Bases of Invasion Biology of Pathogens

- Source: it can be either a pathogen in the native area, possibly with a cryptic phase, or not (weak pathogen or endophyte). In general the larger the populations, the easier they will become a source
- Transport, survival: related to resting structures, distance, season
- Establishment: related to presence of host (if host specific), to host-jumps, to survival as saprobe, to ecological similarity with source area, to lack of competitors/predators. This may be last stage
- If transmission > mortality then organism becomes invasive, although at first unnoticed (lag phase) and populations transported long-distance become founder populations
- Founder populations are all characterized by a strong genetic bottleneck, and at the same time must face completely new environments: purifying selection (loss of alleles that may be costly and not necessary); sexual reproduction especially if multiple introductions occurred from different sources; hybridization with native congeneric species and subsequent gene introgression (major evolutionary leap forward); plasticity, ability to have a variable phenotypic response to different environments…
  - ARE ALL MAJOR MECHANISMS LEADING TO INVASION

Predicting impacts of invasions (I)

$$I = k * i * t_{(hcpsv)}$$

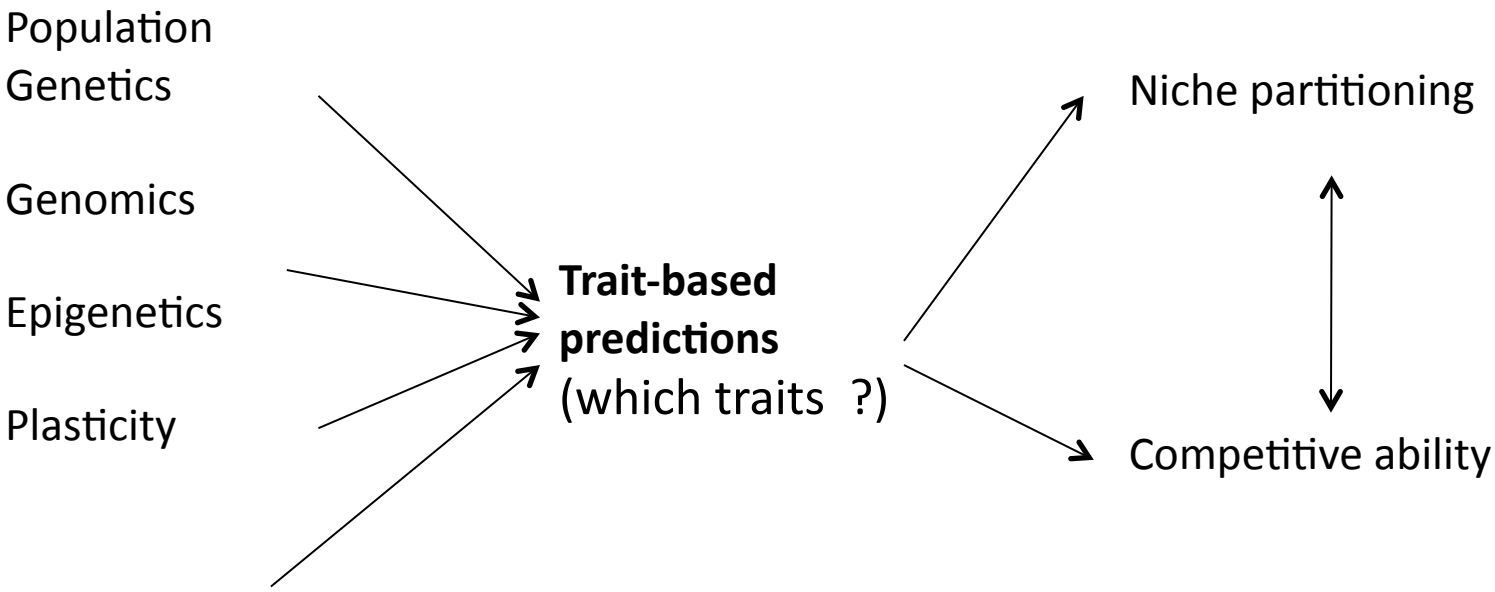
- k = constant depending on the pathosystem
- i = number and source of introduction events
- t = transmission rate

h = density of sporulating hosts

c = permeability of landscape
p = favorability of climatic conditions
s = synchronicity between host susceptibility and pathogen life cycle
v = variation in susceptiility of hosts and virulence pathogen

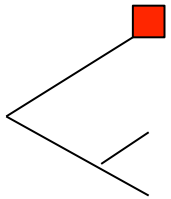
Garbelotto & Pautasso, 2012

<u>Transmission</u> is an overlooked aspect, which includes, but is much more, than just pathogenicity

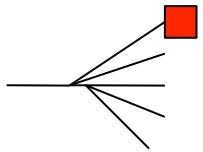


Genetic structure of an invasive microbial species

- Species has **ancient** lineages evolved in isolation (drift, selection, etc.)

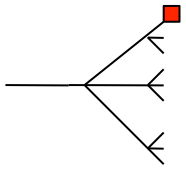


- Introduced population(s) only partial representation of native species

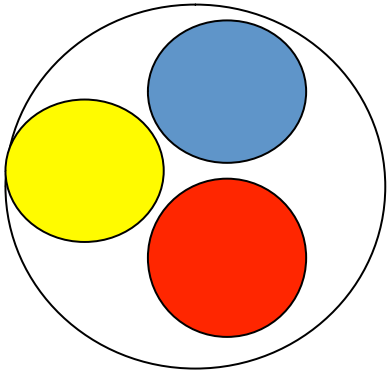


= introduced

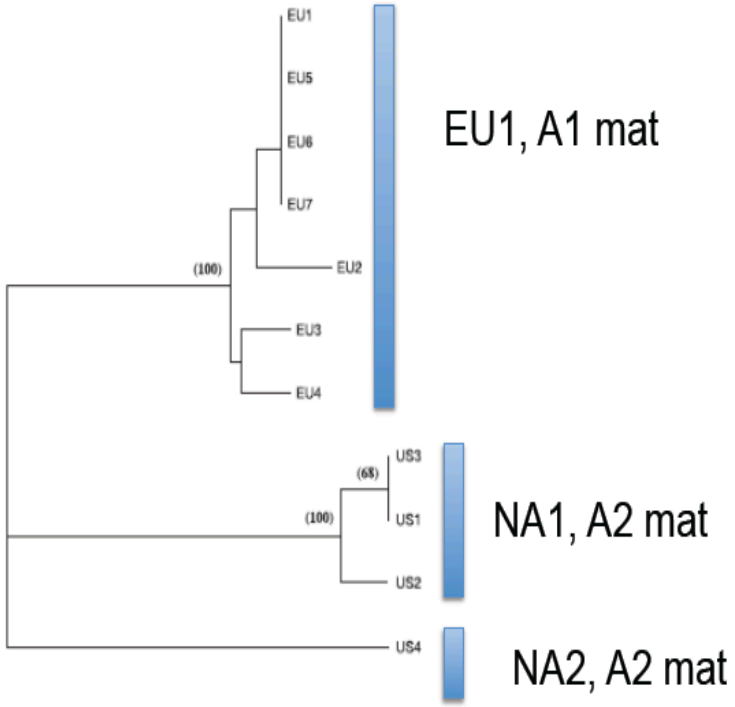
- Specific “recently” emerged genotypes associated with specific phenotypes (single genes or more)



Species has ancient lineages evolved in isolation (e.g. *P. ramorum*)



- Genetic divergence
- Phenotypic diversity:
  - Mating type
  - Growth rate
  - Pathogenicity
  - Host-specificity



Mating type

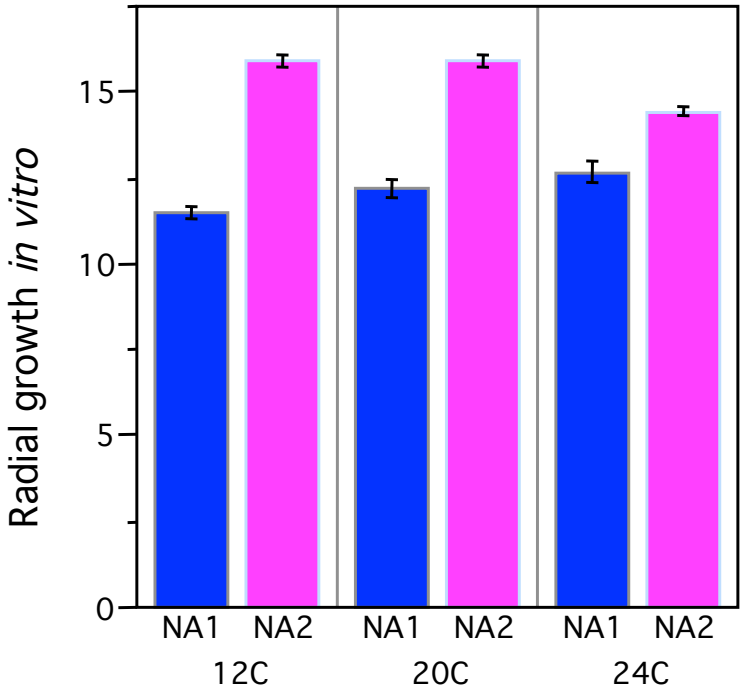
- Loci under selection may be linked to mating type
- Recombination will accelerate evolutionary rate (see *Prediction* section)
- Sexual structures may confer advantage

Sexual structures may confer advantage

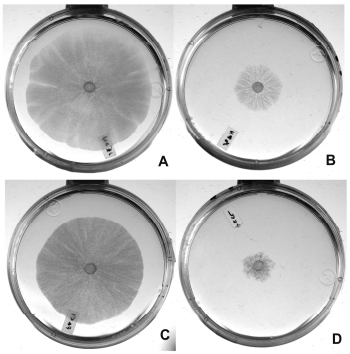
- Sexually produced **oospores** allow for survival in harsher climatic conditions

- Homothallic species such as *P. nemorosa* and *P. pseudosyringae* are less virulent than *P. ramorum* but are also introduced in California (Linzer et al. 2008) and have a **much broader distribution**, why?
  - History (introduced earlier ?)
  - Because homothallic they produce oospores

Growth rate…

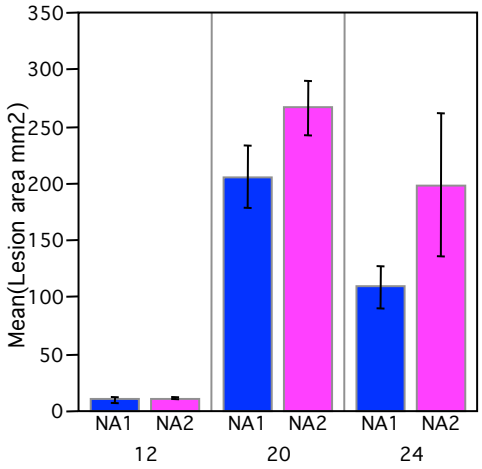


NA2 NA1

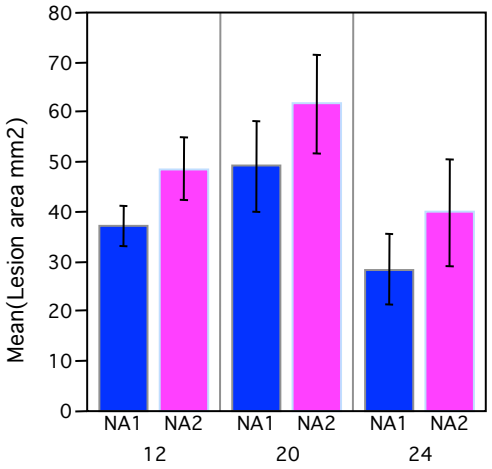


NA2 > NA1

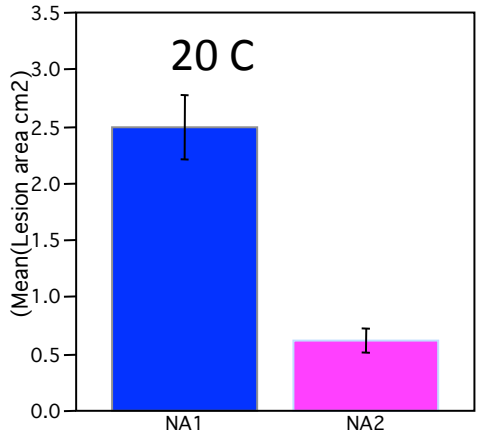
Interaction of lineage, host, and temperature.



Rhododendron sp.
NA1 < NA2, P = 0.05



Umbellularia californica
NA1 < NA2 , P = 0.008

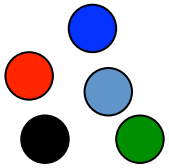


Camellia setsugeken
NA1 > NA2 , P < 0.0001

At 20 C, *Rhododendron* and *Umbellularia* effective hosts more for NA2 and NA1, but *Camellia*, only for NA1

At 12 C, *Rhododendron* not an effective host, *Umbellularia* yes, that may explain why *P. ramorum* limited spread in Northern Europe on *Rhododendron*

Specific genotypes associated with specific phenotypes



- Some specific genotypes carry specific phenotypes
- *Phytopthora cinnamomi*: different genotypes associated with different commodities and transported around the world: are genotypes equivalent?

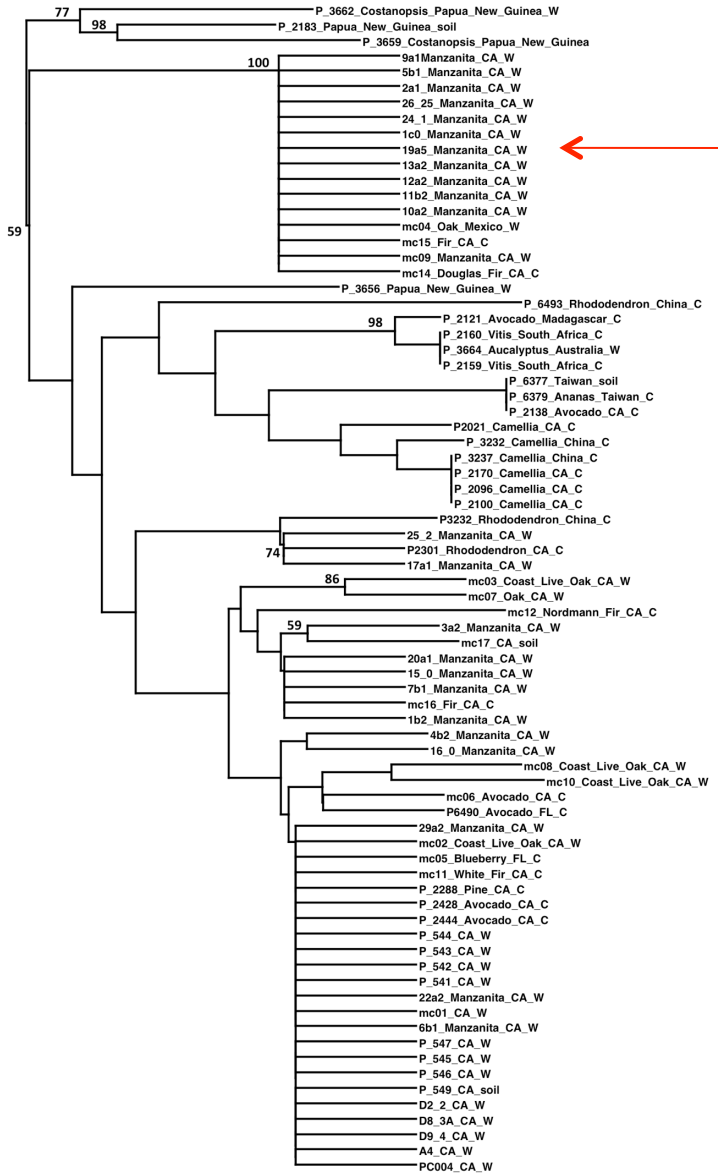
1- Huge oak mortality in Colima, Mexico (Tainter *et al*. 2000)

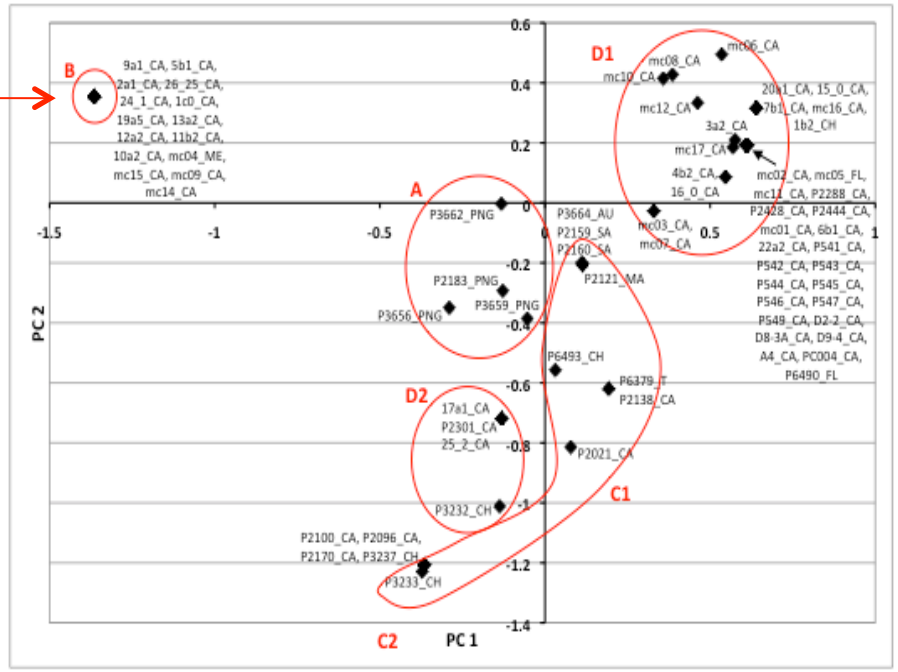
2- New problems in CA Christmas tree nurseries (2002).

3- Quasi-extinction of rare manzanita in CA (Swiecki *et al*. 2004)

- All three recently reported (10 years)
- All three associated with *P. cinnamomi*
- All three associated with the same novel genotype of the pathogen

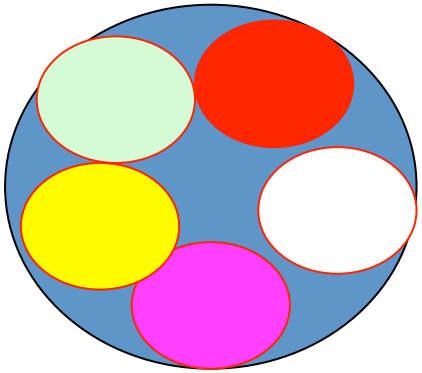
Widespread mortality of lone manzanita caused by *P. cinnamomi*





Newly discovered single genotype now found in different locations, on multiple hosts, and represents a new threat

Introduced population only partially represents phenotypes and genotypes of source

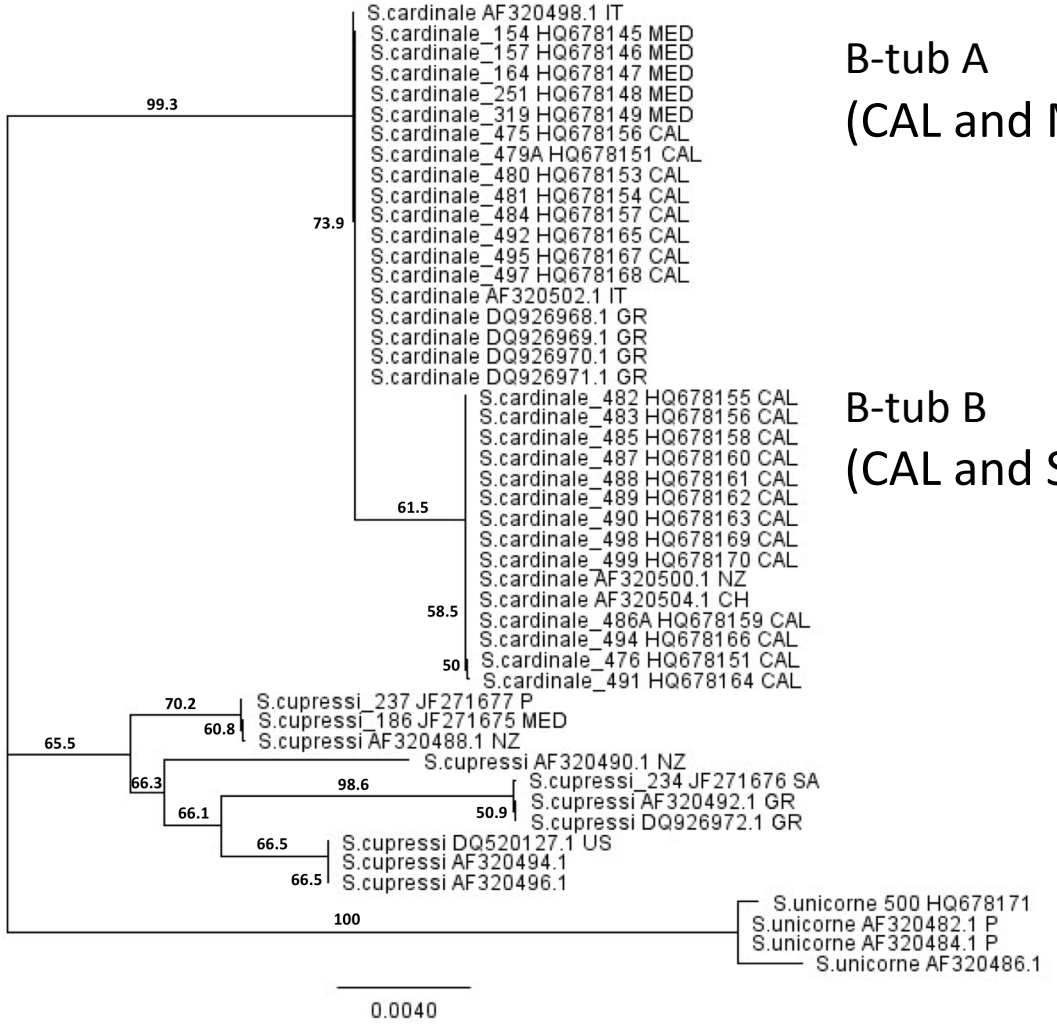


- One or more mating types?
- How low is genetic diversity and how is genetic diversity generated? (Pop size, Different substrates)
- Does low genetic diversity correspond to low phenotypic variability (Plasticity)
- If Host genotype x Pathogen genotype interaction exists, genotypic diversity will affect breeding programs

Cypress canker caused by *Seiridium cardinale*

- Mortality in Mediterranean up to 95% locally
- Introduced in 1930s from California, when Monterey Cypress became popular
- No sex and little genetic variability (one B-tub lineage) observed in Mediterranean
- Two B-tub lineages and sex in California where pops are in equilibrium
- Very large pop sizes and diversity generated by mitotic recombination/mutation in Mediterranean

Significant bottleneck in Med



B-tub A
(CAL and MED)

B-tub B
(CAL and South H.)

Pairwise Si (AFLPs) and Linkage Disequilibrium (SSRs) indicate lack of sex in Med and its presence in Cal

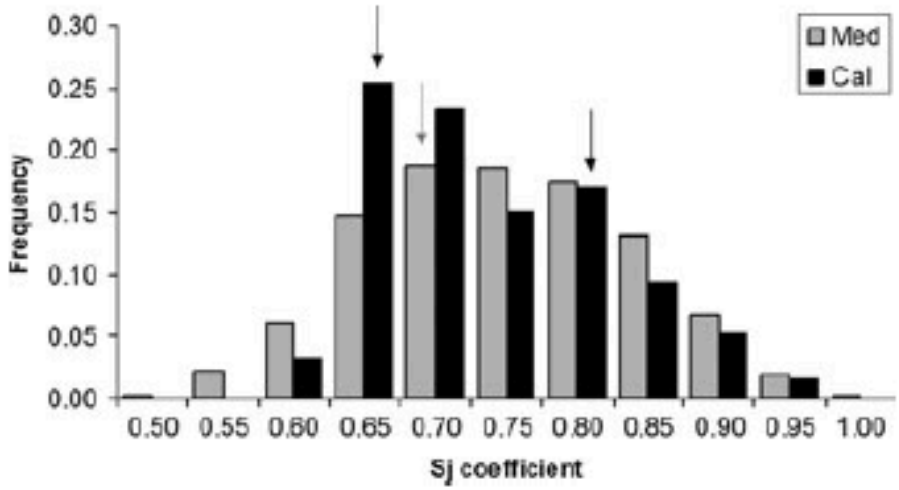


Fig. 1. Distributions of pairwise Jaccard coefficient of similarity (S_J) calculated using AFLP data within Mediterranean and Californian *Seiridium cardinale* populations. Frequency histograms show the distributions of pairwise S_J coefficients for 102 Mediterranean (n = 5151) and 23 California (n = 253) isolates. Arrows point to the maxima for the bimodal California (Cal) and for the unimodal Mediterranean (Med) distributions.

Forest Pathology WILEY-BLACKWELL

For. Path. doi: 10.1111/efp.12019
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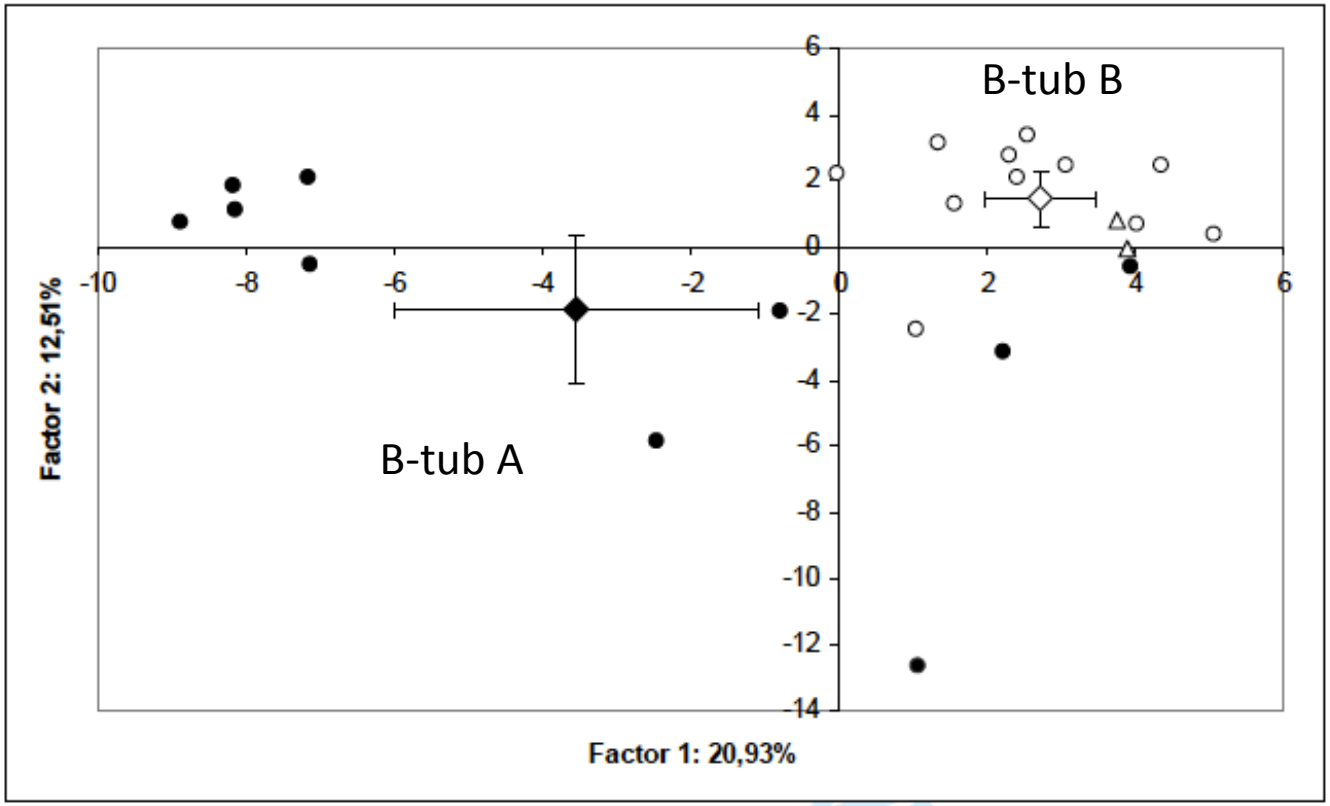
AFLP analyses of California and Mediterranean populations of *Seiridium cardinale* provide insights on its origin, biology and spread pathways

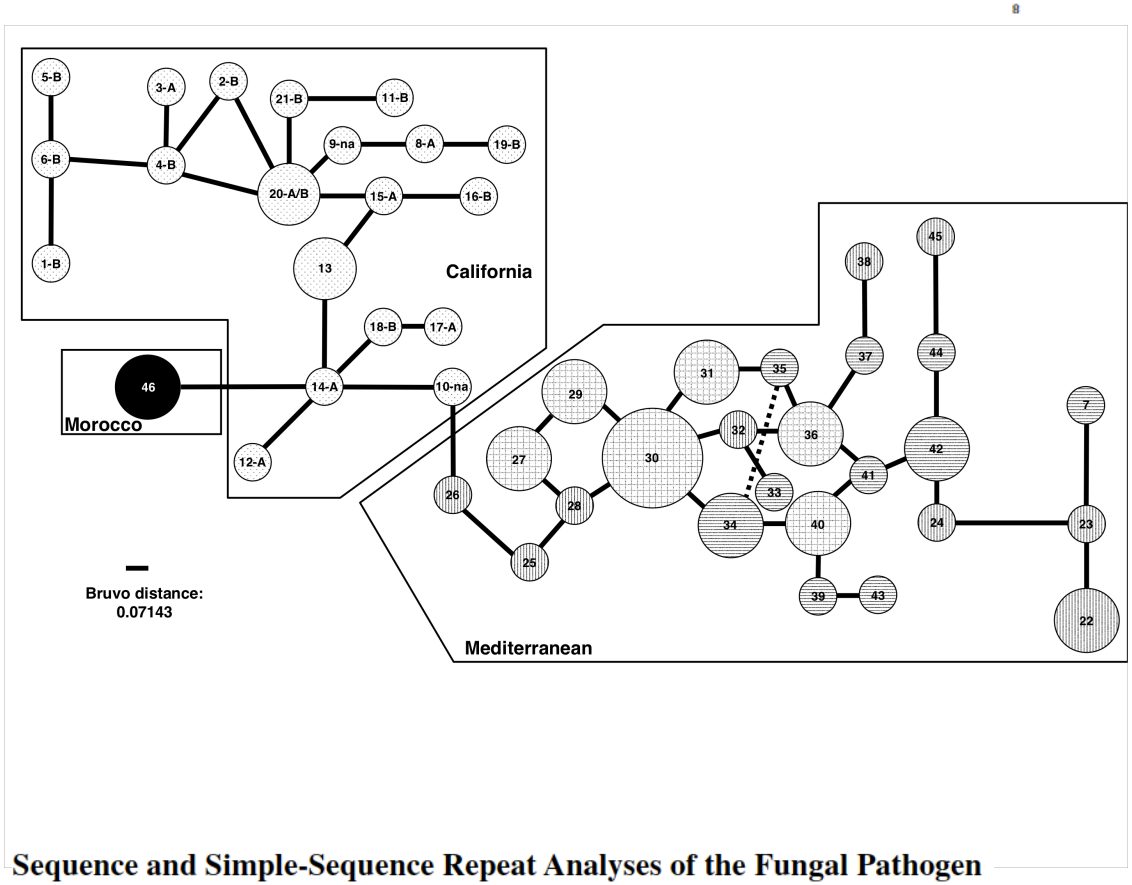
By G. Della Rocca[1], T. Osmundson[2], R. Danti[1], A. Doulis[3], A. Pecchioli[1], F. Donnarumma[4], E. Casalone[4] and M. Garbelotto[2,5]

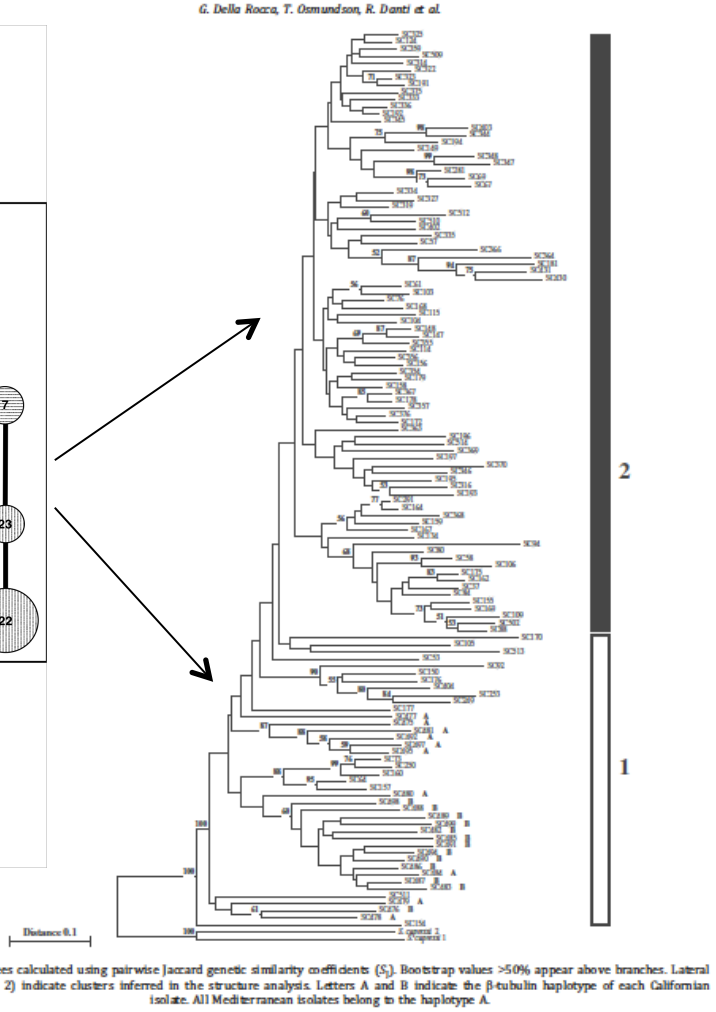
Does B-tub lineage mean more than diversity at one locus?

Fig. 4. Principal Component Analysis based on AFLP profiles of Californian isolates. Filled circles = isolates belonging to β-tubulin haplotype A; open circles = isolates belonging to β-tubulin haplotype B. Rhombs are the centroids of each group of isolates belonging to the two different haplotypes, and bars indicate the standard deviations. Δ=isolates from southern California counties (San Diego).



CAL has sex (Ia) and higher genetic diversity (Gd 0.98 vs. 0.88), is likely source, but MED pop has now differentiated in two, offering a wonderful system for trait-based hypotheses testing





Sequence and Simple-Sequence Repeat Analyses of the Fungal Pathogen *Seiridium cardinale* Indicate California Is the Most Likely Source of the Cypress Canker Epidemic for the Mediterranean Region

G. Della Rocca, C. A. Eyre, R. Danti, and M. Garbelotto

Fig. 2. NJ trees calculated using pairwise Jaccard genetic similarity coefficients (S_J). Bootstrap values >50% appear above branches. Lateral bars (1 and 2) indicate clusters inferred in the structure analysis. Letters A and B indicate the β-tubulin haplotype of each Californian isolate. All Mediterranean isolates belong to the haplotype A.

Pop	CAL	MED1	MED2	Trend
Role	Source	Founder	Invasive	
Genetic Div	H	L	L-M	
Growth Low T	1	0.76	0.63	Dec
Growth Ave T	1	0.81	0.81	Dec/stable
Growth High T	1	0.61	0.62	Dec/stable
Spore size	1	0.94	0.91	Decrease
% germination	1	0.93	0.81	Decrease
Canker size	1	0.89	0.87	Dec/stable
Plasticity Growth	1	1.8	1.5	Inc/Dec
Plast. Spore size	1	1.23	0.23	Inc/Dec
Plast. Canker	1	1.38	0.65	Inc/Dec

- No selection towards increased growth and canker size, especially in limiting conditions (High and Low T)

- Selection towards smaller spores, even if trade off is decrease in germination

-Plasticity increases with invasion and then decreases as species adapts, independent of genetic diversity

-Some CA genotypes much more virulent on Italian cypress: future of resistance breeding in question

Introduction by US troops in 1945 of *H. irregulare* from North America into Italy shows ecology and transmission may be more important than pathogenicity

North American *Heterobasidion irregulare* and pine mortality associated with it

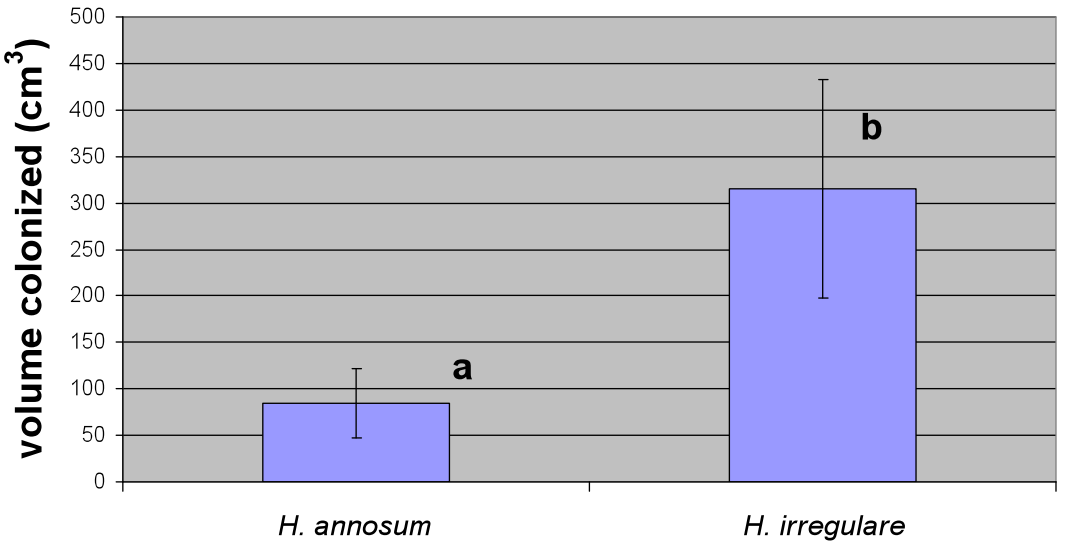
Biol Invasions
DOI 10.1007/s10530-009-9514-4

ORIGINAL PAPER

Comparing the influences of ecological and evolutionary factors on the successful invasion of a fungal forest pathogen

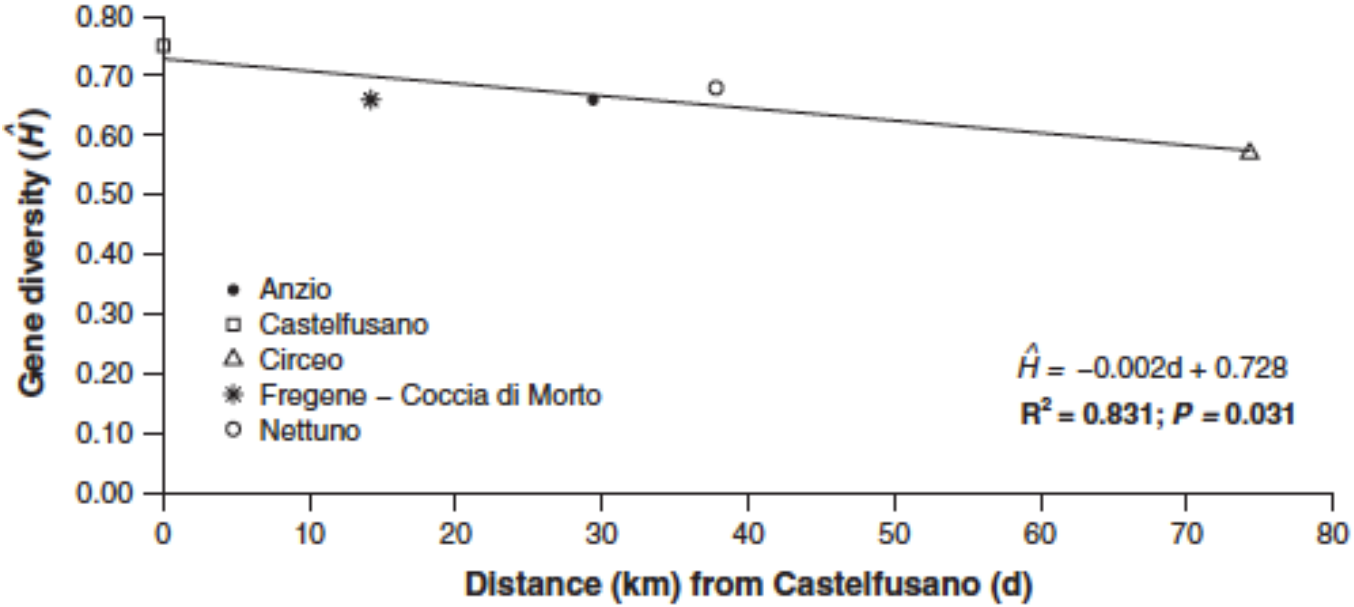
Matteo Garbelotto · Rachel Linzer ·
Giovanni Nicolotti · Paolo Gonthier

- Pathogenicity same between native and exotic spp.
- Sporulation much more significant in exotic sp.
- Saprobic ability higher in exotic species and correlated to fruiting body production. True when co-inoculated



In Press: Biol. Invasions

Due to bottleneck, genetic diversity of invasive *H. irregulare* alleles progressively decreases as distance from introduction point increases, how does invasive sp. maintain viable genome if introduction is single ?



Molecular Ecology (2013)

doi: 10.1111/mec.12452

Population genetics analyses provide insights on the introduction pathway and spread patterns of the North American forest pathogen *Heterobasidion irregulare* in Italy

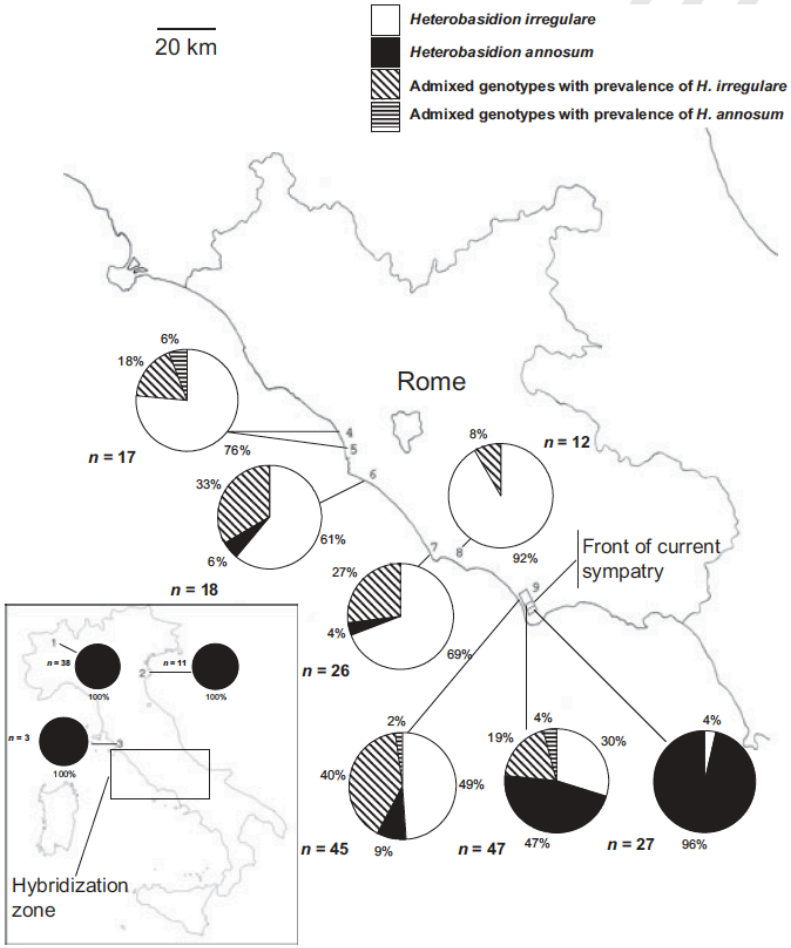
M. GARBELOTTO,* F. GUGLIELMO,† S. MASCHERETTI,* P. J. P. CROUCHER* and P. GONTHIER†

PROOF

Molecular Ecology (2011)

doi: 10.1111/j.1365-294X.2011.05121.x

Amplified fragment length polymorphism and sequence analyses reveal massive gene introgression from the European fungal pathogen *Heterobasidion annosum* into its introduced congener *H. irregulare*



25% of genotypes had admixed genomes

All loci potentially introgressed in first phase of sympatry

About 20% of cases were recombinant novel alleles absent in either species: evolutionary acceleration

Roles of gene introgression

- New combinations of alleles, possibly although rarely leading to phenotypes with ++ traits, that is super-strains. These are often capable to do “stuff” that either parents were incapable of. At times this may result in speciation with “hybrid” species limited to limiting environments
- Generation of new alleles, rapid evolution
- Massive genetic changes in invasive population, because invasive pop is in exponential growth, so any allele that is introgressed, rides the wave of this growth (allele surfing)
- Changes in native species are limited but focused on alleles that are adaptive, so alleles that will make a difference will be selectively introgressed. End effect can be a measurable and inheritable change in the native pop

- Pathogenicity insufficient to explain invasions: need to focus on ecology of transmission of invasive sp. in new niche or competitive ability in same niche as natives. Saprobic ability and sporulation may be driving invasion

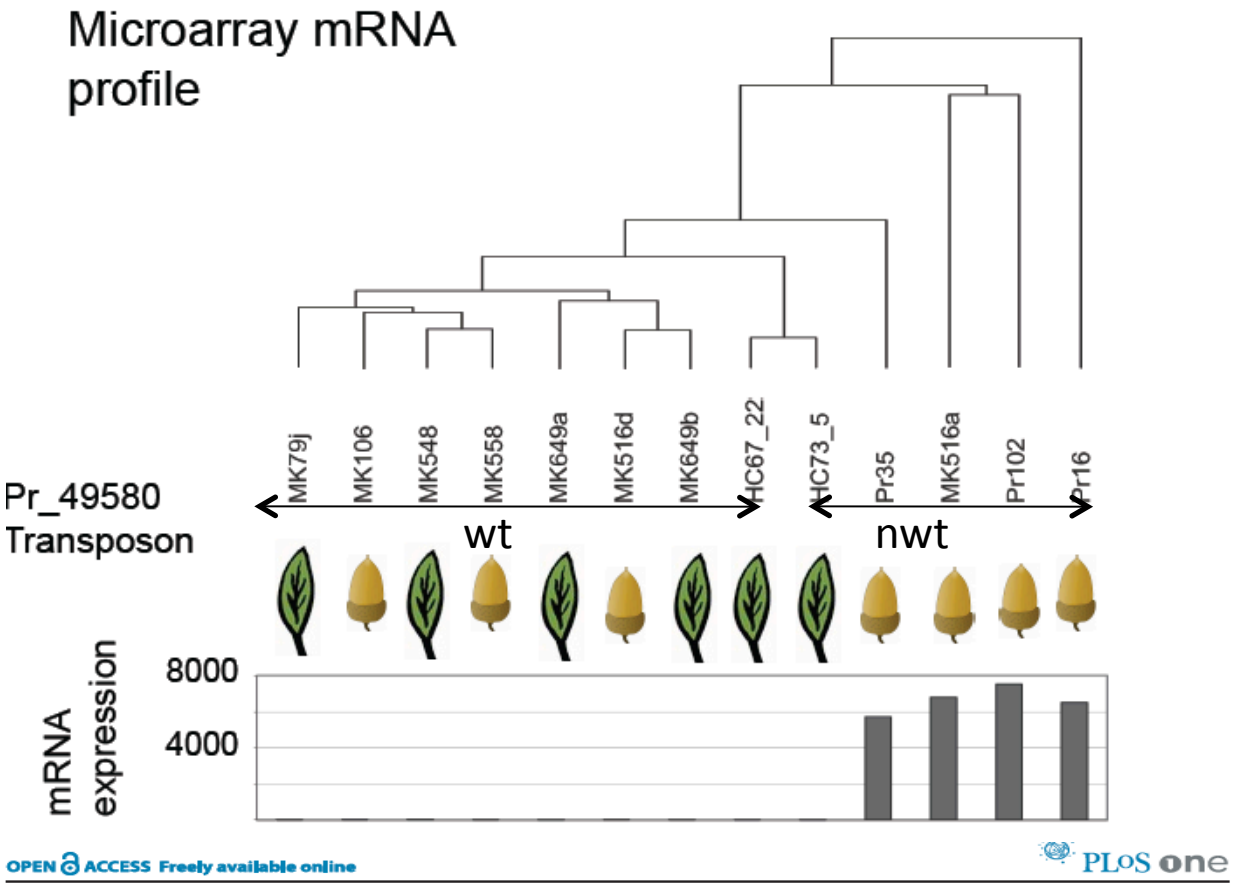
- Pop genetics and genomics need to be linked to functional traits such as spore size, host specificity, temp optima. Ancient lineages or novel genotypes may possess groups of traits that confer a phenotype ,even if recombination occurs. Multiple introductions from different sources need to be avoided, nomenclature issue

- We should be careful in saying that low genetic variability corresponds to low phenotypic variability. Plasticity may play a huge role in invasion. Models needed to study how plasticity drives traits important for invasions

- Hybridization will complicate predictions and accelerate adaptation (basic unpredictable nature of outcome when hybridization is ongoing)

- New evidence from *P. ramorum* suggests individual genotypes with a different history will behave differently permanently (nwt=not wild type, slow growth and early senescence).

13000 genes
Tn = upregulated
CRN= downregulated
Nwt only if from oaks

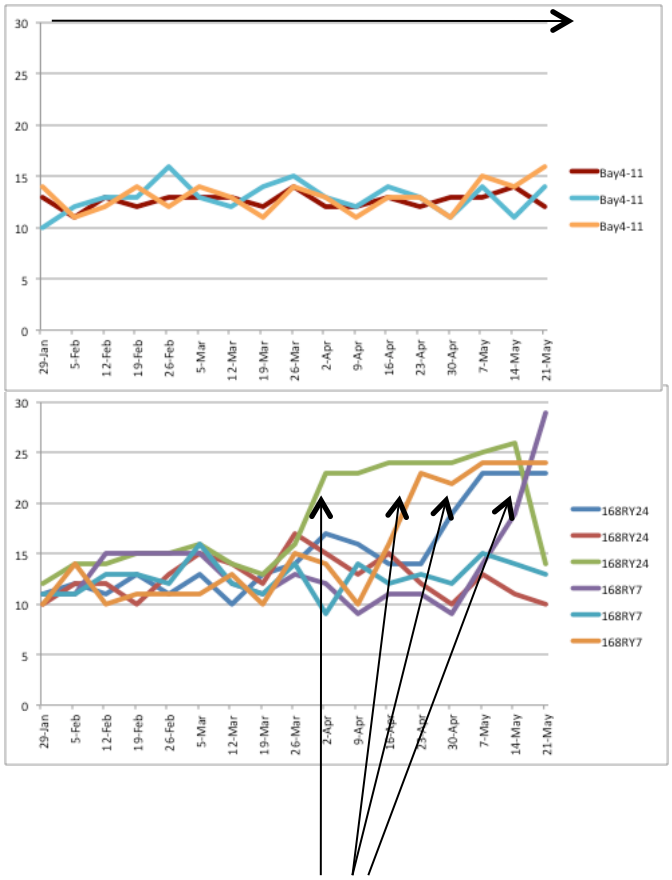


OPEN ACCESS Freely available online
PLoS one

Phenotypic Diversification Is Associated with Host-Induced Transposon Derepression in the Sudden Oak Death Pathogen *Phytophthora ramorum*

Takao Kasuga[1], Melina Kozanitas[2], Mai Bui[1], Daniel Hüberli[2¤], David M. Rizzo[3], Matteo Garbelotto[2*]

Time since re isolation of bay isolate



Inoc. on bay

Inoc. on oak

nwt

We have been able to generate nwt using same isolate and dual inoculations

National Science Foundation
WHERE DISCOVERIES BEGIN

Press Release 12-074

HISTORY IS KEY FACTOR IN PLANT DISEASE VIRULENCE

Where disease-causing microbes have been makes a difference