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Were bed bugs the first urban pest insect? Genome-wide patterns of bed bug demography mirror global human expansion

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There are calls for research into the historical evolutionary relationships between humans and their commensals, as it would greatly inform models that predict the spread of pests and diseases under urban population expansion. The earliest civilizations emerged approximately 10 000 years ago and created conditions ideal for the establishment and spread of commensal urban pests. Commensal relations between humans and pests likely emerged with these early civilizations; however, for most species (e.g. German cockroach and black rat), these relationships have formed relatively recently—within the last 5000 years—raising the question of whether others could have emerged earlier. Following comparative whole genome analysis of bed bugs, *Cimex lectularius*, belonging to two genetically distinct lineages, one associated with bats and the other with humans, coupled with demographic modelling, our findings suggests that while their association with humans dates back potentially hundreds of thousands of years, a dramatic change in the effective population size of the human-associated lineage occurred approximately 13 000 years ago; a pattern not found in the bat-associated lineage. The timing and magnitude of the demographic patterns provide compelling evidence that the human-associated lineage closely tracked the demographic history of modern humans and their movement into the first cities. As such, bed bugs may represent the first *true* urban pest insect species.

1. Introduction

Since the dawn of civilization, approximately 10 000 years ago (ya), the human population has grown from around 5 million to nearly 8.2 billion. With this rapid expansion, urban areas have grown at unprecedented rates and now house over 55% of the global population [1], with further expansion expected following urban population growth [2]. The earliest recorded city of Çatalhöyük, located in the southern Anatolia region of Turkey, dates to the Neolithic period, approximately 9400 ya. Archaeological estimates suggest that it was home to between 800 and 8000 people [3]. In contrast, today's largest cities, by population, house over 30 million people. While

population sizes differ drastically between and among the first cities and those of the present day, many characteristics associated with urbanization are comparable. Notably, urbanization substantially alters the natural landscape, influencing the evolutionary trajectories of flora and fauna [4].

Although the extent of the impact of ancient urbanization on wildlife is unknown, current patterns reveal considerable impacts on the adaptive (i.e. selection) and non-adaptive (i.e. gene flow and genetic drift) evolutionary processes of organisms that live within and near cities [4–7]. Human commensals and urban pests offer particularly useful insights, given the array of selective pressures they experience [4], ranging from factors that influence demography, connectivity, and genomic diversity [6–8], to intense selection and adaptive resistance to control measures (e.g. insecticide resistance) [8,9]. Selective pressures arising from interspecies interactions between commensal and human hosts may leave reciprocal signals of fitness effects on both.

The bed bug, *Cimex lectularius*, has a long association with humans, with the earliest records of infestations dating back to Pharaonic Egypt, approximately 3375 ya [10]. Ancestrally an ectoparasite of bats [11], bed bugs underwent a host transition to hominins approximately 245 000 ya, resulting in two genetically distinct lineages: one associated with bats and found throughout Europe and the Middle East, and a human-associated lineage that is now globally distributed [12]. Despite the potential for secondary contact, gene flow between these lineages appears absent [12–14]. While abundant prior to World War II, the species was seemingly eradicated from modernized countries due to intense selection resulting from the use of the insecticide dichloro-diphenyl trichloroethane (DDT). However, within five years of DDT's introduction, resistant bed bug populations were documented [15]. During the late 1990s, bed bugs underwent a global resurgence, potentially linked to an increase in domestic and international travel and the evolution and spread of mechanisms conferring insecticide resistance [8,9,16], such that they now represent a pest of significant economic and health concern. While our understanding of patterns of contemporary evolution of bed bugs is growing, we know little about their demographic history, thus their early associations with human civilization.

There have been calls for research into historical evolutionary relationships between humans and their commensals, as it would greatly inform models that predict the spread of pests and diseases under urban population expansion, especially considering the additional effects of global climate change [17]. Demonstrating that the global expansion of urban pests like bed bugs may have coincided with human global expansion has implications for the direct role that humans initially played in the emergence and evolution of pest commensals as well as for the types of traits that have co-evolved during the close interactions of humans and pests during urban development. Here, through the comparative analysis of whole genome sequences derived from the genetically diverged bat-associated and human-associated lineages of the bed bug, coupled with demographic modelling, we investigate their evolutionary history and place it in the context of the emergence of global city expansion.

2. Material and methods

Bed bugs (n = 19) were collected from six sites in the Czech Republic in 2014. Human-associated bed bugs were collected from Havířov (n = 3), Beroun (n = 3) and Praha (n = 3), whereas bat-associated samples were collected from Prudká (n = 3), Dubá (n = 3) and Úštěk (n = 4). Previous studies have found the bat- and human-associated lineages collected here to be genetically diverged based on host-associated [12–14]. DNA was extracted using the DNeasy Blood and Tissue kit (Qiagen, Germantown, MD). Individual whole genome libraries were prepared using the KAPA HyperPlus kit (Roche Sequencing, Indianapolis, IN) and 2 × 250 bp paired-end reads were sequenced on an Illumina HiSeq2500 at the Brigham Young University DNA Sequencing Center. Individual genomes were aligned to the haplotype-resolved chromosome-level genome (NCBI BioProject PRJNA1165749; [18]) using Bowtie v. 2.4.1 [19], and variants were inferred using *bcftools* v. 1.11 [20]. Sites with more than 50% missing data were removed using *vcftools* v. 0.1.18 [21].

Genetic diversity, π, was estimated using *vcftools* [21] with a 1 kb sliding window, for all samples combined and for each lineage separately. Between lineages, the statistical significance of differences in genome-wide π was determined with a Student's t-test, performed in R. Given the large number single nucleotide polymorphisms (SNPs) detected (see §3), a principal component analysis (PCA) was performed on the samples using the glPCA function in *adegenet* v. 2.1.10 [22]. Tajima's D [23], a population genetic statistic that distinguishes neutral versus non-neutral DNA sequence evolution, was estimated using *vcftools* [21] with a 1 kb sliding window for each lineage independently. A negative Tajima's D signifies a population expansion, whereas positive values indicate a population bottleneck or balancing selection. To test for the statistical significance of Tajima's D between lineages, a χ^2 test was performed in R. All data were visualized in R v 4.3.0 using *ggplot2* [24].

The demographic history (i.e. changes in effective population size [N_e]) of each lineage was inferred using Stairway Plot 2 [25], assuming a mutation rate of 2.8×10^{-8}, as is commonly used for insect demographic analyses (e.g. [26]). Four different models were tested with generation parameters of 3–6 generations per year for both lineages, representing a potential lower and upper range for the species assuming a generation time of 35–40 days during optimal conditions [27]. The unfolded site frequency spectrum (SFS)—the among-population joint distribution of allele frequencies throughout the genome—for each lineage was generated by the *vcf2sfs* R package [28]. Beta-PSMC [29] was used as a complementary analysis, leveraging whole genome re-sequenced data to confirm the pattern of demographic history obtained from Stairway Plot 2. We specifically selected beta-PSMC as it improves resolution and accuracy for recent population size changes, which is particularly relevant to our study [29]. Our choice of time segmentation (6 + 25*2 + 4 + 4) was designed to provide higher resolution in recent and intermediate time periods (6 + 25*2), as our focus in this study is the demographic history of bed bugs in relation to the expansion of human populations and civilization. We allocated fewer parameters to deeper time (4 + 4) since ancient

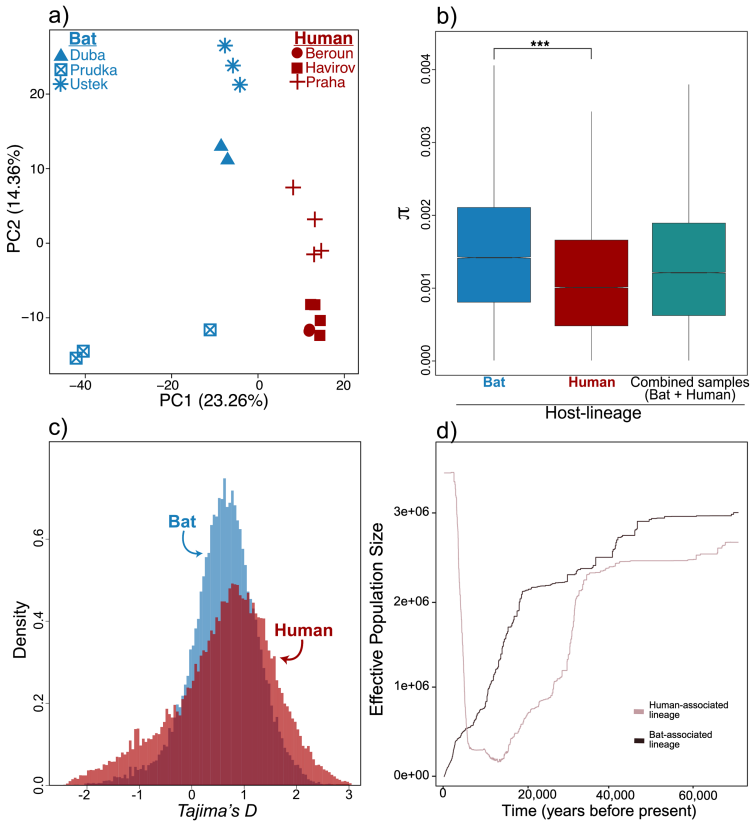

Figure 1. PCA uncovered a principal separation between the two host lineages (a) and these findings were further reflected in our genome-wide distributions of nucleotide diversity with significant differences (two-sample Mann–Whitney *U*-test, $p < 2.2 \times 10^{-16}$) between the two lineages with lower average π in the human lineage overall (b). Investigation of Tajima's D indicates evidence of genetic bottlenecks in both lineages, but more significant in the human-associated lineage (c). Reconstruction of demographic histories for both lineages supports divergent N_e-curves-through time (d). Both lineages have experienced effective population size declines during previous glacial maxima; however, bat-associated lineages continue to decline whereas human-associated bed bugs have experienced exponential population growth, starting around 5000–13 000 ya (d). The period consistent with population declines coinciding with the LGM, whereas the decline in the human-associated lineage was halted coinciding with the emergence of the first cities, and then subsequently experienced a dramatic growth as further civilizations such as the Cucuteni–Trypillia (approx. 7000 ya) and Mesopotamia (approx. 5000 ya) emerged, through to the present day.

demographic history was not the focus. Bcftools [20] was used to remove insertions or deletions (indels) and SNPs in annotated repeats, then Beta-PSMC was run independently for each sample with a time segment pattern of 6 +25*2 + 4 + 4 and the following flags: '-N50 -t20 -r5'.

To test the hypothesis that bed bugs mirrored the demographic history of humans and the expansion of urbanization, we used Hudson's [30] ms program (http://home.uchicago.edu/~rhudson1/source/mksamples.html) to simulate the coalescent process of evolving genealogical trees under different demographic scenarios. Both archeological and genetic studies indicate that the human population rapidly expanded in size approximately 50 000–200 000 ya from an N_e of approximately 10 000 individuals [31,32], which affects genetic diversity by producing a significant genome-wide skew in the SFS towards rare allelic variation. If the bed bug human-associated lineage co-evolved with humans as its preferred host, we may expect a similar genome-wide SFS signature that mirrors the human demographic history of a bottleneck followed by a recent rapid expansion. The estimated timing of the bed bug population decline approximately 40 000 ya is consistent for both bed bug lineages (figure 1c) and supported by prior literature [26,33], thus we fixed this demographic event in our simulations. We also fixed the current N_e in all scenarios, as this estimate comes directly from the observed SNP data. Lastly, our simulations reflected prior published accounts in varying mutation rate [34,35]) and generation time (3–6 per year [36]); however, our results were largely insensitive to the variance in these parameter ranges. Thus, our final results fixed the mutation rate (2×10^{-8}) and generation time (four per year) reflected in our stairway plot analysis. We simulated 1000 coalescent trees based on the sampled number of individuals ($n = 20$ haploid human-associated genome sequences) and nucleotide sites (540 Mbp), for scenarios that each varied the timing (over the last 40 ky) and magnitude (0.5–10% of the current N_e of 3.9 M) of expansion events. We used the MS program to calculate Tajima's D as a proxy of the SFS for each of the 1000 simulations for each scenario. These simulated Tajima's D distributions were then compared to the observed distribution of Tajima's D values from our bed bug human-associated lineage genome-wide data (figure 1c), using chi-squared goodness-of-fit statistical tests. For more details on the rationale using the MS program for our simulations, see the electronic supplementary materials.

3. Results and discussion

Whole genomes were sequenced to a coverage of > 10 × (537.9 Mb across 13 autosomes and two sex chromosomes (X_1X_2) with 9.7 M variant SNP sites). PCA of genome-wide SNP variation supports previous findings that these lineages are genetically distinct [12–14], with PC−1 broadly separating the host-associated lineages (explaining 23.26% of the variance), and PC−2 separating populations of the bat-associated lineage, but not populations of the human-associated lineage (14.36% of variance explained; figure 1a). Across samples, genetic diversity was relatively low (mean π = 0.0013 (figure 1b); however, the mean genomic diversity of the bat-associated lineages is significantly higher than that of the human lineage (π = 0.0015 vs. 0.0011, respectively; two-sample Mann–Whitney *U*-test, W = 1 586 559 054, $p < 2.2 \times 10^{-16}$) (figure 1b). This contrast in diversity is consistent with the placement of the bat-associated lineage as ancestral to the human-associated lineage [12,14].

We investigated genome-wide signatures of population demography using Tajima's D statistic, with negative values being consistent with population expansion, whereas positive values suggest a population bottleneck [23]. Median genome-wide Tajima's D across all samples was positive (0.67); however, when comparing lineages separately, the human-associated lineage exhibited a significantly higher positive value (bat lineage = 0.63 vs. human lineage = 0.73; two-sample Mann–Whitney *U*-test, W = 1 214 681 971, $p < 2.2 \times 10^{-16}$) (figure 1c). The genome-wide signature of a bottleneck is further supported by stairway plot analyses, which indicated that both lineages experienced marked reductions in N_e from 50 000–20 000 ya (figure 1d). During periods of glaciation, including the last glacial maximum (LGM), many organisms experienced population bottlenecks, consistent with the timing of the reductions observed here [26]. While both lineages show declines coincident with the LGM, the human-associated lineage shows a more rapid and earlier decline, possibly as propagules of the human-associated lineage were transported out of caves during the upper Palaeolithic period as modern humans began to lead more nomadic lifestyles. The effective population size of the bat-associated lineage continues to decline towards the present day; likely a result of ongoing habitat loss and fragmentation inhibiting gene flow across the species natural range, as seen in other species [37,38]. Additionally, cimicid insects appear especially prone to the effects of climate change, potentially impacting their ability to survive overwintering conditions [39]. However, N_e of the human-associated lineage experienced an expansion starting at approximately 13 000 ya, then plateaus for approximately 5000 years before experiencing a dramatic increase at approximately 7000 ya, to present day values (figure 1d; electronic supplementary material, figure S1).

Our evidence of a rapid population proliferation in the human-associated lineage coincides with the dawn of civilization and the appearance of the first cities. Demographic modelling of human-associated bed bugs further indicates an older population bottleneck followed by a more recent expansion over the past 40 000 years. Our fitted models suggest that this apparent bed bug expansion is likely to have started between approximately 5000 and approximately 20 000 ya, with an initial diploid population size of 225 000 – 350 000. These simulations indicate that even at the lowest potential *Ne*, the population was sufficiently large (e.g. 225 000) and had sufficient time (e.g. 20 000 years) before expanding to its current *Ne* that is now over a magnitude greater (3.9 M; electronic supplementary material, figure S2). This scenario may explain the apparent relative reduction of genomic diversity in the human-associated lineage that is not significantly skewed towards rare alleles. In contrast, although within the same time interval, the 'Out of Africa' expansion of modern humans experienced a more dramatic reduction in population size relative to its comparatively low N_e prior to expansion. This drastic population bottleneck followed by a rapid expansion resulted in a contemporary global population with significantly lower overall genetic diversity that is significantly skewed towards rare allelic variation in contrast to their African counterparts that maintained a historically larger and more stable population size [31].

Patterns of genomic variation revealed here suggest that the human-associated bed bugs have maintained a close relationship with human society for at least 50 000 years. This scenario is supported by a population decline of the human-associated lineage relative to that of the bat-associate lineage, but also that the decline in the human-associated lineage was halted coinciding with the emergence of the first cities, and then subsequently experienced a dramatic growth as further civilizations such as the Cucuteni–Trypillia (approx. 7000 ya) and Mesopotamia (approx. 5000 ya) emerged, through to the present day (figure 1d); a trend not observed in the bat-associated lineage. The LGM likely resulted in a significant genetic bottleneck that altered genome-wide variation in both the human- and bat-associated lineages with potential implications for genetic diversity and adaptive potential. However, humans provided a vehicle for global expansion into new environments and hence a novel adaptive landscape for the human-associated lineage. These landscapes have shaped the genotypes and phenotypes of human-associated bed bugs such that they now are markedly diverged from the bat lineage. For example, human-associated bed bugs are smaller, less hairy and have larger extremities compared to their bat-associated counterparts [12]. Additionally, human-associated bed bugs have recent mutations linked to insecticide resistance, not found in the bat-associated lineage [13,14]. These adaptations reflect traits that have evolved in response to the host transition from bats to the *Homo* lineage, potentially hundreds of thousands of years ago, but also traits that have evolved rapidly in response to selective pressures in the modern urban environment [9]. These findings support bed bugs as an ideal model system to investigate the impact that host transition has had on rapid and recent trait evolution. Of further potentially unique value is the role this model system will play in our understanding of genes and pathways that have evolved within and between humans and our close commensals across different global cities, for example, those associated with immune response or circadian rhythm [40–43].

The timing and magnitude of bed bug demographic patterns found here provide compelling evidence that the human-associated lineage of bed bugs closely tracked the demographic history of modern humans and their movement into the first cities. Another cosmopolitan urban pest, the German cockroach, *Blattella germanica*, shares many characteristics with the human-associated lineage of bed bugs (e.g. global distribution, multiple mechanisms that confer insecticide resistance and found only in strict association with humans in the indoor environment). However, unlike the bed bug, the German cockroach co-evolved with humans very recently, approximately 2100 ya [7]. Similarly, the black rat, *Rattus rattus*, is believed to have formed a

commensal relationship with humans around 5000 ya [44]. House mice, *Mus musculus*, share a commensal relation with humans that may date back to 15 000 ya [45]; however, whether they strictly rely on humans is questionable [46]. As such, it is possible that bed bugs were one of the first *true* urban pests solely reliant on humans as a host. The strict relationship between bed bugs and humans has continued to the present day, and as a result, their genome has been and will continue to be shaped by global spread and urban-associated selection pressures.

Ethics. This work did not require ethical approval from a human subject or animal welfare committee.

Data accessibility. Sequence data were deposited to NCBI under BioProject PRJNA1176704. Code are available from the Dryad Digital Repository: [47].

Supplementary material is available online [48].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. L.S.M.: conceptualization, data curation, formal analysis, investigation, methodology, writing—original draft, writing—review and editing; B.C.V.: conceptualization, data curation, formal analysis, investigation, methodology, writing—original draft, writing—review and editing; R.A.: formal analysis, investigation, methodology, visualization, writing—review and editing; Y.Z.F.: formal analysis, investigation, methodology, visualization, writing—review and editing; D.C.C.: data curation, formal analysis, methodology, writing—review and editing; O.B.: resources, writing—review and editing; T.A.C.: conceptualization, formal analysis, funding acquisition, investigation, methodology, resources, supervision, writing—review and editing; W.B.: conceptualization, data curation, funding acquisition, investigation, methodology, project administration, resources, supervision, validation, writing—original draft, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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