Host induced phenotypic diversification in *Phytophthora ramorum*

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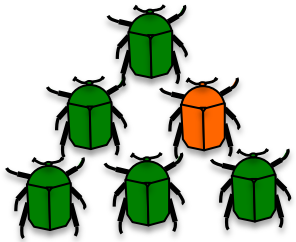
Non-native species are able to evolve rapidly following introduction into new region
(different environment, unfamiliar food, predators ...)
e.g.)
Animals: Increase in leg length of cane toad in Australia (Phillips et. al., 2006)

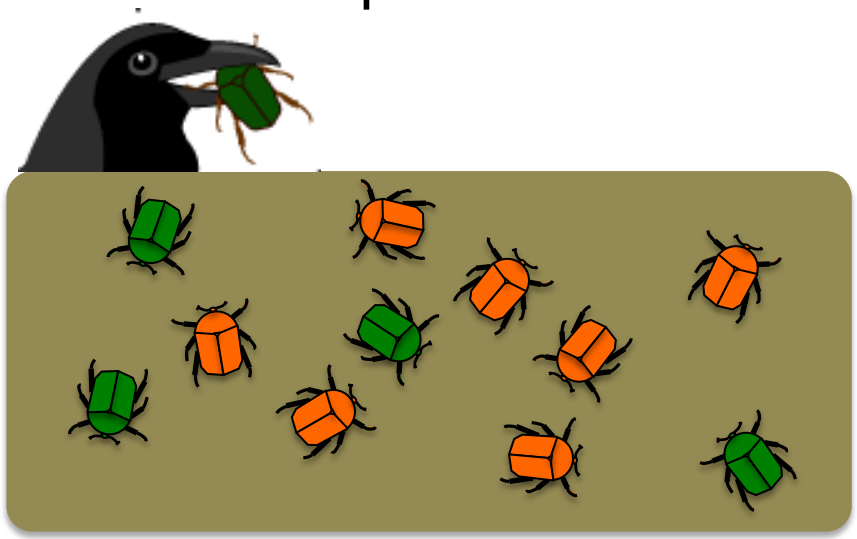
Plants: Changes in leaf size of St. John's wort (Maron et al., 2004)

Oomycetes: Morphological and pathogenic diversification in a clonal lineage of *Phytophthora cinnamomi* (Hüberli et al., 2001)

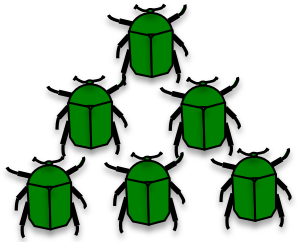
Selection on preexisting variation



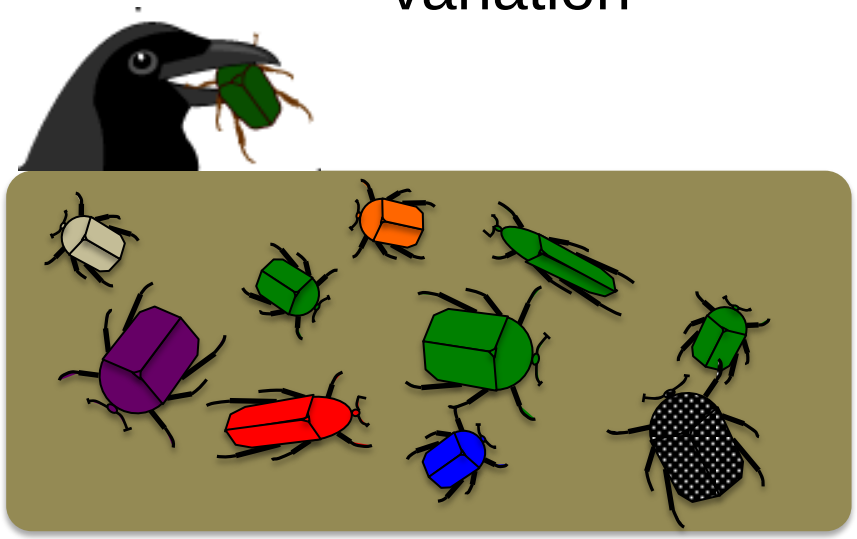
Different selective pressure



Little variation in clonal population



Generation of de novo variation



Generation of de novo variation in response to stress

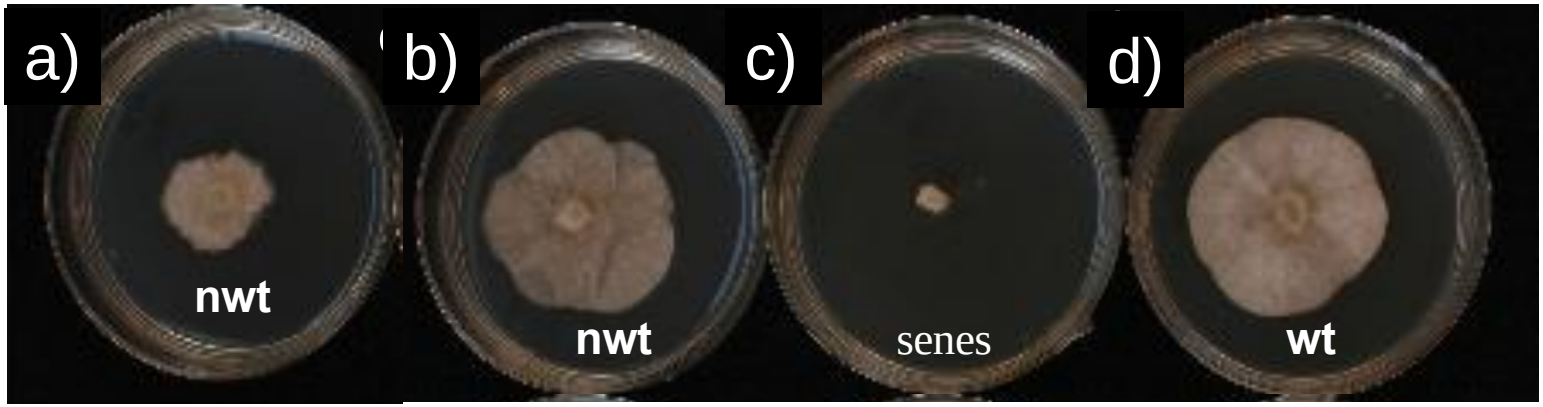
1. Adaptive mutagenesis: an increase in DNA mutation rates by alternative error-prone DNA polymerases.

1. Epigenetic variation: heritable changes in gene regulation by histone modification, DNA methylation, etc.

1. Transposon (mobile genetic element) activation and genome restructuring.

Phytophthora ramorum: Phenotypic diversification in the absence of genetic variation

1. The NA1 lineage displays large variations in growth rate and morphology (Brasier et al., 2006):
   wild type (*wt*) & non-wild type (*nwt*).



4 NA1 isolates

2. *nwt* isolates are less aggressive on leaves of *Rhododendron, Camellia*, etc. (Elliott et al., 2011)

Pathogenicity test on Coast Live Oak

Hüberli & Garbelotto

45 isolates from three hosts (bay laurel, tanoak and coast live oak)

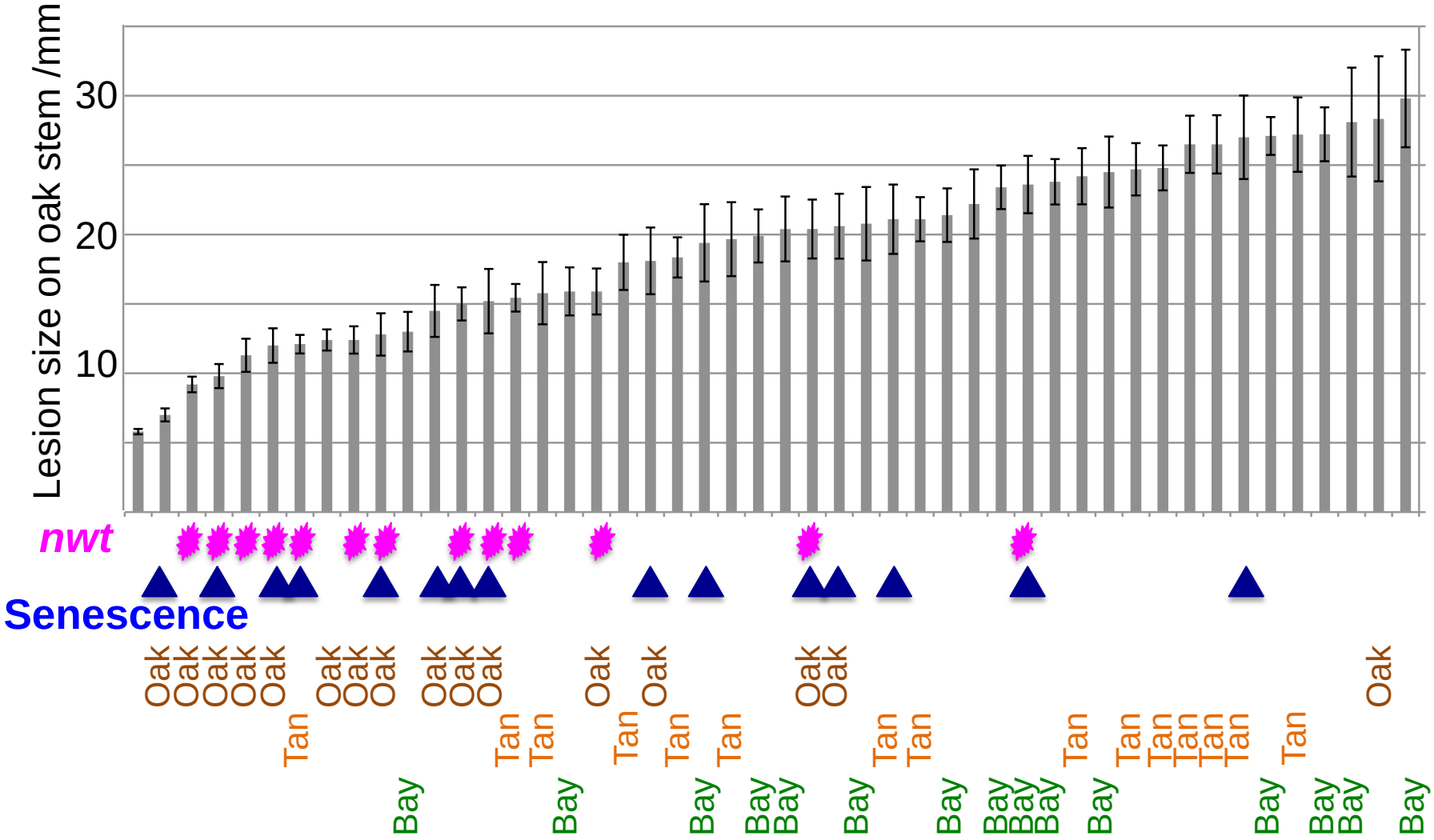
↓

Inoculate oak seedlings, 10 replicates / isolate

↓

average diameter of lesions under the bark

nwt, senescence and reduced virulence are associated with Oak isolates



Kasuga et al., 2012

Host-driven diversification between isolates from bay laurel (*wt*, virulent) and coast live oak (*nwt,* less virulent)?

Simple sequence repeat (SSR) genotyping (Kozanitas):

No genetic differentiation among isolates from oak, bay laurel and tanoak was detected (AMOVA p=0.62)

Epidemiology: no differentiation

Bay laurel is an infective host

Oak is a dead-end host

Hypothesis:

P. ramorum generates de novo variation in oak trees.

Koch's postulates experiment on canyon live oak is underway

Bay isolates

↓

Canyon live oaks

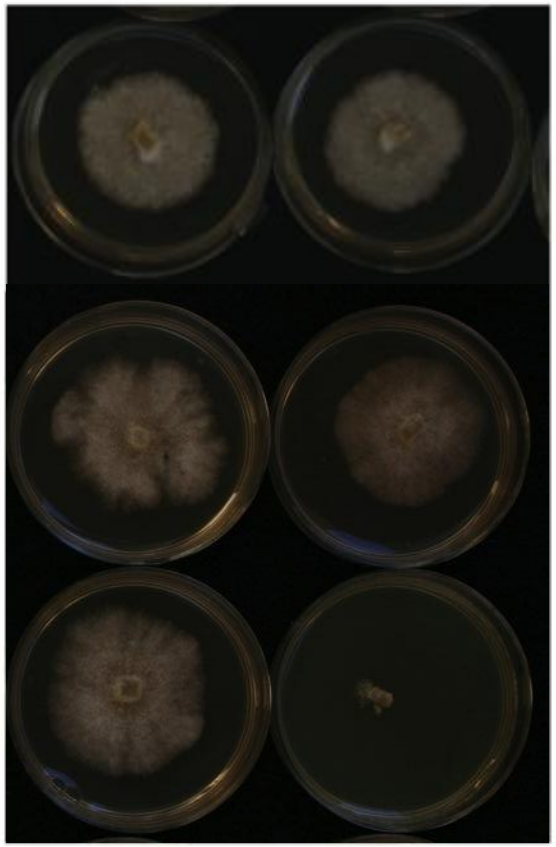
↓

Re-isolation 4 & 9 months after inoculation

Phenotypic conversion in canyon live oak!

□ *nwt*

○ *wt*



Original isolate

Re-isolates
c.a. 20% *nwt*

Hypothesis:

Genetic differentiation between the original isolate and re-isolates should be very small.

Most of the phenotypic differences should be due to gene regulation rather than genetic polymorphism.

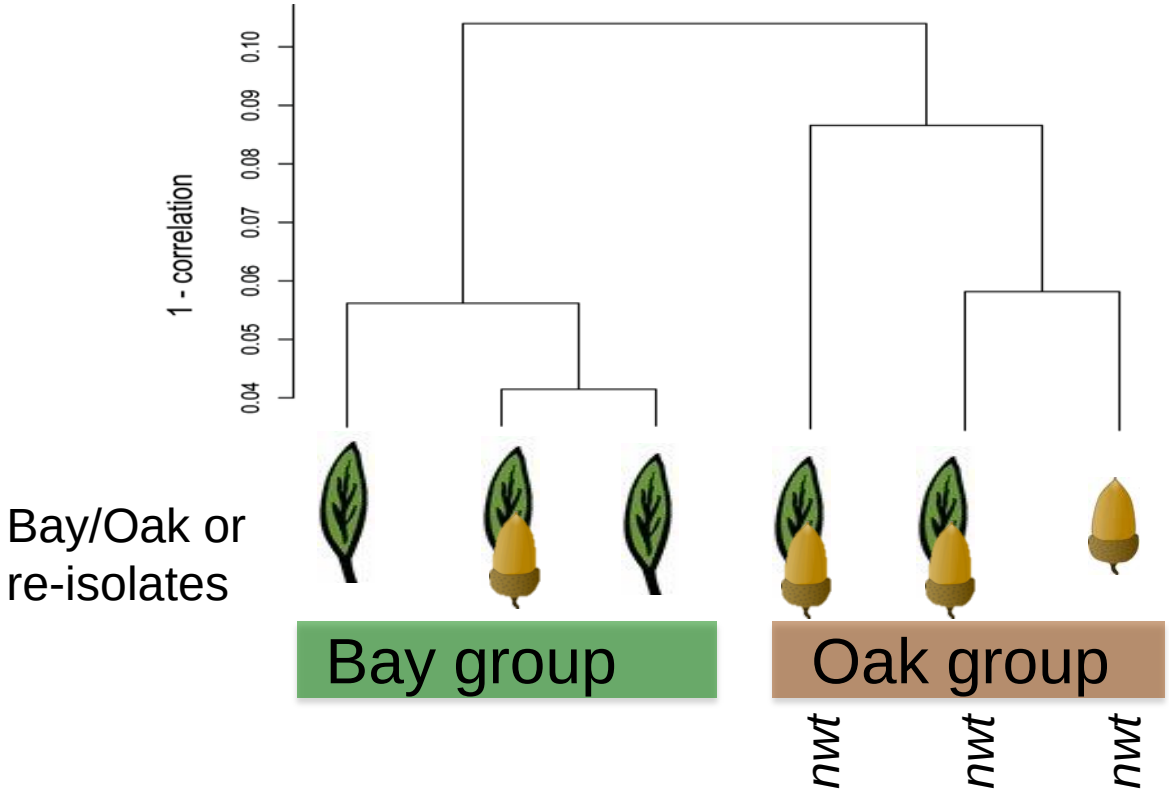
Global mRNA profiling can give a snapshot of expression variation.

Microarray mRNA profiling

2 bay laurel isolates,
1 coast live oak isolate
3 re-isolates from canyon live oak

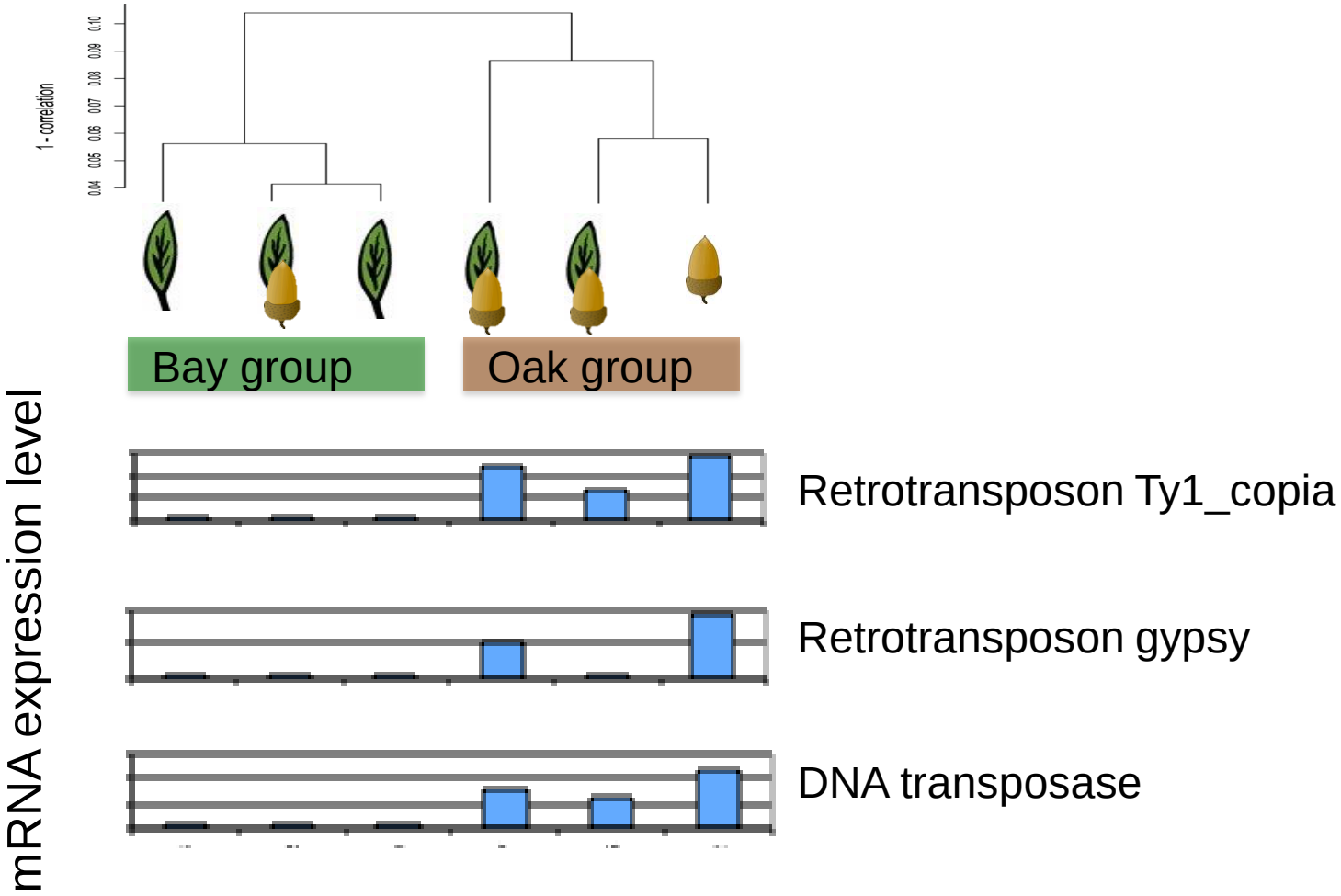
Grew on 1x clarified V8 for 7 days
↓
RNA extraction
↓
cDNA synthesis and hybridization to NimbleGen microarray
↓
Monitor c.a. 16K gene activities

Some re-isolates showed oak-like global mRNA expression profiles

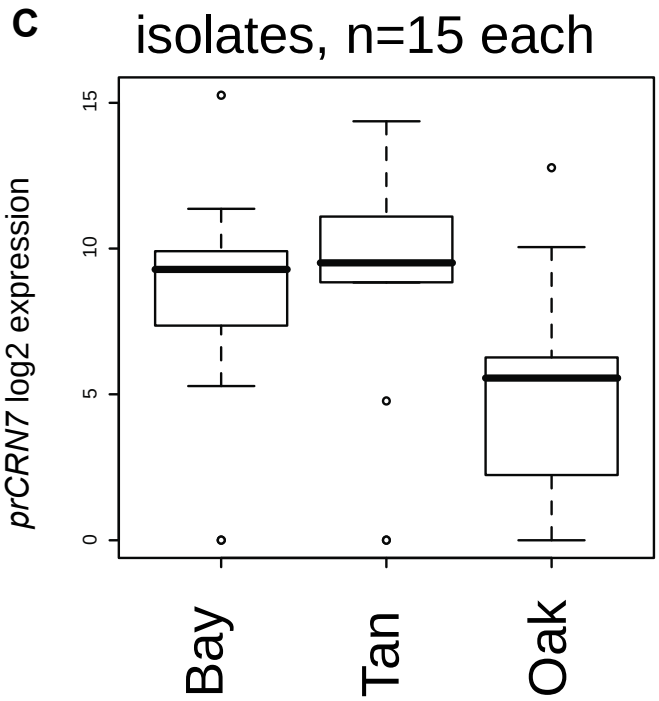


The 6 isolates clustered due to mRNA expression of c.a.12,000 genes

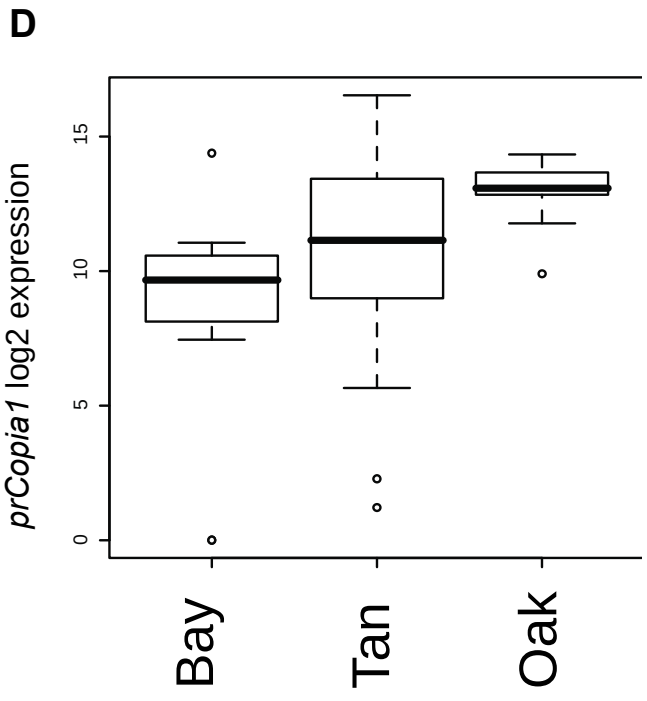
Various types of transposable elements are de-repressed in isolates from oaks



Two qRT-PCR markers differentiate between bay laurel and oak isolates



Crinkler effector gene



Retrotransposon

Conclusions

4 months in canyon live oak was sufficient to convert phenotypes:

nwt colony morphology
senescence
de-repression of transposons

Phenotypic diversification is of epigenetic origin

De-repression of transposons: by-product or cause?

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Rays Jiang (Broad Inst)

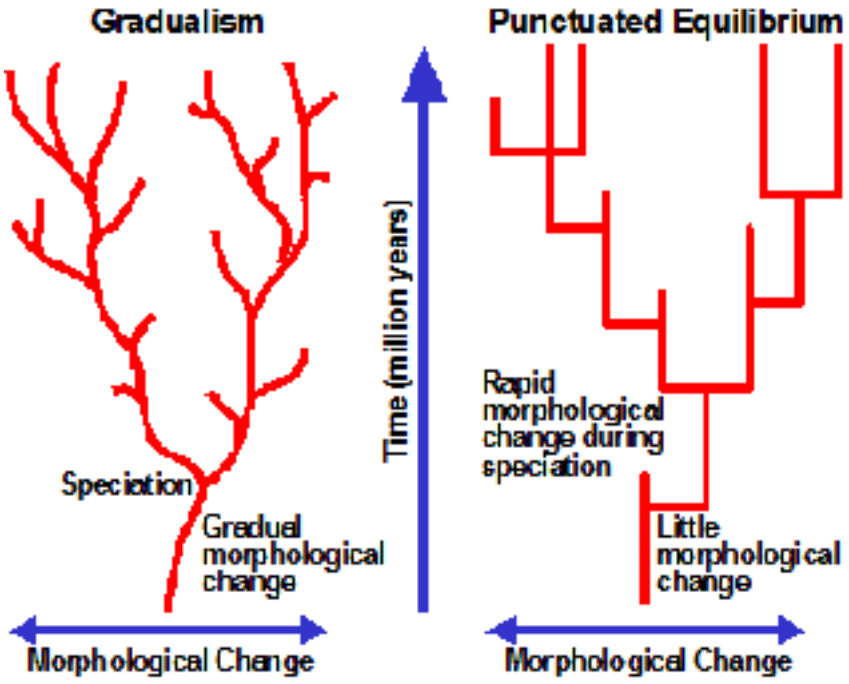
Questions

(1) Oak/Bay phenotypes reversible (epigenetics)?

(2) Cause for de-repression of transposons:

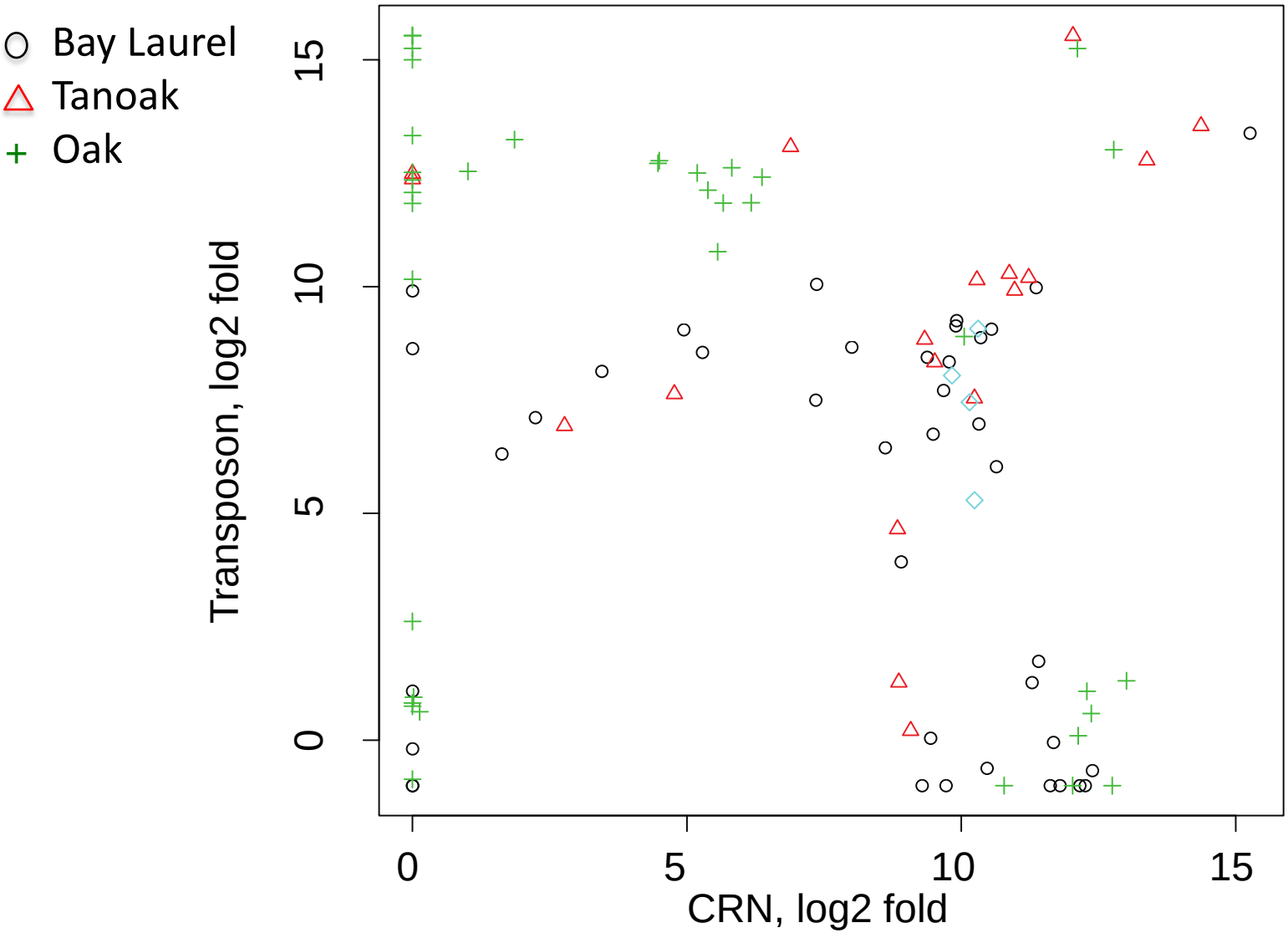
- Viral infection? (dsRNA virus negative, Hacker et al. 2005)

(3) Epigenetic modification and/or relaxation of epigenetic control of transposons for “rapid adaptation to new hosts”?



Morphological change

99 Californian isolates were scored for CRN and Transposon mRNA expressions by RT qPCR



Stability of RT qPCR markers

Isolates on CV8

↓

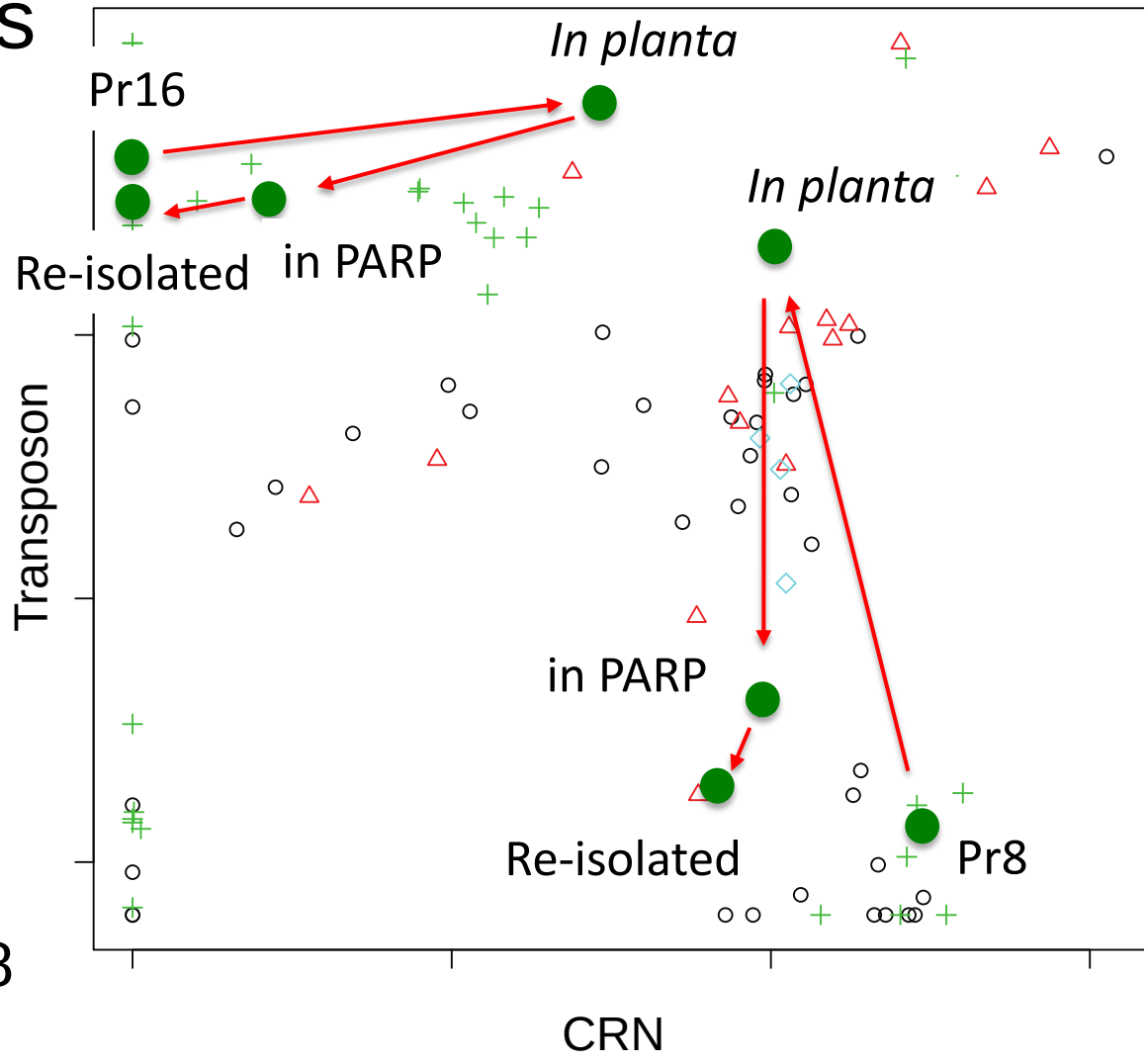
1 week in Rhododendron

↓

Re-isolation on PARP selective medium

↓

Subculture on CV8

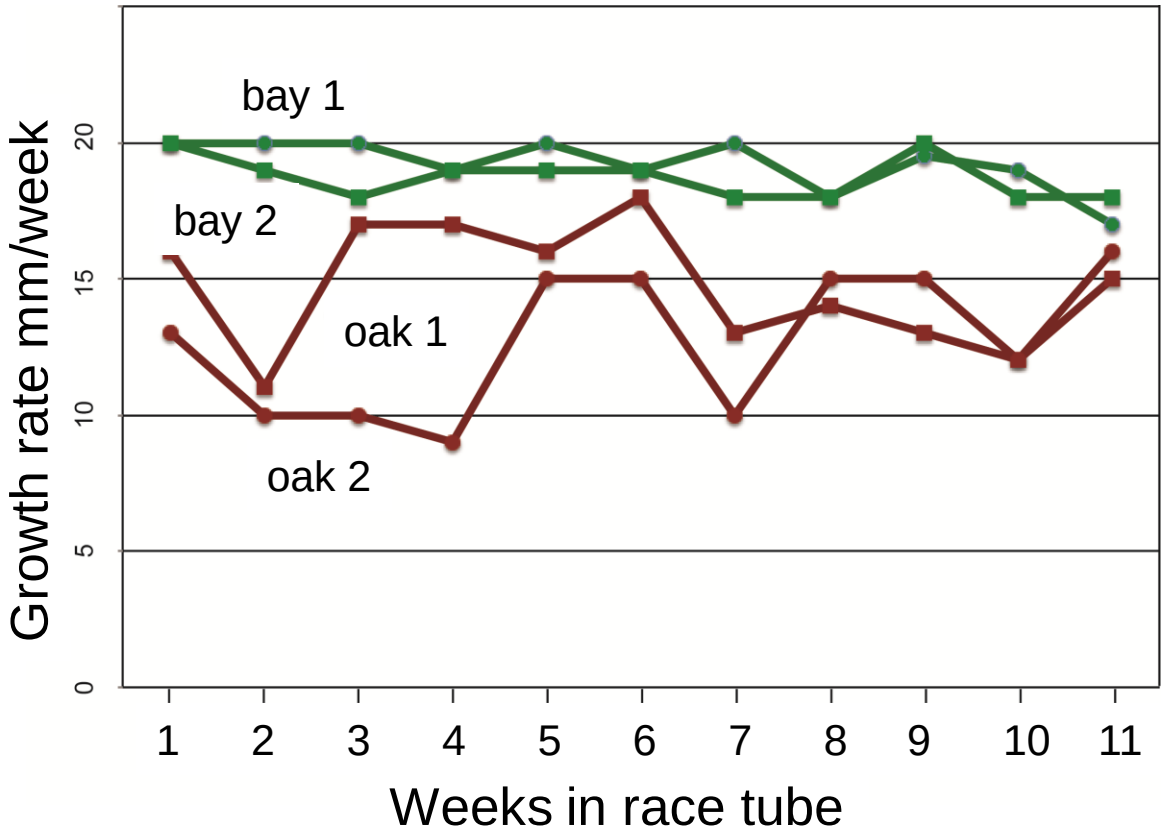


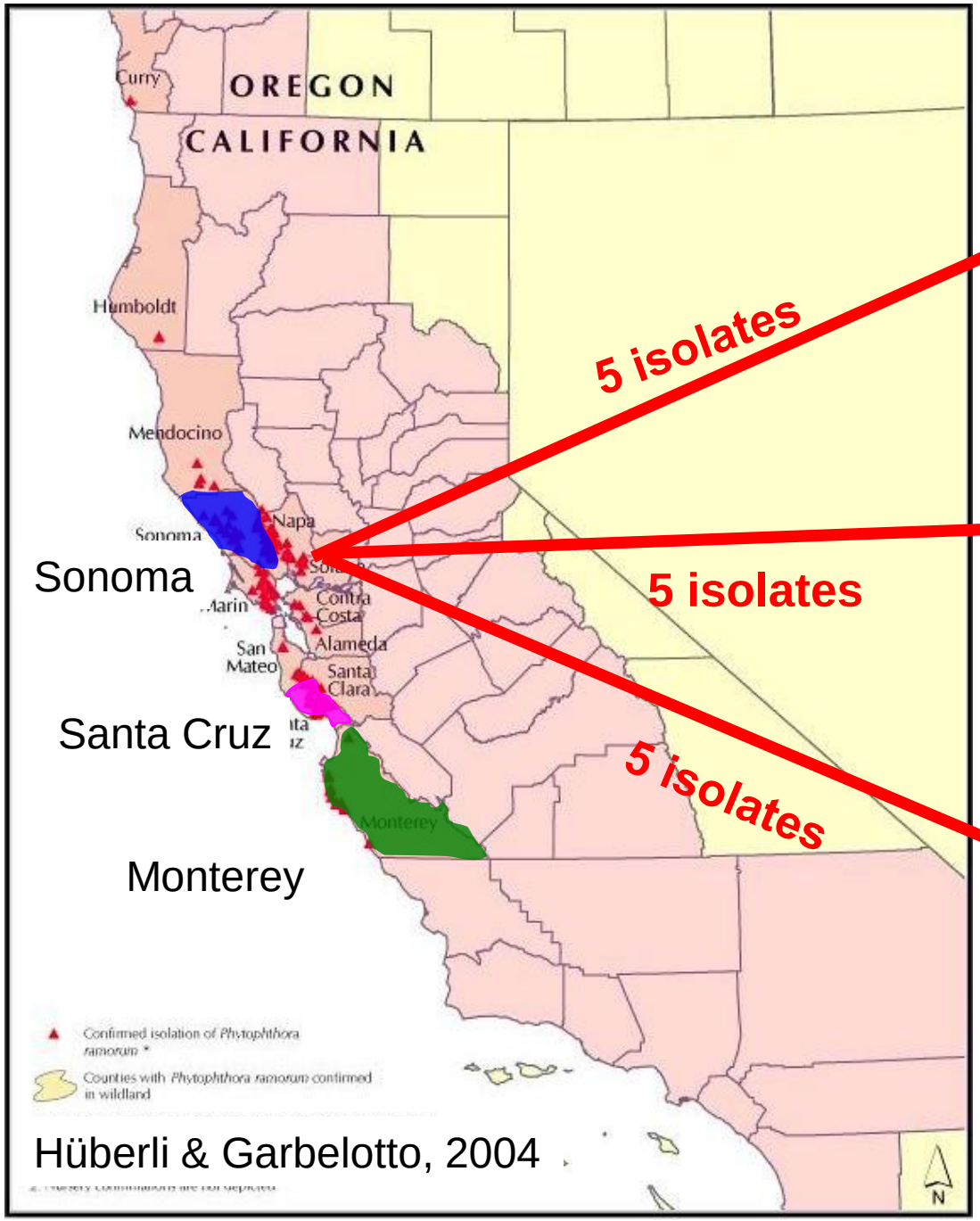
A high rate of death among isolates from coast live oak

The death toll of *P. ramorum* isolated between 2000 and 2002

	# isolates	obs. Dead	exp. Dead	P-value
tanoak	50	24 (48%)	25.5 (51%)	
coast live oak	60	43 (72%)	30.6 (51%)	<0.01
Rhododendron	9	4 (44%)	4.6 (51%)	
bay laurel	61	20 (33%)	31.1 (51%)	<0.01

Oak isolates show punctuated growth rate





Sonoma

Santa Cruz

Monterey

Hüberli & Garbelotto, 2004

5 isolates

5 isolates

5 isolates

Bay Laurel

Tanoak

Coast Live Oak