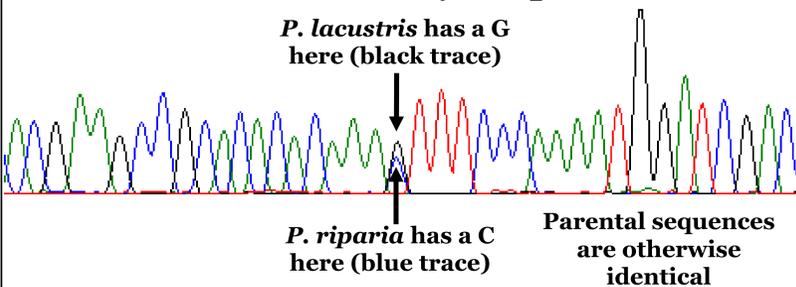


## Detecting *Phytophthora* hybrids

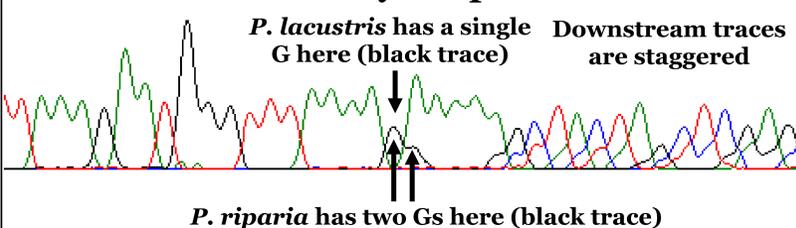
- A common way to determine that an individual organism is a hybrid using DNA sequences is to sequence many different genes, and observe that some genes match species A, while others match species B
- This is laborious, and for many species of *Phytophthora*, there aren't more than two genes available against which to compare
- However, we can observe putative *Phytophthora* hybrids when sequencing a single nuclear barcoding locus (ITS) using standard methods (direct Sanger sequencing)
- When a hybrid *Phytophthora* isolate contains ITS sequences from both parents, these two distinct sequences are simultaneously amplified and sequenced
- Where sequences from the two parents differ, sequencing artifacts appear, which, if properly identified and analyzed, can give evidence that an isolate is a hybrid, as well as information about the putative parents
- Two sequences that are otherwise identical can differ because of a change in identity of a base-pair (a substitution) or the insertion or deletion of a base-pair (an indel) in one of the sequences
- When the parental sequences differ because of a substitution, we observe a double-peak in the sequencing trace, called a substitution polymorphism

### Substitution Polymorphism



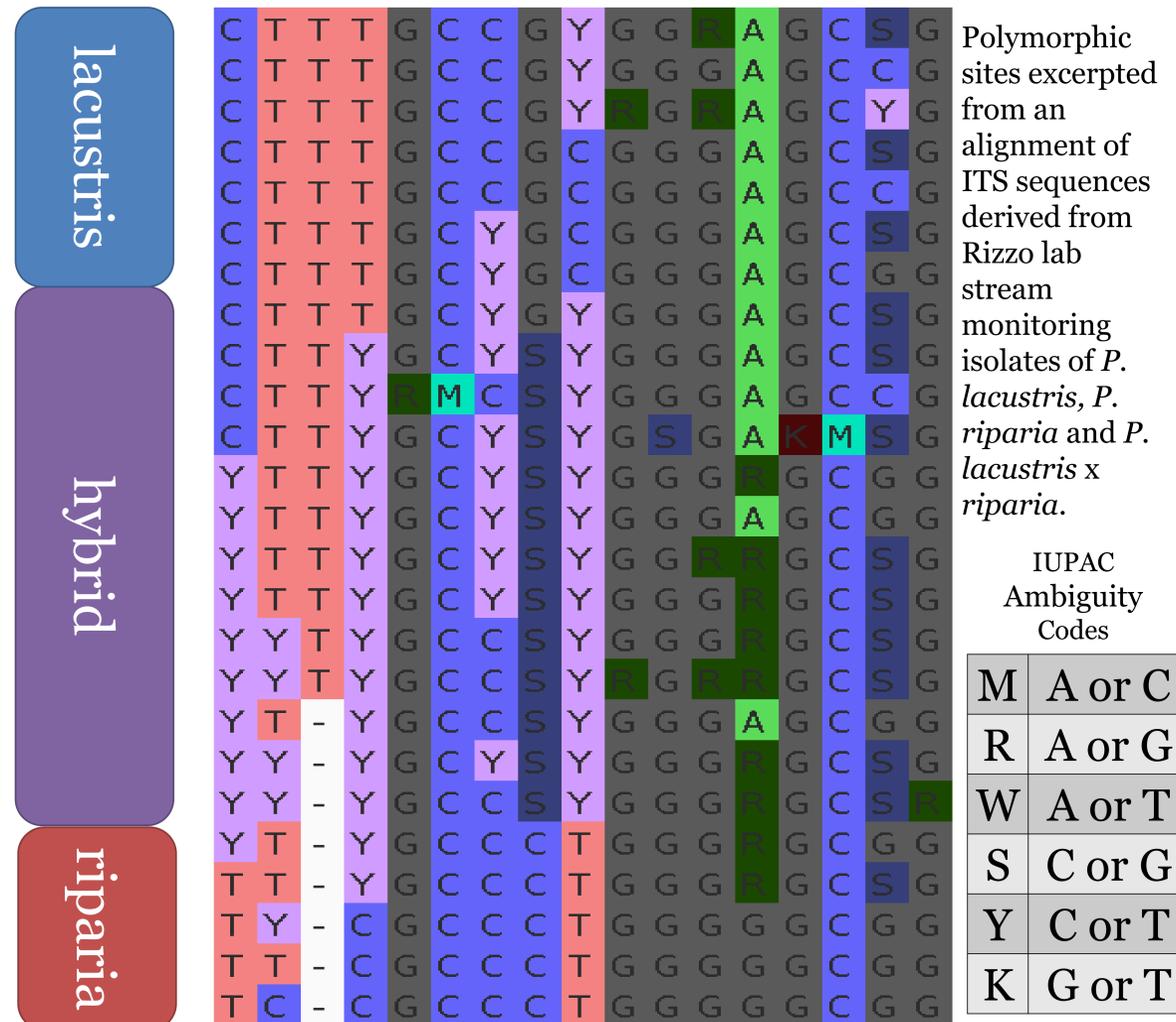
- When the parental sequences differ because of an indel, we observe a staggering of the sequence downstream of the indel polymorphism, which is often interpreted as a failed sequencing run

### Indel Polymorphism



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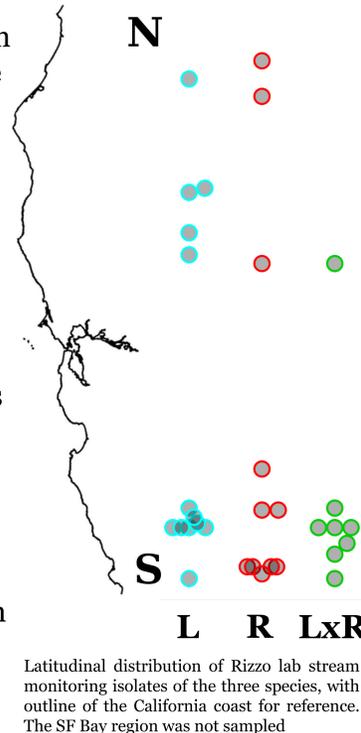
Polymorphic sites excerpted from an alignment of ITS sequences derived from Rizzo lab stream monitoring isolates of *P. lacustris*, *P. riparia* and *P. lacustris x riparia*.

IUPAC Ambiguity Codes	
M	A or C
R	A or G
W	A or T
S	C or G
Y	C or T
K	G or T

- Phytophthora lacustris*, *P. riparia* and a hybrid of the two, *P. lacustris X riparia*, are commonly isolated during the ongoing Rizzo lab sudden oak death stream monitoring project
- ITS sequences generated from direct Sanger sequencing runs were analyzed using a method that interpreted select sequencing errors as evidence of hybridization
- Each species showed great intraspecific variation with regards to ITS sequences; this ITS sequence variation may be a consequence of locally diverse populations, but also may be a consequence of prolonged asexuality, resulting in the breakdown of the rDNA homogenization that occurs during meiosis
- High levels of ITS variation observed between *P. lacustris X riparia* isolates suggest the two species are closely enough related to create numerous homoploid hybrids
- Cloning or high throughput sequencing is needed to better characterize the intragenomic or intra-individual rDNA variation inside these isolates

## *P. lacustris* and *P. riparia*

- P. lacustris* and *P. riparia* are both members of *Phytophthora* clade 6, which includes many members known better as aquatic litter decomposers than terrestrial plant pathogens
- P. lacustris*, formerly known as *P. taxon salixsoil*, may be the most ubiquitous and widely-distributed member of the genus
- P. riparia* has only been reported from North America and China
- Hybridization or introgression between *P. lacustris* and *P. riparia* was documented in the original description of *P. riparia*
- During Rizzo lab stream monitoring activities, the three species have not commonly been baited from the same watershed, although this could be due to insufficient sampling
- Hybrid isolates tend to have mitochondrial sequences that correspond to *Phytophthora lacustris*



## Hybrid swarms

- Different patterns of hybridization may be observed when two species that are not reproductively isolated come into contact
- For more distantly related species, polyploid hybridization is most commonly observed, which results in a hybrid with a different number of chromosomes than either parent
- The polyploid hybrids are typically reproductively isolated from their parents
- For more closely related species, homoploid hybridization may occur, with equal chromosome numbers
- The homoploid hybrids are typically interfertile with their parents and each other, generating great amounts of variation
- This is known as a hybrid swarm

