BRIEF COMMUNICATION

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Isolation, Amplification, and Sequencing of Human Mitochondrial DNA Obtained from Human Crab Louse, *Pthirus Pubis* (L.), Blood Meals*

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ABSTRACT: The ability to identify individual human hosts based on analyses of blood recovered from the digestive tract of hematophagous arthropods has been a long-term pursuit in both medical and forensic entomology. Blood meal individualization techniques can bring important advancements to studies of vector-borne disease epidemiology. Forensically, these analyses may aid in assailant identification in violent crime cases where blood-feeding insects or their excreta are recovered from victims or at crime scenes. Successful isolation, amplification, and sequencing of human mitochondrial DNA obtained from adult human crab lice fed on human volunteers are reported. Adult lice were removed from recruited volunteers frequenting inner city health clinics. Live lice were killed by freezing and subsequently air dried at ambient temperature. A saliva sample was obtained from each volunteer and served as a DNA reference sample. Volunteers were afforded free, approved pediculosis treatment. Individual lice were subsequently processed using procedures developed for the extraction of mitochondrial DNA from human hair, teeth, and bone. The resulting DNA was amplified by the polymerase chain reaction and sequenced. Our results point to valuable avenues for future entomological research.

KEYWORDS: forensic science, DNA typing, mitochondrial DNA, blood meal analysis, louse, insect, forensic entomology, *Pthirus Pubis*

The ability to identify individual human hosts based on the analysis of blood recovered from the digestive tract of hematophagous arthropods has been a continuing pursuit in both medical and forensic entomology. Blood meal individualization techniques can bring important advancements to studies of vector-borne disease epidemiology and tropical disease ecology (1,2). Forensically, such analyses may aid in assailant identification in violent crime cases (e.g., rape, homicide, and child abuse), where blood-feeding insects or their excreta are recovered from victims or at crime scenes (3). Such studies also serve to further validate the reliability of human DNA typing methodologies.

Human mitochondrial DNA (mtDNA) is an extrachromosomal, closed, circular, organelle-specific genome consisting of approximately 16.5 kb (11). Mitochondrial DNA consists of coding sequences for 2 ribosomal RNAs, 22 transfer RNAs, and 13 proteins, and a noncoding region called the displacement loop (D-loop) (12).

Forensically, mtDNA has a distinct advantage over nuclear DNA in terms of its abundance. While each set of nuclear chromosomes is present in only two copies per cell, there are thousands of mitochondrial DNA chromosomes per cell (11). In instances where the amount of extracted DNA is very small or degraded, the probability of obtaining a positive DNA typing result from mtDNA is greater than for nuclear DNA. Techniques have been developed for extracting, amplifying, and directly sequencing mtDNA from human bone, teeth, and hair shafts (13,14).

This short communication describes successful isolation, amplification, and sequencing of human mtDNA obtained from blood meals located within the digestive tract of adult human crab lice, *Pthirus pubis* (L.) that fed on human volunteers. The data demonstrate the sensitivity of current mtDNA sequencing technology and point to valuable avenues for future entomological research.

Materials and Methods

Historically, immunological methodologies have been successfully applied to insect blood meal analysis and have proven useful in determining the vertebrate species on which mosquitoes have fed (4,5). Human blood group markers, including ABO blood groupings (6) and haptoglobin (7) have also been isolated from mosquito blood meals. Molecular biological procedures currently provide scientists the ability to analyze genetic polymorphisms at

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the DNA level. One such technique, the polymerase chain reaction (PCR), has greatly enhanced the specificity and sensitivity of DNA detection and provided the template for accurate analysis of small, degraded DNA samples (8). Recently, human nuclear DNA has been successfully isolated from engorged female mosquitoes (9,10) and from louse excreta (3).

Volunteers gave informed consent and received free, approved treatment for pediculosis at the end of the study. Participants were financially compensated for their participation. All sampling was conducted by the Field Epidemiology Survey Team (FEST) of the University of Miami School of Medicine, and study protocols were preapproved by the University Institutional Review Board for the Protection of Human Subjects.

Adult human crab lice, *Pthirus pubis* (L.), were collected from human volunteers frequenting inner-city clinics in Miami, FL (Fig. 1). Adult lice, removed from the pubic region of recruited volunteers, were immediately placed into cylindrical pill boxes (12 mm high by 24 mm diameter) capped with a fine nylon mesh stocking on each end. These boxes were then taped to the thigh, waist, or calf of the study subject depending on which location was most comfortable. Volunteers were examined each day for ten days. At each visit lice housed within the containers were counted, deceased lice were removed, and free lice congregating outside the boxes were removed from the skin with fine-tipped forceps and placed inside. All remaining lice were removed from the louse houses on day ten. Our sampling design ensured the collection of engorged adult lice of known host origin.

Live lice were killed by freezing and subsequently air-dried at ambient temperature (22°C). A reference saliva sample was obtained from each volunteer and also was air-dried. Desiccated lice and reference saliva samples were forwarded via air courier to the Forensic Science Research and Training Center, FBI Academy, Quantico, VA.

Blood meal mtDNA analyses were conducted in the context of a larger study dealing with louse embryonic development and longevity.

Individual lice were washed in distilled water, sectioned longitudinally along the dorsal midline and suspended in a 1.5 mL fliptop tube containing 300 μL of stain extraction buffer. Stain extraction buffer (SEB) facilitates the breakdown of cellular material and assists in liberating the DNA for further isolation. Stain extraction buffer contains 10 mM Tris, pH 9.0, 100 mM NaCl, 39 mM DTT, and 10 mM EDTA. The suspension was vortexed for 30 s; the resulting homogenate was incubated at 56°C for 2 and then transferred into a sterile 0.5 mL tube for organic solvent extraction. 300 μL of phenol/chloroform/isoamyl alcohol (24:24:1) were added and the resulting mixture centrifuged at 10,000 × g, for 2 min. The aqueous phase was removed and transferred to a Microcon-100 μL concentrator (Amica, Beverly, MA) containing 100 μL of sterile, filtered TE-4 (100 mM Tris, PH8, 0.1 mM

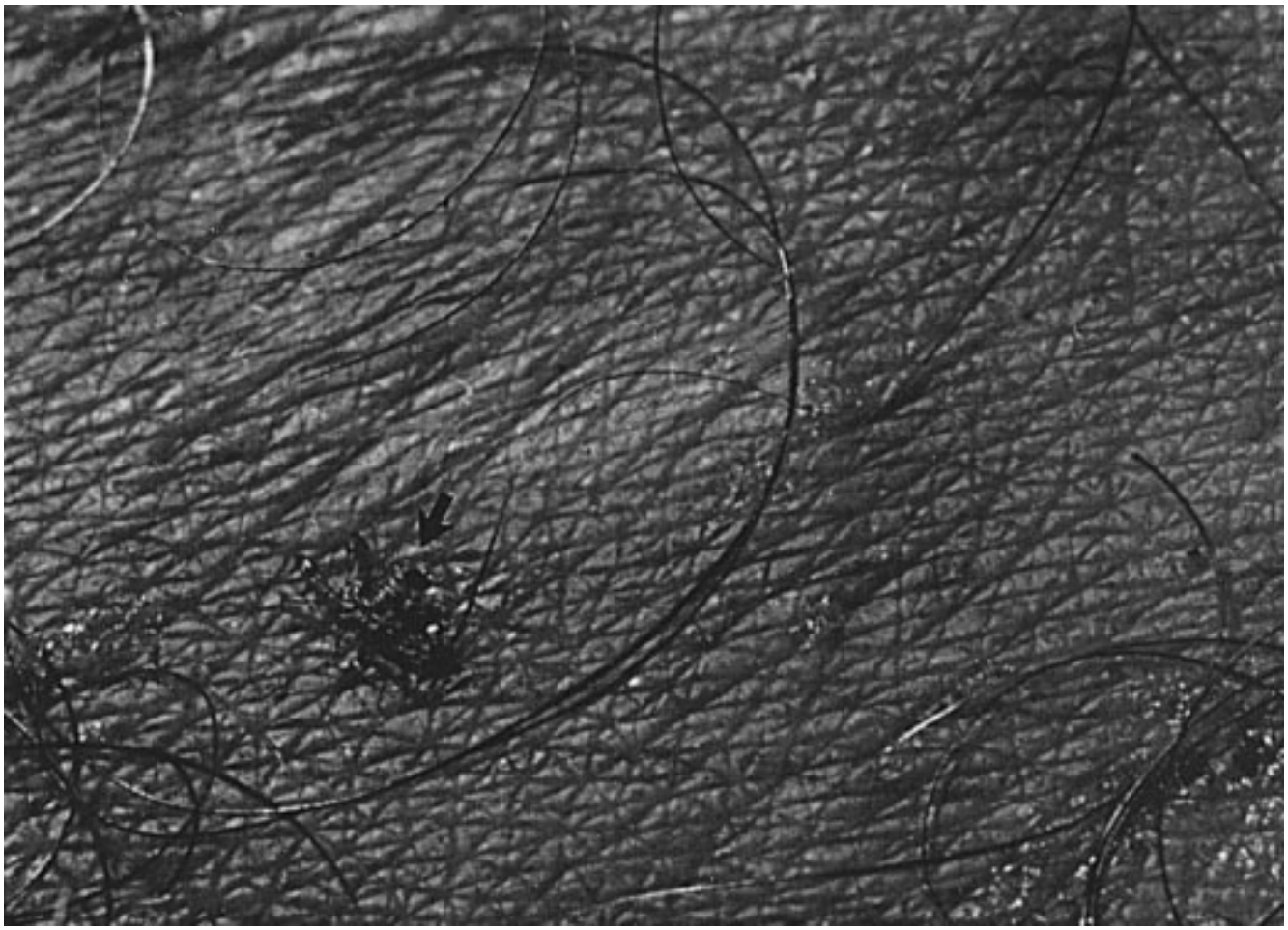

FIG. 1—*Adult human crab louse,* Pthirus Pubis (L.), *attached to the skin of a study volunteer. Volunteers were afforded free, approved pediculosis treatment.*

EDTA). The mixture was then centrifuged at 5000 × g for 5 min. After discarding the wash, an additional 100 μL of sterile, filtered TE-4 were added to the Microcon tube and centrifugation repeated. Following wash disposal, a final 200 μL of sterile TE-4 were added. The Microcon-100 was then vortexed at medium speed for 30 s and inverted into the collection tube provided. The collection apparatus was then centrifuged at 12,000 × g for 3 min. A volume of approximately 200 μL was recovered. Hypervariable region 2 (HV2) of the mtDNA genome (Bases 073-340) was amplified from 10 μL of the extract in a Perkin-Elmer Geneamp PCR system 9600 thermal cycler and subsequently sequenced using a Perkin-Elmer ABI 373A automated DNA sequencer as described by Wilson, et al. (11,13). MtDNA sequences were then compared to the referenced standard established by Anderson et al. (12) and to donor saliva samples that had been similarily processed. Replicates consisting of single lice and groups of three lice were processed by the previously detailed protocol. Single louse replicates were processed individually, whereas grouped lice were pooled together.

Results and Discussion

The ability to identify individual human hosts via the analysis of blood recovered from the digestive tract of hematophagous arthropods is a continuing pursuit in both medical and forensic entomology. Successful blood meal individualization techniques have the potential to create major advancements in vector-borne disease epidemiology and to aid in the identification of assailants and victims of crimes. Coulson et al. (2) demonstrated that human nuclear DNA could be isolated from mosquito blood meals following preservation of the adult mosquitoes by freezing at 70°C or placement in 100% isopropanol. Hawley and Budowle (10) demonstrated that human DNA amenable to AMP-FLP analysis could be obtained from blood meals of human-fed mosquitoes that were killed 10 h after feeding and subsequently preserved in liquid nitrogen. Replogle et al. (3) successfully recovered human nuclear DNA from crab louse fecal materials but were unable to isolate typable human DNA from the lice themselves. Human mtDNA has been recovered and sequenced from beetle larvae collected from human skeletal materials (15).

Herein, human mtDNA was successfully isolated, amplified, and sequenced from adult human crab lice that fed on human volunteers. Both single louse and tri-louse replicates produced positive mtDNA characterizations. All resulting mtDNA sequences matched those obtained from reference saliva samples from respective volunteers. Representative results from a single volunteer are displayed in Table 1. The results clearly illustrate the ability of mtDNA analysis to provide valid, host-specific sequences from desiccated hematophagous arthropods. While our results are promising, it must be emphasized that other aspects of our human crab louse study (i.e., developmental analyses and RFLP typing) left only a small number of lice for mtDNA analysis. A total of only eight lice were analyzed, resulting in a 100% mtDNA recovery. The small number of lice tested render these results preliminary.

TABLE 1—*Human mitochondrial DNA polymorphisms sequenced from crab louse blood meals and host saliva.*

HV 2	Saliva	Lice 1	Lice 3
73	G	G	G
146	C	C	C
153	G	G	G
235	G	G	G
263	G	G	G
315.1	C	C	C

Polymorphisms depicted are those sites that differ from Anderson et al. (12).

In conclusion, the results of our study clearly illustrate the ability of mtDNA analyses to provide individual host characterizations of desiccated and frozen arthropod blood meals. Entomologists, medical and forensic, who are interested in the application of DNA technologies are provided with another example of the stability of human mtDNA and methods by which it can be isolated, amplified, and sequenced. The preliminary results reported here will serve as a guide for more detailed studies on a wide variety of carrion-frequenting and hematophagous arthropods.

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