Population genetics as a tool to understand invasion dynamics and insecticide resistance in indoor urban pest insects

Warren Booth

Many indoor urban pest insects now show a near-global distribution. The reasons for this may be linked to their cryptic behaviors, which make unintentional transport likely, tied to their reliance on human-mediated dispersal that can result in spread over potentially long-distances. Additionally, numerous species exhibit an array of mechanisms that confer insecticide resistance. Using population genetics, it is possible to elucidate the genetic characteristics that define globally successful indoor urban pest insect species. Furthermore, this approach may be used to determine the frequency and distribution of insecticide resistance. Here, I review the recent literature that utilizes population genetic analyses in an effort to identify the characteristics that help explain the success of indoor urban pests.

Address
Department of Entomology, Virginia Polytechnic Institute and State University, 1015 Life Science Circle, 215C Steger Hall, Blacksburg, VA, USA

Corresponding author: Booth, Warren (warrenbooth@vt.edu)

Introduction

We currently live in an increasingly urbanized world, where many urban centers are characterized by elevated densification and expansion. By 2050, it is expected that over two-thirds of the human population will live in urban centers, and as such, patterns of human movement and supply chains will expand. The diversity of transport vectors will increase, as will transport pathways, both nationally and globally. With these changes, opportunities for the introduction and transport of non-native species will increase, including those associated with the indoor environment. In concert, the global spread of many species suggests that indoor environments may be converging on a temperature and humidity range that is conducive to supporting non-native indoor pest species in regions where environmental conditions are otherwise beyond the thresholds for survival. It is therefore increasingly likely that indoor urban pest insects will expand their range through human-mediated processes, with many becoming globally cosmopolitan.

In addition to this proclivity for invasion, insecticide resistance is commonly observed in indoor urban pest insects. In many species, multiple mechanisms have evolved, translating into resistance across a diversity of active ingredients [1,2]. While some mechanisms are difficult to evaluate in large-scale field studies (e.g. cuticular thickening or cytochrome P450 gene expression patterns), others are the result of target-site mutations that can be easily monitored and studied in spatio-temporal contexts (e.g. *kdr*-associated mutations [3–5]).

While the fields of conservation and population management have embraced the use of molecular methods in the formulation of management strategies and the development of policy, its application to urban pest management is yet to be realized, offering an opportunity to expand the technology to this field. Understanding the processes and patterns of invasion and dispersal, which includes infestation/reinfestation dynamics, and the evolution and spread of insecticide resistance, from a genomic perspective, may provide important insights that could prove informative in the development and efficacy of future management strategies for urban pest control. Additionally, such information may further our understanding of how species adapt and evolve to the anthropogenic pressures within the urban realm. In light of this, population genetics is now playing an increasingly important role in studies investigating invasion biology and the frequency and distribution of insecticide resistance in indoor urban pest insects [3–7].

Here, I review the literature on invasion and insecticide resistance of indoor urban pest insects from a population genetic perspective. While a diversity of insects can be found in our homes, I concentrate specifically on species for which data predominate, namely bed bugs (the common bed bug, *Cimex lectularius*, and the tropical bed

bug, *C. hemipterus*), the German cockroach (*Blattella germanica*), cat fleas (*Ctenocephalides felis*), and human-associated lice (*Pediculus humanus* spp.).

Indoor urban pest insects: a brief introduction

The indoor urban pest insects with which we associate, albeit involuntarily, originate from the Middle East (*Cimex* spp.), Asia (*B. germanica*, but note that as no free-living populations have been found outside of human-built structures, the actual origin is unknown), and Sub-Saharan Africa (*C. felis* and *P. humanus*). Owing to their close association with humans, each species now exhibits a near-global, cosmopolitan distribution, the exception being the bed bugs, with the common bed bug, *C. lectularius*, primarily distributed in temperate regions, whereas the tropical bed bug, *C. hemipterus*, is largely found in tropical countries. While all can disperse actively over short distances or within contiguous structures, dispersal over larger distances is host-mediated. The females of each species are highly fecund, capable of producing hundreds (*C, lectularius*, *C. hemipterus*, *B. germanica*, and *P. humanus* spp.) to thousands (*C. felis*) of offspring over their lifetime. While *B. germanica* is a generalist omnivore, primarily associated with low socioeconomic households, restaurants, and hotels, *C. lectularius*, *C. hemipterus*, *C. felis*, and *P. humanus* spp. are hematophagous, requiring frequent bloodmeals from their respective hosts to complete development and reproduction, and are found across all socioeconomic classes. As a result of their ability to either spread disease or cause allergic reactions in their hosts, all are considered species of medical and/or veterinary significance.

Invasion

When considering indoor urban pest insects, invasion can be viewed hierarchically. At its finest scale, studies have revealed the infestation dynamics within single buildings or apartment complexes [8–12], with evidence supporting both single and multiple independent invasions and both passive and active modes of dispersal from one room to another. Alternatively, due to the propensity for human-mediated movement driving the dispersal of many indoor urban pest insects, we may view invasion at greater geographic scales, from regional to intercontinental [6,7,10–13]. To date, it has proven difficult to disentangle local-scale dispersal patterns from long-distance human-mediated movement, largely as a result of insufficient sample sizes and a lack of resolution of the genetic markers resulting from low levels of intra-infestation polymorphism. However, given the ease of sampling thousands of single-nucleotide polymorphisms from across the genome, coupled with a significant reduction in sequencing costs over recent years, these genome-wide high-resolution markers now offer a remarkable opportunity to trace patterns of invasion and spread at multiple geographic scales.

The genomic signature of invasion — founder effects, bottlenecks, and bridgeheads

High propagule pressure and high levels of genetic diversity are two factors often credited to the success of a species invading a new area. Paradoxically, these criteria are rarely met by indoor urban pest insect species. Typically, infestations appear to be founded by few individuals, resulting in a genetic bottleneck upon introduction [14]. This is then exacerbated by subsequent rounds of inbreeding during population establishment. As such, most indoor urban pest insect species are characterized by infestations that are genetically depauperate (i.e. lacking genetic diversity) [7,14,15]. This is particularly true for bed bugs, both *C. lectularius* [9,10,16,17] and *C. hemipterus* [18,19], and human lice, *P. humanus* spp. [7], which exhibit remarkably low levels of intra-infestation genetic diversity, despite high levels of intraspecies diversity [7,14,18]. Additionally, gene flow into established infestations appears to be negligible [7,9,10,18,19], resulting in discrete lineages that propagate and spread through both local and long-distance human-mediated dispersal. The combination of which means that genetic isolation-by-distance may be rarely observed [14].

Population-level data are missing for *C. felis*; however, at broader geographic scales, mitochondrial diversity appears low to moderate, depending on geographic region [20]. At the intra-infestation level, *B. germanica* exhibits low-to-moderate levels of genetic diversity [8,13,21], this may be reduced within infestations that experience insecticide-based management [21]. While population genetic studies that address levels of genetic diversity at the intra-infestation level are biased toward bed bugs, it appears clear that indoor urban pest insects are characterized by low levels of diversity, a propensity toward and resilience to inbreeding, and limited levels of gene flow.

Studies of invasion have frequently identified secondary sources that act as bridgeheads for subsequent spread (i.e. invasive populations that then serve as a source for colonists for future invasions) [22]. Indoor urban pest insects represent an extreme example of this bridgehead model, as each infestation has the latent potential to act as a bridgehead, theoretically seeding many future infestations (Figure 1a); instead of a single or few populations acting as bridgeheads, as is commonly seen in other invasive species [22]. Following invasion, extensive inbreeding among founding individuals may occur, elevating the coefficient of inbreeding [9,10,14–16] (Figure 1b), and decreasing genome-wide heterozygosity [8–10,14–19] (Figure 1c). The rate at which the inbreeding coefficient may rise (Figure 1b) and genome-wide heterozygosity may drop (Figure 1c), within newly established populations, may be rapid due to founding propagules possessing low levels of

Figure 1
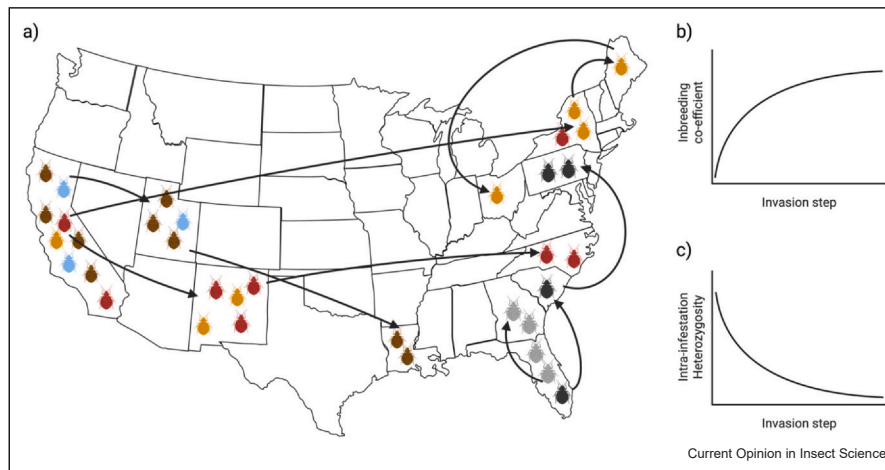
(a) Hypothetical invasion pathways characterized by multiple bridgehead populations. Each population may act as a bridgehead for successive invasions. **(b)** Theoretical change in inbreeding coefficient as the number of invasion steps increases. Each successive population is founded by a small number of related individuals. With negligible gene flow into new populations, the inbreeding coefficient will increase rapidly with each invasion step. **(c)** Theoretical pattern of heterozygosity loss as the number of invasion steps increases. With each step, rare alleles are lost and common alleles become fixed, reducing genome-wide heterozygosity.

intraindividual heterozygosity and high levels of inter-individual relatedness, as is commonly seen in indoor urban pest insect species [14,15]. The success of such genetically depauperate, inbred populations, may result from the genetic purging of deleterious recessive mutations exposed during population foundation at each invasion step, resulting in lineages shaped through selection during prior invasions that possess preadapted gene complexes that promote invasion success to the indoor environment. These lineages may then be successful, both spatially and temporally (i.e. comprising multiple bridgeheads, potentially spread over large geographic areas, and long-lasting), explaining the global distribution and continued spread of many indoor urban pest species.

The distribution and frequency of insecticide resistance

In contrast to the relatively limited number of studies addressing the population and invasion genetics of indoor urban pest insects, studies reporting the distribution and frequency of insecticide resistance-associated mutations are more common [3,5,23–26]. While mechanisms conferring resistance to insecticides are diverse [1,2,4], target-site mutations conferring insensitivity, namely those targeting the γ-amino butyric acid (GABA)-gated chloride ion channel receptor and the voltage-gated sodium channel (VGSC), are easily sequenced, and as a result are frequently studied.

Mutations exist in two forms: a) synonymous, where a mutation that alters the DNA sequence does not result in an amino acid change, and b) nonsynonymous, where

the mutation results in an amino acid change. The former will have no influence on the sensitivity to insecticides (but may be informative when investigating patterns of gene flow), whereas the latter has the potential to alter the conformational structure of the resulting protein and hence its functionality. Across indoor urban pest insect species, studies have identified an array of nonsynonymous mutations, some common across unrelated species (e.g. A302S — GABA-receptor *Rdl*; L993F/L1014F — *kdr*).

γ-Amino butyric acid and the resistance to dieldrin mutation

The resistance to dieldrin (*Rdl)* mutation of the GABA receptor confers target-site insensitivity to insecticides belonging to the cyclodiene (e.g. dieldrin) and phenylpyrazole (e.g. fipronil) families. In several species of indoor urban pest insect, the *Rdl* gene encoding the GABA receptor has been identified and a common nonsynonymous substitution has been found, which results in an alanine (A) to serine (S) switch at amino acid position 302 (A302S) [27,29,30]. While the mutation in the homozygous states confers a high level of resistance, heterozygotes also exhibit an elevated level of insensitivity, relative to the homozygous wild type [31], a factor that may promote spread.

Within both *B. germanica* and *C. felis*, the A302S mutation appears to be common and geographically widespread, reported in the United States, United Kingdom, Europe, Asia, and Australia [27,30–38]. In contrast, while genome sequencing has confirmed the presence of the *Rdl* subunit in both *C. lectularius* [28] and *P. humanus* [29],

population-level studies investigating the frequency and distribution of mutations in this subunit in *P. humanus* are lacking, and only a single study has been published to date for *C. lectularius*. The authors, however, failed to detect the mutation in any of the 10 field-collected strains (9 United States and 1 Czech) examined [39]. Additionally, I am unaware of any study investigating *Rdl*-associated mutations in *C. hemipterus*. Regardless of species, it is clear that there is a paucity of large-scale geographic or temporal studies regarding indoor urban pest insects.

Voltage-gated sodium channel and knockdown resistance

Nonsynonymous mutations have been identified in the gene encoding the VGSC α-subunit of a variety of pest insects, including a number of indoor-associated species [40–42]. Many of these have been shown to alter the functionality of the sodium channel, commonly referred to as knockdown resistance (*kdr*), resulting in insensitivity to pyrethroid, pyrethrin, and organochlorine insecticides.

The number of mutations detected within the VGSC has been found to vary markedly across species, with some exhibiting few (e.g. *C. felis* — 2), while in others, many have been detected (e.g. *B. germanica*/*P. humanus* spp. > 8). Interestingly, this pattern has also been found within a single genus. In *C. lectularius*, three mutations have been reported [40], whereas potentially eight or more have been found in *C. hemipterus* [43–45]. This does not appear to be related to the number of samples screened, where the failure to detect a mutation may result from a small sample size, as Lewis et al. [5] reported *kdr* profiles for 394 unique infestations, detecting only the three previously identified. Furthermore, a caveat of exhibiting multiple mutations is that meta-analyses are complicated when the frequencies of all potential mutations are not reported; either as a result of gene fragments featuring mutations not being sequenced in a given study, or the authors being unaware of additional variable sites in a given fragment. We cannot assume that a mutation is absent if the mutation frequency is not reported for a given study.

With the exception of Hodgdon et al. [3] (*P. humanus*), studies reporting the *kdr*-associated mutation frequencies for indoor urban pest populations on a global scale are lacking. That said, some mutations are common, even across species, and data can be compiled across studies to provide a global picture (Figure 2a–d). For all species discussed here, resistance alleles are globally distributed. However, the frequency of these does vary geographically. For example, within *P. humanus* spp., the 917/929 resistance allele appears less frequent in Asia versus northern Europe (Figure 2c). *Cimex lectularius* homozygous for the 925-/419-resistant

alleles predominates in the United States, whereas in Europe, the Middle East, and Australia, the 419-resistant allele is largely absent (Figure 2b). In contrast, in *C. hemipterus*, I am unaware of any study that documents infestations of wild type (susceptible) at M918I and L1014F, with all infestations reported homozygous for at least one resistant allele, and the majority homozygous resistant at both [43–50].

Few studies report *kdr*-associated mutation frequencies across temporal scales [5,46]. For those that do, or for species for which data can be compared temporally across multiple studies, the trend is for resistant-allele frequency and/or the number of mutations conferring resistance, to increase. The frequency of resistance alleles within populations of *P. humanus* spp. sampled across the United States between 1999 and 2009 was ~84%; however, when considering only those collected between 2007 and 2009, the frequency increased to ~97% [51]. This was corroborated by Gellatly et al. [52] who report a resistant-allele frequency of ~98%, with 132 of 138 U.S. populations sampled between 2013 and 2015 having a resistance allele frequency of 100%. Similarly, in *C. lectularius*, Lewis et al. [5] reported an increase in the 925/419 double mutant from 50% to 84% in samples collected approximately a decade apart (2005–2009 versus 2018–2019). The reasons for this cannot be conclusively identified; however, these trends suggest populations are subjected to anthropogenic pressures that are selecting for resistant alleles, and that susceptible alleles are becoming rare within/among populations. Within populations in which mutations conferring insecticide resistance are fixed, target-site-associated susceptibility will only be recovered when either gene flow occurs from susceptible populations or back mutations to a susceptible state take place. These must also be coupled with an ease in selection pressure (i.e. reduction in insecticide application). However, within indoor urban pest insects, gene flow among populations appears rare, and the frequency at which back mutations take place is unknown.

Temporal studies of population genetics may also play an important role in understanding the evolution of insecticide resistance, through disentangling selection upon standing genetic variation pre-existing within a population before the onset of insecticide introduction versus novel mutation following introduction. Addressing this question is only possible through the use of museum specimens as many insecticides were introduced decades ago (e.g. DDT in the 1940s). A caveat associated with this, is that storage conditions within museums (i.e. frequently in 70% ethanol at room temperature or dry-pinned) often result in specimens exhibiting a high degree of DNA degradation. This will result in short DNA fragments that might not be PCR-amplifiable; thus, next-generation sequencing methods
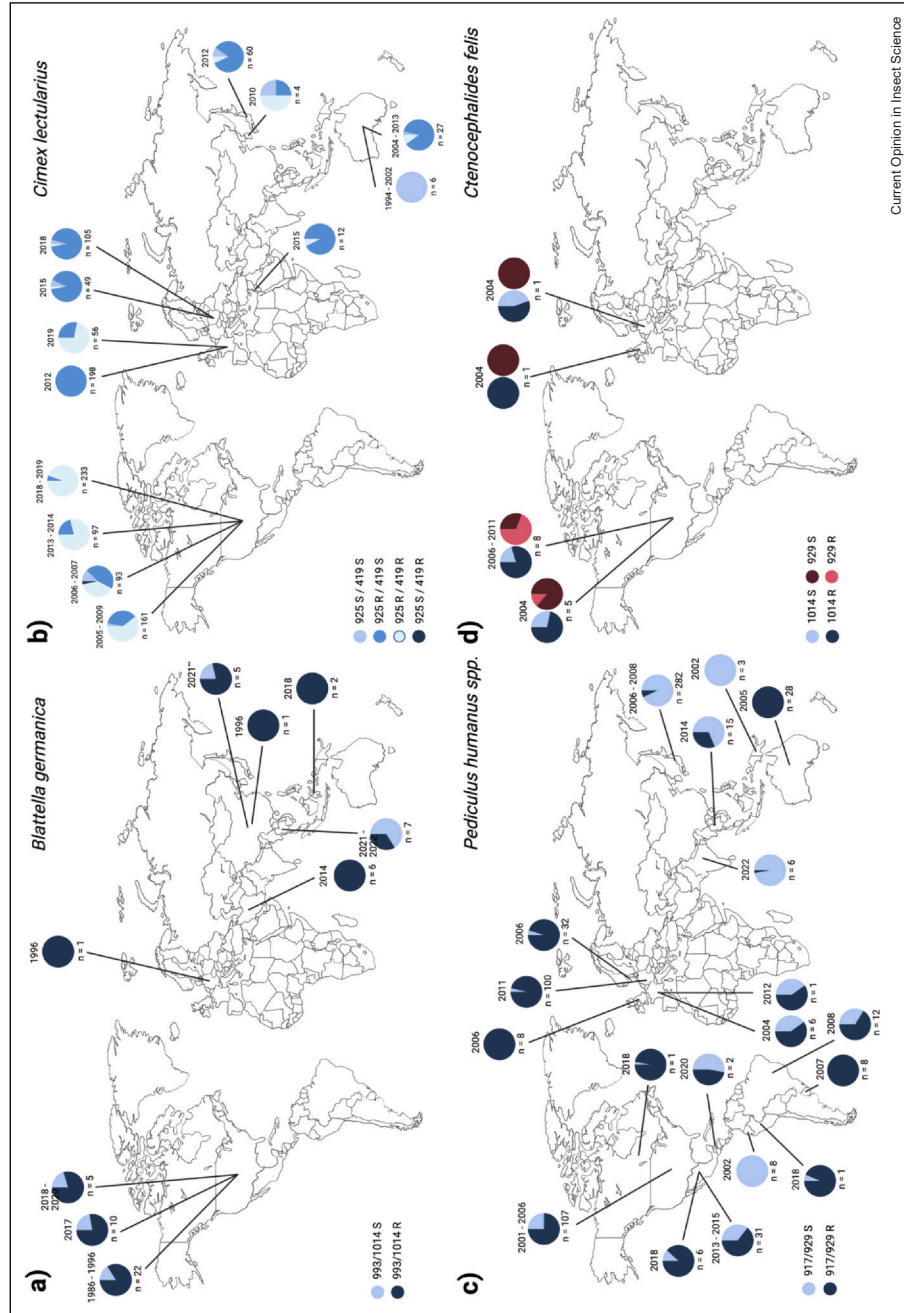
Figure 2

Global frequency and distribution maps for the common *kdr*-associated mutations in four indoor urban pest insect species. References from which data were compiled in parentheses after species name: **(a)** German cockroach, *Blattella germanica* [37,38,42,65–69]; **(b)** common bed bug, *Cimex lectularius* [3,4,41,51–53,57–59,72–74]; **(c)** human head louse, *Pediculus humanus* spp. [5,23–26,40,70,71]; **(d)** Cat flea, *Ctenocephalides felis* [35,60].

may be required. Similarly, without studies over broad geographic scales, linked to studies of gene flow, it is impossible to determine whether selection/mutation occurred independently in multiple regions or once and then spread. Interestingly, while target-site-associated insecticide resistance has been shown to have associated fitness costs [53], 'modifier' loci may alleviate these costs [54]. The relative effects that target-site mutations may confer on fitness in the indoor urban pest insects discussed here require further investigation, as some studies have failed to detect a negative effect [30,55], while others suggest that fitness effects may be significant [56]. The geographically widespread nature of mutations in the GABA receptor and VGSC in these indoor pest species suggests that the fitness costs may be insufficient to eliminate them from the populations, thus, it is likely that mutations will remain common in populations long after specific insecticide classes are removed from use.

Evident following a review of the literature is the lack of information relating to field-collected *C. felis*. Instead, studies report resistance allele frequencies in lab strains originating from samples collected in the field often years earlier [35,60]. Similarly, studies largely fail to report resistance allele frequencies of *P. humanus* spp. per individual, but instead frequencies are derived from sample pools collected across multiple individuals and often multiple collection sites [51,52,57–59,61–63]. This creates a problem when we consider what exactly constitutes a population of an indoor urban pest insect. For example, *P. humanus* spp. infesting a single human may be viewed as a population within a metapopulation framework, the metapopulation being the children/adults that make up a school. Reporting only the allele frequencies of the metapopulation provides no information regarding the dynamics within each single population, and as a result cannot be used to infer source-versus-sink dynamics (i.e. populations with positive growth rates that act as a source of emigrants versus populations with negative growth rates sustained by immigration), in addition to population-specific characteristics. The sources of given variants may be multiple, and indeed not all variants may be present in all populations, thus reporting a metapopulation frequency cannot be used to understand the origin and spread of resistance-associated alleles.

Conclusion

It is clear from this review that studies investigating the temporal and geographic dynamics of indoor urban pest insect invasions are largely lacking. Those published to date, have primarily focused at small scales, from within building [8,9,12] to within regions [10,11,17]. While these have provided important insights into local and regional levels of structure, understanding gene flow across larger geographic scales is essential to understand the dynamics of spread and could prove informative in

the development of control strategies through the identification of source and sink populations. The potential for this has greatly improved in recent years with the generation of reference genomes for several species, including *C. lectularius*, *B. germanica*, *C. felis*, and *P. humanus* spp. These genomic resources provide a basis from which population-level sequencing can be used to understand gene flow, selection, investigating the evolution of insecticide resistance through disentangling standing genetic variation in ancestral populations from the evolution of novel mutations, and identifying novel insecticide targets [28,64]. Given the widespread distribution of resistant *kdr*- and *Rdl*-associated alleles, this is particularly timely.

Through the use of population genetics, four characteristics have emerged that explain the invasion success of indoor urban pests: a) the ability to seed new infestations from small numbers of genetically depauperate founders, b) the ability to resist the potentially harmful effects of prolonged rounds of inbreeding, c) negligible levels of gene flow, and d) populations comprising individuals that possess one or more insecticide resistance mechanisms. Given this, it is easy to understand the near-global distribution of the indoor urban pest species discussed here.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Dang K, Doggett SL, Vera Singham G, Lee C-Y: **Insecticide resistance and resistance mechanisms in bed bugs, *Cimex* spp. (Hemiptera: Cimicidae)**. *Parasit Vectors* 2017, **10**:318, https://doi.org/10.1186/s13071-017-2232-3

2. Scharf ME, Gondhalekar AD: **Insecticide resistance: perspectives on evolution, monitoring, mechanisms and management**. In *Biology and Management of the German Cockroach*. Edited by Wang C, Lee C-Y, Rust MK. CSIRO Publishing; 2021:231-255.

3. Hodgdon HE, Yoon KS, Previte DJ, Kim HJ, Aboelghar GE, Lee SH,
• Clark JM: **Determination of knockdown resistance allele frequencies in global human head louse populations using the serial invasive signal amplification reaction**. *Pest Manag Sci* 2010, **66**:1031-1040, https://doi.org/10.1002/ps.1979.
Here, the authors develop a serial invasive signal amplification reaction method to monitor kdr allele frequencies in the head louse, *P. humanus* capitis. Upon validation of accuracy, the authors screen samples collected across 14 countries, generating the first global map of kdr allele frequency and distribution.

4. Fox K, Larkin K, Sanchez A: **Global trends in genetic markers of *Pediculus humanus capitis* resistance mechanisms**. *Curr Trop Med Rep* 2020, **7**:65-73, https://doi.org/10.1007/s40475-020-00204-3

5. Lewis CD, Levine BA, Schal C, Vargo EL, Booth W: **Decade-long
•• upsurge in mutations associated with pyrethroid resistance in bed bug populations in the United States**. *J Pest Sci* 2023, **96**:415-423, https://doi.org/10.1007/s10340-022-01505-04.
Knockdown resistance has been identified as a potential factor in the global resurgence of the bed bug, *C. lectularius* over the past two decades. Here, the authors screen ~400 unique infestations sampled across two time periods separated by approximately a decade and show an increase in the frequency of the double mutant genotype to near fixation in the recent samples. To my knowledge this is the first study to specifically address the temporal change in kdr allele frequencies over time. The results suggest anthropogenically associated selection.

6. Vargo EL, Crissman JR, Booth W, Santangelo RG, Mukha DV, Schal C: **Hierarchical genetic analysis of German cockroach (*Blattella germanica*) populations from within building to across continents**. *PLoS ONE* 2014, **9**:e102321, https://doi.org/10.1371/journal.pone.0102321

7. House N, Miro-Herrans A, Allen J, Ashfaq M, Boud B, Braig H,
•• Mediannikov O, Mumcuoglu K, Perotti A, Fernando R-R, *et al*.: **Genome-wide analysis of the human head louse (*Pediculus humanus capitis*) reveals geographically structured genetic populations**. *Authorea* 2023,1-22, https://doi.org/10.22541/au.168061521.18272910/v1.
The authors undertake an extensive study of the global population structure of the human head louse, *P. humanus* capitis, generating whole genome sequences for 377 samples collected from 43 countries, spanning five continents and Oceania. Five genetic clusters are identified following Bayesian analyses, corresponding to large geographical regions, either continental or intercontinental. The highest level of genetic variation was found in samples from sub-Saharan Africa. The results suggest that host-mediated global dispersal has shaped and maintained population genetic boundaries.

8. Crissman J, Booth W, Santangelo RG, Mukha C, Vargo EL, Schal C: **Population genetic structure of the German cockroach (*Blattella germanica*) within and among apartments**. *J Med Entomol* 2010, **47**:553-564, https://doi.org/10.1603/me09036

9. Booth W, Saenz VL, Santangelo RG, Schal C, Vargo EL: **Molecular
• markers reveal infestation dynamics of the bed bug (Hemiptera: Cimicidae) within apartment buildings**. *J Med Entomol* 2012, **49**:535-546, https://doi.org/10.1603/me11256.
Using 24 polymorphic nuclear microsatellite loci, this study is the first to show that building-wide infestation of the bed bug, *C. lectularius* can be derived from the introduction of a single gravid female or small propagule of highly related individuals.

10. Saenz V, Booth W, Schal C, Vargo EL: **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* 2012, **49**:865-875, https://doi.org/10.1603/ME11202

11. Akhoundi M, Kengne P, Cannet A, Brengues C, Berenger J-M, Izri A, Marty P, Simard F, Fontenille D, Delaunay P: **Spatial genetic structure and restricted gene flow in bed bugs (*Cimex lectularius*) populations in France**. *Infect Genet Evol* 2015, **34**:236-243, https://doi.org/10.1016/j.meegid.2015.06.028

12. Fan X, Wang C, Bunker DE: **Population structure of German
• cockroaches (Blattodea: Ectobiidae) in an urban environment based on single nucleotide polymorphisms**. *J Med Entomol* 2022, **59**:1319-1327, https://doi.org/10.1093/jme/tjac036.
Here the authors investigate the genetic structure of the German cockroach, *B. germanica*, within and among three low-income

apartment buildings in New Jersey, USA, using single nucleotide polymorphisms. The results indicate significant genetic structure exists among buildings, whereas within buildings variable levels of structure exist, potentially indicating multiple colonization events.

13. Tang Q, Jiang H, Li Y, Bourguignon T, Evans TA: **Population structure of the German cockroach, *Blattella germanica*, shows two expansions across China**. *Biol Invasion* 2016, **18**:2391-2402, https://doi.org/10.1007/s10530-016-1170-x

14. Booth W, Schal C, Vargo EL: **Population genetics of bed bugs**. In *Advances in the Biology and Management of Modern Bed Bugs*. Edited by Doggett SL, Miller DM, Lee C-Y. John Wiley & Sons; 2018:173-182.

15. Vargo EL: **Dispersal and population genetics**. In *Biology and Management of the German Cockroach*. Edited by Wang C, Lee C-Y, Rust MK. CSIRO Publishing; 2021:143-152.

16. Fountain T, Duvaux L, Horsburgh G, Reinhardt K, Butlin RK: **Human-facilitated metapopulation dynamics in an emerging pest species, *Cimex lectularius***. *Mol Ecol* 2014, **23**:1071-1084, https://doi.org/10.1111/mec.12673

17. Narain RB, Lalithambika S, Kamble ST: **Genetic variability and geographic diversity of the common bed bug (Hemiptera: Cimicidae) populations from the Midwest using microsatellite markers**. *J Med Entomol* 2015, **52**:566-572, https://doi.org/10.1093/jme/tjv061

18. Masran SNAS, Majid AHA: **Population genetic structure and breeding pattern of *Cimex hemipterus* (F.) (Hemptera: Cimicidae) in Malaysia**. *J Med Entomol* 2019, **56**:942-952, https://doi.org/10.1093/jme/tjz024

19. Mohammad WNFW, Soh L-S, Ismail WNW, Singham GV: **Infestation pattern and population dynamics of the tropical bed bug, *Cimex hemipterus* (F.) (Hemiptera: Cimicidae) based on novel microsatellite and mtDNA markers**. *Insects* 2020, **11**:472, https://doi.org/10.3390/insects11080472

20. Azrizal-Wahid N, Sofian-Azirun M, Low VL: **New insights into the haplotype diversity of the cosmopolitan cat flea *Ctenocephalides felis* (Siphonaptera: Pulicadae)**. *Vet Parasitol* 2020, **281**:109102, https://doi.org/10.1016/j.vetpar.2020.109102

21. Booth W, Santangelo RG, Vargo EL, Mukha DV, Schal C: **Population genetic structure in German cockroaches (*Blattella germanica*): differentiated islands in an agricultural landscape**. *J Hered* 2011, **102**:175-183, https://doi.org/10.1093/jhered/esq108

22. Blumenfeld AJ, Eyer P-A, Husseneder C, Mo J, Johnson LNL, Wang C, Grace JK, Chouvenc T, Wang S, Vargo EL: **Bridgehead effect and multiple introductions shape the global invasion history of a termite**. *Commun Biol* 2021, **4**:196, https://doi.org/10.1038/s42003-021-01725-x

23. Zhu F, Wigginton J, Romero A, Moore A, Ferguson K, Palli R, Potter
•• MF, Haynes KF, Palli SR: **Widespread distribution of knockdown resistance mutations in the bed bug, *Cimex lectularius* (Hemiptera: Cimicidae), populations in the United States**. *Arch Insect Biochem Physiol* 2010, **73**:245-257, https://doi.org/10.1002/arch.20355.
Here the authors undertake the first large-scale investigation of kdr-associated allele frequencies and distribution across the United States. Of 93 bed bug, *C. lectularius*, infestations screened, 88% were found to possess kdr-associated mutations L925I, V419L, or both. This study is significant as it identified kdr resistance alleles as a potential prominent driver behind the resurgence of the species in the United States.

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

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

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

27. Bass C, Schroeder I, Turberg A, Field LM, Williamson MS: **Identification of the *Rdl* mutation in laboratory and field strains of the cat flea, *Ctenocephalides felis* (Siphonaptera: Pulicidae)**. *Pest Manag Sci* 2004, **60**:1157-1162, https://doi.org/10.1002/ps.937

28. Rosenfeld JA, Reeves D, Brugler MR, Narechania A, Simon S, Durrett R, Foox J, Shianna K, Schatz MC, Gandara J, *et al.*: **Genome assembly and geospatial phylogenomics of the bed bug *Cimex lectularius*** *Nat Commun* 2016, **7**:10164, https://doi.org/10.1038/ncomms10164

29. Lamassiaude N, Toubate B, Neveu C, Charnet P, Dupuy, Debrierre-Grockiego F, Dimier-Poisson I, Charvet CL: **The molecular targets of ivermectin and lotilaner in the human louse *Pediculus humanus humanus*: new prospects for the treatment of pediculosis**. *Plos Pathog* 2021, **17**:e1008863, https://doi.org/10.1371/journal.ppat.1008863

30. Hansen KK, Kristensen M, Jansen K-MV: **Correlation of a resistance-associated *Rdl* mutation in the German cockroach, *Blattella germanica* (L), with persistent dieldrin resistance in two Danish field populations**. *Pest Manag Sci* 2005, **61**:749-753, https://doi.org/10.1002/ps.1059

31. Gondhalekar AD, Scharf ME: **Mechanisms underlying fipronil resistance in a multiresistant field strain of the German cockroach (Blattodea: Blattellidae)**. *J Med Entomol* 2012, **49**:122-131, https://doi.org/10.1603/ME11106

32. Daborn P, McCart C, Woods D, Ffrench-Constant RH: **Detection of insecticide resistance-associated mutations in cat flea *Rdl* by Taqman-allele specific amplification**. *Pestic Biochem Physiol* 2004, **79**:25-30, https://doi.org/10.1016/j.pestbp.2004.02.001

33. Brunet S, Meter CL, Murray M, Soll M, Audonnet J-C: ***Rdl* gene polymorphism and sequence analysis and relation to in vivo fipronil susceptibility in strains of the cat flea**. *J Econ Entomol* 2009, **102**:366-372, https://doi.org/10.1603/029.102.0147

34. Ang L-H, Nazni WA, Kuah M-K, Shu-Chien AC, Lee C-Y: **Detection of the A302S *Rdl* mutation in Fipronil bat-selected strains of the German cockroach (Dictyoptera: Blattellidae)**. *J Econ Entomol* 2013, **106**:2167-2176, https://doi.org/10.1603/EC13119

35. Rust MK, Vetter R, Denholm I, Blagburn B, Williamson MS, Kopp S, Coleman G, Hostetler J, Davis W, Mencke N, *et al.*: **Susceptibility of adult cat fleas (Siphonaptera: Pulicidae) to insecticides and status of insecticide resistance mutations at the *Rdl* and knockdown resistance loci**. *Parasitol Res* 2015, **114**:S7-S18, https://doi.org/10.1007/s00436-015-4512-1

36. González-Morales MA, DeVries Z, Santangelo RG, Kakumanu ML, Schal C: **Multiple mechanisms confer fipronil resistance in the German cockroach: enhanced detoxification and *Rdl* mutation**. *J Med Entomol* 2022, **59**:1721-1731, https://doi.org/10.1093/jme/tjac100

37. Lee S-H, Choe D-H, Scharf ME, Rust MK, Lee C-Y: **Combined metabolic and target-site resistance mechanisms confer fipronil and deltamethrin resistance in field-collected German cockroaches (Blattodea: Ectobiidae)**. *Pestic Biochem Physiol* 2022, **184**:105123, https://doi.org/10.1016/j.pestbp.2022.105123

38. Tisgratog R, Panyafeang C, Lee S-H, Rust MK, Lee C-Y: **Insecticide resistance and its potential mechanisms in field-collected German roaches (Blattodea: Ectobiidae) from Thailand**. *J Econ Entomol* 2023, **116**:1321-1328, https://doi.org/10.1093/jee/toad117

39. González-Morales MA, DeVries Z, Sierras A, Santangelo RG, Kakumanu ML, Schal C: **Resistance to fipronil in the common bed bug (Hemiptera: Cimicidae)**. *J Med Entomol* 2021, **58**:1798-1807, https://doi.org/10.1093/jme/tjab040

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

41. Lee SH, Yoon K-S, Williamson MS, Goodson SJ, Takono-Lee M, Edman JD, Devonshire AL, Clark JM: **Molecular analysis of *kdr*-like resistance in permethrin-resistant strains of head lice, *Pediculus capitis***. *Pestic Biochem Physiol* 2000, **66**:130-143, https://doi.org/10.1006/pest.1999.2460

42. Liu Z, Valles SM, Dong K: **Novel point mutations in the German cockroach *para* sodium channel gene are associated with knockdown resistance (*kdr*) to pyrethroid insecticides**. *Insect Biochem Mol Biol* 2000, **30**:991-997, https://doi.org/10.1016/s0965-1748(00)00074-6

43. Dang K, Toi CS, Lilly DG, Lee C-Y, Naylor R, Tawatsin A, Thavara U, Bu W, Doggett SL: **Identification of putative *kdr* mutations in the tropical bed bug, *Cimex hemipterus* (Hemiptera: Cimicidae)**. *Pest Manag Sci* 2014, **71**:1015-1020, https://doi.org/10.1002/ps.3880

44. Punchihewa R, de Silva WAPP, Weeraratne TC, Karunaratne SHPP: **Insecticide resistance mechanisms with novel '*kdr*' type gene mutations in the tropical bed bug *Cimex hemipterus***. *Parasit Vectors* 2019, **12**:310, https://doi.org/10.1186/s13071-019-3565-x

45. Soh L-S, Singham GV: **Cuticle thickening associated with fenitrothion and imidacloprid resistance and influence of voltage-gated sodium channel mutations on pyrethroid resistance in the tropical bed bug, *Cimex hemipterus***. *Pest Manag Sci* 2021, **77**:5202-5212, https://doi.org/10.1002/ps.6561

46. Lewis CD, Levine BA, Vargo EL, Schal C, Booth W: **Recent detection of multiple populations of the tropical bed bug (Hemiptera: Cimicidae) exhibiting *kdr*-associated mutations in Hawaii**. *J Med Entomol* 2020, **57**:1077-1081, https://doi.org/10.1093/jme/tjaa022

47. Cho S, Shin E-H, Ju HC, Jeong ES, Lee SH, Kim JH: **The first case of *Cimex hemipterus* (Hemiptera: Cimicidae) with *super-kdr* mutations in the Republic of Korea**. *J Med Entomol* 2023, **60**:822-827, https://doi.org/10.1093/jme/tjad038

48. Ghavami MB, Ghahremani Z, Raeisi N, Taghiloo B: **High levels of pyrethroid resistance and *super-kdr* mutations in the populations of tropical bed bug, *Cimex hemipterus,* in Iran**. *Parasit Vectors* 2021, **14**:470, https://doi.org/10.1186/s13071-021-04962-5

49. Zhao Y, Feng X, Li M, Qiu K: **The double-mutation (M918I + L1014F) *kdr* allele is fixed in *Cimex hemipterus* populations in Guangxi, China**. *Bull Entomol Res* 2020, **110**:506-511, https://doi.org/10.1017/S0007485319000877

50. Tiotour M, Shaddel M, Aminianfar M, Mirahmadi H, Barzegar G, Solgi R, Darvishi M: **Identification of knockdown resistance mutations in the *Cimex hemipterus* (Hemiptera: Cimicidae) in Iran**. *Am J Trop Med Hyg* 2022, **107**:204-207, https://doi.org/10.4269/ahtmh.22-0092

51. Yoon KS, Previte DJ, Hodgdon HE, Poole BC, Kwon DH, El-Ghar GE, Lee SH, Clark JM: **Knockdown resistance allele frequencies in North American head louse (Anoplura: Pediculidae) populations**. *J Med Entomol* 2014, **51**:450-457, https://doi.org/10.1603/me13139

52. Gellatly KJ, Krim S, Palenchar DJ, Shepherd K, Yoon KS, Rhodes
•• CJ, Lee SH, Clark JM: **Expansion of the knockdown resistance frequency map for human head lice (Phthiraptera: Pediculidae) in the United States using quantitative sequencing**. *J Med Entomol* 2016, **53**:653-659, https://doi.org/10.1093/jme/tjw023.
This study provides the most comprehensive resistance-allele frequency map generated to date for human head lice, *P. humanus* capitis, infestations sampled across the United States. From 48 states, a total of 138 collections were made between 2013 and 2015. 132 of these sites exhibited a resistance-allele frequency of 100%, whereas only a single site was found to exhibit only the susceptible allele at each of the three mutations screened.

53. Platt N, Kwiatkowaska RM, Diabaté A, Dabire R, Wondji CS: **Target-site resistance mutations (*kdr* and *Rdl*), but not metabolic resistance, negatively impact male mating competitiveness in the malaria vector *Anopheles gambiae***. *Heredity* 2015, **115**:243-252, https://doi.org/10.1038/hdy.2015.33

54. McKenzie JA: **Selection at the diazinon resistance locus in overwintering populations of *Lucilia cuprina* (the Australian sheep blowfly)**. *Heredity* 1994, **73**:57-64, https://doi.org/10.1038/hdy.1994.98

55. Ang LH, Lee C-Y: **Absence of fitness penalty in insecticide-resistant German cockroaches, *Blattella germanica* (L.) (Dictyoptera: Blattellidae)**. *Int J Pest Manag* 2011, **57**:195-204, https://doi.org/10.1080/09670874.2011.563876

56. Gordon JR, Potter MF, Haynes KF: **Insecticide resistance in the bed bug comes with a cost**. *Sci Rep* 2015, **5**:10807, https://doi.org/10.1038/srep10807

57. Yoon KS, Gao J-R, Lee SH, Coles GC, Meinking T, Taplin D, Edman JD, Takano-Lee M, Clark JM: **Resistance and cross-resistance to insecticides in human lice from Florida and California**. *Pestic Biochem Physiol* 2004, **80**:192-201, https://doi.org/10.1016/j.pestbp.2004.07.008

58. Gao J-R, Yoon KS, Lee S-H, Takano-Lee M, Edman JD, Meinking TL, Taplin D, Clark JM: **Increased frequency of the T929I and L932F mutations associated with knockdown resistance in permethrin-resistant populations of the human head louse, *Pediculus capitis*, from California, Florida, and Texas**. *Pestic Biochem Physiol* 2003, **77**:115-124, https://doi.org/10.1016/j.pestbp.2003.08.003

59. Kasai S, Ishii N, Natsuaki M, Fuktomi H, Komagata O, Kobayashi M, Tomita T: **Prevalence of *kdr*-like mutations associated with pyrethroid resistance in human head louse populations in Japan**. *J Med Entomol* 2009, **46**:77-82, https://doi.org/10.1603/033.046.0110

60. Bass C, Schroeder I, Turberg A, Field LM, Williamson MS: **Identification of mutations associated with pyrethroid resistance in the *para*-type sodium channel of the cat flea, *Ctenocephalides felis***. *Insect Biochem Mol Biol* 2004, **34**:1305-1313, https://doi.org/10.1016/j.ibmb.2004.09.002

61. Ponco-Garcia G, Villanueva-Segura K, Trujillo-Rodriguez G, Rodriguez-Sanchez IP, Lopez-Monroy B, Flores AE: **First detection of the *kdr* mutation T929I in head lice (Phthiraptera: Pediculidae) in schoolchildren of the metropolitan area of Nuevo Leon and Yucatan, Mexico**. *J Med Entomol* 2017, **54**:1025-1030, https://doi.org/10.1093/jme/tjx045

62. Ponco-Garcia G, Villanueva-Segura K, Garza-Elizondo K, Villegas-Ramirez HM, Fernandez-Salas I, Rodriguez-Sanchez IP, Dzui-Manzanilla F, Flores-Suarez AE: **Determination of knockdown resistance (*kdr*) allele frequencies (T929I mutation) in head louse populations from Mexico, Canada, and Peru**. *Korean J Parasitol* 2022, **60**:217-221, https://doi.org/10.3347/kjp.2022.60.3.217

63. Larkin K, Rodriguez CA, Jamani S, Fronza G, Roca-Acevedo G, Sanchez A, Toloza AC: **First evidence of the mutations associated with pyrethroid resistance in head lice (Phthiraptera: Pediculidae) from Honduras**. *Parasit Vectors* 2020, **13**:312, https://doi.org/10.1186/s13071-020-04183-2

64. Swale DR: **Perspectives on new strategies for identification and development of insecticide targets**. *Pestic Biochem Physiol* 2019, **161**:23-32, https://doi.org/10.1016/j.pestbp.2019.07.001

65. DeVries ZC, Santangelo RG, Crissman J, Suazo A, Kakumanu ML, Schal C: **Pervasive resistance to pyrethroids in German cockroaches (Blattoidea: Ectobiidae) related to lack of efficacy of total release foggers**. *J Econ Entomol* 2019, **112**:2295-2301, https://doi.org/10.1093/jee/tez120

66. Gholizadeh S, Nouroozi B, Ladonni H: **Molecular detection of knockdown resistance (*kdr*) in *Blattella germanica* (Blattodea: Blattellidae) from Northwestern Iran**. *J Med Entomol* 2014, **51**:976-979, https://doi.org/10.1603/ME13160

67. Liu K, Ma S, Zhang K, gao R, Jin H, Cao P, Yuchi Z, Wu S: **Functional characterization of knockdown resistance mutation L1014S in the German cockroach, *Blattella germanica* (Linnaeus)**. *J Agric Food Chem* 2023, **71**:2734-2744, https://doi.org/10.1021/acs.jafc.2c05625

68. Liu J, Xu Y, Tan A, Zeng J, Liu P, Yu X, Wang M, Wang R, Luo W, Qiu X: **First report of the L993S mutation in the voltage-gated sodium channel in field populations of the German cockroach *Blattella germanica***. *J Econ Entomol* 2022, **115**:297-304, https://doi.org/10.1093/jee/toab238

69. Rahayu R, Masmiwati, Saputra MR: **Detection of voltage-gated sodium channel (VGSC) mutation in deltamethrin-resistant *Blattella germanica* L. from Indonesia**. *Pak J Biol Sci* 2022, **25**:905-910, https://doi.org/10.3923/pjbs.2022.905.910

70. Akhoundi M, Chebbah D, Sereno D, Marteau A, Jan J, Bruel C, Elissa N, Izri A: **Widespread mutations in voltage-gated sodium channel gene of *Cimex lectularius* (Hemiptera: Cimicidae) populations in Paris**. *Int J Environ Res Public Health* 2021, **18**:407, https://doi.org/10.3390/ijerph18080407

71. Cho S, Kim H, Chong S, Klein TA, Kwon DH, Lee SH, Kim JH: **Monitoring of pyrethroid resistance allele frequency in the common bed bug (*Cimex lectularius*) in the Republic of Korea**. *Korean J Parasitol* 2020, **58**:99-102, https://doi.org/10.3347/kjp.2020.58.1.99

72. Brownell N, Sunantaraporn S, Phadungsaksawasdi K, Seatamanoch N, Kongdachalert S, Phumee A, Siriyasatien P: **Presence of the knockdown resistance (*kdr*) mutations in the head lice (*Pediculus humanus capitis*) collected from primary school children of Thailand**. *PLoS Neg Trop Dis* 2020, **14**:e0008955, https://doi.org/10.1371/journal.pntd.0008955

73. Durand R, Millard B, Bouges-Michel C, Bruel C, Bouvresse S, Izri A: **Detection of pyrethroid resistance gene in head lice in schoolchildren from Bobigny, France**. *J Med Entomol* 2007, **4**:796-798, https://doi.org/10.1093/jmedent/44.5.796

74. Kristensen M, Knorr M, Rasmussen A-M, Jespersen JB: **Survey of permethrin and malathion resistance in human head lice populations from Denmark**. *J Med Entomol* 2006, **43**:533-538, https://doi.org/10.1093/jmedent/43.3.533