Genetic Analysis of Bed Bug Populations Reveals Small Propagule Size Within Individual Infestations but High Genetic Diversity Across Infestations From the Eastern United States

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ABSTRACT Bed bugs (*Cimex lectularius* L.) are a resurgent pest worldwide and infestations within the United States are increasing at a rapid rate. Because of the physical and psychological discomfort inflicted by their blood feeding habits, and allergies and secondary infections associated with bites, bed bugs are recognized as a significant public health problem. Although bed bug infestations are spreading and becoming more prevalent, we have a poor understanding of their dispersal patterns and sources of infestation. To help fill this gap, we conducted a genetic study of 21 bed bug infestations from the eastern United States, nearly all of which came from single rooms within residences. We genotyped samples comprised of 8–10 individuals per infestation at nine polymorphic microsatellite loci. Despite high genetic diversity across all infestations, with 5–17 alleles per locus (mean = 10.3 alleles per locus), we found low genetic diversity (1–4 alleles per locus) within all but one of the infestations. These results suggest that nearly all the studied infestations were started by a small propagule possibly consisting of a singly mated female and/or her progeny, or a female mated with multiple males that were highly related to her. All infestations were strongly genetically differentiated from each other (mean pairwise F_{ST} between populations = 0.68) and we did not find strong evidence of a geographic pattern of genetic structure, indicating infestations located in closer proximity to each other were nearly as genetically differentiated as those located hundreds of kilometers away. The high level of genetic diversity across infestations from the eastern United States together with the lack of geographically organized structure is consistent with multiple introductions into the United States from foreign sources.

KEY WORDS *Cimex lectularius*, population genetics, gene flow, introduced species, microsatellite

The recent resurgence of the common bed bug *Cimex lectularius* L. (Hemiptera: Cimicidae) has created an unprecedented demand for research on its biology. Reports of infestations are increasing at a rapid rate in urban centers globally. Outbreaks of this pest have been reported in Australia (Doggett et al. 2004), Europe (Boase 2001, 2004; Bencheton et al. 2011), Asia (Lee et al. 2008, Suwannayod et al. 2010), Canada (Hwang et al. 2005), and the United States, where all 50 states are infested (Gangloff-Kaufmann et al. 2006). Bed bugs affect people of all social and economic levels, and infestations have been found in almost every human-made structure, including hotels, apartments, hospitals, homeless shelters, single family homes, nursing homes, office buildings, and schools (Potter et al. 2010).

The causes of resurgence are not clear but some possible reasons include greater international commerce and travel, increased exchange of second hand furniture, host switching and maintenance on other hosts, lack of knowledge of the pest, and changes in pest control practices and insecticide resistance (Reinhardt and Siva-Jothy 2007, Potter 2005, Romero et al. 2007, Reinhardt et al. 2008). Resistance of *C. lectularius* field populations to commonly used pyrethroid insecticides is widespread in the United States (Romero et al. 2007, Yoon et al. 2008, Zhu et al. 2010, Davies et al. 2012) and may play a particularly important role in the rapid resurgence of this pest. The causes for pyrethroid resistance are unknown as are the geographic locations where resistance developed, but its widespread and rapid emergence suggests introduction from regions where strong selection pressure with pyrethroids is prevalent in sleeping quarters.

Bed bugs are wingless, obligate hematophagous ectoparasites that have lived in close association with humans since ancient times (Usinger 1966). They feed primarily on humans; however, they can feed on a wide range of hosts such as bats, birds, and occasionally various domestic animals (Usinger 1966). They live in cracks and crevices in mixed-sex aggregations or groups comprised of adults and juveniles, and are

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active at night (Usinger 1966, Pinto et al. 2007, Siljander et al. 2008).

Although there is no evidence of bed bugs transmitting disease (Usinger 1966, Pinto et al. 2007, Goddard and deShazo 2009), Delaunay et al. (2011) identified 45 human pathogens that could potentially be transmitted by bed bugs. Moreover, bed bugs have been experimentally infected with one of these potential pathogens, hepatitis B virus, in the laboratory, where the virus was detected in the insects and their feces up to 5 wk after infection (Blow et al. 2001). Besides the threat of infection, bed bugs are the cause of other important medical conditions. Bed bug bites may trigger dermatological reactions that include macules, papules, vesicles, wheals, bullae, or nodules. Systemic reactions such as asthma, urticaria, and anaphylaxis may occur (Goddard and deShazo 2009, Criado et al. 2011). In addition to reactions to bites, anxiety, sleep loss, and psychological trauma have been reported (Pinto et al. 2007). Because of their presence in beds and blood feeding habits that result in an array of physical and psychological discomfort during infestation and post eradication (Usinger 1966, Pinto et al. 2007), bed bugs are now recognized as a significant public health problem (Rossi and Jennings 2010).

Infestation dynamics and dispersal of bed bugs are important aspects of their biology that are not well studied (Reinhardt and Siva-Jothy 2007). Bed bugs have the potential to disperse, both actively and passively (human-mediated). Because bed bugs do not fly, active dispersal is limited to the crawling abilities of the insects. Although all juvenile and adult stages can actively disperse, adults are more likely to disperse than juveniles, and adult females are more likely to disperse away from infestations than males (Reinhardt and Siva-Jothy 2007, Pfiester et al. 2009a, Wang et al. 2010). Passive movement of *C. lectularius*, however, is the most common mode of dispersal (Usinger 1966). Humans can disperse bed bugs in their belongings when they travel and/or when they purchase second hand furniture. While it is recognized that humans play a significant role in their movement across small and large geographic areas, there is little information about patterns of movement, including whether there are certain areas that serve as major sources of bed bugs and whether local infestations within a city are more likely to come from nearby or far away sources.

Molecular genetic markers such as microsatellites are powerful tools for various applications, including determination of geographic origins, population genetic structure, gene flow, and dispersal of pests (Sunnucks 2000, Lee 2002, Avise 2004). Understanding dispersal and gene flow of a pest can help us understand the spatial distribution of insecticide resistance traits (Dunley and Croft 1992, Jeger 1999), which can ultimately aid in the development of effective control strategies. Knowledge of the geographic origins of resurgent populations may help identify source populations, providing valuable clues about how resistance developed and, more importantly, allowing the implementation of tactics to curtail the spread of resistant bed bugs to other locations.

Bed bug populations could come from either numerous local sources (residential or agricultural) that have recently expanded, or they could be the result of one or a few source populations (from inside or outside the United States) that have spread globally through human-mediated transport. To date, there is only one study addressing the genetic structure of bed bug populations. Szalanski et al. (2008) investigated genetic variation within and among 22 populations of *C. lectularius*, mainly from the United States, using two types of molecular markers: the mitochondrial DNA (mtDNA) 16S rRNA gene and a portion of the nuclear RNA internal transcribed spacer one (ITS1) region. These authors found moderate genetic diversity with the mtDNA marker and no genetic diversity with the nuclear marker. Szalanski et al. (2008) hypothesized that bed bug resurgent populations in the United States either arose from nearby local populations that were maintained for several decades on alternate hosts such as poultry or domestic pets and have only recently undergone an expansion, or they originated from international sources. Recent work indicates that while pyrethroid resistance is prevalent in bed bug populations across the United States (Zhu et al. 2010), bed bugs from poultry farms were much more tolerant of pyrethroids (Fletcher and Axtell 1993, Steelman et al. 2008), suggesting that most bed bug populations found in homes may not have originated from poultry farms. Furthermore, one of the most common haplotypes detected by Szalanski et al. (2008) in the United States was also present in Australia and Canada, suggesting an international connection between these populations. Clearly, additional studies are needed to address the geographic sources of resurgent bed bug populations.

On a more local scale little is known about infestation dynamics, such as the propagule size that most often starts infestations and the spread of bed bugs through buildings once they become infested. Doggett and Russell (2008) tracked the spread of bed bug infestations within a multi-story residential building in Australia for 2 yr, and Wang et al. (2010) tracked infestations in a high-rise apartment building in Indianapolis, IN, for 41 mo. In both studies infestations spread through the buildings, but it could not be determined in either case whether the spread was because of an expansion of the initial infestation, the result of new introductions, or both. Wang et al. (2010) captured dispersing bed bugs in traps without lures placed outside apartment doors, suggesting that some active dispersal between apartments occurs; however, these approaches could not determine whether dispersing individuals could successfully reproduce and establish in new apartments, or the relative significance of dispersal versus new introductions.

Our objective was to elucidate patterns of genetic structure, genetic differentiation, gene flow and dispersal of bed bug infestations in the United States. Using high resolution microsatellite markers, we

Table 1. Sources of bed bug samples analyzed

State	City or county	Abbreviation	Source	No. rooms sampled	Year collected
Florida	Broward Co.	FL1	Apartment	NA	2009
	Panama City	FL2	Condo	NA	2009
Washington, DC	Washington, DC	DC	Apartment	Multiple	2008
Massachusetts	Boston	MA1	Apartment	Single	2009
	NA	MA2	Poultry farm	NA	2009
New Jersey	Elizabeth	NJ1	Apartment	Single	2008
	Freehold	NJ2	Apartment	Single	2005
	Trenton	NJ3	Apartment	Single	2008
New York	New York City	NY1	Apartment	Single	NA
	New York City	NY2	Apartment	Single	2009
North Carolina	Raleigh	NC1	Apartment	Single	2009
	Raleigh	NC2	Apartment	Single	2009
	Raleigh	NC3	Apartment	Single	2007
	Raleigh	NC4	House	Single	2009
	Winston Salem	NC5	House	Single	2007
	Winston Salem	NC6	Apartment	Single	2008
	Winston Salem	NC7	Apartment	Single	2009
	Winston Salem	NC8	Apartment	Single	2008
Pennsylvania	Buttler	PA	Hotel room	Single	2008
South Carolina	Myrtle Beach	SC	Motel room	Single	2009
Virginia	NA	VA	Hospital	NA	2009

NA, information not available.

addressed three fundamental questions regarding infestation and dispersal dynamics: 1) How many individuals are generally responsible for founding infestations? 2) What is the degree of genetic diversity across infestations in the eastern United States and what can this tell us about the origins of the resurging populations? and 3) What is the genetic relationship among infestations? Such information should provide important insights into this species' reproductive ability, dispersal patterns, and may give clues about the number and sources of resurgent populations in the eastern United States.

Materials and Methods

Sample Collection. Bed bugs were collected from 21 infested structures in locations spanning the east coast of the United States from Massachusetts to Florida between 2007 and 2009 (Table 1; Fig. 1). Between 8 and 50 insects were collected from each structure. Nearly all samples within infested structures were collected from a single room. In four cases we could not determine the number of rooms sampled by the collectors, and in one instance (DC sample), samples from multiple rooms were combined. We also included samples taken from an organic poultry farm in Massachusetts (MA2). Samples outside North Carolina were generously collected by pest control companies and/or other researchers with specific instructions provided by us. Upon collection, all samples were immediately placed in vials containing 95% ethanol and stored at −20°C until DNA extraction.

DNA Extraction and Microsatellite Genotyping. We extracted total genomic DNA from 8 to 10 adults from each location using a phenol-chloroform protocol (Taggart et al. 1992). We genotyped individuals at nine polymorphic microsatellite loci (*bb4b*, *bb15b*, *bb21b*, *bb28b*, *bb29b*, *bb31b*, *bb38b*, *bb42b*, and *Clec37*) developed by Booth et al. (2012). We used the polymerase chain reaction (PCR) conditions described by Booth et al. (2012). Microsatellite analysis was performed by fluorescent labeling according to the methods of Oetting et al. (1995), which consisted of adding the first 19 bp of the M13 forward sequencing primer (CACGACGTTGTAAAACGAC) to the 5′ end of one of the specific primers in each pair. The M13 tail was attached to the left primer in each pair. A fluorescently labeled M13 primer (M13 F-29 IRD 700 or 800, Li-Cor Biosciences, Lincoln, NE) was included in the PCR, yielding a labeled product. Samples were loaded on 25 cm 6% polyacrylamide gels and run on a Li-Cor 4300 automated DNA sequencer. Fifty to 700-bp IRDye size standards (Li-Cor Biosciences) were loaded every 15 samples for accurate size determination. Allele sizes were scored using Gene Profiler software, version 4.05 (Scanalytics, Rockville, MD).

Genetic Data Analysis. We considered each location a separate population for all genetic analyses. Summary population statistics (mean number of alleles, expected (H_E) and observed (H_O) heterozygosities) were calculated using the Genetic Data Analysis software (GDA) version 1.1 (Lewis and Zaykin 2001). Tests for departure from Hardy–Weinberg (HWE) and linkage disequilibrium were performed using GENEPOP version 4.0 (Raymond and Rousset 1995, Rousset 2008). We applied the Bonferroni correction for multiple tests to each of these analyses (Rice 1989).

Across all populations genetic differentiation was assessed by overall and pairwise F_{ST}-values (Weir and Cockerham 1984) estimated using FSTAT version 2.9.3.2 (Goudet 1995). Additionally, we estimated F_{ST}-values for samples grouped within state and within city. Confidence intervals (CIs) for all F_{ST}-values were obtained by bootstrapping over loci. We performed a test of isolation-by-distance to determine if genetic differentiation and geographic distance were correlated across the 21 populations. This test was performed by regression analysis of the genetic and geo-

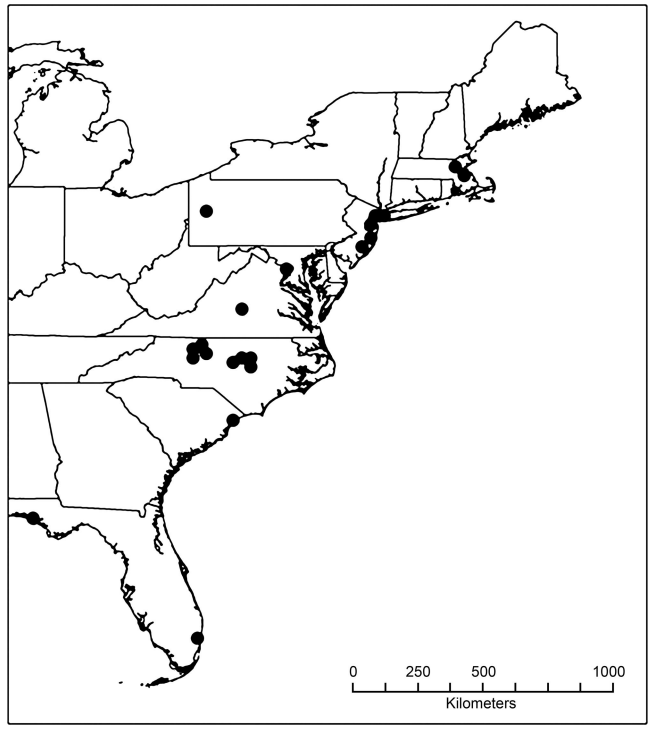

Fig. 1. Locations of the 21 study populations of *C. lectularius*.

graphic distances of each pair of populations using the Mantel test (Mantel 1967) implemented in MANTEL version two (Liedloff 1999), with a total of 10,000 permutations. Geographic distances, calculated as the shortest straight line distance between each pair of populations, were log transformed. Additionally, we tested pairwise genotypic differentiation between samples using the log-likelihood-based *G*-test (Goudet et al. 1996), implemented in GENEPOP. The Markov Chain parameters were set to 1,000 dememorizations, 100 batches, and 1,000 iterations per batch.

We investigated the genetic structure within populations through analysis of inbreeding coefficients (*F*-statistics) and coefficient of relatedness (*r*) among individuals from each population as implemented in the program FSTAT. CIs were determined by bootstrapping over loci.

Results

Summary Statistics. We genotyped a total of 206 individuals at the nine microsatellite loci analyzed. Across all populations, we observed relatively high genetic diversity with 5–17 alleles per locus. The mean expected and observed heterozygosities for all loci were 0.779 and 0.222, respectively (Table 2). The observed heterozygosities were much lower than the expected values in the global analysis because of the strong structuring among populations, with each population containing a limited number of alleles (see Tables 3 and 4). After Bonferroni correction, only one locus (*bb38b*) showed significant deviation from HWE, in a single population, which amounted to <1% of the 189 tests performed (21 populations × 9 loci). The remaining eight loci showed no significant deviations from HWE in any of the populations. Because there was no consistent deviation from HWE for any locus, and there was no evidence for linkage disequilibrium between pairs of loci, we considered the markers to be unlinked and suitable for genetic analysis of the study populations.

Analysis within populations showed that the mean observed and expected heterozygosities for all loci were 0.225 and 0.265, respectively, and did not differ significantly from each other (two-tailed *t*-test, $P > 0.05$; Table 3). The mean number of alleles per locus ranged from 1.1 to 2.7 across the 21 populations (Table

Table 2. Descriptive statistics for nine microsatellite loci across 21 populations of *C. lectularius* (n = 8–10 individuals per population)

Locus	H_E	H_O	NA
bb4b	0.679	0.119	9
bb15b	0.886	0.439	17
bb31b	0.790	0.289	10
bb38b	0.705	0.180	6
bb21b	0.823	0.195	10
bb28b	0.873	0.230	15
bb29b	0.874	0.284	13
Clec37	0.674	0.068	5
bb42b	0.711	0.191	8
Mean	0.779	0.222	10.3

NA, mean no. of alleles; H_E, expected heterozygosity; H_O, observed heterozygosity.

Table 3. Summary statistics for 20 *C. lectularius* collections from residential structures and one collection from an agricultural structure genotyped at nine microsatellite loci

Population	H_E	H_O	NA
FL1	0.021	0.000	1.1
FL2	0.142	0.140	1.3
DC	0.174	0.071	1.8
MA1	0.221	0.110	1.9
MA2	0.430	0.321	2.6
NJ1	0.422	0.342	2.7
NJ2	0.011	0.011	1.1
NJ3	0.139	0.126	1.3
NY1	0.184	0.200	1.6
NY2	0.281	0.225	2.1
NC1	0.375	0.288	2.3
NC2	0.493	0.599	2.6
NC3	0.410	0.513	2.2
NC4	0.447	0.513	2.4
NC5	0.263	0.165	2.3
NC6	0.301	0.279	2.2
NC7	0.311	0.379	1.8
NC8	0.406	0.252	2.1
PA	0.302	0.190	1.7
SC	0.057	0.100	1.1
VA	0.185	0.115	1.6
Mean	0.265	0.225	1.9

NA, mean no. of alleles; H_E, expected heterozygosity; H_O, observed heterozygosity.

3). All but one population (NJ1) had ≤4 alleles present at all nine loci (Table 4). The simplest explanation for such low genetic diversity within individual infestations is that nearly all of them were founded by a single female mated to a single male and/or their progeny that would result in a maximum of four alleles per locus in the case where both the male and female were heterozygous for different alleles. Alternatively, the infestations could have been started by a single female mated to multiple highly related males. The sole exception was population NJ1 (Elizabeth, NJ), which had five alleles at both *bb15b* and *bb29b* loci, a finding that could be explained by an original foundress mated to two or more unrelated males (and/or their descendants), or the introduction of two or more individuals from either different source populations, or from a common genetically diverse source population.

Over all populations, the mean coefficient of relatedness *r*, for individuals within an aggregation was 0.78, and it was significantly >0.5, which is the value expected for siblings and parent/offspring pairs (95% CI = 0.74–0.83). Although the high level of relatedness of individuals within an aggregation suggested that individuals were more related than siblings or parent-offspring relations, the observed high values were because of the strong genetic differentiation among infestations and the apparent lack of gene flow among them (see Genetic Differentiation below). Furthermore, individual bed bugs from different locations do not interbreed because of limited active dispersal. Thus, because each population studied here was a genetically isolated family, these values were elevated over those expected among groups in a large population with frequent mating between groups. The overall standard inbreeding coefficient F_{IS} was 0.15 (95% CI = −0.025–0.313) and was not significantly greater than zero, suggesting that individuals within aggregations were not significantly inbred relative to others within the same aggregation. These results are consistent with each infestation comprising a group of freely mating individuals. However, the small propagule initiating each infestation means that each population, although comprised of randomly mating individuals, consists of close relatives and is therefore highly inbred.

Genetic Differentiation. We found high genetic differentiation of populations at all levels from among

Table 4. Number of alleles per population for nine microsatellite loci for each of the 21 sampled populations

Population	Locus									Average
	bb4b	*bb15b*	*bb31b*	*bb38b*	*bb21b*	*bb28b*	*bb29b*	*Clec37*	*bb42b*	
FL1	1	1	1	1	2	1	1	1	1	1.1
FL2	1	1	1	1	2	2	2	1	1	1.3
DC	2	2	1	2	2	1	2	1	1	1.6
MA1	3	1	1	3	2	3	2	1	1	1.9
MA2	3	4	2	3	2	3	1	1	3	2.4
NJ1	1	**5**	3	2	2	3	**5**	2	1	2.7
NJ2	1	1	1	2	1	1	1	1	1	1.1
NJ3	1	2	2	1	2	1	1	1	1	1.3
NY1	2	2	1	1	2	3	1	1	1	1.6
NY2	2	3	1	1	3	4	2	1	1	2.0
NC1	2	4	2	2	3	2	2	2	1	2.2
NC2	2	2	4	2	3	3	3	2	2	2.6
NC3	2	3	3	2	2	2	2	2	2	2.2
NC4	4	4	2	3	2	1	2	2	2	2.4
NC5	1	2	2	3	3	4	2	1	0	2.0
NC6	2	2	3	1	3	2	3	1	3	2.2
NC7	2	3	2	1	1	2	2	1	2	1.8
NC8	3	2	1	1	3	2	2	2	3	2.1
PA	1	2	2	1	2	2	2	1	2	1.7
SC	1	2	1	1	1	1	1	1	1	1.1
VA	1	1	2	2	1	2	2	1	1	1.4

Values in bold highlight populations that have five alleles at each locus. $N = 8–10$ individuals for each population.

Table 5. *F*-statistics, and associated *G*-test results for *C. lectularius* samples grouped by city, state, and overall population

	City (sample size)	F_{ST} (95% CI)	*G*
Within city	Raleigh ($n = 4$)	0.502 (0.412, 0.593)	<0.001
	Winston Salem ($n = 4$)	0.623 (0.525, 0.723)	<0.001
Within state	North Carolina ($n = 8$)	0.555 (0.504, 0.611)	<0.001
	New Jersey ($n = 3$)	0.742 (0.626, 0.861)	<0.001
	New York ($n = 2$)	0.709 (0.476, 0.901)	<0.001
	Massachusetts ($n = 2$)	0.657 (0.516, 0.791)	<0.001
	Florida ($n = 2$)	0.906 (0.812, 0.979)	<0.001
Total populations	All ($n = 21$)	0.678 (0.630, 0.734)	<0.001

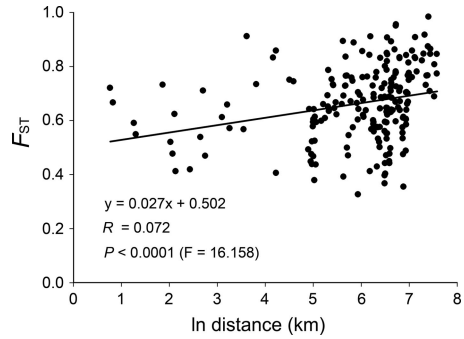

Fig. 2. Isolation by distance analysis of the *C. lectularius* study populations. Pairwise comparisons between populations are plotted as genetic distance (F_{ST}) versus the natural log of geographic distance in kilometers.

states to among locations within a city (Table 5). F_{ST} values for populations in the cities of Raleigh and Winston–Salem, the two cities for which multiple samples were obtained, were >0.5. Pairwise *G*-tests performed among all populations within each city were significant. The very high levels of genetic differentiation among populations that are in close proximity (<10 km apart) suggest that populations within a city may be unrelated to each other and experience very limited or no gene flow between them.

We also found very strong genetic differentiation of populations within states, with F_{ST} values ranging from 0.502 and 0.906, and very similar to the city values (Table 5). Pairwise *G*-tests were significant and confirmed these results. The high levels of genetic differentiation within state samples suggest populations within states are no more similar to each other than are populations located in other states.

Considering all 21 populations, we detected strong genetic differentiation with an overall F_{ST} of 0.678 (Table 5). Population pairwise F_{ST}-values ranged from 0.325 to 0.983. The *G*-tests also yielded significant results between all pairs of populations. Similar to cities and states, there were extremely high levels of genetic differentiation that suggest little if any gene flow among populations.

Isolation by Distance. The pattern of isolation by distance revealed a shallow slope, and across all populations there was a weak but significant positive correlation ($R^2 = 0.072$; $P < 0.0001$) between the natural log of geographic distance and genetic distance (F_{ST}) (Fig. 2). An analysis using the transformed genetic distance ($F_{ST}/(1\text{-}F_{ST})$) gave an even weaker relationship ($R^2 = 0.030$; $P = 0.0124$). The significant isolation by distance pattern suggests that infestations were more likely to have originated from populations within the same or nearby city than from more distant populations. However, the low R^2-value suggests that the probability of this happening is very low.

Discussion

Our results on the genetic structure of bed bug infestations provide the first evidence for the possible size of propagules responsible for starting infestations. The low genetic diversity observed within nearly all infestations (one to four alleles per locus) indicates that most populations are founded by few individuals, possibly only a singly mated female, her descendants, or a female that mated with several males sharing similar genotypes. These results are similar to our preliminary genetic studies of bed bug infestations across the United States (W.B., V.S., E.V., and C.S., unpublished data) and those in multi-story buildings (Booth et al. 2012). Together, these studies show a genetic signature indicating that most bed bug infestations originate from a singly mated female or a female that mated several times to genetically related males. The one exception to this pattern in the current study was the NJ1 population, collected from an apartment, which contained five alleles at two loci but three or fewer alleles at the other seven loci examined. There are at least four possible explanations for the presence of more than four alleles in this population. First, it could have been started by a single female mated to two or more related males (or their progeny). Second, it could have been initiated by two or more related females. The relatively low genetic diversity in this infestation is consistent with both of these possibilities. Third, the infestation could have been started by multiple females introduced from different source infestations, either at the same time or at different times. In support of this latter possibility, the apartment had been treated once previously according to the pest management professional who collected the sample. If the treatment did not eliminate all individuals from the original infestation, and reinfestation of the apartment occurred before the time the sample was collected, the sampled population could have contained individuals originating from both the old and new infestations, possibly with interbreeding between the two groups. Finally, it is possible there was active dispersal of unrelated bed bugs from other apartments in the building that entered the sampled apartment after it had already been infested from introduction of bed bugs from outside the building. Wang et al. (2010) found that some active dispersal between apartments in multistory buildings occurs. Therefore active dispersal cannot be ruled out as a possible cause of the unusually high genetic diversity of this one infestation.

One implication of our finding of low genetic diversity within infestations is that for the most part introductions of bed bugs appear to be relatively rare events. Because the level of genetic diversity in nearly all the studied infestations is consistent with the introduction of a single female mated to a single male, most infestations appear to have resulted from a single introduction event followed by population growth through reproduction by the founding individuals and their descendants. If introduction of bed bugs by a given set of residents occurred frequently from different source populations, we would expect to find much greater genetic diversity than we observed. Thus, it seems that in most cases structures are not under heavy propagule pressure and successful colonizers arrive infrequently. It is also possible that all or most of our collections were from relatively young infestations, and older infestations may be more genetically diverse because of multiple introductions. In either case, each propagule appears to represent highly related individuals of low genetic diversity.

The low genetic diversity we found among infestations, suggesting that infestations may be started by a singly mated female, is surprising considering that female bed bugs are known to be polyandrous. Stutt and Siva-Jothy (2001) reported that females mated on average five times after a bloodmeal under laboratory conditions. However, sperm precedence occurs such that the last male to mate sires most of the offspring, effectively reducing, but not entirely eliminating, the contributions of the females' previous mates (Stutt and Siva-Jothy 2001). In addition, when presented the opportunity to disperse, females will leave aggregations, especially when the density of males is high (Pfiester et al. 2009b), raising the possibility that females may disperse after mating only once or a few times. Because our results show that infestations are composed of highly related individuals, it is certainly possible that colonizing females are mated to multiple related males yielding offspring with limited genetic diversity. Further studies of field caught females are needed to determine the typical number of matings in both dispersing and nondispersing females.

Our findings within single dwelling infestations appear to differ somewhat from those of Szalanski et al. (2008), who found moderate genetic variation within individual infestations using the 16S rDNA mitochondrial marker. Of the six single resident homes studied by Szalanski et al. (2008), they found multiple mtDNA haplotypes in four of the homes, with up to six haplotypes present in one home. Infestations initiated by a single female or her progeny are expected to have a single mtDNA haplotype present. Therefore, finding six different haplotypes in a single infestation suggests that the infestation originated from a number of females representing six distinct genetic lineages. This could result from either a single introduction of multiple females from one source population with high genetic diversity, or from multiple introductions of one or more individuals from several source populations. However, Szalanski et al. (2008) did not indicate whether their samples came from single rooms within each structure or multiple rooms. Thus, it is possible that these populations represented individuals originating from different infestations in the same buildings, or that the genetic structure and/or infestation dynamics of these infestations was very different from those investigated here.

Our findings of a low number of founders, high relatedness of individuals, and limited gene flow among infestations suggest that individual infestations are highly inbred. Inbreeding in natural populations of animals and plants usually results in inbreeding depression, leading to reduced population growth and increased rates of extinction (Keller and Waller 2002, Frankham et al. 2010). Therefore, it is rather surprising to see the proliferation of bed bugs across the United States and elsewhere through the establishment of such highly inbred populations. It would appear that bed bug populations can either tolerate high levels of inbreeding or have mechanisms to purge deleterious mutations. Studies conducted on the invasive species *Harmonia axyridis* (Pallas) reveal that bottlenecks of intermediate size can purge deleterious alleles that cause inbreeding depression (Facon et al. 2011). It is possible that a similar purging mechanism occurs in bed bug populations that experience bottlenecks during introduction events or eradication efforts. Future studies are needed to determine the effects of inbreeding on bed bug populations and to uncover possible mechanisms that allow bed bugs to overcome small propagule size and reduced genetic diversity.

An important question concerning the resurgence of bed bugs relates to the geographic source(s) of the spreading populations. Given that insecticide resistance is widespread among resurging populations (Romero et al. 2007, Zhu et al. 2010), resistance likely developed within the source population(s). There are several possible scenarios for the sources and subsequent spread of insecticide resistant bed bugs. First, resistance could have developed multiple times independently within local populations that only recently spread. Based on their study of mtDNA diversity of 20 populations in the United States using the 16S rDNA gene, Szalanski et al. (2008) proposed that resurgent urban populations came from either local sources that were maintained on alternative hosts, such as pets, bats, and poultry, or they originated from other parts of the world where bed bugs went uncontrolled. Our findings of high genetic diversity across a panel of nine microsatellite loci are consistent with a diverse genetic origin of the bed bug populations we sampled. However, if populations are derived from local reservoirs where bed bugs have persisted for many decades, then we would have expected to find strong genetic structuring across the United States, with nearby populations being more genetically similar than those further away. We did not find such a relationship in our isolation by distance analysis. In fact, in the cities for which we have multiple samples (Winston–Salem and Raleigh, NC), infestations were as genetically differentiated as those located hundreds of kilometers apart, and the populations in each of these cities had over half the genetic diversity found in the entire set of 21

populations (mean = 5.5 alleles per locus for both, Raleigh and Winston–Salem populations). Moreover, such a scenario would require that resistance developed many times nearly simultaneously across the United States and globally, a series of events that seems unlikely. In addition, we had one population from an organic poultry farm in Massachusetts (MA2); it showed the same low genetic diversity as the other infestations and is therefore unlikely to have served as a reservoir of genetically diverse bed bugs for the surrounding area. Admittedly, this is a single observation and additional studies are needed to test the poultry farm source hypothesis further. Finally, the few poultry infestations that have been examined in Arkansas and North Carolina were susceptible to selected pyrethroid insecticides (Fletcher and Axtell 1993, Steelman et al. 2008). Thus, it seems improbable that the main sources of resurging bed bug populations in the United States are simultaneously expanding local reservoir populations. In fact, in their genetic study Szalanski et al. (2008) found that the most common mtDNA haplotype they observed in the United States was also present in Canada and Australia, suggesting international connections between bed bug populations in the United States and elsewhere in the world.

Perhaps a more likely scenario for the source of bed bug populations in the United States is an international location(s) where resistance developed. If the resurgent bed bug populations do indeed come from outside the United States, the high level of genetic diversity we observed would suggest that they either came from multiple geographic locations, or from multiple introductions of a single genetically diverse population, rather than a single introduction that then spread within the United States. A number of authors have suggested the current bed bug infestations likely came from foreign sources that were then spread in the United States and globally through international travel. For example, Boase (2001, 2004) and Kilpinen et al. (2008) noted that in the United Kingdom and other parts of Europe bed bug infestations were reported by people returning from traveling abroad and had stayed in hotels while traveling. Recent collections of the tropical bed bug *Cimex hemipterus* (F.) in temperate regions also implicate the international transport of bed bugs (Reinhardt and Siva-Jothy 2007). If in fact the major source of bed bugs in the United States is international sources, there are three possible scenarios for their introduction. First, they could have been introduced once or a few times from a single genetically diverse source population with high pyrethroid resistance, and then spread around the United States through domestic travel. Second, they could have been introduced multiple times from either the same source population or from different source populations containing pyrethroid resistant bed bugs. The fact that we found relatively high genetic diversity with up to 17 alleles at a single microsatellite locus across populations in the eastern United States makes the first possibility unlikely, because a single introduction from a source population would be expected to show limited genetic diversity. This is especially true in light of the small propagule size we found in this study. Once established, subsequent introductions from the resulting infestations should reduce genetic diversity even further. Thus, it is more likely that either there is a single highly genetically diverse source population that has served as a staging ground for multiple introductions around the world, or there are multiple source populations from which bed bugs have spread. Obviously, the latter is more likely, especially considering pyrethroid resistance has been documented in bed bug populations around the world (Davies et al. 2012), including Europe (Kilpinen et al. 2011), Australia (Lilly et al. 2009), and Asia (Suwannayod et al. 2010), but additional genetic studies of populations representing a broad cross-section around the globe are needed to identify potential source populations. Studies combining population genetic data with insecticide resistance profiles should be particularly fruitful in this regard.

Populations of most invasive species experience a reduction in genetic diversity in the introduced environment compared with the native environment (Caldera et al. 2008, Dlugosch and Parker 2008). During the initial phases of invasion (introduction and establishment), the small number of founder individuals (founder effects) and a small population size over the first few generations may lead to a loss of genetic diversity with respect to the source population (Dlugosch and Parker 2008). However, multiple introductions may increase the genetic diversity of the invasive population particularly when several genetically differentiated source populations contribute to the invasion (Dlugosch and Parker 2008). The relatively high level of genetic diversity present in the 21 bed bug populations studied here (up to 17 alleles at one locus; mean = 10.3 alleles per locus) is comparable to that in other invasive species that were introduced multiple times into new areas as measured with microsatellite markers. For example, the southern house mosquito (*Culex quinquefasciatus* Say) was introduced to Hawaii multiple times where it has 8–11 alleles at four microsatellite loci (mean = 9.25 alleles per locus) (Fonseca et al. 2000). Similarly, the red imported fire ant, *Solenopsis invicta* Buren, has up to nine alleles at seven microsatellite loci (mean = 5.6 alleles per locus) in its introduced range in the United States where it was likely introduced at least three times from its native South America where it displays up to 17 alleles per locus (mean = 14.2 alleles per locus) (Shoemaker et al. 2006, Caldera et al. 2008).

Our results on the bed bug, an insect closely associated with humans, permits some comparisons with recent work on the genetic structure of the German cockroach, *Blattella germanica* (L.), another human commensal with a worldwide distribution because of frequent human-mediated dispersal. In a previous paper, we documented high levels of genetic diversity finding up to 22 alleles per locus (mean = 11.9 alleles per locus) in 12 populations in North Carolina when screened at 10 microsatellite loci (Booth et al. 2007). Such high genetic diversity in this species is not sur-

prising given that *B. germanica* has been spread around by humans for hundreds of years and is frequently moved both short and long distances in shipping containers, boxes, furniture, and electronic equipment (e.g., Mouchtouri et al. 2008, Schal 2011). Similar to bed bugs, German cockroaches are confined to human structures, but they have greater ability to move within buildings through active dispersal. In a study of 18 apartments from six apartment complexes in Raleigh, NC, we found high genetic diversity within apartments, where populations had on average 7.7 alleles per locus (Crissman et al. 2010). Thus, compared with the present results on bed bugs, German cockroach infestations have much higher genetic diversity, suggesting that either propagule size is much larger in the case of *B. germanica* or propagule pressure is greater resulting in frequent introduction into the same building, or both.

The large scale pattern of genetic diversity of bed bug and German cockroach populations in the United States has important similarities and differences. As discussed above, there is high genetic diversity across populations in both species. However, the level of genetic differentiation in *B. germanica* populations within and among cities in the United States was low (mean F_{ST} = 0.099; Crissman 2008, Crissman et al. 2010) compared with an average F_{ST} among bed bug populations in the current study of 0.679. This is because of the lower genetic diversity within *C. lectularius* populations and the lack of extensive mixing among infestations. Despite large differences in the degree of genetic differentiation between *C. lectularius* and *B. germanica* populations in the United States, neither species shows strong signs of genetic structure across large geographic scales (Crissman 2008, Crissman et al. 2010), as expected for populations with extensive human-mediated dispersal that are moved both long and short distances.

We report the results of the first geographic study of bed bug infestations using multiple single locus nuclear DNA markers. Our results can be summarized in two main conclusions regarding bed bug infestation sources and spread in the eastern United States based on 21 populations studied from the East Coast. First, we found evidence that most individual infestations are started by a very small propagule, likely a single inseminated female or her descendants. Second, the high level genetic diversity across the study populations, together with the lack of geographically organized genetic structure, suggests that resurgent bed bugs in the United States likely originated from several introductions from outside the United States. One implication of our findings is that because infestations are generally started by a few individuals and seem to be rather rare events within any given structure, early detection and mitigation of infestations are critical for eradicating bed bug infestations within a property. The source(s) of bed bug populations globally remains an important gap in our understanding of the resurgence of this pest. Detailed studies are needed of bed bug populations globally, especially from areas where bed bugs remained common the last several decades, to identify potential source populations and to reconstruct possible routes of recolonization on a global scale.

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