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Comparative population genetics of two invading ticks: Evidence of the ecological mechanisms underlying tick range expansions

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ABSTRACT

Two species of ixodid tick, *Ixodes affinis* Neumann and *Amblyomma maculatum* Koch, are simultaneously expanding their ranges throughout the mid-Atlantic region of the US. Although we have some understanding of the ecology and life history of these species, the ecological mechanisms governing where and how new populations establish and persist are unclear. To assess population connectivity and ancestry, we sequenced a fragment of the 16S mitochondrial rRNA gene from a representative sample of individuals of both species from populations throughout the eastern US. We found that despite overlapping host preferences throughout ontogeny, each species exhibited very different genetic and geographic patterns of population establishment and connectivity. *I. affinis* was of two distinct mitochondrial clades, with a clear geographic break separating northern and southern populations. Both *I. affinis* populations showed evidence of recent expansion, although the southern population was more genetically diverse, indicating a longer history of establishment. *A. maculatum* exhibited diverse haplotypes that showed no significant relationship with geographic patterns and little apparent connectivity between sites. Heteroplasmy was also observed in the 16S mitochondrial rRNA gene in 3.5% of *A. maculatum* individuals. Genetic evidence suggests that these species rely on different key life stages to successfully disperse into novel environments, and that host vagility, habitat stability and habitat connectivity all play critical roles in the establishment of new tick populations.

1. Introduction

The spread of parasites is a major source of disease emergence for vertebrate taxa, including humans (George, 2008; Mack et al., 2000; Williams et al., 2013). Climate change and anthropogenic landscape alterations, such as fragmentation and suburban sprawl, have been linked to tick range expansions worldwide, resulting in changes to tick community structure and altered tick-borne pathogen dynamics (Childs and Paddock, 2003; Cumming and Van Vuuren, 2006; Gage et al., 2008; George, 2008; Léger et al., 2013).

In North America, the expanded ranges of the lone star tick, *Amblyomma americanum*, and the blacklegged tick, *Ixodes scapularis*, have received much attention because of the importance of these ticks to human health (Brinkerhoff et al., 2009; Childs and Paddock, 2003; Ogden et al., 2008; Springer et al., 2014). More recently, *Ixodes affinis* and the Gulf coast tick, *Amblyomma maculatum*, have invaded the eastern United States (US), changing tick community and pathogen dynamics in the affected areas (Fornadel et al., 2011; Harrison et al., 2010; Nadolny et al., 2011; Paddock and Goddard, 2015; Varela-Stokes et al., 2011; Wright et al., 2011). Because of their small size and vulnerability to the environment when off-host, tick dispersal is intricately linked to movements of their hosts (Falco and Fish, 1991). The ecological processes driving tick invasions require study in order to better predict and mitigate disease emergence and improve understanding of tick behavior.

Coalescent genetic analyses can reveal the ancestry of newly established populations, and offer clues to how organisms disperse over long distances. The use of molecular methods to trace the ancestry of invading species is well established (Templeton et al., 1995; Ibrahim et al., 1996; Le Roux and Wieczorek, 2009). For more than 30 years, molecular methods have been used to investigate tick population genetic structure (Araya-Anchetta et al., 2015). Previous studies have documented the effects of host-mediated dispersal on genetic structure in the seabird tick *Ixodes uriae*, using

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microsatellite markers to determine how gene flow patterns changed depending on the host species exploited for dispersal (McCoy et al., 2001, 2003). Other studies have used phylogenetic analyses to determine recent population expansion, identify founder effects, and examine population structure at the expansion fronts of *A. americanum* and *I. scapularis* (Kelly et al., 2014; Mechai et al., 2013; Ogden et al., 2011). Some broad conclusions reached by this body of work are that tick behaviors and life cycle strategies are as critical as host mobility in understanding tick population genetic structure, particularly as applied to different families of ticks (Araya-Anchetta et al., 2015).

The northward expansions of *I. affinis* and *A. maculatum* into the Mid-Atlantic region of the US have been documented over the last decade (Florin et al., 2014; Fornadel et al., 2011; Harrison et al., 2010; Nadolny et al., 2011; Varela-Stokes et al., 2011; Wright et al., 2011). Both species are recent additions to a diverse assemblage of ticks and have the potential to significantly affect pathogen dynamics (Oliver, 1996; Nadolny et al., 2011, 2014). *I. affinis* is a competent sylvatic vector for *Borrelia burgdorferi* sensu stricto, the bacterial agent of Lyme disease, and *A. maculatum* is the major vector for *Rickettsia parkeri*, the agent of Tidewater spotted fever, and several significant veterinary pathogens (Oliver et al., 2003; Paddock et al., 2004; Paddock and Goddard, 2015). Both species have expanded northward from historic ranges in the southern US and Central America and both parasitize avian and mammalian hosts throughout their ranges (Harrison et al., 2010; Paddock and Goddard, 2015; Teel et al., 2010).

Despite some overlap in the vertebrates parasitized by these two tick species, *I. affinis* and *A. maculatum* are generally found in different habitat types and are expanding their ranges in different geographic patterns. The northern edge of the expansion front for *I. affinis* is currently in Virginia (Nadolny et al., 2011), and individuals can be consistently collected at low densities in woodland habitats (Gaff and Nadolny, unpublished data). *A. maculatum* appears to be expanding in isolated populations in ephemeral successional habitats, with large areas containing few to no *A. maculatum* separating established breeding groups (Gaff and Nadolny, unpublished data). These expansion patterns reflect the distributions of these ticks in their native ranges (Harrison et al., 2010; Teel et al., 2010; Varela-Stokes et al., 2011), but the implications of these two expansion types on population connectivity are largely unexplored.

Several genetic markers have been historically used to characterize population structure in tick populations, most commonly nuclear microsatellites and variation in mitochondrial genes. Microsatellites have been characterized for twelve tick species (Araya-Anchetta et al., 2015). Microsatellites, however, are often unable to achieve cross-species amplification (McCoy and Tirard, 2000) and are also not universally abundant in all tick genomes (Fagerberg et al., 2001). The use of the 16S mitochondrial rRNA gene is pervasive in studies of tick population genetics and tick phylogeny (Black and Piesman, 1994; Kelly et al., 2014; Mixson et al., 2006; Norris et al., 1996; Qiu et al., 2002). This gene mutates at a rate that is generally informative for species-level phylogenetics and broad biogeographic inferences (Araya-Anchetta et al., 2015).

In this study, we used single nucleotide polymorphisms (SNPs) in the 16S mitochondrial rRNA gene to examine patterns of genetic structure in *I. affinis* and *A. maculatum* ticks across the eastern US. This method enabled direct comparison of our results across our two species without the need to characterize two novel sets of species-specific microsatellites. Using a mitochondrial gene was also appropriate for the scale of our research, as we were investigating broad biogeographic patterns of connectivity across many US states (Araya-Anchetta et al., 2015). We used the resulting information on haplotype frequencies, geographic and genetic distance between new populations, and host and habitat associations of each tick species to describe likely routes by which these ticks are expanding their ranges.

Field-dwelling ticks and generalist ticks that parasitize a range of hosts are less likely to exhibit genetic structure than nidicolous (nest-dwelling) ticks, single-host ticks, or ticks that specialize on hosts with small home ranges (Araya-Anchetta et al., 2015). We compare the genetic structure of two non-nidicolous tick species that are expanding their ranges simultaneously across the eastern US, and that share host preferences for avian and mammalian hosts. We discuss how tick ecology and behavior may affect population structure and use this structure to infer likely invasion routes and important life history characters that have facilitated range expansion for each species. We hypothesized that both species would exhibit isolation by distance, with northern populations originating from related southern populations.

2. Materials and methods

2.1. Study sites and tick collection

Questing adult ticks were collected from populations in Virginia, North Carolina, and South Carolina using standard tick collecting methods, as previously described (Nadolny et al., 2011; Wright et al., 2011). Ticks and/or tick DNA extracts from additional populations in states throughout the established US ranges of both ticks were included to provide a more complete analysis of population structure (Supplementary Table 1). After morphological species identification (Keirans and Litwak, 1989; Sonenshine, 1979), ticks were frozen at −80 °C prior to DNA isolation.

2.2. Molecular methods

Prior to DNA extraction, individual ticks were placed in 2 mL microcentrifuge tubes containing 1 and 2.5 mm glass beads and homogenized in a bead-beater (BioSpec Products, Inc., Bartlesville, OK) for 30 s. DNA was then extracted from individual adult ticks using a DNeasy Blood & Tissue Kit (Qiagen Inc., Valencia, CA), eluted in 50 μL of buffer AE and stored at −20 °C until processing.

We used standard PCR to amplify a fragment of the tick mitochondrial 16S rRNA gene using primers 16S + 1 (5′-CTGCTCAATG ATTTTTTAAATTGCTGT-3′) and 16S − 1 (5′-GTCTGAACTCAGATCAA GT-3′) (Macaluso et al., 2003; Nadolny et al., 2011). PCR reactions were performed in 15 μL reaction volumes, with 0.05 U/μL Taq DNA Polymerase (BioPioneer Inc., San Diego, CA), 1 μM each primer, 1.5 mM $MgCl_2$, 1× PCR buffer (Qiagen Inc., Valencia, CA) and 2 μL DNA template. The PCR protocol consisted of an initial 3-min denaturation step at 95 °C followed by 30 cycles of 95 °C for 30 s, 52 °C for 45 s and 72 °C for 1 min, with a final extension at 72 °C for 7 min. PCR products were visualized on 1.5% agarose gels, and purified using ExoSap-IT (Affymetrix Ltd., Santa Clara, CA). Sequencing reactions were performed using the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). Amplicons with overlapping peaks, indicating heteroplasmy, were cloned into the PCR 2.1 TOPO vector using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA), and resulting plasmid inserts were sequenced, as above. DNA sequences were identified by BLAST search (Altschul et al., 1990). Successfully amplified DNA provided unambiguous bi-directional sequence data along the length of the sequence for all samples. All sequences were aligned and consensus sequences were constructed using Geneious R7 (http://www.geneious.com, Kearse et al., 2012).