JOURNAL OF THE KANSAS ENTOMOLOGICAL SOCIETY
82(4), 2009, pp. 316–320

SHORT COMMUNICATION

Lack of Variation in Nuclear Genes Among Isolated Populations of the Sand Dune Restricted Bee *Colletes stepheni* (Hymenoptera: Colletidae)

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Sand dunes in the Mojave and Great Basin Deserts are unique habitats that often harbor distinct and endemic taxa (Ricketts *et al.*, 1999). Epps *et al.* (1998) compared sand dunes to ''habitat islands'' in the desert, which provide habitat for many sand-obligate organisms. Because many of these dunes are distant from other dunes, and often flanked by mountain ranges or alkaline hardpan, isolated populations of dune-restricted organisms are often genetically distinct (e.g., Britten and Rust, 1996; Epps *et al.*, 1998).

We attempted to discover phylogeographic patterns among populations of *Colletes stepheni* Timberlake, a sand dune restricted, oligolectic solitary bee. *Colletes stepheni* nests solely in loose sandy substrates and provisions its nest with pollen only from a limited number of plant species (Hurd and Powell, 1958; Minckley *et al.*, 2000; Andrus, 2003). Populations of *C. stepheni* in the Mojave Desert use pollen only from *Larrea tridentata* (creosote bush; Zygophylaceae), which is a common shrub in the hot deserts of North America, while populations in the Great Basin Desert, a cold desert, use pollen from *Psorothamnus* (indigo bush; Fabaceae) (Andrus, 2003).

Andrus (2003) attempted to find phylogeographic patterns among populations of *C. stepheni* from 13 dune sites across the Mojave and Great Basin Deserts using mtDNA. These attempts uncovered the presence of nuclear mitochondrial pseudogenes (NUMTS), which are mitochondrial genes that have been incorporated into the nuclear genome (Margulis, 1970). The presence of these pseudogenes in *C. stepheni* made the use of mtDNA for phylogeographic analyses difficult, if not impossible.

While the majority of phylogeographic analyses use mtDNA, particularly the gene COI, to investigate phylogeographic patterns, several recent studies employ various nuclear genes to uncover intraspecific variation. For example, a phylogeographic study of sea urchins found that the internal transcribed spacer region 2 was more variable at the population level than COI (Iuri *et al.*, 2007). Studies on several other taxa have also shown that the internal transcribed spacer regions 1 and 2 (ITS1 & ITS2) are variable at the population level (e.g., Mavárez *et al.*, 2002; Gómez-Zurita and Vogler, 2003; Holderegger and Abbott, 2003).

While nuclear genes are not widely used in phylogeographic studies of Hymenoptera, several recent studies have found population-level variation in various hymenopterans. For example, variation has been found among populations of stingless bees (De la Rúa *et al.*, 2007), an encyrtid wasp (Alvarez and Hoy, 2002), an oak gallwasp (Rokas *et al.*, 2001), and several velvet ant species (Wilson and Pitts, 2008, 2009). Other studies on velvet ants (Hymenoptera: Mutillidae), while not specifically investigating intraspecific variation, showed that such variation exists in both the ITS1 and ITS2 sequences (Pilgrim and Pitts, 2006; Pitts *et al.*, 2007; Pilgrim *et al.*, 2008).

Another nuclear gene, Long-wavelength rhodopsin (opsin), has not been widely used in population-level analyses, but has three introns (167 total bp in *C. stepheni*), which have been informative in differentiating between isolated populations of mutillid wasps (Wilson and Pitts, unpubl. data). Also, Almeida and Danforth (2009) in a phylogenetic analysis of colletid bees found that the introns of opsin were too variable to be used in a species-level analysis. This would suggest that the introns might be informative at the intraspecies level. Kuhlmann *et al.* (2007) found population-level differences in the introns of another nuclear gene Elongation Factor 1-Alpha for *Colletes halophilus* as well.

In this study, we attempted to find phylogeographic patterns using three nuclear genes, the two rDNA internal transcribed spacer regions (ITS1 & ITS2), and the long-wavelength rhodopsin gene (Opsin).

Accepted 24 September 2009; Revised 26 October 2009

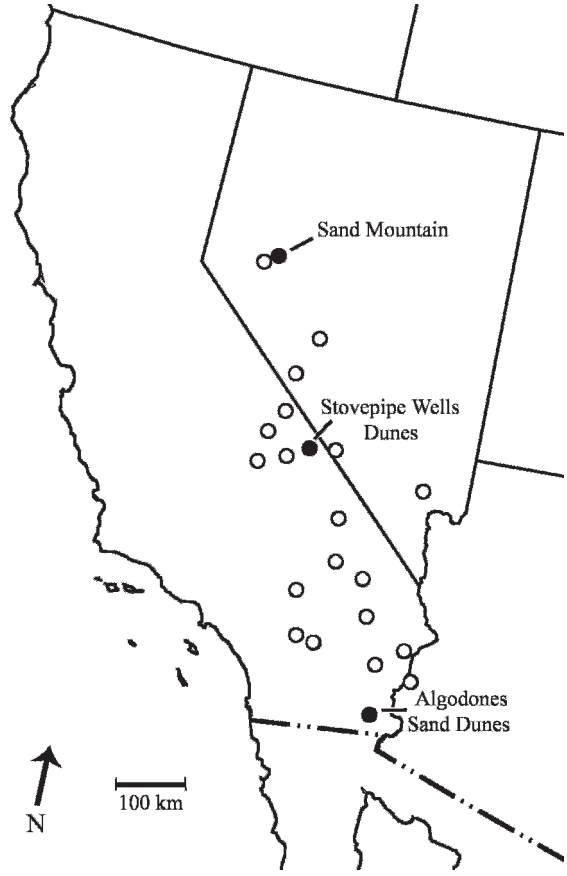

Fig. 1. Map showing the locations of the sand dunes in the Mojave Desert and the surrounding area. Closed circles indicate the dunes from which *Colletes stepheni* DNA was sampled. Open circles indicate other dunes that may provide habitat for *C. stepheni.*

Materials and Methods

Because this study was simply a preliminary assessment of population-level diversity, bees were sampled from populations from three isolated dune systems: one in the southernmost range of the species, the Algodones Sand Dunes in southern California; another in the center of the species' range, the Stovepipe Wells dunes in Death Valley, California; and one in the northernmost portion of the range of the species, Sand Mountain, Nevada (Fig. 1). To determine if genetic differences could be found between dune systems, genetic information was collected from one bee from each of the three sand dunes. Often, when exploring new genes for phylogeographic analyses, researchers will simply sample one individual from distant geographic areas to determine if variation exists at the macrogeographic scale to limit loss of time and funds (e.g., Presa *et al.*, 2002). Additionally, it is common to find evidence of historical barriers to gene flow by only examining one individual from each isolated area (e.g., Tan and Wake, 1995; Devitt, 2006). Even with nuclear genes evidence of historical isolation can be found by simply examining two individuals from separate areas (e.g., Schroth *et al.*, 2002; Gantenbein and Largiadèr, 2003; Wilson and Pitts, 2008, 2009). If variation is not found in a limited number of specimens from widely separated sites, these genes are likely useless for population-level studies. Because of the difficulty in using mtDNA in this system (Andrus, 2003), we chose three nuclear genes that have shown variation in other population-level analyses to test for phylogeographic patterns in *C. stepheni*. The two rDNA genes ITS1 and ITS2 were amplified and sequenced following the protocol described by Pilgrim and Pitts (2006).

We also amplified and sequenced the long-wavelength rhodopsin nuclear gene (opsin) from two widely separated populations from the Algodones dunes and from Sand Mountain (Fig. 1). All sequences were

aligned and compared using Sequencher 4.1 (Gene Code Corp., Ann Arbor, MI). All sequences have been submitted to GenBank (Accession nos. GU132308–GU132315).

Results and Discussion

All of the dunes from which bees were sampled are a minimum of 320 km from each other (Fig. 1). Although other sand dunes intervened between the populations from which we sampled, these dunes are still separated from each other by a minimum of 60–100 km (Fig. 1). More than 2100 base pairs were compared between individuals from isolated populations. All individuals sampled had identical sequences for each of the three genes tested.

This lack of genetic diversity among widespread populations of C. *stepheni* is surprising given the variation found among other hymenopterans. Because the populations we sampled were at least 320 km apart, and isolated from each other by large expanses of inhospitable habitat, it was expected that some level of genetic variation would be observed. Furthermore, we expected to see evidence of divergence due to differences in pollen host; Kuhlmann *et al.* (2007) suggested that when the different populations of a species exhibit different life history strategies (i.e., floral preferences), the populations may belong to different genetic strains. We sampled from populations that utilize different host plants, yet we found no genetic differences between these populations.

There are multiple possible explanations as to why we see no variation among these dune restricted bee populations. One reason could be that the genes we used are not informative at this level. Because we see variation between populations of several other species, this explanation seems unlikely. Another possible explanation for this phenomenon could be that there is significant gene flow between populations isolated on separate dunes, which would suggest that these populations are, in fact, not as isolated as previously thought. It is well established that this species nests solely on loose sandy substrates (Hurd and Powell, 1958), so it is very unlikely that populations are living in the desert areas between dune systems which contain no such substrates. In the Mojave and Great Basin deserts, sand dunes are rare when compared to the more common calcareous and alkali soils (MacMahon and Wagner, 1985; Ricketts *et al.*, 1999). Additionally, while creosote bush, the bee's, host plant, is found throughout the Mojave Desert, it is unlikely that individual bees would disperse across the vast inhospitable distance between dune fields (T. Griswold, pers. comm.). Future analyses using microsatelites possibly could be used to test for gene flow between populations.

Another possible explanation for the genetic congruency is that *C. stepheni* recently speciated and, therefore, not enough time has passed for differences to occur among different populations. The majority of the dunes in western North America are thought to be recently formed, with most less than 10,000 years old (Norris and Norris, 1961; Sharp, 1966; Macdonald, 1970; Mehringer, 1986). Despite the young age of the dune systems, many taxa have diversified extensively on the dunes of western North America, and many animals are endemic to these particular dune systems. For example, the Mexican Fringe-Toed Lizard (*Uma exsul* Schmidt and Bogert) is endemic to a dune system in Coahuila, Mexico, and another lizard, *Uma inornata* Cope, is endemic to the dune systems in the Coachella Valley of California (Stebbins, 2003; Garcia-De la Peña *et al.*, 2007). Given that multiple vertebrate species have evolved into separate, endemic species since the apparent formation of the dunes, it seems likely that separate populations of *C. stepheni* would also develop different mutations in non-coding regions of DNA, especially because insect DNA, including nuclear DNA, generally exhibits a faster rate of change than that of most vertebrates (Caccone and Powell, 1990). Additionally, because *C. stepheni* is only found on the dunes of western Nevada, southern California, and extreme northern Baja California, Mexico, we do not think the lack of genetic variation between isolated bee populations is a result of recent expansion onto the dunes we sampled.

While it is evident that *C. stepheni* is restricted to sand dunes, and that these dunes are separated from each other by expanses of uninviting desert landscapes, there appears to be no sign of genetic isolation, even between populations isolated by over 300 km. Although data from other arthropods isolated on some of these same dunes suggest genetic differentiation (Britten and Rust, 1996), it remains unclear why remote populations of *C. stepheni* do not show any indication of genetic isolation. Because none of the three possible explanations about the lack of genetic diversity in *C. stepheni* seem probable, additional studies are needed to understand this situation. Perhaps uncovering the mechanisms behind the intriguing lack of genetic diversity of *C. stepheni* will aid in our understanding of regulatory mechanisms maintaining the gene pools of other sand dune restricted organisms.

Acknowledgments

We would like to thank Rebekah Andrus Nelson for allowing us to use her extracted DNA from multiple populations of *C. stepheni*. We would also like to thank Terry Griswold for discussions on the

biology of *C. stepheni* and the different dune systems on which *C. stepheni* was collected. Also, we thank the editor and two anonymous reviewers whose comments greatly contributed to this manuscript. This research was supported by the Utah Agricultural Experiment Station, Utah State University, Logan, Utah. Approved as journal paper no. 8012.

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