Lifestyle and compact genome of the body louse provide a unique functional genomics opportunity

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The body louse, with its recently sequenced genome, is now primed to serve as a powerful model organism for addressing fundamental questions relating to how insects interact with their environment. One characteristic of the body louse that facilitates this research is the size of its genome—the smallest insect genome sequenced to date. This diminutive genome must nonetheless control an organism that senses and responds to its environment, reacting to threats of corporal and genomic integrity. Additionally, the body louse transmits several important human diseases compared to its very close relative, the head louse, which does not. Therefore, these two organisms comprise an excellent model system for studying molecular mechanisms associated with vector competence. To understand more fully the development of vector/pathogen interactions, we have developed an in vitro bioassay system and determined that the body louse genome appears to contain the genes necessary for RNAi. The body louse will therefore be useful for determining the set of conditions permissive to the evolution of vector competence.

The estimated 6 million extant species of insects, living in a great diversity of environments, have widely divergent food sources, with life-history patterns to match. Correspondingly, a proliferation of insect genome projects has provided an important opportunity to use functional genomics to determine how variation in gene products and cellular pathways permit insects and other organisms to adapt to a broad range of evolutionary challenges. A few model insect species (such as Anopheles gambiae, Drosophila melanogaster and Tribolium castaneum) have fairly well developed genomic and bioinformatic resources. However, the functional genomics of critical aspects of insect biology is still in its infancy. Functional genomics in non-model insects has not progressed more rapidly in part because many insect species have complex life histories that complicate the process of matching gene function with phenotype. Moreover, in most cases in which genomes of related species have been compared to ascertain functions, the species diverged so long ago that they now occupy very different ecological niches, making it difficult to identify and understand those genes and pathways that were critical for adaptations during the initial stages of species divergence. Yet another problem for functional genomics research is redundancy, the existence within a genome of multiple genes that perform the same or similar function. Redundancy limits the value of RNAi knockdown as a research tool—that is, RNAi knockdown of one gene may be compensated for by other genes or pathways so there may be no detectable effect on phenotype.1,2

Key words: RNAi, genome size, disease vector, functional genomics, insect genomics

Submitted: 11/23/10
Accepted: 11/24/10
DOI: 10.4161/cib.4.2.14279

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Addendum to: Kirkness EF; et al. Genome sequences of the human body louse and its primary endosymbiont provide insights into the permanent parasitic lifestyle. Proc Natl Acad Sci USA 2010; 107:12168–73; PMID: 20566863; DOI: 10.1073/pnas.1003379107.
Problems also confront researchers who study the functional genomics of insects that vector pathogens. Many insects that transmit human diseases have had long co-evolutionary relationships with the pathogens they vector. As noted, a long evolutionary or co-evolutionary history between insect and pathogen makes it difficult to determine the “first steps” that enabled an insect to evolve into a competent vector. Such problems in studying functional genomics may be less serious with the human body louse, Pediculus humanus humanus, and its close relative the head louse, Pediculus humanus capitis, than with many other insects. We suggest that the body louse along with the head louse for comparative studies of vector competence comprise an excellent system for functional genomics research.

For instance, critical questions concerning the molecular mechanisms regulating how organisms sense and respond to the environment and how at least one insect vector maintains and transmits a pathogen can be addressed with this species pairing. One reason the human body louse is an excellent model for functional genomics is that its life history is much simpler than that of most other insects. It is a free-living obligate ectoparasite whose only food is human blood and whose only habitat is human clothing. With respect to vector competence, the human body louse is ideal for studying functional genomics because it is a vector that has only recently diverged from the head louse, which is not a vector. Although some researchers have estimated that strains of body and head lice diverged as recently as 100,000 years ago, other estimates suggest that some body louse groups have emerged even more recently and more frequently from head louse populations under poor hygiene conditions. Regardless of the exact divergence time(s) for the body and head louse, they are very closely related species, sub-species or even potentially different ecotypes of the same species. The most recent head louse genome sequencing revealed that these louse genomes are 99.8% identical with each other in their nucleotide sequences (Lee SH, Clark JM, Pittendrigh PR, unpublished data). The near identity of the head and body louse genomes will facilitate the identification and investigation of critical gene products and pathways that determine differences in their competence as vectors.

Body lice differ from head lice in two significant ways. First, as noted, body lice (but not head lice) can transmit human diseases, including epidemic typhus (Rickettsia prowazekii), relapsing fever (Borrelia recurrentis) and trench fever (Bartonella quintana). The fact that body lice, but not head lice, transmit pathogenic bacteria prompts the question: is a loss of immune system-related ability to control the proliferation of such microbial agents a step to vector competency? Moreover, the genome of the body louse’s primary bacterial endosymbiont has also been sequenced. Thus, the genomic interactions of body louse, its endosymbiont and the bacterial diseases transmitted by body louse also represent a useful model to study the functional genomics of insect-bacteria interactions. Secondly, body lice can survive off the host (in clothing) but head lice cannot. To survive, head lice attach to the hair on the human scalp, where they take frequent blood meals (about every 6 h). In contrast, body lice take larger blood meals; moreover, they can withstand lower humidity and thus are able to survive when separated from the host for 48–72 h. Body lice are also 2–3 times larger than head lice and are more heavily sclerotized. All of these differences in ecological niches, life-history patterns and vector competence between head and body lice are well-defined and experimentally manipulable. Thus, body and head lice provide ideal subjects for comparative functional genomics analysis, with the goal of elucidating the molecular underpinnings of species or biotype divergence and vector competence.

Another advantage for using the body louse in functional genomics research is related to the size of its genome. The recent sequencing of the body louse genome has revealed that, although the body louse genome is sufficient for supporting basic physiological processes, those genes associated with sensing and responding to the environment have been dramatically reduced, with a substantial loss of potential redundancy in many systems. The body louse genome is 108 Mb and contains 10,773 predicted protein-coding genes. Relative to other insect species with sequenced genomes, the body louse genome has a reduced number of odorant-, gustatory- and chemosensory-related genes, has a complete but minimal insulin/target of rapamycin (TOR) pathway, and has a reduced set of detoxification enzyme-coding genes. Additionally, with regard to phase I detoxification enzymes, the body louse has only 37 cytochrome P450s, the smallest number of P450s so far observed in any insect species. This reduced set of genes may permit the successful use of RNAi to identify key genes in sensing and responding to the environment, without the confounding effects of redundancy that stymie the use of RNAi in many other insect systems.

The development of body louse and head louse as a model system requires an in vitro rearing system, which will facilitate controlled laboratory experiments, and a RNAi system to reduce the expression levels of target genes thought to be critical for a particular biological response. An in vitro rearing system has been developed for human body and head lice, and work in progress by the research groups of Clark, Lee and Pittendrigh suggests that the injection of dsRNA results in reduced expression of target genes. Although not specifically identified in the recent body louse genome paper, many of the known genes associated with RNAi in other insects are found in the body louse genome (e.g., Drosophila melanogaster; Table 1). To this end, the Clark, Lee, Berenbaum and Pittendrigh laboratories are now using the head louse/body louse combined system to understand genes and gene pathways that influence vector competence, response to environmental stimuli and detoxification of xenobiotics. Furthermore, the presence of many of the protein components of the small non-coding (nc) RNA pathways (e.g., esiRNA, miRNA, piRNA) strongly suggests that the louse genome also possesses the machinery necessary to mount a response to invasion by nucleic acid parasites. The louse genome is curiously bereft (ca. 1% of the genome) of the “junk” baggage of transposons and other repetitive elements that seem to plague other insect genomes. Perhaps the paucity of evidence for nucleic acid invaders in the louse genome reflects
Table 1. Genes identified in body louse that are homologous to members of the RNAi pathway in the fruit fly Drosophila melanogaster*

| Gene or microRNA name | Symbol | Flybase ID | Body louse | E-value |
|-----------------------|--------|------------|------------|---------|
| Argonaute 1           | AGO1   | FBgn0026611| PHUM617260-PA | 0.00E+00 |
| Argonaute 2           | AGO-2  | FBgn0046812| PHUM004130-PA | 1.00E-137 |
| Argonaute 3           | AGO-3  | FBgn0250816| PHUM411830-PA | 1.00E-162 |
| armitage              | armi   | FBgn0041164| PHUM074090-PA | 1.00E-158 |
| Ars2                  | Ars-2  | FBgn0033062| PHUM507110-PA | 0.00E+00 |
| aubergine             | aub    | FBgn0000146| PHUM653960-PA | 0.00E+00 |
| capsuleen             | csul   | FBgn0015925| PHUM336830-PA | 1.00E-125 |
| Dicer-1               | Dcr-1  | FBgn0039016| PHUM435060-PA | 0.00E+00 |
| Dicer-2               | Dcr-2  | FBgn0034246| PHUM174480-PA | 5.00E-78  |
| drosha                | drosha | FBgn0026722| PHUM524860-PA | 0.00E+00 |
| Fmr1                  | Fmr1   | FBgn0028734| PHUM440700-PA | 1.00E-141 |
| gawky                 | gw     | FBgn0051992| PHUM421980-PA | 9.00E-76  |
| Ge-1                  | Ge-1   | FBgn0032340| PHUM294360-PA | 3.00E-67  |
| Hen1                  | Hen-1  | FBgn0033686| PHUM430970-PA | 1.00E-58  |
| loquacious            | loqs   | FBgn0032515| PHUM559590-PA | 3.00E-90  |
| maternal expression   | me31B  | FBgn0004419| PHUM345900-PA | 0.00E+00 |
| microRNA encoding gene mir-14 | mir-14 | FBgn0046827| Present     | N/A |
| mir-184 (microRNA)    | mir-184| FBgn0067726| Present     | N/A |
| partner of drosha     | pasha  | FBgn0039861| PHUM574170-PA | 1.00E-160 |
| piRNA methyltransferase| Hen-1/pimmet | FBgn0033686| PHUM430970-PA | 1.00E-58 |
| piwi                  | piwi   | FBgn0004872| PHUM653960-PA | 1.00E-167 |
| r2d2                  | r2d2   | FBgn0031951| PHUM504330-PA | 1.00E-12  |
| twin                  | twin   | FBgn0039168| PHUM129580-PA | 5.00E-90  |
| zucchini              | zuc    | FBgn0004056| PHUM318600-PA | 1.00E-16  |
| Hsp90                 | Hsp90  | FBgn0001233| PHUM581090-PA | 0.00E+00 |
| Rm62                  | Dmp68  | FBgn0003261| PHUM521070-PA | 1.00E-176 |
| vasa intronic gene    | VIG    | FBgn0024183| PHUM032630-PA | 4.00E-24  |
| vasa intronic gene-2  | VIG-2  | FBgn0046214| PHUM032630-PA | 9.00E-30  |
| spindle-E (homeless)  | sp-E   | FBgn0003483| PHUM492140-PA | 0.00E+00 |
| Yb                    | Yb     | FBgn0000928| PHUM090360-PA | 1.00E-16  |
| Rhino                 | Rhino  | FBgn0004400| PHUM169860-PA | 3.00E-10  |
| Elp1 (RNA-dependent RNApol) | Elp1  | FBgn0037926| PHUM473060-PA | 1.00E-136 |

*Additionally, two microRNAs potentially associated with RNAi are also given.

a particularly active maintenance function of small ncRNAs bestowing “genetic memory” of resident parasites through gene silencing mechanisms. Again, comparative genomics of the unique aspects of louse life histories, including study of their silencing machinery, may reveal novel and informative adaptations of this organism to its environment.

References
1. Kan L, Kessler JA. New tool for an old problem: can RNAi efficiently resolve the issue of genetic redundancy? Bioessays 2005; 27:14-6.
2. Vavouri T, Semple J, Lehner B. Widespread conservation of genetic redundancy during a billion years of eukaryotic evolution. Trends Genet 2008; 24:485-8.
3. Kirtler R, Kayser M, Stoneking M. Molecular Evolution of Pediculus humanus and the Origin of Clothing. Curr Biol 2004; 14:1414-7.
4. Reed DL, Light JE, Allen JM, Kirkman JJ. Pair of lice lost or parasites regained: the evolutionary history of anthropoid primate lice. BMC Biol 2007; 5:7.
5. Li W, Oriol G, Gimenez G, Reed DL, Pittendrigh BR, Raoul L. Genotyping of human lice reveal multiple emergences of body lice from local head louse populations. PLOS Negl Trop Dis 2010; 4:641.
6. Piesman P, Gates L. Bacterial and ricksettial diseases. Boston, MA: Kluwer Academic, 2000.
7. Kelly DJ, Richards AL, Temenak J, Strickman D, Dasch GA. The past and present threat of rickettsial diseases to military medicine and international public health. Clin Infect Dis 2002; 34:245.
8. Kirkness EF, Haas BJ, Sun W, Braig HR, Perrotti MA, Clark JM, et al. Genome sequences of the human body louse and its primary endosymbiont provide insights into the permanent parasitic lifestyle. Proc Natl Acad Sci USA 107:12168-73.
9. Lee SH, Min JS, Yoon KS, Strycharz JP, Johnson R, Mitapoli O, et al. Decreased detoxification genes and genome size makes the human body louse an efficient model to study insecticide resistance. Insect Mol Biol 2010; 19:599-615.
10. Takano-Lee M, Yoon KS, Edman JD, Mullens BA, Clark JM. In vivo and in vitro rearing of Pediculus humanus capitis (Anoplura: Pediculidae). J Med Entomol 2010; 40:628-35.
11. Yoon KS, Strycharz JP, Gao JR, Takano-Lee M, Edman JD, Pittendrigh BR. Decreased number of detoxification genes makes the human body louse an efficient model to study xenobiotic metabolism and insecticide tolerance. Abstract Book, Fourth International Conference on Phthiraptera. Cappadocia, Turkey: Turkish Society of Parasitology, 2010; 78.
13. Miyoshi T, Takeuchi A, Siomi H, Siomi MC. A direct role for Hsp90 in pre-RISC formation in Drosophila. Nat Struct Mol Biol 17:1024-6.
14. Ishizuka A, Siomi MC, Siomi H. A Drosophila fragile X protein interacts with components of RNAi and ribosomal proteins. Genes Dev 2002; 16:2497-508.
15. Grachev E, Das M, Elgin SC. Drosophila RISC component VIG and its homolog Vig2 impact heterochromatin formation. PLoS One 2009; 4:6182.
16. Pal-Bhadra M, Leibovitch BA, Gandhi SG, Rao M, Bhadra U, Birchler JA, et al. Heterochromatic silencing and HP1 localization in Drosophila are dependent on the RNAi machinery. Science 2004; 303:669-72.
17. Olivieri D, Sykora MM, Sachidanandam R, Mechtler K, Brennecke J. An in vivo RNAi assay identifies major genetic and cellular requirements for primary piRNA biogenesis in Drosophila. EMBO J 2010; 29:3301-17.
18. Klattenhoff C, Xi H, Li C, Lee S, Xu J, Khurana JS, et al. The Drosophila HP1 homolog Rhino is required for transposon silencing and piRNA production by dual-strand clusters. Cell 2009; 138:1137-49.
19. Lipardi C, Paterson BM. Identification of an RNA-dependent RNA polymerase in Drosophila involved in RNAi and transposon suppression. Proc Natl Acad Sci USA 2009; 106:15645-50.
20. Girard A, Hannon GJ. Conserved themes in small-RNA-mediated transposon control. Trends Cell Biol 2008; 18:136-48.