

Whole Genome Sequencing of West Nile Virus and Future Use of PCR Testing



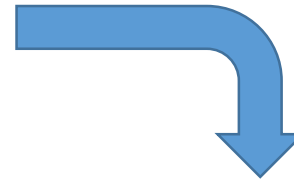
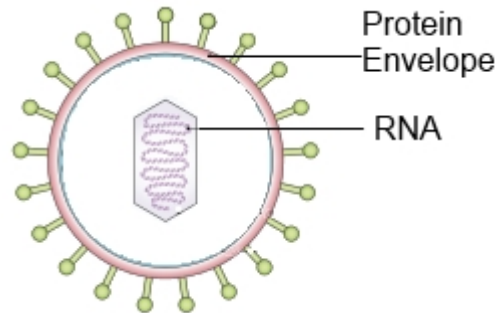
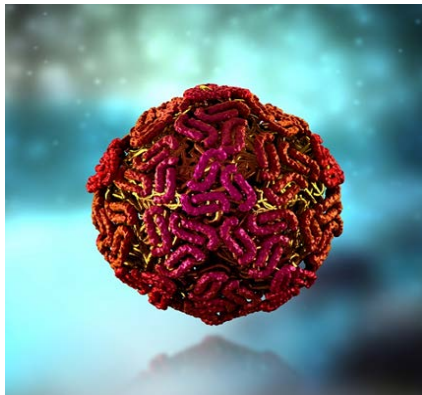
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Genome?



What is the West Nile Virus genome?

- The genome is all the genetic material of an organism.
- WNV genetic material is entirely made of RNA.



Extract the RNA
to sequence the
genome



DNA & RNA

- DNA and RNA make up the genetic codes of all living organisms.
- DNA and RNA are located inside mosquito cells and virus particles.
- Sections of DNA and RNA are unique “barcodes” for mosquitoes and viruses.



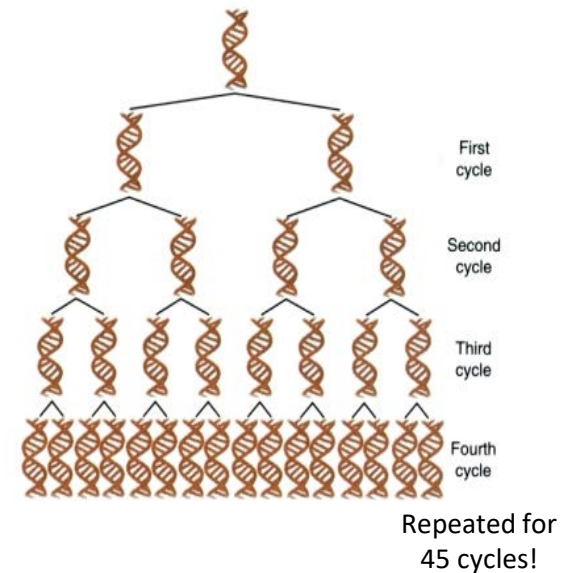
PCR Analysis

- PCR, Polymerase Chain Reaction, makes copies of “barcode” DNA and RNA.



- PCR is a tool that can be used to understand all organisms:

- Vector mosquitoes
- Mosquito borne viruses
 - West Nile Virus



West Nile Virus Surveillance

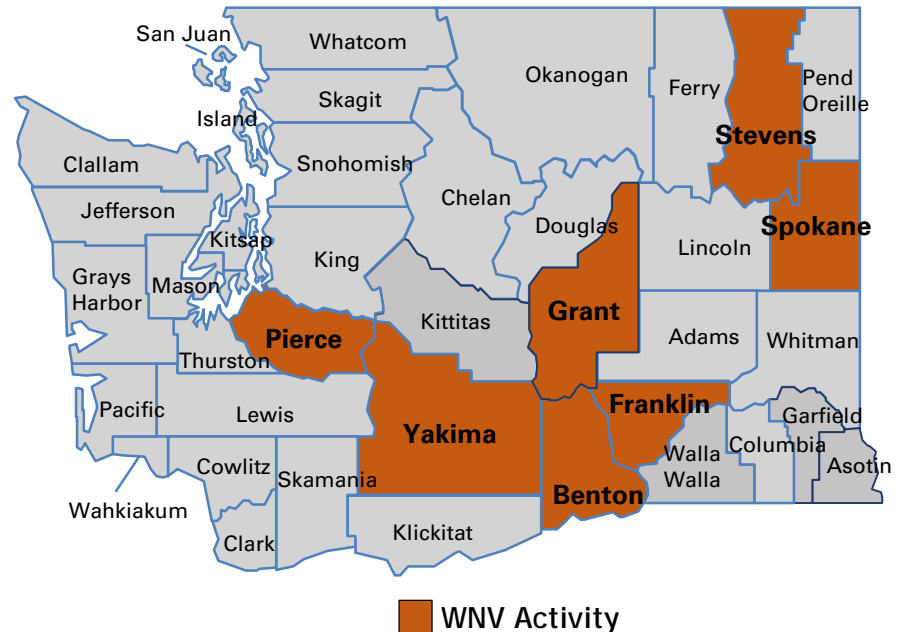
- 2018 is the first year we:
 - Used PCR in our ZD Lab to test vector mosquitoes for West Nile Virus
 - Detected West Nile Virus in mosquitoes west of the Cascade mountains

Mosquito testing by ZD Lab in 2018

County	# Tested*	# Positive
Thurston	22	0
Pierce	56	4
Spokane	15	3
Yakima	9	0
Totals	102	7

*Mosquitoes were tested in pools of up to 50 individuals grouped by species and location.


2018 WNV positive mosquito pools



Washington's 2018 WNV Genomes

Collaborators



 WA WNV Samples
Pierce County – 4
Spokane County – 3



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West Nile Virus Genome Project

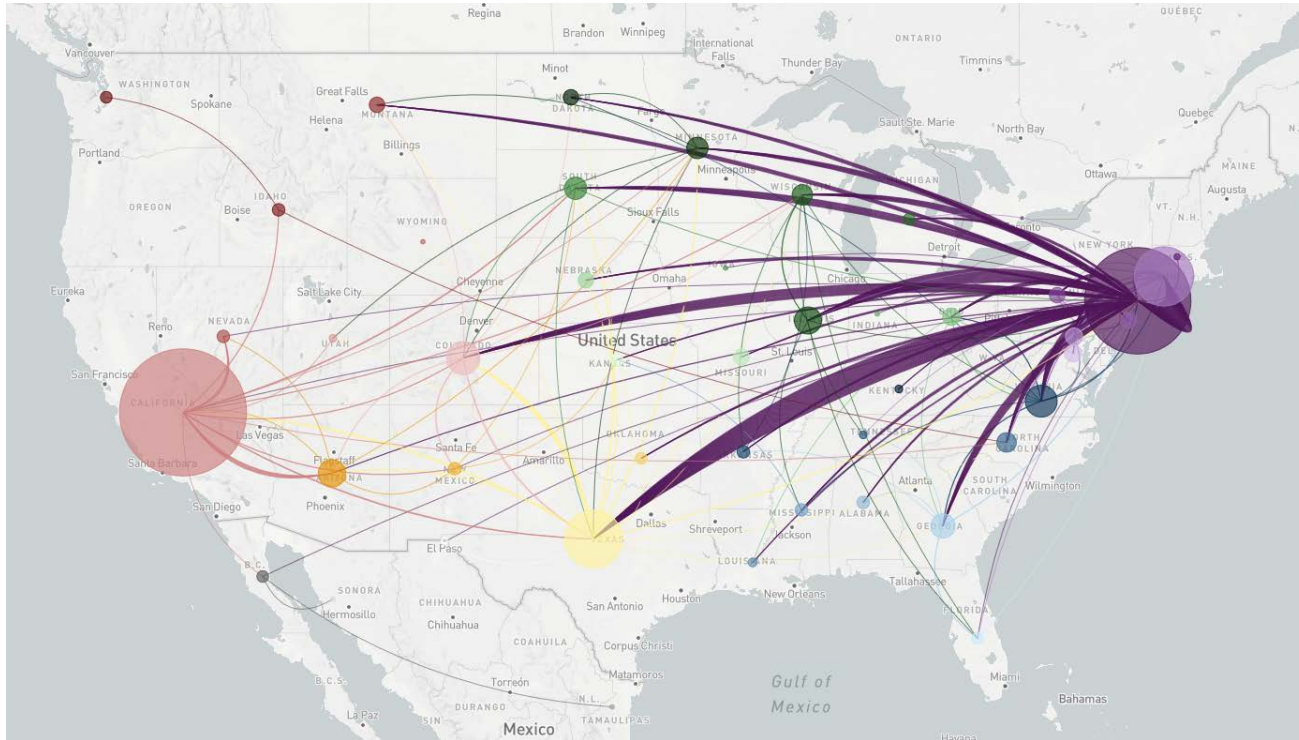
Sequencing the genomes of West Nile Virus allows us understand:

1. How the virus spreads between regions
2. If the virus overwinters locally between seasons
3. Which environmental factors may promote local outbreaks



Twenty Years of WNV in North America

Genomes sampled between 1999 and 2018

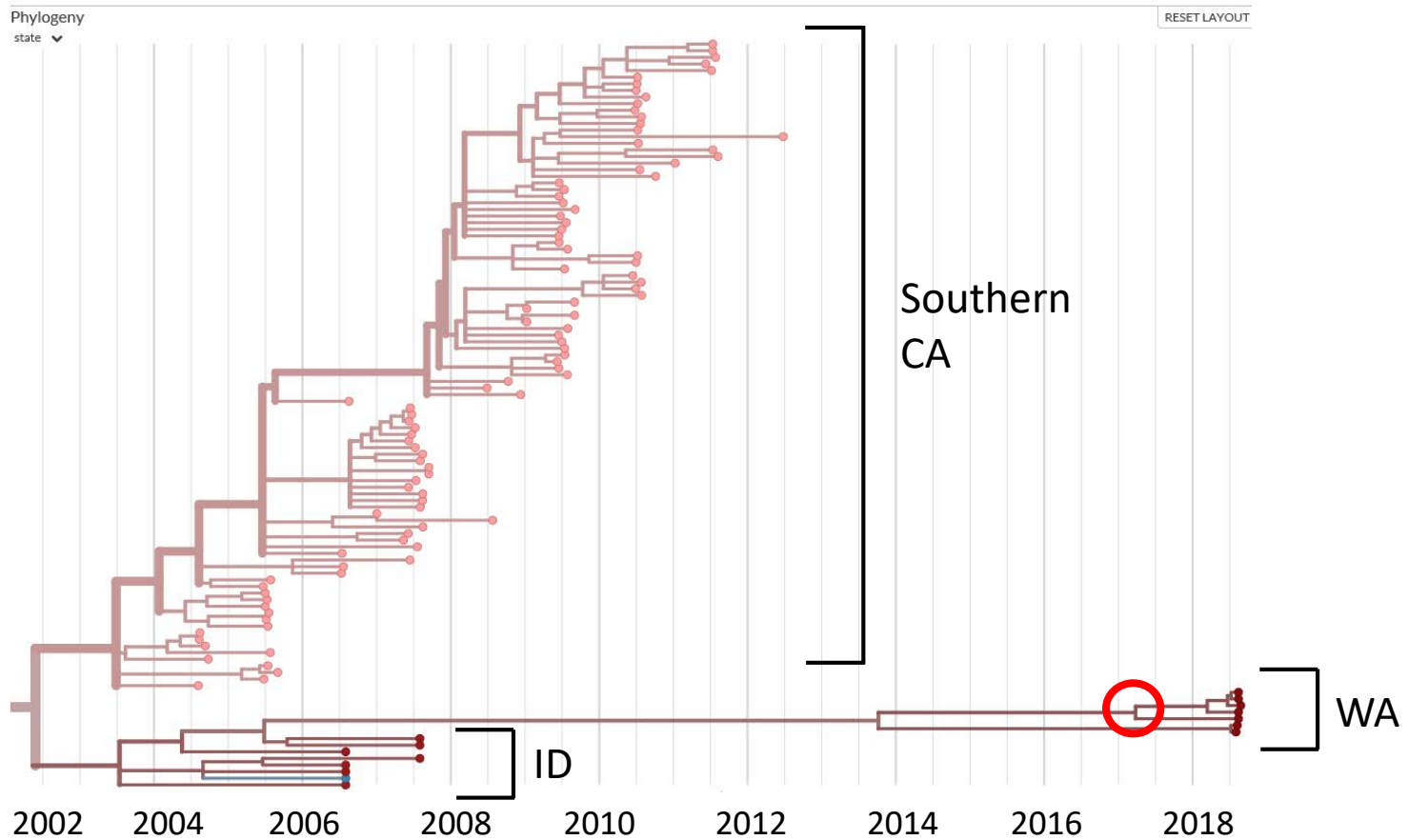


NextStrain.com

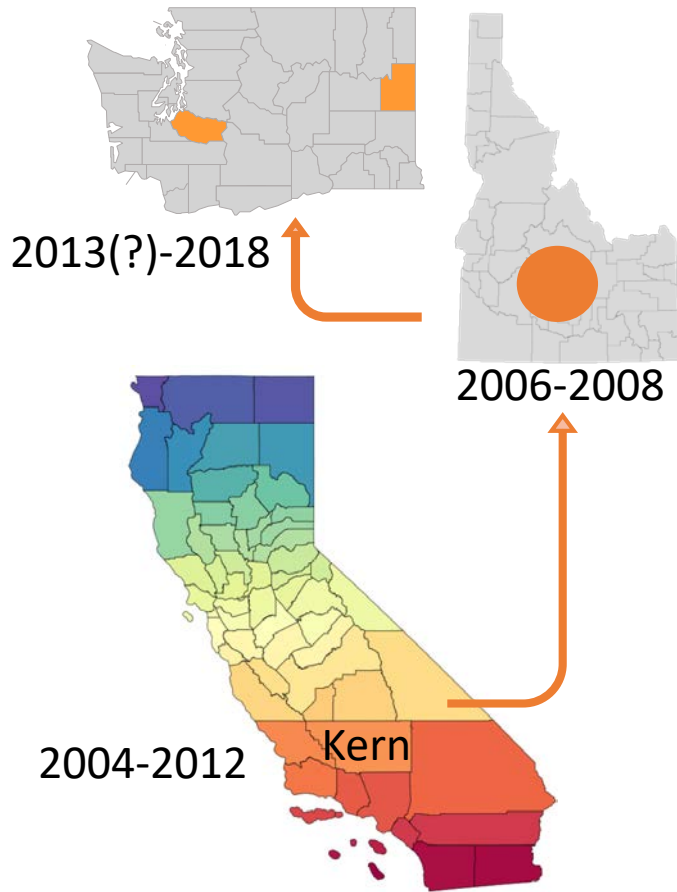
- WA WNV lineages are SW03 which emerged in the Southwest in 2003.
- These sequences were the very first from the Pacific Northwest!

Geographic Origins

Phylogenetic Tree Showing Lineage Divergence of WNV from Washington in 2018



Geographic Origins



- Genome sequences indicate the 2018 WNV strain in WA originally came from southern California.
- This WNV strain was detected in Idaho during a two year study.
- Taxonomic analysis indicates that the 2018 WNV strain has been in WA for at least 5 years

More Questions



- So, where else was the 2018 WA lineage for 5+ years between California, Idaho, and Washington?
- The 2018 sequences suggest that WNV overwinters in Washington mosquitoes.
 - Without additional data, we don't know if and when there were new introductions of WNV into Washington.



Overwintering WNV

- Is WNV overwintering...
 - in hibernating mosquitoes?
 - in dormant egg rafts?
 - In roosting bird populations?



- Overwintering of WNV means there is high potential for emergence of human disease the next year.

Vectors & Climate Change

- Genome data can offer higher resolution for studies on how climate change may effect WNV transmission.



Special Theme – Environment and Health

Climate change and vector-borne diseases: a regional analysis

Andrew K. Githeko,¹ Steve W. Lindsay,² Ulisses E. Confalonieri,³ & Jonathan A. Patz⁴

South America. Climate change has far-reaching consequences and touches on all life-support systems. It is therefore a factor that should be placed high among those that affect human health and survival.

Culex Mosquito Genome

- Genome data from Culex mosquitoes reveals:
 - Which genes have a role in virus transmission.
 - How mosquitos develop insecticide resistance.
 - The genes expressed in mosquito parts that contain virus replication.
- So that new ways to combat and control arboviruses can be developed!

Mosquito Resistance

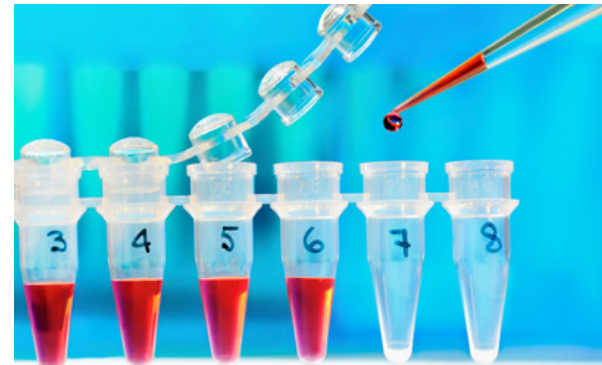
- Resistance is a worldwide concern!
- The longer pesticide applications are done in any area, the higher chance that resistance will develop.

What happens if you continue to apply a pesticide that the mosquito population is known to be resistant to?

- You will have a population that is completely resistant to that pesticide.
- Resistance is passed on to the offspring and the pesticide becomes completely useless.
- Mosquito management becomes ineffective at protecting the public from mosquito borne diseases.

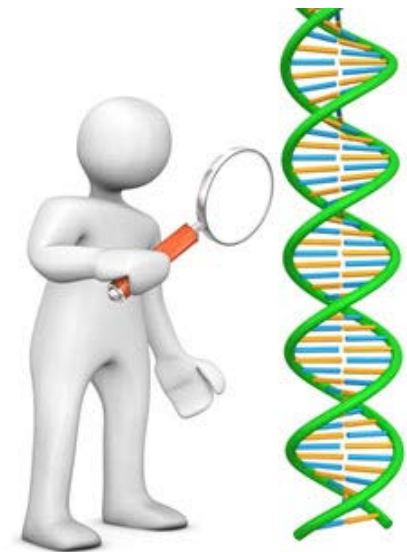
Mosquito Resistance Testing

- Pesticide resistance is evaluated by bottle bioassays.
- Resistance genes can be detected using PCR testing.
 - If you know what you're looking for.



Mosquito Resistance Pilot Project

- Do Washington mosquitoes carry resistance genes?
 - Which genes?
 - For which chemicals?



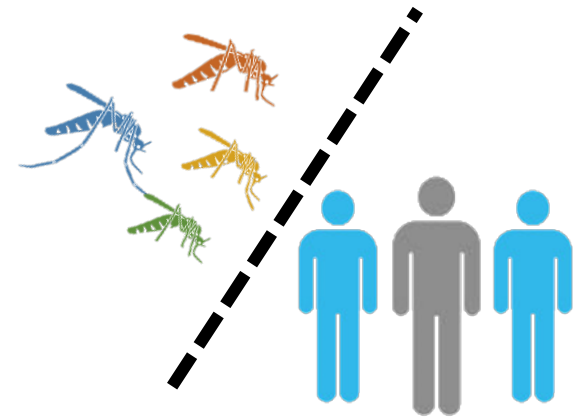
Vector Control Practices

How can WNV Genome data inform vector control practices?

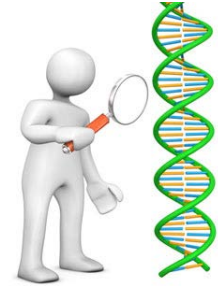
Control methods can be customized based on what we learn.

Example: If there is a distinct local WNV transmission network in Washington...

- local level mosquito control could be effective at suppressing WNV transmission
- If WA is connected the greater Pacific WNV cycle, then control could be coordinated both locally and regionally



Public Health Practices



How can WNV genome data inform public health practices?

Genome data adds more detail to outbreak surveillance & investigations.

Example: What if an outbreak of WNV occurs in Washington?

- Epidemiologists could use genome sequences to narrow down the probable place(s) of exposure.
- Tracing how the virus moves can help public health officials determine what interventions may work to prevent future spread.

Vector Control Practices

How can resistance data inform vector control practices?

Pesticide use can be rotated according to an evidence-based schedule.

Example: If mosquitoes are found to carry resistance genes...

- alternative pesticides can be chosen
- control strategies can be monitored for effectiveness
- molecular methods can help figure out how resistance arose



Knowledge for Protection, Prevention, & Diagnostics

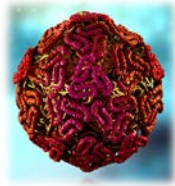
- Medical professional and public awareness means patients are more likely to receive earlier diagnosis and treatment
- People aware of vector-borne diseases are more likely to protect themselves and request diagnostic testing

Knowledge of risks can change people's behavior:

- Exercise caution when risk is highest
- Utilize protective measures when needed



Questions?



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