EMERGENT DISEASES

• Caused by exotic pathogens introduced from a different region of the world. Either pathogen or pathogen+vectors may be introduced.

• Caused by climatic or ecological changes increasing pathogenicity of native microbes:
  – Global warming, strongest effects are at the margin of ranges
  – Anthropogenic alteration of the ecosystem
  – Exotic ecosystem and native pathogens: planting of exotics or planting off site
Emergent diseases

• They are normally infectious, not to be confused with syndromes caused by a combination of abiotic and biotic factors

• They are long lasting or permanent, as opposed to secondary diseases that emerge periodically, for instance during prolonged drought
Drought induced plant health issues
Stunted growth

Fungal twig dieback  
*Cryptoclinae*

Fungal branch dieback  
*Diplodia*

Twig girdlers  
*Agrilicus*

Foliar insects, oak pit scale

Canker rots,  
*Hymenochaetales*

Soilborne Phytophthoras

Trunk insects & associated fungi

Root rots,  
*Armillaria*

Soilborne Phytophthoras

Stem cankers & terminal secondary decay fungi

Canker rots,  
*Hymenochaetales*
Emergent Diseases: temporal patterns are generally different between

- **EXOTIC AGENTS**
  - Rapid outbreaks
  - May cycle down after outbreak

- **NATIVE AGENTS**
  - Progressive, gradual even if dramatic increase
  - Less likely to cycle down
Bases of Invasion Biology of Pathogens

• **Source:** it can be either a pathogen in its native area, possibly with a cryptic phase, or not (weak pathogen or endophyte). In general, the larger the populations of the pathogen, the easier they will become a source.

• **Transport,** survival: related to resting structures, distance, season, substrate (wood, soil, live plants, animal vector)
Biogeographical patterns and determinants of invasion by forest pathogens in Europe

What is being moved

(b)

- Ascomycota
- Basidiomycota
- Oomycota

<table>
<thead>
<tr>
<th>Period</th>
<th>Ascomycota</th>
<th>Basidiomycota</th>
<th>Oomycota</th>
</tr>
</thead>
<tbody>
<tr>
<td>1800-29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1830-59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1860-89</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1890-1919</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1920-49</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1950-79</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1980-2008</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Where from
SUBSTRATE/PATHWAY of introduction
Globalization

Figure 2: The global aviation network
Bases of Invasion Biology of Pathogens

• Establishment success is related to
  – presence of host (if host specific) or of similar host
  – survival as saprobe
  – similarity in climate between home and new region
  – lack of competitors/predators.

• If transmission > mortality then organism becomes invasive
Transmission

• If naïve hosts do not have any resistance then

Transmission=Reproductive potential

If exotic pathogen has a one or a few hosts, spread is usually faster. Gaps in host presence may be less important than dilution of inoculum (some hosts will not be infectious)
Bases of Invasion Biology of Pathogens

- Exotic organisms are all characterized by a strong genetic bottleneck, because only a few individuals make it!! How can a few individuals conquer a new continent
  - Drop traits they do not need (purifying selection)
  - De novo mutation
  - Hybridization with native relative: btw most of the genes go from native to exotic species
  - Mate with other introduced individuals maybe originally from a different area
With a few exceptions

• Most successful exotic invasive species were introduced multiple times. That is why we need not to be complacent about re-introducing what apparently we think is the same pathogen, or even moving introduced pathogens from one site to another

• Eg. Only one sex of exotic forest pathogens is often introduced, that limits the ability of organism to adapt; need to avoid further introductions
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
- 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...

Radial growth in vitro

<table>
<thead>
<tr>
<th></th>
<th>NA1</th>
<th>NA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>12C</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>20C</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>24C</td>
<td>13</td>
<td>14</td>
</tr>
</tbody>
</table>

NA2 > NA1
Interaction of lineage, host, and temperature.

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

• *Phytophthora cinnamomii*: 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. cinnamomomi*
- All three associated with the same novel genotype of the pathogen
Widespread mortality of Ione manzanita caused by *P. cinnamomii*
Newly discovered single genotype now found in different locations, on multiple hosts, and represents a new threat
Predicting impacts of invasions (I)

\[ I = k \times i \times t \]

- \( k \) = 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 susceptibility of hosts and virulence pathogen

Garbelotto & Pautasso, 2012
Transmission is an overlooked aspect, which includes, but is much more, than just pathogenicity.

Population Genetics
Genomics
Epigenetics
Plasticity

Trait-based predictions (which traits ?)

Niche partitioning
Competitive ability
Fungal Invasions: beyond the Lack-of Co-evolution Hypothesis

Matteo Garbelotto
Department of ESPM
U.C. Berkeley
Co-authors

- University of Turin, Italy (*Heterobasidion*)
  - Paolo Gonthier, P.I.
  - Fabiano Sillo, Postdoc
  - Luana Giordano, Postdoc

- Center for National Research Center “CNR” of Florence, Italy (*Seiridium*)
  - Roberto Danti, P.I.
  - Gianni Della Rocca, Postdoc

- U.C. Berkeley
  - Rachel Linzer, Grad Student
  - Maria Friedman, Grad Student
  - Todd Osmundson, Postdoc (UW Lacrosse)
Invasion Biology: Hypotheses

- Enemy Release
- Better utilization of resources than native spp.
- Optimal Ecological Adaptation
Invasive Fungal Pathogens: Hypotheses

- Lack of Co-evolution: naïve host populations have never been exposed to exotic pathogens, hence they have not been selected for resistance. High host susceptibility leading to outbreaks.
Different approaches have rarely been employed

- An organism becomes invasive when: \[ \text{transmission rate} > \text{mortality rate} \]

- We are interested in studying those traits that will affect transmission rate of fungi

Gonthier and Garbelotto 2013, CABI Mini Review
Two scenarios

I Invasive species occupies an empty niche, i.e. there is no inter-specific competition

II Invasive species occupies a niche already occupied by a native species with similar ecological parameters

- COMPETITION
- HYBRIDIZATION AND GENE INTROGRESSION
Experimental Approaches

- Population genetics to understand origin(s) of invasive species and their range expansion history
- Common garden studies to compare various phenotypic traits including virulence, sporulation, temperature adaptation, phenotypic plasticity
- Population genetics to determine if trait variation is random or adaptive (Qst-Fst)
- Comparative genomics to understand the genomic basis that gives the invasive species an advantage over native taxa
Cypress Canker

- Pathogen: *Seiridium cardinale*

- Native Range: Pacific Southwest, where it reproduces sexually and asexually

- Limited host range: one family, *Cupressaceae*

- Invasive outbreaks in the Mediterranean caused by the introduction through nursery stock of a single genotype reproducing clonally

- No competitors, as related taxa (*S. cupressi and S. unicorne*) have extremely low incidence (with exceptions)
Mitospores (conidia)

Lesion and resinosis

Cypress dieback and mortality

Asexual fruiting produced ONLY ON LIVE TISSUE (Including cones)
Wounds

• Mechanical wounds

• Growth wounds, plants that grow too fast (genetic), some fast growing cypresses are very susceptible because of this trait

• Climate induced wounds (frost)

• Insects e.g. *Phloeosinus* spp.
Phloeosinus sp. beetles

Biological life cycle of *Phloeosinus* is among diseased and healthy cypress trees.

1. Overwintering
2. Oviposition (on diseased trees)
3. Feeding (on healthy trees)
4. Oviposition (on diseased trees)

First generation (July)

Second generation (September)

Beetles are contaminated with *S. cardinale* conidia

Spread of infections
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

First author: Fondazione Edmund Mach–Istituto Agrario di san Michele all’Adige, via E. Mach, 38010 San Michele all’Adige (TN), Italy; first and third authors: Institute for Plant Protection–CNR, Via Madonna del Piano 10, I-50019, Sesto Fiorentino (FI), Italy; and second and fourth authors: Department of ESPM, 137 Mulford Hall, University of California, Berkeley 94720. Accepted for publication 3 August 2011.

AFLP analyses of California and Mediterranean populations of *Seiridium cardinale* provide insights on its origin, biology and spread pathways

By G. Della Rocca¹, T. Osmundson², R. Danti¹, A. Doulis³, A. Pecchioli¹, F. Donnarumma⁴, E. Casalone⁴ and M. Garbelotto²,⁵

¹Institute for Plant Protection – CNR, Sesto Fiorentino (FI), Italy; ²Department of Environmental Science, Policy & Management, University of California, 137 Mulford Hall, Berkeley, CA 94720, USA; ³Institute of Viticulture, Floriculture & Vegetable Crops, Laboratory of Plant Biotechnology-Genomic Resources, National Agricultural Research Foundation (NAGREF), Heraklion, Greece; ⁴Department of Evolutionary Biology, University of Florence, Florence, Italy; ⁵Email: matteog@berkeley.edu (for correspondence)
Cypress canker described as early as 1885, in the field book of Jepson.

In central valley where Monterey cypress is not native.
Natural Distribution of Monterey Cypress is coastal. Planted in inland valleys for trade and for agricultural purposes.
Two separate introductions, both from California. The first to Europe matches the high importation of Monterey cypresses from CA Valley nurseries in between the two world wars, and is documented in notes by the owner of the largest nursery in Europe.
Global cypress epidemic traced to CA

Robin Meadows

Massive die-offs of Italian cypresses from Portugal to Greece have been linked to California’s Monterey cypresses (Cupressus macrocarpa), solving a long-standing puzzle in the world of plant pathology and surprising researchers. “When I was a student, this was one of the big mysteries”, says study

1- In CA population explosion of the pathogen due to off site planting of Monterey Cypress in the Valleys

2- Large CA population of pathogen increased likelihood of long distance Transfer

3- EU outbreaks due to fact pathogen is exotic there, but how did it succeed?
1- CA and EU networks are connected by a single link between two genotypes (red to green): introduction of a single genotype likely source of EU outbreak

2- Introduced population becomes invasive with time (green to yellow; increased size of circles)

Fig. 2. Minimum spanning network (MSN) showing relationships between multilocus genotypes (MGs) in Californian and Mediterranean populations. Each node represents a different MG and nodes are scaled to reflect the numbers of individuals sharing the same MGs; small = singleton MG, medium = 2 to 5 individuals with same MG, large = 30 individuals with same MG. Labeling with A, B, or na within nodes relates to whether individuals with that MG were found within clade A or B or not included in the analysis, respectively, in the Sciridium cardinalei neighbor-joining tree (Fig. 1). Dotted line between nodes 34 and 35 indicates where the branch length was unable to be scaled properly without overlap. Distance is the equivalent of the shortest distances in the network; for example, equal length to those between 30 and 32 or 32 and 36.
Invasive EU population derived from founder pop

Source (CA) and Founder (EU from CA) populations
Invasion history (Sequencing, SSRs, and AFLPs)

California Native
- Cal Btub B source
- Morocco

Southern Hemisphere

Mediterranean-Exotic
- Med1 FOUNDER
- Med2 INVASIVE

SOURCE
- Med1
- Med2

FOUNDER
- Med1

Della Rocca et al. 2011, Phytopathology
Della Rocca et al. 2013, Forest Pathology
An increase in transmission-related traits and in phenotypic plasticity is documented during a fungal invasion

Matteo Garbelotto,1 Gianni Della Rocca,2† Todd Osmundson,1,3 Vincenzo di Lonardo,2 and Roberto Danti2

1Department of ESPM, 54 Hilgard Hall, University of California, Berkeley, California 94720 USA
2Institute for Sustainable Plant Protection, CNR, Via Madonna del Piano 10, 50019 Sesto Fiorentino (FI), Italy

Citation: Garbelotto, M., G. Della Rocca, T. Osmundson, V. di Lonardo, and R. Danti. 2015. An increase in transmission-related traits and in phenotypic plasticity is documented during a fungal invasion. Ecosphere 6(9):XXX. http://dx.doi.org/10.1890/ES14-00426.1
Variation in transmission traits in three successive populations

<table>
<thead>
<tr>
<th>Trait</th>
<th>Source CA Pop</th>
<th>Founder Med Pop</th>
<th>Invasive Med Pop</th>
<th>Adaptive Qst-Fst</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro growth rate</td>
<td>High</td>
<td>Low</td>
<td>Low</td>
<td>Yes</td>
</tr>
<tr>
<td>Canker size</td>
<td>High</td>
<td>Low</td>
<td>Low</td>
<td>No</td>
</tr>
<tr>
<td>Fruiting 30 days</td>
<td>High</td>
<td>Low</td>
<td>Low</td>
<td>No</td>
</tr>
<tr>
<td>Fruiting 90 days</td>
<td>High</td>
<td>Medium</td>
<td>High</td>
<td>n/a</td>
</tr>
<tr>
<td>Spore size</td>
<td>High</td>
<td>Medium</td>
<td>Low</td>
<td>Yes</td>
</tr>
<tr>
<td>Spore germination</td>
<td>High</td>
<td>Medium</td>
<td>Medium</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Garbelotto et al. 2015, Ecosphere, In press
Trade off between spore size and germination

$r = 0.44; \ p < 0.01$
Variation in phenotypic plasticity index in three successive populations

Garbelotto et al. 2015, Ecosphere, In press
CA and EU populations are clearly different genetically and phenotypically

Med populations are not suited to grow at low and especially at high temperatures, those traits have been selected in native populations, but probably have been lost due to the bottleneck of the introduction. “Master of some” scenario

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>MED</th>
<th>CAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>15°</td>
<td>3.18 a</td>
<td>4.39 b</td>
</tr>
<tr>
<td>20°</td>
<td>5.10 a</td>
<td>5.94 b</td>
</tr>
<tr>
<td>25°</td>
<td>5.61 a</td>
<td>6.92 b</td>
</tr>
<tr>
<td>30°</td>
<td>2.04 a</td>
<td>3.39 b</td>
</tr>
</tbody>
</table>

In less than 100 years, in spite of large population expansions both in Ca (1920-1940s) and in Europe (1950-1970s), CA and EU pops are phenotypically distinct
Seiridium conclusions

- No selection towards increased virulence. Excessively virulent genotypes may produce less spores due to host death, but within moderate virulence the best performing isolates produce more spores.
- No need to produce spores fast (e.g. in 30 days), because no interspecific competition.
- As invasion progresses, selection for higher overall sporulation (e.g. in 90 days).
- Continued adaptive selection towards smaller spores (further dispersal) in spite of trade-off of reduced germination.
- Plasticity increases in first phase and then decreases.
USA-EU trade

• Populations of the pathogen are clearly distinct both genetically and phenotypically with USA populations displaying sexual reproduction, higher virulence, and better adaptation at low and high temperatures when compared to EU isolates.

• Further USA to EU introductions could be catastrophic.
Native area of CCD

Greenhouse validation of Italian cypress clones resistant to CDD based on a wide selection of genotypes representative of the diverse native California population

Field selection of Italian cypress clones resistant to CDD based only on a single genotype representative of the exotic Mediterranean population
Resistance to Cypress Canker Disease in Italian cypress has desirable effects on disease epidemiology, but may fail against novel genotypes of the pathogen *Seiridium cardinale*.

Gianni Della Rocca\textsuperscript{a}, Roberto Danti\textsuperscript{a}, Tina Popenuck\textsuperscript{b}, Vincenzo Di Lonardo\textsuperscript{a}, Matteo Garbelotto\textsuperscript{b,*}

\textsuperscript{a} Institute for Sustainable Plant Protection IPSP, National Research Council CNR, Via Madonna del Piano 10, 50127, Sesto Fiorentino, Firenze, Italy

\textsuperscript{b} Department of ESPM, 54 Mulford Hall, University of California, Berkeley 94720, CA, USA

<table>
<thead>
<tr>
<th>Clone</th>
<th>Putative phenotype (R/S)</th>
<th>Susceptibility based on LLD (mm)</th>
<th>Susceptibility based on ramets survival (%)</th>
<th>Infectivity index based on n. of acervuli</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM-318</td>
<td>R</td>
<td>24.8 a</td>
<td>58.0 a</td>
<td>2.2 a</td>
</tr>
<tr>
<td>PM-322</td>
<td>R</td>
<td>35.0 ab</td>
<td>22.7 b</td>
<td>2.7 ab</td>
</tr>
<tr>
<td>PM-2440</td>
<td>R</td>
<td>36.2 abc</td>
<td>21.8 b</td>
<td>3.3 ab</td>
</tr>
<tr>
<td>PM-2546</td>
<td>R</td>
<td>50.9 cd</td>
<td>4.0 c</td>
<td>3.7 b</td>
</tr>
<tr>
<td>PM-3947</td>
<td>S</td>
<td>46.8 bcd</td>
<td>29.0 bc</td>
<td>5.2 b</td>
</tr>
<tr>
<td>PM-3954</td>
<td>S</td>
<td>64.0 d</td>
<td>6.1 c</td>
<td>4.2 b</td>
</tr>
<tr>
<td>PM-3962</td>
<td>S</td>
<td>49.8 bcd</td>
<td>10.0 c</td>
<td>2.3 ab</td>
</tr>
</tbody>
</table>
Heterobasidion root rot

- Invasion of Central Italy by Eastern North American species *H. irregulare*, brought by US Army in 1944
- Generalist with preference for *Pinaceae* (pines, true firs, spruces)
- Presence of native sister taxon *H. annosum*
  - Exotic species is much more widespread and damaging in Italy than native species
  - Hybrid swarms and rapid evolution: up to 50% of isolates have admixed genomes and 20% of alleles are chimeric (recombinant)

Gonthier et al. 2008, Molecular Ecology
Gonthier and Garbelotto, 2011, Molecular Ecology
The war on nature

America’s troops came to liberate Italy in the Second World War, but they may have brought with them a deadly fungus that is still killing trees now.

Trees become casualties of war
THE WAR ON NATURE

AMERICA'S TROOPS CAME TO LIBERATE ITALY IN THE SECOND WORLD WAR, BUT THEY MAY HAVE BROUGHT WITH THEM A DEADLY FUNGUS THAT IS STILL KILLING TREES NOW, SAYS ANNA ANELIA.
Distribution of the exotic pathogen

Gonthier et al. 2008, Molecular Ecology

Note admixed genotypes not shown.
Pop gen analysis

- Showed a signal of decreasing diversity from the presumed Castelporziano/Castelfusano introduction site
- Showed three genotypes were introduced but these were distributed everywhere negating independent introductions
- It looks like the pathogen can cross gaps up to 20 km, but more has been historically hard, hence its asymmetric distribution (Northward < Southward)
Is exotic sp. “hypervirulent” due to lack of coevolution?

- Comparative reciprocal inoculation tests show comparable virulence of Eurasian (pink) and North American (yellow) fungal species, independent of host.

- Spore trappings show exotic NA species to have constantly higher sporulation levels than EU species.

Study 1 Garbelotto et al. Biological Invasions 2009

Fig. 2 Results of comparative inoculation on European (Pinus pinea) and North American (P. taeda) host pines. Bars show mean stem length colonized by Eurasian-type Heterobasidion annosum s.s. (□) and North American-type H. annosum P1SG (III). Error bars show 95% confidence intervals. Genotypes not shown.
Which traits differentiate two species?

- 1: Inoculation of logs show NA species to have 4x higher saprobic wood decay ability than EU
- Higher saprobic ability is positively correlated to higher fruiting, with the NA species fruiting much more than the EU one

Study 2 Giordano et al. Biological Invasions 2013
Saprobiic stump infection by airborne spores

Pathogenic phase, by vegetative growth

Saprobiic late phase Leading to sporulation on dead trees and stumps

'Spreading of root and butt rot infection'

Illustration Jan Stenlid

Photos of stumps and dead trees.
Can comparative genomic analysis corroborate previous findings?

- 1 NA sp. genome fully sequenced, we used a NGS approach to sequence 3 more NA and 3 EU isolates

- We analyzed the divergence of three groups of genes between the two species: pathogenicity, saprobic decay, and fruiting/sporulation

- We independently determined those groups of genes that most differed between the two species
Biological materials

Three pure homokarion genotypes of *H. annosum* sampled in Italy and three pure genotypes of *H. irregulare* sampled in the invasion area in Central Italy were selected for the analyses. High quality DNA was extracted and sent to Functional Genomics Laboratory (Berkeley, USA) for sequencing.

Multiplexed Paired-End (PE) 100bp DNA libraries sequenced by using Illumina HiSeq2000.
MATERIALS AND METHODS: NGS sequencing and bioinformatics analysis

NGS sequencing

De novo assembly

Mapping reads to *H. irregulare* reference genome

Putative *H. annosum* variations (shared among *H. annosum* genotypes and absent in *H. irregulare* genotypes)

Phylogenomic analysis

Simple Sequence Repeat survey

Compare size and confirm SVs

Structural Variations (SVs)

Sequence variations (SNPs/InDels)

Genes characterization (identification and characterization of genes inside *H. annosum* SVs and species specific alleles)
### RESULTS: mapping to reference genome

Mapping reads to reference genome: results

Reads were mapped to reference genome by using BWA-MEM. More than 75% of reads of *H. annosum* s.s. mapped on *H. irregulare* reference. A 25% of unmapped *H. annosum* reads appeared to be related to transposable elements.

<table>
<thead>
<tr>
<th></th>
<th><em>H. annosum</em> genotypes</th>
<th></th>
<th><em>H. irregulare</em> genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>137OC</td>
<td>BM42NG</td>
<td>109SA</td>
</tr>
<tr>
<td>Paired End</td>
<td>8018594</td>
<td>8018514</td>
<td>8020180</td>
</tr>
<tr>
<td>Reads sequenced</td>
<td>8018594</td>
<td>8018514</td>
<td>8020180</td>
</tr>
<tr>
<td>Mapped Reads</td>
<td>6148803 (76.68%)</td>
<td>6383225 (79.61%)</td>
<td>6086921 (75.90%)</td>
</tr>
<tr>
<td>Properly mapped</td>
<td>5679959 (70.83%)</td>
<td>5906955 (73.67%)</td>
<td>5650323 (70.45%)</td>
</tr>
<tr>
<td>Singletons</td>
<td>206180 (2.57%)</td>
<td>207745 (2.59%)</td>
<td>188937 (2.36%)</td>
</tr>
<tr>
<td></td>
<td>8036722</td>
<td>8025191</td>
<td>8028750</td>
</tr>
<tr>
<td></td>
<td>7039599 (87.59%)</td>
<td>7313645 (91.13%)</td>
<td>7404292 (92.22%)</td>
</tr>
<tr>
<td></td>
<td>6618004 (82.35%)</td>
<td>6955953 (86.68%)</td>
<td>7051281 (87.83%)</td>
</tr>
<tr>
<td></td>
<td>63510 (0.79%)</td>
<td>57368 (0.71%)</td>
<td>62502 (0.78%)</td>
</tr>
</tbody>
</table>

~ 25% unmapped *H. annosum* reads

Velvet

Assembled in 1104 contigs

ORF prediction and characterization

blastp

79 predicted protein related to retroelements

High percentage of mapped reads of *H. annosum* in *H. irregulare* reference

Intraspecific variation (5-6%)
**RESULTS: phylogenomic analysis**

*H. irregulare* and *H. annosum* are clearly distinct species at genomic level

Phylogenomic analyses on whole genomes confirm that *H. irregulare* and *H. annosum* are clearly distinct species. Interspecific variations seem to be confined on several specific scaffolds.
Results 1: comparison of genes involved in pathogenicity, saprobic decay, and fruiting

- Pathogenicity genes of both species have undergone purifying selection (adaptive reduction in allelic diversity) and are extremely similar between two species.

- Genes involved in saprobic wood decay and in fruiting are distinctively more divergent between the two species than the first group.
The dN/dS ratio and the identification of genes under positive selection

In order to detect genes influenced by speciation events (i.e. natural selection), dN/dS ratio was calculated for each gene. Genes showing a dN/dS ratio > 1 (positive selection) were 153 and were related to mitochondrial components, mitochondrial functions, transcriptional functions and metal homeostasis.
**RESULTS: pathogenicity VS sporulation VS saprobicity**

Genes related to pathogenicity appeared as more conserved than genes involved in saprobic processes and fruiting body formation. A t-test confirmed that only dN/dS of genes related to pathogenesis was significantly different from the average value (difference between means = 0.16, t=-2.6, p-value = 0.009). Previous studies have showed that saprobic ability and fruiting body production may explain the invasiveness of *H. irregulare* rather than pathogenicity (for which *H. irregulare* shows no differences compared to *H. annosum*) (Giordano et al., 2014).
● Results 2: which genes are the most divergent between the two species?

● Peroxidases involved in saprobic wood decay

● Loci involved in gene regulation
**RESULTS: identification of genes located in SVs and rich in SNPs/InDels**

**Genes in inter-chromosomal translocations and CNVs**

14 out of 63 genes in translocations were related to **retrotransposons** (e.g., gag-domain containing proteins). Regions affected by CNV harboured 39 genes also related to mobile elements.

**Genes in regions affected by inversions**

Among 223 genes found in inversions, genes encoding proteins related to **mitochondrial pathways** were identified.

**Species specific alleles (high level of SNPs/InDels)**

707 alleles harboured high SNPs density, showing less than 96% of nucleotide identity between the two fungi. The most significantly over-represented terms in species-specific alleles were related to **oxidation-reduction process** (66) and **heme binding protein** (18).

---

In press Genome Biology and Evolution
• What is happening through hybridization?

- *H. irregulare*, native to North America
- Exotic in Europe

- *H. annosum*, native to Europe

Regulatory genes only

Genes involved in wood decay

Mitochondrion
As a result of hybridization...

• Both species are changing:
  – Invasive specie can regulate its genes better in new environment
  – Native is acquiring genes increasing its fitness
  – Invasive is facilitated and not hindered by presence of native species

Mitonuclear interactions may contribute to fitness of fungal hybrids

Luana Giordano¹,², Fabiano Sillo¹, Matteo Garbelotto³ & Paolo Gonthier³

OPEN

Published: 8 May 2017
Revised: 5 January 2018
Overall Conclusions

- We analyzed two examples of invasion: one (cypress canker fungus) in the absence of a real competitor and one (*Heterobasidion* root rot fungus) with the presence a competing sister taxon. In both cases we knew source of invasion.

- In both cases, high virulence was not key to success of invasion, rather traits enhancing transmission were key, and some were adaptively selected
- Phenotypic plasticity was key
- Planting of species out of their range (or changes due to global warming) can cause outbreaks
- High pathogenicity due to lack of coevolution is not needed
Lack of co-evolution?

● It can’t be universally invoked as the reason for the success of an exotic pathogen!

THANK YOU!!

GO TO
WWW.MATTEOLAB.ORG
FOR PERTINENT PUBLICATIONS
Cypress canker study (absence of competitor)

- One of the first to compare many phenotypic traits in three stages of an invasion (source, founder, invasion)
- Change in traits depends on their evolvability, on bottleneck of introduction (founder effect), and on selection pressure

  - Smaller spore size favoring longer dispersal range is adaptively selected for, even with trade-off of reduced germinability

  - In first “founder” phase of invasion, increased phenotypic plasticity seems to be a universal and key trait for a successful invasion. Interestingly, plasticity is reduced in second invasion stage, as pathogen finds ideal niche

  - No competitor, no selection for fast sporulation, but overall higher sporulation is eventually favorable, because it increases transmission. This trait may be less evolvable, hence it requires more time to change and change occurs in second stage

  - Intermediate pathogenicity is winning strategy as it extends sporulation time and thus transmission
Heterobasidion study (presence of competitor)

- Presence of closely related competitor provides excellent term of comparison to assess what confer invasive advantage to exotic sp.
- One of the first studies to corroborate ecological data using a comparative genomic approach

- Inoculations and field surveys show that pathogenicity is equal between the non invasive native species and the exotic invasive species

- Traits recorded as different between the two species through manipulative experimentation studies and field surveys were sporulation levels, and saprobic wood decay potential

- Both traits make sense considering the biology of the pathogen (most primary infections occur through saprobic decay of stumps initiated by spores)

- Genomic studies identifies genes involved in saprobic decay and sporulation as the most divergent between the species, and also identified regulatory genes as important elements of the difference that presumably explains the invasiveness of the exotic species
“Emergent diseases”:
2: environmental changes

• Forestry and intensive forest use:
  timber production
  tree felling and creation of stumps
  fire exclusion and increase in density
  oversimplified forest composition
  changes in forest composition
  changes in forest structure
**Heterobasidion** root disease

- *Heterobasidion* (a bracket or shelf mushroom) infects trees through wounds and stumps, then it spreads through the roots to neighboring trees.
- With tree felling, stumps and wounds are created, suddenly exponentially increasing infection levels.
Heterobasidion shelf fruit-body
Stump creation and subsequent infection by *Heterobasidion annosum*
Use of molecular genetics:

• Differentiate Heterobasidion on fir/sequoiias (*H. occidentale*) from that on pine/junipiers (*H.irregulare*)

• Show that airborne meiospores are responsible for most infection of *Heterobasidion*

• Show that in pines most infections start on stumps and that in true firs most infections on wounds
Each spore is a genetically different individual:

In pines we found the same genetic individual in stumps and adjacent trees indicating direct contagion between the two.

In true firs and true firs/sequoias we find same individual in adjacent standing trees indicating infection not linked to stumps but to wounds on standing trees.
CONCLUSIONS:

• Logging activities increase *Heterobasidion* infection because of stump creation in pines and because of wounding in true firs sequoias.

• We have shown that in pine stumps *H. irregulare* and *H. occidentale* can both be present and create a new hybrid entity.

• We have shown that in the past these hybridization events have lead to sharing of genes among these two species (Horizontal gene transfers).
New disease of Alpine larch

Fig. 1. Symptomatic larches (left) and a Heterobasidion basidiocarp (top).
*H. Irregulare*-like is a new species generated by hybridization between *H. irregulare* and *H. occidentale* on a host that could be infected by both: alpine larch!!!
Once Heterobasidion is established at high frequency

- Significant loss in timber value
- Dangerous situations (campsites)
- Mortality of valuable species such as sequoia
- With insects and pollution it makes trees more susceptible to mortality
- Can make fires more destructive
Sequoia National Park
Annosus root disease in giant sequoia
Ponderosa pine

Incense cedar
Yosemite Lodge complex
1972

cabin crushed by tree with rotted roots

since 1973
7 fatalities
19 serious injuries
Over $1M property damage
Yosemite Lodge 1975  Root disease centers outlined
Many gaps with very little regeneration and have not closed in
## Change in gap area 1972-1999

<table>
<thead>
<tr>
<th>Year</th>
<th>Area in gaps (m²)</th>
<th>Percent in gaps</th>
</tr>
</thead>
<tbody>
<tr>
<td>1972</td>
<td>6125</td>
<td>3.5</td>
</tr>
<tr>
<td>1999</td>
<td>53,981</td>
<td>31</td>
</tr>
</tbody>
</table>