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GENETIC ANALYSIS OF BED BUG INFESTATIONS AND POPULATIONS

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Abstract Populations of the common bed bug *Cimexlecturalius*are undergoing a huge resurgence, but little is known about their patterns of spread and dispersal. We have begun to address this information gap by conducting population genetic studies of bed bug infestations in the U.S. using microsatellite markers and mtDNA sequence data. In addition, we investigated the frequency of insecticide resistance (*kdr*) alleles in the U.S. Across 17 populations from the eastern U.S., we found high genetic diversity at microsatellite loci, suggesting that these populations likely have heterogeneous origins rather than being introduced from a single source population. However, within individual populations, genetic diversity was low, consistent with each population being started by a small number of individuals, even a single inseminated female. Microscale studies of aggregations within apartments in a single, multi-unit building indicate that the building was likely infested through a single introduction of an inseminated female which founded a population that subsequently expanded and spread to more than 20 apartments. Finally, we found high prevalence of one or both of two mutations for insecticide resistance in 38 populations in the U.S. Together our results provide important insights into the modes of dispersal, levels of genetic diversity and information on the frequency of insecticide resistance in U.S. populations of bed bugs.

Key Words *Cimex lectularius,*microsatellites, mitochondrial DNA, insecticide resistance, dispersal, population genetics

INTRODUCTION

The common bed bug, *Cimex lectularius* L.*,* is currently undergoing a dramatic resurgence in the U.S and other developed countries. A large gap in our understanding of the rapid increase in bed bug infestations is a lack of information on the geographical sources of the resurgent populations and their patterns of dispersal. Resurgent bed bug populations could come from either local sources that have recently expanded, or they could be the result of one or a few source populations that have been spread globally through human transport.

Bed bugs can disperse through both active and passive means (Usinger, 1966; Pinto et al., 2007; Reinhardt and Siva-Jothy, 2007; Wang et al., 2010). It has been assumed that active dispersal is limited to short distances, such as between adjoining rooms or apartments in the human environment. Passive traps (i.e., without lures) placed in front of apartment doors within a heavily infested building in Indianapolis, Indiana captured dispersing insects, primarily adults (Wang et al., 2010), demonstrating that some active dispersal between apartments can occur. However, the distances that such individuals are able to travel on their own and their ability to successfully colonize new apartments have not been established in the field.

Although many factors are no doubt contributing to the upsurge in bed bug infestations, it is becoming increasingly clear that insecticide resistance, particularly to pyrethroid insecticides, is prevalent. Resistance to pyrethroids (and DDT) in the U.S. is widespread and has been shown to be associated with two point mutations (V419L and L925I) in the *para*-type voltage-sensitive sodium channel α-subunit gene, known as knockdown resistance (*kdr*) (Romero et al., 2007, 2009; Yoon et al., 2008; Zhu et al., 2010), with some populations showing >2500× higher tolerance to deltamethrin exposure compared to susceptible populations. While these recent studies shed important new light on the likely mechanisms of insecticide resistance and its prevalence, they do not inform us about where or how resistance developed or the mechanism(s) by which it has spread throughout the U.S. and elsewhere.

In this study, we use two classes of molecular genetic markers to help determine the sources of bed bug infestations, elucidate patterns of their dispersal and spread in infested areas, and clarify the relationship among bed bugs infestations across the U.S.

MATERIALS AND METHODS

Marker Development

We developed 25 polymorphic microsatellite lociusingpyrosequencing technology to identify microsatellite regions in genomic DNA and develop primers for their amplification by PCR(Allentoft et al., 2009). Figure 1 shows a subset of the loci we developed for *C. lectularius*.

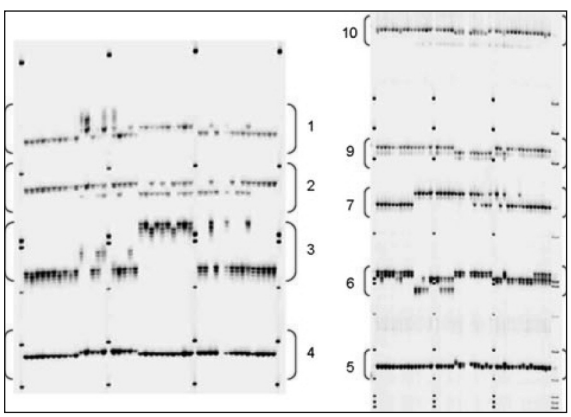

Figure 1. Genetic variation in C. lectulariusat 10 multiplexed microsatellite loci: set 1(left; Loci 1-4) and set 2 (right; Loci 5-10), screened for four apartment samples (n = 10 per sample). Each lane represents a single individual. Individuals were loaded with a marker ladder every 10 lanes.

Sample Collection

Population diversity and differentiation.To investigate the genetic structure within and among aggregations along the East Coast of the U.S., we sampled 17 aggregations by collecting 10 or more individuals from a single room in an infested structure. Figure 2 shows the locations of the study populations.

Microspatial genetic structure and dispersal within buildings. We collected samples from 20 apartments within a single multi-apartment building in Raleigh, North Carolina.

Geographic variation in kdr genotype. Samples were collected in 38 populations in the U.S., mostly in the eastern part of the country (Figure 3).

Genetic Methods

DNA was extracted using the DNEasy Kit (Qiagen, Valencia, California). Microsatellite genotyping was performed on a Li-Cor automated DNA sequencer as described by Booth et al. (2011). We used eight microsatellite loci in the study of population differentiation and 18 loci in the microscale study of a single building apartment. *Kdr* genotype was determined for two genomic regions of the sodium channel α-subunit genes (region 1 = 419; region 2 = 925) by sequencing according to the methods of Yoon et al. (2008). Amplification of the 16S mitochondrial gene was performed following the methods of Szalanski et al. (2008).

Data Analysis

For microsatellite genotypes, the program GenePop on the Web (Raymond and Rousset, 1995) was used to test for Hardy-Weinberg Equilibrium (HWE) and linkage disequilibrium, and to conduct *G*-tests of population differentiation. The program FSTAT (Goudet, 1995) was used to determine genetic diversity, including number of alleles and expected and observe heterozygosities, calculate fixation indexes (*F*-statistics), and estimate the degree of relatedness among individuals within aggregations.

RESULTS AND DISCUSSION

Population Diversity and Differentiation

Across all populations (aggregations collected from a single room), allelic diversity ranged from 6 to 13 alleles per locus with an average of 8.4. Observed heterozygosities ranged from 0.136 to 0.698 per population with an

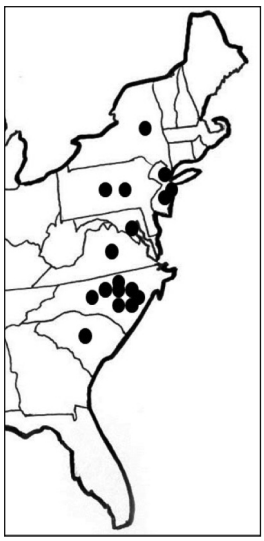

Figure 2. Locations of the 17 populations sampled for the study of population diversity and differentiation.

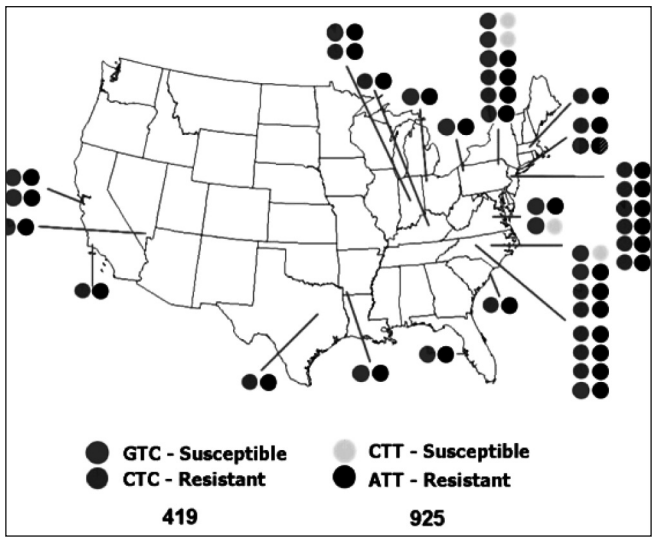

Figure 3. Insecticide resistance profiles (kdr mutations) for 38 populations of bed bugs sampled in the U.S. Each population, represented by 2 side-by-side pie charts (419 region left, 925 region right), consisted of 10 individuals.

average of 0.281, which did not differ significantly from the expected value of 0.301. With one exception, there wereno significant deviations from Hardy-Weinberg Equilibrium. No significant evidence for linkage disequilibrium was detected among possible pair-wise locus comparisons.

Within single collection locations, we detected no more than four alleles in all but one sample, in which we found 5 alleles at one locus. Individual aggregations may therefore represent offspring from a single mated female. Relatedness estimates, based on Queller and Goodnight's (1989)*r*, support these preliminary conclusions (average $r = 0.75$, [0.719 – 0.797, 95% CI]), and suggest that infestations consist of highly inbred populations. These findings raise the question of whether bed bug populations suffer from inbreeding depression, as do natural populations of many organisms (Keller and Waller, 2002), and if so, what the long term consequences of this might be for their persistence and dispersal. This certainly deserves future study.

We found significant genetic structuring among populations (overall $F_{ST} = 0.618$ [0.570 – 0.665, CI]). Within cities, F_{ST}-values were equally high (Raleigh, NC: 0.460 [0.276 – 0.519, CI]; Winston-Salem, NC: 0.738 [0.588 – 0.863, CI]), as were those within states (North Carolina: 0.503 [0.497 – 0.584, CI]; Pennsylvania: 0.337 [0.237 – 0.410, CI]; New Jersey: 0.613 [0.400 – 0.728, CI]). All pair-wise population comparisons were significant ($P < 0.05$). Overall, samples were no more likely to cluster together with other samples from the same state as they were to cluster with samples from other states.

Our preliminary results point to two conclusions regarding the sources of bed bugs in the U.S. and their dispersal. First, the relatively high level of genetic variation across the 17 studied populations in the eastern U.S. (6-13 alleles per locus) make it unlikely that all the current infestations originated from a single introduction from abroad. Second, each individual infestation appears to be started by a single female and her descendants. This conclusion is supported by our results of the fine scale genetic structure within a single building described below.

Microspatial Genetic Structure and Dispersal Within Buildings

Among the 20 apartments sampled in the single multi-unit building, allelic diversity of the 18 microsatellite loci ranged from 1.21 to 2.07 alleles per locus/population (aggregation), with an average of 1.69 alleles. Over all populations within the building, we found an average of 2.78 alleles per locus. Observed heterozygosity across all loci averaged 0.244 and did not deviate significantly from the expected value of 0.260. Only a single aggregation was found to deviate from Hardy-Weinberg expectation. When considered in combination with the number of alleles detected per locus across all samples (<3), our results suggest a single introduction event followed by population expansion and spread throughout the building.

Dispersal pathways within the building do not appear to follow simple routes. For example, populations in adjacent apartments did not necessarily share strong genetic similarity. Of 190 possible pair-wise comparisons among infested apartments, non-significant *G*-tests (i.e., genetically indistinguishable populations) were returned for only eight cases (4%). Of these, none were observed between horizontally adjacent apartments, whereas one was observed in a vertically adjacent pair (Figure 4). Pair-wise comparison of F_{ST} supports the *G*-test results, also identifying the same three groups, within which there was no significant differentiation. Clearly the mechanisms leading to establishment of genetically differentiated aggregations following a single introduction event involving one or a few individuals merits further study.

Geographic Variation in Kdr Genotype

In ourpreliminary screen of 38 populations located primarily in the eastern U.S. for two genomic regions of the sodium channel α-subunit genes, we found a high frequency of *kdr* mutations, and hence likely significantly high levels of insecticide resistance (Figure 3). Of these 38 populations, 20 had both *kdr* mutations, a condition shown to result in approximate 264 fold resistance to pyrethroid insecticides (Yoon et al., 2008). Twelve further populations exhibited a single *kdr* mutation, a condition that is considered to result in a 100 fold resistance to pyrethroid insecticides (Yoon et al., 2008). These results reveal a grim pattern regarding the prevalence of insecticide resistance within bed bug populations in the eastern U.S., emphasizing the importance of additional screening throughout the continental U.S. and Hawaii. Of great concern is the fact that these samples were collected between 2005 and 2009. In that short period of time, given the dramatic resurgence of bed bugs in the U.S., it is entirely possible that the proportion of insecticide resistant populations is now even greater. We are currently investigating the present prevalence of *kdr* alleles across the U.S.

We assessed haplotype variation at the ribosomal 16S subunit of the mtDNA across the 38 populations screened for *kdr* mutations. We detected 16 haplotypes, with 22 variable positions in the 349 bpamplicon. However, we found no concordance between haplotype and either *kdr* insecticide resistance genotype or geographic location suggesting that *kdr* resistance may not have a single geographic origin.

These results are consistent with the high genetic diversity we found across 17 populations in the eastern U.S. We are currently expanding these studies in the U.S. and around the world to get a better handle on the origins, spread and dispersal patterns of bed bugs.

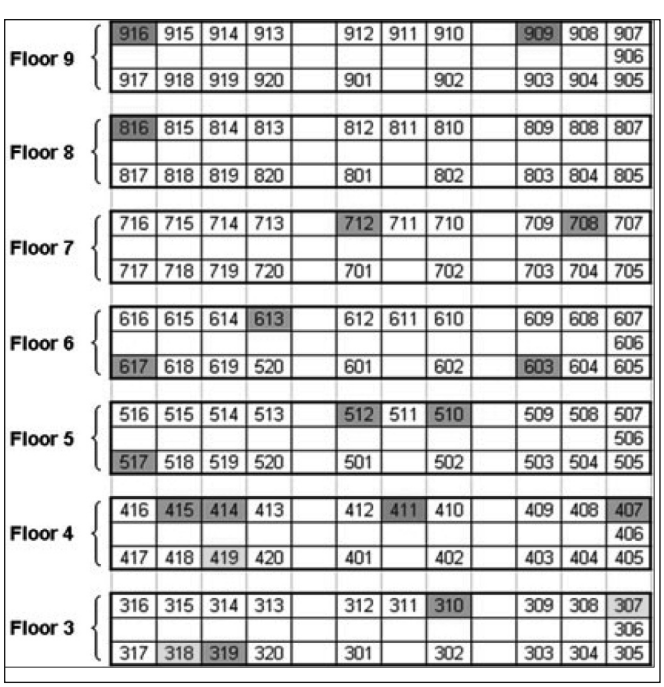

Figure 4. Within building genetic differentiation of bed bugs. Samples collected from floors 3 through 9. Colored boxes represent sampled apartments sampled. Those sharing genetic identity by means of a G-test share the same color.

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