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Simplify, simplify

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.

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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]

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.[3,4] 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 lice groups have emerged even more recently and more frequently from head louse populations under poor hygiene conditions.[3,5] 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.[5] 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*).[6,7] 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.[8] 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.[8] 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.[9] 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,[10,11] 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.[12] Although not specifically identified in the recent body louse genome paper,[8] many of the known genes associated with RNAi in other insects are found in the body louse genome (e.g., *Drosophila melanogaster*; **Table 1**).[13-19] 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.[20] 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.[8] 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	PHUM563960-PA	0.00E+00
capsuléen	*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	PHUM249360-PA	3.00E-67
Hen1	*Hen-1*	FBgn0033686	PHUM430970-PA	1.00E-58
loquacious	*loqs*	FBgn0032515	PHUM559590-PA	3.00E-90
maternal expression at 31B	*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	PHUM563960-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 intonic gene-2	*VIG-2*	FBgn0046214	PHUM032630-PA	9.00E-30
spindle-E (homeless)	*spn-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.

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