

## GEOGRAPHIC DISTRIBUTIONS AND ORIGINS OF HUMAN HEAD LICE (*PEDICULUS HUMANUS CAPITIS*) BASED ON MITOCHONDRIAL DATA

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**ABSTRACT:** Human head lice (*Pediculus humanus capitis*) are subdivided into 3 deeply divergent mitochondrial clades (Clades A, B, and C), each having unique geographical distributions. Determining the evolutionary history and geographic distribution of these mitochondrial clades can elucidate the evolutionary history of the lice as well as their human hosts. Previous data suggest that lice belonging to mitochondrial Clade B may have originated in North America or Asia; however, geographic sampling and sample sizes have been limited. With newly collected lice, we calculate the relative frequency, geographic distribution, and genetic diversity of louse mitochondrial clades to determine the geographic origin of lice belonging to Clade B. In agreement with previous studies, genetic diversity data support a North American origin of Clade B lice. It is likely that lice belonging to this mitochondrial clade recently migrated to other geographic localities, e.g., Europe and Australia, and, if not already present, may disperse further to occupy all geographic regions.

Modern humans dispersed from Africa within the last 80,000 yr, and eventually spread out to occupy all inhabitable landmasses (Forster, 2004). A diverse assemblage of parasites, mutualists, and commensals has accompanied humans around the globe. For example, human head and body lice (*Pediculus humanus*; Phthiraptera: Anoplura) have parasitized their hosts for millions of years (Mumcuoglu and Zias, 1988; Araújo et al., 2000; Reed et al., 2004), and they currently infest millions of people worldwide. Lice, just like their human hosts, can be characterized into several distinct lineages based on mitochondrial DNA (Forster, 2004; Manwaring et al., 2006). Head and body lice can be differentiated into 3 deeply divergent mitochondrial clades, each having unique geographic distributions (Reed et al., 2004; Light et al., 2008; Raoult et al., 2008). Clade A consists of a worldwide distribution of both head and body lice; Clade B consists only of head lice from North America, Central America, Australia, and Europe; and Clade C contains only head lice from Africa and Nepal (Reed et al., 2004; Light et al., 2008; Raoult et al., 2008). Clades A, B, and C are deeply divergent mitochondrial lineages dating back 2 million yr. When mitochondrial and nuclear markers are sequenced for a given group of individual lice, the 3 mitochondrial clades are not recovered by the nuclear genes, likely due to recent recombination in these markers (Light et al., 2008). Thus, the mitochondrial genes, lacking recombination, provide a long-term history of louse evolution that will be obscured in nuclear markers. Additionally, gene flow among Clades A, B, and C is negligible (M. A. Troups, unpubl. obs.), and genetic divergence among these 3 mitochondrial clades is relatively high (Reed et al., 2004; Light et al., 2008; Raoult et al., 2008). The presence of deeply divergent mitochondrial clades is possibly the result of retained ancestral polymorphism, multiple colonization events of lice on their modern human hosts from now extinct archaic hominids (Creer et al., 2001; Reed et al., 2004; Light

et al., 2008), and/or multiple modern human and parasite migration events (Araújo et al., 2008), or a combination.

The evolutionary history and geographic distribution of these mitochondrial clades can give us clues regarding the evolutionary history of the lice as well as their human hosts. Current geographic distributions, as well as phylogenetic and population genetic analyses of the 3 mitochondrial lineages, indicate that lice belonging to Clade B may have originated in North America (Raoult et al., 2008). The exact source of Clade B lice before their diversification is unknown, but it could be Asian resulting from a recent host switch from ancient to modern humans (Reed et al., 2004). However, present mitochondrial clade sizes are generally small and geographic sampling has been limited. Therefore, to better understand the origin of Clade B lice, as well as the evolutionary histories of both lice and humans, it is imperative to sample lice worldwide to determine the number and geographic distribution of each mitochondrial clade.

Herein, we examine lice collected from new geographic localities to determine the relative frequency, geographic distribution, and genetic diversity of louse mitochondrial clades (specifically, the mitochondrial cytochrome *c* oxidase subunit I gene [COI], the most prevalent louse mitochondrial marker available on public databases such as GenBank), and we use these data to determine the geographic origin of Clade B lice. Unlike previous studies that have focused on differentiating human head and body lice (Leo et al., 2002, 2005; Kittler et al., 2003; Yong et al., 2003; Leo and Barker, 2005; Light et al., 2008), we restrict our study to head lice (*P. h. capitis*) because body lice are genetically indistinguishable from head lice (Light et al., 2008) and because head lice encompass the full genetic diversity of the species, whereas body lice do not.

### MATERIALS AND METHODS

#### Data collection

Louse specimens obtained through scientific donations were preserved in 95% ethanol and stored at  $-80^{\circ}\text{C}$ . DNA was isolated from louse specimens using the DNAeasy tissue kit (QIAGEN, Valencia, California) according to louse-specific protocols (Cruickshank et al., 2001; Johnson and Clayton, 2003) that enabled extraction of whole genomic DNA from each louse while retaining the entire louse body as a voucher specimen. Polymerase chain reaction (PCR) amplification of a portion

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of mitochondrial COI (378 bp) was performed using the primers L6625 and H7005 (Hafner et al., 1994) as described in Reed et al. (2004). Additionally, a longer fragment of mitochondrial COI (879 bp) was amplified for a smaller subset of lice using the primers L1718 (Reed et al., 2004) and H7005 (Hafner et al., 1994) as described in Reed et al. (2007). Amplified fragments were purified using ExoSAP-IT (USB Corporation, Cleveland, Ohio), and all sequencing reactions were performed at the University of Florida DNA Sequencing Core Laboratory (Gainesville, Florida) using ABI Prism BigDye Terminator cycle sequencing protocols (Applied Biosystems, Foster City, California). Excess dye-labeled terminators were removed by ethanol precipitation and purified products were dried using SpeedVac® (Thermo Fisher Scientific, Waltham, Massachusetts) and suspended in Hi-Di formamide (Applied Biosystems). Sequencing reactions were performed using POP-7 sieving matrix on 50-cm capillaries in an ABI Prism® 3730 genetic analyzer (Applied Biosystems) and analyzed by ABI Sequencing Analysis software version 5.2 and KB Basecaller.

### Data analysis

The shorter 378-bp mitochondrial COI fragment was sequenced only in 1 direction for the majority of the louse specimens ( $n = 106$ ) and was used to determine mitochondrial clade membership only. Sequences were edited using Sequencher version 4.2.2 (Gene Codes Corporation, Ann Arbor, Michigan) and aligned by eye to a subset of published data that included representatives of each of the mitochondrial clades. For each single-stranded sequence, clade membership was assessed using Basic Local Alignment Search Tool (BLAST) searches on GenBank. Additionally, following Light et al. (2008), a simple distance-based neighbor-joining (NJ) analysis was performed using PAUP\*4.0b10 (Swofford, 2003) to verify clade membership based on phylogenetic groupings. Clades A, B, and C are highly genetically divergent (Reed et al., 2004; Light et al., 2008); therefore, assignment of clade membership using phylogenetic analysis is unambiguous.

For more rigorous phylogenetic and population genetic analyses, the longer 879-bp fragment was sequenced in both directions, edited using Sequencher version 4.2.2, aligned by eye, trimmed in reference to translated protein sequence, and deposited in GenBank (EU493361–EU493447). One outgroup taxon (*Pediculus schaeffi*) and additional head louse data for the mitochondrial COI gene were downloaded from GenBank (AY695999 and EF152558, AY695940–AY695957, AY695975–AY695998, AY316748–AY316752, AY239288, AY589982–AY590041, respectively) and aligned to the sequences obtained via sequencing. The double-stranded and downloaded sequences were subjected to a NJ analysis using PAUP\*4.0b10 (Swofford, 2003) to determine mitochondrial clade membership. Because sequences obtained on GenBank are variable in length, NJ analyses were performed both including and excluding missing data and redundant haplotypes. Nodal support was assessed using nonparametric bootstrap analyses (1,000 NJ pseudoreplicates; Felsenstein, 1985). All executable files are available on TreeBase (<http://www.treebase.org>; study accession number S2056). In addition to phylogenetic analyses, simple population genetic summary statistics and haplotype networks were estimated for all double-stranded louse samples. Standard genetic diversity parameters such as number of haplotypes ( $h$ ), haplotype diversity ( $H_d$ ; Nei, 1987), and nucleotide diversity ( $\pi$ ; Tajima, 1983) were calculated using DnaSP software version 4.10.9 (Rozas et al., 2003). Historical relationships among haplotypes, i.e., genealogies, were reconstructed excluding missing data using statistical parsimony (implemented in TCS version 1.6; Clement et al., 2000). Statistical parsimony assembles the most parsimonious haplotype tree, with linkages between taxa representing mutational events, and estimates a 95% confidence limit of the reliability of those linkages.

## RESULTS

Molecular data from the mitochondrial COI gene were collected from 193 head lice (87 of which were sequenced in both directions) and examined in combination with 109 louse samples available in GenBank. In total, 27 countries were represented by these 302 louse specimens (Table I). NJ analyses of double-stranded sequences (see below) and NJ analyses and

TABLE I. Geographic distribution (by country) of human head lice (*Pediculus humanus*) based on mitochondrial sequence data from the mitochondrial COI gene. Numbers include newly sequenced data from this study as well as all data available on GenBank.

Geographic area	Mitochondrial clade			
	A	B	C	Total
North America				
United States*	44	62	0	106
Total	44	62	0	106
Europe				
England	9	45	0	54
France	1	1	0	2
Germany	0	1	0	1
Norway	2	1	0	3
Portugal	0	1	0	1
Total	12	49	0	61
Central America				
Honduras	3	5	0	8
Panama	2	0	0	2
Total	5	5	0	10
South America				
Argentina†	28	0	0	28
Brazil	2	0	0	2
Colombia	2	0	0	2
Ecuador	3	0	0	3
Total	35	0	0	35
Asia				
China	12	0	0	12
Mongolia	1	0	0	1
Total	13	0	0	13
Africa				
Burundi	5	0	0	5
Ethiopia	0	0	1	1
Kenya	1	0	0	1
Uganda	1	0	0	1
Total	7	0	1	8
Australasia				
Australia	4	6	0	10
Papua New Guinea	10	0	0	10
New Zealand	1	0	0	1
Total	15	6	0	21
Middle East				
Iran	2	0	0	2
Israel	2	0	0	2
Total	4	0	0	4
Southeast Asia				
Philippines	14	0	0	14
Nepal	22	0	3	25
Thailand	3	0	0	3
Total	39	0	3	42
Oceania				
Cook Islands	2	0	0	2
Total	2	0	0	2

\* Sampling in the United States occurred primarily in Florida and Utah.

† Sampling in Argentina included multiple lice from the same 8 hosts.

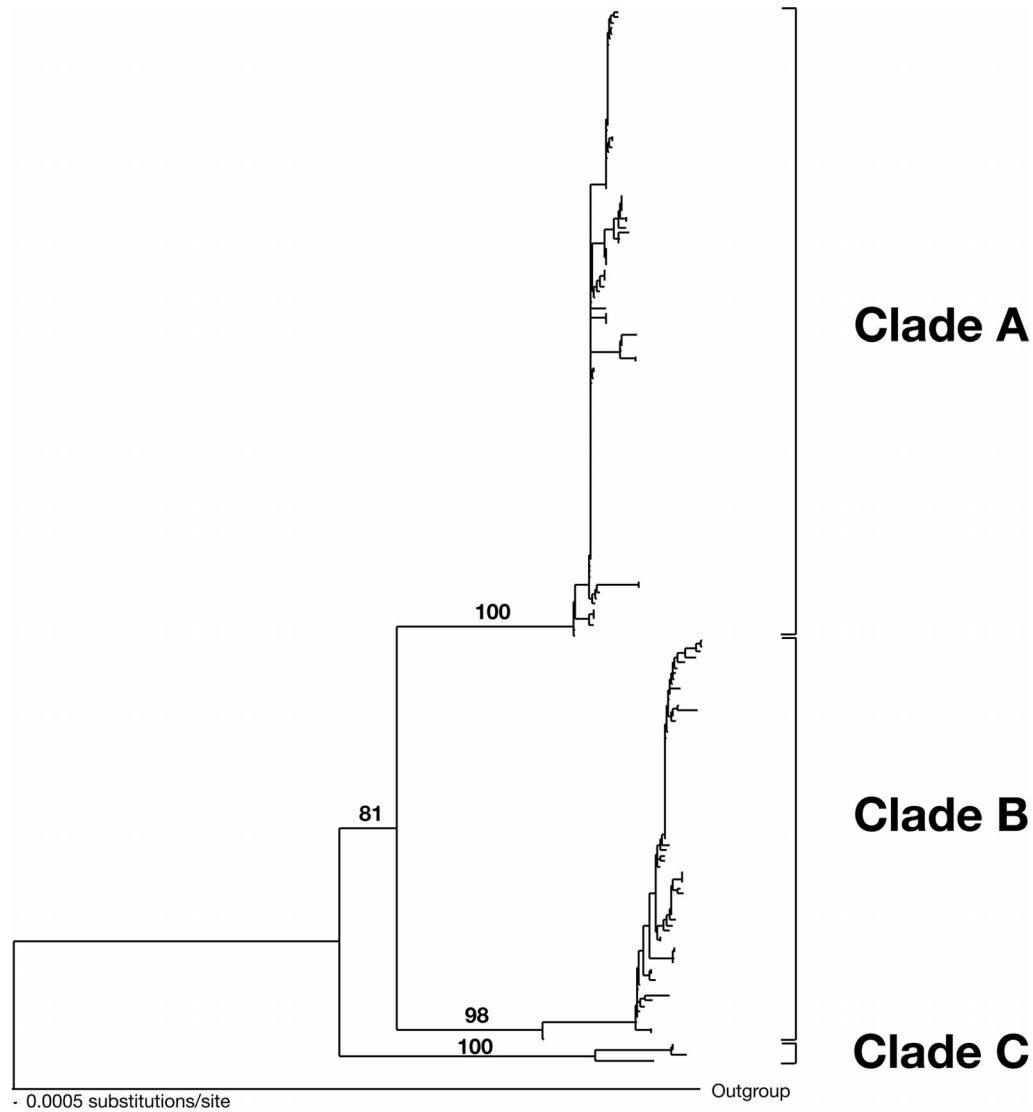


FIG. 1. NJ phylogram resulting from analysis of the double-stranded mitochondrial COI data available on GenBank and collected as part of this study. Bootstrap support values greater than 75 (based on 1,000 NJ bootstrap replicates) are located above the nodes. Mitochondrial clade memberships are indicated to the right of each tree. Head and body lice belonging to Clade A are distributed worldwide; head lice belonging to Clade B are restricted to North and Central America, Europe, and Australia; and head lice belonging to Clade C are restricted to Ethiopia and Nepal.

BLAST searches of single-stranded sequences revealed that lice belonging to Clade A were distributed worldwide and were present in almost all countries examined. Lice belonging to Clade B were most prevalent in the United States (50.8%) and the U.K. (40.2%), although lice from Clade B also were present in several other European countries, Central America, and Australia (Table I). No additional lice belonging to Clade C were found as part of this study; therefore, the geographic distribution of this clade remains restricted to Africa (specifically Ethiopia) and Nepal.

More rigorous analyses of double-stranded samples resulted in 3 distinct and well supported mitochondrial clades (Clades A, B, and C) with lice belonging to Clade C representing the most divergent lineage (Fig. 1). Excluding missing data and redundant haplotypes, genetic divergences within each clade

were small, ranging from 0.538 to 1.502% (uncorrected  $p$  distances), with the highest divergences found within Clade C. Genetic divergences among clades ranged between 5.78 and 7.87%, with the highest divergences observed with comparisons to lice belonging to Clade C. In agreement with phylogenetic analyses, network analyses resulted in 24 haplotypes differentiated into three unconnected subnetworks groups corresponding to Clades A, B, and C (Fig. 2). Clade A and B subnetworks were starlike in structure, with the most prevalent haplotype in the center and distributed across all geographic regions. In general, few mutational steps were present among haplotypes and less prevalent haplotypes, when represented by more than 1 louse, tended to consist of lice from the same geographic region (Fig. 2). Estimates of genetic diversity for the 196 double-stranded sequences of head lice were generally high ( $h = 24$ ,

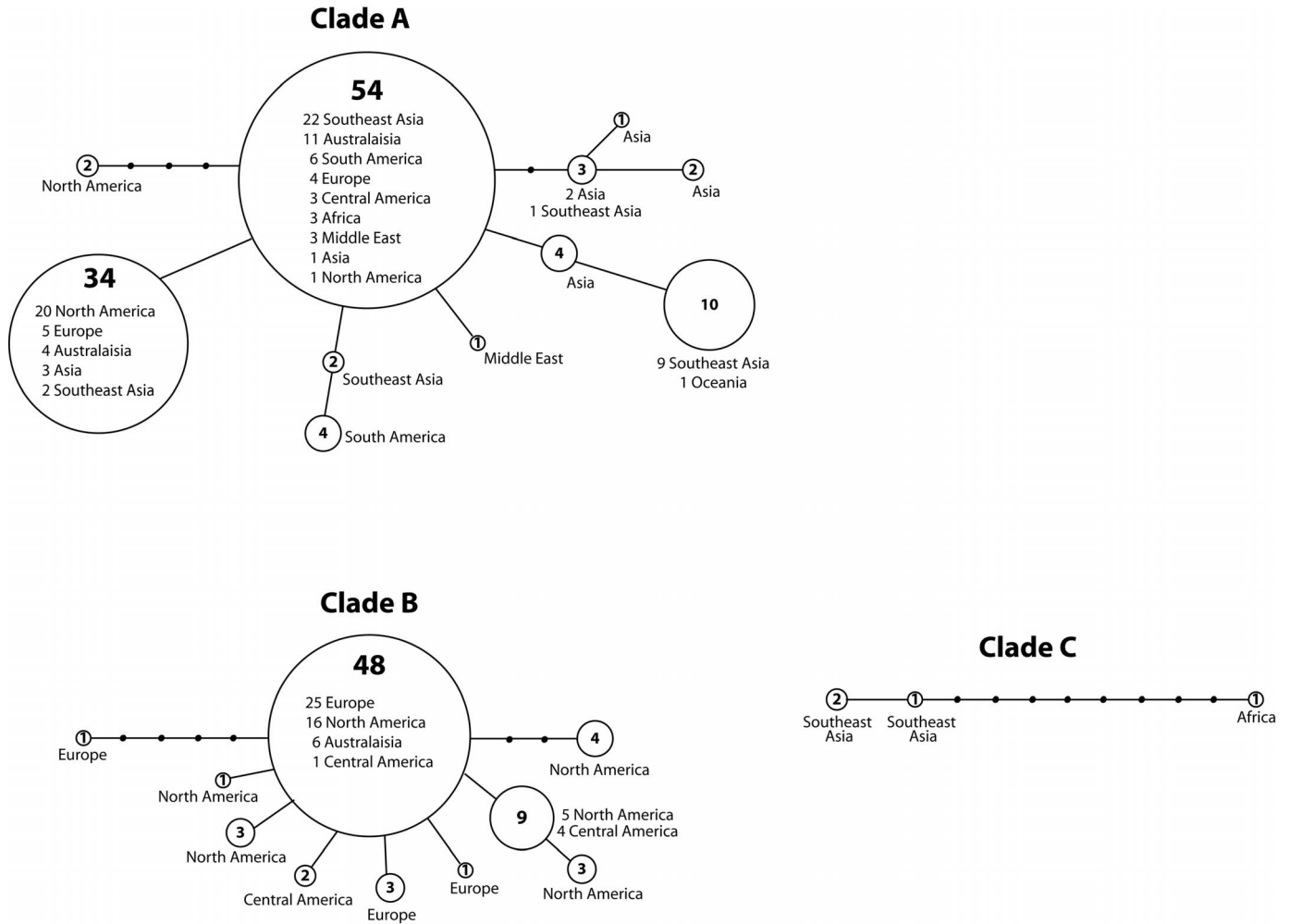


FIG. 2. Statistical parsimony networks for mitochondrial COI Clades A, B, and C. Each connection represents a single mutational step with inferred haplotypes represented by small black circles. Observed haplotypes are shown as large circles with haplotype frequency indicated within the circles. For each haplotype, geographic distribution and frequency is indicated.

Hd = 0.831, and  $\pi = 0.031$ ), with the highest diversity found within Clade C ( $h = 3$ , Hd = 0.833, and  $\pi = 0.01126$ ) compared with Clades A ( $h = 9$ , Hd = 0.563, and  $\pi = 0.0021$ ) and B ( $h = 11$ , Hd = 0.697, and  $\pi = 0.00305$ ).

**DISCUSSION**

Ancient and recent events in human evolutionary history are often poorly understood due to a lack of adequate fossil and molecular data. Host-specific and fast-evolving parasites represent independent markers that can be used to infer human evolutionary history (Ho et al., 1993; Ashford, 2000; Hoberg et al., 2001; Reed et al., 2004; Pavesi, 2005; Wirth et al., 2005; Yogo et al., 2005; Caufield et al., 2007; Holmes, 2007; Linz et al., 2007; Szmargd and Balloux, 2007; Araújo et al., 2008; Kitchen et al., 2008). Human head lice may provide important information regarding human evolutionary history because these lice are fast-evolving insects (Reed et al., 2004) and have cospeciated with their hosts over millions of years (Reed et al., 2004, 2007). Therefore, louse molecular data collected here, as well as data collected with continued worldwide sampling of

head lice, will be invaluable for elucidating the evolutionary history of both lice and their human hosts.

Human head lice, in general, are genetically diverse and occur in 3 deeply divergent mitochondrial clades (Figs. 1, 2). This high genetic diversity facilitates the determination of probable geographic origins for each clade and, for our interests, Clade B. Previous findings have suggested a North American and/or Asian origin of Clade B lice (Reed et al., 2004; Raoult et al., 2008). Although our current sampling does not allow an examination of an Asian origin for this mitochondrial clade (Table I), we can assess a North American origin of Clade B lice using both haplotype frequency and genetic data. Using haplotype frequency data, we expect that the region where Clade B lice are most prevalent (compared with lice belonging to the other mitochondrial clades) represents the geographic origin of Clade B (Donnelly and Tavaré, 1986). Clade B lice characterize the most prevalent louse lineage in both North America and Europe (Table I). In North America, however, the proportion of Clade B lice is not significantly higher than lice belonging to Clade A ( $\chi^2 = 3.06$ ,  $df = 1$ ,  $P > 0.05$ ). Alternatively, in Europe there

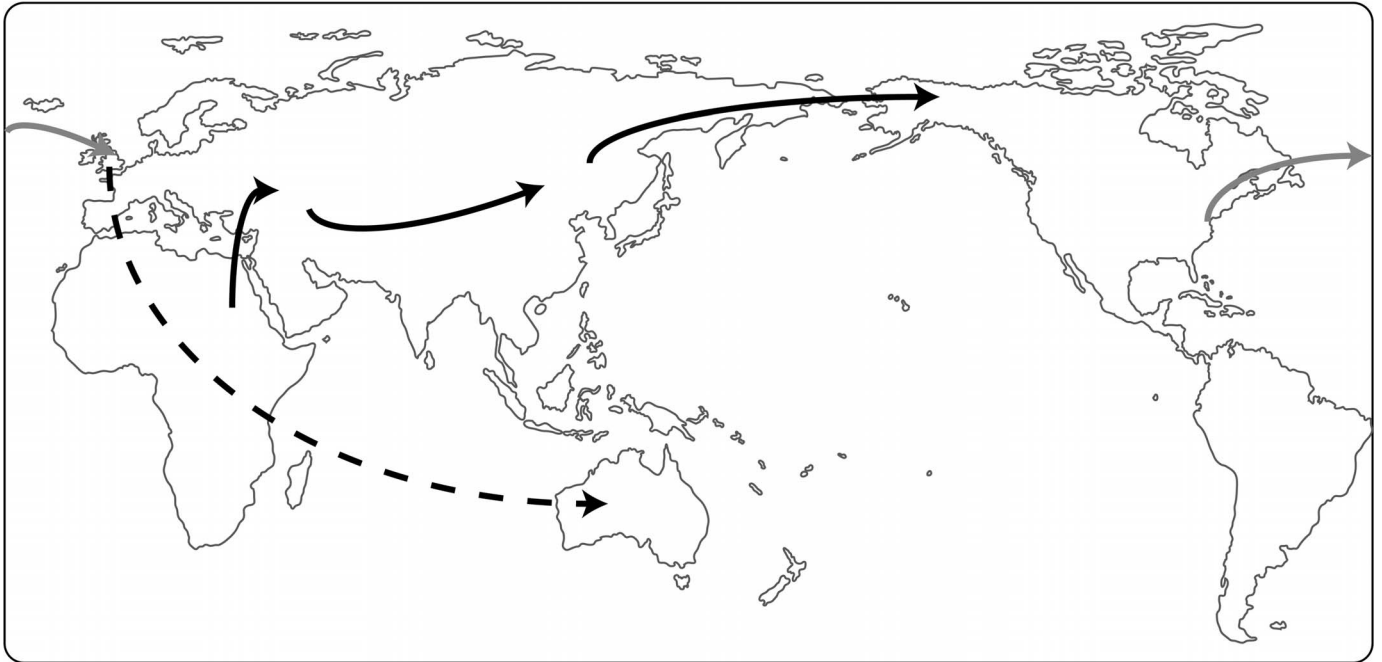


FIG. 3. Proposed migration route of Clade B lice according to a North American origin of the lice collected in this study. Lice may have migrated from Africa to Asia and then subsequently to the New World, perhaps with the first peoples of the Americas where they remained in situ for thousands of years (black arrows). European colonists returning to the Old World from the Americas would have taken Clade B lice back to Europe beginning in the 16th Century (gray arrow). European colonization of Australia roughly 150 yr later may account for the presence of Clade B lice in Australia (dashed arrows) and potentially other geographic regions as well.

are significantly more Clade B lice relative to Clade A ( $\chi^2 = 22.4$ ,  $df = 1$ ,  $P < 0.05$ ), thus indicating a European, rather than a North American, origin of Clade B.

These frequency data, however, are potentially misleading because sample sizes are not equal among the various geographic regions, e.g., 108 lice in North America compared with 61 lice in Europe. Furthermore, the frequency of mitochondrial haplotypes can vary among geographic regions through genetic drift, migration, and other evolutionary forces. Therefore, it may be preferable to use genetic diversity to detect signatures of geographic origination, expecting to see higher diversity in the source population provided the population size has remained high enough to maintain the diversity (Relethford, 2001). Within North America, average genetic diversity of Clade B is 0.37% (uncorrected  $p$  distance), whereas in Europe, average diversity is 0.21%. Genetic variation is, therefore, 1.76 times greater in North America than Europe. Number of haplotypes, haplotype diversity, and nucleotide diversity are significantly higher in Clade B lice found in North America compared with Europe (DnaSP  $\chi^2 = 22.029$ ,  $0.05 < P < 0.01$ ;  $h = 6$ ,  $Hd = 0.701$ , and  $\pi = 0.00324$  in North America;  $h = 4$ ,  $Hd = 0.313$ , and  $\pi = 0.00136$  in Europe). The haplotype network for Clade B also visually indicates greater genetic diversity within North America than Europe (Fig. 2). In North America, there are 16 lice distributed among 5 haplotypes (unique to North America), whereas there are only 5 lice and 3 haplotypes unique to Europe (Fig. 2). Genetic diversity estimates are, therefore, in agreement with haplotype frequency data as well as previous studies supporting a North American origin of Clade B lice. It is likely that lice belonging to both mitochon-

drial Clades A and B were present in North America before the first Europeans arrived (Raoult et al., 20008; this study), a common finding among human parasites (Araújo et al., 2008). Furthermore, Clade B lice likely migrated to other geographic localities, e.g., Europe and Australia, relatively recently (Raoult et al., 2008), and, if not already present, may disperse further to occupy all geographic regions in time (Fig. 3). Our data show that the origin of the living population of Clade B is North American; however, given the past migration patterns of their human hosts, Clade B lice certainly did not arise in this geographic region. The ultimate origin of Clade B lice remains unknown because these lice have never been found in Asia or any other region reputed to have led to the peopling of the Americas. Further sampling from Native Americans and Asians (especially those in Mongolia and Siberia; Torroni et al., 1993; Kolman et al., 1996), as well as additional phylogenetic analyses and estimates of divergence times, will therefore be necessary to determine when and where Clade B lice ultimately originated.

The overall level of mitochondrial diversity observed in head lice seems to be appropriate for tracking older events in human history. For example, the high genetic diversity found within Clade C is consistent with the idea that lice belonging to this clade are ancestral to lice of the other mitochondrial clades, supporting an African or Asian origin of lice and their human hosts (Relethford, 2001). Furthermore, despite a long history of coevolution that would predict similar clade ages for hosts and parasites, previous analyses of louse mitochondrial data have shown that the age of the *P. humanus* is an order of magnitude older than its modern human host (Reed et al., 2004). The pres-

ence of geographically isolated mitochondrial clades within *P. humanus* could possibly be the result of multiple colonization events of lice on their modern human hosts from now extinct archaic hominids, i.e., an Asian origin of Clade B lice (Creer et al., 2001; Reed et al., 2004) and/or multiple modern human and parasite migration events (Araújo et al., 2008). Additional studies of louse mitochondrial markers could shed light on the origin and timing of the symbiotic association between lice and humans, as well as possibly track human and louse migration patterns out of Africa. To address more recent population-level questions, such as the peopling of the Americas, faster molecular markers, such as microsatellites, single-nucleotide polymorphisms, and SNPSTRs (single nucleotide polymorphisms adjacent to a short tandem repeat), will likely be necessary (Light et al., 2008; Reed et al., 2008) and are now being developed as a result of the sequencing of the human louse genome (Pittendrigh et al., 2006).

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