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Population Genetics

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18.1 Introduction

In their review of bed bug biology, Reinhardt and Siva-Jothy (2007) identified population and dispersal ecology as aspects of bed bug biology for which data were sparse. In recent years, however, substantial progress has been made, with notable advances, particularly in our understanding of fine-scale dispersal and population genetics of the Common bed bug, *Cimex lectularius* L. (Szalanski *et al*., 2008; Pfiester *et al*., 2009; Booth *et al*., 2012, 2015; Saenz *et al*., 2012; Fountain *et al*., 2014; Akhoundi *et al*., 2015; Cooper *et al*., 2015; Narain *et al*., 2015; Raab *et al*., 2016); unfortunately, such information is virtually non-existent for the Tropical bed bug, *Cimex hemipterus* (F.). Through a combination of ecological and genetic approaches, insight has been gained into three aspects key to the development of effective management strategies:

- the origins of new infestations
- pathways to invasion
- propagule pressure.

With the array of molecular markers now available, questions previously out-of-reach using purely behavioral or ecological approaches can now be addressed with relative ease (Avise, 2004). To date, studies of bed bug population genetics have focused on two classes of molecular marker: mitochondrial DNA (mtDNA) and nuclear microsatellites. However, with advances in technology and lower costs, next-generation sequencing methods are likely to become prominent in future studies.

It should be noted that this chapter deals exclusively with *C. lectularius*. To date, only a single study has assessed the use of genetic markers to study *C. hemipterus* (Majid and Kee, 2015). With its likely introduction and spread into sub-tropical and temperate regions, genetic studies of this species are warranted.

18.2 The Evolution of Modern Bed Bugs

Cimex lectularius has a long-term association with humans, with evidence suggesting the human-associated lineage evolved from the ectoparasites of cave-dwelling bats (Usinger, 1966; Balvín *et al*., 2015). Data derived from three classes of molecular markers lend support to this hypothesis (Booth *et al*., 2015), with one or more lineages subsequently switching to hominids approximately 245,000 years before present (Balvín *et al*., 2012a). Across the Old World, populations of *C. lectularius* still exist in association with their putative ancestral hosts (Povolný and Usinger, 1966; Usinger, 1966; Balvín *et al*., 2012a), and recent evidence indicates genetic and reproductive isolation between lineages associated with bats and humans within homes (Balvín *et al*., 2012a; Booth *et al*., 2015). Hybridization experiments between the two host-associated

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lineages failed to produce viable offspring, so Wawrocka *et al.* (2015) concluded that post-mating barriers separate these two lineages. Usinger (1966) reported that *C. hemipterus* has been found to utilize birds, humans, and bats as hosts. With a tropicopolitan distribution, the ancestral origin of *C. hemipterus* is currently unknown, but molecular genetic studies of populations on these alternative hosts may clarify the evolutionary history of this species.

18.3 Genetic Variation within Populations

At the infestation level, basic population genetic data (such as number of alleles/haplotypes, levels of expected and observed heterozygosity, and haplotype/nucleotide diversity) provide information relevant to understanding genetic diversity, and thus potentially the number of introductions into a given structure. Careful selection of the most appropriate markers should be made in relation to the question at hand because different markers do not necessarily yield the same information (Avise, 2004).

With some exceptions, notably *C. lectularius* (see Section 18.5), mtDNA has proven useful when inferring propagule pressure of invasive populations, given that the presence of multiple mitochondrial lineages suggests either several distinct introduction events, or the introduction of a single, genetically diverse propagule (Fitzpatrick *et al.*, 2012). Following the sequencing of 22 spatially distinct infestations in the USA, Canada, and Australia at the 16S rRNA gene, Szalanski *et al.* (2008) observed multiple haplotypes within many structures. This finding led the authors to suggest that gene flow among populations was common, and that within these countries bed bugs utilized alternative host species that served as reservoirs prior to their recent resurgence.

Microsatellite DNA – Mendelian inherited, short tandem repeat sequences dispersed throughout the nuclear genome – revealed a contrasting picture. A scenario with four or fewer alleles at each locus within an infestation is possible only when the founding propagule consisted of a single, singly-mated female, a single sexual pair, or a group of highly related individuals (Scenario A). In contrast, observing more than four alleles at two or more loci suggests a single introduction from a genetically diverse source population, or multiple introductions from more than one source population (Scenario B). Note, however, that genetic diversity informs us about the number of *genetically* distinct introductions but not the number of potential *introduction events* from the same source population. Moreover, extensive use of insecticides could selectively eradicate haplotypes that are associated with susceptibility to insecticide, and thus obscure the history of unique introductions, as suggested by Dang *et al.* (2015) in regards to *C. lectularius* in Australia. To date, microsatellite studies have revealed limited within-infestation genetic diversity in human-associated populations (Table 18.1, Figure 18.1a) (Booth *et al.*, 2012, 2015; Saenz *et al.*, 2012; Fountain *et al.*, 2014; Akhoundi *et al.*, 2015; Narain *et al.*, 2015; Raab *et al.*, 2016). As such, with four or fewer alleles observed within most infestations (but see Raab *et al.*, 2016), introductions appear to follow Scenario A, in contrast to the conclusions of Szalanski *et al.*, (2008). Conversely, multiple infestations of the bat-associated lineage follow Scenario B (Booth *et al.*, 2015), a finding not unexpected given their limited exposure to insecticides (see Section 18.5.3), higher prevalence of bed bugs in bat roosts, and greater host density permitting more frequent passive dispersal events (Balvín *et al.*, 2012b; Bartonička and Růžičková, 2013). Therefore, populations of bat-associated *C. lectularius* are likely to be more stable than human-associated populations and likely open to more frequent introductions.

With infestations founded by only one or a few individuals, inbreeding occurs rapidly, elevating genome-wide homozygosity and thus exposing recessive deleterious alleles. This may result in one of two potential outcomes: population extinction or, conversely, the purging of genetic load. Across seven studies for which within-infestation relatedness (r) could be calculated, the average value within human-associated populations was 0.751 (range 0.636–0.822), whereas within the bat-associated lineage, the average value was lower ($r = 0.590$) (Table 18.1). To place the significance of these values in context, within an outbred population r is

Table 18.1 Within and among *Cimex lectularius* infestation population genetic summary statistics

Sample location	No. of loci	*n*	Average N_A	*He*	*Ho*	Average *r*	Reference
Within infestation							
	24	322	2.21	0.229	0.207	0.733	Booth *et al*., 2012
	9	206	1.9	0.265	0.225	0.780	Saenz *et al*., 2012
	8	80	2.83	0.433	0.406	0.636	Narain *et al*., 2015
	6	183	1.81	0.453	NA	NA	Akhoundi *et al*., 2015
	19	156	1.85	0.231	0.194	0.822	Fountain *et al*., 2014 [A,*]
	21	63	1.71	0.288	0.273	0.728	Fountain *et al*., 2014 [B,*]
	20	130	2.42	0.388	0.303	0.590	Booth *et al*., 2015 [C]
	20	525	2.21	0.166	0.129	0.805	Booth *et al*., 2015 [D]
Among infestation							
	24	322	5.75	0.592	0.214	NA	Booth *et al*., 2012
	9	206	10.3	0.779	0.222	NA	Saenz *et al*., 2012
	8	80	12	0.800	0.406	NA	Narain *et al*., 2015
	6	183	NA	0.307	NA	NA	Akhoundi *et al*., 2015
	19	156	4.89	0.566	0.194	NA	Fountain *et al*., 2014 [A,*]
	21	63	5.67	0.680	0.266	NA	Fountain *et al*., 2014 [B,*]
	20	187	10.5	0.721	0.306	NA	Booth *et al*., 2015[C]
	20	525	7.5	0.603	0.130	NA	Booth *et al*., 2015[D]

n, sample size; N_A, number of alleles; H_e, expected heterozygosity; H_O, observed heterozygosity; *r*, relatedness;
* data calculated from DRYAD dataset (doi:10.5061/dryad.cg10d);
[A] five populations across UK and Australia;
[B] within London, UK;
[C] bat-associated lineage;
[D] human-associated lineage.

expected to be ~0 between unrelated individuals, whereas between full-sibling or parent–offspring pairs *r* should be ~0.5. The lower value seen in the bat-associated lineage reflects increased allelic diversity due to more frequent immigration events. However, *r* is still >0.5, suggesting mating among siblings or parent–offspring pairs is common. As expected, where population size is small and consanguineous matings common, within-population heterozygosity is low (Table 18.1, Figure 18.1b). These values sharply contrast with within-population levels of heterozygosity of another significant human-commensal pest, the German cockroach (*Blattella germanica* L.) (Figure 18.1b), highlighting the unique nature of population foundation by the bed bug. Remarkably, heterotic effects experienced by bed bugs resulting from outbreeding appear short-lived (Fountain *et al*., 2015). Therefore, inbreeding may actually promote local adaptation through the purging of deleterious alleles and the fixation of beneficial gene-complexes.

18.4 Genetic Variation among Populations

Regardless of host lineage, dispersal outside of contiguous structures relies exclusively on passive events tied to the respective host. With the increasing frequency and relative ease of both national and international travel, *C. lectularius* now has a distribution spanning the world's temperate regions. The bat-associated

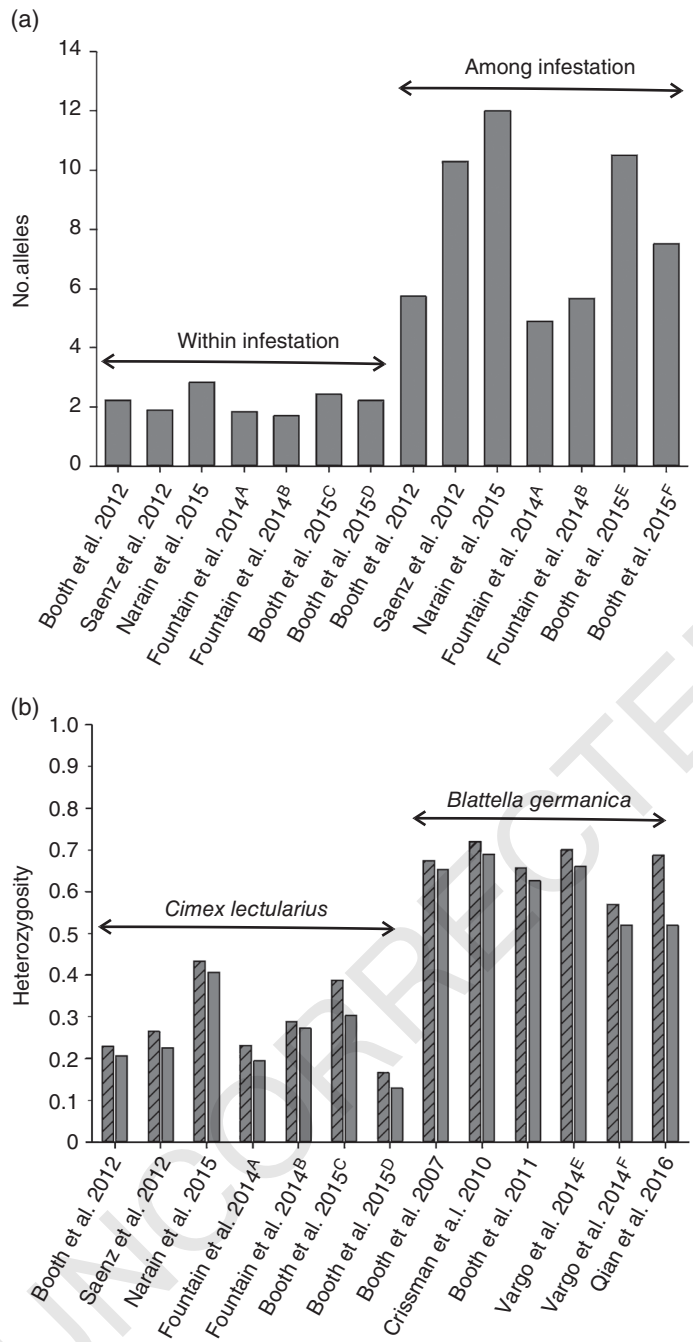

Figure 18.1 Genetic diversity in *Cimex lectularius* populations: (A) Within and among infestation mean number of alleles per locus; (B) Comparison of heterozygosities between the Common bed bug (*C. lectularius*) and the German cockroach (*B. germanica*). Hashed bar, expected heterozygosity; open bar, observed heterozygosity; [A]within-infestation, [B]within city, [C]bat-associated lineage, [D]human-associated lineage. [E]within USA, [F]within Eurasia.

lineage, however, has been recorded only in the Old World (Povolny and Usinger, 1966; Balvín *et al*., 2012b), and with the limited likelihood of transatlantic bat migrations (Constantine, 2003), is unlikely to spread further by bats.

Nevertheless, bats are often found on ships and shipping containers, and their association with human structures could result in eastward and westward dispersal of the bat-associated bed bug lineage with humans. Passive dispersal in association with high population turnover, driven through pest control, has resulted in

human-associated populations existing in highly structured metapopulations, consisting of many island-like populations each founded by a genetically depauperate propagule with limited gene flow among them (Saenz *et al*., 2012; Fountain *et al*., 2014; Booth *et al*., 2015).

As multiple distinct metapopulations likely exist within even limited geographic ranges (say, within a city) (Rosenfeld *et al*., 2016), each founded from genetically distinct sources, high levels of structuring in association with insufficient sampling of multiple populations within a given metapopulation results in exceptionally high values in measures of genetic differentiation, such as F_{ST}. Comparisons with other species are difficult to make as few species exist in such human-centered metapopulations where dispersal is restricted to host-associated movements. The German cockroach and the human louse (*Pediculus humanus* L.) may be the only species to which comparisons are possible. Therefore, when we compare overall F_{ST} values, for example, for *B. germanica*:

- Crissman *et al*., 2010: 0.048
- Booth *et al*., 2011: 0.171
- Vargo *et al*., 2014: 0.026–0.391

with those found in bed bugs (Table 18.1; Range: 0.472–0.718), the latter represent extreme levels of differentiation. Even in *P. humanus*, F_{ST} values in this upper range are only recorded between ecotypes; in other words, head versus body lice ($F_{ST} = 0.409$, Ascunce *et al*., 2013).

When mtDNA genes are considered, diversity appears moderate to high, regardless of scale (regional or continental). For example, haplotype diversity at Cytochrome Oxidase I (COI) is comparable within two US states (0.698 ± 0.052 in Oklahoma and Kansas, Booth and Grant Robison, The University of Tulsa, Department of Biological Science, Tulsa, unpublished results) and across Europe (0.693 ± 0.057; Balvín *et al*., 2012). Variation within the bat-associated lineage shows slightly elevated values (0.797 ± 0.020; Booth *et al*., 2015). A similar pattern of lower diversity within the human-associated lineage versus the bat-associated lineage in Europe is observed at the rRNA 16S gene (Balvín *et al*., 2012a; Booth *et al*., 2015). As bats likely represent the ancestral host, greater genetic diversity in the bat-associated lineage is not surprising (Booth *et al*., 2015).

When allelic diversity across populations is compared over scales ranging from single multi-apartment buildings (Booth *et al*., 2012; Raab *et al*., 2016) to cities or even continents (Saenz *et al*., 2012; Fountain *et al*., 2014; Booth *et al*., 2015; Akhoundi *et al*., 2015; Narain *et al*., 2015), the species-level gene pool is diverse (Figure 18.1a). Following an apparently global population decline in the 1950s, and the resurgence of two species concurrently, it is unlikely that the recent resurgence was driven by a single diverse source population, but instead more likely that multiple propagules from a genetically diverse region in which populations of both species persisted were reintroduced globally. Given that populations of both species have been present in high densities in Africa prior to the global resurgence (Newberry and Jansen, 1986; Newberry *et al*., 1987; Newberry and Mchunu, 1989; Newberry, 1990), it is possible that this continent represents the source of today's resurgence. Future genetic studies should therefore prioritize the collection of samples from across Africa as part of a global investigation. The likelihood that resurgence did not result from multiple local sources is also supported by the absence of detectable patterns of isolation by distance over large geographic scales (Table 18.2, but see Saenz *et al*., 2012 which potentially resulted from samples collected along a frequently travelled interstate route). Locally, a metagenomic single nucleotide polymorphism (SNP) analysis, from environmental swabs collected across New York City, suggests higher relatedness among populations in close proximity (say, linked by subway lines), whereas differentiation occurred among boroughs (Rosenfeld *et al*., 2016).

18.5 Mitochondrial Heteroplasmy

Mitochondrial DNA has long been utilized as an informative marker in studies of phylogeography, population genetics, and invasion biology. This has been due to the inherent properties considered characteristic of mtDNA, specifically a relatively high mutation rate, uniparental inheritance, homoplasmy, and a lack of recombination (Avise, 2000). However, in recent years it has come to light that these characteristics may not

Table 18.2 Among *Cimex lectularius* infestation measures of genetic differentiation.

Sample location	Number of populations	Approx. distance (km)	F_{ST}	Pairwise F_{ST} range	Isolation by distance	Reference
Raleigh, NC and Jersey City, NJ, USA	3	680	0.472	0.176–0.597	NA	Booth *et al*., 2012*
Eastern USA	21	2,000	0.68	0.325–0.983	P <0.0001	Saenz *et al*., 2012
Nebraska and Kansas City, USA	10	265	0.481	0.193–0.742	NA	Narain *et al*., 2015
France	14	900	0.556	0.004–0.862	NS	Akhoundi *et al*., 2015
UK	13	43.4	0.592	–0.035–0.764	NS	Fountain *et al*., 2014**
UK & Australia	5	17,000	0.709	0.492–0.834	NA	Fountain *et al*., 2014**
Europe – Bat lineage	14	2,300	0.468	0.031–0.659	NS	Booth *et al*., 2015
Europe – Human lineage	55	2,300	0.718	0.078–0.982	NS	Booth *et al*., 2015

Approx. distance (km) is approximate distance between most distant infestations;
NA, data not available;
NS, not significant,
* calculated across five STRUCTURE defined populations,
** data calculated from DRYAD dataset (doi:10.5061/dryad.cg10d).

be universal across species, and in cases where these "laws" are violated, the utility of mtDNA as a marker of genetic structure is compromised (White *et al*., 2008). With advances in DNA sequencing technologies and the ability to accurately identify low-frequency variants, reports of heteroplasmy (having two or more distinct mitochondrial lineages present within a cell) are increasing. At the population or individual level heteroplasmy is often rare (White *et al*., 2008); but in the bed bug, it appears to be geographically widespread and pervasive (Robison *et al*., 2015).

18.5.1 Variation in Heteroplasmy across Host Lineages and Among Populations

MtDNA heteroplasmy has been identified across two continents (North America and Europe) and in both host lineages (Booth *et al*., 2015; Robison *et al*., 2015). Among the human-associated lineage it has been found to range from 17% of infestations (south central USA; Robison *et al*., 2015) to 28.8% (Europe; Booth *et al*., 2015), whereas in the bat-associated lineage it was found in 21.6% of populations (Booth *et al*., 2015). Robison *et al.* (2015) further assessed the frequency of heteroplasmic variants within individual bed bugs, and found uncharacteristic patterns in comparison to previous studies of heteroplasmy in other systems. Assessing heteroplasmic individuals from five infestations collected in Oklahoma and Missouri, each were found to possess two mtDNA variants in an approximate 2:1 ratio. Within infestations, some nymphs were also found to be differentially homoplasmic for the variants of heteroplasmic adult females, suggesting that transitions occur from heteroplasmy to homoplasmy. Other individuals have been found to harbor as many as five distinct mtDNA variants, but three variants appeared in low frequency (accounting for 8% of the total variants) (Robison and Booth, unpublished results). It should be noted that the methods for heteroplasmy detection employed here, namely Sanger sequencing of one to two genes, likely underestimates the true rate of heteroplasmy, as variants in other genes may be present. Furthermore, the ability to detect low-frequency variants may be limited. As such, future whole mitogenome studies utilizing next-generation sequencing technology may reveal a rate higher than currently reported. Preliminary studies of "families" generated from differentially homoplasmic laboratory colonies support paternal leakage as the mechanism underlying heteroplasmy (Booth, Vargo and Schal, unpublished results).

18.5.2 Implications of Heteroplasmy

The presence of heteroplasmy has the potential to significantly impact mtDNA-based analyses through the generation of phylogenetic uncertainty resulting from the inability to identify individual haplotypes in a heteroplasmic sequence (White *et al*., 2008). While there are methods to separate haplotypes and thus derive distinct haplotypes from heterozygous genotypes, this is difficult in practice without PCR product cloning and re-sequencing. Analysis of populations may therefore require the exclusion of heteroplasmic individuals (see, for example, Booth *et al*., 2015), the elimination of ambiguous nucleotide sites, or the coding of ambiguous sites as a fifth character state.

In *C. lectularius*, microsatellite studies suggest that the majority of infestations follow Scenario A (see Section 18.3), predicting the occurrence of a single mtDNA haplotype per infestation. However, when samples from a multi-apartment building, previously inferred to have resulted from a single introduction (Booth *et al*., 2012), were sequenced at the COI gene, five mtDNA haplotypes were identified and heteroplasmy was found to be common (Robison and Booth, unpublished results). The strongly supported inference through highly polymorphic nuclear markers of a single introduction, coupled with the understanding that heteroplasmy is pervasive in this species, questions the use of mtDNA for inferring bed bug invasion history and the dating of host-associated lineage divergence.

18.5.3 Insecticide Resistance, *kdr*, and Geographic Variation

Although several mechanisms of insecticide resistance have been documented in *C. lectularius* (see, for example, Adelman *et al*., 2011; Koganemaru *et al*., 2013; see also Chapter 29), knockdown resistance (*kdr*) has been investigated most extensively at the population level. One or more mutations occur in the voltage-sensitive sodium channel α-subunit gene, resulting in the modification of the amino acid sequence and lower sensitivity of the target site to pyrethroid insecticides. In *C. lectularius*, three mutations have been found. Two are geographically widespread: V419L (valine to leucine) and L925I (leucine to isoleucine) (Yoon *et al*., 2008; Zhu *et al*., 2010; Vargo *et al*., 2011; Booth *et al*., 2015; Dang *et al*., 2015; Palenchar *et al*., 2015; Raab *et al*., 2016; Holleman and Booth, The University of Tulsa, Department of Biological Science, Tulsa, unpublished results). These were detected in infestations across the USA, Europe, Middle East, and Australia. A third mutation, I936F (isoleucine to phenylalanine), was identified in Australia and Israel (Dang *et al*., 2015; Palenchar *et al*., 2015). Note that the former amino acid in each is the wild type and is associated with susceptibility, whereas the latter is associated with resistance.

Independent of their contribution to insecticide resistance, it may be possible to view these mutations as informative markers in population genetic studies. For example, within the USA, haplotype B (419 susceptible, 925 resistant) and haplotype C (419 resistant, 925 resistant) are prevalent (32.3% and 57.1%, respectively) (Figure 18.2). In contrast, within Europe, haplotype B dominates (90%); A was found in 4%, C in 2.0%, and the remaining 4% represented populations heterozygous for haplotypes A and B. Within a complex of two high-rise apartment buildings in Paris, France, samples collected from 26 apartments exhibited only haplotype B (Durand *et al*., 2012). Samples collected in Israel were comparable to those in Europe (92% haplotype B, 8% haplotype C). In contrast, haplotype A (419 susceptible, 925 susceptible) is the only haplotype detected in the bat-associated lineage (Booth *et al*., 2015), providing evidence that it represents the ancestral haplotype. This is consistent with microsatellite and mtDNA data, indicating that contemporary gene-flow between host-associated lineages is absent or minimal, and that it is unlikely that bat-associated populations provide propagules for human-associated infestations. Furthermore, the lack of broad homogeneity of the haplotypes across sampled regions suggests that movement between the Old World and New World populations may be limited. Within the USA alone, while samples at present are from only 25 of 50 states and sample size across various states is often small and spatially uneven (Figure 18.2), it is clear that regions such as the east and west coasts exhibit high diversity, with all haplotypes present despite relatively limited sampling. Within the south-central USA, in contrast, only haplotypes B and C have been detected, despite larger sample sizes (Figure 18.2). From this asymmetrical pattern of diversity, we may speculate that patterns of dispersal differ markedly across geographic regions.

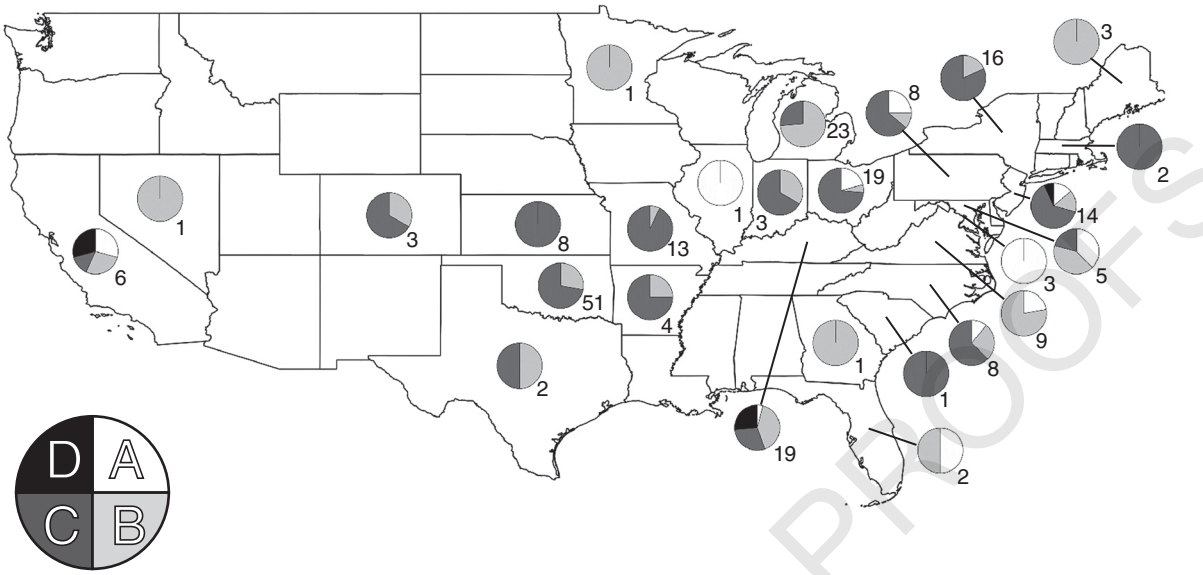

Figure 18.2 *kdr* haplotype distribution in *Cimex lectularius* across the USA. Letter (A–D) refers to haplotype. A: 419 susceptible, 925 susceptible, B: 419 susceptible, 925 resistant, C: 419 resistant, 925 resistant, D: 419 resistant, 925 susceptible. Number below pie is total number of infestations screened. Data combined from Zhu *et al*., (2008); Vargo *et al*., (2011); Holleman and Booth (unpublished results).

18.6 Future Directions in Bed Bug Population Genetics

It is clear that while significant advances have been made in our understanding of the population genetics of *C. lectularius*, these represent the tip of the iceberg. With the recent publication of its genome (Benoit *et al*., 2016; Rosenfeld *et al*., 2016), the bed bug looks poised to become an important model organism for the study of a suite of evolutionary processes. Nevertheless, a number of areas still lack clarity. These include defining genetic associations among globally distributed populations, and understanding the temporal and spatial patterns of *kdr* mutation frequency, from the apartment level to the global scale. Studies using genome-wide markers such as SNPs to identify sources of resurgent populations are critical and may shed light on the evolution of resistance in this species. Taking advantage of the sequenced genome to identify genes underlying host-associated lineage differentiation represents an exciting area for future investigation. Likewise, identifying the mechanisms that underlie heteroplasmy may revolutionize our understanding of eukaryote mitochondrial inheritance.

References

Adelman, Z.N., Kilcullen, K.A., Koganemaru, R., *et al.* (2011) Deep sequencing of pythreroid-resistant bed bugs reveals multiple mechanisms of resistance within a single population. *PLoS ONE*, **6** (10), e26228.

Akhoundi, M., Kengne, P., Cannet, A., *et al.* (2015) Spatial genetic structure and restricted gene flow in bed bugs (*Cimex lectularius*) populations in France. *Infection, Genetics, & Evolution*, **34**, 236–243.

Ascunce, M.S., Toups, M.A., Kassu, G., *et al.* (2013) Nuclear genetic diversity in human lice (*Pediculus humanus*) reveals continental differences and high inbreeding among worldwide populations. *PLoS ONE*, **8** (2), e57619.

Avise, J.C. (2000) *Phylogeography: The History and Formation of Species*, Harvard University Press, Cambridge, MA.

Avise, J.C. (2004) *Molecular Markers, Natural History and Evolution*, Chapman and Hall, New York.
Balvín, O., Munclinger, P., Kratochvíl, L. and Vilímova, J. (2012a) Mitochondrial DNA and morphology show independent evolutionary histories of bedbug *Cimex lectularius* (Heteroptera: Cimicidae) on bats and humans. *Parisitology Research*, **111** (1), 457–169.
Balvín, O., Ševčík, M., Jahelková, H., Bartonička, T., Orlova, M. and Vilímová, J. (2012b) Transport of bugs of the genus *Cimex* (Heteroptera: Cimicidae) by bats in western Palaearctic. *Vespertilio*, **16**, 43–54.
Balvín, O., Roth, S. and Vilímova, J. (2015) Molecular evidence places the swallow bug genus *Oeciacus* Stal within the bat and bed bug genus *Cimex* Linneaus (Heteroptera: Cimicidae). *Systematic Entomology*, **40** (3), 652–665.
Bartonička, T. and Růžičková, L. (2013) Recolonization of bat roost by bat bugs (*Cimex pipastrelli*): could parasite load be a cause of bat roost switching? *Parasitology Research*, **112** (4), 1615–1622
Benoit, J.B., Adelman, Z.N., Reinhardt, K., *et al*. (2016) Unique features of a global human ectoparasite identified through sequencing of the bed bug genome. *Nature Communications*, **7**, 10165.
Booth, W., Santangelo, R.G., Vargo, E.L., *et al*. (2011) Population genetic structure in German cockroaches (*Blattella germanica*): differentiated islands in an agricultural landscape. *Journal of Heredity*, **102**, 175–183
Booth, W., Saenz, V.L., Santangelo, R.G., *et al*. (2012) Molecular markers reveal infestation dynamics of the bed bug (Hemiptera: Cimicidae) within apartment buildings. *Journal of Medical Entomology*, **49** (3), 535–546.
Booth, W., Balvín, C., Vargo, E.L., *et al*. (2015) Host association drives significant genetic differentiation in the common bed bug, *Cimex lectularius*. *Molecular Ecology*, **24**, 980–992.
Cooper, R., Wang, C. and Singh. N. (2015) Mark-release-recapture reveals extensive movement in bed bugs (*Cimex lectularius* L.) within and among apartments. *PLoS ONE*, **10** (9), e0136462.
Constantine, D.G. (2003) Geographic translocation of bats: known and potential problems. *Emerging Infectious Disease*, **9** (1), 17–21.
Crissman, J.R., Booth, W., Santangelo, R.G., *et al*. (2010) Population genetic structure of the German cockroach (Blattodea: Blattellidae) in apartment buildings. *Journal of Medical Entomology*, **47** (4), 553–564.
Dang, K., Toi, C.S., Lilly, D.G., *et al*. (2015) Detection of knockdown resistance (*kdr*) mutations in the common bed bug, *Cimex lectularius* (Hemiptera: Cimicidae) in Australia. *Pest Management Science*, **71** (7), 914–922.
Durand, R., Cannet, A., Berdjane, Z., *et al*. (2012) Infestation by pyrethoids resistant bed bugs in the suburb of Paris, France. *Parasite*, **19** (4), 381–387.
Fitzpatrick, B.M., Fordyce, J.A., Niemiller, M.L. and Reynolds, R.G. (2012) What can DNA tell us about biological invasions? *Biological Invasions*, **14** (2), 245–253.
Fountain, T., Davaux, L., Horsburgh, G., *et al*. (2014) Human-facilitated metapopulation dynamics in an emerging pest species, *Cimex lectularius*. *Molecular Ecology*, **23** (5), 1071–1084.
Fountain, T., Butlin, R.K., Reinhardt, K. and Otti, O. (2015) Outbreeding effects in an inbreeding insect, *Cimex lectularius*. *Ecology and Evolution*, **5** (2), 409–418.
Koganemaru, R., Miller, D.M. and Adelman, Z.N. (2013) Robust cuticular penetration resistance in the common bed bug (*Cimex lectularius* L.) correlates with increased steady-state transcript levels of CPR-type cuticle protein genes. *Pesticide Biochemistry and Physiology*, **106** (3), 190–197.
Majid, A.H.A. and Kee, K.L. (2015) Genotyping of tropical bed bugs, *Cimex hemipterus* F. from selected urban areas in Malaysia inferred from microsatellite marker (Hemiptera: Cimicidae). *Entomology, Ornithology and Herpetology*, **4**, 155.
Narain, R.B., Lalithambika, S. and Kamble, S.T. (2015) Genetic variability and geographic diversity of the common bed bug (Hemiptera: Cimicidae) populations from the Midwest using microsatellite markers. *Journal of Medical Entomology*, **52** (4), 566–572.
Newberry, K. (1990) The tropical bedbug *Cimex hemipterus* near the southernmost extent of its range. *Transactions of the Royal Society of Tropical Medicine & Hygiene*, **84** (5), 745–747.
Newberry, K. and Jansen, E.J. (1986) The common bedbug *Cimex lectularius* in African huts. *Transactions of the Royal Society of Tropical Medicine & Hygiene*, **80** (4), 653–658
Newberry, K. and Mchunu, Z.M. (1989) Changes in the relative frequency of occurrence of infestations of two sympatric species of bedbug in northern Natal and KwaZulu, South Africa. Transactions *of the Royal Society of Tropical Medicine & Hygiene*, **83** (2), 262–264.

Newberry, K., Jansen, E.J. and Thibaud, G.R. (1987). The occurrence of the bedbugs *Cimex hemipterus* and *Cimex lectularius* in northern Natal and KwaZulu, South Africa. *Transactions of the Royal Society of Tropical Medicine & Hygiene*, **81** (3), 431–433.

Palenchar, D.J., Gellatly, K.Y., Yoon, K.S., *et al.* (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. *Journal of Medical Entomology*, **52** (3), 1018–1027.

Pfiester, M., Koehler, P.G. and Pereira, R.M. (2009) Effect of population structure and size on aggregation behavior of *Cimex lectularius* (Hemiptera: Cimicidae). *Journal of Medical Entomology*, **46** (5), 1015–1020.

Povolný, D. and Usinger, R.L. (1966) The discovery of a possibly aboriginal population of the bed bug (*Cimex lectularius* Linnaeus, 1958). *Acta Mus Moraviae, Sci Biol*, **51**, 237–242.

Raab, R.W., Moore, J.E., Vargo, E.L., *et al.* (2016) New introductions, spread of existing matrilines, and high rates of pyrethroid resistance result in chronic infestations of bed bugs (*Cimex lectularius* L.) in lower–income housing. *PLoS ONE*, **11** (2), e0117805.

Reinhardt, K. and Siva-Jothy, M.T. (2007) Biology of the bed bugs (Cimicidae). *Annual Reviews of Entomology*, **52**, 351–374.

Robison, G.A., Balvín, O., Schal, C., *et al.* (2015) Extensive mitochondrial heteroplasmy in natural populations of a resurging human pest, the bed bug (Hemiptera: Cimicidae). *Journal of Medical Entomology*, **52** (4), 734–738.

Rosenfeld, J.A., Reeves, D., Brugler, M.R., *et al.* (2016) Genome assembly and geospatial phylogenomics of the bed bug *Cimex lectularius*. *Nature Communications*, **7**, 10164.

Saenz, V.L., Booth, W., Schal, C. and Vargo, E.L. (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. *Journal of Medical Entomology*, **49** (4), 865–875.

Szalanski, A.L., Austin, J.W., Mckern, J.A., Steelman, C.D. and Gold, R.E. (2008) Mitochondrial and ribosomal internal transcribed spacer 1 diversity of *Cimex lectularius* (Hemiptera : Cimicidae). *Journal of Medical Entomology*, **45** (2), 229–236.

Usinger, R.L. (1966) *Monograph of Cimicidae (Hemiptera – Heteroptera)*, Entomological Society of America, College Park.

Vargo, E.L., Booth, W., Saenz, V., *et al.* (2011) Genetic analysis of bed bug infestations and populations. In: *Proceedings of the Seventh International Conference on Urban Pests, 2011, August, 7–10, São Paulo, Instituto Biológico, São Paulo*.

Vargo, E.L., Crissman, J.R., Booth, W., *et al.* (2014) Hierarchical genetic analysis of German cockroach (*Blattella germanica*) populations from within buildings to across continents. *PLoS ONE*, **9** (7), e102321.

Wawrocka, K., Balvín, O. and Bartonička, T. (2015) Reproductive barrier between two lineages of bed bug (*Cimex lectularius*) (Heteroptera: Cimicidae). *Parasitology Research*, **114** (8), 3019–3025.

White, D.J., Wolff, J.N., Pierson, M. and Gemmell, N.J. (2008) Revealing the hidden complexities of mtDNA inheritance. *Molecular Ecology*, **17** (23), 4925–4942.

Yoon, K.S., Kwon, D.H., Strycharz, J.P., *et al.* (2008) Biochemical and molecular analysis of deltamethrin resistance in the common bed bug (Hemiptera: Cimicidae). *Journal of Medical Entomology*, **45** (6), 1092–1101.

Zhu, F., Wigginton, J., Romero, A., *et al.* (2010) Widespread distribution of knockdown resistance mutations in the bed bug, *Cimex lectularius* (Hemiptera: Cimicidae), populations in the United States. *Archives of Insect Biochemistry and Physiology*, **73** (4), 245–257.