Isolation and Characterization of Human DNA From Bed Bug, *Cimex lectularius* L., (Hemiptera: Cimicidae) Blood Meals[1]

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ABSTRACT The ability to identify individual human hosts based on analyses of blood recovered from hematophagous insects is beneficial for both medical and forensic entomology. Bed bugs, *Cimex lectularius* L. (Heteroptera: Cimicidae), may have several advantages over other blood-feeding arthropods for forensics because they do not remain on the host after their blood-feeding activity and remain in close proximity to a crime scene. Successful isolation, amplification, and sequencing of human DNA obtained from adult bed bugs is reported for the first time from this study. Engorged bed bugs were recovered from a human volunteer and from field collected samples from New York, New York, and Brazos County, Texas. Samples were preserved by drying, stored in 70% ethanol, or freezing at –20°C. DNA was extracted from individual insects, and polymerase chain reaction was conducted using short tandem repeat (STR), human mitochondrial DNA (mtDNA) hypervariable region (HVR1), and insect mtDNA 16S markers. Amplification of a STR marker used in forensic investigations, D18S51, a HVR1 marker, and an insect mtDNA 16S marker was successful. These results demonstrate that DNA isolated from bed bugs is qualitatively and quantitatively sufficient for DNA typing and could be helpful to identify individuals for forensic analysis.

KEY WORDS bed bug, *Cimex lectularius*, forensic entomology, STR typing, hypervariable region

Cimex lectularius L. (Heteroptera: Cimicidae) is a hematophagous insect of humans and has regained worldwide attention as the result of its recent resurgence. Because the species is easily translocated by passive dispersal and adapts to multiple hosts (Usinger 1966, Marshall 1981, Lehane 2005) when preferred food sources are unavailable, it is extremely difficult to isolate the origins of recent infestations, even with genetic tools. However, basic molecular biology tools can be used to elucidate the identity of hosts fed upon by bed bugs, hence providing the potential for forensic applications into host identity from undi-

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gested blood obtained from recently-fed bed bugs. Bed bugs are obligate hematophages, with both sexes feeding on blood and blood meals are required before eclosion into subsequent molts (Usinger 1966). Thus, these insects present an excellent means for criminologists to match human hosts to specific locations in criminal investigations while excluding other hosts (e.g., birds or bats). Their basic biological necessities from hosts make them temporally and spatially predictable, forming gregarious assemblages in enclosed spaces (Reinhardt & Siva-Jothy 2007) providing ample opportunity to quickly locate recently blood-fed samples for examination.

The ability to isolate human DNA and possibly genotype blood from insects may prove invaluable to future forensic cases in which associating the presence of a person to a specific location may be needed. Human DNA has been successfully isolated and characterized from mosquitoes (Kreike & Kampfer 1999), human crab louse, *Pthirus pubis* (L.) (Anoplura: Pthiridae) (Lord et al. 1998), fly larvae (Wells et al. 2001, Zehner et al. 2004), and nitidulid beetles (DiZinno et al. 2002). To date no studies have been conducted on the isolation and characterization of human blood from bed bugs. The goal of this study was to determine if human blood can be isolated and characterized from bed bugs and establish the potential for its use in forensic criminal investigations.

Materials and Methods

In this study, a total of 35 blood-fed bed bugs were subjected to DNA analysis. Thirteen bed bugs were obtained from human dwellings in New York, New York and preserved in 90% ethanol. Seventeen bed bugs were from human dwellings in Brazos County, Texas, and preserved by freezing at –20°C. Five additional bed bugs were allowed to feed on a human volunteer for 15 min and then were maintained at 25°C for 24 h before preservation in 100% ethanol. The bed bugs that fed on the human volunteer are from a laboratory strain maintained at Virginia Tech, Blacksburg, Virginia, and blood feeding was conducted as approved by the Virginia Tech Institutional Review Board (IRB 06–165). A reference saliva sample was obtained from the human volunteer and air-dried.

Adult *C. lectularius* were morphologically identified using descriptions outlined by Usinger (1966), and voucher specimens are deposited in the Arthropod Museum, Department of Entomology, University of Arkansas, Fayetteville, Arkansas, USA.

DNA was extracted from individual insects using a Puregene DNA isolation kit D-5000A (Gentra, Minneapolis, Minnesota). Extracted DNA was resuspended in 50 μL of Tris:EDTA and stored at –20°C. polymerase chain reaction (PCR) was conducted with three primer sets. DNA from the reference saliva sample was extracted from FTA cards (Whatman International Ltd., Maidstone, United Kingdom). Single 2.0-mm punches were taken from the FTA card and prepared for PCR by washing three times with 200 μL of FTA Purification Reagent and twice with 200 μL of TE buffer, as recommended by the manufacturer.

PCR was conducted using two human and one insect marker. PCR primers L15997 and R202 amplify the human mtDNA first hypervariable part (HRV1) of the control region (Cavelier et al. 2000); primers 5′-CAAACCCGACTACCAG-CAAC-3′ and 5′-GAGCCATGTTCATGCCACTG-3′ amplify a 271–343 bp region of

the human D18S51 locus (Barber & Parkin 1996); and primers LR-J-13007 (5′-TTA CGC TGT TAT CCC TAA-3′) (Kambhampati & Smith 1995) and LR-N-13398 (5′-CGC CTG TTT ATC AAA AAC AT-3′) (Simon et al. 1994) amplify a 428-bp fragment of the insect 16S rRNA gene. The PCR reactions were conducted with 2 μL of the extracted DNA following Szalanski et al. (2003). PCR conditions for the D18S51 marker consisted of 35 cycles of denaturing at 94°C for 30 sec, annealing at 55°C for 45 sec, extension at 72°C for 45 sec, and final extension at 72°C for 5 min (Egyed et al. 2006). The HRV1 thermocycler profile consisted of 28 cycles of denaturing at 94°C for 30 sec, annealing at 60°C for 30 sec, with extension at 72°C for 45 sec, and final extension at 60°C for 30 min. The profile for the 16S marker consisted of 35 cycles of 94°C for 45 sec, 46°C for 45 sec and 72°C for 60 sec. The 16S and HVR1 PCR products were resolved on 1% agrose gels, whereas the STR PCR products were separated by 10% PAGE per Taylor et al. (1996). Amplified DNA from the HRV1 marker was purified and concentrated using Microcon-PCR Filter Units (Millipore, Bedford, Massachusetts). Samples were sent to the University of Arkansas Medical School DNA Sequencing Facility (Little Rock, Arkansas) for direct sequencing in both directions using an ABI automated sequencer (Foster City, California). DNA sequences were aligned using BioEdit v5.89 (Hall 1999) and adjusted manually.

Results and Discussion

From the 35 bed bugs subjected to DNA analysis, DNA was successfully isolated from alcohol preserved, air dried, and frozen specimens. The mtDNA 16S marker amplified successfully for all of the bed bugs, whereas the STR D18S51 maker amplified for 32 bed bugs (Fig. 1), and the HVR1 maker amplified for 30 bed bugs. The mtDNA 16S marker can be subjected to DNA sequencing analysis, allowing population genetics studies of the bed bugs themselves (Szalanski et al. 2007). We have conducted this on bed bugs populations from Arkansas, Texas, South Carolina, Michigan, Virginia, and Illinois, and several haplotypes have been observed (unpublished data). From the five blood-fed bed bugs collected from the human volunteer, all of the STR allelic patterns and HVR1 sequences matched those obtained from the reference saliva sample. The STR marker set revealed five distinct allelic patterns from the bed bugs obtained from Brazos Co. Texas, which provides evidence that the bed bugs had fed on at least five individuals. On the basis of our findings, profiling with additional Combined DNA Index System (CODIS) STR primer sets should allow genetic characterization to individuals using bed bug blood meals. In addition, PCR product from the HRV1 amplicon from three field collected bed bugs samples was subjected to DNA sequencing and was confirmed as human DNA using a Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990) search found at the National Centers for Biotechnology Information (NCBI) website http://www.ncbi.nlm.nih.gov/.

Host location is of paramount importance to cimicids: Nymphs of *C. lectularius* die within a few days of emerging from the egg if they do not feed (Usinger 1966). Marx (1955) suggests that *C. lectularius* detects human hosts from as far away as 1.5 m through the use of heat cues, host kairomone(s) and/or CO_2. Laboratory colonies of bed bugs have been shown to feed every 7 d (Usinger 1966, Siva-Jothy 2006). This feeding rate was similar to a study that looked at naturally infested

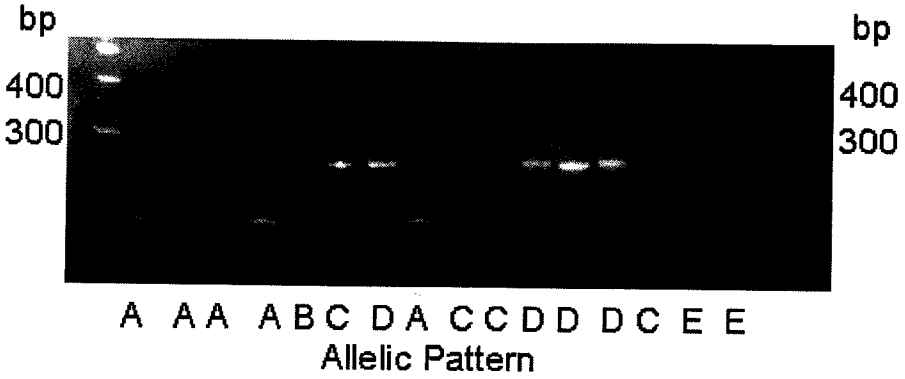

Fig. 1. STR D18S51 allelic patterns from 17 bed bugs resolved on a 5% polyacrylamide gel.

rat cages (Mellanby 1939), but considerably longer than studies of other cimicid species in hotter climates, which were observed feeding daily (Hase 1917). However, field surveys of other cimicid species suggest a feeding cycle that likely occurs between 3 and 7 d, suggesting that cimicids may not be as limited to blood meals in the field (Reinhardt & Siva-Jothy 2007). By evaluating local environmental conditions, the availability of alternative hosts, and the basic biological demands of bed bugs for any given location, the ability to use modern detection techniques such as molecular diagnostics provides yet another tool for forensic specialists to attempt to link hosts with a location. The next logical steps after identifying what hosts are being fed upon would be to attempt to identify the individual by genotyping, as would be common in any forensic investigation, and predict the duration from time of feeding to recovery of viable human DNA. This work is currently under investigation in our research laboratories. Future studies that are attempting to identify geographic relationships are also being evaluated and may yet provide additional important clues (unpublished data). Although our sample size here is small, this study demonstrates that human DNA can be isolated from bed bug blood meals and that the DNA can be characterized using both STR and DNA sequencing methods. Dried bed bugs, as well as samples preserved by freezing and in ethanol, all worked well for DNA profiling. Bed bugs may have several advantages over other blood feeding arthropods for human forensics. For example, bed bugs do not remain on the host after their blood feeding activity, and will remain in close proximity to a crime scene. These findings may have forensic applications in the use of bed bug derived blood meals for host identification and warrants future research into host-specific identification using molecular approaches.

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