Ecological genomics
Isheng Jason Tsai
Lecture objective

- Ecological genomics
- Various approaches and disciplines
- Case studies
Recommended textbooks

- Ecological Genomics: Ecology and the Evolution of Genes and Genomes
  - Christian R. Landry
  - Nadia Aubin-Horth
  - Editors
  - Springer (2014)

- The Ecological Genomics of Fungi
  - Francis Martin
  - Wiley Blackwell
Ecological genomics (EG) - definition

“A unique combination of disciplines is emerging — evolutionary and ecological functional genomics — which focuses on the genes that affect ecological success and evolutionary fitness in natural environments and populations.

- “the focus is on organisms that inhabit natural environments and the goal of researchers is to explain variation in DARWINIAN FITNESS in populations, and variation in size, range, longevity and diversity among populations, species and higher taxa.

- Identify gene or genes of interest.

- This is challenging and requires multiple disciplines (ecology, evolution, functional biology and genomics).

An ideal model organism for EG

Infrastructure
- Large, active and interactive community of investigators
- Physical and virtual community resources
- Interaction with other basic and applied communities

Not many organisms fit all these criteria

Gene discovery and phylogenetic data
- Forward and reverse genetic tools
- Capacity to detect variation, including differences in transcript and protein levels
- Known phylogeny, to enable, for example, historical change in traits of interest to be inferred

Ecological context
- Relatively undisturbed habitats in the native range of the species
- Observable ecology and behaviour in nature
- Genetic differentiation causing local adaptation to a range of abiotic or biotic environments
- Legally protected fieldsites for long-term ecological studies

Molecular data
- Access to genomic sequence and chromosomal maps
- Upstream regulators and downstream targets identified for the gene of interest
- Function of gene product known and its impact on fitness under natural conditions inferred

Variation in sequence and phenotype
- Nucleotide variants in natural populations
- Abiotic and biotic environmental factors correlated with each segregating haplotype
- Evolutionary forces underlying nucleotide variation inferred from molecular evolution analyses
- Characterized phenotypes under natural conditions for each variant
- Impact of variants on fitness, abundance, range and persistence known
- Structure and dynamics of the natural population known

Ecological genomics

Genotypes
- Genotype frequencies
- Genomic variations
- Population genomics
- Comparative genomics

Phenotypes
- Phenotype frequencies
- Phenotype plasticity
- Development

Ecology
- Abiotic
- Biotic
- Short term / long term

Traditional model organisms

Ecologists
Ecological genomics

Genotypes
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Advances in genomics really kick off this field; rather than choose a model species we can ask virtually any questions across all organisms

Traditional model organisms

Ecologists
Conceptual framework for eco genomics

Ecological interactions between the organism, the population and community levels and the ecosystem

Interactions between the levels, with organismal responses affecting and being affected by its genotype, which in turn affects what genes are expressed and at what levels, which in turn has effects on the phenotype of the organism, ultimately leading to its overall response.

Ecological genomic studies seek to integrate these disciplines (orange arrows) through the use of functional genomics approaches.

Some questions in EG

- What are the genes that underlie traits that mediate ecological responses in nature?
- How does environmental variation influence mechanisms underlying organismal response?
- Does adaptation and plasticity involve many genes of small effect or a few genes of large effect?
- How does ecological context influence the evolution of genome structure and function?
- How does genotypic variation within and among species influence evolutionary responses, and (or) population, community, or ecosystem dynamics?
- How do microbial communities shift when environments change and how do these shifts influence ecological processes?

Morgan et al (2018) Genome
Ecological genomics – a fungi perspective
Fungi – a definition

fungus (n.)
1520s, "a mushroom," from Latin fungus "a mushroom, fungus;" used in English at first as a learned alternative to mushroom (funge was used in this sense late 14c.). The Latin word is believed to be cognate with (or derived from) Greek sphongos, the Attic form of spongos "sponge" (see sponge (n.)). "Probably a loanword from a non-IE language, borrowed independently into Greek, Latin and Armenian in a form *sphong- ...." [de Vaan]

“The concept of a “fungus” has developed over many years, and the historic definition of fungi as nonphotosynthetic plants has been shown to be both too simplistic and phylogenetically inaccurate"

Bridge et al (2005) Advances in Botanical Research
https://www.etymonline.com/word/fungus
General Characteristics of Fungi

• Cell wall present, composed of cellulose and/or chitin.
• Food storage - generally in the form of lipids and glycogen.
• Eukaryotes - true nucleus and other organelles present.
• Most fungi require water and oxygen (no obligate anaerobes).
• Fungi grow in almost every habitat imaginable, as long as there is some type of organic matter present and the environment is not too extreme.
• Diverse group, number of described species is \(~70,000\) (estimated 5.1 million species total).
Fungi are eukaryotes
Fungi are heterotrophs (‘other food’)

• i.e., acquire nutrients by absorption

• Three main types:
  • **Saprophytes** or saprobes - absorb nutrients from dead organic material
  • **Parasitic** fungi - absorb nutrients from cells of living hosts; some are pathogenic
  • **Mutualistic** fungi - absorb nutrients from a host, but reciprocate to benefit the host

Credit: UWA 2005
Generalized Life Cycle of a Fungus

- Spore-producing structures (n)
- Dikaryotic stage (n+n)
- Karyogamy (fusion of nuclei)
- Dikaryotic stage (n+n)
- Asexual reproduction
- Sexual reproduction
- Spores (n)
- Mycelium (n)
- Spores (n)
- Spore-producing structures (n)

Haploid (n)  
Dikaryotic (n+n)  
Diploid (2n)
Fungal diversity in different environments

Ecological impacts of fungi

• **Beneficial Effects of Fungi**
  • Many organisms depend on/utilise fungi
  • Decomposition - nutrient and carbon recycling.
  • Biosynthetic factories. Can be used to produce drugs, antibiotics, alcohol, acids, food (e.g., fermented products, mushrooms).
  • Traffic network for microorganisms and host
  • Model organisms for biochemical and genetic studies.

• **(Host) Harmful Effects of Fungi**
  • Destruction of food, lumber, paper, and cloth.
  • Animal and human diseases, including allergies.
  • Toxins produced by poisonous mushrooms and within food (e.g., grain, cheese, etc.).
  • Plant diseases.
How fungi are engaged in ecosystem processes

### Fungi in Ecosystem Processes (2nd Edition) CRC Press

<table>
<thead>
<tr>
<th>Ecosystem Service</th>
<th>Fungal Functional Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil formation</td>
<td>Rock dissolution</td>
</tr>
<tr>
<td>Particle binding</td>
<td>Lichens</td>
</tr>
<tr>
<td>Providing fertility for primary production</td>
<td>Saprotrophs</td>
</tr>
<tr>
<td>Decomposition of organic residues and nutrient mineralization</td>
<td>Mycorrhizae</td>
</tr>
<tr>
<td>Soil stability (aggregates)</td>
<td>Saprotrophs</td>
</tr>
<tr>
<td></td>
<td>(Ericoid and ectomycorrhizae)</td>
</tr>
<tr>
<td>Primary production</td>
<td>Direct production</td>
</tr>
<tr>
<td>b. Other fungal/faunal interactions</td>
<td>Mycorrhizae</td>
</tr>
<tr>
<td>c. Providing food for both vertebrate and invertebrate animals</td>
<td>Endophytes</td>
</tr>
<tr>
<td>a. Polluton</td>
<td>Plant yield</td>
</tr>
<tr>
<td>b. The built environment</td>
<td>Defense against pathogens</td>
</tr>
<tr>
<td></td>
<td>Mycorrhizae</td>
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</tr>
<tr>
<td>c. Providing food for both vertebrate and invertebrate animals</td>
<td>Endophytes</td>
</tr>
</tbody>
</table>

### Notes:
- Fungal groups in parentheses are regarded as being of lesser importance in that function.
1. Endophytes can be beneficial to plant growth, especially in harsh environments.

2. Mycorrhizas. Key benefits to terrestrial ecosystems, including: (i) enhanced nutrient uptake, (ii) soil structure; and (iii) carbon sequestration.
Mycorrhizae

• “Fungus roots”
• Mutualism between:
  • Fungus (nutrient & water uptake for plant)
  • Plant (carbohydrate for fungus)
• Several kinds
  • Zygomycota – hyphae invade root cells
  • Ascomycota & Basidiomycota – hyphae invade root but don’t penetrate cells
• Extremely important ecological role of fungi!
Main types of Mycorrhizae and their plant partners

<table>
<thead>
<tr>
<th>MYCORRHIZAL TYPE</th>
<th>PLANT PARTNER</th>
<th>FUNGAL PARTNER</th>
<th>MAIN ECOSYSTEMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ectomycorrhizal fungi</td>
<td>2.2% of plant species, especially woody species; Pinaeae (e.g. pine, spruce, fir, larch) and angiosperms (e.g. beech, oak, chestnut, hazelnut, rockrose)</td>
<td>20,000 Basidiomycota and Ascomycota species</td>
<td>Temperate, boreal, mediterranean, and some tropical forests</td>
</tr>
<tr>
<td>Arbuscular mycorrhizal fungi</td>
<td>78% of plant species; Herbs, shrubs, trees, liverworts, hornworts, lycopods and ferns</td>
<td>300–1,600 Mucoromycota (Glomeromycotina) species</td>
<td>Tropical and temperate forests, grasslands, savannas, shrublands, deserts, agricultural crops</td>
</tr>
<tr>
<td>Ericoid mycorrhizal fungi</td>
<td>1.5% of plant species; Ericaceae (e.g. heather, rhododendron, blueberry) and liverworts</td>
<td>&gt;150 Ascomycota and some Basidiomycota species</td>
<td>Heathlands, tundra, boreal and temperate forests</td>
</tr>
<tr>
<td>Orchid mycorrhizal fungi</td>
<td>10% of plant species; Orchidaceae (orchids)</td>
<td>25,000 Basidiomycota and some Ascomycota species</td>
<td>Tropical, temperate, mediterranean</td>
</tr>
</tbody>
</table>
Emerging fungal threats to animal, plant and ecosystem health

Matthew C. Fisher¹, Daniel A. Henk¹, Cheryl J. Briggs², John S. Brownstein³, Lawrence C. Madoff⁴, Sarah L. McCraw⁵ & Sarah J. Gurr⁵

<table>
<thead>
<tr>
<th>Plant pathogens</th>
<th>Sugar</th>
<th>Sugar</th>
<th>Biotroph</th>
<th>Sexual</th>
<th>20</th>
<th>6.7</th>
<th>Repeat-rich gene clusters that encode effector candidates</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sporisorium scitamineum</em> (Ssc18)</td>
<td>cane</td>
<td>cane</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ustilago maydis</em> (S21)</td>
<td>maize</td>
<td>maize</td>
<td></td>
<td></td>
<td>20</td>
<td>6.7</td>
<td>Repeat-rich gene clusters that encode effector candidates</td>
</tr>
<tr>
<td><em>Microbotryum lycopersici-dioicae</em> (pIA1 Lamole)</td>
<td>red campion</td>
<td>red campion</td>
<td></td>
<td></td>
<td>33</td>
<td>14</td>
<td>Repeat-rich gene clusters that encode effector candidates</td>
</tr>
<tr>
<td><em>Melampsora larici-palulina</em> (98AG31)</td>
<td>poplar and larch</td>
<td>poplar and larch</td>
<td></td>
<td></td>
<td>101</td>
<td>45</td>
<td>ND*</td>
</tr>
<tr>
<td><em>Puccinia graminis f.sp. tritici</em> (CDL75-36)</td>
<td>wheat</td>
<td>wheat</td>
<td></td>
<td></td>
<td>89</td>
<td>45</td>
<td>Highly polymorphic effector candidates</td>
</tr>
<tr>
<td><em>Zymoseptoria tritici</em> (IPSO25)</td>
<td>wheat</td>
<td>wheat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Leptosphaeria biglobosa</em> 'canadensis' (J154)</td>
<td>mustard</td>
<td>crucifers</td>
<td></td>
<td></td>
<td>32</td>
<td>3.9</td>
<td>ND*</td>
</tr>
<tr>
<td><em>Leptosphaeria maculans</em> 'brassicace' (p33.1.3)</td>
<td>oilseed rape</td>
<td>crucifers</td>
<td></td>
<td></td>
<td>45</td>
<td>35.5</td>
<td>Enrichment of effector candidates and chromatin-mediated effector-candidate regulation in AT-isochores</td>
</tr>
</tbody>
</table>

### Plant pathogens

<table>
<thead>
<tr>
<th>Pathogen/Host</th>
<th>Plant</th>
<th>Lifestyle</th>
<th>Life Cycle</th>
<th>Effector(s)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Blumeria graminis</em> f. sp. <em>hordei</em> (DH14)</td>
<td>Barley</td>
<td>Various</td>
<td>Biotroph</td>
<td>Sexual and asexual</td>
<td>120 64</td>
</tr>
<tr>
<td><em>Magnaporthe oryzae</em> (70–15)</td>
<td>Rice</td>
<td>Various crops and wild grasses</td>
<td>Hemi-biotroph</td>
<td>Sexual and asexual</td>
<td>41 10</td>
</tr>
<tr>
<td><em>Ophiostoma novo-ulmi</em> (H327)</td>
<td>Elm</td>
<td>Elm</td>
<td>Necrotroph</td>
<td>Sexual and asexual</td>
<td>32 3.4</td>
</tr>
<tr>
<td><em>Verticillium dahliae</em> (VdLS17)</td>
<td>Lettuce</td>
<td>Various</td>
<td>Necrotroph</td>
<td>Asexual</td>
<td>37 12</td>
</tr>
<tr>
<td><em>Fusarium solani/Nectria haematococca</em> MPVI (77–13–4)</td>
<td>Pea</td>
<td>Various</td>
<td>Hemi-biotroph</td>
<td>Sexual and asexual</td>
<td>54</td>
</tr>
<tr>
<td><em>Fusarium graminearum</em> (PH-1)</td>
<td>Wheat</td>
<td>Wheat and barley</td>
<td>Hemi-biotroph</td>
<td>Sexual and asexual</td>
<td>36 &lt;3</td>
</tr>
<tr>
<td><em>Fusarium oxysporum</em> f. sp. <em>lycopersici</em> (4287)</td>
<td>Tomato</td>
<td>Various</td>
<td>Hemi-biotroph</td>
<td>Asexual</td>
<td>60</td>
</tr>
</tbody>
</table>

*LS*, lineage-specific; ND, no data; TEs, transposable elements. *Genome size and repeat content refer to the respective reference isolate. Isolate-specific hosts refer to the host plant from which the reference isolate was collected. In some cases, other isolates of the same species infect other hosts. *Genome characteristics have been inferred from comparative genomics analyses.

Corn smut *Ustilago*

Photo by David Cohen/flickr/CC BY 2.0

Nutritional Requirements of the Edible Gall-producing Fungus *Ustilago esculenta*

黑胡桃網路閣 [https://blackwalnut.npust.edu.tw/archives/325221](https://blackwalnut.npust.edu.tw/archives/325221)
## Animal pathogens

<table>
<thead>
<tr>
<th>Host</th>
<th>Pathogen (Phylum)</th>
<th>Disease dynamics leading to mass mortality in animal and plant hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphibian species (for example, the common midwife toad, <em>Alytes obstetricans</em>)</td>
<td><em>Batrachochytrium dendrobatidis</em> (Chytridiomycota)</td>
<td>Worldwide dispersal of a hypervirulent lineage by trade(^\text{64}). Ultra-generalist pathogen manifesting spillover between tolerant/susceptible species. Extent of chytridiomycosis is dependent on biotic and abiotic context(^\text{15,82}).</td>
</tr>
<tr>
<td>Coral species (for example, the sea fan, <em>Gorgonia ventalina</em>)</td>
<td><em>Aspergillus sydowii</em> (Ascomycota)</td>
<td>Sea-fan aspergillosis caused by a common terrestrial soil fungus(^\text{21,66}). Epizootics are associated with warm-temperature anomalies. Coral immunosuppression is probably a factor causing decline.</td>
</tr>
<tr>
<td>Bee species (for example, the hive of the domestic honeybee (<em>Apis mellifera</em>) suffering colony collapse disorder)</td>
<td><em>Nosema species</em> (Microsporidia)</td>
<td>Microsporidian fungal infections are associated with colony collapse disorder and declining populations. Pathogen prevalence is probably a part of a multifactorial phenomenon that includes environmental stressors and polyparasitism(^\text{87,88}).</td>
</tr>
</tbody>
</table>

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Worldwide emergence of resistance to antifungal drugs challenges human health and food security

Matthew C. Fisher,¹* Nichola J. Hawkins,² Dominique Sanglard,³ Sarah J. Gurr⁴,⁵*

The recent rate of emergence of pathogenic fungi that are resistant to the limited number of commonly used antifungal agents is unprecedented. The azoles, for example, are used not only for human and animal health care and crop protection but also in antifouling coatings and timber preservation. The ubiquity and multiple uses of azoles have hastened the independent evolution of resistance in many environments. One consequence is an increasing risk in human health care from naturally occurring opportunistic fungal pathogens that have acquired resistance to this broad class of chemicals. To avoid a global collapse in our ability to control fungal infections and to avoid critical failures in medicine and food security, we must improve our stewardship of extant chemicals, promote new antifungal discovery, and leverage emerging technologies for alternative solutions.
Fungal species with reported antifungal resistance, by country

Fisher et al. (2018) Science
Evolutionary drivers of antifungal resistance

So how do we study all of these traits at once?

Genome reference
  • Genome assembly

Compare different species (inter-species comparisons)
  • Phylogenomics
  • Comparative genomics
    • Gene family evolution
      • Gain/loss
      • Expansion/Contraction

Compare different strains (intra-species comparisons)
  • Population genomics
  • QTL
  • GWAS

Ecological modeling
First – you need to sequence genomes
Advances in sequencing

Cost per Raw Megabase of DNA Sequence

Moore’s Law

NIH National Human Genome Research Institute

genome.gov/sequencingcosts
Fungal genomes

**Goal:** Sequencing 1000 fungal genomes from across the Fungal Tree of Life will provide references for research on plant-microbe interactions and environmental metagenomics.

**FIGURE 1: CUMULATIVE NUMBER OF FUNGAL SPECIES WITH WHOLE-GENOME SEQUENCES**

[Data collated from online genome databases including NCBI Genome database (ncbi.nlm.nih.gov/genome), JGI Genome Portal: MycoCosm (genome.jgi.doe.gov/fungi), and EnsembleFungi (fungi.ensembl.org)]

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**SCIENTIFIC REPORTS**

**The Genome Sequences of 90 Mushrooms**

Huiying Li², Song Wu², Xiao Ma², Wei Chen², Jing Zhang³, Shengchang Duan³, Yan Ge³, Ling Kai³, Wenli Huang², Peng Wu², Ruyao Shi², Yifan Li³, Yuanzheong Wang³, Jianqun Li³, Xiang Guo³, Xiaoli Luo², Qiang Li³, Chuan Xiong³, Honggao Liu³, Mingying Gu³, Jun Sheng³ & Yang Dong³³⁴³⁵³⁶
Second – assign orthology and phylogenetic position of the species
The term **homology** was first coined before Darwin

“the same organ in different animals under every variety of form and function” – Richard Owen

Owen 1843, p.379

Darwin later reformulated homology as a result of “descent with modification”, i.e., share ancestry

http://darwin-online.org.uk/
Refining *how* homologous genes are related

Distinguishing homologous from analogous proteins

(1970)

WALTER M. FITCH

Fig. 1.—Distinguishing convergent from divergent types of nucleotide replacement patterns. Given are two groups of species (related within each group as shown by the solid lines) together with the nucleotide present at a specific position of the gene for each member species as shown at the branch tips. Given also the requirement that the ancestral nucleotide must permit the descendant nucleotides to be obtained in the minimum number of replacements, the ancestral nucleotide of the upper two groups must be set as C, with the required replacements indicated by the arrows. Were one to postulate a common ancestor for the two groups, no new mutations would need to be assumed; hence, this kind of pattern is called the divergent type. The lower two groups are identical except for rearranging the nucleotides at the branch tips, but now, in order to account for descendants in only four nucleotide replacements, the ancestral nucleotide of the lower two groups must be A and C. To postulate a common ancestor for these two groups would require, unlike the upper pair, an additional mutation. This situation shows different ancestral characters apparently converging toward the same descendant character, and hence it is called the convergent type. One can calculate the frequency with which one might expect each type to be found in examining a large number of such nucleotide positions and compare that value to what is in fact found for a particular set of proteins. An abnormally large number of either type is evidence favoring that type of relation between the two groups examined.
From homology to orthology

Homologues are sequences derived from a common ancestor...

• What are then orthologues? and paralogues?


"Where the homology is the result of gene duplication so that both copies have descended side by side during the history of an organism, (for example, alpha and beta hemoglobin) the genes should be called paralogous (para = in parallel).

Where the homology is the result of speciation so that the history of the gene reflects the history of the species (for example alpha hemoglobin in man and mouse) the genes should be called orthologous (ortho = exact)."
Search for similarity, collinearity, conservation of morphological characters

**Search for similarity**

One of the most frequent activity in Bioinformatics

**Common ancestor**

Two genes are homologs if and only if they derive from the same ancestor

Gene1

Gene2

Homology is almost uniquely inferred by sequence similarity
How to detect orthologous genes?
- The most intuitive way: **Best Reciprocal Hit (RBH)**
Sequence by clustering

**mcl: The Markov Cluster Algorithm**
http://micans.org/mcl/ (Stijn Van Dongen)

Produce clusters (gene families) using different inflation parameter
Orthology prediction methods

**Similarity**
Rely on genome comparisons and clustering of highly similar genes to identify orthologous groups *(suitable for large genome datasets)*

**Phylogeny**
use candidate gene families determined by similarity and then rely on the reconciliation of the phylogeny of these genes with their corresponding species phylogeny to determine the subset of orthologs *(Good and more interpretable for small set of genomes)*

**Others**
Combination of (1) and (2)
Some uses synteny
From gene trees to species tree

Supertree methods infer the best topology for each gene (using the same phylogenetic method for each), and then a topological consensus is obtained. Such methods are able to make consensus trees even if the number of leaves among gene trees differs but overlaps to some extent, for example when a gene has not been sequenced for some taxa.
A step beyond supertrees is the use of methods that take into consideration specific evolutionary processes that may be responsible for differences in gene topologies, and then estimate the species tree which would most likely have generated such gene trees, under different scenarios.
Importance of genomics in fungi - Phylogeny

The accelerating pace of fungal genome sequencing by a number of large-scale sequencing projects **paved the way for assembling larger and taxon-specific datasets that clarified some of the puzzling fungal relationships.**

- sister relationship between the Leotiomycetes and Sordariomycetes
- resolved the position of the Xylonomycetes, a small class of leaf endophytes, as a sister group to the Lecanoromycetes and Eurotiomycetes.

Resolving ancient divergences poses significant challenges even for phylogenomic datasets. For example, the definition of the fungal kingdom and the placement of the Microsporidia as fungi or nonfungal eukaryotes have been debated. (to be discussed in the next lecture)

Nagy and Szollosi (2017) Fungal Phylogenetics and Phylogenomics
3rd – Comparative genomics
Why comparative genomics?

Compare multiple genomes now a norm
Similarity and differences between genomes
Reveal the evolutionary relationships among species
Link evolutionary processes with function

Use genomes to study evolution of these species:
• At various resolution (whole genome, chromosomes, regions, genes, base pairs)
• Identify the genomic basis of key phenotypes
Comparing genomes

- **Alignment of homologous regions**
  - *Inter-genomic*: aligning genomic sequences from *different* species
  - *Intra-genomic*: aligning genomic sequences from the *same* species

- **Different levels of resolution**
  - Comparative mapping (markers)
  - Synteny (~ gene content)
  - Colinearity (gene content + order conservation)
  - DNA-based alignments (base-to-base mapping)
Evolutionary processes include:

- **Expansion**: Duplication, Segmental dup., Whole genome dup.
- **Phylogeny**:
- **Genesis**:
- **HGT**: introgression
- **Exchange**: Rearrangements
- **Reduction**: Loss

Evolution process of a genome

Tekaia (2016)
Sources of gene innovation  
(Intuitive as genome gain genes of new functions)

**Gene duplication (GD)**

Any duplication of a region of DNA that contains a gene

- Plant organic material decay
- Starch catabolism
- Degradation of host tissues
- Toxin production

**Horizontal gene transfer (HGT)**

Exchange of genes between organisms other than through reproduction

- Xenobiotic catabolism
- Toxin production
- Degradation of plant cell walls
- Wine fermentation

Slides of Antonis Rokas
Importance of genomics in fungi - Comparative

- Comparative genomics analysis of *S. cerevisiae* and closely related species has contributed to our understanding of **how new species emerge and has shed light on the various mechanisms that contribute to reproductive isolation**.

- Genomic analysis of *Saccharomyces* yeasts has provided a better understanding of the mechanisms underlying large-scale genomic changes, such as polyploidy, and their consequences for genome evolution and cell physiology.

- Genomic approaches are increasingly contributing to our understanding of how budding yeasts adapt to natural environments by identifying the genes that are involved in adaptation within natural substrates.

Dujon and Louis (2017) Genetics
Importance of genomics in fungi - Comparative

- Fungi contain a remarkable diversity of both primary and secondary metabolic pathways involved in ecologically specialized or accessory functions.

- Genes in these pathways are frequently physically linked on fungal chromosomes, forming metabolic gene clusters (MGCs).

- Improved knowledge of the evolutionary life cycle of MGCs will advance our understanding of the ecology of specialized metabolism and of the interplay between the lifestyle of an organism and genome architecture.

Importance of genomics in fungi - Comparative (example)

**Highlights**

- 332 genomes, including 220 newly sequenced, covering ~1/3 of known budding yeasts
- Genome-scale inference of robust phylogeny and time tree of budding yeast subphylum
- Reconstruction of 45 metabolic traits infers complex budding yeast common ancestor
- Reductive evolution of traits and genes is a major mode of evolutionary diversification

Shen *et al* (2018) *Cell*
The genomes of fungal plant pathogens can vary in size and composition, even between closely related species. Differences in the content of transposable elements cause variation in genome architecture.

Variation in genome architecture results from differences in population genetic factors, including effective population size and the strength of genetic drift.

During periods of low effective population size, non-adaptive mutations, such as transposable elements, can invade genomes and shape their architecture.

Transposable elements contribute to the establishment and maintenance of rapidly evolving genome compartments that can comprise virulence genes. High mutation rates in these compartments support the evolution of new virulence phenotypes.
Importance of genomics in fungi – plant pathogens

- The genomes of fungal plant pathogens can vary in size and composition, even between closely related species. Differences in the content of transposable elements cause variation in genome architecture.

Importance of genomics in fungi – plant pathogens

- The genomes of fungal plant pathogens can vary in size and composition, even between closely related species. Differences in the content of transposable elements cause variation in genome architecture.

Moving onto intraspecies variation - population genomics
Importance of genomics in fungi - Population

- Population genomics and comparative genomics of *Saccharomyces* yeasts have revealed that hybridization occurred frequently throughout, and has had substantial effects on, yeast evolution. Hybridization could itself be a mechanism of adaptation and speciation.

- Genomic analysis of yeast strains associated with humans has revealed the history of yeast domestication and the mechanisms that have contributed to its adaptation to anthropogenic environments.

Once collection is available: genetic mapping

(A) QTL

(B) BSA

(C) GWAS

Finding the gene of interest

Sardi and Gasch (2017) FEMS Yeast Research
Genome-wide association study (GWAS) in microbes

Falush (2016) Nature Microbiology

Need to control for possible confounding effects of genomic relatedness
Quantitative trait locus (QTL) mapping in yeast

Wilkening et al (2013) Genetics

Reciprocal hemizygosity scanning
Quantitative trait locus (QTL) mapping in yeast

Wilkening et al (2013) Genetics
Community and microbial ecology
(already taught by previous lectures)
Main steps in a fungal metabarcoding project

Biogeography and emerging views of fungal diversity

The evolution of new species or genetic diversity in fungi is often associated with dispersal or migration into new habitats.

Moving towards functions
An ideal model organism for EG

Not many organisms fit all these criteria

Model organisms

• Easy to maintain and breed in a laboratory setting.
• Many model organisms can breed in large numbers.
• Some have a very short generation time, which is the time between being born and being able to reproduce, so several generations can be followed at once
• Mutants allow scientists to study certain characteristics or diseases.
• Easy and cheap genetic manipulation
• Some model organisms have orthologs to humans.
• Model organisms can be used to create highly detailed genetic maps.
• Or they may occupy a pivotal position in the evolutionary tree

https://www.yourgenome.org/facts/what-are-model-organisms
Research in other model fungi

- All have genome sequence available
- Most are animal/plant pathogens
- So much emphasis put on study of virulence

Perez-Nadales *et al* (2014) Fungal Genetics and Biology
Research in model yeast Saccharomyces cerevisiae

Yeast: An Experimental Organism for 21st Century Biology

David Botstein* and Gerald R. Fink†

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- Biotechnology
- Fermentation
- Synthetic biology
- High-throughput / Systematic
- Light sensing

Functional Genomics: Gene–Protein–Function Association via Mutants
Databases and Gene Ontology
Gene Expression and Regulatory Networks
Protein Interaction Networks
Gene Interaction Networks
Integrating Co-expression and Protein and Gene Interaction Networks
Leveraging Diversity to Understand Complex Inheritance
Strengths and Weaknesses of Genome-Scale Experimentation and Inference: Experimental Validation Is Essential
Evolution
- Evidence for the theory of duplication and divergence
- Experimental evolution studies with yeast
Human Disease

Case studies: Origin of *Saccharomyces cerevisiae*
S. cerevisiae arguably one of the most domesticated species for its fermentation product
Revealing a 5,000-y-old beer recipe in China

Jiajing Wang\textsuperscript{a,b,1}, Li Liu\textsuperscript{a,b}, Terry Ball\textsuperscript{f}, Linjie Yu\textsuperscript{d}, Yuanqing Li\textsuperscript{c}, and Fulai Xing\textsuperscript{f}

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Edited by Dolores R. Piperno, Smithsonian Institution, Fairfax, VA, and approved April 26, 2016 (received for review January 27, 2016)
True ecology of Saccharomyces cerevisiae?

FEMS Yeast Research, 15, 2015, fov009
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Advance Access Publication Date: 27 February 2015

Commentary

Saccharomyces cerevisiae: a nomadic yeast with no niche?

Matthew R. Goddard¹,²,* and Duncan Greig³,⁴

Role of social wasps in Saccharomyces cerevisiae ecology and evolution

Irene Stefanini¹, Leonardo Dapporto², Jean-Luc Legras²,³, Antonio Calabretta²,³, Monica Di Paola⁵, Carlotta De Filippo⁶, Roberto Viola⁷, Paolo Capretti⁸, Mario Polsinelli⁹, Stefano Turillazzi¹¹, and Duccio Cavalieri¹²,¹³

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Edited by Nancy A. Moran, Yale University, West Haven, CT, and approved July 5, 2012 (received for review May 18, 2012)
Genome evolution across 1,011 *Saccharomyces cerevisiae* isolates

Jackson Peter1,6, Matteo De Chiarat1,6, Anne Friedrich1, Jia -Xing Yue2, David Pfieger1, Anders Bergströmk, Anastasie Sigwart1, Benjamin Barre2, Kelle Freet1, Agnès Lloredg, Coralie Cruaud2, Karine Labadie1, Jean -Marc Aury1, Benjamin Istace1, Kevin Lebrimagd1, Pascal Barbry1, Stefan Engelen1, Arnaud Lemaitrè1, Patrick Wincker1,2,7, Gianni Liti1,2,8 & Joseph Schacherer1,2

“The Taiwanese wild lineage represents the most divergent population that has yet been described (average of 1.1% sequence divergence to non-Taiwanese strains).”
The origin and adaptive evolution of domesticated populations of yeast from Far East Asia

Shou-Fu Duan\textsuperscript{1,2}, Pei-Jie Han\textsuperscript{1}, Qi-Ming Wang\textsuperscript{1}, Wan-Qiu Liu\textsuperscript{1}, Jun-Yan Shi\textsuperscript{1,2}, Kuan Li\textsuperscript{1}, Xiao-Ling Zhang\textsuperscript{1} & Feng-Yan Bai\textsuperscript{1,2}
Contrasting evolutionary genome dynamics between domesticated and wild yeasts

Jia-Xing Yue1, Jing Li1, Louise Aigrain2, Johan Hallin3, Karl Persson4, Karen Oliver5, Anders Bergström6, Paul Coupland2, Jonas Warringer2, Marco Cosentino Lagomarsino7, Gilles Fischer4, Richard Durbin2 & Gianni Liti1

- long-read sequencing to generate end-to-end genome assemblies for 12 strains representing major subpopulations of the partially domesticated yeast Saccharomyces cerevisiae and its wild relative S. paradoxus.

Contrasting evolutionary genome dynamics between domesticated and wild yeasts

- enable precise definition of chromosomal boundaries between cores and subtelomeres
- S. paradoxus shows faster accumulation of balanced rearrangements (inversions, reciprocal translocations and transpositions), S. cerevisiae accumulates unbalanced rearrangements (novel insertions, deletions and duplications) more rapidly.

- Such striking contrasts between wild and domesticated yeasts are likely to reflect the influence of human activities on structural genome evolution.
A fungal wheat pathogen evolved host specialization by extensive chromosomal rearrangements

Fanny E Hartmann, Andrea Sánchez-Vallet, Bruce A McDonald and Daniel Croll

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Fungal pathogens can rapidly evolve virulence towards resistant crops in agricultural ecosystems. Gains in virulence are often mediated by the mutation or deletion of a gene encoding a protein recognized by the plant immune system. However, the loci and the mechanisms of genome evolution enabling rapid virulence evolution are poorly understood. We performed genome-wide association mapping on a global collection of 106 strains of *Zymoseptoria tritici*, the most damaging pathogen of wheat in Europe, to identify polymorphisms linked to virulence on two wheat varieties. We found 25 distinct genomic loci associated with reproductive success of the pathogen. However, no locus was shared between the host genotypes, suggesting host specialization. The main locus associated with virulence encoded a highly expressed, small secreted protein. Population genomic analyses showed that the gain in virulence was explained by a segregating gene deletion polymorphism. The deletion was likely adaptive by preventing detection of the encoded protein. Comparative genomics of closely related species showed that the locus emerged *de novo* since speciation. A large cluster of transposable elements in direct proximity to the locus generated extensive rearrangements leading to multiple independent gene losses. Our study demonstrates that rapid turnover in the chromosomal structure of a pathogen can drive host specialization.
Genetic polymorphism, population structure, and virulence phenotypes of 106 *Zymoseptoria tritici* isolates

Hartmann *et al* (2017) ISME
No loci were shared between two hosts

Hartmann et al (2017) ISME
Population genomic analyses for the most significant GWAS locus associated with virulence of *Z. tritici*
The evolutionary history and structural variation at the major virulence locus detected by GWAS.
Case study II

RESEARCH ARTICLE

Genome-wide association across \textit{Saccharomyces cerevisiae} strains reveals substantial variation in underlying gene requirements for toxin tolerance

Maria Sardi$^{1,2,\ast}$, Vaishnavi Paithane$^1$, Michael Place$^1$, De Elegant Robinson$^2$, James Hose$^3$, Dana J. Wohlbach$^{1,b}$, Audrey P. Gasch$^{1,3,\ast}$

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Sardi et al (2018) PLOS Genetics
Complex trait - hydrolysate-toxin (HT) tolerance

“One significant hurdle with regards to microbial fermentation is the presence of toxic compounds in the processed plant material, or hydrolysate, including weak acids, furans and phenolics released or generated by the pretreatment process.”

“We used genome-wide association (GWA) in *S. cerevisiae* strains responding to synthetic hydrolysate (SynH), both to identify new genes and processes important for HT tolerance and to explore the extent to which genetic background influences mechanism.

We tested 20 genes associated with HT tolerance and swapped alleles across strains to validate several allele-specific effects. However, in the process of allele exchange we discovered striking differences in gene contributions to the phenotype: out of 14 gene knockouts tested in two strains with opposing phenotypes, 8 (57%) had a statistically significant effect on HT tolerance in one of the backgrounds but little to no significant effect in the other background.

In most of these cases, the specific allele had little observable contribution to the phenotype. Thus, although GWA successfully implicated new genes and processes involved in HT tolerance, the causal variation in the tested strains is not at the level of the allele but rather whether or not the gene’s function is important for the phenotype in that background.

This raises important implications for considering natural variation in functional networks to explain phenotypic variation”

Sardi et al (2018) PLOS Genetics
Strain-specific difference for SynH and HT tolerance

Sardi et al (2018) PLOS Genetics
Fig 3. Distribution of SNP alleles. (A) A heat map of the 38 SNPs found in the GWA analysis (columns) in each strain (rows), where the alleles associated with the sensitive or resistance phenotypes are color-coded according to the key. Strains were organized from tolerant (top) to sensitive (bottom). (B) Percent glucose consumed in SynH + HTs was plotted against the number of sensitive alleles identified in each strain. Correlation of the two is indicated by the $R^2$ and linear fit line.

Sardi et al (2018) PLOS Genetics
Knockout effects of genes containing SNPs found in GWA

Extensive background effects influence gene involvement in SynH tolerance

This indicates substantial epistatic interactions with the genetic background, such that the gene is important in one strain and but dispensable in another.

Sardi et al (2018) PLOS Genetics
Adaptive differentiation coincides with local bioclimatic conditions along an elevational cline in populations of a lichen-forming fungus

Francesco Dal Grande¹*, Rahul Sharma², Anjuli Meiser¹, Gregor Rolshausen¹, Burkhard Büdel³, Bagdevi Mishra¹, Marco Thines¹, Jürgen Otte¹, Markus Pfenninger¹,² and Imke Schmitt¹,²*

Dal Grande et al (2017) BMC Evolutionary Biology
Here we report on the population genomics of a lichen-forming ascomycete along an altitudinal gradient in the Mediterranean region. As model, we chose *Lasallia pustulata* (Umbilicariaceae), a species with a distribution from southern Europe to northern Scandinavia, which forms dense populations on exposed, siliceous rocks [30]. Using genomic data from geographically close populations along a steep altitudinal gradient in northern Sardinia (Italy), we analyzed whether genetic clusters were present, and whether relatedness between clusters was correlated with signatures of local adaptation. Heat, drought, and radiation stress constitute determining factors in the composition of biological communities inhabiting rocky outcrops and boulders in Mediterranean mountains [31]. Therefore we tested the hypothesis that environmental factors shape genome-wide population differentiation in lichenized fungi which occur across different bioclimatic regions. Specifically, we addressed the following questions: i) what is the genome-wide population structure and connectivity between geographically close populations along an elevation gradient?, ii) what are putative functions of highly differentiated genes between the genetic clusters?, iii) what are putative functions of the genes showing strong correlation with local climatic factors?, and iv) do individuals belonging to different genetic clusters (and environments) display fitness differences?
Strong genetic structure separating lower altitude populations and rest

Dal Grande et al (2017) BMC Evolutionary Biology
Photosynthetic CO2 gas exchange of *L. pustulata* highland (population 6; blue) and lowland population (populations 1 to 5; red) related to thallus water content (TWC).

The genetic separation coincided with differences in physiological responses to thallus water content (WC).

“Future studies …, and quantitative trait locus mapping experiments of the candidate genes in controlled and field settings will help to elucidate the drivers of local adaptation in this and other fungal species.”

Dal Grande *et al* (2017) BMC Evolutionary Biology
### Genotypes
- Genotype frequencies
- Genomic variations
- Population genomics
- Comparative genomics

### Phenotypes
- Phenotype frequencies
- Phenotype plasticity
- Development

### Ecology
- Abiotic
- Biotic
- Short term / long term

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What’s next?
SOIL ECOLOGY

The role of multiple global change factors in driving soil functions and microbial biodiversity

Matthias C. Rillig\textsuperscript{1,2,*†}, Masahiro Ryo\textsuperscript{1,2,*}, Anika Lehmann\textsuperscript{1,2}, Carlos A. Aguilar-Trigueros\textsuperscript{1,2}, Sabine Buchert\textsuperscript{1,2}, Anja Wulf\textsuperscript{1,2}, Aiko Iwasaki\textsuperscript{1,2}, Julien Roy\textsuperscript{1,2}, Gaowen Yang\textsuperscript{1,2}

Rillig et al (2020) Science
Factor in soil ecology experiments

Rillig et al (2020) Science
the number of global change factors alone might predict general trends in changes of biodiversity and ecosystem processes
Effects on the soil fungal community of different global change factors applied singly and using different numbers of factors

Rillig et al. (2020) Science
Predicting species’ responses to climate change

promising strategy to use genomics for predictions: fast enough and for a broad taxon spectrum

ecosystem management & conservation strategies

When abundance of all species is not possible – identify keystone species first.
Questions and knowledge gaps

**KEY UNRESOLVED QUESTIONS:**
- What is the relative importance of fungal adaptation, migration and acclimatisation?
- How does climate change affect the yield of fungal spore-bearing structures?
- How does climate change affect fungal growth and activity?
- How do fungi mediate ecosystem responses to climate change?
- How do changes in the phenology of spore-bearing structure production reflect changes in activity, abundance, biomass and distribution?
- Can fungi track climate space shifts?

**KEY INFORMATION GAPS:**
- Long-term data
- Large-scale data
- Data from tropical, subtropical and warm temperate ecosystems
- Experimental data from fungi associated with trees, rather than seedlings
- Fungal response and effect traits
- Data from multiple simultaneous drivers of change (nitrogen deposition, carbon dioxide, ozone, UV, temperature, drought, fire)
Not covered but very important – phenotypic plasticity

Morris and Rogers (2014) Ecological Genomics
More references

# Some nice papers
https://www.nature.com/subjects/fungi

# Fungal Phylogenetics and Phylogenomics
https://www.sciencedirect.com/bookseries/advances-in-genetics/vol/100/suppl/C

# The Impact of Molecular Data in Fungal Systematics

# Genome Diversity and Evolution in the Budding Yeasts (Saccharomycotina)
http://www.genetics.org/content/206/2/717

# Evolutionary biology through the lens of budding yeast comparative genomics
https://www.nature.com/articles/nrg.2017.49

# Dimensions of biodiversity in the Earth mycobiome
https://www.nature.com/articles/nrmicro.2016.59

# Evolution and genome architecture in fungal plant pathogens
https://www.nature.com/articles/nrmicro.2017.76
Ecological genomics: understanding gene and genome function in the natural environment
https://www.nature.com/articles/6800992