1	32.5907	– 95.1833
Central	Hawkins	Hawkins	Texas	1	32.62615	– 95.2143
Central	Lindsey Park	Smith	Texas	9	32.3105	– 95.3777
Central	Camp Maxey	Lamar	Texas	1	33.78166	– 95.535
North	Arkansas Oak Nature Reserve	Nevada	Arkansas	6	33.65808	– 93.1723
North	Miller County Sandhills	Miller	Arkansas	6	33.19354	– 94.0278
North	Norman	Norman	Oklahoma	5	35.20912	– 97.5037

(mtDNA). We used the following primers: C1-J2195 (alias CO1-RLR; 5′-TTGATTTTTTGGTCATCCAGAAGT-3′); and C2-N-3661 (alias Barbara; 5′- CCACAAATTTCTGAACATTGACCA-3′ (Simon et al. 1994). Polymerase chain reaction (PCR) mixtures and cycling profiles were identical to those used in prior studies (Seal et al. 2011; Seal et al. 2015; Matthews et al. 2021). PCR mixtures were as follows: 4 µL (~ 20 ng µL^{-1}) of DNA, 2 µL of 10×PCR buffer, 1.6 µL of 1 mM deoxyribonucleotide triphosphates (dNTPs), 1.6 µL of MgCl2, 1.6 µL of bovine serum albumin (BSA), 0.2 µL (1U) of *Taq* polymerase, and 1.2 µL of 10 µM primer. The PCR cycles involved an initial denaturation for 2 min at 94 °C; 38 cycles of 94 °C for 1 min, 50 °C for 1 min, and 68 °C for 2 min; and a final extension at 72 °C for 5 min.

PCR products were purified and sequenced at the University of Texas at Austin's DNA Sequencing

Facility on an Applied Biosystems 3730 DNA Analyzer. Chromatograms were manually checked and resolved in Geneious v10.2.3 (Kearse et al. 2012), and sequences were aligned in MEGA v6.06 (Tamura et al. 2013) using the ClustalW algorithm (Thompson et al. 1994). New sequences were deposited into NCBI GenBank under accession numbers MH193071–MH193170.

With our COI mtDNA alignment, we examined haplotype diversity and calculated basic measures of genetic polymorphisms across and within regions using DNAsp v6 (Rozas et al. 2017). We then reconstructed a haplotype network for the COI data using the TCS method (Clement et al. 2000) in PopArt v1.7 (Leigh and Bryant 2015). We calculated haplotype and nucleotide diversities and tested for population expansion using Tajima's *D* neutrality test and Harpending's *h* statistic. Expanding populations typically differ from neutrality. Negative Tajima's *D* values indicate a lower frequency of polymorphism than expected by chance, which often indicate population bottlenecks or recent expansions, whereas a positive value indicates population contraction.

Results

COI sequences exhibited variation with regard to the region sampled. Haplotypes were most diverse in the southernmost regions in central Texas and least diverse in the northernmost populations in Arkansas and Oklahoma (Fig. 3, Table 2). For example, every second haplotype collected in central Texas was unique and these were never found outside the South region, whereas some identical haplotypes were found throughout the northern and northwestern region. Northern and Central haplotypes appear to be closely related to three separate haplotypes found in central Texas (Fig. 3). The tests of population expansion indicate that the populations are not

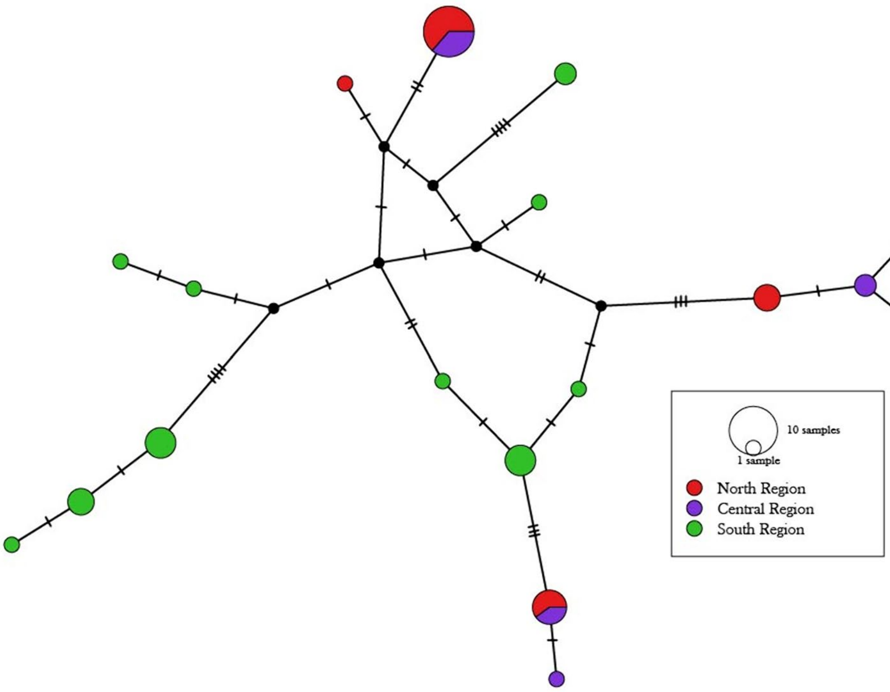

Fig. 3 A TCS haplotype network for *Pogonomyrmex comanche* constructed with COI mitochondrial DNA sequences. Shaded circles represent unique haplotypes and their relative size is proportional to the number of individuals with that haplotype; black circles represent predicted intermediate haplotypes. Shading corresponds to sampling sites (regions) in Fig. 1. Tick marks on branches indicate the number of mutations between haplotypes

Table 2 Population genetic diversity indices and tests of population expansion of *Pogonomyrmex comanche* ants across the region studied n.s. = not significant $\alpha = 0.05$, * corresponds to $0.10 > p > 0.05$ and ** corresponds to $p < 0.05$

Region	*n*	*h*	hd (SD)	π (SD)	*k*	Tajima's *D*
All	48	18	0.919 (0.023)	0.00923(0.00697)	7.165	$1.065^{n.s.}$
South	19	10	0.906 (0.04)	0.00749 (0.006)	5.183	$1.016^{n.s.}$
Central	12	5	0.833 (0.07)	0.00959 (0.00683)	7.439	1.7448*
North	17	5	0.779 (0.07)	0.00836(0.00534)	6.485	2.614**

Abbreviations include: sample size (*n*), number of unique haplotypes (*h*), haplotype (hd) and nucleotide (π) diversities with standard deviation (SD), and the average number of nucleotide differences (*k*) expanding in the South region, whereas the significant test in the North population was positive and could be evidence of population contraction (Table 2).

Discussion

The most striking finding in this study is the presence of significant genetic variation throughout the range of *P. comanche* and there is no evidence of recent expansion. Rather, there is potential evidence of population contraction along the northern portions of its range; the significant positive Tajima's D statistic suggests an excess of common alleles and an absence of rare alleles. This pattern is unlike the phylogeographic structure of a distantly related co-occurring ant species, *Trachymyrmex septentrionalis*, which is characterized by little haplotypic variation across its range in the western Gulf Coastal Plain and negative Tajima's D statistic (Seal et al. 2015), which is all the more surprising since the latter study used a longer section of COI than in the current study on *P. comanche*. Presumably, this reflects a longer phylogeographic history of *P. comanche* in this region. Alternatively, this pattern may be related to differences in dispersal biology. It seems likely that female *T. septentrionalis* queens can disperse up to 100 km or more (Matthews et al. 2021), whereas *Pogonomyrmex* queens are much larger and may have more restricted movements (Suni and Gordon 2010). Our results support limited dispersal of *P. comanche* queens because the haplotypes of *P. comanche* appear to be regionally structured. Congener *P. badius* is also known to exhibit significant mtDNA geographic structuring in a relatively small region (Strehl and Gadau 2004), which may be a result of queens mating on mounds and dispersing locally instead of dispersing over longer distances to mate (J Seal, unpublished observations). A better understanding of the dispersal biology of *P. comanche* and comparative approach with *P. badius* could help to explain the phylogeographic patterns observed in the present study.

The factors behind dispersal limitation need to be explored in greater detail, but there are several potential non-exclusive factors that may contribute to *Pogonomyrmex* dispersal and their genetic diversity. Some have remarked potential drivers in the loss of *Pogonomyrmex* populations in the form of invasive fire ants (*Solenopsis invicta*.), but fire ants are not known to occur in northern populations and are also known to avoid dry, xeric habitats typical for *Pogonomyrmex* (Tschinkel 1988; King and Tschinkel 2008; Warriner 2011; King and Tschinkel 2015). An alternate explanation may be related to habitat fragmentation or land use change. Habitat fragmentation is thought to be an important factor in driving ant community structure (Crist 2009) and has been directly implicated in the loss of genetic variation and reductions in population sizes of European wood ants (*Formica* spp.) (Mäki-Petäys et al. 2005; Mäki-Petäys and Breen 2007; Dekoninck et al. 2010). Changes in land use and habitat fragmentation could be an important factor related to dispersal behaviors, since *Pogonomyrmex* typically prefer hot, open environments. For example, experimental shading of *P. badius* colonies caused more frequent nest relocations, possibly because the shading cooled soil (Carlson and Gentry 1973). In another study, *P. badius* foraging increased after a prescribed fire; however, it was unclear whether this behavior was due to a stress associated with incinerated food or stimulated by an increase of seeds (food) released after a fire (McCoy and Kaiser 1990). If fires are an important mechanism to free up open space, in addition to droughts, among other mechanisms that could clear up space, then future studies should examine the impact of local environments on colony-level performance and growth (Seal and Tschinkel 2006; Seal and Tschinkel 2010). Food limitation might be another explanation limiting their growth in isolate population fringes (Smith 2007). Generally, dispersal limitation coupled with a dependence on hot, open environments and mechanisms that to create open spaces could be an explanation for why *P. comanche* populations are not expanding and may instead be contracting.

Future genetic work should involve diploid markers such as microsatellites or whole genome approaches to examine for gene flow among populations. Attempts to use microsatellites developed from other species (Volny and Gordon 2002; Gadau et al. 2003) on these populations of *P. comanche* were not successful as the alleles were not sufficiently polymorphic (Romo 2018). However, novel microsatellite markers could be developed (Matthews et al. 2020) or high-throughput whole genome approaches such as genotyping-by-sequencing could be implemented (Beigel et al. 2021). One promising strategy (using either molecular genetic approach) may be to explore the population genetics of both *P. badius* and *P. comanche* in a comparative framework. Since both species live in similar environments (the southeastern coastal plain), a congeneric comparative approach could help determine whether both are experiencing range contractions or if these processes apply only to *P. comanche* or if an 'umbrella' approach could be used to protect *Pogonomyrmex* species, generally, as advocated in conservation measures for *Formica* wood ants (Balzani et al. 2022).

We believe our results are robust despite known issues with mtDNA. For example, some properties of mtDNA such as pseudogenes or nuclear insertions (numts) can complicate conclusions regarding the evolutionary relationships among organisms (Martins et al. 2007; Beckenbach 2009; Moreau 2009; Toews and Brelsford 2012; Cristiano et al. 2014). While we recognize these limitations, mtDNA sequences can be readily obtained and their associated problems can be easily examined, whereas the problems associated with biparentally inherited nuclear markers are not always as clear (Moreau 2009; Bowen et al. 2014). Consequently, mtDNA sequences are robust tools to assess phylogenetic patterns (Mikheyev et al. 2008; Seal et al. 2015; Matthews et al. 2021). Nevertheless, we examined for stop codons in our sequences and found none. We also noted that the sequences in this study were long and frequently readable at > 800 bp and contained variation, which numts usually lack as they are subject to DNA proofreading mechanisms in the nucleus.

A tragic finding in this study is that the southernmost region of our study (Central Texas) appears to be the most genetically rich area of *P. comanche* and should be under prime consideration for conservation efforts; however, central Texas is among the fastest growing areas of Texas. However, harvester ants are a major food of the state reptile (Burgess et al. 2018; Schmidt 2019), the Texas Horned Lizard (*Phrynosoma cornutum*), which is also threatened (McIntyre 2003). As a result, conservation programs could be developed that target both species, such as by increasing awareness of the role of habitat loss, among other mechanisms that could be involved in the loss of these two charismatic and important species.

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Data availability All sequences were deposited into NCBI GenBank under accession numbers MH193071–MH193170.

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