The
Wolbachia
Project

Discover the Microbes Within!

Lab 5: Bioinformatics III

Wolbachia Identification & Naming

Project Guide
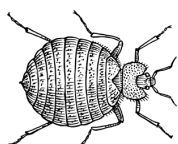
The Wolbachia Project

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Wolbachia Identification

Wolbachia is a bacterial genus that was first identified in 1924 by entymology student Marshall Hertig and his advisor, S. Burt Wolbach, as *Rickettsia*-like microorganisms that infect insects (Hertig & Wolbach, 1924). In 1936, Hertig officially proposed the name *Wolbachia pipientis* because it was found to be present in every germ cell of both male and female *Culex pipiens* mosquitoes throughout all stages of development (Hertig, 1936). It has since been detected in numerous arthropods (including insects, spiders, mites, and crustaceans), filarial nematodes, and plant-parasitic nematodes. *Wolbachia* are obligate intracellular endosymbionts, meaning they live within the cells of other organisms. Unlike free-living bacteria, such as *E. coli*, they cannot be cultured on a petri dish or studied in cell-free media. In order to determine morphology and distinguishing characteristics, scientists rely on tools such as transmission electron microscopy (Fig 5.1), scanning electron microscopy (Fig 5.2) and, most commonly, DNA sequencing and genetic analysis.
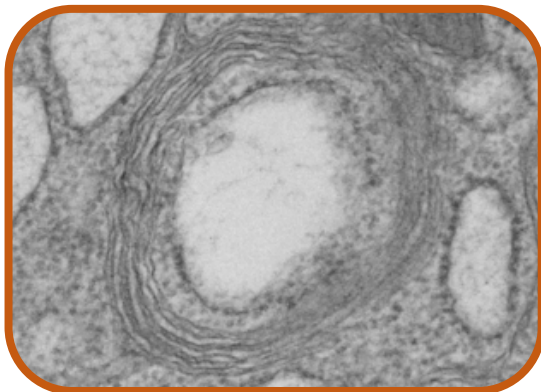

Figure 5.1. Transmission electron micrograph shows a single *Wolbachia* cell enclosed by multiple eukaryotic-derived membranes within an insect host.
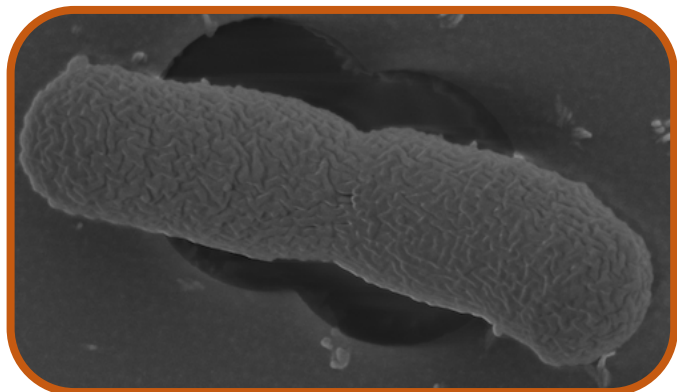*Image credit:* Sarah Bordenstein

Figure 5.2. Scanning electron micrograph shows a *Wolbachia* cell, purified from an insect host, that is in the process of dividing.
Image credit: Dennis Kunkel and Sarah Bordenstein

One of the most widespread genetic tools to identify *Wolbachia* is the **16S rRNA gene** marker. This phylogenetic marker is commonly used to identify bacteria at the species level and to differentiate between closely related species. While 16S rRNA provides an introductory level of classification, additional genes such as *wsp* and a set of multilocus sequence typing (MLST) genes - *ftsZ, gatB, coxA, hcpA*, and *fbpA* - have also been used to further resolve evolutionary relationships.

References

Hertig, M., & Wolbach, S. B. (1924). Studies on Rickettsia-Like Micro-Organisms in Insects. *The Journal of Medical Research*, 44(3), 329–374.7.

Hertig, M. (1936). The Rickettsia, *Wolbachia pipientis* (gen. et sp.n.) and Associated Inclusions of the Mosquito, *Culex pipiens*. *Parasitology,* 28(4), 453-486.

Wolbachia Naming

As discussed in Lab 1, the Linnean system of taxonomy classifies all organisms into genus and species. Beyond this, many species are further classified into subcategories. Using domestic dogs as an analogy, we know that they are all classified as one taxonomic species - *Canis familiaris* - yet there are dramatic differences between a German Shepherd and a Dalmatian (Fig 5.3). Society therefore categorizes domestic dogs into breeds. If you've ever watched an AKC dog show, you'll notice that individual breeds can also be organized based on the historical work associated with each type: sporting group, hound group, working group, terrier group, toy group, herd group, and non-sporting group.
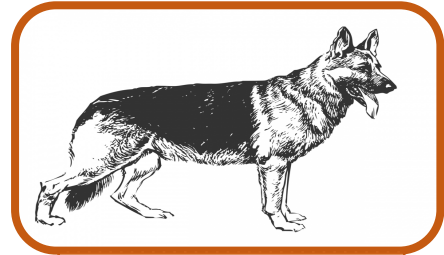
Canis familiaris
Breed: German Shepherd
Group: Herding group

Wolbachia are currently classified into one species: *Wolbachia pipientis*. Within this species designation, they are further categorized into **strains**. Strains are generally written as a lower-case *w* (to indicate *Wolbachia*) followed by a two to three letter abbreviation of the host species. The first letter following *w* is always capitalized. Below, the three letters are derived from just the species name.
- *w*Mel is the <u>*Wolbachia*</u> endosymbiont of *Drosophila melanogaster*
- *w*Kue is the <u>*Wolbachia*</u> endosymbiont of *Ephestia <u>kue</u>hniella*
- *w*Meg *is the* <u>*Wolbachia*</u> endosymbiont of *Chrysomya <u>meg</u>acephala*
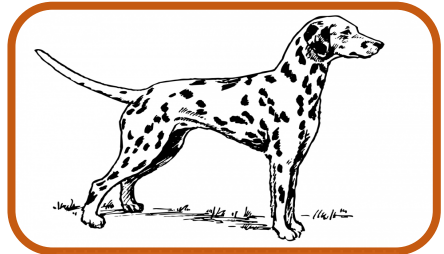
Canis familiaris
Breed: Dalmatian
Group: Non-sporting group

Figure 5.3

Alternatively, the capitalized letter may refer to genus and the following letters identify the species.
- *w*Cle is the <u>*Wolbachia*</u> endosymbiont of <u>*Ci*</u>*mex <u>le</u>ctularius*
- *w*Bm *is the* <u>*Wolbachia*</u> endosymbiont of <u>*B*</u>*rugia <u>m</u>alayi*
- *w*Ov *is the* <u>*Wolbachia*</u> endosymbiont of <u>*O*</u>*nchocerca <u>v</u>olvulus*

In many cases, multiple *Wolbachia* strains might be associated with a single host species. Rather than have *w*Sim1 and *w*Sim2 for *Wolbachia* endosymbionts of *Drosophila simulans*, for example, standardized strain designations can be replaced by an abbreviation for the geographic location of its host.
- *w*Ri is the *Wolbachia* endosymbiont of *Drosophila simulans* from <u>Ri</u>verside, CA
- *w*Au is the *Wolbachia* endosymbiont of *Drosophila simulans* from <u>Au</u>stralia

While both strains infect *Drosophila simulans* and share high genetic similarity, it is important to differentiate between the two because *w*Ri induces cytoplasmic incompatibility whereas *w*Au is not associated with a reproductive phenotype in its host.

In addition, abbreviated descriptors might be added to the end of a strain name.
- wCobs-BR is the <u>*Wolbachia*</u> endosymbiont of <u>*C*</u>*ardiocondyla <u>obs</u>curior* from <u>Br</u>azil
- wCobs-JP is the <u>*Wolbachia*</u> endosymbiont of <u>*C*</u>*ardiocondyla <u>obs</u>curior* from <u>J</u>apan
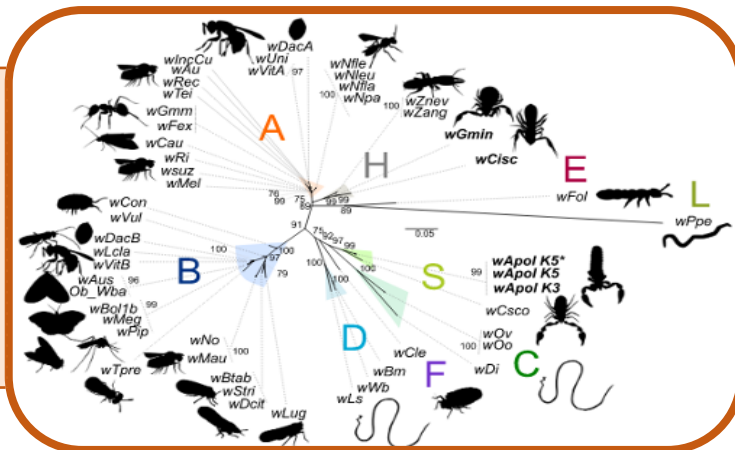
Wolbachia Supergroups

In 1998, **Supergroups** were proposed in order to group genetically related strains of *Wolbachia* (Zhou et al., 1998). The designation often, but not always, correlates with host type. The first described Supergroups A and B are associated with arthropods whereas Supergroups C and D are associated with filarial nematodes; Supergroup E has only been described in springtails; Supergroup F is found in both termites and filarial nematodes; and so on. Because different *Wolbachia* strains can co-infect the same host, Supergroup designations are often added to the end of the strain name:

- *w*VitA is the Supergroup A-*Wolbachia* endosymbiont of *Nasonia vitripennis*
- *w*VitB is the Supergroup B-*Wolbachia* endosymbiont of *Nasonia vitripennis*
- *w*AlbA is the Supergroup A-*Wolbachia* endosymbiont of *Aedes albopictus*
- *w*AlbB is the Supergroup B-*Wolbachia* endosymbiont of *Aedes albopictus*
- *w*CauA is the Supergroup A-*Wolbachia* endosymbiont of *Cadra cautella*
- *w*CauB is the Supergroup B-*Wolbachia* endosymbiont of *Cadra cautella*

Figure 5.4. Unrooted phylogenetic tree of *Wolbachia* Supergroups based on 2 genetic markers, *ftsZ* and *coxA*. *Image credit: Lefoulon et al., 2020*

Supergroups were originally based on DNA sequence homology of the *wsp* (*Wolbachia* surface protein) gene and have since been expanded to encompass multi-locus phylogenies, where a phylogenetic tree is constructed from more than one genetic marker (Fig 5.4). Using multiple markers across the genome allows for resolution of closely related species and helps to delineate Supergroups. For the purpose of this lab activity, we will construct a tree based solely on a short segment of the **16S rRNA gene** marker.

> *Pros:* This method is easy and affordable. For less than $5 per sequence, we can determine a *putative* Supergroup designation. A phylogenetic tree of one gene can be created using freely available software with no required background experience in bioinformatics.

> *Cons:* Using only a small segment (i.e., about 300-400 bp) of a single 16S rRNA gene (>1,400 bp) may not resolve closely related strains and/or clearly differentiate between some *Wolbachia* Supergroups. More advanced phylogenomic assays (involving genome data and evolutionary reconstructions) may be required to definitively identify proper grouping.

References

Lefoulon, E., Clark, T., Borveto, F. *et al.* (2020). *Pseudoscorpion Wolbachia* symbionts: diversity and evidence for a new supergroup S. *BMC Microbiol* **20,** 188.

Zhou, W., Rousset, F., & O'Neil, S. (1998). Phylogeny and PCR-based classification of *Wolbachia* strains using *wsp* gene sequences. *Proceedings. Biological Sciences*, **265**(1395), 509–515.

Pre-Lab Question

Complete the following table to match each *Wolbachia* strain name to the corresponding host species.

wAlbB	wCauA	wNfe
wAna_India	wCauB	wNfla
wAu	wFex	wPipJHB
*w*Bif	*w*Kue	*w*Rec
wBt	*w*Mel	wVitA

Host species (*Wolbachia* Supergroup)	*Wolbachia* strain name
Drosophila recens	
Drosophila bifasciata	
Drosophila melanogaster	
Cadra cautella (Supergroup A)	
Cadra cautella (Supergroup B)	
Bemisia tabaci	
Nomada ferruginata	
Nomada flava	
Culex pipiens Johannesburg	
Ephestia kuehniella	
Drosophila simulans from Australia	
Drosophila ananassae from India	
*Aedes albopictus (*Supergroup B)	
Formica exsecta	
*Nasonia vitripennis (*Supergroup A)	

Lab Activity 1: *Wolbachia* Discovery

> **Lab Pre-requisite -** Before beginning this lab, complete the following modules:
> - Bioinformatics I: Sanger Sequencing Analysis
> - Bioinformatics II: NCBI Taxonomy & BLAST Searching

> If your arthropod did not have a *Wolbachia* infection, select one of the
> "Example *Wolbachia* Sequences" from Module III:
> https://www.vanderbilt.edu/wolbachiaproject/lab-5-dna-sequences

The goal of this activity is to assess your *Wolbachia* discovery in context of previous research. Follow the steps below to see if *Wolbachia* has been identified in your arthropod(s).

Wolbachia Project Database
- Open a browser window and go to: https://wolbachiaprojectdb.org/
- Select 'Database >> Browse by Taxonomy' from the navigation menu.
- Scroll through the available taxa – is yours listed?

1. Is your arthropod (or a closely related species) represented in the *Wolbachia* Project Database? If so, how do your results compare to previous results?

NCBI
- Open a browser window and go to: https://www.ncbi.nlm.nih.gov/taxonomy
- Enter *Wolbachia* in the top Taxonomy search bar and select Search.
- Select *Wolbachia*.
- Scroll through the alphabetized list to see if *Wolbachia* has been described in your arthropod.
- *Optional -* If your arthropod is present, click on the link. The taxonomy page links all NCBI records (see upper right-hand corner for nucleotide and protein links).

2. Is *Wolbachia* listed for your arthropod?

Google Scholar
- Open a browser window and go to: https://scholar.google.com
- In the search bar, enter Wolbachia and your arthropod name. This will provide a list of journal articles and books that discuss your arthropod. Be careful, some papers report negative (uninfected) data. To the best of your ability, scroll through the article to determine status.

3. Has anyone previously detected *Wolbachia* in your arthropod?

Lab Activity 2: *Wolbachia* Naming

The goal of this activity is to follow standard *Wolbachia* nomenclature in order to name your strain. This name will be used as the taxon label for phylogenetic trees. Refer to page 5 for more information.

To the best of your knowledge, based on Activity 1, has *Wolbachia* been previously identified in your arthropod host?

YES

Add a unique identifier to the established name. For example, *w*RecTN for *Wolbachia* from *Drosophila recens* (*w*Rec) in Tennessee.

NO

Do you know the exact species of your arthropod host?

YES

The strain name will be an italicized lowercase '*w*' follwed by the first two to three first letters of species name. The first letter is capitalized. For example, *w*Mel for *Wolbachia* of *Drosophila melanogaster*.

NO

Using *Wolbachia* nomenclature (lowercase italicized '*w*' followed by two to three descriptive letters) select a name that best represents your strain. For example, you might use *w*Phe for a strain from the genus Pheidole. Or you might select *w*Ks for a genus of Pheidole ants from Kansas.

1. What is your *Wolbachia* strain name? _____

2. Open a browser window and search for "*Wolbachia w*XXX" (insert strain name). Has this name already been used?

3. If your name has already been used by scientists, revise the name by adding a descriptor at the end of the strain. For example, if your name is *w*Sim (for *Wolbachia* of *Drosophila simulans*) and you collected the fly from the city of Forest Hills, you might use *w*SimFH (for *Wolbachia* of *Drosophila simulans* from Forest Hills).

It is very possible that your *Wolbachia* strain could be identical to one that has already been described. Unfortunately, we are not able to conclusively assess *exact* genomic identity with just a partial 16S rRNA sequence. More detailed analyses, such as genome sequencing, are required. However, contributing the 16S rRNA sequence will inform scientists about the strain infecting your particular arthropod.