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# Becoming a Friend Instead of a Foe: An Attempt to Create Mutualistic Symbiosis

*by*
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## Part I – A Likely Symbiotic Progenitor: *Sodalis praecaptivus*

You are a student working for a principal investigator (PI) in a lab that specializes in insect-bacteria symbiotic relationships. The PI assigns you the task of reviewing several papers regarding the discovery of a bacteria species that is related to several bacteria symbionts. After reading the papers, you summarize the information for the PI as described below.

The discovery of *S. praecaptivus* revealed intriguing details about this free-living bacterial species. Phylogenetic analysis revealed similarities between *S. praecaptivus* and a *Sodalis* clade of insect symbionts (Figure 1). However, genomic sequencing of *S. praecaptivus* revealed that it had a large genome (~5.16 millions of base pairs, or ~5.16 Mbp), when compared to the symbionts *S. glossinidius* (~4.3Mbp) and *Sitophilus oryzae* primary endosymbiont (SOPE ~4.5 Mbp) (Clayton *et al.*, 2012).

The size difference between the genomes of *S. praecaptivus* and the *Sodalis*-clade symbionts was due to genome degeneration, a biological phenomenon that regularly occurs with symbionts (Enomoto *et al.*, 2017). The insect host provides the symbiont with energetic resources and protection. This relaxes the selective pressure on the bacteria and mutations that arise. These mutations that were detrimental and lethal outside the insect host are no longer catastrophic for the new bacteria symbiont. More mutations accumulate over time and eventually result in the reduction or even deletion of genes within the symbiont genome.

You are intrigued by the Enomoto article combined with previous work (Chari *et al.*, 2015), and you sketch the phylogenetic tree shown in Figure 1. Because *S. praecaptivus* is a free-living *Sodalis* species with a large and intact genome, it is plausible that it is the likely progenitor to the *Sodalis*-clade symbionts of today.

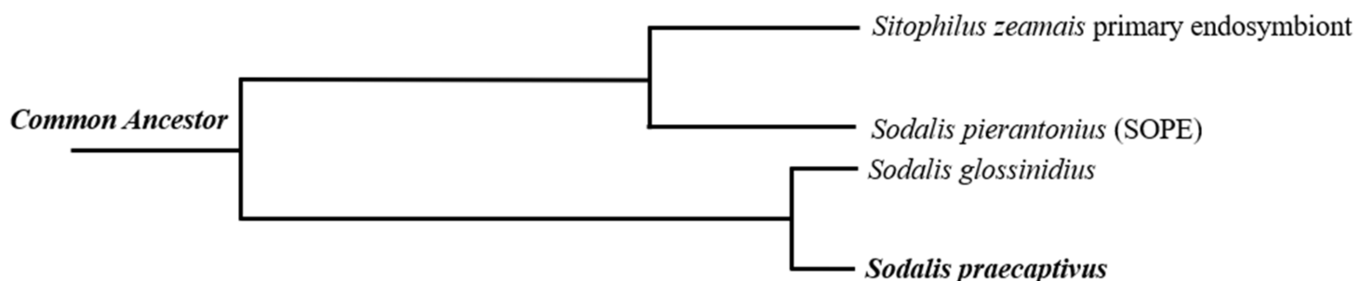


*Figure 1.* Phylogenetic tree of *Sodalis*-clade symbionts

You realize that the recent discovery of *S. praecaptivus* has opened new avenues for exploration. Little information exists as to the genesis of bacteria-insect symbiotic relationships. Your PI is intrigued by the discovery of *S. praecaptivus* and seeks to write a research proposal for further study. The proposal is based upon a hypothesis: if *S. praecaptivus* is the free-living progenitor to *Sodalis*-clade symbionts, is it a viable candidate to start a symbiotic relationship with another insect species? To address this, she wants you to ponder a few questions.

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## *Questions*

1. Would you categorize *S. praecaptivus* as an opportunistic pathogen or as a symbiont? Explain.

2. Explain why the genome of the symbionts *S. glossinidius* and SOPE are much smaller than the free-living *S. praecaptivus.* Is a larger genome beneficial for symbiosis?

3. Consider the phylogeny shown in Figure 1. Would *S. praecaptivus* be a likely candidate for symbiosis? Explain your answer.

## Part II – Using *Drosophila melanogaster* as a Host Organism

You decide to investigate potential symbiotic relationships. To test these, you will need to identify a host organism. You recall reading about the experiments using weevils (Enomoto *et al.*, 2017) and wonder whether similar relationships exist across other hosts. After chatting with your postdoctoral resident, you identify several criteria. Potential hosts will need to be able to reproduce in large numbers, be easily maintained, and provide several opportunities to repeat your experiment.

This leads you to consider *Drosophila melanogaster*, the common fruit fly. *Drosophila* is an ideal organism and meets all of your criteria. The species is easy to maintain in a controlled environment, easily fed, and it reproduces in large numbers. The flies generate a large population that will allow you to accurately detail the results of experimentation at low cost, low maintenance, and high-yield benefits.

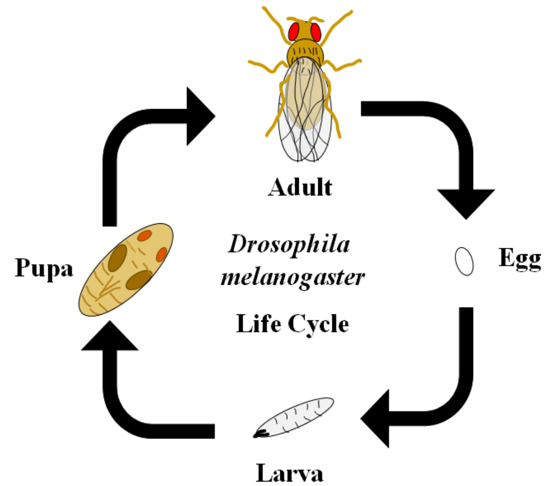


*Figure 2.* The life cycle of *D. melanogaster.*

You search your textbook and find a diagram (Figure 2), from which you realize that fruit flies have an open and observable lifecycle. *D. melanogaster* lay their eggs on the surface of their food, detectable by the human eye, and then hatch into larvae that are easily identifiable. You will have a host that you can study at any point of the life cycle from the fertilized egg to the adult.

With a host in mind, you query the literature and realize that *D. melanogaster* does not have a symbiotic history with any specific bacterial symbiont, including any members of the *Sodalis* clade. Since *S. praecaptivus* is an opportunistic pathogen that is known to establish infection throughout the lifespan of a host, you think it could possibly infect *D. melanogaster* and hypothetically lead to a new symbiotic relationship. You can't wait to share your findings with the team as you begin designing an experiment to test this novel hypothesis.

### *Questions*

1. Why is *D. melanogaster* an ideal specimen to use for this specific experimentation?

2. What method(s) would you use to infect *D. melanogaster* with *S. praecaptivus*? Explain.

3. *D. melanogaster* has a very efficient innate immune system against pathogens. Could this be problematic for *S. praecaptivus* infection and your proposed experiment? Explain.

## Part III – The *D. melanogaster* and *S. praecaptivus* Symbiotic Experiment

In designing the experiment, it is helpful to take into consideration how insects become infected with pathogens. You once again ask the postdoc for advice. He suggests either ingestion or microinjection to infect the flies. Ingestion seems the simplest technique. *D. melanogaster* eats decaying fruit that is high in sugar content, a required component for ideal bacterial growth. Adding *Sodalis* to the media would be feasible. Microinjection sounds scary to you at first but could also work.

In this experiment, you decide to use several available strains (wild type and mutants) of *S. praecaptivus* to infect *D. melanogaster.* This will enable you to analyze how each strain of *S. praecaptivus* compares to one another in the novel host, and how they impact *D. melanogaster* compared to *Sodalis* and the grain weevil (Enomoto *et al.*, 2017). You have plenty of *Drosophila* available and generate a chart to summarize the available *Sodalis* strains that you can use in the experiment.

The available wild type and *Sodalis* mutant strains are listed below:

- Wild type (WT): This is the strain most commonly observed in nature without any mutations.
- marR: Synthesizes antimicrobial peptides (AMP) to protect against immune responses.
- phoP: Required for the synthesis of antimicrobial peptides.
- *ypeI:* Quorum sensing (QS) gene that regulates *ypeR* and *yenR* genes.
- *ypeR:* QS gene that regulates the synthesis of toxins.
- *yenR:* QS gene that regulates the synthesis of toxins.

If the genes listed above are mutated or knocked out (Δ), their function is severely impacted and potentially results in the death of *S. praecaptivus* or the host organism. Double mutants are used to determine if there is synergy between the genes.

Your PI directs you to perform experiments infecting *D. melanogaster* with the various strains of *S. praecaptivus* using both ingestion and microinjection. You collect data and prepare the following charts for interpretation. Using these figures (Figure 3 and 4), prepare for the next laboratory meeting by answering each question.

***Ingestion:*** The first attempted method to infect *D. melanogaster* was oral ingestion. Each individual strain of *S. praecaptivus* was grown on separate agar media. Then, flies were added to each individual strain of *S. praecaptivus* agar and allowed to feed for a period of two days. At the conclusion of two days, a sample of live flies from each population were homogenized and tested to determine if any were infected with *S. praecaptivus*. The remaining population was transferred to normal fly media, free of any *S. praecaptivus*. At Day 14, the remaining flies from each population were homogenized and tested to determine if *S. praecaptivus* established a persistent infection (Figure 3). An infection is considered persistent if over 60% of *D. melanogaster* population are infected with *S. praecaptivus* at Day 14.

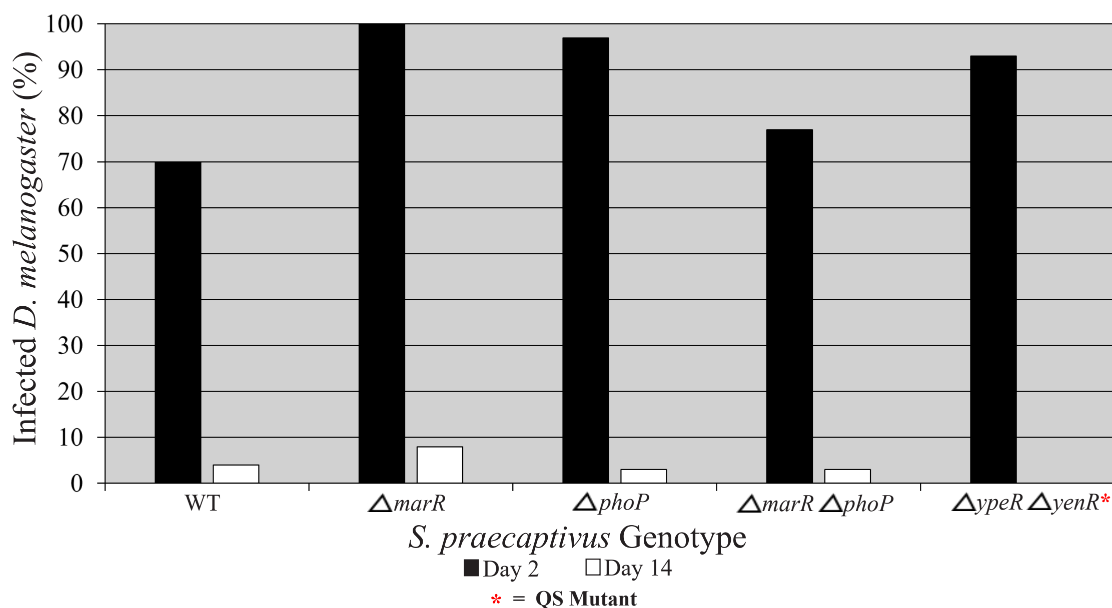


*Figure 3.* Infection percentage of *D. melanogaster* via ingestion.

## Questions

1. From Figure 3, was *D. melanogaster* infected by *S. praecaptivus* at Day 2? Explain your answer.

2. Did *S. praecaptivus* establish a persistent infection at Day 14? Explain.

3. What is a possible reason why *S. praecaptivus* could not establish a 60% persistent infection in the fly populations?

***Microinjection:*** The second method used to infect *D. melanogaster* was microinjection. Separate fly populations were microinjected with individual strains of *S. praecaptivus* and immediately placed on normal fly media. Two days post infection, a sample size from each fly population was homogenized and tested for infection. At 14 days post infection, the remaining flies from each population were homogenized and tested for *S. praecaptivus*. Infection percentages remained high for the WT, Δ*ypeI*, and the double mutant Δ*ypeR*-Δ*yenR* strain (Figure 4). The AMP mutants ΔmarR and ΔphoP had a significantly lower infection percentage, less than 60% at Day 14.

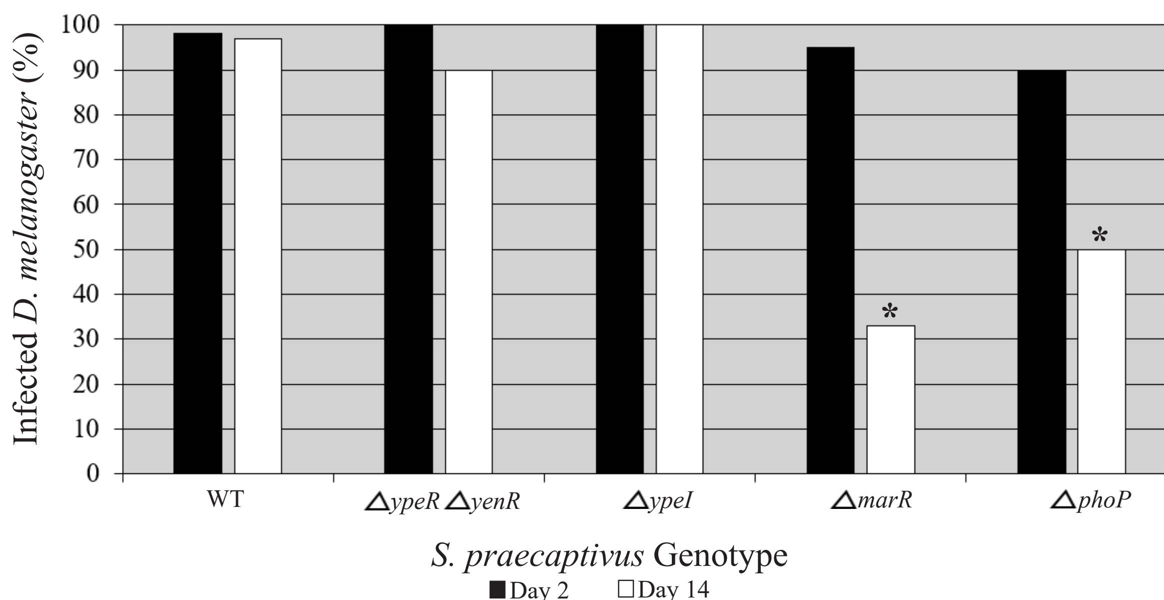


*Figure 4*. Infection percentage of *D. melanogaster* via microinjection.

## Questions

4. Which *S. praecaptivus* strain(s) established a persistent infection by Day 14? Explain.

5. Explain why the WT, Δ*ypeR*-Δ*yenR*, and Δ*ypeI* mutants had a higher infection percentage than that of the ΔmarR and ΔphoP mutants.

6. Is microinjection a more successful infection method compared to ingestion? Explain.

## Part IV – Pathogenesis of *S. praecaptivus*

Following the lab meeting, your PI was particularly interested in infection via microinjection. She asks you whether the microinjection data was comparable to other published information on the grain weevil (Enomoto *et al.*, 2017), which you recall from your earlier reading. She is also concerned whether the mutant strains have a negative impact on the life expectancy of the fly population. To address this, you decide to extend the observation of the infected fly population from two weeks (14 days) to four weeks (28 days).

After successfully infecting *D. melanogaster* with *S. praecaptivus* via microinjection, you observe the fly population for 28 days. At two weeks post-infection, the *S. praecaptivus* infection began to have lethal effects on several fly populations (Figure 5).

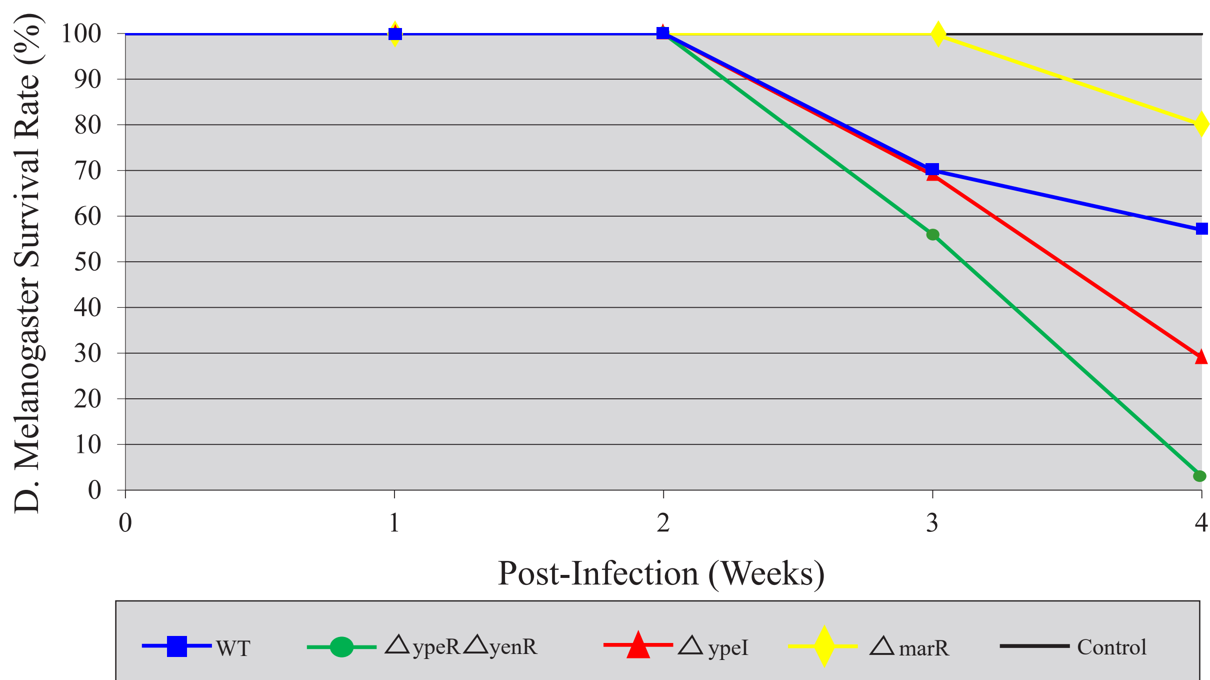


*Figure 5*. Survival rate of *D. melanogaster* at four weeks.

### *Questions*

1. What *S. praecaptivus* strain(s) were most lethal to *D. melanogaster*? How does this compare with the grain weevil data from Enomoto *et al.*, 2017?

2. How does infection of the WT strain at four weeks compare between *D. melanogaster* (your data) and the grain weevil (Enomoto *et al.*, 2017)?

3. Based upon these results, is *S. praecaptivus* (WT) a viable candidate to further explore the symbiotic relationship with *D. melanogaster*? Explain.

## References

Chari, A., K. Oakeson, S. Enomoto, D. Jackson, M. Fisher, and C. Dale. 2015. Phenotypic characterization of *Sodalis praecaptivus* sp. nov., a close non-insect-associated member of the *Sodalis*-allied lineage of insect endosymbionts. *International Journal of Systematic and Evolutionary Microbiology* 65: 1400–5. <https://doi.org/10.1099/ijs.0.000091>

Clayton, A., K. Oakeson, M. Gutin, A. Pontes, D. Dunn, A. von Niederhausern, R. Weiss, M. Fisher, and C. Dale. 2012. A novel human-infection-derived bacterium provides insights into the evolutionary origins of mutualistic insect-bacterial symbioses. *PLoS Genetics* 8(11): e1002990. <https://doi.org/10.1371/journal.pgen.1002990>

Enomoto, S., A. Chari, A. Clayton, and C. Dale. 2017. Quorum sensing attenuates virulence in *Sodalis praecaptivus*. *Cell Host Microbe* 21(5): 629–636.e5. <https://doi.org/10.1016/j.chom.2017.04.003>