USING POPULATION GENETICS OF HUMAN HEAD AND CLOTHING LICE TO ELUCIDATE HUMAN EVOLUTION

By

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To my family.

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Parasites are often used as proxies for investigating their host evolution. This is particularly common in the study of human evolution. In this study, I use the genetics of human head and clothing lice to address the origin of clothing use in humans and develop new microsatellite loci to further the study of louse population genetics.

Parasitic head and clothing use may be used to address the origin of clothing use because the divergence between these taxa is indicative of a minimum age of clothing use. When humans lost their body hair 1.2 million years ago, lice that once roamed the entire body were isolated on the head, and recolonized the body with the advent of clothing use. Coalescent analyses of multilocus data indicate that head and clothing lice diverged between 650,000 and 700,000 years ago, indicating that clothing was an innovation of archaic hominids, not modern humans as previously thought. This is consistent with several other lines of evidence for an archaic origin of clothing, such as the presence of archaic hominids in high latitudes during this time period and the appearance of tools used to scrape hide 300,000 years ago. Furthermore, our analyses indicate that only a small proportion of head lice initially colonized clothing.

Finally, I developed eleven polymorphic microsatellite loci for head and clothing lice. All loci were tested on eighteen head louse individuals, and the number of alleles ranged from three to seven. These loci will be valuable for further study of louse population genetics.

CHAPTER 1
THE NATURAL HISTORY OF THE HUMAN HEAD AND CLOTHING LOUSE, *Pediculus humanus*

Introduction

At least half of the organisms that exist today are parasitic (Price 1980). The parasitic lifestyle has been hypothesized to be a key innovation leading to the diversification of insects within several different orders (Farrell 1998; Johnson et al. 2004; Whitfield 1998). The insect order Phthiraptera consists of parasitic lice, which are permanent, obligate ectoparasites of vertebrates. Phylogenetic analysis of Phthiraptera demonstrates that this order is polyphyletic and indicates that there are two origins of parasitic lice (Johnson et al. 2004). One origin of parasitism unites all members of one suborder of chewing lice, Amblycera, and the second origin of parasitism unites two suborders of chewing lice, Ischnocera and Rhynchophthirina, with the suborder of sucking lice, Anoplura.

Anoplurans spend their entire life cycle on their mammalian hosts and cannot survive without their host for more than 24 to 36 hours. Fertilized eggs of sucking lice are referred to as nits, which subsequently develop through three nymph stages (1^{st}, 2^{nd}, and 3^{rd} instar) before achieving adulthood. Sucking lice are hematophagous and contain endosymbiotic bacteria in specialized mycetome cells (Allen et al. 2007; Perotti et al. 2007; Sasaki-Fukatsu et al. 2006) that supplement the louse's diet with essential vitamins (Buchner 1965). Removal of endosymbionts from a louse or from louse eggs results in louse and larval mortality (Aschner 1932).

Parasitism of vertebrates requires a variety of adaptations to meet the challenges of remaining attached to the host, feeding on the host, and escaping the host defenses (Clayton and Johnson 2003). For example, lice have a single tarsal claw on each leg that clings to host hair. Anoplurans also have specialized mouthparts to suck blood from their mammalian hosts. Such morphological, behavioral, and physiological specializations often restrict each species of

parasitic louse to a single host species (Price et al. 2003). This often results in cospeciation between louse and host, such as the twenty-five million year coevolution between primates and their sucking lice (Reed et al. 2007; Reed et al. 2004).

Members of two louse families parasitize humans. The family Phthiridae includes the human pubic louse, *Pthirus pubis*, and its sister taxon, the gorilla louse, *Pthirus gorillae*. *P. pubis* likely switched from a gorilla-like host to an archaic hominid approximately 3 million years ago (Reed et al. 2007). The family Pediculidae includes the species *Pediculus humanus*, which exists as two morphotypes, the human head louse and human clothing louse, *Pediculus humanus capitis* and *P. humanus humanus*, respectively. *Pediculus humanus* is thought to have diverged from chimpanzee lice, *Pediculus schaeffi*, at the chimp-human divergence 5-7 million years ago.

Ecology of *P. humanus*

Human head and clothing lice are found on every continent. Head lice are common worldwide, infesting millions of school children every year. This resistance is due, in part, to the evolution of resistance to insecticidal shampoos that are used to treat pediculosis (Burgess 1995). Clothing lice are less prevalent, but are potentially more harmful because they are known vectors of at least three bacterial pathogens in humans: *Rickettsia prowazekii* (epidemic or louse- borne typhus; but see Robinson et al. 2003), *Borrelia recurrentis* (louse-borne relapsing fever) and *Bartonella quintana* (trench fever; Buxton 1946; but see Sasaki et al. 2006). The incidence of clothing lice is determined by weather, humidity, poverty, and a lack of hygienic conditions (Raoult and Roux 1999). Clothing lice, and consequently their bacterial pathogens, are most common in areas with cold weather, where people wear multiple layers of clothes, and in areas stricken by poverty or political and social unrest that prevent inhabitants from having multiple sets of clothes (Raoult and Roux 1999). They are also becoming increasingly common among

the homeless populations in developed countries in the United States and Western Europe (Brouqui et al. 1996; Drancourt et al. 1995; Koehler et al. 1997; Raoult and Roux 1999).

As their names imply, head and clothing lice are spatially segregated on their human hosts. Head lice are found on the head and attach their eggs to the base of hair shafts, most often behind the ears, whereas clothing lice are found on the body and in clothing and prefer to attach their eggs to the pleats and seams of clothing, particularly the undergarments, rather than body hair (Burgess 1995; Buxton 1946; Maunder 1982; Nuttall 1917). In the absence of clothing, clothing lice will infest beads and necklaces (Buxton 1946; Busvine 1978). Clothing lice are believed to have evolved from head lice, invading the body region only recently with the advent of clothing use in modern humans (Burgess 1995; Kittler et al. 2003; Kittler et al. 2004). Head lice require more frequent blood meals than do clothing lice (Alpatov and Nastjukova 1955). Head and clothing lice tend to return to their preferred ecological habitat when displaced, and clothing lice are known to be especially attracted to areas that are occupied by other clothing lice or clothing louse feces (Wigglesworth 1941; Mumcuoglu et al. 1986). Morphologically, clothing lice (and their eggs) are generally larger than head lice, most notably in the length of the tibia on the second pair of legs (Schöll, 1955; Busvine 1978; Reed et al. 2004). However, these morphological differences are small, and were apparent to Reed et al. (2004) only when assessed with discriminant function analysis.

Taxonomic Debate

The species status of human *Pediculus* has been a topic for debate for over a century. Ferris (1951) and Burgess (1995) provide detailed accounts of this taxonomic confusion. Nuttall (1917, 1919a, 1919b, 1920) and Ferris (1935) strongly argued that head and clothing lice represented, at most, subspecies, and that the morphological, behavioral, and ecological differences between these two louse groups represented natural intraspecific variation. Buxton

(1946) presented an argument that head and clothing lice are probably too similar to be considered distinct species, but may represent "species in the making." Fahrenholz (1915, 1916), Busvine (1944, 1978), and Schaefer (1978), on the other hand, argued that the differences observed between head and clothing lice were more than sufficient to recognize these taxa as distinct species. This taxonomic confusion regarding the species status of head and clothing lice continues today, primarily because of the ecological differences.

In recent years, the specific status of head and clothing lice has also been addressed in studies examining louse isozymes (Amevigbe et al. 2000), louse primary endosymbionts (Allen et al. 2007; Perotti et al. 2007; Sasaki-Fukatsu et al. 2006), and louse bacterial pathogens (Sasaki et al. 2006; but see Parola et al. 2006). While the results of the endosymbiont and bacteria work indicate that head and clothing lice are conspecific, the results of the isozyme work indicate that genetic differentiation may exist between these louse forms. There also has been a series of DNA-based studies that have directly addressed the specific status of head and clothing lice (Kittler et al. 2003; Leo and Barker 2005; Leo et al. 2002; Reed et al. 2004; Yong et al. 2003). The majority of these molecular-based studies have concluded that head and clothing lice are not distinct species because of the lack of reciprocal monophyly between head and clothing lice.

Reed et al. (2004) found three deeply divergent mitochondrial clades of lice, all of which contained head lice, whereas only one of which contained clothing lice. Furthermore, all three clades had unique geographic distributions: 1) one clade was composed of a worldwide distribution of both head and clothing lice, 2) one clade consisted only of head lice from North America, Central America, Australia, and Europe, and 3) a second clade that contained only head lice from Africa and Nepal (further sampling is necessary to determine the geographic range of lice belonging to this third mitochondrial lineage). These mitochondrial results, the presence of

three deeply divergent clades and the finding that clothing lice arose from only a subset of head lice, were novel compared to previous studies based on both mitochondrial and nuclear data (Kittler et al. 2003; Leo et al. 2002; Yong et al. 2003) and resulted in several follow-up studies examining these results (Leo and Barker 2005; Light et al. 2008).

Light et al. (2008) assessed the taxonomic status of head and clothing lice using both phylogenetic and population genetic methods and the most diverse geographic and molecular sampling available. The analyses resulted in reticulated networks, gene flow, and a lack of reciprocal monophyly, all of which indicate that head and clothing lice do not represent genetically distinct evolutionary units. Based on these findings, as well as inconsistencies of morphological, behavioral, and ecological variability between head and clothing lice, Light et al. (2008) state that no known species concept would recognize head and clothing lice as separate species.

Medical Importance of *P. humanus*

Parasitism by lice is a recognized medical condition known as pediculosis. Most patients are infested by only a few individuals (Raoult and Roux 1999), however, some patients can be infested with up to hundreds or thousands of lice (Raoult and Roux 1999). When the louse punctures the host's skin to feed, it injects the host with an anticoagulant and an anesthetic (Burgess 1995). After three to four weeks of infection, the host begins to have an allergic reaction, which leads to pruritis. In a clothing louse infestation, areas of the host that are heavily parasitized may darken, which is referred to as vagabond's disease (Burgess 1995). Heavy infestation of lice can also cause dull headache, drowsiness, joint pain, rash, and a mild fever (Buxton 1946).

Louse Transmission

The transmission of head and clothing lice has been studied extensively (Burkhart and Burkhart 2007; Canyon et al. 2002; Takano-Lee et al. 2005). Though lice can disperse at any stage, adults are most likely to transfer from one head to another (Takano-Lee et al. 2005). Canyon et al. (2002) explored the spatial and kinetic factors of direct head-to-head contact in transmitting head lice. A variety of factors were explored, including the angle of the hairs, the directionality of hair movement in relation to the louse, and the speed at which the hair moved relative to the louse. Of the 240 lice that Canyon et al. (2002) tested, zero transferred to hairs that were placed in a 90° angle to their body. However, when lice were presented with a hair parallel to their bodies, a small proportion transferred to the new hair. The exact proportion of lice that transferred was determined by the direction the hair was moving in and the speed at which the hair was moving. Canyon et al. (2002) demonstrated that lice prefer hair that is moving laterally (as opposed to dorsal-ventral or reverse) from slow-moving (~4m/min) tail towards the head (as opposed to head to tail). Canyon et al. (2002) also observed that all lice that successfully transferred hairs first grasped it with their tarsal claw on their first leg. The precise kinetics required for lice to transfer from one hair to another indicates that transferring of lice between hosts through brief head-to-head contact is likely rare (Canyon et al. 2002). The authors also suggest that because such precise kinetics is necessary, the role of fomites (objects that may indirectly move louse from one individual to another, such as towels, brushes, etc.) in transmission may be overestimated (Canyon et al. 2002).

Takano-Lee et al. (2005) investigated the role of fomites in louse transmission. They demonstrated that air movements, combing, or toweling easily dislodges lice, and lice also passively transfer to fabric. Both Takano-Lee et al. (2005) and Burkhart and Burkhart (2007)

argue that these findings are evidence for fomite transmission. However, how dislodged lice reattach to their host is not addressed by these studies. Given the findings of Canyon et al. (2002), it seems that such specific requirements for louse transmission are unlikely to be met during the 24-36 hour period that a louse can survive off the host.

Clothing Lice as Vectors of Bacterial Pathogens

The clothing louse vectors three deadly bacterial pathogens. It is generally assumed that head lice cannot vector diseases (Burgess 2004), though head lice can vector these pathogens in a laboratory setting (Goldberger and Anderson 1912). Both *R. prowazekii*, which causes epidemic typhus, and *B. quintana*, which causes trench fever, are members of the α subgroup of Proteobacteria, and *B. recurrentis*, which causes relapsing fever, is a spirochete (Raoult and Roux 1999).

Rickettsia prowazekii

Zinsser (1935) argued that epidemic typhus is likely responsible for more deaths of soldiers than all wars in history. During the twentieth century alone, typhus outbreaks were associated with both world wars, and more recently, outbreaks of typhus have occurred in Burundi, Peru, and Russia in the 1990s (Raoult and Roux 1999). Charles Nicolle demonstrated that the clothing louse was the typhus vector in 1909 and was awarded the Nobel Prize in 1928 for this pioneering work (Gross 1996).

There are two competing hypotheses for the origin of epidemic typhus. Some researchers contend that typhus has an Old World origin (Zinsser 1935) and became established in Spain in the fifteenth century. Zinsser (1935) speculated that a number of historical epidemics, including the Athens plague, were possibly caused by typhus. However, given the generality of the recorded symptoms of these events, it impossible to definitively know if *R. prowazekii* was the responsible agent (Raoult et al. 2004). Others argue that typhus has a New World origin,

because the only known nonhuman reservoir, the flying squirrel (*Glaucomys volans volans*), is native to Mexico (Bozeman et al. 1975). Historically, this has been challenged by the lack of evidence that clothing lice successfully accompanied the Amerindians in the migration to the New World. However, a recent ancient DNA study by Raoult et al. (2008) demonstrated that head lice belonging to a pre-Columbian Peruvian mummy belonged to the same clade of lice that contains clothing lice, suggesting that clothing lice likely colonized the New World along with the Amerindians. This finding weakens the argument that clothing lice did not exist in the pre-Columbian New World. However, the first definitive evidence of typhus occurred in the fifteenth century in Spain and sixteenth century in Mexico (Raoult et al. 2004).

Rickettsia prowazekii is a pathogen to the louse as well as its human host. Clothing lice contract typhus through feeding on an infected person, though transmission rate is not 100% (Wolbach et al. 1922). Once ingested, *R. prowazekii* infects the midgut epithelial cells of the louse and multiplies (Raoult and Roux 1999). These cells become enlarged and burst, which releases the bacteria into the gut lumen (Raoult and Roux 1999). *R. prowazekii* is then excreted from the louse in large quantities in the feces (Raoult and Roux 1999). Since the ruptured epithelial cells of the gut are not replaced, infection with *R. prowazekii* eventually kills the louse (Raoult and Roux 1999). After the cells rupture, the ingested blood will flow freely through the intestine and occasionally cause the louse to turn red (Bozeman et al. 1975). Interestingly, *R. prowazekii* cannot be transmitted vertically from mother to egg (Buxton 1940; Houhamdi et al. 2002).

The salivary glands of the louse do not contain *R. prowazekii* (Arkwright and Bacot 1923), and therefore *R. prowazekii* is not transmitted to the human host through the louse bite. *R. prowazekii* is transmitted to the human host when infective feces are rubbed into open sores

(often caused by louse bites) in the host's skin or into conjunctivae or mucus membranes (Raoult and Roux 1999). Infective bacteria can persist in the louse excrement for over 100 days (Raoult and Roux 1999).

Once a person is infected with *R. prowazekii*, the bacterium spreads throughout the host's body through the bloodstream and damages the endothelial cells, compromising the host's vascular system and causing hemorrhages (Raoult and Roux 1999). During this time, the patient will likely experience fever and headaches, and may also experience a variety of other symptoms, including cough, nausea, a rash on the trunk of the body, and myalgias (Raoult and Roux 1999). Many patients develop some abnormalities of the central nervous system (Raoult and Roux 1999). Without antibiotic treatment, 10-30% of all *R. prowazekii* infections are fatal (Raoult and Roux 1999). However, a single dose of 200mg of doxycycline often cures the patient of epidemic typhus (Raoult and Roux 1999).

Borrelia recurrentis

B. recurrentis, the agent of louse-borne relapsing fever, is closely related to *B. duttonii,* the agent of East African tick-borne relapsing fever (Cutler et al. 1997; Raoult and Roux 1999). It is suspected that the ancestor of *B. recurrentis* diverged from its tick-borne relatives after it became associated with clothing lice (Raoult and Roux 1999).

Unlike *R. prowazekii, B. recurrentis* does not cause illness in its louse host. However, similar to *R. prowazekii, B. recurrentis* is not transmitted to the human host through the louse bite (Raoult and Roux 1999). Until recently, it was thought that *B. recurrentis* was only to transmitted to its human host through louse crushing. However, a recent experiment by Houhamdi and Raoult (2005) showed evidence of *B. recurrentis* in louse feces, suggesting an additional route of infection. Like *R. prowazekii, B. recurrentis* may infect its human host through infective louse feces rubbed into open sores of the host or into conjunctivae or mucus

membranes (Houhamdi and Raoult 2005).

Humans are the only host of *B. recurrentis.* Infection of *B. recurrentis* is characterized by an initial stage of fever, chills, and headaches, which is often the most severe, followed by a period of recovery, and a less severe relapse approximately a week later. This cycle continues and is caused by the cyclic antigenic response of the bacteria. Gene rearrangements within the bacterium allow new serotypes to be serially expressed. After several relapses, the genetic variation within the bacterium is often exhausted, and the immune system is able to prevent further relapses (Raoult and Roux 1999). In the absence of antibiotic treatment, 10-40% of relapsing fever cases are fatal. However, treatment with antibiotics reduces fatality to 2-4% (Southern and Sanford 1969).

Bartonella quintana

Members of the genus *Bartonella* are specialized to infect mammalian hosts and are transmitted through a variety of arthropod vectors (Raoult and Roux 1999). Infection with *B. quintana* is known as trench fever because the first records of infection with *B. quintana* occurred in both Allied and German forces during WWI (Raoult and Roux 1999). Currently, the only identified reservoir host is humans, and the only known vector for *B. quintana* is the clothing louse (Raoult and Roux 1999).

Bartonella quintana does not cause illness in the louse host. A louse becomes infected with *B. quintana* through feeding on an infected host (Raoult and Roux 1999). *B. quintana* then multiples within the louse intestine and is transmitted to the human host through infective louse feces rubbed into broken skin or conjunctivae or mucus membranes (Raoult and Roux 1999).

Once infected, a person experiences severe fever, headache, shin pain, and dizziness (Foucault et al. 2006). Infection with *B. quintana* also occurs in cycles, with the first cycle being the most severe. A small proportion of infected patients are asymptomatic, though some

experience bacteremia (Foucault et al. 2006). Infection with *B. quintana* is the least fatal of the known louse borne diseases, with a mortality rate of <1% (Raoult and Roux 1999). Little is known about the effectiveness of antibiotic treatment of trench fever, since most data from infections occurred prior to the use of antibiotics (Raoult and Roux 1999). However, a combination of doxycyline and gentamicin has been shown to be effective (Raoult and Roux 1999).

Sequencing of the Clothing Louse Genome

The sequencing and annotation of the clothing louse genome was recently completed, although its final publication has not occurred. The completed genome will provide important genetic information for the biomedical community. The bacterial genomes for pathogens carried by clothing lice, *R. prowezii*, (Anderson et al. 1998), *B. quintana* (Alsmark et al. 2004), and two species of *Borrelia* (Fraser et al. 1997; Glockner et al. 2004) have already been sequenced. The sequenced genome of *P. humanus* will provide insights into host-vector-pathogen interactions (Pittendrigh et al. 2006). The discovery of genes that are involved in vitellogenin production or olfactory receptors involved in locating potential hosts may provide targets for louse-specific pesticides (Pittendrigh et al. 2006). The development of oligonucleotide arrays and RNAi may aid in gene discovery. Further, oligonucleotide arrays may aid in determining which genes are induced or repressed during changes in louse behavior and environment, such as between head lice and clothing lice (Pittendrigh et al. 2006). Finally, sequencing of the *P. humanus* genome has the potential to determine if head lice are able to serve as vectors for bacterial pathogens (Pittendrigh et al. 2006).

In addition to the importance of the *P. humanus* genome to the biomedical community, the genome will also provide insights to the evolutionary biology community. *P. humanus* will be the first genome sequenced of a hemimetabolous insect. Already, the small size (105MB) of

the *P. humanus* genome relative to other insect genomes, such as *Drosophila* (175MB) and *Anopheles gambiae* (260MB), is raising important evolutionary questions, particularly because most other hemimetabolous insects are generally estimated to have large genomes (2000MB-16,300MB) (Pittendrigh et al. 2006). In order to explain such a small genome size, several questions related to genome composition are raised, such as the number and size of noncoding regions and the number and types of repetitive elements (Pittendrigh et al. 2006). Further, is the reduction in genome size a result of human parasitism, and if so, is it a result of selection for metabolic or replication efficiency or ecological specialization for the human host (Pittendrigh et al. 2006)? In addition to interesting biological questions raised by the small genome size, evolutionary biologists can also use the *P. humanus* genome to search for genes that help resolve relationships within Phthiraptera. Further, genes involved in parasitism of vertebrate hosts will be identified (Pittendrigh et al. 2006).

The *P. humanus* genome project has achieved 8x coverage. Preliminary analyses indicate that the genome is AT rich, with a GC content of only 27.5%. However, 56% of genes of *P. humanus* occur in GC rich regions, indicating a slight bias for genes to occur in GC rich areas. Currently, 11,214 genes have been identified, and 90% of those (10,187) share sequence similarity to genes in other insects. Of the 11,214 genes, 9698 contain introns, of which the average length is 303 base pairs and the average exon length is 238 base pairs. Only 1% of the *P. humanus* genome consists of transposable elements, and both class I and class II elements are present. When compared with other insect genomes, such as *Apis mellifera, Drosphila melanogaster, Anopheles gambiae,* and *Tribolium castaneum, P. humanus* shows a reduction in gene families associated with detoxification, such as the p450, GST, and esterase gene superfamilies. The reduction in detoxification mechanisms is consistent with the life-history of

P. humanus. Because *P. humanus* is so closely ecologically tied to its human host, it is not exposed to secondary compounds produced by plants. Similarly, the *P. humanus* genome also has a reduction of gene families involved in sensory functions, such as opsin and chemoreceptor genes.

In addition to the clothing louse genome, the primary endosymbiont and mitochondrial genomes of *P. humanus* have also been sequenced. The reduced size of the primary endosymbiont, *Candidatus Riesia pediculicola,* is consistent with the genome reduction that occurs when a bacterium is sequestered as an endosymbiont. Phylogenetic analysis of the *Candidatus Riesia pediculicola* sequence indicates that it is placed among the basal gamma-proteobacteria.

The mitochondrial genome of *P. humanus* displays a unique organization. Unlike the other >1000 bilaterian animals with sequenced mitochondrial genomes, the *P. humanus* genome does not consist of a single circular 16kb chromosome. Rather, it consists of 18 minicircular chromosomes, each of which has a length of 3kb and 1-3 genes. Analysis of the mitochondria of the pubic louse (*Pthirus pubis*), chimp louse (*Pediculus schaeffi*), and the langur louse (*Pedicinus ancoratus*) indicates that these also have minicircular mitochondrial genomes, and that the most recent common ancestor of these lice that parasitize primates likely had a similar mitochondrial genome organization 22.5 million years ago.

Lice as Markers of Human Evolutionary History

Sucking lice complete their entire life cycle on their mammalian host, which selects for high host specificity and leads to cospeciation between parasite and host. Primates and their parasitic sucking lice have been cospeciating for the last 22.5 million years (Reed et al. 2004). Researchers have exploited this property to learn more about primate evolution (Reed et al. 2004, Reed et al. 2007). Reed et al. (2004) analyzed molecular data from sucking lice of anthropoid

primates and confirmed the date of the human-chimp divergence to be 5-7 million years ago. Another study by Reed et al. (2007) dated the divergence between two sister taxa of lice, the gorilla louse, *Pthirus gorillae*, and the human pubic louse, *Pthirus pubis*, to be three million years. This date is significantly earlier than the human-chimp ancestor and gorilla divergence of 7-8 million years ago (Reed et al. 2007), suggesting a host switch from gorillas to humans.

Sucking lice, particularly *P. humanus*, has also provided insights into the population genetics of archaic and modern humans. Reed et al. (2004) confirmed that head and clothing lice went through a population bottleneck and subsequent expansion out of Africa with modern humans. Reed et al. (2004) also determined that there are three mitochondrial lineages of *P. humanus*, one that contains head and clothing lice and two that contain only head lice. Also, ancient DNA from *P. humanus* of a 1000 year-old Peruvian mummy indicates that the clade containing head and clothing lice was in the New World prior to European colonization (Raoult et al. 2008). This discovery supports the hypothesis that the bacterial agent of typhus, *Rickettsia prowazekii*, may be the result of a New World origin that involves a host switch of the bacterium from its reservoir host, the flying squirrel, *Glaucomys volans volans* (Raoult et al. 2008).

Population genetics of *P. humanus* has the potential to address several other questions relating to human evolutionary history. In order to address the origin of clothing use, Kittler et al. (2003, 2004) used unspecified phylogenetic methods to date the age of the clade that contained both head and clothing lice to 107,000 years ago. Though the phylogenetic methods used by Kittler et al. (2003, 2004) may have been inappropriate for a population-level question, lice may provide insight into the origin of the clothing use through coalescent methods to date the population divergence between head and clothing lice. Also, the taxonomic status of head and clothing lice may be addressed using molecular techniques. Further, the deep divergences

that occur among three lineages of *P. humanus* are potentially informative about the relationships between archaic hominids and modern humans (Reed et al. 2004).

Currently, evolutionary biologists and anthropologists studying lice rely on three nuclear markers, EF1-alpha, RPII, and 18S, four mitochondrial markers, COI, CytB, ND4, and control region, and five microsatellite loci (Leo and Barker 2005) to draw inferences about the population genetics of head and clothing lice. With the release of the *P. humanus* genome, a variety of new molecular tools will soon be available, such as a variety of nuclear genes, SNPs, microsatellites, and oligonucleotide arrays. These new tools will allow researchers to address biological and anthropological questions whose answers have been elusive thus far.

CHAPTER 2
POPULATION GENETIC ANALYSIS OF *Pediculus humanus* PROVIDES INSIGHT INTO THE ORIGIN OF HUMAN CLOTHING

Introduction

Biologists studying organisms with reduced genetic variation have begun to regularly exploit host parasites and pathogens to track events in the evolutionary history of the host. This is particularly true in the fields of conservation (Whiteman and Parker 2005), invasive species (Meusnier et al. 2001), and human evolution research (reviewed in Wirth et al. 2005), where host genetic variation is greatly reduced. In such scenarios, the parasite may generate more genetic diversity than the host due to its generally shorter generation time, which increases its evolutionary rate. Additionally, parasites often have larger effective population sizes, and therefore maintain more genetic variation through bottleneck events. The faster evolutionary rate of the parasite and increased genetic variation may therefore capture evolutionary events that are not coded in host DNA, allowing biologists to study the evolutionary history of the host through analysis of parasite DNA. This has been used most often in studies of human history (reviewed in Wirth et al. 2005), where JC virus, herpes simplex virus 1, human papillomaviruses, and *Helicobacter pylori* have been used to track human evolution.

Microparasites (bacteria and viruses) often reproduce clonally, and are thus highly susceptible to selective sweeps at a few loci producing patterns of genomic diversity that do not reflect the history of the host (Grenfell et al. 2004; Nieberding et al. 2006; Rich et al. 1998). Conversely, macroparasites (such as parasitic arthropods) often reproduce sexually and may therefore track host evolutionary history more accurately because selective sweeps affect only closely linked loci in their genomes (Grenfell et al. 2004). For this reason, the use of macroparasites, such as pinworms (Araujo et al. 2008) and lice (Raoult et al. 2008; Reed et al. 2004), has become increasingly common in the study of human evolution.

Sucking lice (Phthiraptera: Anoplura) have cospeciated with their primate hosts for at least the last 22.5 million years (Reed et al. 2004). These lice are permanent, obligate ectoparasites that require direct contact between their primate hosts for transmission (Burgess 2004; Canyon et al. 2002). Molecular examination of these lice has confirmed events known from primate history, such as the human-chimp divergence 5-7 million years ago (Reed et al. 2004). Additionally, the study of human lice (*Pediculus humanus*) has confirmed events in human demographic history, such as the population expansion of anatomically modern humans 100,000 years ago (Reed et al. 2004). These findings indicate that human lice have great utility for the study of human population history.

Two varieties of human lice, head and clothing lice (both *P. humanus*; see Light et al. 2008), may also provide insights into human evolution not recorded in human genetic data, specifically the origin of clothing use. Determining the origin of clothing use has been primarily inferential in anthropological literature as most materials used for clothing (furs, skin, cloth) degrade rapidly and are thus unknown in the archaeological record of the Late Pleistocene. Archaeological evidence for the use of *tailored* clothing, such as needles, first appears ~40,000 years ago (Delson et al. 2000). Earlier evidence for tools used to scrape hide appears ~300,000 years ago (Toth and Schick 1993); however, there is no definitive evidence that these tools were used to make even rudimentary clothing. The inability of the archaeological record to answer this question suggests that an innovative approach is necessary.

The biology of human head and clothing lice makes them potentially useful markers to infer the origin of clothing use in humans. Molecular data suggest that humans lost their body hair approximately 1.2 million years ago (Rogers et al. 2004). Prior to body hair loss, lice in the genus *Pediculus* likely occurred more uniformly across the body, but upon body hair loss

became restricted to the head. As humans started wearing clothing, a portion of head lice colonized this new habitat and became the ancestors of clothing lice (Burgess 1995; Kittler et al. 2003; Kittler et al. 2004). These lice then became specialized for the clothing niche, and at present spend more time in clothing than on the body, only returning to the body to feed once or twice per day. Of the three mitochondrial lineages of *P. humanus* (Leo et al. 2002; Raoult et al. 2008; Reed et al. 2004), two lineages contain only head lice and the third contains both head and clothing lice. Kittler et al. (2003, 2004) used phylogenetic methods to date the age of the lineage that contained both head and clothing lice and determined the origin of clothing use to be 107,000 years ago.

Kittler et al.'s date of 107,000 years (2003, 2004) suggests a recent origin of human clothing use. However, phylogenetic methods are inappropriate for answering this population-level question since several researchers have shown that head and clothing lice do not form reciprocally monophyletic clades (Leo et al. 2002; Reed et al. 2004; Yong et al. 2003). Furthermore, a recent study has demonstrated significant gene flow between head and clothing lice (Light et al. 2008), suggesting that recent head and clothing lice evolution is best understood as a population genetic process.

In this study, we exploit recent advances in coalescent methodology using multiple loci to analyze louse genetic data and address the origin of clothing use in humans. We first use a relaxed clock method (Drummond et al. 2006) to estimate mutation rates for our multilocus dataset. We then employ an isolation-with-migration coalescent method that jointly estimates divergence times, population sizes and migration rates from our multilocus dataset (Hey 2005; Nielsen and Wakeley 2001) to date the divergence of, and migration rates between, human head and clothing louse populations.

Materials and Methods

Molecular Data

All available DNA sequence data for the following genes were downloaded from GenBank (see Supplemental Table 1): mitochondrial gene COI (166 sequences) and the nuclear genes 18S rRNA (22 sequences), EF-1α (40 sequences; not including any of the sequences from (Yong et al. 2003), which were shown by Light et al. (2008) to be contaminants, and RPII (53 sequences). Sequences for the outgroup taxon *Pediculus schaeffi* (parasitic on chimpanzees) were downloaded from GenBank for each of the four genes (Supplemental Table 1). All COI, EF-1α and RPII sequences were aligned by hand using MacClade (Maddison and Maddison 2005) and manually edited to maintain proper reading frames using Se-Al v2.01a11. All 18S rDNA sequences were aligned manually in reference to secondary structure (Gillespie et al. 2004; Gillespie et al. 2005); alignment available at the jRNA web site) and ambiguously aligned sites were removed before analysis.

EvolutionaryRate Calculation

Phylogenies and evolutionary rates (μ) for each of the four genes were co-estimated under a "relaxed" (uncorrelated lognormal distribution) molecular clock using BEAST v.1.4.6 (Drummond et al. 2006; Drummond and Rambaut 2007). Estimates of μ were calibrated under an assumption of host-louse cospeciation, which has been demonstrated by Reed et al. (2004) for Hominidae. Specifically, an exponential prior distribution with a lower bound of 5MYA and mean of 5.5MYA was placed on the divergence of *P. humanus* and *P. schaeffi* that reflects current estimates for the divergence of their human and chimpanzee hosts (Kumar and Hedges 1998), respectively. All analyses were performed with a GTR + I + Γ nucleotide substitution model and a Yule birth-death prior on the tree topology. All Markov chains were run for

10,000,000 generations with samples taken every 1,000 generations and the first 1,000,000 generations were discarded as burn-in.

Isolation with Migration Analysis

A Bayesian isolation-with-migration coalescent analysis (Hey and Nielsen 2004; Nielsen and Wakeley 2001) was performed on the multilocus louse dataset using the program IM (Hey 2005). The isolation-with-migration coalescent model assumes that an ancestral population of effective size N_A diverges at some time *t* into two daughter populations of initial size sN_A and $(1-s)N_A$, both of which then experience independent exponential growth and migration between populations (with rates m_1 and m_2). All IM analyses were performed with a Hasegawa-Kishino-Yano (HKY) nucleotide substitution model for all four loci, and the upper bound of the prior distribution for the time of divergence was set to 5.5MYA, following best estimates for the human-chimpanzee divergence (see above). Estimates of μ from the evolutionary rate calculation above were used to convert population mutation parameter estimates (i.e. *t*) from mutational units into absolute units. All other priors were conservatively estimated from preliminary runs with very broad uniform prior distributions to facilitate adequate Markov chain mixing. All Markov chains were run for 600 million generations and sampled every 600 generations. Ten replicate runs with unique seed values were performed to ensure Markov chain convergence.

Results

Independent runs of IM analyses converged on similar posterior distributions (summarized in Figure 1). Peaks in the distribution were taken as point estimates of population genetic parameters. At the time of completion, all estimates of effective sample size exceeded 400. Estimates of θ and *t* were converted to effective population size and absolute time-since-

divergence, respectively, using estimates of μ obtained with BEAST v.1.4.6 (Drummond et al. 2006; Drummond and Rambaut 2007).

The distributions of current effective population sizes of head and clothing lice were contained within the priors. Point estimates were 2.5 million individuals for head lice and 1.8 million individuals for clothing lice. Estimates of the splitting parameter *s* indicate that only a small proportion of the ancestral population (<1%) initially colonized clothing, whereas the majority (>99%) remained head lice, which is thought to be the ancestral condition. Estimates of bidirectional migration (*m*) showed very high migration from head lice to clothing lice (*m* = 1.16), but virtually no migration in the opposite direction (*m* = 0.0040).

The posterior distribution for the time of population divergence peaks between 650,000-700,000 years ago in all IM replicates. The effective population size for the common ancestor of head and clothing louse peaked at the lowest bin and decreased thereafter. There are two potential explanations for this. First, the effective population size of the common ancestor was smaller than our analysis could detect. Alternatively, because head and clothing lice diverged long ago and have likely been through the same population bottlenecks as their human host, there may be too little information in the data to effectively estimate this parameter.

Discussion

Pediculus humanus diverged from its sister taxon, the chimpanzee louse *P. schaeffi*, at the human-chimp divergence roughly 5.5 million years ago (Reed et al. 2007; Reed et al. 2004). It is likely that lice in the genus *Pediculus* parasitized all subsequent hominid lineages given their history of cospeciation. Because *P. schaeffi* is found throughout the body of their chimpanzee hosts it is likely that the human parasite species of *Pediculus* also utilized much of the human body prior to the general loss of body hair in humans. At the time humans lost their body hair,

Pediculus likely became restricted to head hair, and only invaded the body niche recently with the advent of clothing use (Busvine 1978; Burgess 1995; Kittler et al. 2003; Kittler et al. 2004). This is inferred primarily from the current natural history of clothing lice. At present, clothing lice spend the majority of their time in clothing, and only return to the body to feed. Clothing lice prefer to attach their eggs to the pleats and seams of clothing, particularly the undergarments, rather than body hair (Burgess 1995; Buxton 1946; Nuttall 1917). In the absence of clothing, clothing lice will infest beads and necklaces (Busvine 1978; Buxton 1946). When displaced, clothing lice tend to return to the clothing and are particularly attracted to areas that are occupied by other clothing lice or clothing louse feces (Mumcuoglu et al. 1986; Wigglesworth, 1941).

Our results support the assertion that the head was likely the ancestral habitat for *P. humanus*. The splitting parameter *s* indicates that a large proportion of the ancestral louse population (>99%) was of the head lice type at the time of divergence, and that only a small proportion (<1%) moved to the clothing niche as a founder population. Furthermore, our results indicate a lack of migration from clothing louse to head louse populations, suggesting that clothing lice are ecologically distinct and possibly unable to survive under head louse conditions. This finding is consistent with empirical transplantation experiments. When head lice are reared as clothing lice in the laboratory (in pill boxes worn upon the skin), morphological and behavioral changes take place such that the head lice gain the morphological and natural history characteristics of clothing lice within a few generations (Alpatov and Nastjukova 1955; Bacot, 1917; Levene and Dobzhansky 1959; see Busvine, 1948 for a single known exception). Further, when clothing lice are displaced, they return to clothing, particularly areas occupied by other clothing lice and clothing louse feces (Mumcuoglu et al. 1986; Wigglesworth 1941). However,

there is no evidence to suggest that clothing lice can successfully colonize the head louse niche, which provides further support for unidirectional gene flow.

Coalescent methods that use multiple loci for population genetic inference reduce uncertainty in estimates of divergence time related to genealogical stochasticity (Edwards and Beerli 2000). This allows us to estimate the divergence of head and clothing lice, as opposed to dating the origin of the clade of head and clothing lice using phylogenetic methods (Kittler et al. 2003; Kittler et al. 2004). By using population genetic methods to date the divergence, we are able to obtain a more precise estimate of when populations of *P. humanus* began to colonize the clothing niche. Furthermore, our analyses uncovered a high rate of migration (m = 1.16) from head to clothing lice, further indicating that the divergence between head and clothing lice is a population genetic process.

Our point estimate of 650,000 years ago (90% HPD 225kya-6mya) indicates that head and clothing lice diverged significantly earlier than previously thought and suggests that the use of rudimentary clothing originated with archaic hominids, not anatomically modern humans, which arose only within the last 120,000 years (Pakendorf and Stoneking 2005). Also, it is important to note that the divergence of human head and clothing lice provides a minimum date for the origin of clothing use; it is possible that the clothing niche existed for some time before parasitic lice successfully colonized clothing.

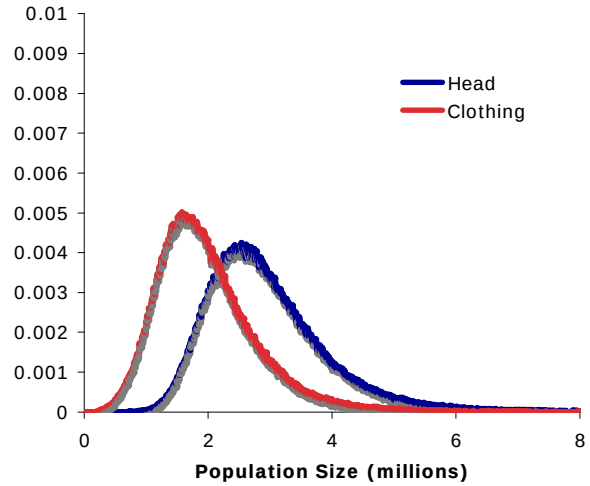
There are both adaptive and neutral explanations for why and how clothing use arose. Clothing may have arisen in Africa because it conferred an advantage to archaic hominids living in high altitudes. Alternatively, clothing use may have arisen for decorative purposes. Though our study cannot disentangle these alternatives, it can provide some insight as to where clothing use arose.

The mitochondrial clade of *P. humanus* that contains both head and clothing lice has the genetic signature of a population expansion that occurred 100,000 years ago (Reed et al. 2004) suggesting that these lice accompanied modern humans out of Africa. Because the date of divergence of head and clothing lice only provides a minimum estimate for the origin of clothing use, the technology to make clothing may have existed for some time prior to 650,000 years ago.

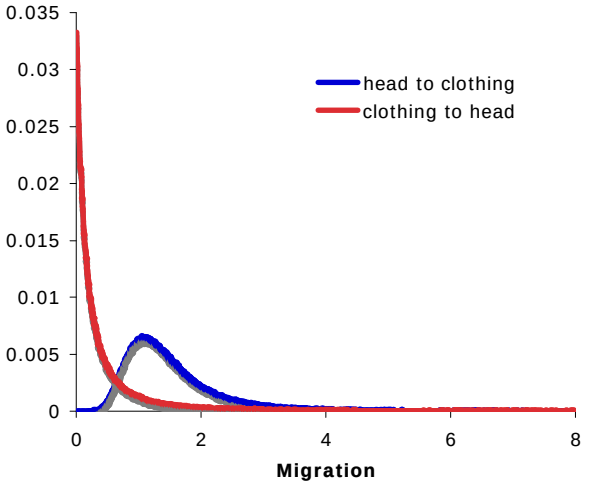
Anthropologists recognize multiple dispersals of hominid species out of Africa (Anton and Swisher 2004; Dennell 2003; Finlayson 2005), with the first dispersal of *H. erectus* occurring approximately 2 million years ago (Anton and Swisher 2004; Dennell 2003; Finlayson 2005). Most of the evidence suggests that *H. erectus* populations outside of Africa were largely confined to Southeast Asia, though there is some evidence of *H. erectus* in northern Asia around this time (Anton and Swisher 2004; Dennell 2003; Finlayson 2005). The permanence of these earliest dispersals into northern latitudes has been questioned (Dennell 2003). Interestingly, our estimate approximately coincides with the first evidence of permanent archaic hominid settlements into high latitude regions of Europe and Central Asia between 800,000 and 500,000 years ago (Ascenzi et al. 1996; Carbonell et al. 1995; Dennell 2003; Ranov 1995). It is possible that the archaic hominids that dispersed and successfully colonized colder climates 800,000 to 500,000 years ago may have possessed the knowledge to use furs and skins as rudimentary clothing, which would have allowed them to survive and persist in the temperate climates of Europe and Central Asia. Alternatively, it is conceivable that clothing use originated with archaic hominids in Europe and Central Asia, and spread to archaic hominid populations in Africa via intermittent contact. However, this is less likely, considering that archaic hominid populations in Central Asia and Europe were likely spatially and temporally discontinuous

during this time period (Dennell 2003), making transfer of technology between populations difficult.

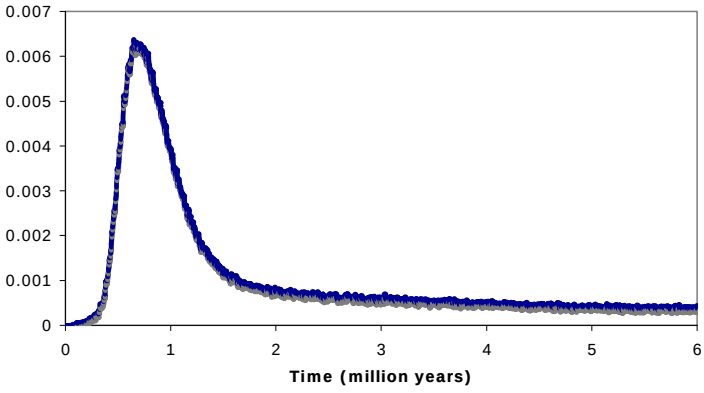
The natural history of clothing lice suggests that they are ecologically distinct from head lice, and therefore it is assumed that clothing lice only arose with the advent of clothing use (Burgess 1995; Busvine 1978; Kittler et al. 2003; Kittler et al. 2004). Our analyses indicate that only a small proportion of lice initially colonized clothing, but that migration from head to clothing has been considerable (m = 1.16) since. Further, our analyses suggest that clothing use originated with archaic hominids in Africa 650,000 years ago, not modern humans, and may have played a role in the archaic hominid colonization of Central Asia and Europe.



A



B



C

Figure 2-1: Marginal posterior probability distributions for model IM parameters in demographic units. (A) Effective population size. (B) Migration rates (*m*). (C) Time since divergence.

Table 2-1: Estimates of evolutionary rate (μ) in substitutions/locus/year for each locus.

Locus	μ
EF1-alpha	0.00000285
COI	0.0000163
18S	0.0000118
RPII	0.000041

CHAPTER 3
CHARACTERIZATION OF ELEVEN POLYMORPHIC MICROSATELLITE LOCI FROM THE HUMAN HEAD AND CLOTHING LOUSE, *Pediculus humanus*

Human head and clothing lice, *Pediculus humanus capitis* and *Pediculus humanus humanus*, respectively, have parasitized humans for thousands of years (Araujo et al. 2000). Currently, head lice are pandemic, infesting millions of schoolchildren worldwide. Clothing lice, though less prevalent, are common among areas stricken by poverty or political and social unrest that prevent inhabitants from having multiple sets of clothes (Raoult and Roux 1999). Clothing lice (also called body lice) are also becoming increasingly common among the homeless populations in developed countries such as the United States and Western Europe (Brouqui et al. 1996; Drancourt et al. 1995; Koehler et al. 1997). Clothing lice are more harmful parasites because they can vector three bacterial pathogens: *Rickettsia prowazekii* (epidemic or louse-borne typhus; but see (Robinson et al. 2003), *Borrelia recurrentis* (louse-borne relapsing fever) and *Bartonella quintana* (trench fever; Buxton 1946; but see Sasaki et al. 2006).

In addition to their importance in the biomedical field, human head and clothing lice are of interest to evolutionary biologists and anthropologists. Head and clothing lice are part of a group of anoplurans that have cospeciated with primates for 22.5 million years (Reed et al. 2004), and therefore have been used to provide insights into human evolutionary history (Kittler et al. 2003; Kittler et al. 2004, Raoult et al. 2008; Reed et al. 2007; Reed et al. 2004). For example, head and clothing lice have been used to estimate the origin of clothing use in humans (Kittler et al. 2003; Kittler et al. 2004). The use of population level markers, such as microsatellites, may provide insights into the epidemiology and evolutionary history of one of the oldest human parasites.

Microsatellite loci were mined from the Human Body Louse Genome Project (Johnston et al. 2007) using Tandem Repeat Finder (Benson 1999). Of the initial microsatellite search, loci

were selected that contained a minimum of eight repeats and at least 200 base pair flanking regions on each side that was free of other repetitive elements. These loci were further screened when designing primer pairs using PRIMER3 (Rozen and Skaletsky 2000) for formation of hairpins, self-dimers, hetero-dimers, and optimal primer GC content of 40-60%. Primer pairs were designed for a total of 48 loci, of which 11 have been assessed for polymorphism using 18 head louse individuals, each from a separate host individual, from West Palm Beach, Florida.

DNA amplifications were carried out in 25μL reactions containing 11.5μL of distilled water, 10μL of 5Prime HotMaster Mix (Eppendorf), 1μL of 1μM M13 labeled forward primer, 1μL of 10μM reverse primer, and 1μL of 10μM FAM label, and 0.5 μL of DNA template. Thermocycling conditions for all loci were identical and are as follows: 94° for 3 min, followed by 10 cycles of 94° for 30 s, 52° for 30 s, 65° for 45 s, then 30 cycles of 94° for 30 s, 48° for 30 s, 65° for 45 s, and a final extension of 65° for 10 min. Fragment lengths of PCR products were detected using ABI 3730 Automated Sequencer and scored using GENEMAPPER 3.0 software (Applied Biosystems).

Data for loci analyzed are available in Table 1. The number of alleles ranged from 3-7 per locus. Observed and expected heterozygosities were calculated using MICROSATELLITE ANALYSER 4.05 (Dieringer and Schlotterer 2003) and ranged from 0.056-0.278 and 0.303-0.820, respectively. Linkage disequilibrium with a Bonferroni correction was tested using GENEPOP version 3.4 (Raymond and Rousset 1995) and no linkage disequilibrium was identified. Deviations from Hardy-Weinberg equilibrium were tested using GENEPOP version 3.4 (Raymond and Rousset 1995). Only two loci, M2_19 and M3_9 are in Hardy-Weinberg equilibrium, and M2_19 is only marginally so (p=0.050). Because 9 of 11 loci are polymorphic

and exhibit heterozygote deficiency,it is likely the population tested that is out of Hardy-Weinberg equilibrium, and not the loci.

Our results indicate that these microsatellites are clearly polymorphic in this population, though not in Hardy-Weinberg equilibrium. Several possible explanations exist for why the population may be out of Hardy-Weinberg equilibrium, such as high population substructure or nonrandom mating. Additional populations should be screened to determine if this is specific to the population of head lice in West Palm Beach, Florida, or if it is characteristic of the species in general. Alternatively, further sampling of the population in West Palm may reveal a high level of population substructure. The polymorphic microsatellites identified in this study may be useful in examining differences between head and clothing lice, in examining fine-scale host migrations, and characterizing local populations worldwide.

Table 3-1. Characteristics of eleven microsatellite loci for the human head and clothing louse, *P. humanus*, including locus name, primer sequence, repeat motif, allele size range, number of alleles (N_a), observed heterozygosity (H_O), expected heterozygosity (H_E), and probability associated with Hardy-Weinberg equilibrium.

Locus	Primer Sequence	Repeat Motif	Allele Size (bp)	N_a	H_O	H_E	P
M2_2	F: TCTTGCAGTGTGTCTCTTTGC	GA	392-412	4	0.056	0.678	0.000
	R: CTATCGGAAATGTGCAGAGC						
M2_3	F: TGATATTTTAGGCGCACAACC	GA	364-370	4	0.059	0.497	0.000
	R: GTCTCAATTCGGCCACTTCT						
M2_4	F: TTCAGGATCTTCTGCCCAAC	GA	308-316	4	0.056	0.662	0.000
	R: GGGTTCGCAAAAAGGTGAC						
M2_16	F: TAACGACCGCTTTTCGAGTT	GA	242-266	6	0.111	0.697	0.000
	R: GGGGTGAACTGGATGTTTCA						
M2_17	F: GCCTAGCGAAAGCTCTGAAA	TA	235-253	6	0.188	0.714	0.000
	R: GAAGTATCATTTCGGCGTGA						
M2_19	F: GGTGGCAAAAACCACTAATGA	TA	182-198	4	0.278	0.486	0.050
	R: TCCGTTAAAAATGGCAAAGG						
M3_3	F: TGTTCCTGCTGGTAAACTTGC	TAA	238-295	7	0.176	0.820	0.000
	R: GAACGATCAATCTGCTTCTGC						
M3_9	F: CCCGTAAAATATCCACGCTGT	TTA	284-302	3	0.278	0.332	0.507
	R: CTGGTCGGCTATGTTTTGCT						
M3_11	F: CTCCTAACGGGAGCAAAGAA	TTA	299-357	6	0.167	0.749	0.000
	R: CCATACATATTAGTCGCCTTCCA						
M3_18	F: CGTCGGAGGAATGTATAGGG	GAC	275-299	4	0.111	0.303	0.011
	R: GACAGTGACGGATCGAACG						
M3_19	F: TTAAGAGCTGATGCCACGTC	CAT	346-358	4	0.118	0.405	0.000
	R: TCTGGAAAAGGACGAAAGGA						

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BIOGRAPHICAL SKETCH

Melissa Toups was born and raised in New Orleans, Louisiana. After graduating from Archbishop Chapelle High School in 2000, she attended Tulane University and majored in ecology and evolutionary biology. While at Tulane, she participated in the Junior Year Abroad program and attended the University of Bristol. She graduated Tulane in the Spring 2004 magna cum laude. She began her graduate career in August 2005 in the Zoology Department at the University of Florida and received her M.S. in August 2008.