Letter: High-level of Heteroplasmy in the Mitochondrial *Cox1*-Minichromosome of the Human Body Louse, *Pediculus humanus*, and the Human Head Louse, *Pediculus capitis*

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Abstract: The mitochondrial (mt) genome of bilateral animals typically consists of a single chromosome, ~16 kb long, and contains 37 genes. Variation in mt gene sequence within an individual, i.e. heteroplasmy, is common in bilateral animals but usually occurs at very low levels. In the lineage that led to the human body louse, the typical mt chromosome has fragmented into 18 minichromosomes: each minichromosome is 3 to 4 kb long and contains 1 to 3 genes and a non-coding region. To understand the presence and the level of heteroplasmy in fragmented mt genomes, we cloned and sequenced seven copies of full-length *cox1*-minichromosome of a human body louse and a human head louse. We found 17 heteroplasmic sites in the coding region and 118 heteroplasmic sites in the non-coding region of the *cox1*-minichromosome. The level of heteroplasmy in the human lice appears to be much higher than that in other animals that have the typical mt genome. We propose that recombination between different minichromosomes of the fragmented mt genome may contribute to the high-level of heteroplasmy in the human lice.

Keywords: *Cox1,* genome, heteroplasmy, mitochondrial minichromosome, *Pediculus capitis*, *Pediculus humanus.*

INTRODUCTION

Mitochondrial (mt) genomes of bilateral animals usually consist of a single circular chromosome, which is ~16 kb in size and contains 37 genes [1]. In the lineage that led to the human body louse, *Pediculus humanus*, however, the single mt chromosome typical of animals has fragmented into 18 minichromosomes [2]. Each minichromosome is 3 to 4 kb long and has 1 to 3 genes and a non-coding region (NCR). Analyses of the sequence-reads generated by the Human Body Louse Genome Project revealed substantial sequence variation in the NCRs and numerous polymorphic sites in the coding regions of mt minichromosomes [2]. It is not known, however, whether the observed sequence variation and the polymorphic sites are between different individuals or within an individual, i.e. heteroplasmy, because the Human Body Louse Genome Project used the DNA extracted from ~100 nymphs [3]. In the present study, we sequenced entirely seven copies of the *cox1*-minichromosome from an individual human body louse and an individual human head louse. We report here the remarkable level of heteroplasmy in the *cox1*-minichromosome of these lice.

MATERIALS AND METHODS

Total DNA was extracted from an adult human body louse of the Orlando strain and an adult human head louse from Brisbane with DNeasy Tissue kit (QIAGEN). Total DNA and TaKaRa LA Taq enzyme were used in PCR amplification. A forward primer, PHC1F(KH) (5'-CAGCC-TTTTTATTATTACTGTCACTTCCAG-3'), and a reverse primer, LC1RA (5'-GACTGCCTTTATTTTGCTGGAGA-GTGTTGG-3'), were used in PCRs to amplify the entire *cox1*-minichromosome except a 3-bp gap between the two primers (Fig. **1A**). The PCR conditions were: 1 min at 96°C, then 35 cycles of 30 sec at 96°C, 30 sec at 58°C and 4 min at 68°C, followed by 8 min at 72°C. Negative controls were always included to ensure no amplicons were contaminated. PCR products were purified with S.N.A.P.™ UV-Free Gel Purification Kit (Invitrogen). Purified PCR products were cloned with a pGEM-T Easy Vector System and *Escherichia coli* JM109 Competent Cells (Promega). *E. coli* colonies were screened by PCR with M13F and M13R primers for DNA inserts of expected size (3-4 kb). We selected at random seven *E. coli* colonies that contained DNA inserts of expected size and sequenced these inserts entirely by primer walking. Five of these inserts were from the *cox1*-minichromosome of the human body louse and two inserts were from the *cox1*-minichromosome of the human head louse. The sequences of these seven inserts were deposited in GenBank under accession numbers HM357241 to HM357247. A direct comparison of the heteroplasmy between *Pediculus humanus* and *Pediculus capitis* was not conducted, due to the differing number of clones, however, Graphpad software (Graphpad software Inc.) was used to conduct a pairwise T test analysis of the quantity of heteroplasmic sites detected in lice compared to other insects.

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RESULTS

The five copies of *cox1*-minichromosome we sequenced from the human body louse are 3554, 3557, 3558, 3559 and 3560 bp long, respectively. Each copy of the *cox1*-minichromosome has a coding region, which contains only *cox1* gene, and a non-coding region (Fig. **1A**). The coding regions of the five copies of *cox1*-minichromosome have the same length, 1572 bp; the non-coding regions, however, vary slightly in length from 1982 bp to 1988 bp. Among the five copies of *cox1*-minichromosomes, there are 13 heteroplasmic sites in the coding region (Fig. **1B**), i.e. in the *cox1* gene, and 73 heteroplasmic sites in the non-coding region (Fig. **1C**). Four of the 13 heteroplasmic sites in the *cox1* gene are at the first codon position, one heteroplasmic site is at the second codon position, and eight heteroplasmic sites are at the third codon position (Fig. **1B**).

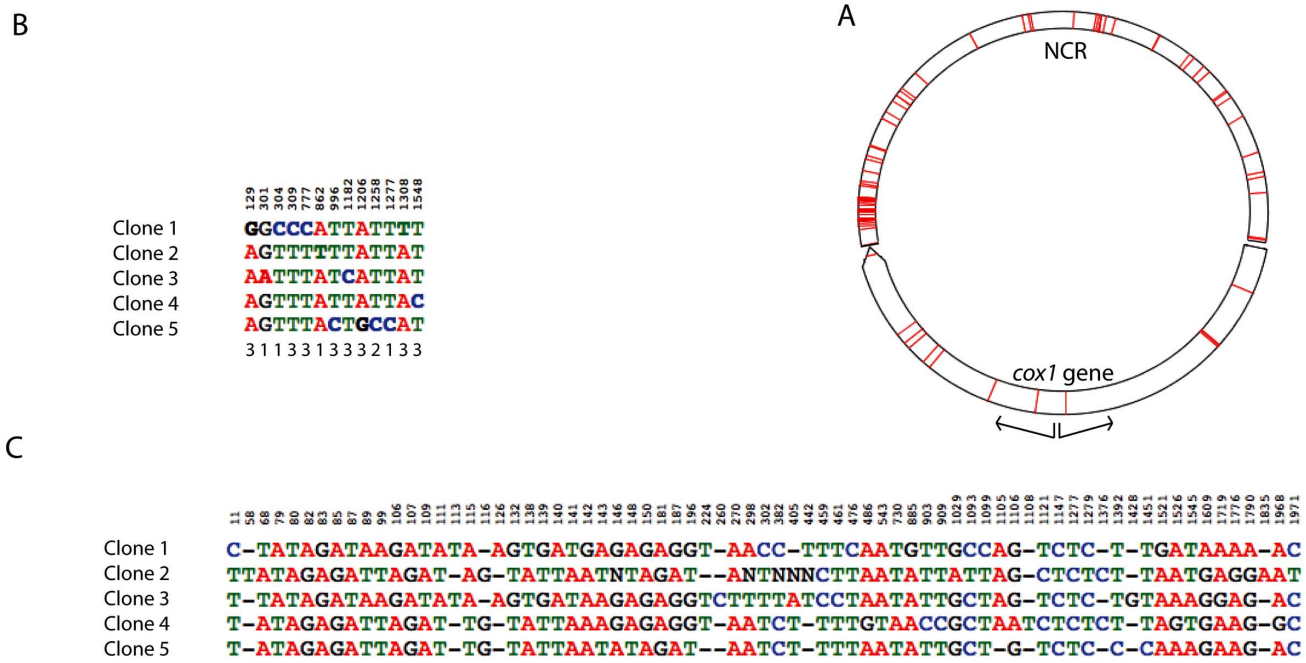

Fig. (1). Heteroplasmic sites in five copies of the *cox1*-minichromosome of an individual human body louse, *Pediculus humanus*. (**A**) The circular structure of the *cox1*-minichromosome; red lines indicate the distribution of heteroplasmic sites. (**B**) Heteroplasmic sites in the coding region, ie. the *cox1* gene. Numbers on the top row indicate the positions of these sites in the *cox1* gene. First, second and third codon positions are labeled as 1, 2 or 3. (**C**) Heteroplasmic sites in the non-coding region. Numbers on the top row indicate the positions of these sites in the non-coding region.

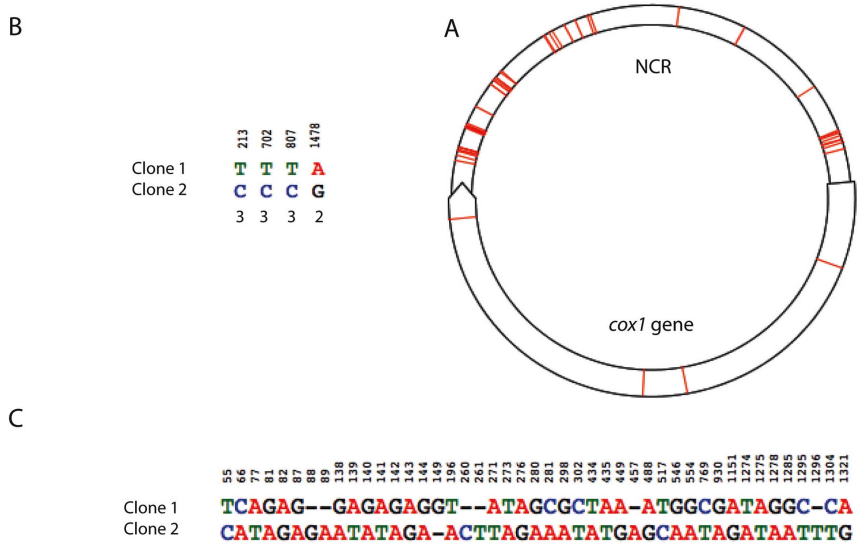

Fig. (2). Heteroplasmic sites in two copies of the *cox1*-minichromosome from an individual human head louse, *Pediculus capitis*. (**A**) The circular structure of the *cox1*-minichromosome; red lines indicate the distribution of heteroplasmic sites. (**B**) Positions of heteroplasmic sites in the coding region, ie. the *cox1* gene. Numbers on the top row indicate the positions of these sites in the *cox1* gene. Second and third codon positions are labeled as 2 or 3. (**C**) Heteroplasmic sites in the non-coding region. Numbers on the top row indicate the positions of these sites in the non-coding region.

The two copies of *cox1*-minichromosome we sequenced from the human head louse are 2965 and 2970 bp in size. As in the human body louse, each copy of the *cox1*-minichromosome of the human head louse has a coding region, which is 1572 bp long and contains only *cox1* gene, and a non-coding region (Fig. **2A**). The non-coding regions of the *cox1*-minichromosomes of the human head louse are 1393 and 1398 bp long, respectively, which are ~500 bp shorter than the non-coding regions of the human body louse (see above). Between the two copies of the *cox1*-minichromosome of the human head louse, there are four heteroplasmic sites in the coding region (Fig. **2B**), i.e. *cox1* gene, and 45 heteroplasmic sites in the non-coding region (Fig. **2C**). One of the four heteroplasmic sites in the *cox1* gene is at the second codon position and the other three are at the third codon position (Fig. **2B**).

DISCUSSION

Variation in mt genome sequence within an individual, i.e. heteroplasmy, is common in animals [4]. In arthropods, heteroplasmy has been reported previously in both non-coding regions [5, 6] and genes [7, 8]. Five heteroplasmic size variants were detected in the non-coding region of bark weevils [5], and at least two heterplasmic size variants have been detected in the non-coding region of *Drosophila melanogaster* [6]. Two heteroplasmic haplotypes, differentiated by four SNPs which are related to insecticide resistance, have been detected in the *cytochrome b* gene (*cob*) in mites [8]. Frey and Frey (2004) measured the level of heteroplasmy in the *cox1* gene of *Thrips tabaci*; they sequenced 64 clones of the *cox1* gene and detected 33 heteroplasmic sites, i.e. an average of 0.5 heteroplasmic sites per clone [7]. In this study, we sequenced seven clones of the *cox1* gene of the human lice but we detected 17 heteroplasmic sites, i.e. 2.4 heteroplasmic sites per clone. The number of heteroplasmic sites we detected in the human lice was significantly higher than that in *Thrips tabaci* ($p = 0.001$).

The level of heteroplasmy has been studied most, however, in humans. Pereira *et al*. (2009) compared the entire mt genome sequences of 5140 human individuals and found, in total, 120 variable sites [9]. Furthermore, He *et al*. (2010) sequenced the entire human mt genomes in extremely high coverage (on average 16,700 times) and found 34 heteroplasmic sites in 10 human individuals; only one of these 34 sites was in *cox1* gene [10]. In the present study, we detected, however, 17 heteroplasmic sites in *cox1* gene and 118 heteroplasmic sites in the non-coding region of the *cox1*-minichromosome of the human lice. Clearly, the level of heteroplasmy in the human lice is much higher than that in humans and other animals.

Why do human lice have high level of heteroplasmy? Observed variations may be sequencing errors introduced by DNA polymerase during PCR process, or may be from nuclear integration of mt sequences [7]. However, these two factors cannot account for the high level of heteroplasmy observed in our study. First, the Takara La Taq DNA polymerase we used in this study has a very high accuracy: the error-rate of this polymerase is 1.7×10^{-5} or 1.7 errors in 100,000 base pairs. At this rate, less than 0.5 mutations can be introduced into the 25,000 bp we sequenced. Second, the nuclear genome of the human body louse has been sequenced and there is no evidence for nuclear integration of mt DNA sequence in this louse [3]. We propose that the high level of heteroplasmy observed in the human lice may be linked to the recombination between different minichromosomes of the fragmented mt genome. Shao *et al*. (2009) reported unequivocal evidence for recombination between five pairs of mt minichromosomes in the human body louse [2]. This recombination in the human body louse was attributed to the extraordinary mt genome architecture of 18 minichromosomes [2]. Recombination between mt minichromosomes in the human body louse led to sequence changes at a substantial number of positions in the coding regions [2]. Although there is no evidence yet for recent recombination between the *cox1*-minichromosome and other minichromosomes in the human body louse, we have evidence for recent recombination between the *cox1*-minichromosome and the *nad4L*-minichromosome in the human pubic louse, *Pthirus pubis*, which is a close relative of the human body louse and the human head louse (Shao & Barker, unpublished).

Sequences of the mt genes, in particular *cox1* gene, have been used in many phylogenetic studies of the human body louse and the human head louse [11-17]. Phylogenetic studies that use *cox1* gene sequences usually assume, explicitly or implicitly, that all copies of *cox1* gene in an individual animal have identical sequence, i.e. homoplasmy. Or, if only a small number of heteroplasmic sites are present, as in humans, the heteroplasmy does not confound the inference of phylogeny. The present study indicates that the human body louse and the human head louse have far more heteroplasmic sites in their *cox1* genes than in other animals. Whether the high level of heteroplasmy in the human lice may affect the inference of phylogeny remains to be studied.

CONFLICT OF INTEREST

Declared none.

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