Proposal for the Sequencing of a New Target Genome: White Paper for a Human Body Louse Genome Project.

Barry R. Pittendrigh, John M. Clark, J. Spencer Johnston, Si Hyeock Lee, Jeanne Romero-Severson, and Gregory A. Dasch

Contact information: Ph. 765-494-7730; Fax 765-496-1219; pittendr@purdue.edu

Summary – The human body louse*, Pediculus humanus humanus*, is the primary vector which transmits the bacterial agents of louse-borne relapsing fever, trench fever, and epidemic typhus. Epidemic typhus, one of the most significant historical diseases of humans, is caused by *Rickettsia prowazekii*, a category B bioterrorism agent that can cause persistent human infection. Besides its notoriety as the agent of the recurrent chronic disease, trench fever, *Bartonella quintana* can cause endocarditis and is a common infection among the homeless. *Borrelia recurrentis* causes another recurrent fever in central and Eastern Africa that is characterized by significant morbidity and mortality. The genome sequences of *R. prowazekii* and *B. quintana* have been determined as well as those of two species of *Borrelia* so that determining the body louse genome will enhance studies of host-vector-pathogen interactions. Body lice and the closely related human head louse, *Pediculus humanus capitis,* belong to the hemimetabolous order Phthiraptera. Head lice represent a major economic and social concern in North America, because head lice infestations are often associated with school-aged children, who miss substantial school days during this critical learning period. Resistance to traditional pesticides used to control head and body lice have developed. It is imperative that new molecular targets are discovered to aid in development of novel compounds to control these insects. No complete genome sequence has been obtained for a hemimetabolous insect species. In large part, this is because hemimetabolous insects often have large (2000 Mb) to very large (up to 16,000 Mb) genomes. Recently, we discovered that the body louse genome is one of the smallest known in the insect world, 107.6 $\pm$ 0.6 Mb for males and 105.4 $\pm$ 0.7 Mb for females, making it an ideal hemimetabolous insect for a genome sequencing project. We currently have made EST libraries for both body and head lice which are being sequenced by a consortium of scientists from Purdue University, University of Massachusetts at Amherst, and Seoul National University. Additionally, we have available (i) a highly inbred strain of body lice that can be used in a genome sequencing project, (ii) the financial resources to help with the creation of a BAC library if needed for the genome project, and (iii) means to generate EST libraries of body lice after exposure to bacterial pathogens and pesticides.

Table of Contents **Page**

A. Specific biological rationales for utility of new sequence data

1. Body lice – A human disease vector associated with three diverse bacterial agents including a potential bioterrorism agent.

Lice are highly species-specific parasites. Lice have been identified on Egyptian mummies and phylogenetic evidence suggests they adapted to humans about 5.6 million years ago when the ancestors of chimpanzees and humans diverged [1]. Body lice live in close contact with humans and are often spread by shared bedding or by close personal contact [2]. This pest typically lives in the seams of the host's clothing and infestations are associated with wearing the same clothing for prolonged periods of time without washing (*e.g.*, wartime, natural disasters or poor personal hygiene). A louse typically feeds five times a day and molts three times in 10 days after hatching 6-9 days after being laid. The adult survives for about 20 days. Lice can lay 200 eggs but require humidity levels above 40% for survival and survive best at 29-32^{o}C.

Body lice can be a serious public health problem because they transmit three highly diverse and specialized bacteria which cause the human diseases, louse-borne relapsing fever, trench fever, and epidemic typhus. Epidemic typhus is caused by *Rickettsia prowazekii*, which is a category B bioterrorism agent and an obligately intracellular alphaproteobacteria. Symptoms of epidemic typhus include headache, fever, chills, exhaustion, and skin rashes; untreated cases are often fatal but the agent also causes persistent chronic infection of humans which are the interepidemic reservoir. The disease is spread because the louse leaves febrile hosts with epidemic typhus but it eventually succumbs from rupture of the gut by growth of the rickettsia. In the preantibiotic era, epidemic typhus was a major scourge of mankind as it killed millions. However, in 1986 more than 50,000 were infected in Burundi and the disease is reemergent in other areas of the world. The agent is acquired from ill individuals by feeding but is transmitted primarily by aerosol and dermal inoculation of highly infected louse feces. *Borrelia recurrentis* is closely related to the tick-borne relapsing species *B. duttonii* and is thought to have diverged from this spirochete after its adaptation to the louse. In WWII, a million cases were observed in North Africa with a fatality rate of 10%. Lice feeding on infected blood become infected for life after the agent passes from the gut to the coelomic cavity but lice cannot transmit the agent by feeding nor transmit it transovarially. The *Borrelia* readily invade skin or mucosal tissues from crushed lice. *Bartonella quintana*, a facultative intracellular alphaproteobacteria most closely related to *Brucella,* causes a five day relapsing fever with long bone shooting pains and was responsible for more than a 1,000,000 cases in WWI on all European fronts. The agent is widespread in the homeless of developed countries and can cause persistent bacteremia and endocarditis and bacillary angiomatosis in HIV-positive individuals. Lice infected via blood meal remain persistently infected and *Bartonella* are excreted in large amounts in the intestine but it can also apparently be transmitted during feeding.

2. Allergenic effects.

Body lice will often cause intense itching in humans due to an allergic reaction in the host against components found in the louse saliva. The bites of body lice may first appear as small red dots and may later cause rashes. Repeated bites by body lice can ultimately lead to a generalized skin eruption or inflammation. This intense itching can lead to injury to the skin caused by scratching by the host and to secondary infections as well as serving as a major mechanism for self-inoculation of *R. prowazekii, B. recurrentis,* and *B. quintana.* The nature of components in louse feces which facilitate preservation of viable *R. prowazekii* and *B. quintana* are incompletely known but this material readily causes aerosol infections with these agents.

3. Smallest known genome size for a hemimetabolous insect.

To the authors' knowledge, the human body louse genome is the smallest known genome size for any hemimetabolous insect. The genome size has been measured at 107.6 $\pm$ 0.6 Mb in males and 105.4$\pm$ 0.7 Mb in females. In fact, the body louse genome is only slightly larger than that of *C. elegans* (100.4 Mb*).* For comparison*, Drosophila melanogaster's* genome is 175 Mb, the mosquito genomes of *Anopheles gambiae* and *Aedes aegypti* are 220 and 800 Mb respectively, and the ticks, *Ixodes scapularis* and *Boophilus microplus*, are 1080 and 7100 Mb, respectively.

4. The value of a small insect genome, a hemimetabolous insect genome, and a parasitic insect genome.

The taxonomic grouping Paraneoptera (Acercaria or hempteroid assemblage) includes the medically important disease vectors in the Triatominae (Hemiptera) and lice (Phthiraptera), plus economic pests such as aphids, white flies, leaf hoppers (Hemiptera), and thrips (Thysanoptera). The other two hemimetabolous insects that will soon be completely sequenced are the pea aphid, *Acyrthosiphon pisum* (Hemiptera: Aphididae) and the triatomid bug, *Rhodnius prolixus*. The relationship of the aphid, *Rhodnius* and louse will provide critical comparative genomic tools to identify regions that are conserved among the homoptera (*e.g.*, exons and regulatory regions). Comparative genomics among these three sequences will also aid discovery of changes associated with the small genome size and the derived parasitic life history of the louse.

Lice are probably the most important single hemimetabolous group from the standpoint of human health. Many other hemimetabolous insects, nevertheless, have great impacts on the quality of life, including the migratory locust, aphids, bedbugs, and other pests. Additionally, the bloodsucking hemimetabolous insects may share significant novel bioeffector molecules not found in other arthropods of medical importance, such as ticks and mosquitoes. As such, the louse sequence will provide important sources of genomic homology and contrast.

Parasites in general tend to have small genomes, yet the louse genome is exceptional even among these [3]. Much as the relatively small fugu genome provided a contrast to the larger genomes of all other fish, the louse genome will show how insect genomes can be reduced. Are introns smaller or fewer? Are genes lost? Are control regions simplified? Are repetitive elements less numerous or less common? All will be of interest for comparative genomics. Thus, the value of other funded hemimetabolous arthropod genome sequencing projects will be enhanced by completion of the small louse genome sequence.

5. Head louse – A close relative of the body louse.

Sequencing of the body louse genome will provide important insights into another major pest species, the human head louse. Head and body lice are closely related. There is an ongoing debate as to whether head and body lice are separate species or separate sub-species. Those that consider them separate species respectively refer to them as *Pediculus capitis* and *Pediculus humanus*. Those that consider them separate subspecies respectively refer to them as *Pediculus humanus capitis* and *Pediculus humanus humanus*. Further genomic information on these two organisms will likely provide critical evidence to help bring resolution to this debate. They can mate and produce offspring, but their progeny are infertile, just like matings between horses and donkeys. They are presently classed as subspecies, and the best guess is that they shared common ancestry no more than 5 million years ago. Thus, what we learn about the genome of body lice will likely provide us with important information in regards to head lice. This information may include, but certainly is not limited to, target sites (such as olfactory receptors) for the development of novel control agents. An additional low coverage (1X - 3X) of the head louse genome as part of the proposed effort would provide valuable information in identification of genes and regulatory regions in body lice.

6. Pediculosis.

Pediculosis, caused by the human head louse, is the most prevalent parasitic infestation of humans in the United States. Lice are ubiquitous in most developing countries [4] and children 3-12 yr old are most affected. Acquired immunity likely reduces louse density and impact on chronically exposed older juveniles and adults. Thus, louse outbreaks may constitute one of many opportunistic infections associated with a depressed immune system (*e.g.*, people with HIV). Unlike body lice, head lice have not been formally incriminated in the biological transmission of pathogens but are known to become infected. However, it has been suggested that they may transmit *Rickettsia prowazekii* [5, 6] and head lice are known to mechanically transmit *Staphylococcus auereus* and *Streptococcus pyogenes* [7].

Although head louse infestations are irritating and can lead to infection, the social, mental, and economic consequences are very substantial. Most people find head lice intolerable and often repeatedly and prophylactically apply costly pediculicides (insecticides) without realizing the potential harm and lethality if misused or overused. This impacts children in particular due to their small size and higher sensitivity to the toxic effects of these pediculicides.

7. Need for new practices for controlling pediculosis.

There are two ways to combat pediculosis: proactive prevention or post-infestation treatment. Emphasis is increasingly on prevention (education) and physical removal (combing or shaving), because a crisis exists in the chemical management of pediculosis. The pediculicide arsenal is limited and shrinking; and health providers are spending an increasing and inordinate amount of time and resources dealing with infestations. Effective management information is lacking and few, if any, alternatives exist when standard pesticide treatments fail. Thus, there is a need for the discovery of new target sites in lice (such as olfactory receptors or genes for vitellogenin production) that could be used for the development of biocides that would selectively rid humans of these pests but not target humans. A genome project for body lice would provide the necessary core information about novel target sites for improved head and body louse control.

8. Resistance to traditional control methods.

Treatment failures are common but the cause is uncertain (*e.g*., resistance, improper application, misdiagnosis, formulation changes, etc.) [8]. Louse resistance to most pediculicides is common and increasing in frequency [4, 9-19], particularly to DDT, the pyrethrins and the pyrethroids [8-11, 20-27]. Dr. Clark's research group has established that knockdown resistance is a major factor [25] in all permethrin-resistant lice studied to date in the U.S. and supports the claim that treatment failure is, in part, due to resistance. An observer-blind study on NIX® (1% permethrin crème rinse treatment) effectiveness validated these findings [28]. In a recent survey of pharmacists, 78-82% of patients treated with synergized pyrethrins or permethrin remained infested and 63% treated themselves more often or at higher doses than mandated by the manufacturer [29]. Predisposition of resistance to pyrethroids in DDT-resistant lice impacts current control measures that rely on natural pyrethrum or permethrin-amended shampoos. Thus, there is certainly a need for development of novel control agents for this pest.

U.S. pediculicide sales were last estimated at >$150 million just for over-the-counter (OTC) remedies (1997, NPA, Newton, MA). Infestation rates range from 6 -12 million cases annually. It is estimated that 2.6 million households are affected with 8% of all school children infested [4]. The overall cost of infestations is > $367 million annually [4]. The long-term impact of the days of lost learning by almost 1 in every 10 school-aged children due to the No-Nit policy and the lack of effective control options overshadows these cost estimates.

The pending loss of pyrethroids [25] and synergized pyrethrins [12] as effective pediculicides due to resistance is not trivial. Pyrethroids (*e.g*., permethrin) are the safest and most effective class of insecticides available and are the principal choice for veterinary and medically important pests. Loss of control associated with resistance, however, has resulted in dangerous overuse [30-33]. Incidences of injury and death increase as people resort to "home remedies," such as kerosene and gasoline. Without options, people overuse registered pediculicides, use combinations or resort to unregistered insecticides [17]. Malathion (e.g. Ovide) is prescribed most often when pyrethroids fail. Malathion is one of the safest organophosphorous insecticides but is still an irreversible cholinesterase inhibitor. It causes a variety of chronic neuropathies, including organophosphate-induced delayed neuropathy, at near lethal dosages.

The current problems with louse control and resistance underscore the need for development of novel control strategies for body and head lice. A genome project in body lice would open the door to the possibility of discovery of louse-unique target sites for the development of new pediculicides.

9. Understanding genes involved in louse behavior and disease transmission.

Detection, treatment, and avoidance measures could be improved by gaining a greater understanding of molecular biology of how lice perceive the human body and head as a living habitat. The complete sequencing of the body louse genome would open the door to development of cDNA or oligoarray gene chips that would allow us to gain a greater understanding of those genes that may be induced or repressed in lice in response to changes in their environments.

Body and head louse behavior could be altered and their populations reduced by exploiting target sites that impact the louse's behavioral patterns. For example, body lice exhibit a "homing instinct" towards their eggs [34,35] and feces [36], indicating that

the response to ovipositional attractants are an important behavior that could be disrupted The *Drosophila* genome project showed that olfactory receptors are species specific and are represented by a diverse and rich set of gene families. Any and all of these could be targets for specific control. Similarly, vitellogenins are the major product from the blood meal in mosquito and are probably also produced at high levels in blood-fed mature lice as well. The gene and regulatory regions of the genes involved in vitellogenesis will also be potential control sites produced by the sequencing effort. Sequencing of the louse genome and cDNA or oligoarray chips from this knowledge will provide the resources to understand the molecular mechanisms of disease transmission. Up and down regulation of specific genes should reveal changes involved in these processes. The availability of louse gene oligoarrays will greatly facilitate subsequent projects to determine how infection with *R. prowazekii, B. recurrentis*, and *B. quintana* affects gene expression in their common louse host. Both unique and common features of the louse to the presence of these agents will be of interest.

Additionally, we also propose to do a 1X - 3X coverage of the head louse genome to generate tools necessary for us to understand how parasites adapt to different parts of the human body. Sequencing of a genome of a subspecies so closely related to body louse will help us to identify genes and their regulatory regions. Further, it will allow the identification of regions subject to strong selection during the process of adaptation to the different parts of the human body.

10. Chromosome structure and meiotic failure.

The centromere of the human chromosome remains an area of very active interest. Nondisjunction leading to polyploidy, monosomy, and trisomy, remains the single largest contributor to infertility and mental retardation in man. Here the louse genome can make a unique contribution. Louse chromosomes are holocentric and behave as if the centromere is distributed throughout the genome. Given the small size of the louse genome, this cannot be the answer. A comparison of the louse genome to that of all other sequenced genomes, including man, should provide fundamental information on the genomic basis of pairing and disjunction. Of potentially equally importance, male meiosis in the louse is achiasmatic, as is the case in *Drosophila* and in portions of the X and Y chromosomes of man. This would seem to be parallel evolution, but it will be valuable to contrast the genomic basis of this process in different organisms.

B. Strategic issues

1. Demand for body louse genome sequence.

The community of body louse investigators is relatively small, with about 100-200 scientists working on pediculosis and the biology of louse species. If, however, one extends this community to include those working on organisms that are more closely related to the louse than to any other sequenced genome (which includes aphids, *Rhodnius* and even ticks), this becomes a large community indeed. Additionally, sequencing of the body louse genome will have broad implications for those considerably larger groups of researchers working on the disease organisms transmitted by body lice, and human researchers interested in the molecular biology of the body louse-human interactions. Moreover, the number of individuals that may be impacted by medical applications of improved pediculicides and improved understanding of human lice and their bacterial pathogens in substantial. The louse sequence will be important for those doing comparative genomics across insect taxa, as well as those interested in co-evolution of humans and their parasites.

2. Suitability of the organism for experimentation: Table 1

Genome and Basic Biology	
Haploid Chromosome #	10-12 - Estimate based on Golub & Nokkala. [3] (Actual number to be determined summer or fall of 2006)
Genome size	107.6 $\pm$ 0.6 Mb for the males and 105.4 $\pm$ 0.7 Mb for the females (heterochromatin levels not known)
Generation Time	Approximately 30 days
Cultivation	Can be reared in laboratory colonies and using an *in vitro* rearing system

Table 1 (Continued)

Genetic Resources/Tools	
Fraction sequenced	<<1%. [37]
cDNA/EST resources	One body lice library and two head lice libraries (all life stages included in each library). The combined libraries are expected to have in excess of about 20,000 ESTs. Currently there are between 3000-5000 sequenced ESTs from body and head lice libraries. Most of these sequences, as of yet, have not been posted at the public databases.
BAC libraries	Creation of library should begin in summer of 2005 – coverage will be either a 5X or 20X depending on resources
Transfect/transgenic	No
Gene inactivation	No
Mutants	None
Germplasm storage	None to date
Special Strengths	(i) One of the smallest known genome sizes for an insect species. (ii) Body lice, and closely related head lice, are health and nuisance problems both within the U.S. and Worldwide. (iii) Body lice transmit diseases, including a category B bioterriorism agent. (iv) In an order of insect that are important parasites, but we know almost nothing about their genomes. (v) Highly inbred strain
Weaknesses	(i) No mutant stocks (ii) No immortal cell culture

---

3. Databases & web resources.

Depending on the availability of funding support, the relational database created for the honeybee by Dr. Chris Elsik at Texas A&M University can be expanded to include a similar relational database for the louse. Included will be a genome browser, blast site, and map viewer. This database will not replace the NCBI database, but rather (as has been the case for FlyBase and for Beebase) it will provide additional tools and visibility for the completed genome. Among the advantages of the relational database have been that gene predictions from NCBI, Ensemble and Heidelberg can be displayed together on the TAMU relational database. For honeybee, this comparison resulted in significant refinements in gene predictions at all three centers and at BeeBase. Because the database will be relational, it can be updated as tools and data are added. EST data can be overlaid and displayed against nucleic acid sequences and predicted protein sequences. Additionally, as the genome is assembled, the map viewer displays the assembled information so that areas of interest are quickly found and queried. Eventually, the database will provide a home for annotation efforts as well as integration of the genetic, physical, and chromosomal maps.

Number of Investigators Approximately 100-200 investigators work directly in louse biology, louse molecular biology, or both.

4. Rationale for complete sequence.

A full genome sequence is required to use body lice to address a wide range of health-related problems: efficient discovery of target sites for potentially novel and louse-specific biocides; discovery of genes involved in disease propagation and

transmission; understanding of human-louse interactions; understanding of putative allergens in lice that impact the human immune system; understanding of human-louse co-evolution; discovery of genes involved in attraction and/or repellency; provide genetic resources for those working on other parasitic Phthiraptera; and, since there is relatively little information in the literature on the genomes of hemimetabolous insects when compared to holometabolous insects, this sequence will provide important information to those studying evolution of insect and other arthropod genomes. We also propose to do a 1X to 3X coverage of the head louse genome for a comparative genomic analysis between these two subspecies.

5. Cost and readiness.

Collaborative work between Drs. Johnson, Clark and Pittendrigh has lead to the recent discovery that body lice have a small genome size. Dr. Clark has developed an *in vitro* rearing strategy that will allow him to produce sufficient quantities of inbred strains of body lice and head lice for genomic efforts. His laboratory also has the capacity to rear field-collected strains. EST libraries have been made for both head and body lice and by mid-August we should have upwards of 2000 body and head lice ESTs annotated, including those already published by the Pittendrigh laboratory [37]. Drs. Pittendrigh and Clark are currently in the process of making arrangements for the creation of a body louse BAC library (between 5X to upwards of 20X coverage). Dr. Pittendrigh currently has the financial resources for the creation of a BAC library for body lice.

6. Basic sequencing strategies.

Pediculus pediculus for this project will be provided from inbred laboratory cultures. BACs and BAC end sequences BES to produce megabase scaffolds will be produced with existing funds.

The Baylor College of Medicine Human Genome Sequencing Center (BCM) has expressed interest in sequencing the body louse. Specific methods will depend on which Center is assigned this project, but we anticipate that this will be a pure whole genome shotgun (WGS) sequencing effort, with assembly from subclone and fosmid libraries. BAC end sequences will be used for scaffolding, in order to create Mb-size scaffolds. We are aware that the NHGRI-supported sequencing centers are experimenting with new low-cost approaches for the sequencing pipeline and are open to getting involved in that for *Pediculus*.

Assembly of the genome can follow the techniques used at BCM for the honey bee (and other organisms) using ATLAS whole genome assembly tools. The annotation of the bee genome was successfully performed in collaboration with the EBI using Ensembl. The Ensembl pipeline has now been installed at BCM and provides a proven platform for insect genome annotation.

BAC ends of contigs can be readily mapped onto the linkage map generated based on the surplus males. As the vast majority of the reads will be short insert pUC18 subclones from sheared genomic DNA, the contig coverage will be fully random as was the case for the honeybee sheared sequencing libraries. Assembly of the honeybee whole genome shotgun sequence has been straightforward using the ATLAS whole genome assembly tools developed at the BCM-HGSC for the assembly of the rat, *D. pseudoobscura*, honey bee, sea urchin and numerous bacteria.

The BCM-HGSC is constantly improving and re-evaluating its sequencing pipeline for the reduction of costs, evaluating the possibilities of new sequencing technologies as they emerge. One such technology is 454 Inc's (Branford, CT) massively parallel pyro-sequencing. We will closely follow and evaluate the possibility of using this technology with *P. humanus* if the scientific requirements can be fulfilled.

We propose to sequence *P. humanus humanus* to sufficient coverage (6-8X) to determine the size of introns, exons, and control regions. If possible, we would also sequence *P. humanus capitus* to 2-3X coverage, as the comparison of the two subspecies will not only help identify loci unique to these pests, but also alleles responsible for differences between the two subspecies.

7. Other (partial) funding sources.

Funding for a BAC library has been provided by a grant from the state of Indiana (21st Century Funds) to the Indiana Center for Insect Genomics, of which Drs. Pittendrigh and Romero-Severson are members. This source of funding will also provide Drs. Pittendrigh and Clark with the resources to sequence >2000 head or body louse ESTs. Dr. Si Hyeock Lee currently has resources to sequence another 1,500 ESTs from either the current head lice EST libraries or from the new body louse EST libraries that Drs. Pittendrigh and Clark will be constructing this summer. The resources to host the database are in place at TAMU with anticipated additional funding from NHGRI.

References

1. Pennisi, E. (2004). Louse DNA suggests close contact between early humans. Science 306,210.
2. Raoult, D., and Roux, V. (1999). The body louse as a vector of reemerging human diseases. Clin. Infect. Dis. 29,888-911.
3. Golub, N., and Nokkala, S. (2004). Chromosome numbers of two sucking louse species (Insecta, Phthiraptera, Anoplura). Hereditas *141*, 94.
4. Gratz, N.G. (1997). Human Lice: Their Prevalence, Control and Resistance to Insecticides (Switzerland: World Health Organization).
5. Goldberger, J. (1912). The transmission of typhus fever with especial reference of transmission by the head louse (*Pediculus capitis*). Public Health Reports *27*, 297-307.
6. Murray, E.S., and Torrey, S.B. (1975). Virulence of *Rickettsia prowazeki* for head lice. Ann. NY Acad. Sci. *266*, 25-34.
7. Meinking, T.L. (1999). Infestations. Curr. Prob. Dermatol. *11*, 73-120.
8. Pollack, R.J., Kiszewski, A., Armstrong, P., Hahn, C., Wolfe, N., Rahman, H.A., Laserson, K., Telford, S.R., III, and Spielman, A. (1999). Differential permethrin susceptibility of head lice sampled in the United States and Borneo. Arch. Pediatr. Adolesc. Med. *153*, 969-973.
9. Wright, J.W., and Brown, A.W.A. (1957). Survey of possible insecticide resistance in body lice. Bull. WHO *16*, 9-31.
10. Wright, J.W., and Pal, R. (1965). Second survey of insecticide-resistance in body-lice, 1958-1963. Bull. WHO *33*, 485-501.
11. Picollo, M.I., Vassena, C.V., Casadio, A.A., Massimo, J., and Zerba, E.N. (1998). Laboratory studies of susceptibility and resistance to insecticides in *Pediculus capitis* (Anoplura; Pediculidae). J. Med. Entomol. *35*, 814-817.
12. Cole, M.M., and Clark, P.H. (1961). Development of resistance to synergized pyrethrins in body lice, and cross resistance to DDT. J. Econ. Entomol. *54*, 649-651.
13. Cole, M.M., Couch, M.D., Burden, G.S., and Gilbert, I.H. (1957). Further studies on resistance of human body lice to insecticides. J. Econ. Entomol. *50*, 556-559.
14. Clark, P.H., and Cole, M.M. (1964). Laboratory studies on resistance of the body louse to insecticides. J. Econ. Entomol. *57*, 205-210.
15. Clark, P.H., and Cole, M.M. (1967). Resistance of body lice to carbaryl. J. Econ. Entomol. *60*, 398-400.
16. Miller, R.N., Wisseman, C.L., Sweeney, G.W., Verschueren, A., and Fabrikant, I.B. (1972). First report of resistance of human body lice to malathion. Amer. J. Trop. Med. Hyg. *66*, 372-375.
17. Blommers, L., and Van Lennep, M. (1978). Head lice in the Netherlands: susceptibility for insecticides in field samples. Entomol. Exp. App. *23*, 243-251.
18. Sinniah, B., and Sinniah, D. (1982). Resistance of head louse (*Pediculus humanus capitis* de Geer) to DDT in Malaysia. Trans. Roy. Soc. Trop. Med. Hyg. *76*, 72-74.
19. Maunder, J.W. (1991). Strategic aspects of insecticide resistance in head lice. J. Roy. Soc. Hlth., 24-26.
20. Hurlbut, H.S., Altman, R.M., and Nibley, C., Jr. (1952). DDT resistance in Korean body lice. Sci. *115*, 11-12.
21. Mumcuoglu, K.Y., Hemingway, J., Miller, J., Ioffe-Uspensky, I., Klaus, S., Ben-Ishai, F., and Galun, R. (1995). Permethrin resistance in the head louse *Pediculus capitis* from Israel. Med.Vet. Entomol. *9*, 427-432.
22. Rupes, V., Moravec, J., Chmela, J., Ledvinka, J., and Zelenkova, J. (1995). A resistance of head lice (*Pediculus capitis*) to permethrin in Czech Republic. Centr. Eur. Publ. Hlth. *3*, 30-32.
23. Downs, A.M.R., Stafford, K.A., and Coles, G.C. (1999). Head lice: prevalence in schoolchildren and insecticide resistance. Parasitol. Today *15*, 1-4.
24. Chosidow, O., Chastang, C., Brue, C., Bouvet, E., Izri, M., Monteny, N., Bastuji-Garin, S., Rousset, J.-J., and Revuz, J. (1994). Controlled study of malathion and d-phenothrin lotions for *Pediculus humanus* var *capitis*-infested schoolchildren. Lancet *344*, 1724-1727.

25. Lee, S.H., Yoon, K.-S., Williamson, M.S., Goodson, S., J., Takano-Lee, M., Edman, J.D., Devonshire, A., and Clark, J.M. (2000). Molecular analysis of kdr-like resistance in permethrin-resistant strains of head lice, *Pediculus capitis*. Pestic. Biochem. Physiol. *66*, 130-143.
26. Meinking, T.L., Entzel, P., Villar, M.E., Vicaria, M., Lemard, G.A., and Porcelain, S.L. (2001). Comparative efficacy for treatments for pediculosis capitis infestations: update 2000. Arch. Dermatol. *137*, 287-292.
27. Meinking, T.L., Serrano, L., Hard, B., Entzel, P., Lemard, G., Rivera, E., and Villar, M.E. (2002). Comparative in vitro pediculicidal efficacy of treatments in a resistant head lice population in the United States. Arch. Dermatol. *138*, 220-224.
28. Meinking, T.L.C., C.M., Chen, C., Kolber, M.A., Tipping, R.W., Furtek, C.I., Villar, M.E. and Guzzo, C.A. (2002). An observer-blinded study of 1% permethrin cream rinse with and without adjunctive combing in patients with head lice. J. Pediatr. 1*41,* 665-670.
29. Pray, W.S. (2003). Pediculicide resistance in head lice: A survey. Hosp. Pharm. 3*8,* 241-246.
30. Rose, G.P., and Dewar, A.J. (1983). Intoxication with four synthetic pyrethroids fails to show any correlation between neuromuscular dysfunction and neurobiochemical abnormalities in rats. Arch. Toxicol. 5*3,* 297-316.
31. Aldridge, W.N. (1980). Mode of action of pyrethroids in mammals: summary of toxicity and histological, neurophysiological and biochemical studies. In Pyrethroid Insecticides: Chemistry and Action, Volume 37, J. Mattieu, ed. (Table Ronde Roussel), p. 45.
32. LeQuesne, P.M., Maxwell, I. C., and Butterworth, S. T. G. (1980). Transient facial sensory symptoms following exposure to synthetic pyrethroids: a clinical and electrophysiological assessment. Neurotoxicol. 2*,* 1-11.
33. Kaloyanova, F.P., and El Batawi, M.A. (1991). Human Toxicology of Pesticides (Boca Raton, Florida: CRC Press). .
34. Bacot, A. (1917). A contribution to the bionomics of *Pediculus humanus* (vestimenti) and *Pediculus capitis*. Parasitol. 9*,* 228-258.
35. Nuttall, G.H.F. (1917). The biology of *Pediculus humanus*. Parasitol. 1*0,* 5-185.
36. Wigglesworth, V.B. (1941). The sensory physiology of the human louse *Pediculus humanus corporis* De Geer (Anoplura). Parasitol. 3*3,* 67-109.
37. Pedra J.H., Brandt, A., Li, H.M., Westerman, R., Romero-Severson, J., Pollack, R.J., Murdock, L.L., and Pittendrigh, B.R. 2003. Transcriptome identification of putative genes involved in protein catabolism and innate immune response in human body louse (Pediculicidae: *Pediculus humanus*). Insect Biochem. Mol. Biol. 33(11):1135-43.

Barry R. Pittendrigh
Purdue University
901 West State Street
West Lafayette, IN 47907-2089, USA

Dear Barry Pittendrigh,

We, the undersigned, representing international members of the research community working on parasitic lice, write to enthusiastically support your proposal to sequence the human body louse genome. We recognise the tremendous implications for human health, human biology, entomology and ecology that these data would yield and express our strong endorsement of this project.

Parasitic lice are insects of major medical and veterinary importance, as parasites, pests, and vectors of animal and human diseases. Human lice in particular have had a tremendous impact on the course of human history, historically as vectors of louse borne typhus, and more prominently in recent times through the epidemic of head lice infecting school age children. Despite this status there are no large-scale genome efforts dedicated toward this group. This is a major impediment to fundamental louse research and ultimately to the development of new louse and louse-borne disease control strategies. Among the many potential insights from this project will be opportunities to identify novel biologically active molecules such as louse salivary gland proteins to develop new vaccine targets; help unravel the complicated molecular mechanisms that underpin louse drug resistance; and provide a major boost to current efforts to develop markers for phylogenetic analysis to help solve outstanding questions regarding the evolution of vectoring capabilities in lice. These efforts together with the genome sequence will promote complementary studies of louse morphology, molecular systematics, phylogeny and population biology.

The louse research community has conducted many decades of research on various aspects of louse biology, physiology, genetics, population biology, ecology, pathogen transmission and the control of lice and louse-borne disease. We endorse this exciting proposal, and are poised to make use of these data for informed interpretation of the function, mechanism, organization and evolution of this parasites genome.

Sincerely,

Signed by 65 scientists working at research institutions in 24 countries.

Names, research specialization, institution, country and e-mail addresses as attached. Original e-mail messages from signees available from Vincent S. Smith (vsmith@inhs.uiuc.edu) upon request.

Centre Collaborateur OMS
WHO Collaborative Center

UNITE DES RICKETTSIES

UPRES A 6020

Centre National de Référence
Rickettsial Reference and Research

Didier Raoult	PU-PH - Directeur
Jean-Louis Mege	PU-PH
Michel Drancourt	PU-PH
Philippe Brouqui	PU-PH
Andreas Stein	PU-PH
Bernard La Scola	PU-PH
Georges Grau	PR
Jean-Marc Rolain	PR
Marie-Noelle Mallet	MCU-PH
Hubert Lepidi	MCU-PH
Véronique Roux	MCF
P-Edouard Fournier	MCU-PH
Florence Fenollar	MCU-PH
Catherine Tamalet	PH
Hervé .Tissot- Dupont	PH
Jean-Paul Casalta	PH
Pierre.Yves Levy	PH
Christian Capo	CRI
Marie Suzan	CRI
Pablo Gluschenkof	CRI
Patricia Renesto	CRI
Eric Ghigo	CRI

Barry Pittendrigh
Purdue University
901 West State Street
West Lafayette, IN 47907-2089
U. S. A.

Marseille, 27 June 2005

Dear Barry,

I am very please to write a letter of support of your proposal for a louse genome project. The human louse has been the vector of large and terrifying outbreaks. It is very efficient in transmitting Typhus, relapsing fever and trench fever, and causes millions of deaths during the XIX and XX centuries. We have laboratory evidences that it is able to transmit other bacterial diseases and to determine new outbreaks.

It keeps its epidemic potential in developpping country and causes a giant outbreak of Typhus and trench fever beginning in 1996 and involving more than 100.000 peoples. It is also a threat in the homeless population in rich countries. In my home town, Marseille, 30% of homeless have body lice and 5% a louse borne chronic infection caused by Bartonella quintana.

A better understanding of the louse physiology based on the study of its genome can definitely help to fight this threat.

Sincerely yours.

Professor D. RAOULT

Faculté de Médecine - 27, boulevard Jean Moulin – 13385 Marseille Cedex – France
☎ 33 (0)4 91 32 43 75 – 33 (0)4 91 38 55 17 ✉ 33 (0)4 91 83 03 90
Didier.Raoult@medecine.univ-mrs.fr

Barry Pittendrigh
Purdue University
901 West State Street
West Lafayette
IN 47907-2089
USA

Vincent S. Smith
Illinois Natural History Survey
607 East Peabody Drive
Champaign, IL, 61820-6970
USA

http://darwin.zoology.gla.ac.uk/~vsmith/

June 23, 2005

Dear Barry,

The letter is to acknowledge the enthusiastic support of Rod Page (University of Glasgow), David Reed (University of Florida) and myself (Vince Smith, Illinois Natural History Survey) for the Human Body Louse Genome Project. The data generated from this sequencing effort will have a profound impact on the research programs of ourselves and our collaborators, opening avenues of study that have hitherto not been feasible.

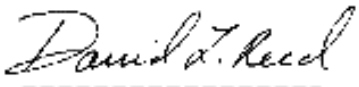
This project will provide the first genomic overview of a non-holometabolus insect, generating a comparative framework that will improve our gene prediction capabilities. Specifically, these data will enable us to identify conserved insect specific genes, divergent orthologs, and differentially expanded paralogous gene families amongst the Arthropoda. Our experience with the molecular biology of parasitic lice leads us to expect that the human body louse genome will be highly unusual. All lice possess long indels in their ribosomal genes, generating secondary structures within some louse genera that exhibit more variation than is known from the rest of the class Insecta. This leads to dramatically elevated rates of sequence substitution in many louse taxa including human lice. Present data suggests that compared to other insects, louse genomes are G-C rich, potentially simplifying the task of assembly.

As the first obligate parasitic insect for genomic sequencing, human body lice are uniquely placed to study the molecular mechanisms underpinning the acquisition and transmission of bacterial pathogens. Body lice transmit epidemic typhus (*Rickettsia prowazekii*), trench fever (*Bartonella quintana*) and louse-borne relapsing fever (*Borrelia recurrentis*) to humans, yet their close relatives, the human head lice, do not vector these diseases. The proposed additional 3X coverage of the head louse genome may help to establish why this is the case. Furthermore, *R. prowazekii* is the closest extant relative to the ancestor of mitochondria in eukaryotic cells, forming a group of alpha-proteobacteria that are closely related to another bacterial symbiont (*Wolbachia*) present in most insects. Understanding the molecular interactions between these bacteria in the louse genome may shed light of the evolution of these ancient bacterial associations.

Finally, we are each involved in the development of web based databasing tools to facilitate the integration and discovery of taxonomic, molecular, and phylogenetic data (http://www.biocorder.org/). We welcome this opportunity to synthesize this genomic component within our databasing efforts, and look forward to future interactions with your group and the insect research community in the analysis and annotation of the human louse genome.

Sincerely yours,





Dr. Vincent S. Smith
INHS
Research Scientist

Prof. Roderic D.M. Page
Professor in Taxonomy and Editor Elect,
Systematic Biology

Dr. David L. Reed
Curator of Mammals at FL
Museum of Natural History

ILLINOIS NATURAL HISTORY SURVEY

UNIVERSITY of GLASGOW

UNIVERSITY OF FLORIDA

INTEGRATIVE BIOLOGY

June 23rd, 2005

Stephen Cameron
Department of Integrative Biology
401 Witdsoe Building
Brigham Young University
Provo, Utah 84602
Office: (801) 422-1396
Lab: (801) 422-5363

Barry R. Pittendrigh,
Purdue University,
901 West State Street,
West Lafayette, IN 47907-2089,
USA.

Dear Prof. Pittendrigh,

We are writing to give our enthusiastic support to the proposal to sequence the nuclear genome of the human louse, *Pediculus humanus*. The sequencing of this genome will greatly improve aid our efforts to understand mitochondrial (mt) genomic evolution in both lice and in general. We have recently been funded by the NSF to investigate mt genome evolution within lice (DEB 0444972) because lice have the highest rates of mt genomic rearrangement yet documented in any animal group. Additionally lice exhibit high rates of mt nucleotide substitution, block deletions of portions of the mt genome, mitochondrial recombination and the macroevolutionary persistence of heteroplasmic mt genome populations. All of these phenomena challenge basic notions of mt inheritance and several are responsible for, or associated with, degenerative age-related diseases in humans. Lice are therefore an excellent system in which to examine nuclear-mitochondrial genomic interactions and how mt genomic stability is achieved. Comparative genomics between *P. humanus* and the other insect genomes which are available, will allow us to examine how the loss or modification of genes responsible for mt genomic homeostasis has led it's breakdown in lice. By extension these insights can be able to be applied to human mt genomic maintenance and greatly advance our understanding of the underlying causes of mt diseases or those with an mt genomic component such as Alzheimer's and Parkinson's.

We are greatly encouraged by your attempt to sequence the louse nuclear genome and feel that this information will have great impacts on our understanding of both insect and general biology.

Sincerely,

Stephen L. Cameron
Department of Integrative Biology
Brigham Young University

Kevin P. Johnson
Illinois Natural History Survey

INTEGRATIVE BIOLOGY

June 23rd, 2005

Barry R. Pittendrigh,
Purdue University,
901 West State Street,
West Lafayette, IN 47907-2089,

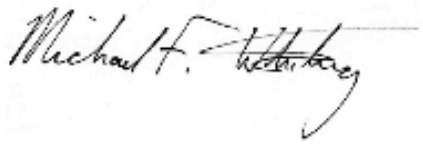
Dear Prof. Pittendrigh,

I am writing to give my enthusiastic support for your proposal to sequence the nuclear genome of the human louse, *Pediculus*. Apart from the obvious usefulness of this project to fight an important human pathogen and vector of serious disease, this research will be incredibly useful to the insect systematics community. We have already seen that the publication of the first two insect genomes, *Drosophila* and *Anopheles*, have been a boon to scientists working on fly evolution, allowing very efficient discovery and application of new genes to phylogenetic study (Weigmann and colleagues, the Diptera Tree of Life consortium). The draft sequence of the honeybee, *Apis*, and pending publication of a beetle, *Tenebrio*, will provide comparable stimulus to workers in those orders, and allow comparative studies across the Holometabola, the most highly derived and speciose insect group. What is desperately needed to enhance comparative genomics across the insects are genomes from earlier branching groups such as *Pediculus*. The sequencing of *Pediculus* will greatly extend the range over which comparative genomics can be applied to insect evolution.

Additionally, a *Pediculus* genome would be an important step towards insect developmental evolution. All current insect genomes are from holometabolous insects, which have a complete metamorphic life cycle (i.e., egg, larve, pupa, adult). There is immense comparative value of genome sequences from non-holometabolous insects, which do not undergo the major rearrangement of their body form in the pupa. The opportunity to examine the evolution of insect developmental genetics from a full genomics perspective will provide new insights into insect evolution and diversification.

The sequencing of the *Pediculus* genome is timely, will greatly aid all branches of entomology, and has my full and enthusiastic support.

Sincerely,



Michael F. Whiting
Associate Professor and Curator
Department of Integrative Biology
Brigham Young University

BRIGHAM YOUNG UNIVERSITY • DEPARTMENT OF INTEGRATIVE BIOLOGY
401 WIDTSOE BUILDING • PROVO, UTAH 84602-5181
(801) 422-2582 / FAX: (801) 422-0090