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# Gone to Texas: phylogeography of two *Trachymyrmex* (Hymenoptera: Formicidae) species along the southeastern coastal plain of North America

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Ants are widely recognized as ecologically important members of many low- to mid-latitude ecosystems. Surprisingly, there is very little phylogeographical information on ants at regional scales. We examine here the phylogeography of two partially sympatric species of *Trachymyrmex* (*Trachymyrmex septentrionalis* and *Trachymyrmex turrifex*) ants in southeastern North America. We test the hypothesis that all *Trachymyrmex* species found in the USA expanded into North America from refugial populations located in northern Mexico as the post-Pleistocene climate warmed. Phylogeographical theory predicts that these northward-expanding species should exhibit higher genetic diversity in regions closer to Mexico and less diversity in more northern regions. We also examine, in the widely distributed *T. septentrionalis*, the hypothesis of vicariance that occurred at the formation of the Mississippi Embayment. Phylogeographical patterns indicate that *T. septentrionalis* has an eastern origin because diversity was highest east of the Mississippi, whereas *T. turrifex* probably has a Mexican origin because it lacked mitochondrial DNA (mtDNA) variation throughout its range and is currently absent from eastern North America. Both species are characterized by reduced haplotypic variation in the western coastal plain of the Gulf of Mexico (Texas and Louisiana), which may indicate recent expansion and/or bottlenecks associated with increased aridity and drought in these western regions. 

ADDITIONAL KEYWORDS: Attini – Florida – Gulf Coastal Plain – 'Lost Pines' of Central Texas – Mississippi Embayment.

## INTRODUCTION

Most of the modern flora and fauna of eastern North America were profoundly influenced by Pleistocene glaciations. The northern half of the continent was directly impacted by an ice sheet several kilometres thick. However, most of the south-east of North America is thought to have escaped the direct effects of glaciation and to have had cooler and drier conditions during that time, as well as an increase in the surface area of land because of contraction of the Gulf of Mexico (Watts, 1980; Watts & Hansen, 1994; Carroll *et al*., 2002). The flora and fauna that later invaded the glaciated North America is thought to have resided and flourished in refugia in Florida or in Mexico (Avise, 2000), which expanded northward as the climate warmed about 10 000 years ago (Hawkins & Porter, 2003).

The south-eastern coastal plain of North America has long been of interest to naturalists and biologists because of its subtropical characteristics and pronounced endemism (Platt, 1999; Sorrie & Weakley, 2001, 2006). The conditions found in this region are largely a result of its proximity to the Gulf of Mexico, a source of humidity and important rainfall, which sets it apart from other areas of similar latitude (e.g. the Sahara Desert; Chen & Gerber, 1990). Broadly

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defined, the area extends north from the Gulf of Mexico to 38°N and from the Atlantic coast westward to 100°W. Most of the expanse is former seabed of relatively low relief (< 250 m in elevation) (Sorrie & Weakley, 2001; Soltis *et al*., 2006) and physiographically divided into several main regions that correspond to major rivers and landscape features (Sorrie & Weakley, 2001, 2006). The easternmost region corresponds to the Atlantic Coastal Plain that extends from southern New England to peninsular Florida (Fig. 1). The Gulf Coastal Plain occurs along the Gulf of Mexico and is divided by the Mississippi River Embayment into eastern (Eastern Gulf Coastal Plain) and western (Western Gulf Coastal Plain) portions (Fig. 1). The western parts of the Western Gulf Coastal Plain tend to be much drier owing to their proximity to the more arid Great Plains and the Chihuahua Desert (Sorrie & Weakley, 2001, 2006; Soltis *et al*., 2006).

To our knowledge, there exists surprisingly very little information about the phylogeographical history of insects found in the south-eastern USA. Of the > 150 species highlighted in a recent review of the phylogeographical patterns of south-eastern animals, plants, and fungi, phylogeographical patterns were known for only four insects (Soltis *et al*., 2006). Although ants are important members of south-eastern ecosystems by occupying key ecological roles (Tschinkel *et al*., 2012; King, Warren & Bradford, 2013), the phylogeographical structures and histories of these insects are virtually unknown.

Conspicuous members of the south-eastern coastal plain are fungus-gardening ants in the genus *Trachymyrmex*. Of the two *Trachymyrmex* species found in the eastern USA, *Trachymyrmex septentrionalis* is by far the most widespread, with a distribution that extends from central Texas to coastal New York and New Jersey (Rabeling *et al*., 2007) (Fig. 1), a distribution that makes it likely to be a coastal-plain endemic, using the criteria of Sorrie & Weakley (2001) for plants. *Trachymyrmex septentrionalis* appears to be strongly associated with pine forests throughout the entire eastern USA and is considered an indicator species, if not an ecosystem engineer of these forests, as it moves annually > 1 metric ton of soil (Seal & Tschinkel, 2006, 2010). Compared with the other *Trachymyrmex* species in the USA, *T. septentrionalis* is the only species found solely in the USA, usually in sandy soils (Rabeling *et al*., 2007). *Trachymyrmex turrifex* is found only west of the Mississippi River, in a variety of sandy and clayey soils and environments (Rabeling *et al*., 2007). Like most of the other *Trachymyrmex* species of North America, the distribution of *T. turrifex* extends into


**Figure 1.** Regions and collecting sites within the south-eastern coastal plain of North America. The Mississippi (bold line) divides the Gulf Coastal Plain into western and eastern halves. Circles and squares correspond to the collecting sites of, respectively, *Trachymymrex septentrionalis* and *Trachymymrex turrifex*.

northern Mexico (Nuevo León) (Rabeling *et al*., 2007; Sanchez-Peña, 2010).

Two phylogeographical hypotheses could explain the current distribution of *T. septentrionalis* and *T. turrifex*. The first is a dispersal hypothesis, suggested by Rabeling *et al*. (2007), which postulates that all *Trachymyrmex* species found in the USA expanded into North America from refugial populations located in northern Mexico as the post-Pleistocene climate warmed. Rabeling *et al*. (2007) noted that seven of the nine species of *Trachymyrmex* are found in northern Mexico and arid parts of the USA (Texas, New Mexico, and Arizona), which suggested to them that the US *Trachymyrmex* species were adapted to arid environments. This hypothesis was further supported by Seal & Tschinkel (2006, 2010), who reported that *T. septentrionalis* is distributed in the driest soils of north Florida and that populations increase during exceptional droughts. The second hypothesis applies only to *T. septentrionalis* by involving a vicariant event that occurred as the Mississippi Embayment formed and increased in size and became an effective dispersal barrier as river flows increased. The implication here is that *T. septentrionalis* has been a longer resident of eastern North America than *T. turrifex* and survived the Pleistocene in refugia located along the Gulf of Mexico or Florida, as did other sympatric species of ants and trees (Al-Rabab'ah & Williams, 2004; Strehl & Gadau, 2004; Jaramillo-Correa *et al*., 2009). This hypothesis was partly supported by Mikheyev, Vo & Mueller (2008), who reported two distinct haplotype clades largely found on either side of the Mississippi River. Unfortunately, the small amount of mitochondrial DNA (mtDNA) [cytochrome *c* oxidase subunit I (COI)] sequenced (< 500 bp) prevented full evaluations of this hypothesis.

These two generalized hypotheses – the dispersal hypothesis and the vicariance hypothesis – make different predictions. If both *T. septentrionalis* and *T. turrifex* expanded northward from Mexico, as assumed by the dispersal hypothesis, less derived haplotypes should be found along the receding edges in the south-west (e.g. Texas) and reduced genetic variation along invasion fronts in northern and eastern regions of their respective ranges (Hewitt, 2000; Hewitt & Nichols, 2005; Sexton *et al*., 2009). On the other hand, if *T. septentrionalis* survived the Pleistocene in refugia in the southern Mississippi River valley, as assumed by the vicariance hypothesis, then both the eastern and western Gulf coastal plains should be characterized by a mixture of both ancestral and derived haplotypes as populations in each half of the valley became isolated and subsequently evolved independently.

In the following analyses, we found that *T. turrifex* lacks phylogeographical structure throughout its distribution in Texas and Louisiana, which suggests recent expansion from Mexico to the USA. In contrast, *T. septentrionalis* appears to have an eastern origin because diversity was highest east of the Mississippi. Both species were characterized by reduced variation in the western coastal plain of the Gulf of Mexico, which may indicate recent expansion and/or bottlenecks associated with increased aridity and drought in these regions.

## MATERIAL AND METHODS

### Sampling

Ants were collected from colonies in a region that ranged from Texas in the south-west to Long Island, New York, in the north-east (see Fig. 1 and Supporting Information, Tables S1 and S2, for collecting sites). Only one ant from each colony was sequenced because all individuals in a colony will have an identical haplotype (mitochondria are maternally inherited).

### DNA sequencing

Total DNA was extracted from whole individual ants using the Qiagen Micro Kit for animal tissues (Qiagen, Valencia, CA, USA). A 1423-bp sequence was obtained from the cytochrome oxidase COI-tRNA Leucine-COII region of mtDNA. In *T. septentrionalis*, this region was sequenced using two primers: C1-J-2195 (alias CO1-RLR) (5′-TTGATTTTTTGGTCAT CCAGA AGT-3′); and C2-N-3661 (alias Barbara) (5′-CCACAAATTTCTGAACATTGACCA-3′). These primers have been used in other ants (Brandt *et al*., 2007; Seal *et al*., 2011) and seem to work well for other North American *Trachymyrex* species and *Atta texana* (J. N. Seal, unpubl. data). On the other hand, neither of these primers worked for *T. turrifex* and thus slightly different primers were employed, which produced a 1540-bp sequence. For *T. turrifex*, the COI end was amplified using the primer C1_J-2183: 5′-CAA CAT TTA TTT TGA TTT TTT GG-3′ (alias Jerry), whereas the other end was sequenced using A8-N-3914: 5′-TCA TTT TAT AGG TAT TAT TTG AGG-3′. The latter primer is located about 300 bp downstream of the same region as C2-N-3661 and contains part of the coding region for ATPase 8 (Crozier & Crozier, 1993; Simon *et al*., 1994). Sequences were deposited to GenBank under accession numbers KP282865–KP283014.

Polymerase chain reaction (PCR) mixtures were as follows: 4 μL (~ 20 ng $\mu L^{-1}$) of DNA, 2 μL of 10× PCR buffer, 1.6 μL of 1 mM deoxyribonucleotide triphosphates (dNTPs), 1.6 μL of $MgCl_2$, 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 were identical to those used by Seal *et al*. (2011): 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.

Although mtDNA is known to have several properties that make evolutionary conclusions problematic, such as pseudogenes, frameshifts, nuclear insertions, and duplications (Martins *et al*., 2007; Beckenbach, 2009; Toews & Brelsford, 2012; Cristiano, Cardoso & Fernandes-Salomão, 2014), the sequences obtained in this study did not display telltale signs, such as stop codons (other than at the COI – tRNA Leucine transitions), insertions or double peaks, a finding similar to other studies that sequenced the same region of the mitochondrial genome (Beibl *et al*., 2007; Brandt *et al*., 2007; Seal *et al*., 2011). Sequences were also long and readable at > 800 bp. Moreover, a recent review highlighted that traditional (i.e. mtDNA) methods have been very valuable at providing an initial evaluation of phylogeographical patterns in a number of species (Bowen *et al*., 2014).

### Phylogeographic methods

The sequences were analysed using a combination of phylogenetic and population-genetic techniques (Posada & Crandall, 2001; Freeland, 2006). We constructed a network produced by the minimum spanning method using HAPSTAR to investigate patterns of haplotypic diversity (Teacher & Griffiths, 2011). To root the haplotype network and to identify possible phylogenetic relationships, the sequences were analysed using the Bayesian algorithm implemented in MrBayes (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). jMODELTEST was used before Bayesian analysis to identify the best model of sequence evolution, which in this case was HKY+G (a gamma distribution of rates) using Bayesian Information Criterion (Posada, 2008). The MrBayes analysis was run for 5 million generations with a sampling frequency of 1000 (burnin = 1250). The standard deviation of split frequencies was < 0.005 at this point. This topology was then compared with that obtained by the maximum likelihood analysis obtained from Garli, version 0.951 (Zwickl, 2006). The tree was estimated using default settings and the HKY+I+G model of sequence evolution (the best model according to Akaike Information Criterion in jModelTest). Ten trees with the highest likelihood score were chosen and bootstrap support was evaluated in 500 pseudoreplicates. In these analyses, the entire sequence was analysed as a single partition because neither the COI nor the COII sequences was explained by different models of sequence evolution.

We used BEAST (Drummond & Rambaut, 2007) to determine approximate divergence dates on the two major radiations revealed by the above analyses. Because sequence evolution was determined, using PAUP (Swofford, 2003), to be not clock-like, the strict fixed-clock model was rejected ($\chi^2 = 191.3$, d.f. = 80, $P < 0.0001$). Thus, divergence dates of the major radiations were estimated using a log-normal relaxed clock model in the software package BEAST (Drummond *et al*., 2006). This analysis was conducted for 25 million generations (burnin = 10 000, effective sample sizes exceeded 200), under the HKY+G model of sequence evolution and a tree prior of coalescence (constant size) was employed. We used a mutation rate of 1.5% (bp per Myr), which approximates those obtained from a variety of arthropods, including ants (DeSalle *et al*., 1987; Schubart, Diesel & Hedges, 1998; Quek *et al*., 2004; Resende *et al*., 2010; Seal *et al*., 2011), even though we recognize that there is disagreement over mutation rates and calibration methods of mitochondrial sequences (Pulquérioa & Nichols, 2007; Moreau, 2009; Ho & Lo, 2013). Owing to this uncertainty, we restricted our use of the dating analysis as an inferential tool to estimate the relative ages of the radiations occurring on either side of the Mississippi Embayment. To affix dates to possible radiation of *T. septentrionalis* in the western Gulf Coastal Plain, we estimated divergence dates on eastern and western clades. To determine the relative placement of the unresolved lineages containing haplotypes from Arkansas, north-east Texas and North Carolina (Fig. 2), we conducted three sets of analyses. The first analysis combined all eastern lineages into a single clade and did the same for western lineages, but excluded the unresolved lineages. In the second analysis we placed the unresolved groups in the eastern lineage and compared divergence dates, whereas in the third analysis the unresolved groups were placed in the western lineage.

### Population genetic analyses

We investigated the amount of genetic variation exhibited across the geographical range of both species. A primary focus was on the structure of *T. septentrionalis* populations on either side of the Mississippi because of the marked differentiation reported previously by Mikheyev *et al*. (2008) for a smaller data set. We examined the amount of variation on haplotypes found on either side of the Mississippi using DnaSP version 5 (Librado & Rozas, 2009). 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 contrac-

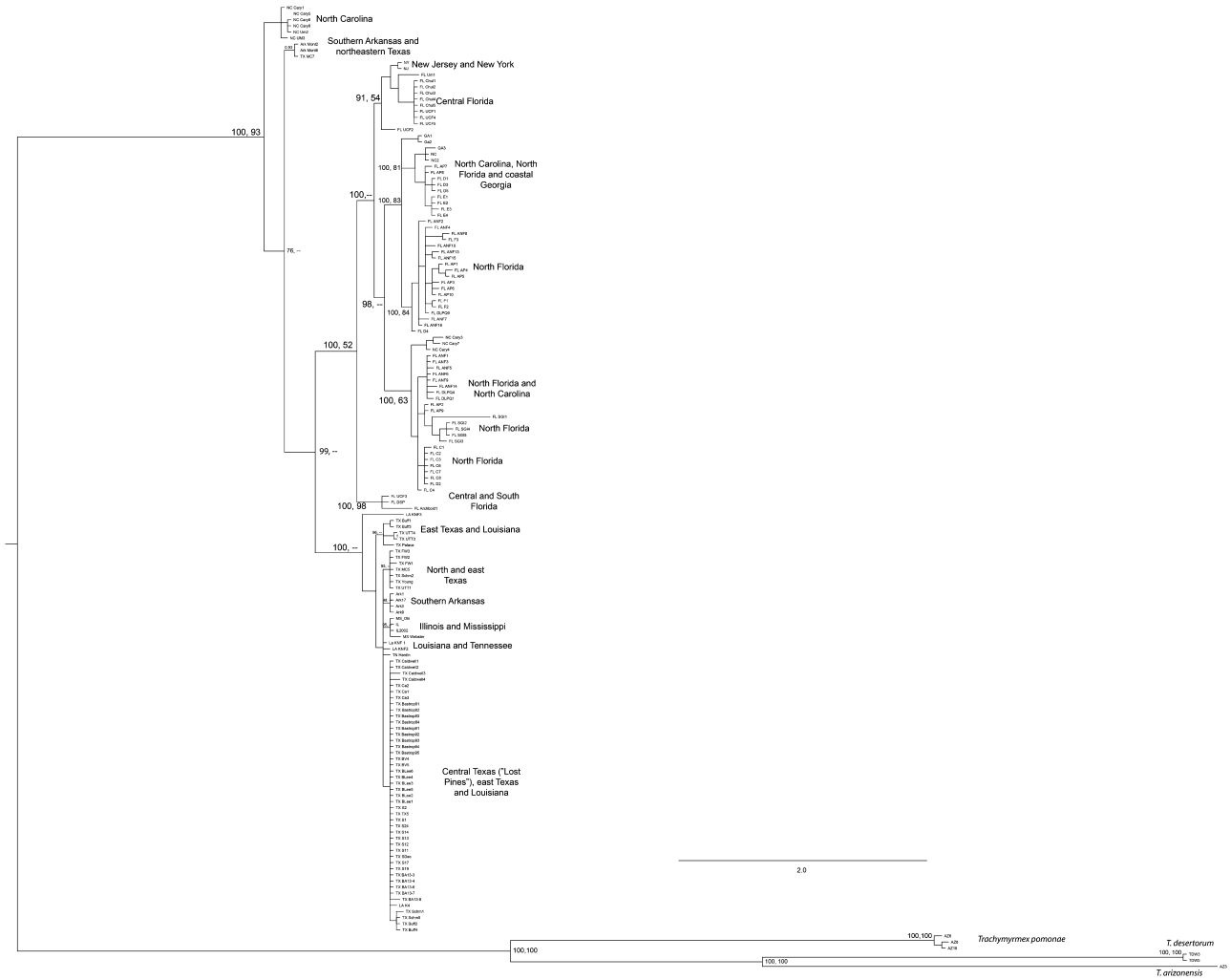


**Figure 2.** Phylogram based on a 1421-bp partial sequence of COI-tRNA Leucine COII of *Trachymyrmex septentrionalis*. Numbers at each node indicate Bayesian posterior probabilities and bootstrap values obtained from maximum likelihood analyses. Values of small clades with values of less than 50 were omitted. The scale bar indicates the number of substitutions per site. The topology shown is from Bayesian Inference. The tree was rooted with sequences from *Trachymyrmex arizonensis*, *Trachymyrmex desertorum* and *Trachymymrex pomonae* (see Supporting Information, Table S1).

tion. A significant Harpending's *h* statistic indicates deviance from the expected null hypothesis of a unimodal distribution of haplotype pairwise differences or a star-like phylogeny. Thus, an expanding population will be supported by a nonsignificant Harpending's *h* statistic.

## RESULTS

### Phylogenetic analysis

*Trachymyrmex turrifex* sequences from throughout Texas and western Louisiana were 100% identical. As a result, all subsequent analyses addressed only the variation found in *T. septentrionalis*. In contrast to *T. turrifex*, *T. septentrionalis* exhibited significant genetic variation across its range. Most variation was found east of the Mississippi, where the majority of individuals clustered into four distinct clades. Western haplotypes clustered primarily into a single clade (Figs 2, 3). Exceptions to this were haplotypes collected in Illinois, Mississippi, and Tennessee, which clustered with individuals collected in Texas and Louisiana. Also, some individuals collected in North Carolina, north-east Texas, and southern Arkansas did not form a group with any of the clades found in the eastern and western coastal plains.

The first BEAST analysis [where unresolved lineages were excluded (Fig. 2)] indicated that the western haplotypes had descended from a common ancestor within the last 390 000 years (390 ± 0.47 kyr),

whereas the eastern individuals could be traced to a common ancestor that lived more than 702 000 years ago (702 ± 0.49 ka). The unresolved haplotypes appeared to represent basal lineages because their presence in either the eastern or the western clades increased the age of that clade. For example, when combined with the eastern haplotypes, the divergence date of the eastern clade increased to 1.314 ± 0.012 Mya, whereas the age of the western clade increased to 1.03 ± 0.02 Mya when it contained the unresolved haplotypes.

### Population genetic analysis

Eastern populations were significantly more diverse than were populations west of the Mississippi River, and haplotype frequencies did not differ from neutrality (Table 1). Not only were western populations less diverse, haplotype frequencies deviated from those expected under neutral conditions by exhibiting signatures of recent expansion (Table 1). The lack of variation was pronounced in the Lost Pines region of central Texas where nearly all individuals had identical haplotypes, except for three individuals collected at the southernmost site known of the entire range of *T. septentrionalis* (Caldwell County; Table 1; Supporting Information, Tables S1 and S3; Figs 2, 3).

## DISCUSSION

The most striking result in this study is that the Western Gulf Coastal Plain is an area of limited genetic diversity for both of the surveyed *Trachymyrmex* species. These patterns suggest recent expansion into this region, but also that each species has done so from different origins. The Mexican origin hypothesis, suggested by Rabeling *et al*. (2007), would appear to explain only the pattern found in *T. turrifex*, which exhibited no variation throughout Louisiana and Texas, including collections near the US–Mexican border. This finding suggests that the *T. turrifex* population inhabiting the USA probably expanded northward from Mexico where this species is known to occur (Sanchez-Peña, 2010). This prediction could be tested in the future by including specimens from Mexico.

In contrast, *T. septentrionalis* has a centre of mtDNA diversity in the eastern coastal plain. The greater diversity of *T. septentrionalis* in eastern North America possibly reflects an eastern origin or eastern Pleistocene refugium from which only few lineages have colonized western regions so far. Western populations were significantly less diverse, the most extreme example of which was found in the Lost Pines of central Texas (the south-western-most population of *T. septentrionalis*), where nearly all haplotypes were identical. Only three haplotypes, which were collected at the most south-western site (Caldwell County), differed from the other Lost-Pines collections by three distinct point mutations. This is the strongest evidence against a Mexican origin of *T. septentrionalis*. Less clear is the significance of the poorly resolved lineages containing some of the haplotypes collected in north-east Texas, southern Arkansas, and North Carolina, although these may represent an eastern clade that managed to reach east Texas and southern Arkansas. Unravelling the finer aspects of the phylogeography of *T. septentrionalis* may require a greater understanding of their dispersal biology.

Phylogeographical patterns do not unambiguously support the hypothesis that the Mississippi Embayment was an effective barrier during the post-pleistocene dispersal of *T. septentrionalis* (Mikheyev *et al*., 2008) because haplotypes collected in Tennessee, Mississippi, and Illinois (localities east of the Mississippi Embayment) clustered with those in

**Table 1.** Sample size, number of unique haplotypes, haplotype and nucleotide diversities, and population expansion (using Harpending's *h* and Tajima's D neutrality test) of the three main populations in this study

| Region | Sample size | Number of unique haplotypes | Haplotype diversity (SD) | Nucleotide diversity (SD) | Harpending's *h* | Tajima's D |
|---|---|---|---|---|---|---|
| Western Gulf Coastal Plain | 68 | 18 | 0.695 (0.0037) | 0.00295 (0.00082) | 0.1495 $P = 0.92$ | **–2.07** ***P* = 0.02** |
| 'Lost Pines' of Central Texas | 40 | 4 | 0.146 (0.006) | 0.00021 (0.00011) | 0.7696 $P = 0.85$ | **–2.101** ***P* < 0.0001** |
| Eastern Gulf and Atlantic Coastal Plains | 84 | 55 | 0.980 (0.0004) | 0.0131 (0.0009) | **0.004** ***P* = 0.038** | –0.828 $P = 0.23$ |

The Western Gulf Coastal Plain includes all regions west of the Mississippi River (and the Lost Pines); the Eastern Coastal Plain includes the regions east of the Mississippi River. Western populations exhibit signatures of recent expansion and less haplotypic diversity than those in the east.
Bold text indicates significant differences (a = 0.05).

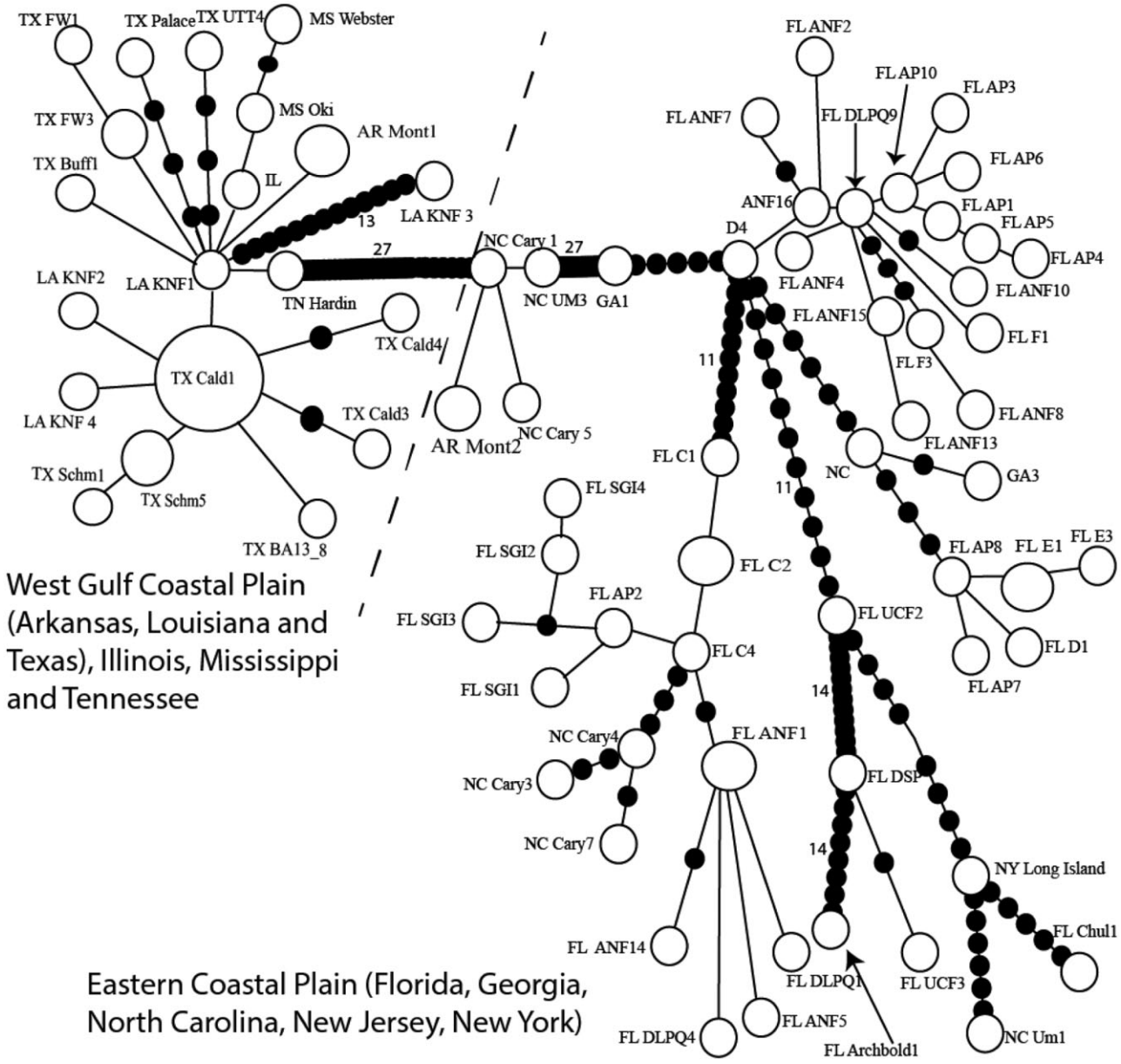


**Figure 3.** Minimum-spanning haplotype network of *Trachymyrmex septentrionalis*. Open circles indicate individual haplotypes. A single bar connecting two haplotypes corresponds to a single base-pair mutation. Solid circles indicate single base-pair mutations. Numbers indicate the number of base-pair mutations between haplotypes inferred in the analysis. Haplotypes found within the western coastal plain tend to be less diverse than those collected in the east. Western haplotypes are separated from eastern haplotypes by one of the largest number of mutational steps ($n = 27$) inferred in the network. Circle size corresponds to frequency of collection (see Supporting Information, Table S3 for frequencies of each haplotype). AR, Arksansas; FL, Florida; GA, Georgia; IL, Illinois; LA, Louisiana; MS, Mississippi; NC, North Carolina; NJ, New Jersey; TN, Tennessee; TX, Texas. Collection IDs refer to collection information in Supporting Information (Table S1).

Texas. Possible explanations for this include that these samples might be an example of dispersal eastward across the more narrow northern Mississippi valley or, alternatively, that the river is not an important phylogeographical barrier, as reported for other taxa; rather, features associated with the Appalachian mountains and the Apalachicola River basin might have been important in structuring late-Pleistocene or early-Holocene populations (Soltis *et al*., 2006).

One possible barrier to westward dispersal of *T. septentrionalis* is an increasingly arid climate. Texas exhibits a pronounced rainfall gradient from east to west, which ranges from > 150 cm to < 25 cm year$^{-1}$, respectively, or an average loss of about 10 cm of rainfall per 100 km (Mueller *et al*., 2011). The western edge of *T. septentrionalis* corresponds to the edge of the loblolly pine distribution and other species typical of the humid south-east (Sorrie & Weakley, 2001; Al-Rabab'ah & Williams, 2004), which corresponds to an average rainfall of approximately 90 cm. In Florida, *T. septentrionalis* inhabits the driest parts of a wet habitat (Seal & Tschinkel, 2010) but it paradoxically exhibits relatively low resistance to desiccation (Hood & Tschinkel, 1990). Although *T. septentrionalis* is found nearly always in xeric, sandy soils, significant expanses of sandy soils can be found outside its distribution in south and central Texas (Fulbright *et al*., 1990; Forman, Gomez & Pierson, 2009), and these appear to lack *T. septentrionalis* but often contain *T. turrifex* (Rabeling *et al*., 2007; U. G. Mueller and J. N. Seal unpubl. data). This finding is consistent with an expansion of *T. septentrionalis* from east to west, which may be influenced by the strong rainfall gradient found in Texas. However, it is unclear if *T. turrifex* is similarly limited by rainfall because it is found much further west in more arid regions of Texas (Rabeling *et al*., 2007). If the distributions are any indicator, then *T. turrifex* should be more resistant to arid environments than *T. septentrionalis*. Accordingly, the inability of *T. turrifex* to expand eastward and northward might be because the Mississippi River is an effective dispersal barrier or because of inadequate adaptation to humid and cooler environments, or both.

Geological and pollen core evidence suggest that the western and eastern Coastal Plains experienced different climates during the Pleistocene (*c*. 2.5 Mya to 11 ka). For example, Texas is thought to have had a wetter and cooler climate during the Pleistocene than today, but by the Holocene (*c.* 11 000 BP), the climate had transitioned to a warmer and drier state with several extreme droughts *c.* 2500–5000 BP (Toomey, Blum & Valestro, 1993; Musgrove *et al*., 2001). Locations further east along the coastal plain, such as Florida, were thought to have had a relatively cooler but drier climate, which became warmer and wetter in the Holocene (Watts, 1980; Watts & Hansen, 1994). Assuming that both *T. septentrionalis* and *T. turrifex* have similar dispersal capabilities and a similar mitochondrial mutation rate, the lack of variation exhibited by *T. turrifex* suggests that this species dispersed into Texas and Louisiana more recently than *T. septentrionalis*, which exhibited some variation in the western coastal plain (Fig. 2, Table 1). However, the lack of variation in *T. turrifex* prevents any conclusions when it invaded Texas. Obtaining more samples of *T. turrifex*, including samples from Mexico, would inform when *T. turrifex* invaded the southern USA. The phylogenetic dating analyses suggested that two major radiations of *T. septentrionalis* occurred in the late Pleistocene, with the radiation containing the individuals from the western coastal plain taking place about 300 000 years after that in the east. However, these dates significantly precede the last glacial maximum (> 20 ka) by several hundred thousand years. Caution is warranted, however, in interpreting these findings because the mitochondrial mutation rates were calibrated using fossils from other species, which may overestimate divergence dates (Pulquérioa & Nichols, 2007; Campbell-Staton *et al*., 2012; Tollis *et al*., 2012; Tollis & Boissinot, 2014).

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Table S1.** Collection information of individual *Trachymyrmex septentrionalis* specimens used in this study. GPS coordinates of sites under private ownership have been omitted, but may be provided upon request.
**Table S2.** Collection information of individual *Trachymyrmex turrifex* specimens used in this study. GPS coordinates of sites under private ownership have been omitted.
**Table S3.** Collection information of *Trachymyrmex septentrionalis* haplotypes obtained in this study. Haplotype codoes refer to collection information in Table S1.