Molecular Markers Reveal Infestation Dynamics of the Bed Bug (Hemiptera: Cimicidae) Within Apartment Buildings

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J. Med. Entomol. 49(3): 535–546 (2012); DOI: http://dx.doi.org/10.1603/ME11256

ABSTRACT The bed bug, *Cimex lectularius* L. (Hemiptera: Cimicidae), has experienced an extraordinary global resurgence in recent years, the reasons for which remain poorly understood. Once considered a pest of lower socioeconomic classes, bed bugs are now found extensively across all residential settings, with widespread infestations established in multiapartment buildings. Within such buildings, understanding the population genetic structure and patterns of dispersal may prove critical to the development of effective control strategies. Here, we describe the development of 24 high-resolution microsatellite markers through next generation 454 pyrosequencing and their application to elucidate infestation dynamics within three multistory apartment buildings in the United States. Results reveal contrasting characteristics potentially representative of geographic or locale differences. In Raleigh, NC, an infestation within an apartment building seemed to have started from a single introduction followed by extensive spread. In Jersey City, NJ, two or more introductions followed by spread are evident in two buildings. Populations within single apartments in all buildings were characterized by high levels of relatedness and low levels of diversity, indicative of foundation from small, genetically depauperate propagules. Regardless of the number of unique introductions, genetic data indicate that spread within buildings is extensive, supporting both active and human-mediated dispersal within and between adjacent rooms or apartments spanning multiple floors.

KEY WORDS bed bug, microsatellite, population genetic structure, 454 pyrosequencing, inbreeding

In recent years, the application of high-resolution molecular markers has provided important new insight into the population genetic structure and infestation dynamics of many insect pest species of public health concern (Conn and Mirabello 2007, Fitzpatrick et al. 2008, Paupy et al. 2008, Endersby et al. 2009, Crissman et al. 2010, Booth et al. 2011, Pérez de Rosas et al. 2011). New molecular tools now make it feasible to not only accurately identify the number of populations actively infesting a building (Pizarro et al. 2008, Crissman et al. 2010) but also to elucidate dynamics and characteristics essential for understanding infestation patterns and history, e.g., levels of genetic diversity (a measure often associated with population health; Paupy et al. 2008, Piccinali et al. 2009), temporal stability of populations after pest control efforts (Pérez de Rosas et al. 2007), and the presence or absence of genetic mutations associated with insecticide resistance (Yoon et al. 2008, Zhu et al. 2010). Such molecular genetic information is likely to represent a significant step forward in the identification of genetically distinct pest populations, followed by the development and evaluation of intervention strategies aimed at the effective eradication of pest infestations. Despite the availability of powerful genetic tools to shed light on the biology and management of insect pests, the infestation dynamics of those primarily commensal within human dwellings remains largely unaddressed.

The bed bug, *Cimex lectularius* L. (Hemiptera: Cimicidae), a wingless hematophagous insect thought to have evolved from the ectoparasites of cave-dwelling mammals, has a long history of association with humans (Usinger 1966, Panagiotakopulu and Buckland 1999). Although considered common in the years before World War II, after the extensive application of organochlorine, organophosphate, and carbamate insecticides (Usinger 1966, Snetsinger 1997, Cooper 2006, Harlan 2006), reports of infestations became extremely sparse in industrialized countries after the 1950s. Recently, a global resurgence of both *C. lectularius* and *Cimex hemipterus* (F.) has occurred (Pinto et al. 2007, Reinhardt and Siva-Jothy 2007, Doggett et al. 2011). Once considered a pest of the lower socioeconomic classes, *C .lectularius* can now be found in hotels, apartments, college dormitories, health care facilities, public transportation systems, and in middle- and upper-class single-family residences (Pinto et al. 2007). The reasons for the rapid increase and spread of bed bugs are not clear, but among the possible

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factors are the reduction in the use of broad-spectrum residual pesticides, increased exchange of secondhand goods and furniture, increased international traffic, and the evolution of insecticide resistance either in the United States or internationally (King et al. 1989, Boase 2001, Romero et al. 2007, Zhu et al. 2010).

Although *C. lectularius* is not known to vector disease (Blow et al. 2001, Goddard 2003), it is nonetheless a significant reemerging public health pest because it affects the human host in many other ways, including swelling and welts resulting in pruritus and secondary infection, the elicitation of immune responses that cause discomfort and psychological stress, and social ostracism (Ryckman 1979, Ryckman and Bentley 1979, Thomas et al. 2004, Hwang et al. 2005, Ter Poorten and Prose 2005). The economic impact of bed bug infestations is dramatic. Annually, infestations result in millions of dollars in damages through both treatments and lawsuits within the hospitality industry and other residential settings (Davies 2004, Doggett and Russell 2007, Reinhardt and Siva-Jothy 2007).

C. lectularius is capable of dispersing both passively (Boase 2001, Doggett et al. 2004) and actively (Mellanby 1938, 1939). Although the primary means through which *C. lectularius* spreads are poorly understood, active dispersal, according to Reinhardt and Siva-Jothy (2007), is an area of research in which the least progress has been made. Early studies by Mellanby (1938, 1939) and Johnson (1941) report that first instars walk readily (cf. Pfiester et al. 2009a) and that adult females are slightly more active walkers than males. Recent evidence by Pfiester et al. (2009b) suggests females exhibit male avoidance behaviors in an effort to escape injury or death by traumatic insemination, an unusual mating system found in cimicids and some other arthropods. *C. lectularius* aggregation behavior seems to be chemically mediated, both through the production of aggregation pheromones (Siljander et al. 2008) and male/nymph–emitted contact pheromones (Siljander et al. 2007). Although the presence of an aggregation pheromone may attract females, this behavior only attracts virgin females, thereby aiding uninseminated females to find a mate-searching male (Siljander et al. 2007, 2008). Some movement away from aggregations has been recorded in males, but solitary individuals are most often female (Pfiester et al. 2009a,b). As a result of the apparent inability of females to produce contact pheromones, females may leave the aggregation without attracting males, resulting in colonization of nearby rooms (Pfiester et al. 2009a). This retreat in turn may stimulate males to undergo active dispersal within or between rooms in an effort to locate potential mates. The ability to survive prolonged periods of starvation and extensive pyrethroid resistance (Romero et al. 2007, Zhu et al. 2010) may permit these mate-seeking males and male-avoiding females to traverse distances of tens of meters, even over insecticide-treated surfaces, potentially among apartments within a building. Although Wang et al. (2010) reported detecting *C. lectularius* in the process of dispersal within a multiapartment building through the use of intercepting traps, dispersal distance itself has not been directly documented nor has the ability of such dispersing individuals to successfully colonize new rooms. However, in a laboratory study of the tropical bed bug, *C. hemipterus*, a species commonly associated with humans in warmer climates, recently blood-fed adult females were found to disperse up to 42.3 m over a 120-h period (How and Lee 2010). It is therefore likely that *C. lectularius* may possess similar dispersal ability. Although recent studies have used molecular markers to elucidate patterns of geographic structuring of *C. lectularius* at the macrogeographic scale (i.e., within cities and across states; Szalanski et al. 2008; Saenz et al. 2012), little is known regarding the genetic structure at the microgeographic scale (i.e., within buildings). With the recent discovery of insecticide resistance within *C. lectularius* populations (Romero et al. 2007, Yoon et al. 2008, Zhu et al. 2010), and the potential difficulty in treating infestations (Pinto et al. 2007), it is vital that we understand how populations within buildings are related and how, upon colonization, *C. lectularius* disperse.

Because of their abundance throughout the eukaryote genome, biparental inheritance, ease and precision of detection through polymerase chain reaction (PCR), and the high level of polymorphism within individuals, microsatellite DNA markers have emerged as extremely versatile molecular tools for population genetic analysis (Avise 2004). Once considered difficult and costly to develop, recent advances in DNA pyrosequencing technology now enable the rapid identification and characterization of large numbers of microsatellite loci with relative ease and minimal cost (Abdelkrim et al. 2009, Allentoft et al. 2009, Copeland et al. 2011). Populations of many pest insect species, including those of both economic and public health importance (e.g., mosquitoes, ticks, cockroaches, kissing bugs, termites) have been effectively studied with microsatellite markers (DeMeeûs et al. 2002, Aluko and Husseneder 2007, Fitzpatrick et al. 2008, Parman and Vargo 2008, Paupy et al. 2008, Crissman et al. 2010, Booth et al. 2011). Thus, in contrast to techniques such as allozyme electrophoresis or random amplified polymorphic DNA, microsatellite markers provide superior levels of resolution regarding colonization and dispersal previously only possible through intricate mark–recapture experiments and thus represent an ideal molecular tool for unraveling the infestation dynamics of many household insect pests.

Here, we describe the development and characterization of 24 high-resolution microsatellite markers for *C. lectularius* through next generation 454 pyrosequencing. We then apply these markers to elucidate patterns of fine-scale genetic differentiation, aggregation characteristics, and infestation dynamics of *C. lectularius* within three heavily infested multiunit apartment buildings in the United States.

Materials and Methods

Microsatellite Characterization. From five *C. lectularius* specimens, selected from geographically distant

sampling locations, DNA was extracted using the DNEasy Blood & Tissue kit (QIAGEN, Valencia, CA). Before extraction, specimens were starved for 3 wk to minimize DNA contamination resulting from undigested bloodmeals. DNA quality and concentration from each specimen were determined using the NanoDrop 1000 spectrophotometer (NanoDrop, Wilmington, DE). Pooled DNA from the five specimens was then subjected to shotgun sequencing using the Roche 454 Genome Sequencer FLX (Roche Applied Science, Penzberg, Germany) with the Titanium Sciences kit XLR 70, performed at the Genomic Sequencing Laboratory located at the North Carolina State University. Sequencing was performed on a 1/8 GS-FLX PTP.

In total, 259,579 reads were obtained, with an average read length of 266 bp and a total of 69,132,484 bp. Using MSATCOMMANDER version 0.8.2 (Faircloth 2008), we screened all unassembled sequences for di-, tri-, and tetranucleotides by using default settings within the program. Primers were designed using the PRIMER3 software (Rozen and Skaletsky 2000), implemented within the MSATCOMMANDER program, and tagged with a 19-bp M13 forward label (CACGACGTTGTAAAACGAC). Amplification products were chosen to be within a 100–400-bp range (including M13 tag), with an optimal annealing temperature of 59°C (range, 57–63°C), an optimal GC content of ≈50%, low levels of self- or pair-complementarity, and a maximum stability of 8.0 (Faircloth 2008). After the removal of duplicate sequences, 1,679 sequences in total were found to contain tandem repeats within the desired criteria with sufficient flanking region for primer design: 1217 di-, 327 tri-, and 135 tetranucleotide microsatellites with at least 10, 5, and 5 repeats, respectively. Of these, 105 primer pairs were tested.

Primer pairs were optimized using 10 individual *C. lectularius* from distinct geographic sample locations collected within the United States (data not shown). PCRs were carried out in 12-μl total volumes, each containing 1× PCR buffer, 1.75 mM $MgCl_2$, 100 mM dNTPs, ≈20 ng of DNA template, 0.5 U of Apex *Taq*DNA polymerase (Genesee Scientific, San Diego, CA), and double-distilled H_2O to 12 μl. Primer concentration varied between 0.3 and 3 pmol (Table 1), with the forward primer end-labeled with an M13 F-29/IRD700 or 800 IRDye tag (LI-COR Biosciences, Lincoln, NE). PCR cycling conditions were comprised of an initial denaturation stage of 3 min at 95°C, followed by 28 cycles consisting of 30-s denaturation at 95°C, 30 s at optimal annealing temperature of 59°C, and 30-s extension at 72°C. After PCR, 5 μl of stop solution (95% formamide, 20 mM EDTA, 0.1% bromophenol blue) was added to each reaction. Reactions were subsequently denatured at 95°C for 4 min before loading onto a 25-cm 6% polyacrylamide gel, by using either 50–350- or 50–700-bp IRDye standards (LI-COR Biosciences) for accurate product sizing. Results were analyzed using the GeneProfiler software (Scanalytics, Inc., BD Biosciences Bioimaging, Rockville, MD). Upon selection for population screening, PCR multiplexing was performed to maximize efficiency (see Table 1 for multiplexed groups).

Sample Collection and DNA Extraction. From three high-rise multiapartment buildings, one building located in Raleigh, NC, and two buildings within a single apartment complex in Jersey City, NJ (JC-A and JC-B), *C. lectularius* were collected from 17 apartments in total in Raleigh and 14 unique apartments in Jersey City (JC-A, nine apartments; JC-B, five apartments) (Figs. 1 and 2). Where possible, 10 individuals (all life stages included, except eggs) were collected per room. An effort was made to collect samples from adjacent apartments (vertical or horizontal) and from multiple rooms within apartments (living room and bedroom collections were made from two apartments within JC-A). All samples were immediately preserved in 95% ethanol and stored at −20°C pending DNA extraction. From 322 specimens in total ($n = 167$ for NC; 106 [JC-A] and 49 [JC-B] for NJ), genomic DNA was extracted using the DNEasy Blood & Tissue kit (QIAGEN).

Genetic Data Analysis. Summary statistics (mean number of alleles [N_A], expected [H_E] and observed [H_O] heterozygosity) were calculated using Genetic Data Analysis software (Lewis and Zaykin 2001). Tests for departures from Hardy–Weinberg equilibrium and linkage disequilibrium were performed using GENEPOP version 4.0 (Raymond and Rousset 1995, Rousset 2008). Bonferroni correction for multiple tests was applied to each test. MICRO-CHECKER version 2.2.3 software (Van Oosterhout et al. 2004) was used to determine whether null alleles, scoring error, or large allele dropout were evident at any of the loci screened.

Population Genetic Structure. Genotypic differentiation between pairs of populations (samples derived from a single room) was tested using the log likelihood based *G*-test (Goudet et al. 1996). The Markov chain parameters were set to 2,000 dememorizations, 200 batches, and 2,000 iterations per batch. Where a nonsignificant *G*-test is returned, a given pair of populations can be considered part of the same panmictic population (Waples and Gaggiotti 2006). Upon partitioning based upon membership to a panmictic population (see Results), partitioning of genetic diversity was then assessed through an analysis of molecular variance (AMOVA), performed using the software ARLEQUIN version 3.01 (Excoffier et al. 2005). We predicted that the greatest level of differentiation would be observed between building samples. Genetic differentiation based on F_{ST} (Weir and Cockerham 1984), both overall and between buildings, was then estimated using FSTAT version 2.9.3.2 (Goudet 2001). Significance of F_{ST} values was determined through permutation. The Bayesian clustering algorithm implemented in STRUCTURE version 2.2.3 (Pritchard et al. 2000) was then used to determine whether the sampled populations within each building could be subdivided into K genetic clusters (where K is unknown), with no a priori assumption of population genetic structure. Under this method, individuals are probabilistically assigned to each genetic cluster based on the proportion of their genome that

Table 1. Characteristics of 24 microsatellite DNA loci developed for the bed bug and screened for a total of 332 specimens collected in Raleigh, NC, and Jersey City, NJ (buildings JC-A and JC-B)

Locus	Primer sequences (5′-3′)	Repeat motif	μM each primer	No. alleles detected				Allele size range	Mplex group[a]	GenBank accession no.
				Raleigh	JC-A	JC-B	N_A[b]			
BB6B	TCTGAAATTTGTAATTGCCTCAA CCCCCGTGGGTTTATTATTT	$(GA)^{21}$	1.2	3	5	4	8	137–163	H	JQ639329
BB15B	TTCAAAGAAAGGCAAAGTCAAA TACGACGTTTCATGGTGAGG	$(GA)^{30}$	1	3	8	3	11	129–187	C	JQ639330
BB21B	CTAGAAGTGCCTGGAATGGA TGTAATAATGGGCTCTTTCCAA	$(GA)^{28}$	3	4	6	3	11	114–194		JQ639331
BB28B	GGGCGAGAGAGAGTGATACC TTGTAGCGCCCTCTTCAACT	$(GA)^{38}$	1.2	5	8	6	12	115–189	D	JQ639332
BB29B	GCTTCGCCTAACACAAGGA CACCTGTACATCTAAAACAGGATACC	$(GA)^{33}$	1	3	9	7	14	178–268	D	JQ639333
BB31B	GGTAATCAGAGAGTGAGAGTCAGAA GGGAATTGATTTCGTGACAG	$(GA)^{27}$	1	3	5	4	8	196–218	A	JQ639334
BB38B	GACCAAATTAACGCTCCACA CAGTGACAGACAGAAATATAAAGACG	$(TC)^{16}$	0.8	3	4	2	4	131–165	A	JQ639335
BB42B	AAAGTTGATTGTGCGTTTGC CCTAACTTTTGCGTGTATTTTGA	$(GATA)^{11}(GA)^{16}$	1.2	4	4	3	6	137–151	B	JQ639336
Clec6	GATACCTGCAACAACC CGTGTCACCCTTGGCTTTC	$(TATC)^{6}$	1	1	2	2	2	306–310	A	JQ639337
Clec11	GCCACTCTCGGTTCAATGC CTTAGCGGACACCAGTCCC	$(GAA)^{6}$	0.8	2	2	2	4	261–272	A	JQ639338
Clec15	GTTTGCAACCGGCACTGG AGTTAGCACGGTAAGCCCG	$(ACC)^{6}$	1	2	2	2	2	225–231	H	JQ639339
Clec21	CCATTCCAACCCTGCTTACTG ACGACTAAACATGTCCAAACTC	$(ATT)^{6}$	0.6	2	1	1	3	278–284	C	JQ639340
Clec37	GGGTTCGATTCCCACCGTC ACGGACTTCATTAAACATCTAGTGC	$(AAT)^{8}$	0.5	1	5	4	5	208–268	B	JQ639341
Clec43	AGAGATACCCATTTACCTCGGTG GGCAGAAACAAGCCTGTCC	$(TTC)^{5}$	0.5	1	2	2	3	437–443	C	JQ639342
Clec45	GCCTTTGATGGTGTGTCCC ACCAGAAGCGGAATGTTGG	$(AAC)^{5}$	1.2	2	2	2	2	166–169	F	JQ639343
Clec48	CCGGTTCGCCCATTGAAAC TGCATGGCAACTGATGTAACTG	$(AAT)^{6}$	0.8	2	2	2	2	243–246	C	JQ639344
Clec90	TTGGATGTTAGGGCATGAAA CGCAACATACCCTACGCTCT	$(AAT)^{10}$	0.4	2	4	3	5	201–228	F	JQ639345
Clec91	ATAATACCCGATCCCGTTCC CATGATCCCGGAAGCACTAT	$(CTT)^{12}$	0.5	1	7	4	7	157–184	G	JQ639346
Clec96	ATTGGCGTGCGCAATTAT CAGCCGGGACATCTAAAAAG	$(ACT)^{8}(CCT)^{5}(ACT)^{14}$	0.5	2	3	2	4	117–195	E	JQ639347
Clec97	AACCCTTCTGAGTGCTACGC ATGAGCCTATGCTGGGATTG	$(AAT)^{13}$	0.5	2	4	3	4	295–316	E	JQ639348
Clec98	GGACGGCAATCCTGGTATATT AGGCGTGAAATGACCTTAGC	$(ATT)^{12}$	0.5	3	4	3	5	240–261	G	JQ639349
Clec99	ACGAAAAGCAGGAGTGGAGA CGCGAGATCTCCTACGAAGT	$(ATC)^{10}$	0.5	2	5	3	5	138–153	F	JQ639350
Clec104	TCCCTACCAATCGGACATTC GCCCCCTTCCAGTTTTATGT	$(AAT)^{13}$	0.3	1	4	1	4	236–266	E	JQ639351
Clec105	CCTCAGACAATCCACCCAGT AAAGGAGAACCGAGCTGTGA	$(AAAT)^{8}$	0.5	1	5	4	5	306–326	G	JQ639352
Mean (SEM)				2.29 (0.22)	4.29 (0.44)	3.00 (0.29)	5.67 (0.69)			

[a] Mplex group is PCR multiple group.
[b] N_A is total number of alleles detected.

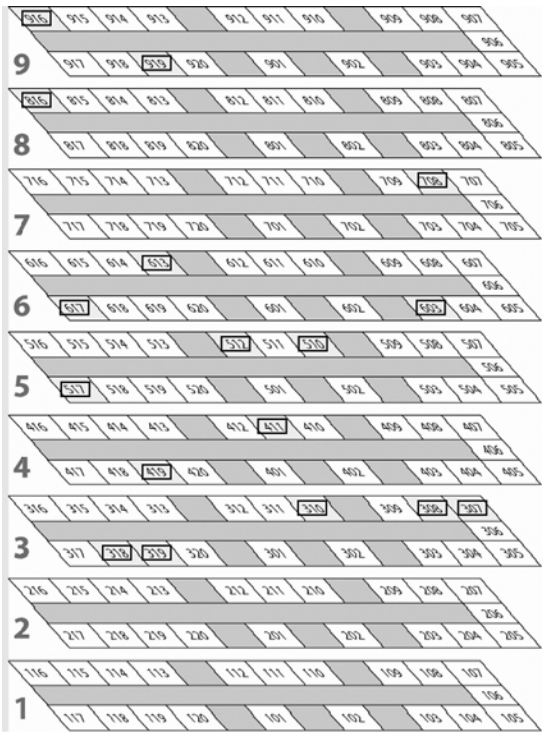

Fig. 1. Building floor plans: Raleigh. Sampled rooms indicated in light gray (outlined with solid black rectangle).

matches that cluster. To determine the true *K*-value, ΔK, a statistic developed by Evanno et al. (2005) was selected. This statistic has been found to accurately identify the uppermost hierarchical level of structure across tested scenarios (Evanno et al. 2005). The presence of large numbers of closely related individuals within a data set can lead to overestimation of the true *K*-value (Vonholdt et al. 2010). Therefore, due to the high level of relatedness within population samples (see Results), single specimens from each population were used for STRUCTURE analysis. To ensure accuracy, 10 independent runs were performed with individuals from within each population selected randomly in each run. STRUCTURE analysis was performed assuming the admixture model with allele frequencies correlated. Runs were based on 200,000 iterations after an initial 50,000 burn-in period of the Markov chain. *K* was set from one to the maximum number of sampled apartments within a building, and replicated three times to check concordance of the data. ΔK was determined using the STRUCTURE HARVESTER version 0.56.3 online software (Earl et al. 2011). *Pr* matrices generated during each replicate run were aligned using the program CLUMPP version 1.1.1 (Jakobsson and Rosenberg 2007) under the GREEDY algorithm with 10,000 random permutations.

Genetic Relatedness and Inbreeding. Average genetic relatedness was estimated at three hierarchical levels: within apartment; within genetic subgroup (where identified); and within building. Estimates were generated using the program Relatedness version 5.0.8 (Queller and Goodnight 1989). The 95% confidence intervals were obtained by jackknifing over loci. Levels of inbreeding (F_{IS}) within each aggregation were estimated using FSTAT (Goudet 2001). These analyses were performed on 10 resampled data sets, each consisting of a single individual selected at random from each sampled room within a given genetic subgroup identified by STRUCTURE.

Results

Microsatellite Characterization. Of the 105 primer pairs tested, 66 amplified unambiguous products within the expected size ranges. Of these, 24 loci exhibiting polymorphism when screened across geographically distinct samples were selected for population analysis (data not shown). When screened across samples collected within the three multiapartment buildings, the number of alleles observed ranged from 2 to 14 (Table 1). After MICROCHECKER analysis, no loci were found to exhibit the genetic signature of null alleles, allelic dropout, or scoring error because of stutter bands. After Bonferroni correction, no significant evidence of linkage disequilibrium was detected.

Summary Population Statistics. Among the studied multiapartment buildings, considerable variation was detected in the levels of allelic diversity: Raleigh, 2.29 ± 0.22 [mean ± SEM]; range, 1–5; Jersey City, JC-A: 4.29 ± 0.44; range, 1–9; JC-B: 3.00 ± 0.29, range, 1–7) (Table 1). Despite this difference in allelic diversity, the mean number of alleles detected within single apartments was comparable in the three buildings (Raleigh, 1.60; JC-A, 2.08; JC-B, 1.37) (Table 2). Within single apartments, overall estimates of expected and observed heterozygosity across loci were low (Raleigh, H_E: 0.198, H_O: 0.190; JC-A, H_E: 0.344, H_O: 0.288; JC-B, H_E: 0.129, H_O: 0.129) (Table 2). After Bonferroni correction, no single apartment population was found to deviate significantly from Hardy–Weinberg expectations. When samples within buildings were combined, however, significant deviations were observed, indicating that random breeding occurs within populations, but the building does not represent a single panmictic unit. Between pairs of populations, nonsignificant *G*-test results were returned in only two instances, and in both cases it involved two rooms in the same apartment within building JC-A (602 LR and 602 BR, and 613LR and 613 BR, respectively), suggesting that each apartment can be considered a single population. As predicted, AMOVA analysis revealed the greatest level of variation to be among buildings (40.90%; $P \leq 0.001$). However, significant variation also was found within buildings (26.69%; $P \leq 0.001$) and within populations (32.41%; $P \leq 0.001$), indicating the existence of substructuring within buildings. Overall F_{ST} between the three buildings was found to be significant, with a value of 0.410 (95% CI, 0.312–0.515). Pairwise estimates revealed the highest level of genetic similarity between the two buildings in Jersey City (0.179), with

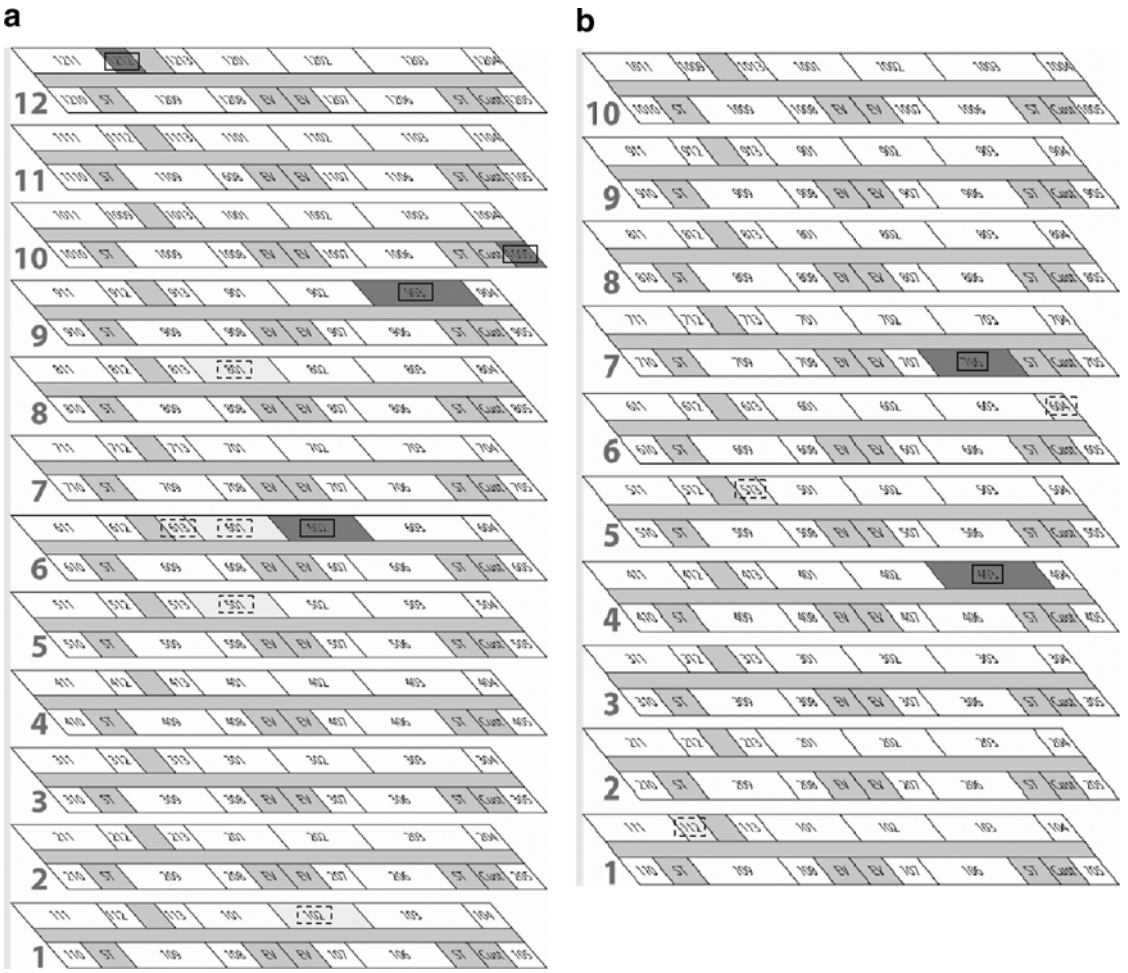

Fig. 2. Building floor plans: Jersey City: (a) JC-A and (b) JC-B. Sampled rooms indicated by shading. JC-A and JC-B cluster 1, dark gray (outlined with solid black rectangle); cluster 2, light gray (outlined with dashed black rectangle).

both exhibiting comparably high values in pairwise comparisons with *C. lectularius* populations in Raleigh apartments (0.433 [JC-A] and 0.463 [JC-B]) (Table 3).

Within Building Population Genetic Structure. Following the method of Evanno et al. (2005) within the Raleigh building, K was identified as 2, with a modal value of 231.86 (averaged across independent runs). No additional ΔK peaks were present. However, Evanno et al. (2005) warn that this method should not be used exclusively as an indicator for K, as ΔK cannot determine the true K if $K = 1$. Assessing the proportion of membership to each cluster within the $K = 2$ runs, no consistent genetic clusters were formed, with significant admixture across clusters common (data not shown). Thus we conclude that *C. lectularius* in the Raleigh building represent a single genetic cluster, and therefore originated from a single introduction event. This is supported by the limited allelic diversity observed across all apartments within the building (Tables 1 and 2).

In contrast to the Raleigh samples, multiple analyses supported the presence of genetic substructure within both buildings sampled in Jersey City. For each, the optimal K-value was determined to be two (Fig. 2), with modal values of ΔK being 60.19 and 34.30 for JC-A and JC-B, respectively (averaged across runs). With the identification of an optimal value of K, STRUCTURE analysis was rerun with all individuals assuming a K of 2 to determine the proportion of membership of *C. lectularius* in each apartment to each genetic cluster (Table 4). In all but one instance, the assignment of individuals to genetic cluster was $\geq$96.7% (Table 4). The single exception with a lower level of assignment was found in building JC-A (apartment 1212, located on the 12th floor), with an assignment of 89.3% to cluster 1. Because no additional apartments were sampled from the 11th or 12th floor of that building, it is impossible to determine whether the lower assignment was due to introgression from nearby populations. However, within that apartment a fifth allele was detected at a single locus, suggesting that the infestation may have multiple origins. Within JC-A, cluster 1 exhibited an average of 3.04 alleles per locus (range, 1–5), and cluster 2 an average of 3.88

Table 2. Summary statistics per apartment sampled within Raleigh, NC (Ral), and Jersey City, NJ (JC-A and JC-B)

Apartment room no.	Avg no. individuals genotyped per locus	A	H_E	H_O	F_{IS}	r (SEM)
Ral 307	9.71	1.67	0.133	0.142	−0.069	0.787 (0.057)
Ral 308	10.00	1.71	0.259	0.263	−0.015	0.323 (0.085)
Ral 310	9.58	1.75	0.242	0.182	0.260	0.393 (0.103)
Ral 318	9.75	1.79	0.146	0.138	0.059	0.716 (0.044)
Ral 319	9.92	1.46	0.174	0.162	0.073	0.699 (0.106)
Ral 411	9.54	1.58	0.191	0.165	0.143	0.544 (0.066)
Ral 419	9.54	1.33	0.097	0.107	−0.111	0.867 (0.051)
Ral 510	9.58	1.67	0.185	0.195	−0.056	0.644 (0.109)
Ral 512	9.79	1.83	0.261	0.222	0.155	0.374 (0.110)
Ral 517	9.83	1.75	0.279	0.266	0.046	0.356 (0.067)
Ral 603	9.96	1.46	0.224	0.194	0.141	0.381 (0.094)
Ral 613	6.75	1.67	0.204	0.144	0.312	0.414 (0.112)
Ral 617	9.83	1.63	0.291	0.279	0.046	0.148 (0.057)
Ral 708	8.46	1.17	0.056	0.068	−0.243	0.924 (0.034)
Ral 816	9.71	1.5	0.179	0.217	−0.229	0.648 (0.076)
Ral 916	9.92	1.67	0.224	0.253	−0.140	0.422 (0.075)
Ral 919	9.42	1.58	0.227	0.236	−0.040	0.473 (0.090)
Mean	9.49	1.60	0.198	0.190	0.043	0.562 (0.042)
JC-A 102	9.88	1.54	0.202	0.170	0.165	0.749 (0.074)
JC-A 501	9.67	2.25	0.430	0.394	0.090	0.244 (0.052)
JC-A 601	9.75	2.33	0.391	0.342	0.133	0.357 (0.075)
JC-A 602 LR	9.83	2.46	0.424	0.39	0.085	0.360 (0.072)
JC-A 602 BR	9.50	2.33	0.403	0.298	0.271	0.322 (0.067)
JC-A 613 BR	9.67	1.96	0.360	0.262	0.285	0.318 (0.035)
JC-A 613 LR	9.67	1.88	0.278	0.302	−0.090	0.558 (0.078)
JC-A 801	6.29	2.13	0.373	0.28	0.268	0.183 (0.065)
JC-A 903	9.63	1.75	0.286	0.241	0.164	0.599 (0.086)
JC-A 1005	8.83	2.04	0.304	0.256	0.165	0.551 (0.077)
JC-A 1212	9.29	2.17	0.337	0.236	0.309	0.405 (0.061)
Mean	9.27	2.08	0.344	0.288	0.171	0.432 (0.028)
JC-B 112	9.72	1.17	0.055	0.050	0.096	0.700 (0.128)
JC-B 403	8.71	1.25	0.117	0.104	0.115	0.782 (0.056)
JC-B 513	9.88	1.75	0.210	0.217	−0.034	0.100 (0.167)
JC-B 604	9.83	1.29	0.110	0.086	0.230	0.525 (0.170)
JC-B 706	9.75	1.42	0.154	0.190	−0.245	0.848 (0.046)
Mean	9.58	1.37	0.129	0.129	0	0.719 (0.040)

Apartment room number (LR, living room; BR, bedroom), average number of individuals genotyped per collection, mean number of alleles per locus (A), expected (H_E) and observed (H_O) heterozygosity, inbreeding coefficient (F_{IS}), and relatedness coefficient (r).

alleles per locus (range, 1–8). Genetic clusters within JC-B, in contrast, exhibited lower levels of allelic diversity, with cluster 1 exhibiting an average of 2.04 alleles per locus (range, 1–5), and cluster 2 an average of 2.21 alleles per locus (range, 1–3) (Table 5). Structure analysis indicates that at least two introduction events from unique populations have occurred for each of the two Jersey City buildings.

Estimates of relatedness revealed comparable results within single apartments among the three buildings, with average within apartment r values equivalent to those expected where consanguineous mating events are common. In spite of this, within the Raleigh building, F_{IS} did not deviate significantly from zero; thus, mating of *C. lectularius* within apartments seems random. In contrast, within JC-A, F_{IS} was found to

Table 3. Population pairwise F_{ST} values (below diagonal) and 95% CI (above diagonal)

	JC-A	JC-B	Raleigh
JC-A	—	0.100–0.272	0.324–0.548
JC-B	0.179	—	0.336–0.585
Raleigh	0.433	0.463	—

Table 4. Proportion of membership, according to the Bayesian method STRUCTURE, for each predefined population (apartment) to each of two inferred clusters identified by *K* within buildings JC-A and JC-B (Jersey City)

Apartment room no.[a]	Inferred cluster		No. individuals
	1	2	
JC-A 102	0.004	0.996	10
JC-A 501	0.011	0.989	10
JC-A 601	0.007	0.993	10
JC-A 602BR	0.978	0.022	10
JC-A 602LR	0.990	0.010	10
JC-A 613BR	0.004	0.996	10
JC-A 613LR	0.002	0.998	10
JC-A 801	0.033	0.967	7
JC-A 903	0.998	0.002	10
JC-A 1005	0.993	0.007	9
JC-A 1212	0.893	0.107	10
JC-B 112	0.999	0.001	10
JC-B 403	0.002	0.998	9
JC-B 513	0.979	0.021	10
JC-B 604	0.998	0.002	10
JC-B 706	0.002	0.998	10

[a] LR, living room; BR, bedroom.

Table 5. Number of alleles observed per locus (and average) detected in each of two STRUCTURE-defined clusters within buildings JC-A and JC-B (Jersey City)

Locus	JC-A-1	JC-A-2	JC-B-1	JC-B-2
BB6B	4	4	3	3
BB15B	5	6	2	2
BB21B	3	6	2	2
BB28B	5	6	3	3
BB29B	4	8	5	3
BB31B	3	5	2	3
BB38B	4	3	2	2
BB42B	3	4	1	3
Clec6	2	2	2	1
Clec11	2	2	2	2
Clec15	1	2	1	2
Clec21	1	1	1	1
Clec37	5	5	4	2
Clec43	2	1	2	2
Clec45	2	2	1	1
Clec48	2	2	1	1
Clec90	3	4	2	3
Clec91	4	6	2	2
Clec96	1	3	2	2
Clec97	3	4	2	3
Clec98	3	4	2	3
Clec99	5	4	2	3
Clec104	2	4	1	1
Clec105	4	5	2	3
Avg	3.04	3.88	2.04	2.21

border on significance within subgroups JC-A-1 ($P = 0.052$; t-test), and was found to diverge significantly from zero in subgroup JC-A-2 ($P = 0.001$; t-test). Within JC-B, F_{IS} did not deviate significantly from zero in either subgroup. Within buildings an r value of 0.562 was observed for *C. lectularius* in the Raleigh building and values of 0.432 and 0.719 for JC-A and JC-B, respectively. When calculated for each of the STRUCTURE defined subgroups within the Jersey City buildings, r values of 0.428 ± 0.037 (SEM) (group 1) and 0.454 ± 0.039 (group 2) were recorded for JC-A and of 0.815 ± 0.066 (group 1) and 0.492 ± 0.115 (group 2) for JC-B. These results inform us that significant inbreeding was occurring in the study populations.

Discussion

This study is the first to address the infestation dynamics of *C. lectularius* within multiapartment buildings specifically through the development and application of high-resolution microsatellite DNA markers. Whereas previous studies have demonstrated that *Cimex* spp. exhibit significant potential for dispersal across contiguous habitats (Pfiester et al. 2009a, How and Lee 2010, Wang et al. 2010), none have used molecular techniques to address this. Thus, the ability of infestations to rapidly spread throughout multiapartment buildings has not been demonstrated because spread could not be differentiated from multiple unrelated introduction events. Despite the contrasting patterns of genetic diversity observed between the Raleigh and Jersey City buildings, the overall levels of polymorphism detected at the loci screened provide insight into the infestation dynamics of these three buildings, revealing that through the introduction of a small founding propagule, infestations can establish and rapidly spread to multiple apartments throughout the building. Although the internal arrangement of apartments within buildings may differ, infestation patterns revealed through microsatellite screening indicate that dispersal occurs both through active dispersal, probably to apartments immediately adjacent (vertically or horizontally) or within a short distance of an infested apartment, and through passive dispersal, probably through human-mediated movement as indicated by infestations detected across multiple noncontiguous apartments or floors.

Genetic Diversity Within Apartments. A pattern observed in all buildings was that of limited genetic diversity within sampled apartments. Within the Raleigh building, genetic diversity was low across all apartments. With the exception of a single locus, all others had no more than four alleles. Indeed, the locus exhibiting a fifth allele did so in a single apartment, with the fifth allele observed in only two individuals. There are several potential scenarios to explain the low genetic diversity we observed in the Raleigh building. First, the initial population from which the founders were derived probably exhibited limited genetic variability. Because no more than four alleles per locus are expected in populations founded by a single female mated to a single male (or to a male and one or more of his full-siblings), introduction may therefore have occurred in the form of a small propagule of individuals, possibly a single female, her progeny, or both. Second, an additional female may have been introduced into the building and then subsequently mated with unrelated males after introduction. Finally, the additional allele observed may have arisen through the process of mutation. The mutation rate of microsatellite loci is considered relatively high, often $\approx 10^{-3}$ to 10^{-4} per locus per gamete per generation (Primmer et al. 1996). Given that this was observed in a single apartment, at a single locus of 24 screened, and in only two individuals, this latter explanation seems to be the most plausible. Within aggregations of *C. lectularius* very rarely are more than four alleles observed. Of 21 infestations collected along the east coast of the United States, 20 were found to exhibit no more than four alleles per locus, including eight populations that also were collected in North Carolina (Saenz et al. 2012). Thus, the likelihood that the population from which the Raleigh building infestation was founded was genetically diverse, if local, is extremely slim. Also, given the high levels of divergence observed between populations as a result of sequential founder effects (i.e., a population that gives rise to a second population that in turn gives rise to a third population, and so on), it is highly unlikely that an introduction occurred from a second source population with matching alleles at all loci plus bearing the additional allele at the single locus.

The results observed here of limited allelic diversity within infestations contrast with the findings of Szalanski et al. (2008), who reported multiple mitochondrial DNA haplotypes within four of six residential dwellings collected within the United States. The detection of multiple maternal lineages within single

collections suggests infestations originate from several genetically distinct founders. Detailed sampling data are not provided by Szalanski et al. (2008), so it is not known whether samples in that study represented different locations within a building. However, in our recent screening of populations collected across the United States and Europe (W. B., C. S., and E.L.V.; unpublished data), we found evidence of common mitochondrial heteroplasmy, raising the possibility that at least some of the variability previously reported within infestations could be due to multiple DNA haplotypes within individuals.

Our use of highly polymorphic microsatellite markers permitted us to estimate the genetic relatedness of each *C. lectularius* population (i.e., collected within each apartment) to all populations within the building and to infer how many unique introduction and colonization events occurred over time, something previously not possible. We found important differences between the Raleigh and Jersey City buildings. These differences may represent geographic variation, construction and locale dissimilarities, or differences in the age and recent colonization events in the respective sites. Whereas the Raleigh building represents spread of *C. lectularius* after an apparent single introduction, the buildings in Jersey City each seem to have been infested by at least two genetically distinct sources. This number may be conservative given the elevated number of alleles observed across loci within genetic clusters. Within JC-A cluster 2, as many as eight alleles were observed, indicating that a minimum of four genetically distinct individuals have contributed to the genetic diversity within that cluster. It is therefore possible that within each building, two separate lineages became established. Subsequently, further introductions occurred that then bred with dispersing males or females, resulting in increased levels of allelic diversity. Within single apartments, populations in different rooms are genetically identical, supporting localized active dispersal as reported by Wang et al. (2010) or frequent short-scale passive dispersal by apartment residents. Given the geographic proximity of this location to New York City, widely reported as one of the most heavily infested cities in the United States, it is possible that the propagule pressure in these buildings was significantly greater than that of the Raleigh building. Thus, the detection of multiple genetic subgroups in the Jersey City buildings is not surprising, based on geographic and locale differences.

As in the Raleigh building, a fifth allele was detected in *C. lectularius* in a single apartment in JC-A (apartment 1212). Given the absence of increased allelic diversity (more than four alleles per locus) within this apartment at the other 23 loci examined, this also may be explained by a mutational event. An alternative explanation is reproduction with an individual from another population that shared a common ancestor within the building but possessed additional alleles through outbreeding. Scanning composite genotypes, no population sampled conclusively explains where this reproductive event may have occurred; however, it was not possible to sample every apartment within each building and no other apartments on the 11th or 12th floors were sampled. It is therefore possible that additional genetic subgroups existed within each building. Regardless, the infestation dynamics within these buildings is remarkable, with rapid spread upon introduction both to rooms vertically and horizontally adjacent to those harboring infestations and also to those lacking obvious physical connections.

Genetic Structure of Infestations

The ability of *C. lectularius* to rapidly infest large multiapartment buildings after the introduction of a small founding propagule raises questions concerning the evolutionary and behavioral processes that counter the potentially detrimental effects of inbreeding. Although relatedness within genetic subgroups is in general elevated to a level indicative of consanguineous mating events, *C. lectularius* reproduction within individual apartments in Raleigh and both JC-B subgroups seems random based upon F_{IS} values. Within the JC-A apartments, F_{IS} is elevated in both subgroups with cluster 1 bordering on significance, and cluster 2 significantly different from zero, potentially indicative of some recent infestation history in which there has been little opportunity for intergenerational breeding. In more established within-apartment populations, the age structure will be more stable allowing for greater multigeneration breeding, resulting in low values of F_{IS} due to panmixia. It is therefore likely that over time, with a failure to sufficiently control the JC-A populations, population sizes within apartments may increase, resulting in decreased F_{IS} values to levels comparable to those seen within apartments in Raleigh and both JC-B subgroups.

The ecological and evolutionary factors permitting *C. lectularius* to successfully infest properties and expand rapidly are currently unknown. Many studies, across a wide range of species, have reported a correlation between levels of observed heterozygosity at apparently neutral microsatellite markers and survival (Calleri et al. 2006), fecundity (Radwan 2003), and lifetime reproductive success (Slate et al. 2000). In *C. lectularius,* values of observed heterozygosity are extremely low (range, 0.129–0.288) compared with other gregarious insect pests (e.g., cockroaches; Crissman et al. 2010, Booth et al. 2011). Yet, aggregations seem to thrive, as evidenced by the extensive spread within buildings observed here and elsewhere (Doggett and Russell 2008, Wang et al. 2010). Although the reduction in heterozygosity is often associated with inbreeding depression resulting from the expression of deleterious recessive alleles (Charlesworth and Charlesworth 1999), there is some empirical evidence that suggests such deleterious genes can be purged from populations demonstrating significant ancestral inbreeding (Crnokrak and Barrett 2002; Swindell and Bouzat 2006a,b; Facon et al. 2011). It is possible, therefore, that the success and rapid spread of *C. lectularius*, both within apartment buildings and globally, may be attributed to such gene purging, either through selection or genetic drift, given the ge-

netic evidence of bottlenecks occurring during colonizations presented here and elsewhere.

Microsatellite Development Through 454 Pyrosequencing. The results presented here on the infestation dynamics were made possible by the development of a large panel of microsatellite markers. Several recent studies have highlighted the potential of 454 pyrosequencing techniques for the detection and characterization of microsatellite DNA, yielding hundreds if not thousands of markers with minimal effort and cost (Abdelkrim et al. 2009, Allentoft et al. 2009, Copeland et al. 2011). Using this technique we successfully isolated 1,679 microsatellite loci, of which 105 were tested and 24 selected for population screening. The potential therefore exists for the optimization of many additional loci should the need arise. With the suite of microsatellite loci selected, genetic diversity was high despite the limited overall sample size. Thus, the loci described here are likely to prove informative at all geographic scales, from local aggregations to global genetic structure.

Conclusions and Implications. The results of this study support the previous findings of Doggett and Russell (2008) and Wang et al. (2010) that infestations, once established within an apartment, have the potential for extensive spread within multiapartment buildings. Remarkably, as demonstrated in the Raleigh building, it seems that only a single introduction of an inseminated female (or her progeny) is capable of establishment and subsequent spread buildingwide. Despite being capable of receiving multiple inseminations after a bloodmeal (Stutt and Siva-Jothy 2001), levels of genetic diversity within aggregations indicate that successful inseminations by multiple males from genetically distinct lineages are exceptionally rare. This pattern is supported after the analysis of 63 additional populations across the United States and Canada (Saenz et al. 2012). The Jersey City buildings may represent a situation more realistic of future infestations within buildings, especially in areas of high propagule pressure (i.e., those in proximity to heavily infested cities or buildings): multiple introductions followed by buildingwide spread. The concern in such instances is the introduction of individuals exhibiting resistance to commonly applied insecticides, such as pyrethroids. Yoon et al. (2008) identified target-site mutations in the voltage-gated sodium channel α-subunit gene responsible for knockdown resistance (*kdr*) to pyrethroid insecticides. The possession of such mutations can result in resistance ratios hundreds, if not thousands, of times greater than populations lacking these mutations (Zhu et al. 2010). Romero et al. (2009) reported metabolic resistance after the inhibition of cytochromes P450 monooxygenase activity with piperonyl butoxide, although only a limited number of populations were assayed. Cytochromes P450 alone, however, were not considered solely responsible for resistance within the populations examined. Recently, after transcriptome analysis, Adelman et al. (2011) documented the significant overexpression of several candidate cytochromes P450 and carboxylesterase genes in a population exhibiting both metabolic and *kdr* resistance to pyrethroids. With resistance arising from multiple evolutionary origins, as the number of unique infestations increases within a building, so does the risk of introduction of resistance. It is therefore vital that new infestations be detected early, ideally through the use of both trained canines and monitoring devices capable of detecting small, early infestations or viable eggs, or a combination (Pfiester et al. 2008, Wang et al. 2010). Our results, revealed through genetic analysis, provide insight into the infestation dynamics of *C. lectularius* within multiapartment buildings. In concert with molecular screening of *kdr*-associated point mutations, this method may educate pest control operators about the methods of treatment most likely to prove effective under such conditions. In addition to informing us of the patterns of infestation and establishment, these methods may prove valuable in the evaluation of treatment success, as well as potentially determining whether reinfestations of treated premises are due to resurgence of untreated individuals due to incomplete pest control treatment or to new introductions of *C. lectularius* from other source populations.

Acknowledgments

We thank the residents within the multiapartment buildings for permitting collections. We are grateful to Paul Labadie and Annette Deferrari for technical assistance and to C. Apperson and W. Watson for valuable research advice and comments on the manuscript. This project was supported by Agriculture and Food Research Initiative Competitive grant 2008-35302-04565 from the USDA National Institute of Food and Agriculture, a grant from the National Pest Management Foundation, the Blanton J. Whitmire endowment at North Carolina State University, and scholarships from the North Carolina Pest Management Association and the Structural Pest Management Training and Research Facility at North Carolina State University.

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Received 21 November 2011; accepted 12 March 2012.