3rd Annual Citrus Health Research Forum

Use of Psyllid genome information for HLB bio-control

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Acknowledgements: We thank Dr. Robert Shatters and Dr. W. Evan Braswell for generously sharing information and providing suggestions on this topic.

Why is the Psyllid Genome information important?

Preventing the spread of insect-borne diseases. Plant, animal, and microbe genomes are already being sequenced. By adding insects to the mix, scientists can better understand the relationships between host, insect, and pathogen--and potentially stop disease transmission. "So if you think about mosquitoes, we currently have three genomes of mosquitoes that are vectors of malaria and other diseases. But there are other very similar, related mosquitoes that do not act as vectors, so we want to sequence non-vector species as well so we can determine what makes an arthropod a vector or not a vector," explained Daniel Lawson, a coordinator at the European Bioinformatics Institute in an interview with American Entemologist.

Assessing psyllid movement via genetic markers and then implementing interdicting procedures.

Modifying the psyllid to be a non-transmitter of Liberibcter asiaticus.

Design and use of genetic insecticides.

Modifying psyllid microbes to kill or negatively impact *D. citri*, or its ability to be a vector.

These are the days of genome sequencing opportunities.

Next generation sequencing opportunities/targets.

1. The Genome: The DNA sequence, usually generated through shotgun *de* novo sequencing (cloning randomly fragmented DNA into a high copy plasmid) or through selected target amplification for targeted resequencing. This information when properly assembled can be used as a blueprint of regulatory pathways of an organism.

2. The Transcriptome: The transcriptome (RNA) is the quantitative and qualitative set of transcripts in a cell. Transcriptomic data can help us interpret the functional elements of the genome thus revealing the molecular constituents of cells and tissues, and even temporally.

3. The Small RNA "ome": This allows discovery of small RNAs involved in gene regulation and in defense. The small RNA data when combined with layers of data from transcriptomic and shotgun sequence data can offer details on the gene regulation at localized regions that are responsible for global changes (miRNA regulations).

Numbers 2 and 3 also offer the opportunity to identify new genes, but also genes of associated and even undiscovered microbes including bacteria (Liberibacter) and viruses.

The Human Genome Project

Completed in 2003, the Human Genome Project (HGP) was a 13-year project coordinated by the U.S. Department of Energy and the National Institutes of Health. During the early years of the HGP, the Wellcome Trust (U.K.) became a major partner; additional contributions came from Japan, France, Germany, China, and others. See our history page for more information. Project goals were to:

- *identify* all the approximately 20,000-25,000 genes in human DNA,
- *determine* the sequences of the 3 billion chemical base pairs that make up human DNA,
- *store* this information in databases,
- *improve* tools for data analysis,
- *transfer* related technologies to the private sector, and
- *address* the ethical, legal, and social issues (ELSI) that may arise from the project.

Battelle Report: $3.8 Billion Investment in HGP Drove $796 Billion in Economic Impact, Created Jobs, and Launched Genomic Revolution (May 2011);
See Response From *Nature* (May 11, 2011)

But why should we sequence the psyllid (*D. citri*) genome and how can the information be used?

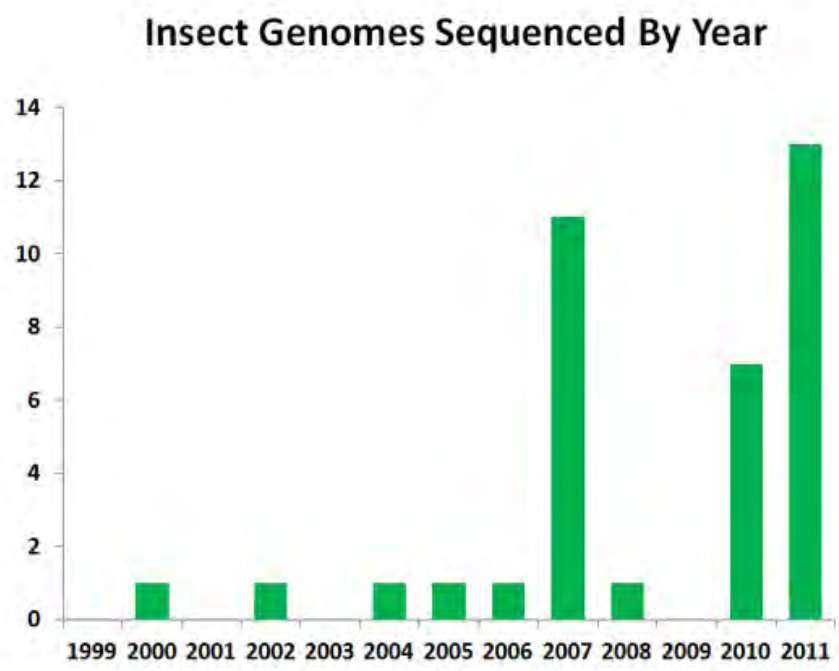
There is lots of interest now in many insect genomes, not just that for the ACP, and no one questions the potential benefit of the resulting information.

Having the psyllid genome will benefit many types of research and HLB control efforts.

Arthropoda

Acromyrmex echinatior
Acyrthosiphon pisum
Aedes aegypti
Anopheles darlingi
Anopheles gambiae
Anopheles gambiae str. PEST
Apis florea
Apis mellifera
Atta cephalotes
Bombus impatiens
Bombus terrestris
Bombyx mori
Camponotus floridanus
Culex quinquefasciatus
Danaus plexippus
Daphnia pulex
Drosophila ananassae
Drosophila biarmipes
Drosophila bipectinata
Drosophila elegans
Drosophila erecta
Drosophila eugracilis
Drosophila ficusphila
Drosophila grimshawi
Drosophila kikkawai
Drosophila melanogaster
Drosophila miranda
Drosophila mojavensis
Drosophila persimilis
Drosophila pseudoobscura
Drosophila pseudoobscura pseudoobscura
Drosophila rhopaloa
Drosophila sechellia
Drosophila simulans
Drosophila takahashii
Drosophila virilis
Drosophila willistoni
Drosophila yakuba
Harpegnathos saltator
Heliconius melpomene melpomene
Ixodes scapularis
Lepeophtheirus salmonis
Linepithema humile
Lutzomyia longipalpis
Manduca sexta
Mayetiola destructor
Megachile rotundata
Mengenilla moldrzyki
Metaseiulus occidentalis
Nasonia giraulti
Nasonia longicornis
Nasonia vitripennis
Pediculus humanus corpo
Phlebotomus papatasi
Pogonomyrmex barbatus
Priacma serrata
Rhipicephalus microplus
Rhodnius prolixus
Solenopsis invicta
Strigamia maritima
Tetranychus urticae
Tribolium castaneum
Varroa destructor

Currently sequenced and available arthropod genomes, many more are in progress.



Lanham, MD; June 15, 2011 – It's been called "the Manhattan Project of Entomology," an undertaking that has the potential to revolutionize the way we think about insects.

The i5k Initiative, also known as the 5,000 Insect Genome Project, was recently launched with a letter to *Science* from ten signers known as the i5k Ad Hoc Launch Group. Now the latest issue of *American Entomologist* features an **interview** with four of the signers about the project's origins, purpose, and goals.

The Initiative aims to sequence the genomes of 5,000 insects and other arthropods over the next five years in order to "improve our lives by contributing to a better understanding of insect biology and transforming our ability to manage arthropods that threaten our health, food supply, and economic security."

"We hope that generating this data will lead to better models for insecticide resistance, better models for developing new pesticides, better models for understanding transmission of disease, or for control of agricultural pests," said Daniel Lawson, a coordinator at the European Bioinformatics Institute. "Moving into the genetics era revolutionizes what you can do, what you can try to assay in your species, what you can infer from your experiments."

According to Gene E. Robinson, a professor at the University of Illinois at Urbana-Champaign, "This will provide information that breeders would need to look for ways of dealing with insect resistance to pesticides. It would also provide geneticists with information on what might be vulnerable points in an insect's makeup, which could be used for novel control strategies."

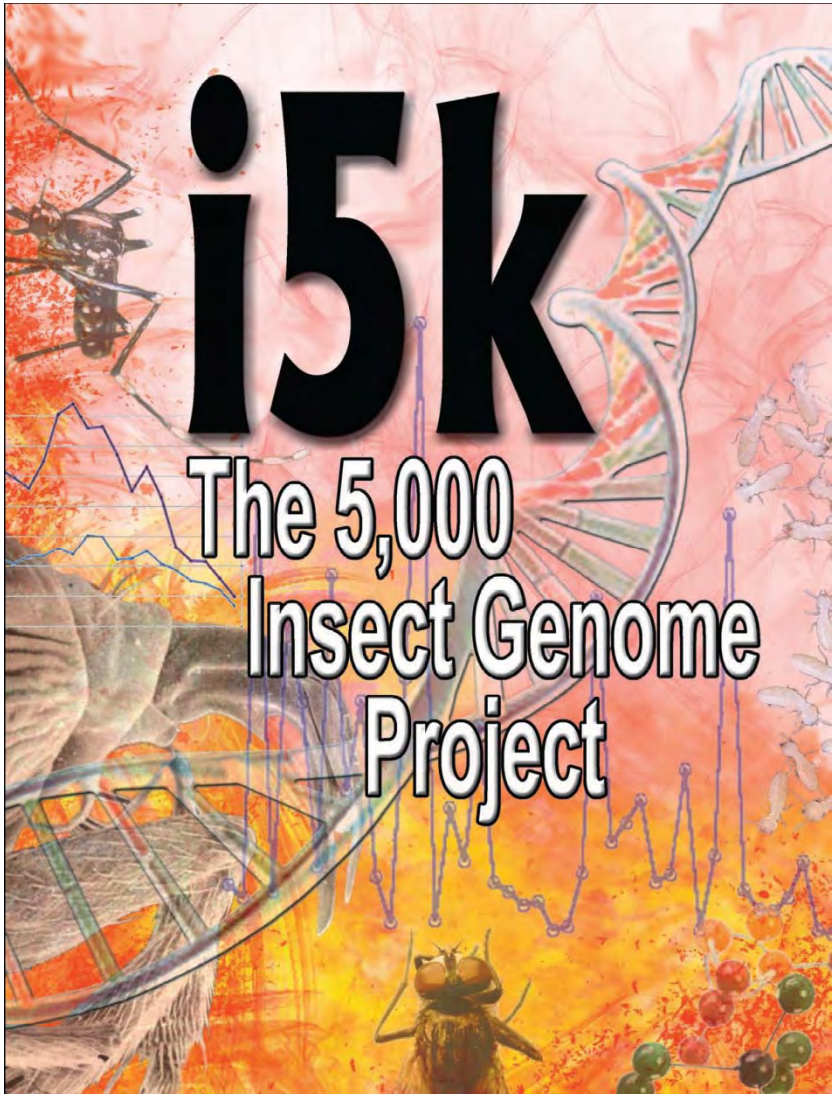
As the costs of genomic sequencing continue to fall due to technological improvements, it will soon become feasible to cheaply sequence the genomes of 5,000 insects of medical and agricultural importance, and then to mine the genomes for data which could lead to better insect control and management products and techniques.

"For example, we could mine data for cytochrome p450 detox genes. Those genes are involved with detoxifying chemicals that are inside insects, so if we know about those genes from one insect to another, we can use that information to actually kill the insects," said Kevin J. Hackett, a national program leader at the USDA Agricultural Research Service. "Or if you take beneficial insects like honey bees, which do not have as many detoxifying genes and are more susceptible to chemicals, that kind of information could be used to help protect bees."

The leaders of the i5k Initiative invite entomologists around the world to sign up and to create wiki pages at http://arthropodgenomes.org/wiki/i5K in order to recommend which insect genomes should be sequenced in the future, report which insect genomes are already being sequenced, and to start conversations with other scientists who are working on similar projects.

"We're trying to find out who's working on what insects, and if they feel that having genomic information about their insects would help," said Susan J. Brown, a professor at Kansas State University. "Quite a few researchers are probably working on transcriptomics, looking at the genes that are transcribed under certain contexts, environmental conditions or life stages. Looking at the whole genome will help us understand these comparatively and not just in one organism."

"We want this to be a broad-based, inclusive effort," said Dr. Robinson. "We want all people to be involved, we want all insects of agricultural importance, all insects of medical importance, and so forth. Workshops will be organized and held, and there will be opportunities for further input, interactions, and the ability to shape the project."



The Insect Genome Project Can Save Us From Disease

Now that we've sequenced human DNA, we move on to our six-legged friends, whose genes might yield important and lucrative discoveries in many fields.

The Human Genome Project was a $3.8 billion undertaking that has so far yielded over $800 billion in economic output. The 5,000 Insect Genome Project (i5k), an initiative that aims to sequence the genomes of 5,000 insects and arthropods over the next five years, could reap similar rewards--but for a fraction of the price.

The initiative's launch group, which includes researchers from the USDA, the European Bioinformatics Institute and Kansas State University, believes that it can sequence all 5,000 genomes for $5 million (and handle bioinformatics and data mining for another $10 million). This is just a fraction of the million or so insect species in existence, of course, but DNA sequencing costs are dropping constantly--so a goal of 5,000 insects is just the start, according to American Entemologist. What might we look forward to in a world where insect genomes have been decoded?

Better pesticides. Researchers can use sequencing information, computational analysis, and bioinformatics to figure out what changes in the genome make insect species resistant to certain pesticides. By mining data for the genes involved with detoxifying chemicals that make their way inside insects, for example, researchers could figure out how to target them. Researchers could also figure out how insect immune systems change in response to the use of biopesticides. We can already hear Monsanto salivating.

Protection for more vulnerable species. The same data about those detoxifying genes could be used to protect honeybees (which don't have as many detox genes to begin with) from being affected by pesticides--and that could help prevent our entire food system from crashing down.

Preventing the spread of insect-borne diseases. Plant, animal, and microbe genomes are already being sequenced. By adding insects to the mix, scientists can better understand the relationships between host, insect, and pathogen--and potentially stop disease transmission. "So if you think about mosquitoes, we currently have three genomes of mosquitoes that are vectors of malaria and other diseases. But there are other very similar, related mosquitoes that do not act as vectors, so we want to sequence non-vector species as well so we can determine what makes an arthropod a vector or not a vector," explained Daniel Lawson, a coordinator at the European Bioinformatics Institute in an interview with American Entemologist.

Development of detection devices for biodefense. If scientists can replicate insects' sharp sensory receptors, we could see all sorts of DARPA-like nanotech military spy cameras.

http://www.fastcoexist.com/1678171/the-insect-genome-project-can-save-us-from-disease

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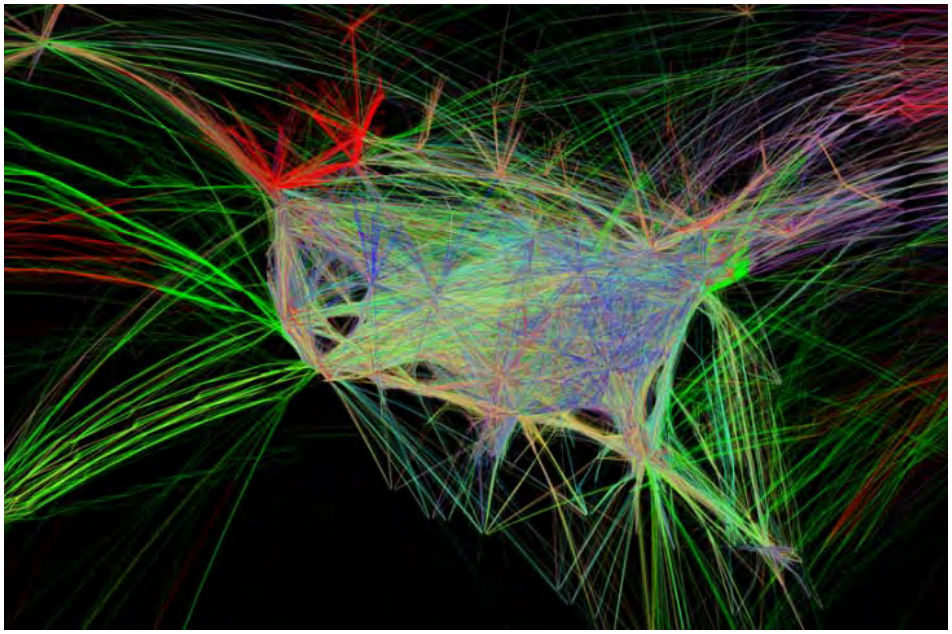
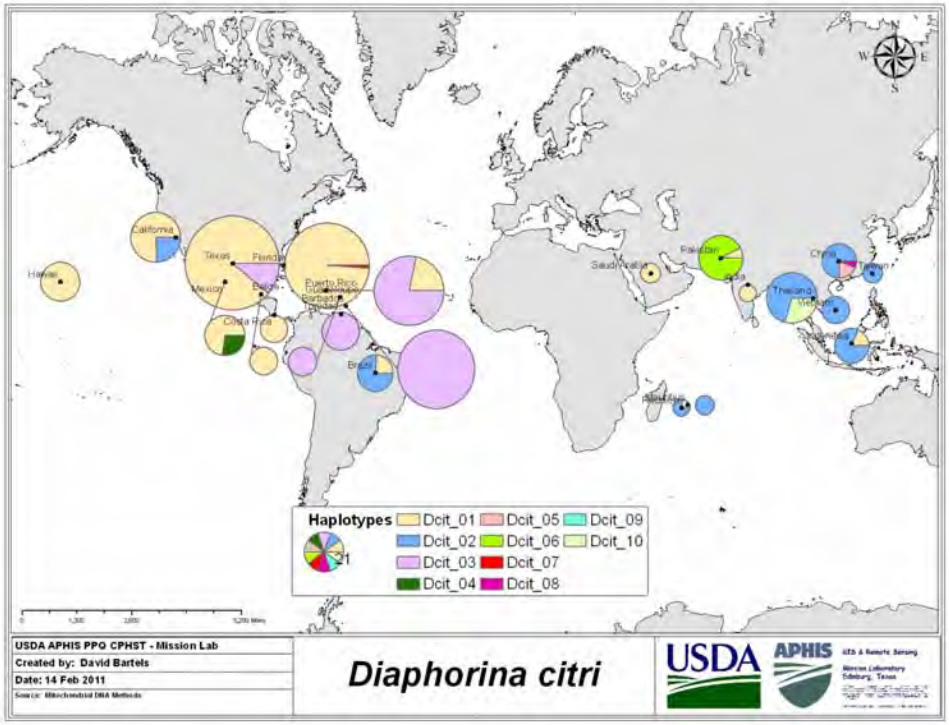
Molecular Pathway Analysis of Asian Citrus Psyllid

Evan Braswell
USDA APHIS PPQ CPHST Mission Laboratory

- Our ability to trace invasive populations is limited by the genetic variation we can detect.
- The ability to scan the ACP genome for variable sites will greatly improve our resolution for molecular pathway analysis.





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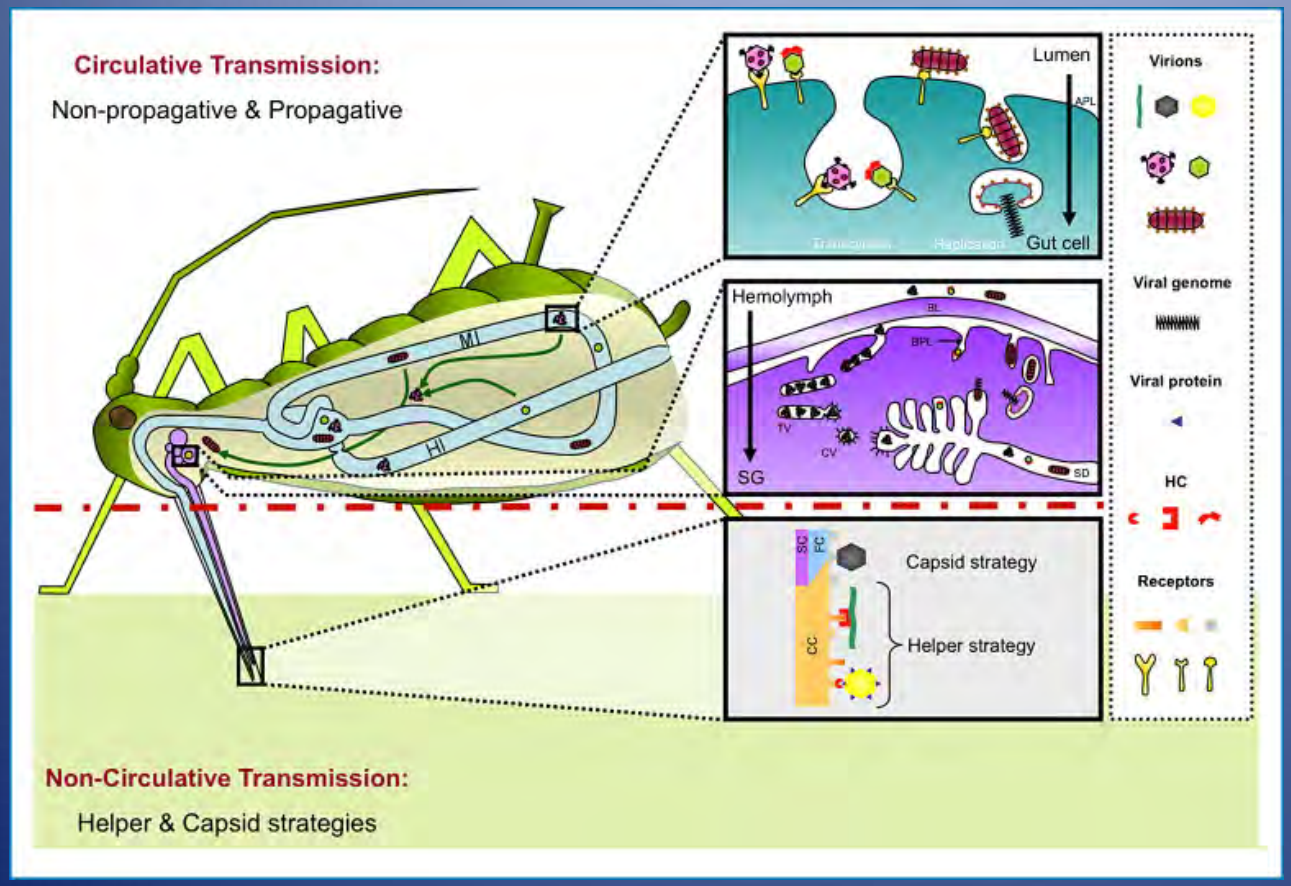
CAPS Grant: Blocking HLB Spread: Inhibiting Circulative Transmission



Using psyllid genome to identify receptors that aid bacterial movement within the psyllid

Véronique Brault et al, 2010. Virology 333: 524-538

Courtesy of Bob Shatters

Engineering RNA interference-based resistance to dengue virus type 2 in genetically modified *Aedes aegypti*

Alexander W. E. Franz*†, Irma Sanchez-Vargas*†, Zach N. Adelman‡, Carol D. Blair*, Barry J. Beaty*§, Anthony A. James¶‖, and Ken E. Olson*§

*Arthropod-Borne and Infectious Diseases Laboratory, Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO 80523; ‡Department of Entomology, 320 Price Hall, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061; and Departments of ¶Molecular Biology and Biochemistry and ‖Microbiology and Molecular Genetics, University of California, Irvine, CA 92697

4198–4203 | PNAS | March 14, 2006 | vol. 103 | no. 11

A. aegyptii has been sequenced and it can be transformed. It is a vector of several important human-infecting viruses including *Dengue virus.*

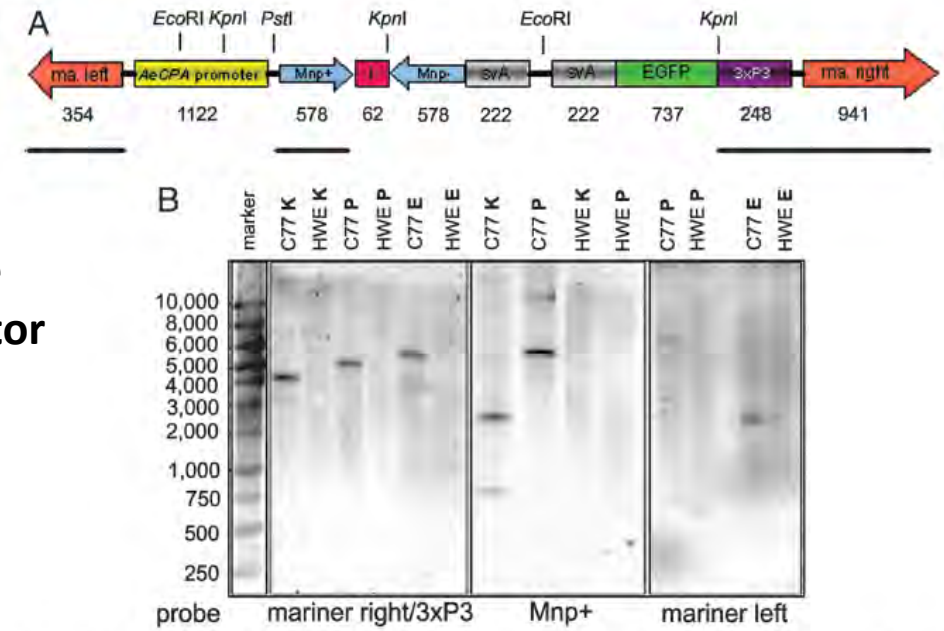


Fig. 1. Southern blot analysis of total DNA extracted from whole body mosquitoes. (*A*) Schematic representation of the Mos-carb/Mnp+/I/Mnp−/svA construct with restriction sites of endonucleases used for DNA digestions. ma. left/ma. right, left/right arms of *mariner MosI*; *AeCPA* promoter, *A. aegypti carboxypeptidase A* promoter; Mnp+, Mnp−, 578-bp cDNAs of the DENV-2 prM protein encoding region in sense and antisense orientations, respectively; I, minor intron of *A. aegypti sialokinin I* (44); svA, polyadenylation signal of Simian virus 40 VP1 gene. Numbers below indicate the sizes of the DNA fragments in base pairs. Black solid bars show the positions of the probes "mariner left," "Mnp+," and "mariner right/3xP3." (*B*) Southern blots after hybridization of EcoRI (E), KpnI (K), or PstI (P) digested total DNA of Carb77 (C77) and HWE with [α^{32}P] dCTP-labeled random-primed DNA probes complementary to the *mariner MosI* right arm/3xP3 fragment (blot on the left), Mnp+ (blot in the center), or *mariner* left arm (blot on the right). Blots were hybridized overnight at 45°C. The marker indicates DNA sizes in base pairs.

The transformed *A. aegyptii* are non-transmitters of *Dengue virus*

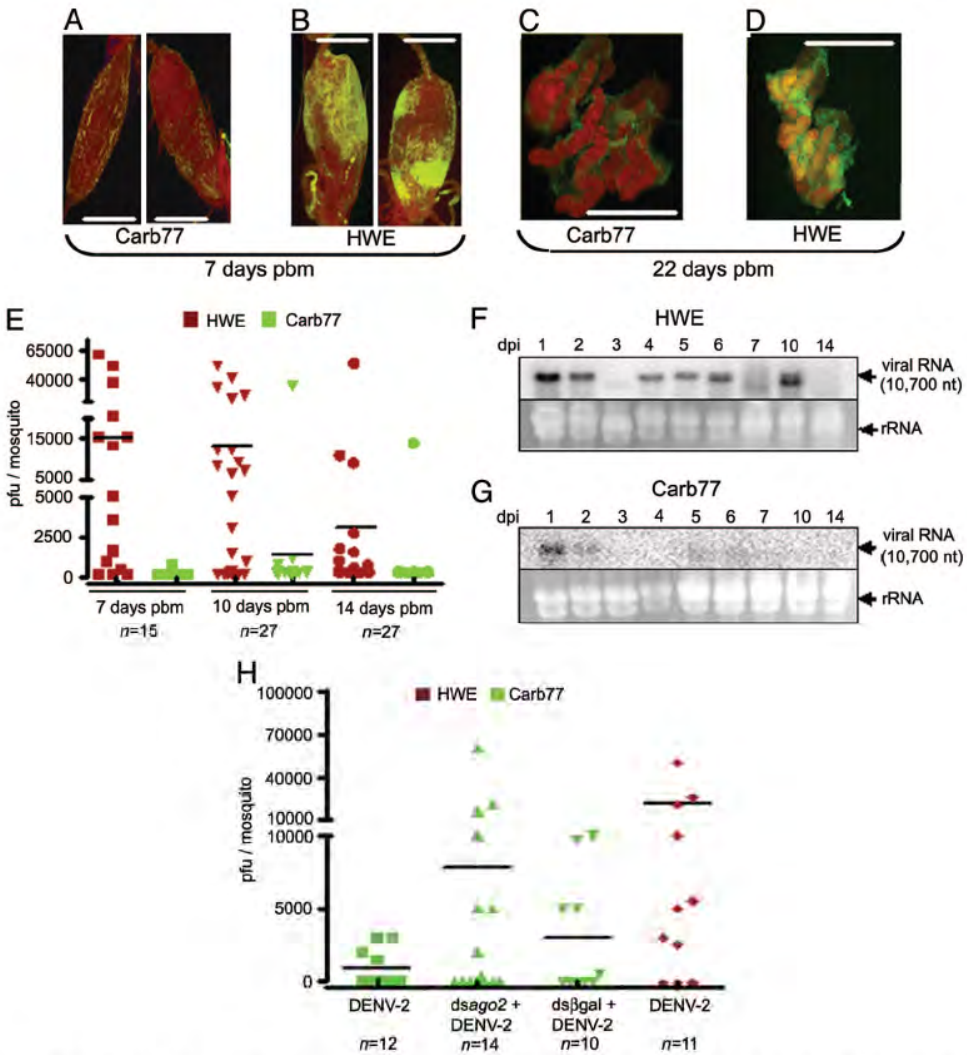


Fig. 3. Characterization of the Carb77 DENV-2 resistance phenotype. Detection of DENV-2 antigen in midguts (scale bars, 0.5 mm) (*A* and *B*) and in salivary glands (three salivary glands/image; scale bars, 0.5 mm) (*C* and *D*) of Carb77 and HWE by IFA by using mAb 3H5 recognizing an epitope of DENV-2 E. Images represent typical infection patterns. (*E*) DENV-2 plaque titrations of single whole body Carb77 and HWE mosquitoes 7, 10, and 14 days pbm (bars indicate mean values of titers). Plaque assays were performed in LLC-MK2 monkey kidney cells at 10-fold dilutions. (*F* and *G*) Detection of DENV-2 viral RNA in midguts of HWE (*F*) and Carb77 (*G*) 1–14 days pbm by Northern analysis by using "Mnp+" probe. Ribosomal RNAs are shown (*Lower*) to indicate amounts of RNA loaded per lane. (*H*) Transient silencing of *A. aegypti ago2* in Carb77 and HWE followed by challenge with a DENV-2-containing bloodmeal. Four days before bloodfeeding, 1 μg dsRNA was injected into 15 3-day-old females. β-gal dsRNA was used as a control. Virus titers of single mosquitoes were assessed 7 days pbm by plaque titration in LLC-MK2 cells (bars indicate mean values of titers).

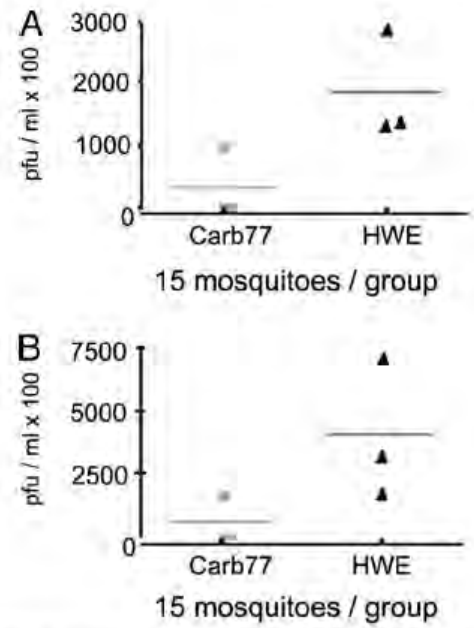


Fig. 4. *In vitro* transmission assay of DENV-2 by Carb77 and HWE 14 days pbm. Three batches of 15 mosquitoes each (Carb77 or HWE) were allowed to feed for 1–2 h on a solution that was placed between two parafilm membranes stretched over a glass feeder. The solution and fed mosquitoes were collected after feeding and separately analyzed for infectious DENV-2 by plaque assays in LLC-MK2 cells (bars indicate mean values of titers). (*A*) Virus titers in feeding solutions after exposure to mosquitoes. (*B*) Virus titers of the three groups of mosquitoes after feeding on the solutions analyzed in *A*.

Thus, *A. aegyptii* is resistant to DV infection and does not efficiently transmit DV.

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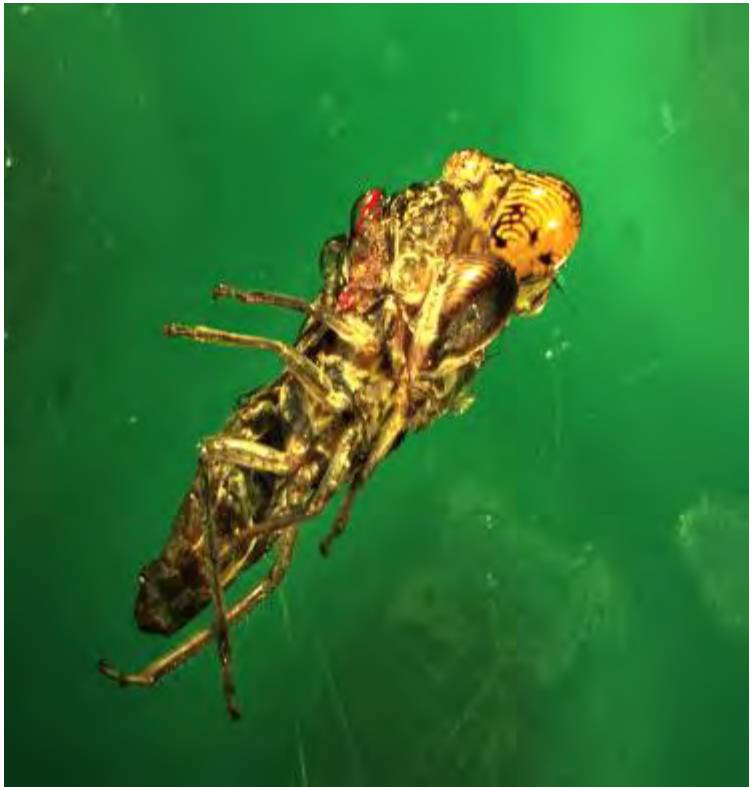


RNA interference (RNAi)
Actin dsRNA effects on GWSS nymphs

Thus, the correct dsRNA, delivered at correct stage, in the correct amount can induce a desired phenotype.

Asian Citrus Psyllid and Tomato Psyllid

1. The adult psyllid is a small insect (about 3.2mm)
2. The adults have white or yellowish markings on the thorax, clear wings, and lines on the abdomen that separate segments.
3. Newly emerged adults remain green for a day or so before turning darker.

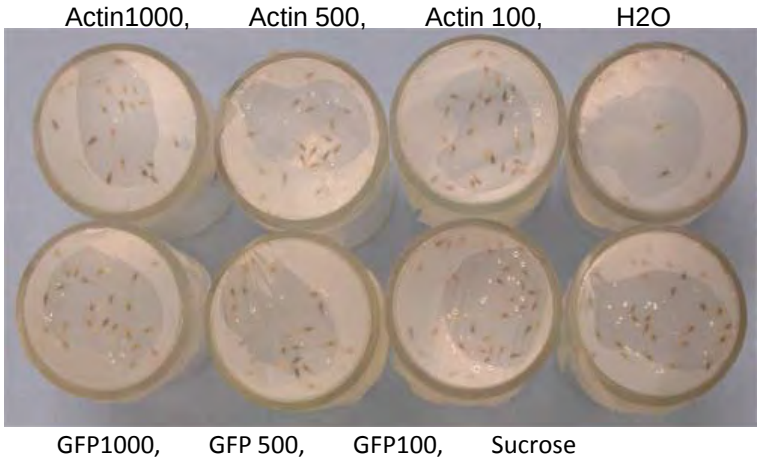
1. Nymph have scalelike flattened, oval, yellowish green to orangish bodies with red eyes and three pairs of short legs.
2. Older nymphs are greenish and fringed with hairs and have wing buds.
3. While feeding, psyllid nymphs excrete small, waxy beads of "psyllid sugar," which resembles granulated sugar. This material may cover leaves during heavy psyllid infestations.
4. The nymph stage usually lasts from 14 to 22 days.

1. Eggs are small, 0.8mm long.
2. Orange-yellow and supported by small stalks.
3. Frequently deposited along leaf margins but may occur on either leaf surface.
4. Hatch in 6 to 10 days.

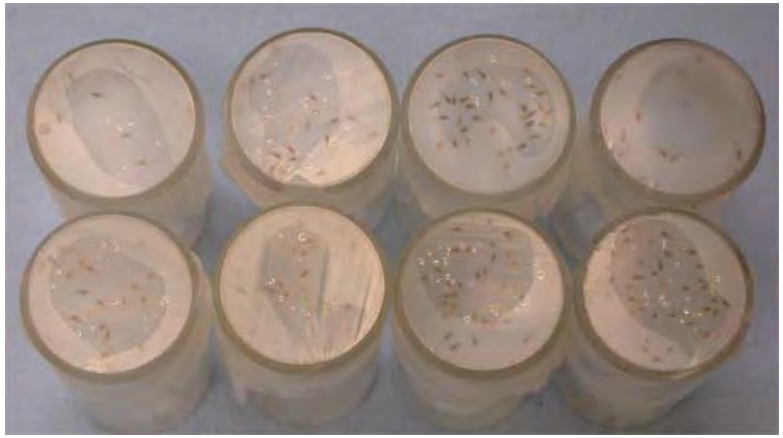
What are the potential genetic insecticides for *D. citri*?

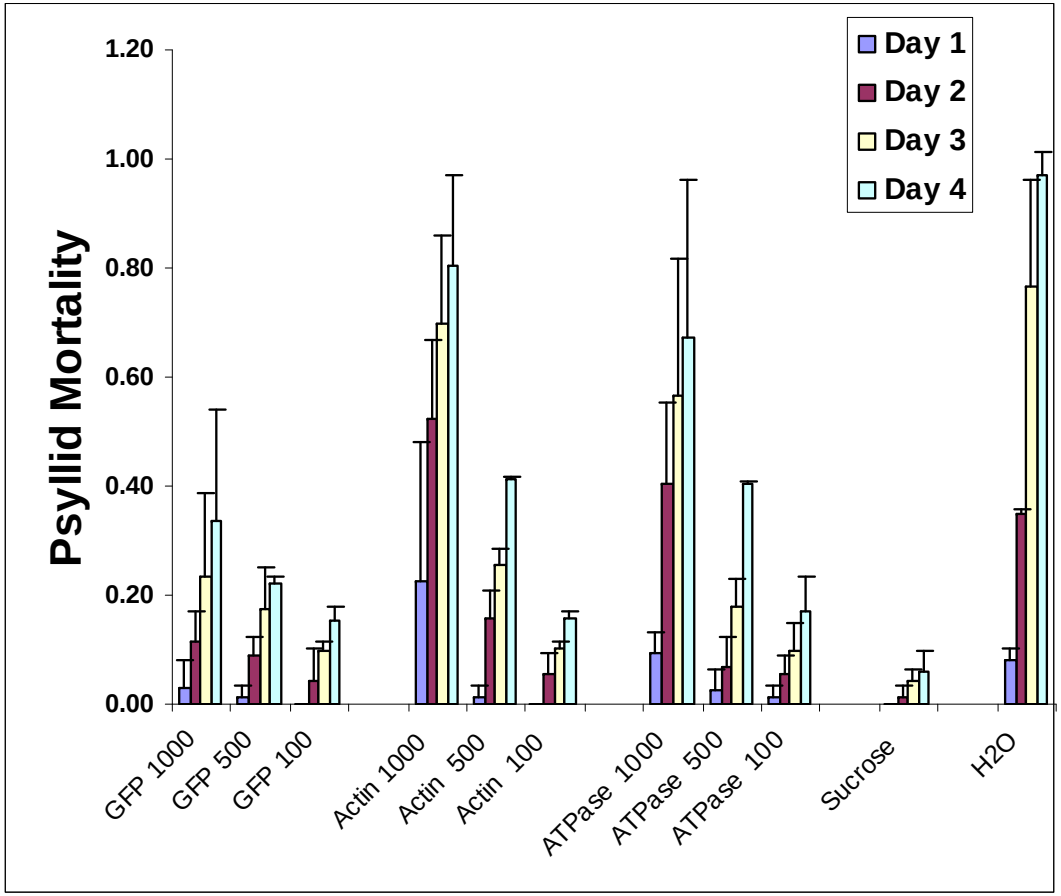
Ds-RNA concentration and sequence-dependent mortality of psyllids

Day1



Day3

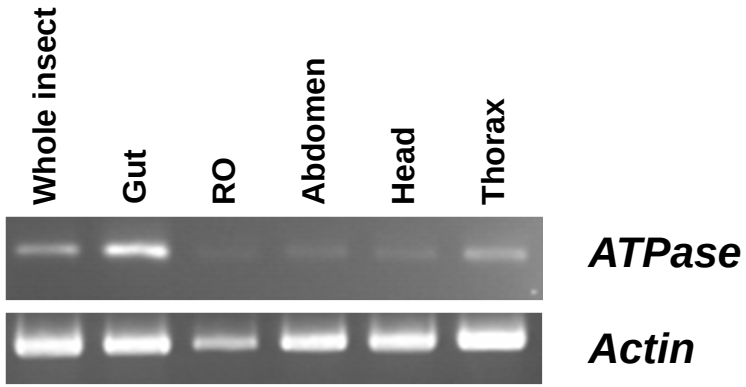


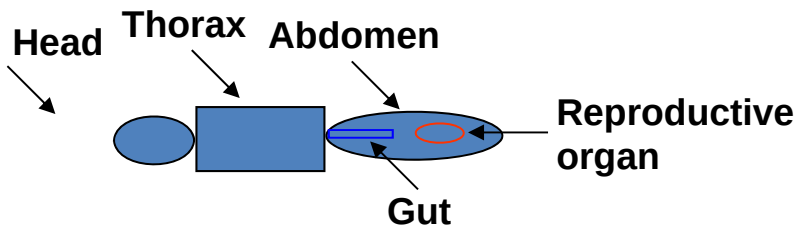


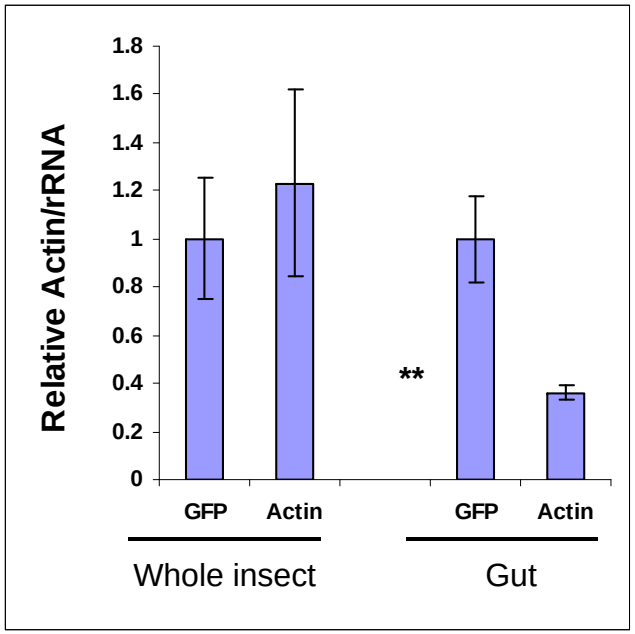
Effects are more obvious at high concentrations of dsRNA effectors.

Endogenous mRNA Reduction

-Small RNA Detection



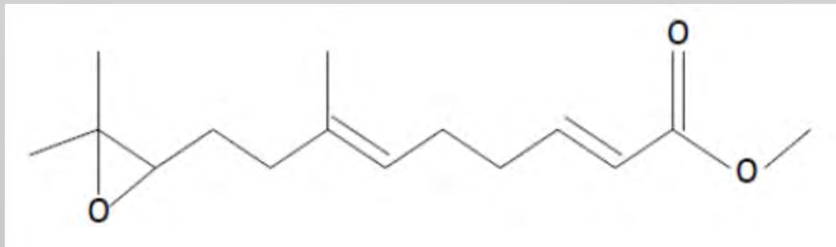




All mRNA targets are not susceptible in all tissues. Effects are not equal in all tissues.

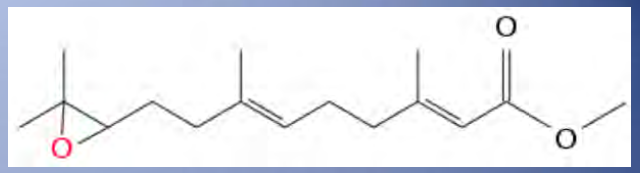
Targeting juvenile hormone metabolic genes in the Asian citrus psyllid (*Diaphorina citri*) as a strategy to reduce the spread of citrus greening disease

Juvenile hormone III



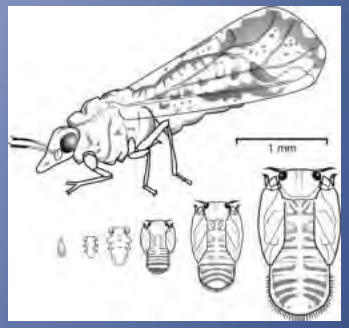
Evelien Van Ekert, D. Borovsky, C. A. Powell, R. D. Cave, R. T. Alessandro, R. G. Shatters, Jr.

Juvenile Hormone = JH

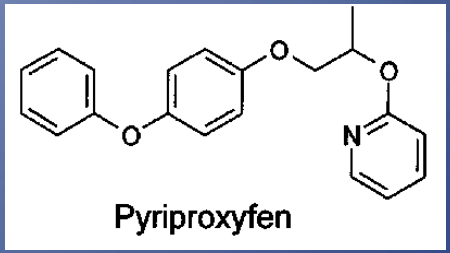


I. JH regulates <u>many physiological processes</u> in all insects

- Molts and metamorphosis
- Reproduction
- Behavior



II. Boina and colleagues (2010):
Application of a JH analogue, pyriproxyfen, to the ACP caused ovicidal/nymphicidal effects, morphological abnormalities, and reduced fecundity



III. Interdiction at JH to Specifically target the ACP

- Perturbations in temporal and spactial regulation of JH impacts insect development and survival
- JH levels are regulated by its biosynthesis and degradation
- Genes that encode biosynthetic and degradative enzymes have a unique sequence in each insect species
- Silence these particular genes with RNA interference → ? Effects on ACP ?

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Which microbes might be avaialble for use in *D. citri*?

Citrus Greening-HLB Genome Resources Group: A bioinformatics resource for diverse projects related to the biology and diagnosis of citrus greening/HLB

Surya Saha[1], Wayne Hunter[2], and Magdalen Lindeberg[1]

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Phylogeographic analysis of Asian citrus psyllid, a vector of citrus greening disease

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Outcome Group 2

Functional and comparative genomics of Liberibacters

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Outcome Group 3

We know that Clas is present and very important, but there are more.

Psyllid microbes are going to be abundant and offer an untapped resource which can be discovered by transcriptome and/or small RNA sequencing.

Bacteria

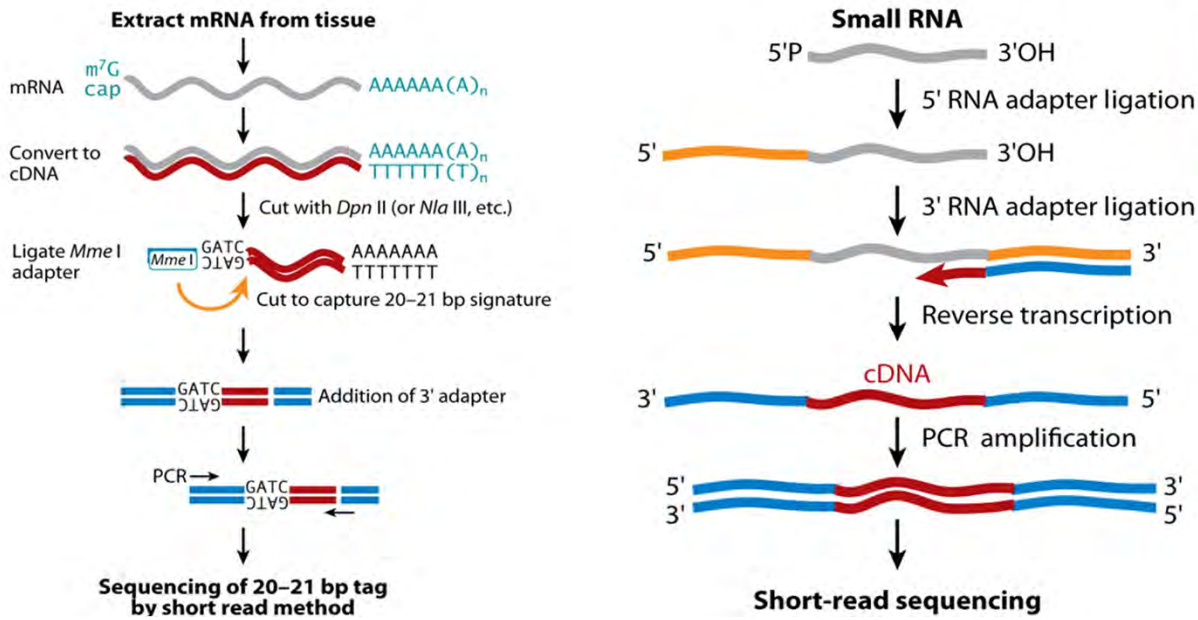
Populations of microbes (such as bacteria and yeasts) inhabit the skin and mucosa. Their role forms part of normal, healthy human physiology, however if microbe numbers grow beyond their typical ranges (often due to a compromised immune system) or if microbes populate atypical areas of the body (such as through poor hygiene or injury), disease can result.
It is estimated that 500 to 1000 species of bacteria live in the human gut[2] and a roughly similar number on the skin.[3][4] Bacterial cells are much smaller than human cells, and there are at least ten times as many bacteria as human cells in the body (approximately 10^{14} versus 10^{13}).[5][6] Though members of the flora are found on all surfaces exposed to the environment (on the skin and eyes, in the mouth, nose, small intestine), the vast majority of bacteria live in the large intestine.

Viruses

Viruses are intracellular, molecular obligate parasites and are the most abundant biological entities on earth with estimates as high as 10^{31} .

Which microbes might be avaialble for use in *D. citri*?

Transcriptome and small RNA sequencing



- **We have sequenced GWSS with total sequencing depth of 22 million reads for small RNA sequencing that generated 4 million unique small RNA sequences to GWSS genome.**

- **Similarly, our transcriptome sequencing of GWSS adult insects yielded 65 million reads of data (51bp) with 46% of the sequences being unique. We were able to generate 52,000 contigs from those sequences that corresponded to approximately 28000 genes.**

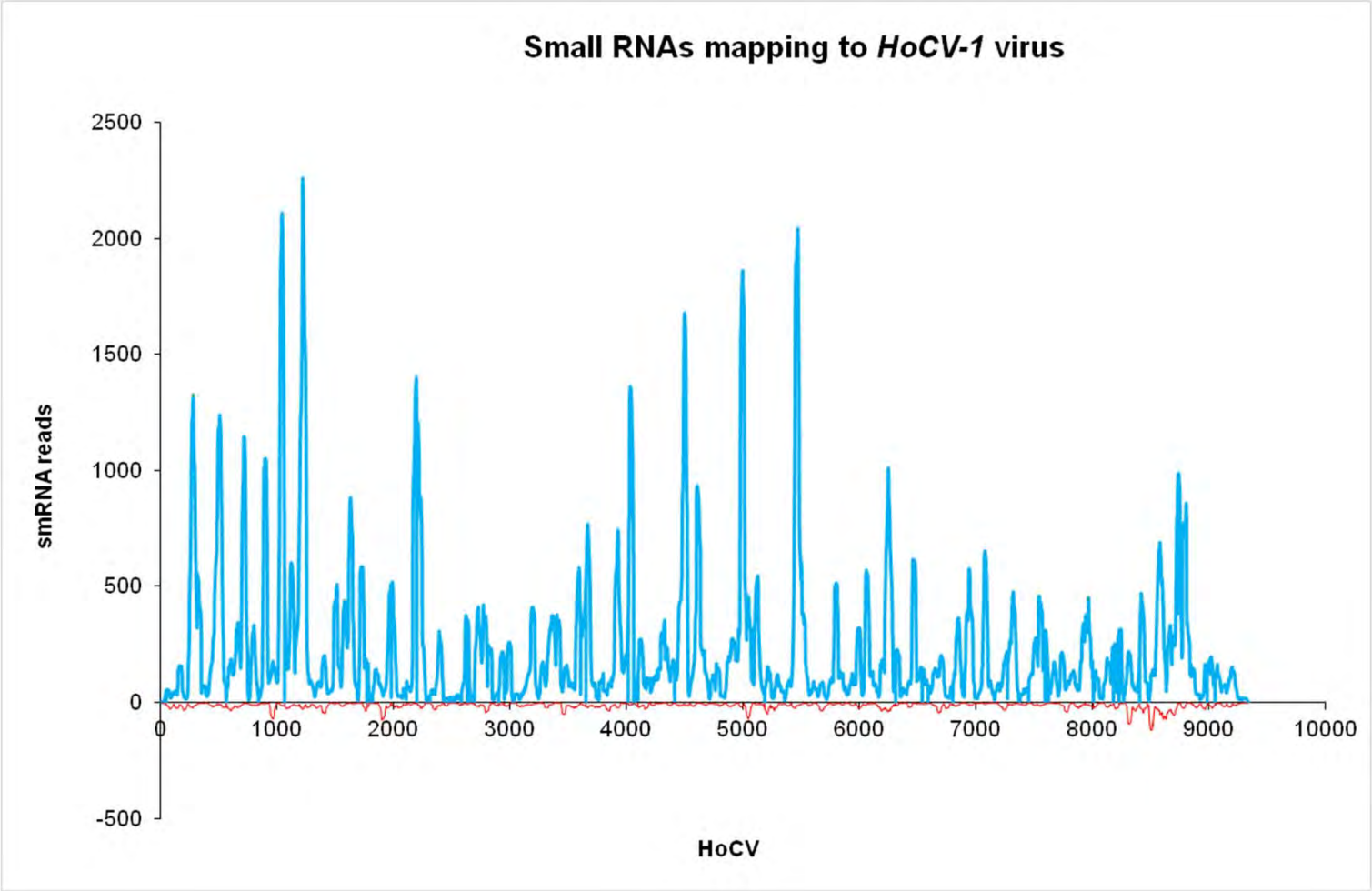
- **We were able to validate few of the selected genes and some of the genes were chosen to be universal targets for gene knockdown experiments.**

- **More importantly, we were able to discover information on the host interactions with other micro-organisms such as viruses, bacteria and fungi that were present residually inside the adult insects.**

Glassy-winged sharpshooter sequencing.

N-ID	lgth	Reference ID	Reference Name	Ref-length	E-value	Identical
N25	204	gi|226423325|ref|NC_012537.1|	Homalodisca vitripennis reovirus segment 3	3180	9e-93	339
N68	163	gi|226423329|ref|NC_012539.1|	Homalodisca vitripennis reovirus segment 5	2521	1e-63	242
n76	151	gi|226423335|ref|NC_012542.1|	Homalodisca vitripennis reovirus segment 8	1434	1e-63	242
N83	135	gi|226423337|ref|NC_012543.1|	Homalodisca vitripennis reovirus segment 9	1187	2e-61	234
N90	230	gi|226423344|ref|NC_012546.1|	Homalodisca vitripennis reovirus segment 12	1040	e-128	456
N93	234	gi|226423333|ref|NC_012541.1|	Homalodisca vitripennis reovirus segment 7	1702	e-122	438
N101	244	gi|226423325|ref|NC_012537.1|	Homalodisca vitripennis reovirus segment 3	3180	e-126	450
N101	352	gi|226423339|ref|NC_012544.1|	Homalodisca vitripennis reovirus segment 10	1205	0	660
N114	125	gi|226423341|ref|NC_012545.1|	Homalodisca vitripennis reovirus segment 11	1234	1e-65	248
N117	231	gi|96980777|ref|NC_008029.1|	Homalodisca coagulata virus	9345	e-117	420
N118	246	gi|226423337|ref|NC_012543.1|	Homalodisca vitripennis reovirus segment 9	1187	e-124	444
N123	194	gi|226423321|ref|NC_012535.1|	Homalodisca vitripennis reovirus segment 1	4475	e-106	385
N125	145	gi|226423323|ref|NC_012536.1|	Homalodisca vitripennis reovirus segment 2	3522	1e-53	208
N130	94	gi|226423333|ref|NC_012541.1|	Homalodisca vitripennis reovirus segment 7	1702	7e-39	159
N132	128	gi|226423337|ref|NC_012543.1|	Homalodisca vitripennis reovirus segment 9	1187	5e-50	196
N144	387	gi|96980777|ref|NC_008029.1|	Homalodisca coagulata virus	9345	0	761
N145	457	gi|226423339|ref|NC_012544.1|	Homalodisca vitripennis reovirus segment 10	1205	0	850
N147	186	gi|226423341|ref|NC_012545.1|	Homalodisca vitripennis reovirus segment 11	1234	1e-88	325
N148	111	gi|226423325|ref|NC_012537.1|	Homalodisca vitripennis reovirus segment 3	3180	3e-57	220
N155	275	gi|96980777|ref|NC_008029.1|	Homalodisca coagulata virus	9345	e-141	500
N157	174	gi|96980777|ref|NC_008029.1|	Homalodisca coagulata virus	9345	1e-82	305
N159	119	gi|226423335|ref|NC_012542.1|	Homalodisca vitripennis reovirus segment 8	1434	9e-36	149
N160	96	gi|226423339|ref|NC_012544.1|	Homalodisca vitripennis reovirus segment 10	1205	3e-44	176
N200	433	gi|226423327|ref|NC_012538.1|	Homalodisca vitripennis reovirus segment 4	2559	0	858

Figure 3, Nandety *et al.*



Can obtain the complete genome sequence of known and unknown microbes.

Can we discover new viruses and engineer them to effect NuPsyllid action?

Genetically Engineered Resistance to Dengue-2 Virus Transmission in Mosquitoes

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The control of arthropod-borne virus diseases such as dengue may ultimately require the genetic manipulation of mosquito vectors to disrupt virus transmission to human populations. To reduce the ability of mosquitoes to transmit dengue viruses, a recombinant Sindbis virus was used to transduce female *Aedes aegypti* with a 567-base antisense RNA targeted to the premembrane coding region of dengue type 2 (DEN-2) virus. The transduced mosquitoes were unable to support replication of DEN-2 virus in their salivary glands and therefore were not able to transmit the virus.

SCIENCE • VOL. 272 • 10 MAY 1996

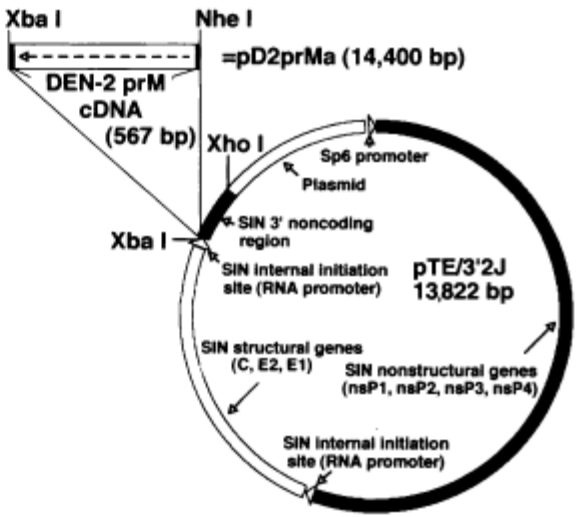

Fig. 1. Plasmid map showing construction of pD2prMa from the parent dsSIN construct pTE/3′2J. To insert the antisense sequence into pTE/3′2J, we synthesized primers to amplify DEN-2 prM cDNA by polymerase chain reaction from p30-VD2 (*24*), which contained cDNA of the structural coding region of DEN-2 (Jamaica) virus. Amplified prM cDNA was TA-cloned (*25*) into a modified Bluescript II SK+ plasmid (Stratagene) that contained an Nhe I site in the polylinker. The prM cDNA was excised with Nhe I and Xba I restriction endonucleases and inserted at the Xba I site downstream of the second RNA promoter of pTE/3′2J. Insert orientation was confirmed by sequence analysis. The dsSIN DNA templates were linearized at the Xho I site and transcribed in vitro from the bacteriophage SP6 promoter (*26*). RNA products were electroporated (BTX, San Diego, CA) into BHK-21 cells at 500 V, 100 μF, and 720 ohms for a duration of approximately 0.8 ms. The dsSIN viruses were harvested from the medium 20 hours later and titrated in BHK-21 cells with the use of an end-point assay (*27*).

Table 1. Interference of transmission of DEN-2 virus in *Ae. aegypti* by transduction of mosquitoes with an antisense RNA targeted to the prM coding region of DEN-2 virus. The number of saliva samples positive for DEN-2 virus after injection of saliva into mosquitoes is indicated as the first number in the second column; the total number of saliva samples injected into mosquitoes, by the second number. In trial 1, one mosquito injected with saliva from a mosquito transduced with prM antisense RNA in trial 1 was weakly positive for DEN-2 virus (fourth column); ND, not done. The total number of saliva samples assayed was produced from three separate trials. Mosquitoes were injected with L-15 medium and DEN-2 virus or co-injected with D2prMa or TE/3′2J virus and DEN-2 virus as described (*16*). Fourteen days after infection, saliva samples were collected from mosquitoes for detection of DEN-2 virus.

Trial	Saliva samples from mosquitoes infected with		
	DEN-2 virus + L-15 medium	DEN-2 + TE/3′2J viruses	DEN-2 + D2prMa viruses
1	8/8	7/9	1/8
2	ND	7/10	0/10
3	ND	6/7	0/8
Total	8/8	20/26	1/26

Summary

- **The resulting sequence data from small RNA profiling and transcriptome profiling of psyllids can help us for many different efforts for ACP and HLB control. These range from identifying sources of ACP to designing genetic insecticides.**

- **The data can help us in furthering applications such as "bio-defense", protection from vulnerable species, and to design better pesticides without the need for off-target effects.**

- **The plethora of information when combined with the available information from other sources can be used for conserved gene target knock downs that can be used to control a pest complex rather than directing the control to one particular pest.**

- **The discovery and identification of new microbes including viruses can help us design the experiments to modify the newly identified viruses for targeting insect vectors.**