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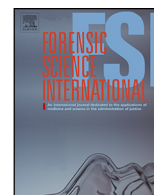
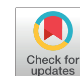


Isolation, identification, and time course of human DNA typing from bed bugs, *Cimex lectularius*



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ABSTRACT

Bed bugs (*Cimex lectularius* L.) are ectoparasitic wingless insects that feed on the blood of mammals, typically in residential settings. The objectives of this study were to establish a time-course of human DNA quantitation from bed bugs and to generate human DNA profile(s) of a host and/or multiple hosts from a bed bug that fed on human blood. Female human genomic DNA concentrations ranged from 18.370 to 0.195 ng/bed bug at 0–108 h post blood meal (PBM), male human genomic DNA concentrations ranged from 5.4 to 0.105 ng/bed bug, and pooled human female and male blood ranged from 5.49 to 0.135 ng/bed bug at 0–96 h PBM. Human autosomal STR complete profiles were obtained until 72 h PBM for female, male, and pooled human blood. These results reveal that identification of multiple human hosts is possible from a single bed bug. However, the ratio of each contributor may be variable depending on the amount of blood ingested from each individual and the time difference of blood consumed from each subject. Average peak heights for three STR markers of low (D3S1358), medium (D13S317), and high molecular weight (D2S1338), were also compared over time. Peak heights were consistently higher for the low molecular weight marker over all time intervals. These data suggest that some markers can be successfully recovered more than three days PBM. Hence, bed bugs can serve as physical evidence in temporal and spatial predictions to match suspects and/or victims to specific locations in criminal investigations.

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1. Introduction

Bed bugs (*Cimex lectularius* L.) are ectoparasitic insects that feed on the blood of mammals and are typically found in groups within residential settings [1]. The enormous global increase in bed bug infestations in the last two decades can be attributed to increased international commerce and travel, popularity of second-hand furniture and thrift shops, and insecticide resistance. Bed bugs usually aggregate in close proximity to sleeping and resting areas such as beds, sofas, and recliners. They are easily translocated by passive dispersal and adapt to multiple host species [1–4].

The recent resurgence of bed bugs, especially in North America, has garnered interest in the field of forensics. Identifying an individual based on blood or tissue isolated from insects can be used to implicate a suspect in the time and place of a crime. However, human DNA isolated from an insect would have to be (1) stable and intact long enough to be useful in a forensic investigation and (2) unambiguously identifiable to an individual host. Researchers have successfully isolated, amplified, and profiled human mitochondrial DNA (mtDNA) from blood-feeding insects, including bed bugs [5] and human crab louse (*Pthirus pubis*) [6], and from maggots of the shiny blue bottle fly (*Cynomyopsis* [=*Cynomya*] *cadaverina*) feeding on human tissues [7,8]. Additional research has been conducted to isolate and profile human nuclear DNA from the blood meals of other insects such as human lice (*Pediculus humanus capitis*) [9] and mosquitoes (Culicidae) [10], and from maggots [11–14].

Experiments with the human crab louse (*P. pubis*) and human head louse (*P. humanus capitis*), both obligate human ectoparasites, found that blood-fed lice and their excreta could yield DNA from single or multiple human hosts [6,9,15]. Human DNA has been extracted and identified from individual mosquitoes for up to 15 h

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post blood meal (PBM) [16] and subsequent studies have confirmed the sensitivity and reproducibility of using DNA fingerprinting of mosquito blood meals to identify individual human hosts [17]. Short Tandem Repeat (STR) identification was used to determine the feeding preferences of *Aedes aegypti*, the yellow-fever mosquito and vector of dengue virus, thus demonstrating the role played by a migrating population in spreading the virus [18,19]. A more recent study determined that Culicinae mosquitoes can be relevant to a criminal investigation when present at a crime scene, as human DNA could be successfully typed from these insects 56 h after a blood meal had been taken [20]. In another case, almost all 15 STR loci were generated in a victim's profile obtained from a mosquito discovered at the crime scene, confirming the presence of the victim in close proximity to the suspect [10]. Lastly, human STR profiles could be obtained from maggots feeding on corpses 2–4 days later depending on the level of starvation the maggots had experienced [11]. Furthermore, it is suggested that STR profiles were best obtained when the maggots were immediately preserved after feeding [14].

The forensic use of bed bugs has several advantages, compared with other blood-feeding arthropods. Bed bugs are wingless insects; therefore, they remain in close proximity to their feeding location. Although a small fraction of a bed bug population can walk relatively long distances, most remain near the host. Hence, any long-range displacement, especially between buildings, can be attributed to human-mediated translocation. This makes bed bugs useful for validating the location of a suspect or a victim in legal and/or forensic investigations. Bed bugs are hemimetabolous, like lice, so all life stages feed on blood, whereas only adult female mosquitoes and fleas (holometabolous) do so. This increases the probability of finding some recently fed bed bugs. Bed bugs also feed infrequently, generally every 4–7 days, which allows genotyping single host blood DNA profiles. In addition, bed bugs have a relatively long lifespan and may survive for longer periods without blood meals compared to mosquitoes, fleas and lice.

Szalanski et al. [5] demonstrated that DNA could be isolated from recently blood-fed bed bugs and it is qualitatively sufficient for DNA genotyping. However, to date, there are no documented reports about successful human blood identification and/or full human STR typing from a bed bug fed on human female, male, or pooled (female:male) blood. Moreover, it is not known whether the identification of multiple humans can be made from a bed bug that had fed on multiple hosts such as male/female, male/male, and female/female combinations.

The objectives of this study were to establish a time-course for human blood identification, to quantify human DNA from bed bugs, and to generate DNA profile(s) of a host and/or multiple hosts from a bed bug that fed on human blood. We demonstrate that sufficient quality and quantity of human blood and human DNA can be isolated and typed from a bed bug. Hence, bed bugs can serve as physical evidence both in terms of temporal and spatial predictions for criminologists to match suspects and/or victims to specific locations in criminal investigations and homeland security.

2. Materials & methods

2.1. Human blood feeding to bed bugs

C. lectularius colonies of the Harold Harlan strain (HH; also known as Fort Dix, collected in 1973), were maintained in an incubator at 27 °C, ~50% relative humidity, and 12:12 h light:dark in the Urban Entomology lab at North Carolina State University. Colonies were fed defibrinated rabbit blood in an artificial feeding system maintained at 37 °C, as described in Romero and Schal [21]. Newly emerged adult bed bugs were starved for two weeks to clear the rabbit blood and guarantee complete engorgement on human blood. Starved adult males were separated into three feeding containers and allowed to feed in the dark on human defibrinated blood (Bioreclamation IVT, New York, NY). Bed bugs in the three different containers were fed female human blood (n = 100), male human blood (n = 100), and pooled blood (1 female:1 male, vol:vol) (n = 90). Bed bugs were given 30 min to feed, and fully fed adult males (Fig. 1) were individually placed into labeled 2.0 mL Eppendorf tubes. Ethanol (95%) was added to each Eppendorf tube at 12-h intervals PBM, beginning at 0 h (immediately after feeding) up to 108 h, and tubes were stored at −80 °C. The samples were then transported to Fayetteville State University (FSU), and stored at −80 °C in the Forensic Science laboratory for further testing.

2.2. Isolation of blood from bed bugs

Ethanol was removed from the tube, leaving only the engorged bed bug. Approximately 500 μL of dH_2O was added to the tube. To remove traces of ethanol, the tube was gently vortexed. Distilled water was removed from the tube, and the cleaning process was repeated twice more. After the bed bug was cleaned, 10 μL of Rapid Stain Identification (RSID) kit universal buffer was added to the tube. A clean probe was used to vigorously homogenize the bed bug. Once homogenized, all fluid containing blood (approximately 10 μL) was removed from the tube and placed onto a labeled Flinders Technology Associates (FTA) blood card (GE Healthcare Bio-Sciences Corp., Piscataway, NJ). Special care was taken to ensure that cuticle fragments were excluded from the fluid. This process was repeated twice more for each bed bug sample. When the last of the fluid from the bed bug sample tube was blotted, the FTA blood card was placed inside a sterile hood in the dark to air dry overnight.

2.3. Isolation of DNA from FTA cards

Each FTA card containing the dried blood from a single bed bug was cut into smaller pieces and transferred into a labeled 2 mL Eppendorf tube. Each FTA card sample, including reference female and male blood samples, was soaked in 500 μL of digestion buffer (10 mM Tris-HCl, 10 mM EDTA, 50 mM NaCl, 2% SDS and 20 μg/mL proteinase K) and incubated at 56 °C for a minimum of 3 h. As a quality control measure, a reagent blank, negative control, and positive control were included. Subsequently, DNA was extracted from each sample using phenol: chloroform: isoamyl alcohol (25:24:1) [22]. The aqueous phase of the DNA extraction was transferred to an Amicon® Ultra-4 Centrifugal Filter Device (Millipore, Billerica, MA) for DNA concentration, and the manufacturer's recommended protocol was followed. The tube contents were washed twice with TE buffer (10 mM Tris HCl, 10 mM EDTA, pH 8) and centrifuged at 5000 g for 15 min. The recovered DNA, in approximately 15–20 μL, was stored at −20 °C.

Fig. 1. Photographs depicting bed bugs: (A) nonengorged, unfed bed bug (B) engored, fully fed bed bug on human blood.

2.4. DNA quantitation

Total human genomic DNA as well as human male DNA were quantified from each bed bug using the Quantifiler® Duo Kit (Thermo Fisher Scientific, Waltham, MA) run on the ABI 7500 Real-Time PCR System (Thermo Fisher Scientific). The manufacturer's validated quantitation protocol was followed.

2.5. DNA amplification and genotyping

Polymerase Chain Reaction (PCR) reactions were prepared according to the manufacturer's recommended protocol using the AmpFlSTR® IdentiFiler® PCR Amplification Kit (Thermo Fisher Scientific). The DNA template was 1 ng in a final volume of 25 μL for each PCR reaction. PCR tubes were loaded into a 96-Well GeneAmp® PCR System 9700 (Bio-Rad, Hercules, CA) for amplification. All amplification reactions were accompanied by negative and positive controls.

2.6. Human specific STR genotyping

Following PCR amplification, the ABI PRISM® 310 Genetic Analyzer (Thermo Fisher Scientific) was employed for electrophoretic separation of amplified products. For ABI 310 sample preparation, 24.5 μL Hi-Di™ Formamide (Thermo Fisher Scientific), 0.5 μL GeneScan™ 500 LIZ® Size Standard (Thermo Fisher Scientific), and 1 μL of PCR amplified product or AmpFlSTR® Identifiler™ Allelic Ladder (Thermo Fisher Scientific) were added to each sample. The reaction tubes were heated at 95 °C for a 3 min denaturation step, immediately snap-cooled on a freezer block for 3 min, and then subjected to capillary electrophoresis. The samples were separated on a 47 cm × 50 μm capillary tube (Thermo Fisher Scientific).

Amplified products were electrokinetically injected for 5 s and fractionated on an ABI Prism® 310 Genetic Analyzer using POP-4® Polymer (Thermo Fisher Scientific). Data were analyzed using a peak detection threshold of 100 relative fluorescence units (RFU) for all dyes with GeneMapper® ID v3.2.1 software (Thermo Fisher Scientific).

Human autosomal STR alleles generated from each bed bug at 0–108 h PBM were divided by the total number of alleles from the reference (female and/or male) blood samples. For example, at 0 h female blood fed bed bug gives 16 STR alleles plus an amelogenin, gender marker, total 17 alleles. This number (17 alleles) is divided by total number of alleles from the respective female reference sample alleles, which are 29 human autosomal STR alleles plus X, an amelogenin marker, total 30 alleles. Hence, percentage of correct alleles at 0 h female PBM is (17/30) × 100 = 56.6%.

The Institutional Review Board (IRB) proposal for this study was approved by the Fayetteville State University Human Rights in Research Committee (IRB # 2013-P-0039).

3. Results

3.1. Recovered liquid from fed bed bugs

The volume of liquid recovered from each bed bug approximately represented the volume of human blood and bed bug blood. Freshly fed and fully engorged bed bugs fed on female, male, or pooled human blood yielded 3–4 μL. The post-feeding volume dropped to 1–2 μL after 60 h.

3.2. Human genomic DNA quantitation from bed bugs

Human genomic DNA concentrations for female DNA ranged from 1.225 to 0.013 ng/μL, with a total amount of 18.370– 0.195 ng of human genomic DNA per bed bug (ng/bed bug) at 0–108 h PBM. Male human genomic DNA concentrations ranged from 0.36 to 0.007 ng/μL, with a total of 5.4–0.105 ng/bed bug and pooled human (female and male) blood ranged from 0.366 to 0.009 ng/μL, totaling 5.49–0.135 ng/bed bug at 0–96 h PBM (Table 1). Average total human DNA recovered from a bed bug fed on female, male, and pooled blood trend shows that it is highest at 12 h from bed bugs fed on female and male blood but highest at 24 h from bed bugs fed on pooled blood. Subsequently average total DNA concentration starts declining with a few inconsistencies. Interestingly, the average total human DNA recovered from a bed bug fed on human female blood is 3.4–5.5 times higher than human DNA recovered from bed bugs fed on human male and pooled blood (Table 1). Pure human defibrinated female and male blood was used as reference.

3.3. Human specific STR genotyping

Human specific STR genotyping profiles were generated from amplified human genomic DNA isolated from human blood obtained from each bed bug. Human autosomal STR profiles from each bed bug sample were compared with reference female and male blood DNA autosomal STR profiles for complete and/or partial match or no profile at various 12-h intervals PBM. Percentage of correct autosomal STR alleles were calculated by counting all the human autosomal STR alleles successfully amplified from the bed bug (0–108 h at 12 h, PBM intervals) and divided by the total number of alleles from the respective reference sample. Complete human female blood fed bed bugs were the only samples who generated autosomal STR profiles that matched with female

Table 1
Human DNA recovered from a bed bug fed on human female, male, and pooled (female and male) blood at 12-h intervals from 0 h–108 h with reference (female and male) samples. Pure human defibrinated female and male blood was used as reference.

Time post blood feeding sacrifice of bed bug (Hrs)	Isolated DNA volume (μL)	Average human female DNA (ng/μL)	Total human female DNA from a bed bug (ng/Bb)	Average human male DNA (ng/μL)	Total human male DNA from a bed bug (ng/Bb)	Average pooled human DNA (ng/μL)	Total pooled human DNA from a bed bug (ng/Bb)
0	15	0.202	3.035	0.111	1.665	0.019	0.278
12	15	1.225	18.370	0.36	5.4	0.220	3.306
24	15	0.965	14.480	0.155	2.325	0.366	5.49
36	15	0.741	11.110	0.21	3.15	0.196	2.94
48	15	0.502	7.526	0.149	2.235	0.075	1.131
60	15	1.118	16.765	0.075	1.119	0.072	1.082
72	15	0.267	4.012	0.014	0.206	0.043	0.645
84	15	0.048	0.718	0.041	0.615	0.011	0.167
96	15	0.152	2.284	0.007	0.106	0.009	0.137
108	15	0.013	0.198	0.012	0.186	N/A	N/A
Reference blood	15	1.4	21	0.71	10.65	1	15

reference autosomal STR profiles until 96 h PBM (Fig. 2). However, 72 h PBM male and pooled samples generated completely concordant profiles with the respective reference samples (Fig. 2). Though complete profiles were not consistently obtained, partial profiles were successfully obtained from female and male fed blood samples for all time intervals and for pooled samples until 96 h PBM (Fig. 3).

Average peak heights for three STR autosomal markers of low (D3S1358), medium (D13S317), and high molecular weight (D2S1338), were also compared over time (Fig. 4). Peak heights were consistently higher for the low molecular weight marker compared to the medium and high molecular weight markers (D13S317 and D2S1338) over all time intervals. These data suggest that some markers can be successfully recovered more than three days PBM; however, most success will be found using smaller autosomal STR markers.

4. Discussion

This study confirms the application of forensic DNA methods for the recovery of good quality and sufficient quantity of human blood from bed bugs fed on either female, male, or pooled blood. The ability to generate human STR profile(s) depends on the volume of the initial blood ingested and the rate of its breakdown in the bed bug. We assert that bed bugs may be better suited for human genotyping than other blood-feeding species found at crime scenes due to their larger post-feeding volume, feeding and digestion kinetics, life cycle and ecology. Bed bugs take infrequent large blood meals punctuated with relatively long periods of starvation [1]. Therefore, starving bed bugs for two weeks to maximize the volume of their blood meal was consistent with their normal ecology. The volume of a fully engorged bed bug on the artificial feeding system was 3.92 ± 0.21 μL (SEM) [23] whereas sand flies (*Phlebotomus argentipes*) ingest only 0.63–0.73 μL (20), and the volumes of blood meals in engorged mosquitoes range between 2–3 μL [24,25]. Cat flea (*Ctenocephalides felis*) females, which are common indoor pests, ingest about 1 μL each during a 3 h feeding trial [26]. Moreover, unlike other blood-feeding arthropods in the indoor environment, all life stages of the bed bug feed on host blood, and while adult females ingest larger amounts of blood, juveniles would ingest appreciably smaller volumes of host blood. Also, unlike other blood feeders, fully engorged bed bugs can use a single blood meal to mature to their next life stage and to produce eggs for 10–15 days [27]. Therefore, the likelihood of extensive blood mixing from different hosts is lower than with other blood feeders.

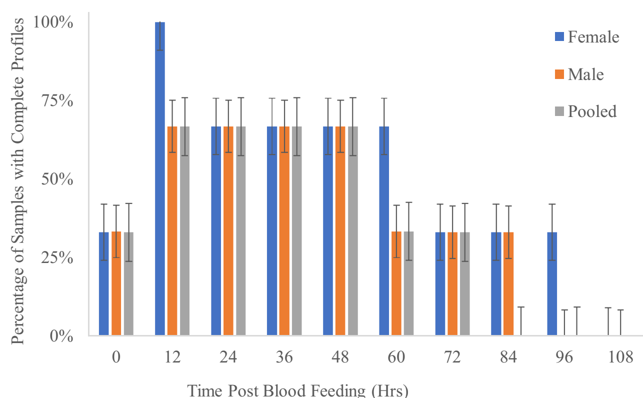

Fig. 2. Percentage of complete human autosomal STR profile matched with female, male, and pooled blood DNA extracted from bed bugs. Data below (100 RFU) analytical threshold is included. Bars represent standard error of the mean (SEM).

Importantly, changes in the volume of ingested blood cannot be accurately inferred from a simple homogenization of the bed bug because this procedure does not differentiate the bed bug's own blood and water content from host blood. The critical consideration for forensic investigations, however, is not the volume of the PBM host blood but its DNA content, quality and detectable human specific STR markers. Our results show that human DNA content and quality decline dramatically over time PBM, as with other blood feeding insects. Thus, our ability to generate human DNA profile from bed bugs depends not only on the size of the ingested blood, but the time-course of its digestion in the bed bug. Crime scene technicians should be trained to recognize the difference between an engorged and nonengorged bed bug (Fig. 1), because recently fed bed bugs provide better quality and quantity of human DNA.

We have found that the ability to obtain readable human DNA fingerprints from bed bugs depended on the kinetics of its digestion. To understand the blood meal digestion rate in bed bugs, and hence, to understand how long DNA profiles can be obtained that match a reference and/or search in a database, we analyzed three representative autosomal STR markers; D3S1358, D13S317, and D2S1338 are low, medium, and high molecular weight IdentiFiler® STR markers, respectively. Peak heights at these markers demonstrated the degradation of DNA over time (Fig. 4). As expected, the high molecular weight marker, D2S1338 showed higher rates of degradation over time compared to the medium (D13S317) and low molecular weight markers (D3S1358) (Fig. 4). The degradation rate was similar in female, male, and pooled blood PBM.

The goal of this study was to establish positive human identification from a bed bug at 12 h intervals PBM until 108 h. Our data suggest that correct (100%) positive identification can be established up to 96 h PBM from bed bugs fed on female blood, up to 84 h PBM from bed bugs fed on male blood, and up to 72 h PBM from bed bugs fed on pooled blood (Fig. 2). Presumably, the combined activities of digestive enzymes and nucleases degraded the ingested human pooled blood so that complete human autosomal STR profiles could not be obtained after 72 h from pooled PBM and 84 h from human male PBM. However, ingested female blood did not show a complete drop out of the STR markers until 108 h PBM, 24 h longer compared to male and 36 h longer compared to pooled PBM respectively. This difference should be viewed with caution until replicated with multiple cohorts of bed bugs and multiple sources of human blood, including naturally fed bed bugs. Nevertheless, these results might suggest that the rate of human blood digestion might differ depending upon the source of the host blood. Future investigations are warranted to determine whether various blood types impact bed bug feeding preferences, ingested blood volume, and rate of digestion.

The low percentage (~55%) of correct allele calls immediately after the ingestion of blood (0 h) for female, male, and pooled human blood corresponded to low amounts of human DNA recovered (Table 1) from the bed bug (average female blood = 0.202 ng/μL; average male blood = 0.111 ng/μL; and average pooled blood = 0.019 ng/μL). This low amount of DNA obtained at 0 h is in contrast to results obtained from maggots, where optimal DNA genotyping results occurred for maggot samples that were immediately preserved after feeding [14]. The low DNA recovery in this study could be attributed to partial inhibition of DNA polymerase during and shortly after feeding. This unknown inhibitory factor would later (after 12 h) become inactivated, yielding higher amounts of DNA (Table 1) and higher percentage of correct alleles (Fig. 3). This time-course suggests that perhaps salivary secretions associated with the blood-feeding event might be involved. PCR inhibitors have been detected in the heads of various insect species, and it is often recommended that heads be

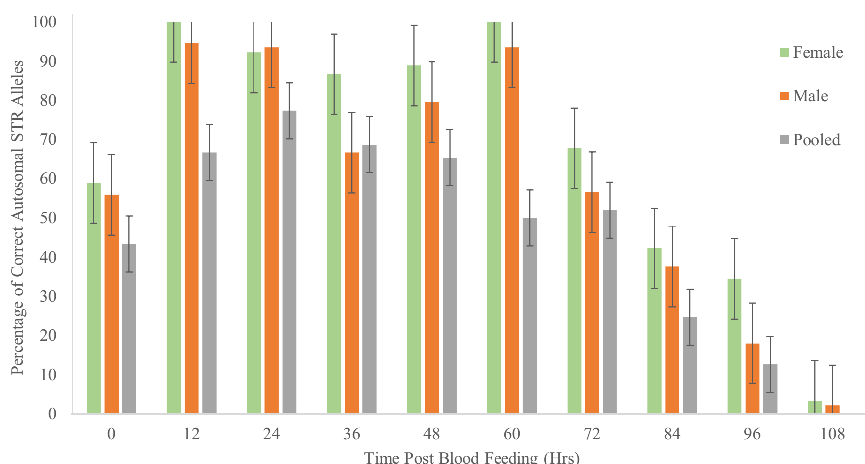

Fig. 3. Percentage of correct autosomal STR allele calls for female, male, and pooled blood extracted from bed bugs. Data below (100 RFU), analytical threshold is included. Bars represent standard error of the mean (SEM).

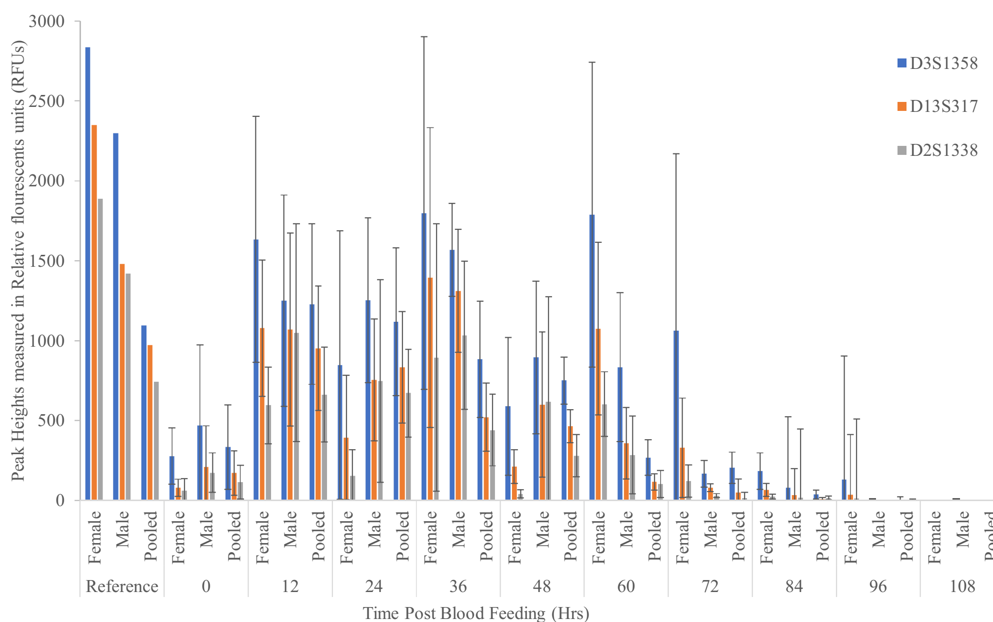

Fig. 4. Average peak heights at low (D3S1358), medium (D13S317), and high (D2S1338) molecular weight autosomal STR markers for female, male, and pooled bloodmeal samples. Data demonstrates the degradation of DNA over time. Data below (100 RFU) analytical threshold is included. Bars represent standard error of the mean (SEM).

removed for PCR reactions that do not require the head [28]. We are investigating if this low amount of human male-specific, Y chromosome DNA as well as low percentage of correct Y STR allele calls are observed at 0 h PBM in bed bugs fed on human male blood.

A second goal of this study was to determine if human blood meal sources could be identified from a mixture of host blood. Bed bugs, unlike some other blood-feeders, are quite stealthy feeders. They feed at night while the host sleeps, and usually complete a blood meal in a single feeding bout with no disturbance to the host. At times, however, feeding may be disrupted, and the bed bug may resume feeding on a different host, resulting in pooled blood from two hosts within the bed bug. Previous research demonstrated that human DNA was successfully detected up to 20 h after blood consumption by two body lice fed on pooled blood meals [9]. Here we have demonstrated that a mixed DNA profile can be obtained after feeding pooled blood from a female host and a male host to a bed bug. The mixed profiles matched with their reference blood profiles up to 72 h post feeding (Fig. 2). These results indicate that identification of multiple human hosts is possible from a single bed bug. However, the ratio of each contributor may be variable depending on the amount of blood ingested from each individual and the time difference of blood consumed from each subject. With all the advantages bed bugs have over other hematophagous insects used for human identification, crime scene technicians should strive to locate and collect them for use as physical evidence in forensic investigations.

5. Conclusion

This study demonstrates for the first time that the use of forensic biology and entomology methods enable identification of human blood sources from bed bugs. It is also evident that single source as well as multiple human hosts can be positively genotyped up to 72 h PBM. However, DNA degradation is a concern as time elapses; therefore, we recommend that crime scene technicians collect multiple blood fed bed bugs from the crime

scene to maximize the recovery of human blood and human-specific DNA and increase the chances of generating complete DNA profile(s) from bed bugs.

Authors contributions

This is a brief detail of each author's contribution in the research design, methods development, methods validation, data production, data analysis, literature review, manuscript writing, edits, revision, and project administration. curation

1. **Coby Schal, Ph.D.:** Dr. Lodhi had the original research idea using bed bugs for human identification. He presented the experimental design and details of research and requested Dr. Schal's collaboration for feeding human blood to bed bugs at Dr. Schal's entomology laboratory at North Carolina State University. Dr. Schal agreed and provided bed bugs for preliminary experiments. Hence, Dr. Schal was involved from the original conceptualization and experimental design. He contributed in data analysis, data interpretation, manuscript writing, review and edits, revision, and project administration.
2. **Natalia Czado, MS:** Ms. Czado was involved in data analysis, figures preparations, data interpretation, manuscript writing, review & edits, and revision.
3. **Richard Gamble, BS:** Richard was involved during the DNA quantitation, DNA amplification, figures preparations, and data analysis.
4. **Amy Barrett, BS:** Amy was involved during the experimental designs and validation: Amy later helped in data collection, figures preparations, data analysis, and literature review.
5. **Kiera Weathers, BS:** Kiera was involved during the experimental designs and validation. She helped in data collection, figures preparations, data analysis, references citation, and literature review.
6. **Khalid Mahmud Lodhi, DSc.:** Dr. Lodhi had the original research idea using bed bugs for human identification. He presented the experimental design and details of research and requested Dr. Schal's collaboration for feeding human blood to bed bugs at Dr. Schal's entomology laboratory at North Carolina State University. Therefore Dr. Lodhi's role in: Funding acquisition, conceptualization, experiment design, methods validation, experimental work, data analysis, data interpretation, manuscript writing, edits, revision, and project administration.

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References

[1] R.L. Usinger, Monograph of Cimicidae (Hemiptera, Heteroptera), vol. 7, Thomas Say Foundation Publications, Entomological Society of America, College Park, Md, 1966 585 p..
[2] W. Booth, et al., Molecular markers reveal infestation dynamics of the bed bug (Hemiptera: Cimicidae) within apartment buildings, J. Med. Entomol. 49 (3) (2017) 535–546.
[3] A.G. Marshall, The Ecology of Ectoparasitic insects, (1981) .
[4] M. Lehane, The Biology of Blood-Sucking in Insects, 2nd ed., (2005) .
[5] A.L. Szalanski, et al., Isolation and characterization of human DNA from bed bug, *Cimex lectularius* L., (Hemiptera: Cimicidae) blood meals, J. Agric. Urban Entomol. 23 (3) (2006) 189–194.
[6] W.D. Lord, et al., Isolation, amplification, and sequencing of human mitochondrial DNA obtained from human crab louse, *Pthirus pubis* (L.), blood meals, J. Forensic Sci. 43 (5) (1998) 1097–1100.
[7] K. Reinhardt, M.T. Siva-Jothy, Biology of the bed bugs (Cimicidae), Annu. Rev. Entomol. 52 (2007) 351–374.
[8] J.D. Wells, et al., Human and insect mitochondrial DNA analysis from maggots, J. Forensic Sci. 46 (3) (2001) 685–687.
[9] K.Y. Mumcuoglu, et al., Use of human lice in forensic entomology, J. Med. Entomol. 41 (4) (2004) 803–806.
[10] S. Spitaleri, et al., Genotyping of human DNA recovered from mosquitoes found on a crime scene, Int. Congr. Ser. 1288 (2006) 574–576.
[11] D.G. Njau, et al., STR analysis of human DNA from maggots fed on decomposing bodies: assessment of the time period for successful analysis, Egyptian J. Forensic Sci. 6 (3) (2016) 261–269.
[12] M. de Lourdes Chávez-Briones, et al., Identification of human remains by DNA analysis of the gastrointestinal contents of fly larvae, J. Forensic Sci. 58 (1) (2013) 248–250.
[13] R. Zehner, J. Amendt, R. Krettek, STR typing of human DNA from fly larvae fed on decomposing bodies, J. Forensic Sci. 49 (2) (2004) 1–4.
[14] C.P. Campobasso, et al., Forensic genetic analysis of insect gut contents, Am J. Forensic Med. Pathol. 26 (2) (2005) 161–165.
[15] J. Replogle, et al., Identification of host DNA by amplified fragment length polymorphism analysis: preliminary analysis of human crab louse (Anoplura: Pediculidae) excreta, J. Med. Entomol. 31 (5) (1994) 686–690.
[16] R. Coulson, et al., Amplification and analysis of human DNA present in mosquito bloodmeals, Med. Vet. Entomol. 4 (4) (2017) 357–366.
[17] E. Michael, et al., Quantifying mosquito biting patterns on humans by DNA fingerprinting of bloodmeals, Am. J. Trop. Med. Hyg. 65 (6) (2001) 722–728.
[18] E. Chow-Shaffer, et al., Laboratory and field evaluation of polymerase chain reaction-based forensic DNA profiling for use in identification of human blood meal sources of *Aedes aegypti* (Diptera: Culicidae), J. Med. Entomol. 37 (4) (2000) 492–502.
[19] J. De Benedictis, et al., Identification of the people from whom engorged *Aedes aegypti* took blood meals in Florida, Puerto Rico, using polymerase chain reaction-based DNA profiling, Am. J. Trop. Med. Hyg. 68 (4) (2003) 437–446.
[20] G. Curic, et al., Identification of person and quantification of human DNA recovered from mosquitoes (Culicidae), Forensic Sci. Int. Genet. 8 (1) (2014) 109–112.
[21] A. Romero, C. Schal, Blood constituents as phagostimulants for the bed bug *Cimex lectularius L*, J. Exp, Biol. 217 (2014) 552–557.
[22] T. Maniatis, E.F. Fritsch, J. Sambrook, Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring, 1982.
[23] A. Sierras, C. Schal, Comparison of ingestion and topical application of insecticides against the common bed bug, *Cimex lectularius* (Hemiptera: Cimicidae), Pest Manag. Sci. 73 (3) (2017) 521–527.
[24] A. Ogunrinade, The measurement of blood meal size in *Aedes aegypti* (L.), Afr. J. Med. Med. Sci. 9 (1–2) (1980) 69–71.
[25] B.C. Redington, W.T. Hockmeyer, A method for estimating blood meal volume in *Aedes aegypti* using a radioisotope, J. Insect Physiol. 22 (7) (1976) 961–966.
[26] M.W. Dryden, S.M. Gaafar, Blood consumption by the cat flea *Ctenocephalides felis* (*Siphonaptera*: *Pulicidae*), J. Med. Entomol. 28 (3) (1991) 394–400.
[27] Y.K. Matos, J.A. Osborne, C. Schal, Effects of Cyclic feeding and starvation, mating, and sperm condition on egg production and fertility in the common bed bug (Hemiptera: Cimicidae), J. Med. Entomol. 110 (2017) 1483–1490.
[28] J.F. Beckmann, A.M. Fallon, Decapitation improves detection of Wolbachia pipientis (Rickettsiales: Anaplasmataceae) in culex pipiens (Diptera: Culicidae) mosquitoes by the polymerase chain reaction, J Med Entomol 49 (5) (2012) 1103–1108.