Wolbachia infection among mosquito species of metro Atlanta

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Wolbachia

- Intracellular, maternally inherited symbiotic bacteria
- Found in ~20% of insect species
- Many different symptoms of infection



Phenotypes of Wolbachia

- Mutualistic:
 - Nematodes
- Parasitic:
 - Sex ratio distortion:
 - Parthenogenesis induction
 - Feminization of genetic males
 - Male killing
 - Reproductive barrier:
 - Cytoplasmic incompatibility



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Cytoplasmic Incompatibility (CI)

 Eggs from uninfected females fertilized with sperm from infected males cannot develop







Application (CI)

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Wolbachia Enhances West Nile Virus (WNV) Infection in the Mosquito *Culex tarsalis*

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Consequences of CI Wolbachia

- Selection pressure on uninfected females
- Wolbachia infection sweeps to fixation (Turelli et al. 1992)
- Eventual speciation due to reproductive barrier (Bordenstein et al. 2001)

Predictions

- Reduced gene flow between uninfected and differentially infected populations
- Linkage disequilibrium: mitochondria and *Wolbachia* inherited together
- Co-evolution between mitochondrial and *Wolbachia* genomes

Significance of Study

- Mosquito-vectored diseases:
 - Arboviruses
 - Parasitic helminths: heartworm
 - Protozoans: malaria



Smart Science: Wolbachia Bacteria Might Stop Zika and Dengue Viruses (Forbes 2016)

Collection and Morphological Identification





Anopheles punctipennis

 CDC light trap baited with dry ice as a source of CO₂



Culex spp.

Molecular Identification & Phylogenetic Analysis

- DNA sequencing
- Alignment and neighborjoining analysis



Species/Abbrv	* * * *	* * * *	* * * *	* *	* *	* * *	*	*	*	*		* *	* * *	* *	* *	*	* *	3
12. B1 A. vexans	AGTTT	ТТСААТ	ATTACC	TCCT	тстт	TAAC	СТ	A C T A	CTTT	<mark>C T A G T</mark>	TCA	ATAG	TAGA	A A A 1	GGA	GC	GGC	i A
13. C49 A. vexans	AGTTT	ТТСААТ	ATTACC	ТССТ	тстт	TAAC	СТ	АСТА	СТТТ	<mark>C T A G T</mark>	TCA	ATAG	TAGA	AAA	GGA	GC	GGC	i A
14. C51 A. vexans	AGTTT	ТТСААТ	ATTACC	СССТ	тстт	TAAC	СТ	ТСТА	СТТТ	СТАСТ	TCA	ATAG	TAGA	AAA	GGA	GC	GGC	i A
15. B6 Anopheles punctipennis	AGATT	ТТСААТ	ATTACC	СССТ	тстт	TGAC	СТ	ТТТ 🖊	ATTT	СТАСТ	A G T	ATAG	TAGA	A A A 1	GGA	GCC	GG	i A
16. H38 Anopheles punctipennis	AGATT	ТТСААТ	ATTACC	тсст	тстт	TGACT	СТ	ТТТ 🖊	ATTT	СТАСТ	A G T	ATAG	TAGA	AAA	GGA	GCC	GG	s /
17. C50 Coquillettidia perturbans	AGATT	ТТСААТ	ACTTCC	TCCC	ТСАТ	TAAC	СТ	сстс	сттт	C T <mark>G</mark> G <mark>G</mark>	G G T	ATAG	TAGA	A A <mark>G</mark> (GGG	GC	GGC	s A
18. A6 Culex quinquefasciatus	AGTTT	ТТСААТ	ACTACC	TCCT	ТСАТ	TGAC	СТ	АСТА	СТТТ	CAAGT	AGT	TAG	TAGA	AAA	GGA	GC	GGC	s A
19. A7 Culex pipiens	AGTTT	ТТСААТ	ACTACC	тсст	ТСАТ	TGAC	СТ	АСТА	СТТТ	C A A G T	AGT	TAG	TAGA	A A A 1	GGG	GC	GGC	, A
20. C39 Culex erraticus	AGTTT	ТТСААТ	ACTACC	ACCG	ТСАТ	TAAC	ТТТ	АТТА	ТТАТ	C <mark>A</mark> A G T	AGT	TTAG	TAGA	AAA	GGA	GC	GG	۸ A
21. C40 Culex erraticus	AGTTT	ТТСААТ	ACTACC	ACCG	ТСАТ	TAAC	ТТТ	АТТА	ТТАТ	C <mark>A</mark> A G T	AGT	TTAG	TAGA	AAA	GGA	GC	GG	۸ A
22. H39 Culex erraticus	AGTTT	ТТСААТ	ACTACC	ACCG	ТСАТ	TAAC	ТТТ	АТТА	ТТАТ	C A A G T	AGT	TTAG	TAGA	ΑΑΑ	G G <mark>G</mark>	GC	GG	۸ A
23. F1 Ochleratatus triseriatus	AGTTT	СТБААТ	ATTACC	тсст	тстт	TAAC	ТТТ	АСТС	сттт	C <mark>G</mark> A G T	AGT	A T <mark>G</mark> G 1	TAGA	AAA	GGA	ТС	GG	۸ A
24. E4 A. albopictus	AGTTT	ТТСААТ	ATTACC	cccc	тстт	TAAC	СТ	G С Т С	сттт	СТАСТ	ТСТ	ATAG	TAGA	A A A 🕻	GGA	GC	GG	۸ A
25. D2 A. albopictus	AGTTT	ТТСААТ	ATTACC	cccc	тстт	TAAC	СТ	G С Т С	сттт	СТАСТ	ТСТ	ATAG	TAGA	A A A 🕻	GGA	GC	GG	۸ A
26. B2 A. albopictus	AGTTT	ТТСААТ	ATTACC	cccc	тстт	TAAC	СТ	GCTG	сттт	СТАСТ	T <mark>C</mark> T	ATAG	TAGA	A A A 🕻	GGA	GC	GG	۸ A
07.14.4	. O T T T			0000	TOTT	T A A O	<u>о т</u>		• • • •	<u>от кот</u>	TOT	A T A O				0.0		Ē,

Mitochondrial diversity



Aedes diversity



Aedes diversity

DNASequences	Translated Protein Sequences	es	
Species/Abbrv	* * * * * * * * * * * *	* <th>* * * * * * * *</th>	* * * * * * * *
1. A1 A. vexans	ΤΑΤΑΤΤΑΤΤΑΑΟ	; T G A T C G A A A T T T A A A T A C T T C <mark>C</mark> T T <mark>C</mark> T T T G A <mark>C</mark> C C A A T T G G A G G <mark>A</mark> G G A G A T C C <mark>A</mark> A [] T <mark>C</mark> T T T A .	ТСААСАТТТ
2. A2 A.vexans	ΤΑΤΑΤΤΑΤΤΑΑ <mark></mark>	; T G A T C G A A A T T T A A A T A C T T C C T T C T T T G A <mark>C</mark> C C A A T T G G A G G A G G A G A C C C A A T T C T T T A	TCAACATTT
3. A3 A.vexans	ΤΑΤΑΤΤΑΤΤΑΑΟ	; T G A T C G A A A T T T A A A T A C T T C C T T C T T T G A <mark>C</mark> C C A A T T G G A G G A G G A G A C C C A A T T C T T T A	TCAACATTT
4. A4 A. vexans	ΤΑΤΑΤΤΑΤΤΑΑ <mark></mark>	; T G A T C G A A A T T T A A A T A C T T C <mark>C</mark> T T <mark>C</mark> T T T G A <mark>C</mark> C C A A T T G G A G G <mark>A</mark> G G A G A C C C <mark>A</mark> A T T <mark>C</mark> T T T A	TCAACATTT
5. A5 A. vexans	ΤΑΤΑΤΤΑΤΤΑΑ <mark></mark>	; T G A T C G A A A T T T A A A T A C T T C <mark>C</mark> T T <mark>C</mark> T T T G A <mark>C</mark> C C A A T T G G A G G <mark>A</mark> G G A G A T C C <mark>A</mark> A T T <mark>C</mark> T T T A .	TCAACATTT
6. A8 A. vexans	ΤΑΤΑΤΤΑΤΤΑΑ <mark></mark>	; T G A T C G A A A T T T A A A T A C T T C <mark>C</mark> T T <mark>C</mark> T T T G A <mark>C</mark> C C A A T T G G A G G <mark>A</mark> G G A G A C C C <mark>A</mark> A T T <mark>C</mark> T T T A	TCAACATTT
7. C3 A. vexans	ΤΑΤΑΤΤΑΤΤΑΑ <mark></mark>	; T G A T C G A A A T T T A A A T A C T T C <mark>C</mark> T T <mark>C</mark> T T T G A T C C A A T T G G A G G <mark>A</mark> G G A G A C C C <mark>A</mark> A T T <mark>C</mark> T T T A	TCAACATTT
8. C4 A. vexans	ΤΑΤΑΤΤΑΤΤΑΑ <mark></mark>	; T G A T C G A A A T T T A A A T A C T T C <mark>C</mark> T T <mark>C</mark> T T T G A <mark>C</mark> C C A T T G G G A G G <mark>A</mark> G G A G A T C C <mark>A</mark> A T T <mark>C</mark> T T T A	TCAACATTT
9. C5 A. vexans	Τ Α Τ Α Τ Τ Α Τ Τ Α Α C <mark>Τ</mark>	; T G A T C G A A A T T T A A A T A C T T C <mark>C</mark> T T <mark>C</mark> T T T G A <mark>C</mark> C C A A T T G G <mark>G</mark> G G G G A G A T C C <mark>A</mark> A T T <mark>C</mark> T T T A	CCAACATTT
10. C6 A. vexans	T A T A T T A T T A A C <mark>T</mark>	; T G A T C G A A A T T T A A A T A C T T C <mark>C T T C</mark> T T T G A <mark>C</mark> C C A A T T G G A G G A G A G A C C C <mark>A</mark> A T T <mark>C</mark> T T T A	TCAACATTT
11. C7 A. vexans	T A T A T T A T T A A C <mark>T</mark>	; T G A T C G A A A T T T A A A T A C T T C <mark>C T T C</mark> T T T G A <mark>C</mark> C C A A T T G G A G G <u>G</u> G G A G A C C C <mark>G</mark> A T T <mark>C</mark> T T T A	TCAACATTT
12. B1 A. vexans	T A T A T T A T T A A C T	; T G A T C G A A A T T T A A A T A C T T C <mark>C T T C</mark> T T T G A <mark>C</mark> C C A A T T G G A G G <mark>A</mark> G G A G A C C C <mark>A</mark> A T T <mark>C</mark> T T T A	TCAACATTT
13. C49 A. vexans	T A T A T T A T T A A C T	; T G A T C G A A A T T T A A A T A C T T C C T T C T T T G A C C C A A T T G G A G G A G G A G A T C C A A T T C T T T A	TCAACATTT
14. C51 A. vexans	TATATTATTAAC	; T G A T C G A A A T T T A A A T A C T T C C T T C T T T G A <mark>C</mark> C C A A T T G G A G G <mark>A</mark> G G A G A T C C <mark>A</mark> A T T C T T T A	TCAACATTT
15. E4 A. albopictu	: T A T A T T A T T A A C A	A G A C C G A A A T T T A A A T A C A T C T T T T T	TCAACATTT
16. D2 A. albopictu	Т А Т А Т Т А Т Т А А <mark>С</mark> А	; A G A C C G A A A T T T A A A T A C A T C T T T T T	TCAACATTT
17. B2 A. albopictu	. T A T A T T A T T A A C A	; A G A C C G A A A T T T A A A T A C A T C T T T T T	TCAACATTT
18. I1 A. albopictus	Т А Т А Т Т А Т Т А А С А	; A G A C C G A A A T T T A A A T A C A T C T T T T T	TCAACATTT
19. B3 A. albopictu	. T A T A T T A T T A A C A	; A G A C C G A A A T T T A A A T A C A T C T T T T T	TCAACATTT
20. D1 A. albopictu	ТАТАТТАТТААСА	; A G A C C G A A A T T T A A A T A C A T C T T T T T	TCAACATTT
21. C1 A. albopictu	ТАТАТТАТТААСА	; A G A C C G A A A T T T A A T A C A T C T T T T T T	TCAACATTT
22. D3 A. albopictu	ТАТАТТАТТААСА	; A G A C C G A A A T T T A A A T A C A T C T T T T T	TCAACATTT
23. E1 A. albopictu	TATATTATTAACA	; A G A C C G A A A T T T A A A T A C A T C T T T T T	TCAACATTT
24. E2 A. albopictu	TATATTATTAACA	; A G A C C G A A A T T T A A A T A C A T C T T T T T	TCAACATTT
25. H1 A. albopicti		; A G A C C G A A A I I I A A I A C A I C I I I I I I	ICAACAIII
26. G1 A. albopicti		; A G A C C G A A A I I I A A I A C A I C I I I I I I	ICAACAIII
27. E3 A. albopictu		; A G A C C G A A A I I I A A I A C A I C I I I I I I	TCAACATIT
28. G2 A. albopicti		; A G A C C G A A A T I I A A A I A C A I C T I I I I I I G A I C C A A I I G G A G G G G G G A C C C C I A I I I A I A	
29. C2 A. albopictu	TATATTATTAACA	; A G A C C G A A T T T A A T A C A T C T T T T T T G A T C C A A T T G G A G G G G G G G A G A C C C T A T T T A T A	I C A A C A I I T T

• Alignment of all *Aedes* sequences illustrates difference in genetic variation between mosquitoes uninfected and infected with *Wolbachia*

Culex infected vs. uninfected



Infected mosquito diversity



Observed *Wolbachia*-positive mosquito diversity

Infected mosquito diversity



Observed *Wolbachia*-positive mosquito diversity

Expected Wolbachia diversity

Infected mosquito diversity



Observed *Wolbachia*-positive mosquito diversity

Observed Wolbachia diversity

Conclusions

- Selective sweep among infected *Aedes* mosquitoes
- Wolbachia influences evolution of infected mosquitoes so they appear more related to other infected mosquitoes than uninfected mosquitoes within the same genus
- Potential for horizontal transmission of *Wolbachia* (water mites)

Future Research

- More mosquito samples and sequences to further support/reject coevolution and relatedness of infected species
- Nuclear genes or strain specific primers to clarify effect of *Wolbachia* on mosquito evolutionary history
- Possible future screens for arboviruses in locally collected mosquitoes