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Unravelling the evolution of the head lice and body lice of humans

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Abstract Recent studies of mitochondrial genes of the head and body lice of humans indicate that present-day lice comprise two lineages that diverged before the evolution of modern humans. To test if this was a locus-specific phenomenon, we studied two nuclear genes, elongation factor-1 α (*EF-1 α*) and small subunit ribosomal RNA (*ssu* rRNA). Our *ssu* rRNA phylogeny was concordant with the phylogenies from mitochondrial genes, but the *EF-1 α* phylogeny was not concordant either with the mitochondrial phylogenies or with the *ssu* rRNA phylogeny. So both nuclear (*ssu* rRNA) and mitochondrial data indicate that there are two lineages of lice: one lineage with head lice only (H-only lineage) the other lineage with head and body lice (H+B lineage). Thus, body lice apparently evolved from just one of the two main lineages of lice. However, the date of divergence and geographical origins of the two lineages are controversial. Kittler et al. (Curr Biol 13:1414–1417, 2003; Curr Biol 14:2309, 2004) proposed that these two lineages diverged 0.77 mya, whereas Reed et al. (PLoS Biol 2:e340, 2004) proposed that they diverged 1.18 mya and suggested that one of the lineages, the H-only lineage, evolved in the New World on *Homo erectus*. We discuss this hypothesis in light of our results from *ssu* rRNA.

Keywords *Pediculus humanus capitis* · Phthiraptera · Small subunit ribosomal RNA · Phylogeny

Introduction

There have been five studies of the genetics and evolution of the head lice and body lice of humans in the last 3 years (Leo et al. 2002; Kittler et al. 2003; Yong et al. 2003; Reed et al. 2004; Leo et al. 2005). Phylogenetic analyses of the mitochondrial genes *cytb* and *nd4* (Kittler et al. 2003; Reed et al. 2004) indicate that the head lice and body lice of humans comprise two lineages: a head-lice-only lineage (H-only lineage) and a head-lice-plus-body-lice lineage (H+B lineage). This hypothesis, however, is based only on mitochondrial genes and thus may be a locus-specific phenomenon.

Further, the date of divergence of the two lineages of lice is controversial. Both Kittler et al. (2003, 2004) and Reed et al. (2004) used nucleotide sequences of mitochondrial genes from *Pediculus schaeffi*, the putative closest relative of head and body lice from humans, to root their trees and calibrate their molecular clocks. Kittler et al. (2003) proposed a divergence of these two lineages at ~0.5 mya, whereas Reed et al. (2004) proposed a divergence at 1.18 mya. Reed et al. (2004) suggested the *cytb* sequence used by Kittler et al. (2003) was not a genuine *P. schaeffi* sequence, which explained the difference in date of divergence estimates. Kittler et al. (2004) subsequently confirmed this and revised their date to ~0.77 mya, after they replaced their *cytb* sequence with that from Reed et al. (2004). Thus, the date of divergence for these two lineages of lice remains unresolved. Given their date of divergence and the geographical distribution of the two lineages of lice, Reed et al. (2004) suggested that the H-only lineage may have evolved on *Homo erectus* and then infested modern humans. We tested this hypothesis with nuclear DNA.

Two nuclear genes from these lice were investigated prior to our study: elongation factor-1 α (*EF-1 α*) (Kittler et al. 2003; Yong et al. 2003) and small subunit ribosomal RNA (*ssu* rRNA) (Yong et al. 2003; Reed et al. 2004). Yet

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the phylogenies from these genes were unrooted, or the trees were not available. We infer rooted phylogenies from *EF-1 α* and *ssu* rRNA to test the phylogeny inferred from mitochondrial genes.

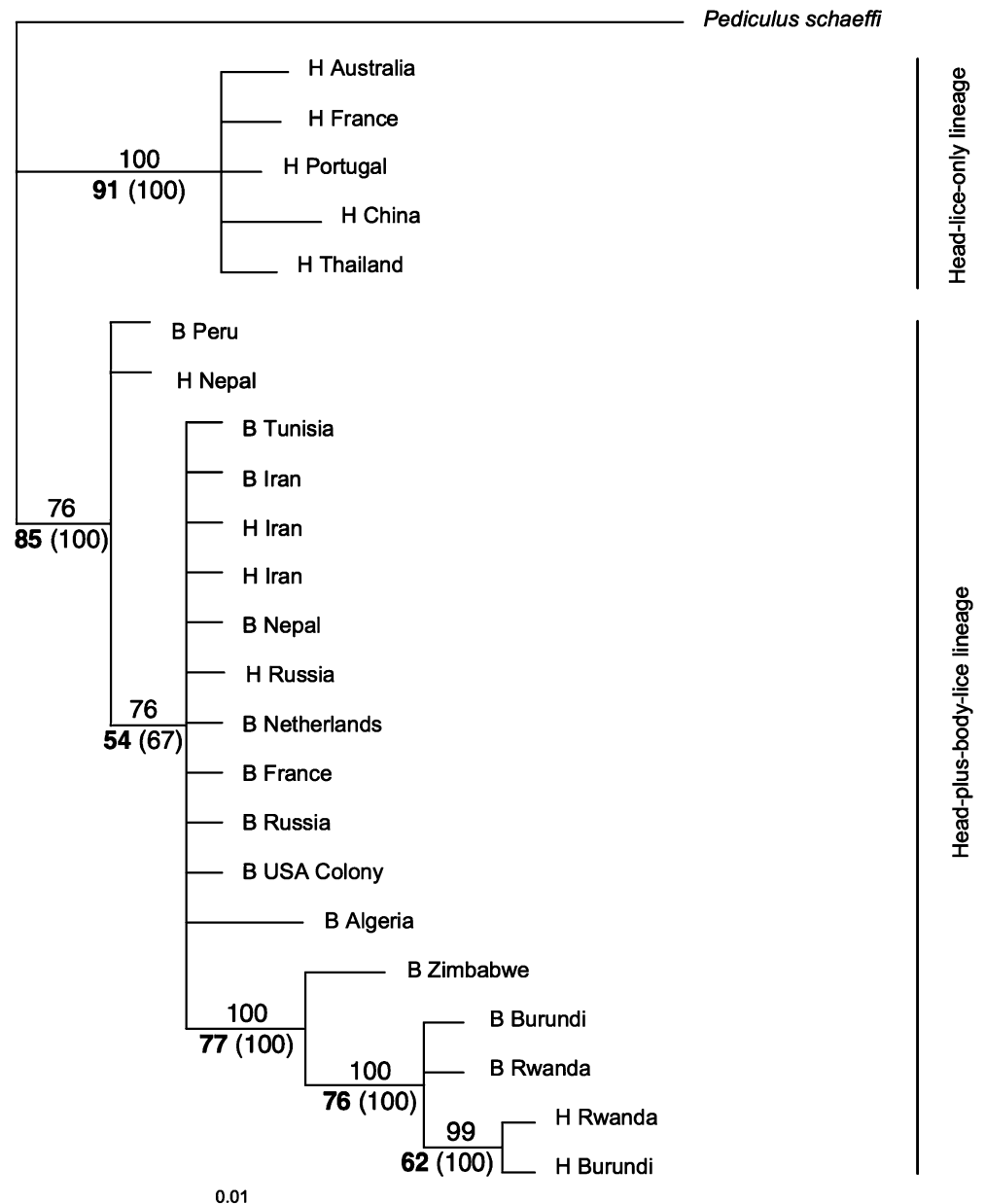
Materials and methods

ssu rRNA

We extracted DNA from whole lice as per Leo et al. (2002). We obtained partial *ssu* rRNA sequences from two head lice and one body louse from Iran, and one head louse and one body louse from Nepal (GenBank accession Nos. AY589938–AY589942). We also sequenced *P. schaeffi* ex chimpanzees, *Pan troglodytes schweinfurthii* (Barker lab reference # B1387,

GenBank accession No. AY589943), and used this sequence to root our trees. The forward primers NS1 (5' GTAGTCAT ATGCTTGTCTC 3') and NS10 (5' AGGCTCTGCAATCG GAATG 3') were used with the reverse primers NS2a (5' CGCGGCTGC TGGCACCAGACTTGC 3') and NS11 (5' GTCAAATTAAGCCGCAAGC 3'), respectively. The cycling conditions for PCR were one cycle of 95°C for 3 min; 10 cycles of 93°C for 30 s, 40°C for 30 s and 72°C for 1 min 30 s; 25 cycles of 93°C for 30 s, 50°C for 30 s and 72°C for 1 min 30 s; and one cycle of 72°C for 5 min. Eighteen partial *ssu* rRNA sequences from head and body lice from Algeria (GenBank accession No. AY236411), Australia (AY077775), Burundi (AY236413, AF139486), China (AY236417), France (AY236410, AF139478), Peru (AF139481), Portugal (AY236414), Russia (AF139484, AF139479), Rwanda (AY236415, AY236412), Thailand (AY236418), a laboratory

Fig. 1 The phylogenetic tree inferred by MRBAYES from *ssu* rRNA sequences from *P. humanus* and *P. capitis*, with *P. schaeffi* as the out-group. The probability of each clade from the Bayesian analysis (MRBAYES) is shown above branches. Below each branch, bootstrap support is shown for NJ analysis and in parentheses for MP analysis



colony kept in the United States (AF139480) and Zimbabwe (AF138482) were retrieved from GenBank.

Nucleotide sequences were aligned with Clustal X (Jeanmougin et al. 1998), and the alignments were checked by eye. Due to the large number of insertions and deletions in the 1,554-bp alignment, we used the program Gblocks v. 0.91b (Castresana 2000) to remove ambiguous sites from the alignment. The final alignment of 1,195 bp was put into MODELTEST (Posada and Crandall 1998). The model of DNA substitution used for the phylogenetic analysis in MRBAYES was number of substitution types=1; gamma distribution (shape parameter=0.0137); proportion of invariable sites=0. We executed 1,500,000 generations in MRBAYES (Huelsenbeck and Ronquist 2001), with 3,000 trees discarded as burn-in. Trees were inferred by phylogenetic analysis using parsimony (PAUP) v. 4.0b10 (Swofford 2002) with neighbour-joining (NJ) and maximum parsimony (MP) methods as for COI. Five hundred cycles of bootstrap re-sampling were used to test the robustness of branches in phylogenetic trees.

Elongation factor 1 α (*EF-1 α*) trees

We retrieved from GenBank the *EF-1 α* sequences of (Yong et al. 2003) and (Kittler et al. 2003) of *Pediculus capitis*, *Pediculus humanus* and *P. schaeffi* (GenBank accession Nos. AY239271–AY239284 and AY316794–AY316834). PAUP was used to infer NJ and MP trees. One hundred cycles of bootstrap re-sampling were used to test the robustness of branches. The model of DNA substitution generated from MODELTEST and used for the phylogenetic analysis in MRBAYES was number of substitution types=6; gamma distribution; proportion of invariable sites=0. We executed 1,500,000 generations in MRBAYES, with 3,000 trees discarded as burn-in.

Results

ssu rRNA phylogeny

The alignment of *ssu* rRNA was 1,195 bp long. Trees from Bayesian, NJ and MP (consensus tree of 12 shortest trees) analyses had the same two main lineages (only the Bayesian tree is shown, Fig. 1): an H-only lineage and an H+B lineage. The only differences among the MP, NJ and Bayesian phylogenies were some polytomies in the topology of the H-only lineage, and the NJ phylogeny had the body louse from Peru and the head louse from Nepal as sister-group to the body louse from Iran and the rest of the H+B lineage.

EF-1 α phylogeny

It was surprising that our *EF-1 α* tree had a topology that was very different to the trees inferred from *cytb*, *nd4*, *col* and *ssu* rRNA, which were concordant with each other

(Fig. 1 and Electronic Supplementary Material). Two possible explanations for the results from *EF-1 α* are that the universal primers used in PCR may have amplified a gene other than *EF-1 α* , or perhaps there has been a gene duplication in the ancestor of head and body lice that resulted in more than one gene tree for the *EF-1 α* gene. We could not eliminate either possibility since all sequences seemed to be coding sequences.

Discussion

Our phylogenies from *ssu* rRNA indicate that there are at least two main lineages of lice: an H-only lineage and an H+B lineage. This is concordant with the topologies of phylogenies inferred from the mitochondrial genes *cytb* and *nd4* (Kittler et al. 2003; Reed et al. 2004). The topology of *EF-1 α* tree was not concordant with any of the trees from *cytb*, *nd4* or *ssu* rRNA. The principle of genealogical concordance (Avice and Ball 1990) is that concordant phylogenies from independent loci are more likely to be true than a phylogeny from a single locus. Thus, from here on, we consider only the trees of the *ssu* rRNA nuclear gene and the *cytb* and *nd4* mitochondrial genes.

The phylogenies of the *cytb*, *nd4* and *ssu* rRNA genes are concordant; all had two lineages of lice; one with H-only lineage and one with H+B lineage. This indicates that body lice evolved from just one of the two lineages of lice. From the phylogenies of mitochondrial genes and *ssu* rRNA, Reed et al. (2004) hypothesised that these two lineages diverged when lice infested *H. erectus* 1.18 mya, well before modern humans evolved. The hypothesis of Reed et al. (2004) was drawn from their estimate of the date of divergence of the H-only and H+B lineages of lice, and from the regions where they found lice from the H-only lineage, Honduras and the USA, i.e. the New World. However, this argument is not supported by results from other studies. First, Kittler et al. (2004) estimated a very different date of divergence for the two lineages of lice (0.77 mya) even after they replaced their *P. schaeffi* *cytb* sequence with that of Reed et al. (2004). It is noteworthy that Reed et al. (2004) reported a mean estimate of 1.70 \pm 1.10 mya for the divergence of H-lineage and H+B lineage, which includes the 0.77 mya of Kittler et al. (2004). Second, our analyses of *ssu* rRNA (Fig. 1) and the *cytb/nd4* phylogeny of Kittler et al. (2003) show that lice from the H-only lineage occur in Europe, Asia, Africa and Australia. So, when all three studies are considered (Reed et al. 2004; Kittler et al. 2003; and the present study), the H-only lineage has a cosmopolitan distribution; it is not restricted to the New World. Since the H-only lineage is clearly cosmopolitan, the argument of Reed et al. (2004) that H-only lineage evolved on *H. erectus* in Asia is weakened (refer to Fig. 5 of Reed et al. 2004).

The cause of the divergence of the H-lineage and the H+B lineage of lice is unclear from current evidence. Both lineages have a cosmopolitan distribution, and the estimated divergence date is different even when calculated with genes from the same locus (i.e. the mitochon-

drial genome). Indeed, different mitochondrial genes have produced different dates-of-divergence for humans and chimps (Jensen-Seaman et al. 2001), the hosts of head lice, body lice and *P. schaeffi*. Preliminary analyses of our *ssu* rRNA data gave date of divergence estimates with such large standard errors that the dates were negative values (results not shown). We found that the standard error for the date of divergence of the two lineages of human lice was magnified by the error from the estimate of the human/chimp split used to calibrate the clock.

Molecular clocks are usually calibrated with dates from fossil evidence that can only give a minimum date of divergence, so these dates need to be reviewed as new evidence comes to light. For example, following the publication of Reed et al. (2004), who used the cercopithecoid–hominoid divergence date of 22.5 mya to date their human/chimp split, Steiper et al. (2004) reported the cercopithecoid–hominoid divergence date to be 29.2–34.5 mya. This new, older date was calculated using calibration points within cercopithecoids and within hominoids. Steiper et al. (2004) suggested that a poor fossil record is responsible for the previous underestimate of the cercopithecoid–hominoid date of divergence. Since the fossil record for lice is extremely poor, we must rely on the assumption of host–parasite co-evolution and host fossil records for calibration of lice molecular clocks.

To conclude, we found that the phylogeny inferred from the nuclear gene, *ssu* rRNA, was concordant with the phylogeny from the mitochondrial genes *cytb* and *nd4* (Kittler et al. 2003; Reed et al. 2004), but that the phylogeny from nuclear gene, *EF-1 α* , was not concordant with either the mitochondrial phylogenies or the *ssu* rRNA phylogeny. Thus, information from two independent loci (nuclear *ssu* rRNA and mitochondrial genes) indicates that there are at least two main lineages of lice: one lineage has head lice only and a geographic distribution that includes the Americas, Europe, Asia and Australia; the other lineage has both head lice and body lice and a worldwide distribution. This, and uncertainty over the date of divergence for the two lineages of lice, weakens the argument that the H-only lineage evolved on *H. erectus*. A precise date of divergence for the two lineages of lice will be difficult to obtain so long as host divergence dates are still debated. As more genes are analysed, and more reliable dates of divergence for host species become available, we can look forward to better estimates of divergences in lice. Until then, it seems we have only just scratched the surface of the evolutionary history of these lice.

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