Current Biology, Vol. 13, 1414–1417, August 19, 2003, ©2003 Elsevier Science Ltd. All rights reserved. DOI 10.1016/S0960-9822(03)00507-4

Molecular Evolution of *Pediculus humanus* and the Origin of Clothing

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Summary

The human head louse (*Pediculus humanus capitis*) and body louse (*P. humanus corporis* or *P. h. humanus*) are strict, obligate human ectoparasites that differ mainly in their habitat on the host [1, 2]: the head louse lives and feeds exclusively on the scalp, whereas the body louse feeds on the body but lives in clothing. This ecological differentiation probably arose when humans adopted frequent use of clothing, an important event in human evolution for which there is no direct archaeological evidence. We therefore used a molecular clock approach to date the origin of body lice, assuming that this should correspond with the frequent use of clothing. Sequences were obtained from two mtDNA and two nuclear DNA segments from a global sample of 40 head and body lice, and from a chimpanzee louse to use as an outgroup. The results indicate greater diversity in African than non-African lice, suggesting an African origin of human lice. A molecular clock analysis indicates that body lice originated not more than about 72,000 ± 42,000 years ago; the mtDNA sequences also indicate a demographic expansion of body lice that correlates with the spread of modern humans out of Africa. These results suggest that clothing was a surprisingly recent innovation in human evolution.

Results and Discussion

We sequenced portions of the mtDNA *ND4* (579 bp) and *CYTB* (440 bp) genes from 26 head and 14 body lice from 12 different geographic regions. We also included a chimpanzee louse (*Pediculus schaeffi*; [3]); assuming that human and chimpanzee lice cospeciated with their hosts, as is frequently found to be the case [4], then the divergence time of 5.5 million years between humans and chimpanzees [5] also corresponds to the *P. humanus-P. schaeffi* divergence, and hence was used as a calibration point for molecular clocks.

Phylogenetic trees constructed separately for the *ND4* and *CYTB* sequences were nearly identical in topology and branch length, so the sequences were concatenated for further analysis (Figure 1). The topology of the tree, with the deepest clades containing only head lice sequences, indicates that body lice originated from head lice, as expected. The head and body lice sequences are not reciprocally monophyletic, but one clade contained all of the body lice sequences and 16 head lice sequences. The root of this clade is estimated to be 72,000 ± 42,000 years old. Since all body lice sequences are subsumed within this combined head/body lice clade, this date is an upper bound for the origin of body lice; the fact that body lice do not form a separate clade from head lice most likely reflects ancestral polymorphism [6]. The mtDNA sequences thus associate the origin of body lice (and, by inference, clothing) with modern humans. For the body lice, Tajima's D value [7] is negative ($D = -1.27$) and approaches, but does not reach, statistical significance ($p = 0.11$). Negative values of the D statistic, as observed for the body lice, are indicative of either directional selection or recent demographic expansion. For head lice, there was no such indication of recent expansion ($D = 0.39$, $p = 0.73$). In addition, the diversity among African lice is significantly greater than among non-African lice (Table 1; one-tailed t test, $p = 0.04$), even though the African lice come from a single location (Ethiopia) whereas the non-African lice represent a global sample (Figure 1). Genetic diversity is also greater in African than in non-African populations of humans [8–10], which is considered evidence for an African origin of modern humans. Thus, the greater diversity in African lice implies an African origin for lice.

To verify the above results based on mtDNA sequences, we also sequenced portions of two nuclear genes, elongation factor-1α (*EF-1*α, 485 bp) and RNA polymerase II (*RPII*, 601 bp). Phylogenetic analysis of the nuclear DNA sequences is complicated by recombination; however diversity analyses indicate that for both *EF-1*α and *RPII* there is both more diversity in African than non-African lice, and more diversity in head lice than in body lice (Table 1), as was found with the mtDNA sequences.

Overall, the greater diversity in Africa, recent origin, global distribution, and indication of population expansion for body lice all suggest a correlation with the global expansion of modern humans out of Africa in the last 100,000 years [11, 12]. Moreover, if the origin of body lice indeed reflects the development of clothing, then these results imply that clothing was a surprisingly recent innovation, associated with the spread of early modern humans out of Africa and into cooler regions.

Alternative interpretations of the results should be considered. In particular, if some or all of the lice mtDNA sequences are actually nuclear inserts of mtDNA [13], our conclusions could be in error. However, several lines of evidence suggest that the lice mtDNA sequences we obtained are not nuclear inserts. First, the sequences exhibit a strong transition to transversion bias (average among clades = 8.3), and the ratio of nonsynonymous to synonymous substitution rates has an average among clades of 0.08, which suggests that these are coding sequences and not nuclear inserts. Second, there are two deletions in the *ND4* gene of the chimpanzee louse compared to human lice; both are 3-bp deletions that result in the deletion of a single amino acid and preserve

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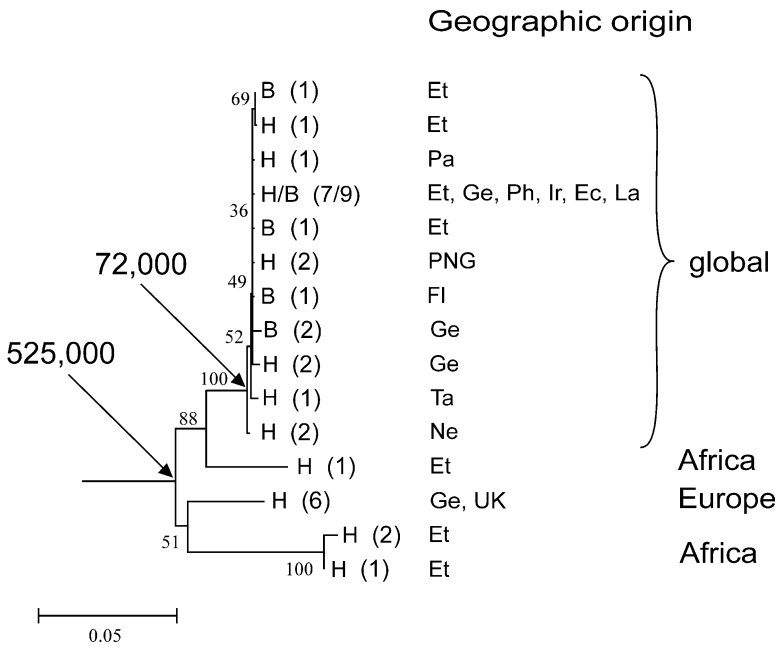

Figure 1. Neighbor-Joining Tree Based on Kimura-2-Parameter Distances for the Concatenated Sequences of *ND4* and *CYTB* from 40 Lice

Identical topologies were obtained for maximum parsimony and minimum evolution trees for these sequences (results not shown). The tree was rooted with the corresponding sequence of *P. schaeffi*; alternative placements of the root at any of the first three deepest branches (with three African, 6 European, and one African head lice sequence, respectively) are not significantly different and do not alter any conclusions. Bootstrap values (500 replications) are indicated on each interior branch. The arrows indicate the estimated age of particular nodes of the tree, based on Poisson-corrected amino acid distances. The tree based on amino acid distances (not shown) is virtually identical in topology to the tree shown, except for some sequences that differ only by silent substitutions. B: body louse, H: head louse; the frequency of a haplotype is indicated in brackets. Geographic origin of lice: Et: Ethiopia, Pa: Panama, Ge: Germany, Ph: Philippines, Ir: Iran, Ec: Ecuador, La: Laos, PNG: Papua New Guinea, Fl: Florida (USA), Ta: Taiwan, Ne: Nepal, UK: United Kingdom.

the reading frame. Preservation of the reading frame would not be expected for random deletions in a nuclear insert. Third, the same tree topology is observed for the *ND4* and *CYTB* sequences when they are analyzed separately, which means that in order for any of the sequences to be from a nuclear insert, two different primer pairs must have amplified the nuclear insert rather than the authentic mtDNA genome. As a further test, we also analyzed a segment of the cytochrome oxidase subunit 1 (*COX1*) gene that was recently studied in a global sample of 56 head and body lice [14]. We amplified and sequenced the same region of *COX1* for five lice that included the major lineages in the *ND4-CYTB* tree; the resulting tree for *COX1* has a topology identical to that of the *ND4-CYTB* tree (data not shown). Since the trees reconstructed from three independently amplified fragments of mtDNA show a similar topology, and the substitution patterns between the different lineages are characteristic for mtDNA, we conclude that the sequences we obtained are indeed authentic mtDNA sequences and not nuclear inserts.

A critical assumption is that the origin of body lice reflects the origin of clothing; it is possible that clothing existed for some time before lice exploited this new ecological niche, in which case the origin of clothing could be much more ancient than the origin of body lice. While we cannot exclude this possibility, the colonization of a new ecological niche usually occurs rapidly after it becomes available. Since modern humans and archaic humans such as Neandertals diverged about 250,000–500,000 years ago [11], in order to associate clothing with archaic humans, clothing would have had to exist for hundreds of thousands of years before the origin of body lice, which seems improbable. Moreover, archaeological evidence does not contradict an association of clothing specifically with modern humans, as the only tools that can be definitely associated with clothing, such as needles, are only about 40,000 years old [15]. Earlier tools, such as scrapers, may have been used to prepare hides for clothing [16], but may also have been used to scrape flesh for food or some other purpose. Indeed, clothing may have allowed early modern humans to colonize more extreme latitudes than their archaic predecessors, and hence might have been a factor in the successful spread of modern humans out of Africa.

Experimental Procedures

Samples and DNA Extraction

Human lice were obtained from parasitologists and physicians, or were collected by one of us (M.K.). Chimpanzee lice were obtained from the Ngamba Island Chimpanzee Sanctuary in Uganda. The

Table 1. Nucleotide Diversity for Human Lice Based on mtDNA and Nuclear Loci

	African Lice		Non-African Lice		Head Lice		Body Lice	
	n	θ_w (%)	n	θ_w (%)	n	θ_w (%)	n	θ_w (%)
MtDNA	16	3.31 ± 1.21	24	1.76 ± 0.60	26	3.42 ± 1.11	14	0.19 ± 0.10
*EF-1*α	20	0.29 ± 0.13	48	0.10 ± 0.10	50	0.23 ± 0.10	18	0.18 ± 0.10
RPII	20	0.94 ± 0.21	50	0.56 ± 0.14	50	0.93 ± 0.19	20	0.61 ± 0.17

n, number of sequences.

identification of the samples as head, body, or chimpanzee lice was confirmed by morphological analysis by E. Mey (Thüringer Landesmuseum Heidecksburg). DNA was extracted with a modified salting-out procedure [17] from single lice that were either stored at −20°C in 96% ethanol or had been air-dried.

Amplification and Sequencing

Universal and/or partially degenerate primers to several segments each of the *ND4*, *CYTB*, *EF-1*α and *RPII* genes were designed from comparisons of sequences from various insects (data not shown). For those segments that by sequence analysis gave authentic products, specific primers were designed for subsequent use; details concerning the primers and PCR conditions used for the three mtDNA and two nuclear gene segments are given in the Supplemental Data available with this article online. PCR cycling conditions were: denaturation at 95°C for 11 min, then 40 cycles consisting of 30 s denaturation at 94°C, 30 s at the annealing temperature (Table S1 in the Supplemental Data), and elongation for 45 s at 72°C, followed by a final extension step for 10 min at 72°C. PCR reactions contained 1 μl DNA extract, 0.2 μM of each primer, 0.2 mM dNTPs (Pharmacia), and 2.5 units of AmpliTaq Gold (Perkin Elmer) with the supplied buffer, in a 50 μl volume. PCR products were sequenced directly, except for *CYTB* in the chimpanzee louse, for which the partially degenerate primers (see the Supplemental Data) gave nonspecific products in addition to the expected product. In this case, the expected product was gel-purified, reamplified, cloned using the TOPO-TA cloning kit (Invitrogen), and several clones were sequenced using the M13 universal primer. Sequences were obtained with the BigDye cycle sequencing kit (Applied Biosystems) and an ABI 3700 automated DNA sequencer (Applied Biosystems); all positions were determined from both strands. Sequence trace files were analyzed with the program Sequence Analysis (DNAStar) and aligned with ClustalW [18]; all polymorphic positions were confirmed by manual inspection of the trace files. The sequences have been deposited with GenBank (accession numbers AY316748–AY316929).

Data Analysis

Neighbor-joining trees were constructed, using Kimura two parameter distances, with MEGA 2.1 [19]. Tajima's D and the nucleotide diversity based on the number of polymorphic sites, θ_W [20] were calculated with DnaSP 3.51 [21]. Because of the large distance between the mtDNA sequences of human lice and the chimpanzee louse outgroup (0.34, based on the number of nucleotide differences), we tested the accuracy of different distance models for dating, using mtDNA sequence data from taxa with known divergence dates [5] and similar sequence distances. For these data the Poisson correction distance based on amino acid substitutions [22] yielded reliable results. The relative rate test of Tajima [23], as implemented in MEGA 2.1, was applied to the Poisson amino acid distances (and to the nucleotide sequences) for the lice mtDNA, and the null hypothesis of a constant rate of evolution was not rejected. We therefore used the Poisson amino acid distances to date particular nodes in the mtDNA tree by the linearized tree method [24].

Supplemental Data

Supplemental Data including PCR primer sequences, product length, and annealing temperature for the lice mtDNA and nuclear DNA loci are available at http://www.current-biology.com/cgi/content/full/13/15/1414/DC1/.

Acknowledgments

We are indebted to E. Mey for his expertise in lice systematics. We thank S. Brauer, B. Höffner, C. Mühle and C. Schwarz for technical assistance; D. Altschuler, P.C. Fan, A. Gharanini, I. Iglisch, D. Reißig, K. Schwettmann, U. Sellenschlo, K. Stafford, R. Trusch and D. Taplin for contributing human lice samples; D. Cox and R. Ssuna (Chimpanzee Sanctuary & Wildlife Conservation Trust, Uganda) for contributing chimpanzee lice samples; K. C. Kim, G. Poinar and G. Weiss for useful discussion, and the Max Planck Society for financial support. R.K. was supported by the German National Merit Foundation (Studienstiftung des deutschen Volkes).

Received: April 24, 2003
Revised: June 13, 2003
Accepted: June 13, 2003
Published: August 19, 2003

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Accession Numbers

The *ND4*, *CYTB*, *COX1*, *EF-1*α, and *RPII* sequences from the head, body, and chimpanzee lice have been deposited in GenBank with accession numbers AY316748–AY316929.