Journal of Pest Science (2026) 99:15
https://doi.org/10.1007/s10340-025-01964-5

RESEARCH

Widespread fixation of *kdr*-associated mutations in temporal samples of *Cimex lectularius* collected from multi-unit buildings

Jin-Jia Yu[1] · Warren Booth[2] · Changlu Wang[1]

Received: 18 June 2025 / Accepted: 12 November 2025

Abstract

The widespread resistance of the common bed bug, *Cimex lectularius* L., to pyrethroid insecticides poses major challenges to effective control. Resistance can be attributed to the presence of target-site DNA mutations and the upregulation of genes associated with metabolic detoxification; the former being easily assessed through sequencing of the *para*-type voltage-gated sodium channel. While studies have documented *kdr*-associated mutation frequencies, temporal investigations are lacking at a scale finer than the continental United States level. To address this knowledge gap, we sequenced 227 populations of *C. lectularius*, primarily collected over a 15 y period (2010–2024) from low-income, multi-unit buildings in New Jersey, to investigate the distribution and temporal dynamics of three *kdr*-associated mutations: V419L, L925I, and I936F. The V419L mutation was present in 95.3–100% of populations sampled across New Jersey, while it was absent from the five populations sampled in Indiana. Post 2014 the V419L mutation was fixed in all sampled populations. Across all temporal and regional samples, the L925I mutation was fixed (100%), whereas the I936F mutation was absent. Our results indicate that the double mutant, commonly referred to as haplotype C, is the predominant genotype across all populations, with haplotype B (L925I mutation only) absent after 2014. The prevalence of *kdr*-associated mutations emphasizes the need for continued resistance monitoring in concert with research into the evolution of resistance mechanisms to support future bed bug management.

Keywords Bed bug · Knockdown resistance · Cimicid · DNA sequencing · Target-site DNA mutation · Invasive species

Introduction

The modern resurgence of bed bugs (*Cimex* spp.) presents a significant challenge to pest management professionals and results in considerable economic and health associated burdens (Doggett and Lee 2023). Over the past three decades, bed bugs have become common pests of the indoor urban environment, including residential and those associated with hospitality, healthcare, and tourism (Lee et al. 2023). Ancestrally, the common bed bug, (*Cimex lectularius* L.) was an ectoparasite of Old-World bats (Booth et al. 2015); however, a human-associated lineage has been associated with urban life since the dawn of civilization (Miles et al. 2025a). This lineage is now globally distributed, primarily in temperate regions, and is the predominant bed bug species found in the United States. While the species is now continentally widespread following its resurgence in the late 1990's/early 2000's, despite over two decades of awareness of bed bugs and their management, infestations continue to be reported frequently (Abbar et al. 2022; Hacker et al. 2022). For instance, reports have increased in four cities in New Jersey since 2011 (Wang et al. 2016), a trend likely mirrored across its introduced range.

Multiple strategies are available for bed bug control, including both non-chemical and chemical treatments (Doggett and Lee 2023). However, managing infestations in multi-unit buildings is particularly challenging (Romero et al. 2017). In multi-unit buildings, bed bugs readily disperse between units through both active and passive means, leading to building wide infestations (Wang et al. 2010; Booth et al. 2012; Cooper et al. 2015). In an effort to suppress populations, chemical control using pyrethroid-based insecticides is frequently implemented by tenants and pest

Communicated by Maohua Chen.

✉ Changlu Wang
changluw@rutgers.edu

[1] Department of Entomology, Rutgers University, 96 Lipman Drive, New Brunswick, NJ 08901, USA

[2] Department of Entomology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA

Published online: 05 December 2025

management professionals (Wang et al. 2016, 2019). However, the frequent use of pyrethroids has led to the development of resistance, either through selection for resistance-associated mutations (Lewis et al. 2023) or the upregulation of genes linked to metabolic detoxification (Adelman et al. 2011); ultimately resulting in control failures. Understanding the distribution and frequency of insecticide resistance of *C. lectularius* at a building wide scale is therefore crucial for the development of appropriate and effective management strategies.

Insecticide resistance mechanisms have been well-characterized in *C. lectularius* populations, especially those associated with pyrethroids (Dang et al. 2017). One key mechanism is target-site-associated DNA mutations in the *para*-type voltage-gated sodium channel (VGSC), commonly referred to as knockdown resistance (*kdr*) mutations (Lee 2025). In *C. lectularius*, three key *kdr* mutations resulting in nonsynonymous amino acid substitutions have been identified: a valine (susceptible) to leucine (resistant) change at position 419 (V419L), a leucine (susceptible) to isoleucine (resistant) at position 925 (L925I), and an isoleucine (susceptible) to phenylalanine (resistant) at position 936 (I936F) (Yoon et al. 2008; Dang et al. 2015). Various haplotypes have also been designated to describe the occurrence of these *kdr* mutations (Zhu et al. 2010; Dang et al. 2015; Holleman et al. 2019; Lewis et al. 2023). These are based upon four key haplotypes (haplotype A—wild-type 419/ wild-type 925; haplotype B—wild-type 419/mutant 925; haplotype C—mutant 419/mutant 925; and haplotype D—mutant 419/ wild-type 925) (Zhu et al. 2010), with additional notation in later studies to denote heterozygosity and the inclusion of the I936F mutation (Dang et al. 2015; Holleman et al. 2019; Lewis et al. 2023). Reviewing studies of *kdr*-associated resistance in U.S. populations of *C. lectularius*, haplotype C has shown an upsurged trend in recent years (Lewis et al. 2023), with approximately 95% of populations sampled after 2018 exhibiting both mutations. More recently, Yu et al. (2025) also documented haplotype C at a high frequency (80–100%) in five out of eight populations of *C. lectularius* sampled in New Jersey between 2012 and 2021. Combined, these findings imply that haplotype C may dominate among *C. lectularius* populations in the U.S.

While numerous studies have taken advantage of the relative ease with which target-site-associated DNA mutations can be screened, few to date have investigated the temporal dynamics of these within and among populations (Booth 2024, but see Lewis et al. 2023, Block et al 2025), and none at a scale finer than the continental level. Given the propensity for low-income communities to harbor persistent and often chronic *C. lectularius* infestations (Wang et al. 2016), understanding the frequency and distribution of mutations within multi-unit buildings, especially those considered low-income, is particularly timely. This is accentuated by the establishment and spread potential of the species, given the widespread distribution of mutations and its ability to rapidly infest multi-unit structures (Doggett and Russell 2008; Wang et al. 2010; Booth et al. 2012; Saenz et al. 2012; Booth 2024). The limiting factor to such temporal studies is the availability of physical samples for DNA sequencing, as temporal infestation data are typically based on reports from pest management professionals or tenants without sample collection.

To address the knowledge gap regarding temporal changes in *kdr*-associated mutations in bed bug populations within multi-unit buildings, *C. lectularius* populations were sampled across three U.S. states between 2010 and 2024. All populations were collected from multi-unit buildings belonging to low-income housing, primarily in New Jersey, except for two samples in New York. Each sample was sequenced to document the frequency of the V419L, L925I, and I936F mutations.

Materials and methods

Sample collection

A total of 227 unique *C. lectularius* populations were collected between 2010 and 2024 from 13 cities or municipalities across three U.S. states (New Jersey: 11 cities, New York: 1 city, Indiana: 1 city) (Fig. 1). In New Jersey, 220 populations were sampled from 29 multi-unit buildings (range: 1–62 populations per building), while only one population was from a single house (Aberdeen) (Table S1). For this study, bed bug samples collected from different apartments within the same building were considered separate populations. The sampled buildings in New Jersey ranged from ~ 5 to ~ 95 km apart (Euclidean distance). In New York, two populations were collected from two separate apartments in a high-rise building; while in Indiana, five populations were collected from five different apartments in a high-rise building. With the exception of the apartment building sampled in New York, all buildings belong to low-income housing. Upon collection, samples were preserved in 95% ethanol and stored at − 20 °C until DNA extraction.

DNA extraction and sequencing

Genomic DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit (Qiagen LLC, Germantown, MD, USA). From each population, a single bed bug was extracted and sequenced. This is justified due to the highly inbred nature of *C. lectularius* populations, which are often founded by a single or small group of highly related individuals that then undergo extensive rounds of inbreeding (Booth et al. 2012, 2018; Saenz et al. 2012; Booth 2024). Therefore, a

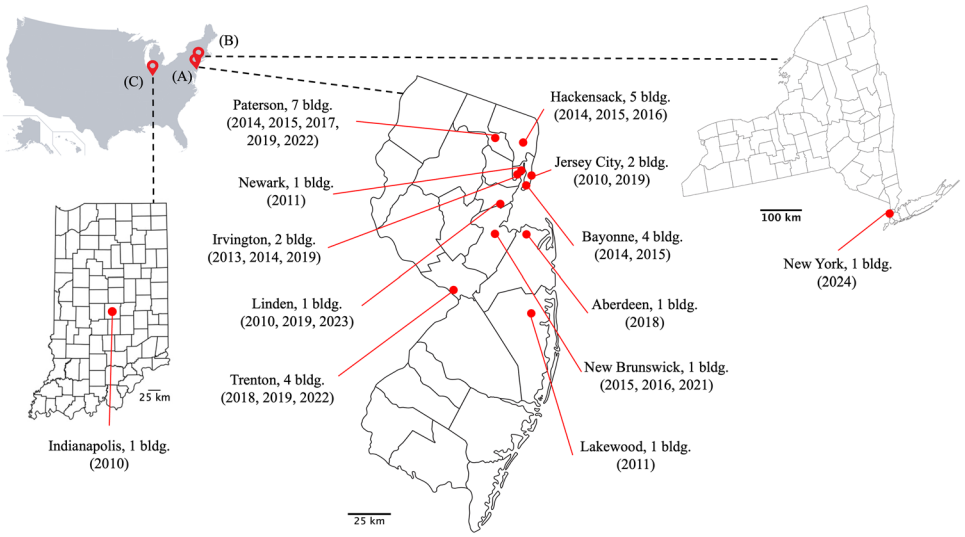

Fig. 1 Cities in (A) New Jersey, (B) New York, and (C) Indiana states where C. lectularius specimens were collected, with the number of buildings sampled listed. Collection years are shown in parentheses

single individual is representative of the genetic diversity within a given population.

Three *kdr*-associated mutations were amplified in two genomic fragments using primer pairs of BBParaF1/BBParaR1 (V419L) and BBParaF3/BBParaR3 (L925I and I936F) (Zhu et al. 2010), following the methods of Holleman et al. (2019). PCR products were purified using Exo-SAP-IT (Thermo Fisher Scientific Inc., Waltham, MA, USA) and sequenced with primers BBParaF1 for V419L and BBParaR3 for L925I and I936F, using the BigDye Terminator v3.1 cycle sequencing kit (Thermo Fisher Scientific Inc., Waltham, MA, USA). Sequencing reactions were purified using the BigDye Xterminator purification kit (Thermo Fisher Scientific Inc., Waltham, MA, USA) and sequenced on an Applied Biosystems SeqStudio Genetic Analyzer System (Thermo Fisher Scientific Inc., Waltham, MA, USA). The resulting chromatograms were visualized using Geneious Prime 2024.0 bioinformatic software (https://www.geneious.com, accessed on 10/20/2024). The presence or absence of mutations was scored.

Detection of VGSC mutations and data analysis

Individuals are identified as susceptible or resistant as follows: V419L—GTC = valine (susceptible), CTC = leucine (resistant); L925I—CTT = leucine (susceptible), ATT = isoleucine (resistant) (Yoon et al. 2008); I936F—ATT = isoleucine (susceptible), TTT = phenylalanine (resistant) (Dang et al. 2015). Heterozygotes are identified as overlapping peaks at the respective nucleotide position. Haplotypes for *kdr* mutations were designated following Dang et al. (2015). For instance, haplotype A^b represents wild-type V419L and L925I, and the resistant I936F.

The variant frequency was calculated by the number of resistant mutations (including both homozygous and heterozygous resistant) divided by the total tested populations. A chi-square test was performed to compare the distribution of the *kdr* haplotypes among populations sampled in the same collection year using R version 4.3.1 (R Core Team 2023).

Results

From the 227 *C. lectularius* populations analyzed, resistance-associated mutations were found at both the 419 and 925 amino acid positions. The I936F mutation was not detected among samples sequenced in this study. Most populations (215/227; 94.7%) exhibited the V419L mutation in the homozygous resistant state, with only twelve samples exhibiting susceptible genotypes (Paterson: 2; Bayonne: 1; Irvington: 2; Linden: 1; Newark: 1; Indianapolis: 5) (Tables 1 and S1). One population from Irvington in 2013 exhibited V419L heterozygosity (Table 1). At the city level, except for Indianapolis, the V419L mutation had a frequency of 95.3–100% (Table 1). In contrast, all populations were fixed for the L925I resistance-associated mutation.

No individual *C. lectularius* was found to exhibit either haplotype A or haplotype D. The dominant haplotype was C across all sample locations (Table 2). In New Jersey, haplotype B was found in five cities, including Paterson, Bayonne, Irvington, Linden, and Newark, from 2010 to 2014, with frequencies ranging from 5.9 to 33.3% (Table 2). However, C was the dominant haplotype in these cities: Paterson (Building 1) in 2014 ($n = 12$, $\chi^2 = 10.7$, df = 1, $p = 0.001$), Irvington (Building 2) in 2014 ($n = 17$, $\chi^2 = 26.5$, df = 1, $p < 0.001$), and Newark in 2011 ($n = 10$, $\chi^2 = 12.8$, df = 1, $p < 0.001$). Populations from Bayonne (Building 2) in 2014, Irvington (Building 1) in 2013, and Linden in 2010 were excluded from statistical analysis due to small sample

Table 1 Collection information of *C. lectularius* populations and the number of samples showing resistant mutation at V419L and L925I

State	City	No. of populations	V419L Resistant (homo/hetero)	L925I Resistant (homo/hetero)
New Jersey	Hackensack	19	19/0 (100%)	19/0 (100%)
	Paterson	62	60/0 (96.8%)	62/0 (100%)
	New Brunswick	6	6/0 (100%)	6/0 (100%)
	Bayonne	30	29/0 (96.7%)	30/0 (100%)
	Trenton	12	12/0 (100%)	12/0 (100%)
	Irvington	43	40/1 (95.3%)	43/0 (100%)
	Linden	31	30/0 (96.8%)	31/0 (100%)
	Jersey City	5	5/0 (100%)	5/0 (100%)
	Newark	10	9/0 (90%)	10/0 (100%)
	Lakewood	1	1/0 (100%)	1/0 (100%)
	Aberdeen	1	1/0 (100%)	1/0 (100%)
New York	New York	2	2/0 (100%)	2/0 (100%)
Indiana	Indianapolis	5	0 (0%)	5/0 (100%)

Percentage data is shown in parentheses. No resistant mutation was found at I936F among all samples

size ($n < 10$). The frequency of haplotype B was low, being found in only eight (3.6%) of 220 populations sequenced. Of these, a single population (0.5%) was found in the heterozygous state ($V419L^{het}$/L925I). Post 2014, all samples exhibited haplotype C. In New York, both populations exhibited haplotype C, whereas all samples in Indiana were found to be haplotype B ($n = 5$) (Table 2).

Discussion

This study conducted a temporal survey of *kdr*-associated mutations across *C. lectularius* populations sampled from low-income, multi-unit buildings. The results demonstrate that resistant V419L and L925I mutations have been prevalent in these *C. lectularius* populations since 2010, whereas the I936F mutation has been absent. Importantly, across the samples collected in New Jersey, the susceptible V419L variant has been absent post 2014.

The V419L and L925I mutations are well-characterized VGSC-associated nucleotide polymorphisms linked to pyrethroid resistance in *C. lectularius* (Yoon et al. 2008; Zhu et al. 2010). When these mutations are combined, and in the absence of increased metabolic activity of glutathione transferases, esterases, and 7-ethoxycoumarin O-deethylase linked to detoxification, they have been shown to be responsible for a 264-fold increase in resistance to the pyrethroid deltamethrin (Yoon et al. 2008). Where bed bug populations may also exhibit increased metabolic detoxification, resistance may increase to thousands-fold (Romero et al. 2007). In the U.S., the V419L and L925I mutations have been reported since 2005 with prevalence subsequently increasing over time (Zhu et al. 2010; Holleman et al. 2019; Lewis et al. 2023; Porras-Villamil et al. 2025). In the current study, V419L and L925I were also found with high frequency (94.7% and 100%, respectively) across all cities from 2010 to 2024. While the I936F mutation has been documented in Australia (Dang et al. 2015), Europe (Balvín and Booth 2018; Porras-Villamil et al. 2025), and Colombia (Porras-Villamil et al. 2025), only two studies reported its presence in the U.S., and at low frequencies (1–2%) (Holleman et al. 2019; Lewis et al. 2023). The I936F mutation was not detected in the current study.

As seen previously in populations sampled across several south-central U.S. states (Holleman et al. 2019), individuals heterozygous at VGSC mutations were rare in the studied populations, with only a single occurrence. This may be attributed to a combination of factors, including founder effects, treatment induced genetic bottlenecks, and negligible gene flow among populations; as is common for this species (Booth et al. 2012, 2015, 2018; Booth 2024; Saenz et al. 2012). As such, populations are likely formed from propagules already fixed for the mutations or rapidly lose susceptible alleles following early treatment with pyrethroids. Additionally, as seen in the southern cattle tick (*Boophilus microplus*) (Aguilar-Tipacamú et al. 2008; Li et al. 2008) and the sub-Saharan mosquito (*Anopheles gambiae*) (Corbel et al. 2004), the survival of heterozygous individuals may be reduced relative to homozygous mutants following pyrethroid-based treatment.

Lewis et al. (2023) compared the temporal change in *kdr*-associated mutation frequency in *C. lectularius* based on samples collected in the U.S. between 2005 and 2009 and 2018–2019. The results revealed a nationwide shift in genotype from the equifrequent distribution of haplotypes B (L925I only) and C (both L925I and V419L), to haplotype C dominating. Haplotype A (wild-type susceptible) was only found in samples collected in 2005–2009, albeit in low

Table 2 Haplotype information for temporal *C. lectularius* populations collected across New Jersey, New York, and Indiana states

State	City	Building	Year	No. of populations	No. of *kdr* haplotypes (%)	
					Haplotype B	Haplotype C
New Jersey	Hackensack	1	2014	1		1 (100)
			2016	1		1 (100)
		2	2014	6		6 (100)
		3	2014	3		3 (100)
			2015	2		2 (100)
		4	2014	2		2 (100)
		5	2014	4		4 (100)
	Paterson	1	2014	12	2 (16.7)	10 (83.3)
			2016	3		3 (100)
		2	2014	6		6 (100)
		3	2014	9		9 (100)
			2016	1		1 (100)
		4	2014	5		5 (100)
			2016	1		1 (100)
		5	2014	3		3 (100)
			2016	1		1 (100)
			2019	3		3 (100)
			2022	1		1 (100)
		6	2014	6		6 (100)
			2016	2		2 (100)
		7	2014	7		7 (100)
			2016	2		2 (100)
	New Brunswick	1	2015	4		4 (100)
			2016	1		1 (100)
			2021	1		1 (100)
	Bayonne	1	2014	12		12 (100)
			2015	2		2 (100)
		2	2014	3	1 (33.3)	2 (66.7)
		3	2014	4		6 (100)
			2015	3		3 (100)
		4	2014	4		4 (100)
		5	2014	2		2 (100)
	Trenton	1	2019	6		6 (100)
		2	2018	1		1 (100)
			2019	1		1 (100)
		3	2022	3		3 (100)
		4	2019	1		1 (100)
	Irvington	1	2013	6	2* (33.3)	4 (66.7)
			2014	11		11 (100)
			2019	1		1 (100)
		2	2014	17	1 (5.9)	16 (94.1)
			2019	8		8 (100)
	Linden	1	2010	3	1 (33.3)	2 (66.7)
			2019	16		16 (100)
			2023	12		12 (100)
	Jersey City	1	2019	2		2 (100)
		2	2010	3		3 (100)
	Newark	1	2011	10	1 (10.0)	9 (90.0)
	Lakewood	1	2011	1		1 (100)
	Aberdeen	1	2018	1		1 (100)
New York	New York	1	2024	2		2 (100)
Indiana	Indianapolis	1	2010	5	5 (100)	

* One heterozygote and one homozygote of resistant mutation at position 419.

frequency. Similarly, Porras-Villamil et al. (2025) reported that haplotype C was the predominant genotype among 14 populations collected between 2008 and 2022. In this study, haplotype C is the dominant genotype (212 out of 220) in New Jersey populations from 2010 to 2023 (Table 2); in contrast, all five Indiana populations sampled in 2010 exhibited haplotype B (L925I only). The failure to detect haplotype B in samples collected post 2014 (this study; Holleman et al. 2019; Cho et al. 2020, 2024; Lewis et al. 2023) suggests that populations are under strong selection pressure from the application of insecticides which act on the VGSC. While the L925I mutation is common and widespread, the V419L mutation is likely to spread rapidly upon its introduction due to the enhanced resistance it confers in combination with L925I. This selective advantage would lead to the elimination of individuals homozygous for the wild-type allele at this position. If these mutations act in an incomplete dominant fashion with no fitness cost being associated with zygosity state, over time selection will then favor the homozygous mutant, resulting in population-level fixation, as seen in the current study. Given the strong selection pressure exerted from continuous building wide insecticide applications, once populations are fixed for haplotype C, the introductions of unrelated haplotypes with reduced resistance (haplotypes A, B, and D) are unlikely to establish due to reduced fitness over haplotype C. Further investigation into the invasion dynamics and temporal stability of populations that take advantage of genome-level resources (e.g., single nucleotide polymorphisms) (Miles et al. 2025b) will benefit our understanding of the role that external sources play in shaping local, regional, and national-level *kdr*-associated haplotypes.

Few studies have documented haplotype A (wild-type susceptible genotype) in the U.S. When found, these were in low frequency (< 13%) (Zhu et al. 2010; Lewis et al. 2023; Porras-Villamil et al. 2025; Yu et al. 2025). In the current study, haplotype A was absent, supporting the nationwide replacement of the susceptible genotype among field-collected *C. lectularius* populations in the U.S, as reported by Lewis et al. (2023). Haplotype D also appears to be rare in *C. lectularius* populations, with its only detection being three samples in the U.S. in 2006 (Zhu et al. 2010). Indeed, based on current publications, the V419L mutation is rare outside of the U.S. (Durrand et al. 2012; Tomita et al. 2012; Booth et al. 2015; Pelenchar et al. 2015; Dang et al. 2017; Booth and Balvin 2018; Cho et al. 2020; Vander Pan et al. 2020; Akhoundi et al. 2021; Castex et al. 2025; Porras-Villimil et al. 2025), suggesting that it evolved within the U.S. and subsequently spread (Balvín and Booth 2018; Booth et al. 2018). Furthermore, the failure of other studies to detect haplotype D, despite significant sampling effort and the temporal span over which samples were collected, suggests that the V419L mutation evolved within a L925I resistant U.S.

population, which may hint that this haplotype is the result of a technical error.

In this study, we hypothesized that the frequency of *kdr*-associated mutations in *C. lectularius* populations would exhibit temporal change toward the double mutant (haplotype C) across the 15 y collection period in response to selection due to the continued application of pyrethroid-based insecticides. This has previously been observed in *C. lectularius* by Lewis et al. (2023), and in *Anopheles sinensis* (Wang et al. 2015). However, our results revealed both consistently high frequencies and widespread prevalence of mutations at both the V419L and L925I mutations. Most samples sequenced here were collected from low-income, multi-unit buildings, where the contracted pest management professionals primarily used pyrethroid-based insecticides to control bed bugs (Wang et al. 2019). Such practices impose strong selection pressure on *kdr*-associated alleles in *C. lectularius* populations. Given the potential for *C. lectularius* to establish building wide infestations following the introduction of a very small founding propagule (Saenz et al. 2012), which rapidly establishes a populations through inbreeding (Booth et al. 2018) then spreads to multiple units within a building (Booth et al. 2012; Wang et al. 2010), this may also explain the low diversity of the *kdr*-associated genotypes observed within the same building in the current study.

Considering the potential factors leading to the fixation of *kdr* alleles in bed bug populations, the exclusive use of pyrethroid-based insecticides is not recommended for managing infestations, particularly in multi-unit buildings where spread may occur rapidly (Doggett and Russell 2008; Wang et al. 2010; Saenz et al. 2012; Cooper et al. 2015). Instead, a building wide cooperation combined with alternative approaches, such as non-pyrethroid insecticides (desiccant dusts) and those with novel active ingredients for which resistance has not been recorded (Saran et al. 2025; Pan et al. 2025), in addition to non-chemical treatment is likely to provide more long-term suppression of resistant bed bug populations (Wang et al. 2019; Wang and Cooper 2023).

To our knowledge, our findings represent the first temporal survey of knockdown resistance genotype frequencies in *C. lectularius* populations specifically focused on low-income multi-unit buildings. Contrary to our hypothesis, haplotype C was the predominant genotype across all populations, with haplotype B absent after 2014. The consistently high prevalence of *kdr*-associated mutations suggests significant challenges for bed bug control. While chemical control remains a cornerstone of bed bug management, these findings emphasize the importance of understanding population-level insecticide susceptibility, ideally through temporal monitoring of resistance mechanisms, in order to develop sustained and effective control strategies.

Author contribution

All authors contributed to the study conception and design. J.Y. and W.B. conducted experiments and analyzed data. J.Y. wrote the first draft of the manuscript. W.B. and C.W. reviewed and revised the manuscript. All authors read and approved the final manuscript.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10340-025-01964-5.

Acknowledgements We thank the current and past members of the Urban Entomology Lab at Rutgers University for assisting with bed bug collections. We appreciate the assistance from the staff of various low-income communities in providing access to bed bug-infested apartments. This is New Jersey Experiment Station publication number D-08-7006476-05-25.

Funding The study was partially funded by the United States Department of Housing and Urban Development Healthy Homes Technical Studies grant program (grant number NJHHU0039-17), and by the National Institute of Food and Agriculture, U.S. Department of Agriculture, Hatch accession no 7006476. Additional funding was provided to W.B. by the Joseph R. and Mary W. Wilson Urban Entomology Endowment and State Research Project VA-137474.

Declarations

Conflict of interests The authors declare no conflict of interests.

References

Abbar S, Cooper R, Ranabhat S, Pan X, Sked S, Wang C (2022) Prevalence of cockroaches, bed bugs, and house mice in low-income housing and evaluation of baits for monitoring house mouse infestations. J Med Entomol 59(3):940–948. https://doi.org/10.1093/jme/tjac035

Adelman ZN, Kilcullen KA, Koganemaru R, Anderson MAE, Miller DM (2011) Deep sequencing of pyrethorid-resistant bed bugs reveals multiple mechanisms of resistance within a single population. PLoS ONE 6:e26228. https://doi.org/10.1371/journal.pone.0026228

Aguilar-Tipacamú G, Miller RJ, Hernández-Ortiz R, Rodriguez-Vivas RI, Vásquez-Paláez C, García-Vázquez Z, Olvera-Valencia F, Rosario-Cruz R (2008) Inheritance of pyrethroid resistance and sodium channel gene mutations in the cattle tick *Boophilus microplus*. Parasitol Res 103(3):633–639. https://doi.org/10.1007/s00436-008-1024-2

Akhoundi M, Chebbah D, Sereno D, Marteau A, Jan J, Bruel CE, Izri A (2021) Widespread mutations in voltage-gated sodium channel gene of *Cimex lectularius* (Hemiptera: Cimicidae) population in Paris. Int J Environ Res Public Health 18(2):407. https://doi.org/10.3390/ijerph18020407

Balvín O, Booth W (2018) Distribution and frequency of pyrethroid resistance-associated mutations in host lineages of the bed bug (Hemiptera: Cimicidae) across Europe. J Med Entomol 55(4):923–928. https://doi.org/10.1093/jme/tjy023

Block CJ, Miles LS, Lewis CD, Vargo EL, Schal C, Booth W (2025) First evidence of the *Rdl* insecticide resistance mutation in populations of the bed bug, *Cimex lectularius* (L.) (Hemiptera: Cimicidae) in North America. J Med Entomol 62:740–744. https://doi.org/10.1093/jme/tjaf033

Booth W (2024) Population genetics as a tool to understand invasion dynamics and insecticide resistance in indoor urban pest insects. Curr Opin Insect Sci 62:101166. https://doi.org/10.1016/j.cois.2024.101166

Booth W, Saenz VL, Santangelo RG, Wang C, Schal C, Vargo EL (2012) Molecular markers reveal infestation dynamics of the bed bug (Hemiptera: Cimicidae) within apartment buildings. J Med Entomol 49(3):535–546. https://doi.org/10.1603/ME1125

Booth W, Balvin O, Vargo EL, Vilímová J, Schal C (2015) Host association drives genetic divergence in the bed bug, *Cimex lectularius*. Mol Ecol 24:980–992. https://doi.org/10.1111/mec.13086

Booth W, Schal C, Vargo EL (2018) Population genetics. In: Doggett SL, Miller DM, Lee C-Y (eds) Advances in the biology and management of modern bed bugs. Wiley, Oxford, pp 173–182

Castex C, Perrin A, Clément L, Perréaz P, Goudet J, Christe P (2025) Genetic characterization of the bat and human lineages of the common bed bug (*Cimex lectularius*) at a local scale. Patasitol. https://doi.org/10.1017/S0031182025000575

Cho S, Kim H-C, Chong S-T, Klein TA, Kwon DH, Lee SH, Kim JH (2020) Monitoring of pyrethroid resistance allele frequency in the common bed bug (*Cimex lectularius*) in the Republic of Korea. Korean J Parasitol 58(1):99. https://doi.org/10.3347/kjp.2020.58.1.99

Cho S, Kim HC, Eom H, Lee JR, Ko CH, Shin E-h, Lee WK, Lee SH, Kim JH (2024) Species identification and pyrethroid resistance genotyping of recently resurgent *Cimex lectularius* and *Cimex hemipterus* in Korea. Parasites Hosts Dis 62(2):251. https://doi.org/10.3347/PHD.24002

Cooper R, Wang C, Singh N (2015) Mark-release-recapture reveals extensive movement of bed bugs (*Cimex lectularius* L.) within and between apartments. PLoS ONE 10(9):e0136462. https://doi.org/10.1371/journal.pone.0136462

Corbel V, Chandre F, Brengues C, Akogbéto LF, Hougard JM, Guillet P (2004) Dosage-dependent effects of permethrin-treated nets on the behavior of *Anopheles gambiae* and the selection of pyrethroid resistance. Malar J 3:22. https://doi.org/10.1186/1475-2875-3-22

Dang K, Toi CS, Lilly DG, Bu W, Doggett SL (2015) Detection of knockdown resistance mutations in the common bed bug, *Cimex lectularius* (Hemiptera: Cimicidae), in Australia. Pest Manag Sci 71(7):914–922. https://doi.org/10.1002/ps.3861

Dang K, Doggett SL, Veera Singham G, Lee C-Y (2017) Insecticide resistance and resistance mechanisms in bed bugs, *Cimex* spp. (Hemiptera: Cimicidae). Parasit Vectors 10:1–31. https://doi.org/10.1186/s13071-017-2232-3

Doggett SL, Russell RC (2008) The resurgence of bed bugs, *Cimex* spp. (Hemiptera: Cimicidae) in Australia. In: Robinson WH, Bajomi D (eds) Proceedings of the 6th international conference on urban pests, 13–16 July 2008, Budapest, Hungary. OOK-Press, Veszprém, Hungary, pp 407–425

Doggett SL, Lee C-Y (2023) Historical and contemporary control options against bed bugs, *Cimex* spp. Annu Rev Entomol 68(1):169–190. https://doi.org/10.1146/annurev-ento-120220-015010

Durand R, Cannet A, Berdjane Z, Bruel C, Haouchine D, Delaunay P, Izri A (2012) Infestation by pyrethroids resistant bed bugs in the suburb of Paris, France. Parasite 19(4):381. https://doi.org/10.1051/parasite/2012194381

Hacker KP, Greenlee AJ, Hill AL, Schneider D, Levy MZ (2022) Spatiotemporal trends in bed bug metrics: New York City. PLoS ONE 17(5):e0268798. https://doi.org/10.1371/journal.pone.0268798

Holleman JG, Robison GA, Bellovich IJ, Booth W (2019) Knockdown resistance-associated mutations dominate populations of the common bed bug (Hemiptera: Cimicidae) across the south central United States. J Med Entomol 56(6):1678–1683. https://doi.org/10.1093/jme/tjz105

Lee C-Y (2025) Global perspective of insecticide resistance in bed bugs and management options. Entomol Res 55(4):e70038. https://doi.org/10.1111/1748-5967.70038

Lee C-Y, Wang C, Su N-Y (2023) Perspective on biology and management of bed bugs: introduction. J Econ Entomol 116(1):1–4. https://doi.org/10.1093/jee/toac141

Lewis CD, Levine BA, Schal C, Vargo EL, Booth W (2023) Decade long upsurge in mutations associated with pyrethroid resistance in bed bug populations in the USA. J Pest Sci 96(1):415–423. https://doi.org/10.1007/s10340-022-01505-4

Li AY, Davey RB, Miller RJ, Guerreri FD, George JE (2008) Genetics and mechanisms of permethrin resistance in the Santa Luiza strain of *Boophilus microplus* (Acari: Ixodidae). J Med Entomol 45(3):427–438. https://doi.org/10.1093/jmedent/45.3.427

Miles LS, Verrelli BC, Adams R, Francioli YZ, Card DC, Balvin O, Castoe TA, Booth W (2025) Were bed bugs the first urban pest insect? Genome-wide patterns of bed bug demography mirror global human expansion. Biol Lett 21(5):40425045. https://doi.org/10.1098/rsbl.2025.0061

Miles LS, Adams R, Franciolo YZ, Card DC, Castoe TA, Booth W (2025) A chromosome-level reference genome for the common bed bug, *Cimex lectularius*, with identification of sex chromosomes. J Hered 116:382–388. https://doi.org/10.1093/jhered/esae071

Palenchar DJ, Gellatly KJ, Yoon KS, Mumcuoglu KY, Shalom U, Clark JM (2015) Quantitative sequencing for the determination of kdr type resistance allele (V419L, L925I, I936F) frequencies in common bed bug (Hemiptera: Cimicidae) populations collected from Israel. J Med Entomol 52:1018–1027. https://doi.org/10.1093/jme/tjv103

Pan K, Sarker S, Wang C (2025) Laboratory evaluation of a novel insecticide, isocyloseram, against the common bed bug (*Cimex lectularius* L.) (Hemiptera: Cimicidae). Insects 16(2):200. https://doi.org/10.3390/insects16020200

Porras-Villamil JF, Hansen IA, Uranga LA, Pinch M, Schal C, Sáez-Durán S, Bueno-Marí R, Trelis M, Fuentes MV, Gaire S, Romero A (2025) Target site mutations and metabolic detoxification of insecticides in continental populations of *Cimex lectularius* and *Cimex hemipterus* (Hemiptera: Cimicidae). J Med Entomol 62(1):130–145. https://doi.org/10.1093/jme/tjae118

R Core Team (2023) R: a language and environment for statistical computing. R Foundation for Statistical Computing. Austria, Vienna

Romero A, Potter MF, Potter DA, Haynes KF (2007) Insecticide resistance in the bed bug: a factor in the pest's sudden resurgence? J Med Entomol 44(2):175–178. https://doi.org/10.1093/jmedent/44.2.175

Romero A, Sutherland AM, Gouge DH, Spafford H, Nair S, Lewis V, Choe D-H, Li S, Young D (2017) Pest management strategies for bed bugs (Hemiptera: Cimicidae) in multiunit housing: a literature review on field studies. J Integr Pest Manag 8(1):13. https://doi.org/10.1093/jipm/pmx009

Saenz VL, Booth W, Schal C, Vargo EL (2012) Genetic analysis of bed bug populations reveals small propagule size within individual infestations but high genetic diversity across infestations from the eastern United States. J Med Entomol 49(4):865–875. https://doi.org/10.1603/ME11202

Saran RK, Hoppé M, Mayor S, Long C, Blakely B, Eppler L, Cartwright B, Scherer CW (2025) Efficacy and utility of isocyloseram a novel isooxazoline insecticide against urban pests and public health disease vectors. Pest Manag Sci 81(2):978–989. https://doi.org/10.1002/ps.8497

Tomita T, Komagata O, Kasai S, Itokawa K, Watanabe M, Yaguchi N, Adachi M, Yoshida M, Kimura G, Kobayashi M (2012) Nationwide survey on pyrethroid-susceptibility of the bed bug, *Cimex lectularius*. Jpn Soc Med Entomol Zool 63:85. https://doi.org/10.11536/jsmez.64.0_85_2

Vander Pan A, Kuhn C, Schmolz E, von Samson-Himmelstjerna G, Krücken J (2020) Detection of target-site and metabolic resistance to pyrethroids in the bed bug *Cimex lectularius* in Berlin, Germany. Int J Parasitol Drugs Drug Resist 14:274–283. https://doi.org/10.1016/j.ijpddr.2020.11.003

Wang C, Cooper R (2023) Environmentally sound bed bug management solutions. In: Dhang P (ed) Urban pest management: an environmental perspective, 2nd edn. CABI, Oxford, pp 11–35

Wang C, Saltzmann K, Chin E, Bennett GW, Gibb T (2010) Characteristics of *Cimex lectularius* (Hemiptera: Cimicidae), infestation and dispersal in a high-rise apartment building. J Econ Entomol 103(1):172–177. https://doi.org/10.1603/EC09230

Wang Y, Yu W, Shi H, Yang Z, Xu J, Ma Y (2015) Historical survey of the *kdr* mutations in the populations of *Anopheles sinensis* in China in 1996–2014. Malar J 14:1–10. https://doi.org/10.1186/s12936-015-0644-0

Wang C, Singh N, Zha C, Cooper R (2016) Bed bugs: prevalence in low-income communities, resident's reactions, and implementation of a low-cost inspection protocol. J Med Entomol 53(3):639–646. https://doi.org/10.1093/jme/tjw018

Wang C, Eiden A, Cooper R, Zha C, Wang D (2019) Effectiveness of building-wide integrated pest management programs for german cockroach and bed bug in a high-rise apartment building. J Integr Pest Manag 10(1):33. https://doi.org/10.1093/jipm/pmz031

Yoon KS, Kwon DH, Strycharz JP, Hollingsworth CS, Lee SH, Clark JM (2008) Biochemical and molecular analysis of deltamethrin resistance in the common bed bug (Hemiptera: Cimicidae). J Med Entomol 45(6):1092–1101. https://doi.org/10.1093/jmedent/45.6.1092

Yu J-J, Lee S-H, Lee C-Y, Wang C (2025) Multiple mechanisms associated with deltamethrin and imidacloprid resistance in field-collected common bed bug, *Cimex lectularius* L. Pestic Biochem Physiol 210:106357. https://doi.org/10.1016/j.pestbp.2025.106357

Zhu F, Wigginton J, Romero A, Moore A, Ferguson K, Palli R, Potter MF, Haynes KF, Palli SR (2010) Widespread distribution of knockdown resistance mutations in the bed bug, *Cimex lectularius* (Hemiptera: Cimicidae), populations in the United States. Arch Insect Biochem Physiol 73(4):245–257. https://doi.org/10.1002/arch.20355