

True syntopy between chromosomal races of the *Cryptocercus punctulatus* wood-roach species complex

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Abstract In this paper, genetic evidence for local co-occurrence (syntopy) between members of two chromosomal races within the *Cryptocercus punctulatus* species complex from the Southern Appalachian Mountains is presented. This phenomenon has not previously been detected, despite extensive sampling and numerous molecular studies of this the sub-social wood-roach spanning the past 20 years. The incidence of syntopy seems rare; it was detected in one of 70 rotting logs sampled across eight states in the southern United States. However, the location where it occurs could become a key focus of research aimed at resolving taxonomic uncertainty in this group.

Keywords Chromosomal race · Cockroach · Geographic range · Southern Appalachian Mountains

Introduction

Saproxyllic (dead wood-dependent) insects are instrumental ecosystem service providers that make major contributions to decomposition and nutrient cycling in forests (Ulyshen

2016). Among the invertebrate detritivores, wood-feeding *Cryptocercus* cockroaches contribute significantly to the breakdown of coarse woody debris (Nalepa et al. 2002). Members of this taxon are also excellent model organisms for reconstructing landscape history, particularly in the Appalachian Mountains (Nalepa et al. 2002; Garrick 2011), and they are a key group for understanding early evolution of termites (Klass et al. 2008).

Despite the ecological, biogeographic and evolutionary importance of *Cryptocercus*, the taxonomic status and geographic distribution of Appalachian Mountain lineages (“*C. punctulatus* species complex”) remains uncertain. Briefly, Kambhampati et al. (1996) first reported the existence of four chromosomal races within the complex, and Burnside et al. (1999) subsequently described them as separate species. However, the basis for their taxonomic subdivision has been questioned, and so it has not been widely adopted (Nalepa et al. 2002; Lo et al. 2006; Everaerts et al. 2008). There are also conflicting reports on the spatial distributions of the four races, which have been described as either strictly allopatric (Burnside et al. 1999), parapatric (Nalepa et al. 2002; Everaerts et al. 2008), or sympatric (Steinmiller et al. 2001). One likely reason for this uncertainty is because the majority of previous studies with dense (i.e., population-level) sampling have based race assignment on just one individual per site (e.g., Steinmiller et al. 2001; Aldrich et al. 2004).

Here, sampling of 70 rotting logs spanning eight states (Fig. 1a; Online Resource 1), coupled with mitochondrial DNA (mtDNA) sequencing of multiple (3) cockroaches from each log, led to the discovery that individuals from one log exhibited very high levels of sequence divergence (see “**Results and discussion**”). This prompted further analyses to determine whether they represented members of different chromosomal races.

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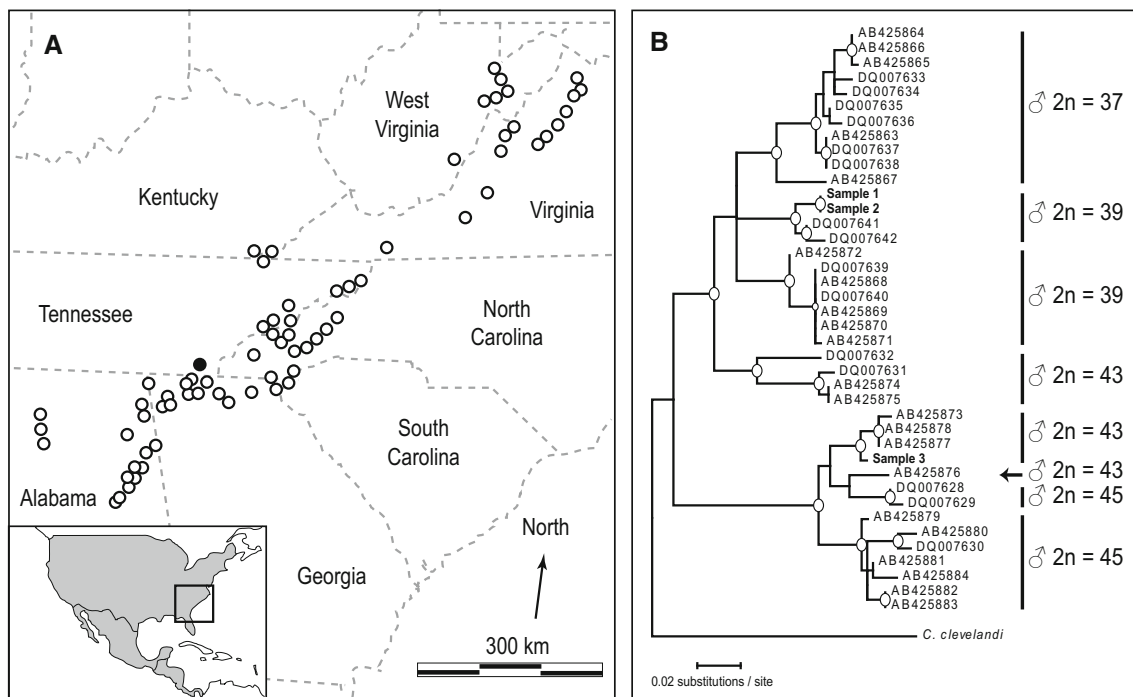


Fig. 1 Evidence for syntopy between chromosomal races. **a** Geographic distribution of 70 rotting logs across the southern United States. The *solid circle* represents the location in Polk County, Tennessee, where members of two different races were sampled from the same rotting log (*inset*: map of North and Central America, showing location of the study region). **b** Assignment of chromosomal

race for three individuals from the Polk County rotting log, based on phylogenetic comparison to 37 COII sequences in a reference dataset derived from GenBank. Terminals on the maximum likelihood tree are labeled with GenBank accession numbers (samples 1–3 are from the present study). Well-supported nodes (bootstrap values >70 %) are marked with *white circles*

Materials and methods

Procedures used for DNA extraction from three adults per rotting log, and polymerase chain reaction amplification and sequencing of mtDNA *cytochrome oxidase subunit I* (COI) and *subunit II* (COII) genes, are given in Online Resource 2. Initially, the level of mtDNA sequence divergence among individuals from the same rotting log was estimated using uncorrected *p* distances, calculated in MEGA v6.06 (Tamura et al., 2013). Previous molecular phylogenetic studies have shown that divergent lower-level monophyletic clades on mtDNA gene trees include members of only a single chromosomal race, even though the races themselves may not be monophyletic (Fig. 1b; Kambhampati et al. 1996; Burnside et al. 1999; Steinmiller et al. 2001; Lo et al. 2006; Everaerts et al. 2008). Thus, phylogenetic approaches can be used to assign an “unknown” individual to a chromosomal race. The COII region sequenced here is directly comparable to sequence data generated by two previous studies (Lo et al. 2006; Everaerts et al. 2008) in which the chromosomal race of 37 individuals was directly determined. This “reference dataset” enabled chromosomal race to be assigned to each of the three individuals with large mtDNA sequence divergences that were sampled from the

same log in the present study (samples 1, 2 and 3 herein; GenBank accessions: KU609620–KU609625). Assignments were performed via phylogenetic analyses of the 40 COII sequences (i.e., reference plus unknown samples) using two approaches: estimation of a maximum likelihood tree, and construction of a haplotype network (Online Resource 3).

Results and discussion

One rotting log in Polk County, Tennessee (coordinates: 35.10896, –84.62477; elevation: 530 m; Fig. 1a), sampled in August 2012, contained individuals that differed in their mtDNA sequences by up to 18 % (COI + COII uncorrected *p* distance). This level of divergence far exceeds any previously reported within-site differences (typically ≤ 1 % for 12S + 16S rRNA genes; Burnside et al. 1999). Based on maximum likelihood phylogenetic analysis, two individuals (samples 1 and 2, both females) were assigned to the ♂ $2n = 39$ chromosomal race, and the other individual (sample 3, a male) was a member of the ♂ $2n = 43$ race (Fig. 1b). The haplotype network-based analysis returned the same assignments (Online Resource 4).

Several previous studies have uncovered geographic regions where two or more chromosomal races of the *C. punctulatus* species complex occur in close proximity. For example, based on collections for which chromosomal race was directly determined, members of the $\delta 2n = 37, 39$ and 45 races all come within ~ 6.5 km of one another near Clingmans Dome in Great Smoky Mountains National Park (Nalepa et al. 2002; Everaerts et al. 2008). Phylogenetically assigned chromosomal races have been used to identify other locations where two races might co-occur. For instance, Aldrich et al. (2004) identified three potential locations of co-occurrence, one of which was within ~ 30 km of the focal rotting log identified in the present study. However, despite extensive sampling and numerous molecular studies spanning the past 20 years, the present study is the first to detect true syntopy in the *C. punctulatus* species complex.

Currently, evidence for or against reproductive compatibility among races of the *C. punctulatus* species complex remains equivocal, and so the main finding of the present study is important for two reasons. First, it overturns previously held ideas about the lack of syntopy between races, such that the opportunity for hybridization is now established. Second, it identifies the location of a “testing ground” for assessing the Biological Species Concept. This information, when coupled with the development of versatile co-dominant nuclear genetic markers (e.g., Garrick et al. 2010), should facilitate future studies aimed at resolving taxonomic uncertainty surrounding this group.

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