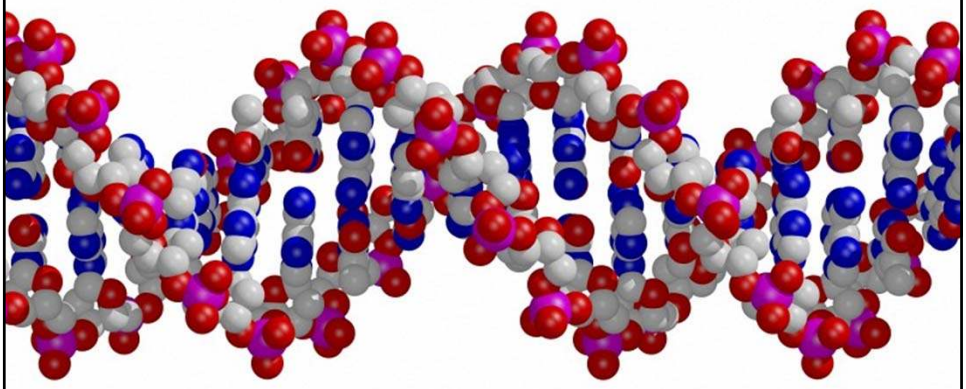
Evolutionary Genomics



Antonis Rokas
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Vanderbilt University

http://as.vanderbilt.edu/rokaslab

Lecture Outline

- **Introduction to evolutionary genomics**
- **Brief summary of high-throughput DNA sequencing & applications**
- **Benchmarking *de novo* transcriptome sequencing for functional and evolutionary genomics**

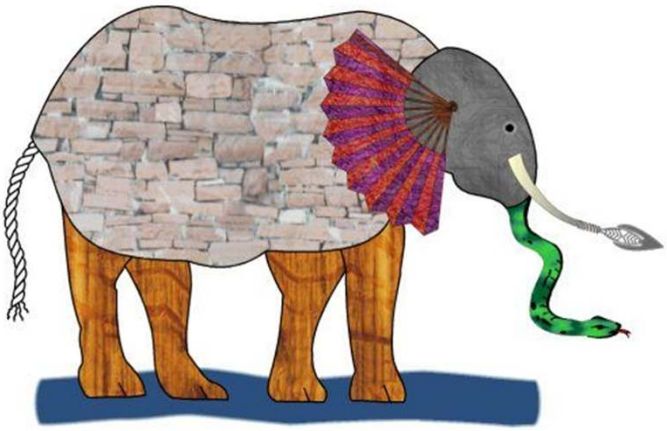
----------Coffee Break----------

- **Phylogenomics**
- **Population Genomics**

----------Coffee Break----------

- **Comparative & Functional Genomics**

What is an Elephant Like?



What is a Genome Like?

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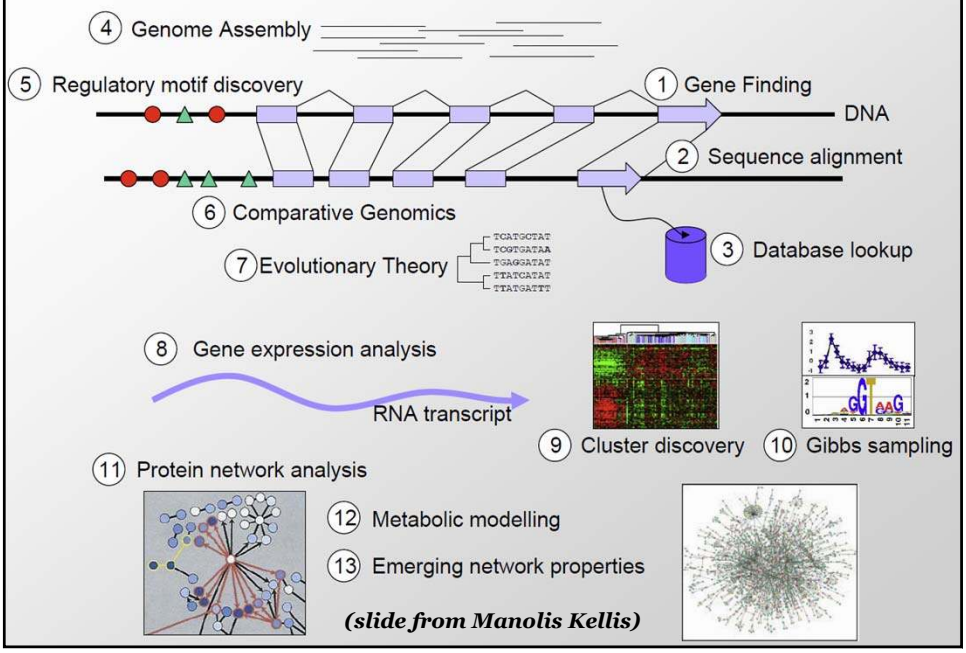
Understanding Sequences Requires Tools and Evolution



What is a Genome Like?

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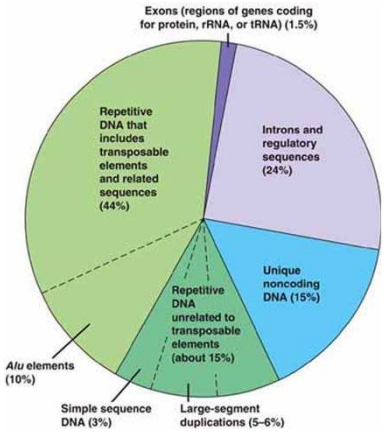
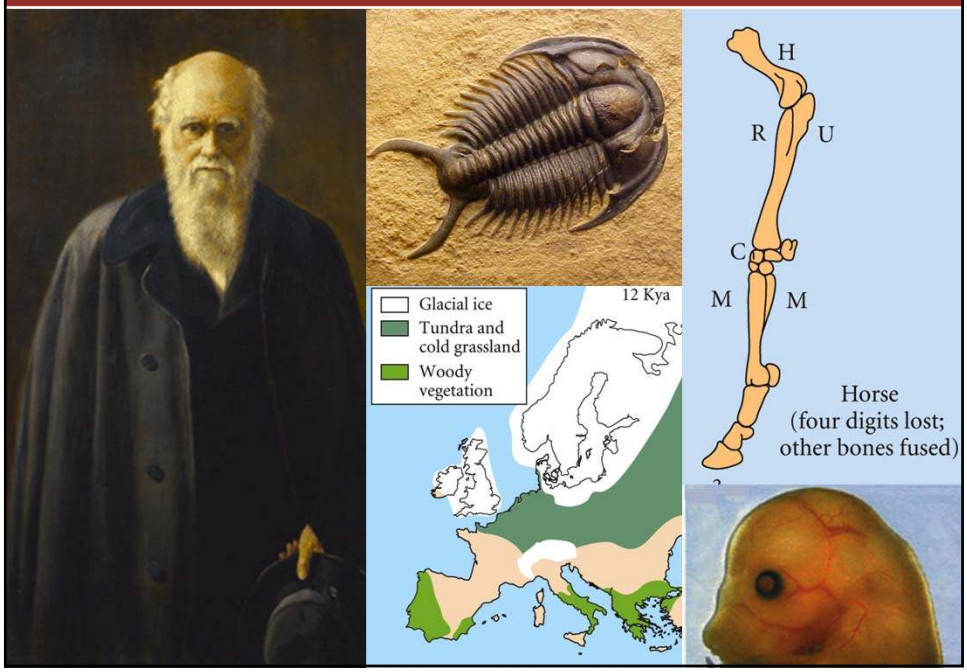
Transposon Protein Binding Site Exon Intron

Organization of the Human Genome

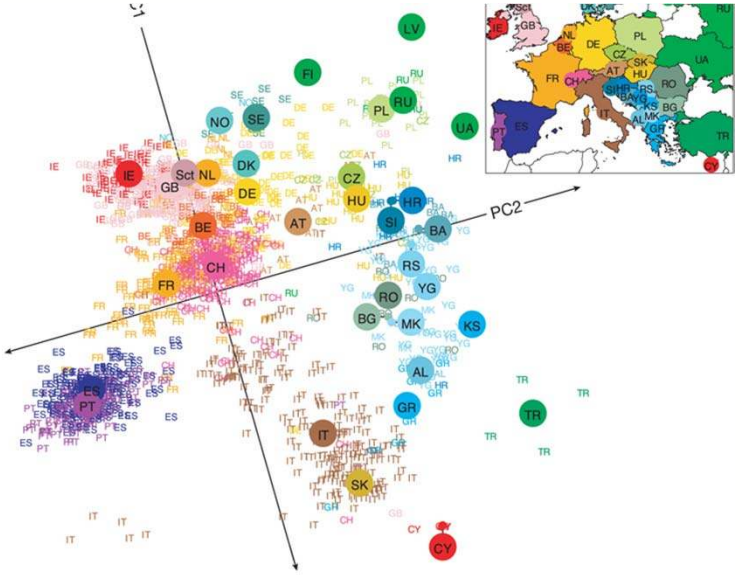


http://www.bio.miami.edu/~cmallery/150/gene/c7.19.14.human.genome.jpg

The DNA Record



Human Genes Mirror Geography



Novembre et al. (2008) Nature

Recent Positive Selection in Human Populations

in the Asian Population, involved in hair follicle development

The twenty-two strongest candidates for natural selection

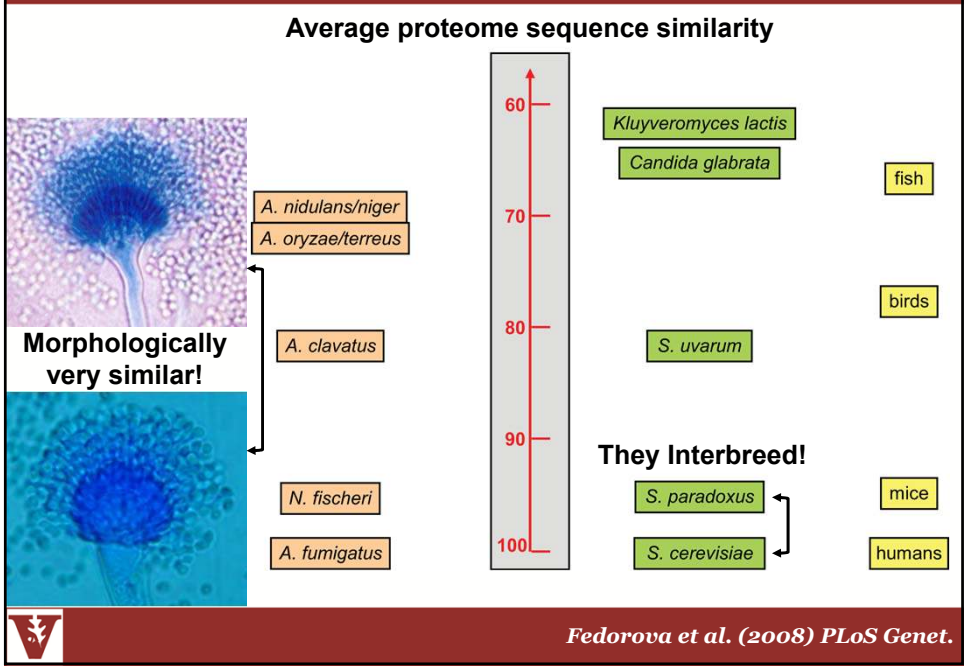
Chr:position (MB, HG17)	Selected population	Long Haplotype Test	Size (Mb)	Total SNPs with Long Haplotype Signal	Subset of SNPs that fulfil criteria 1	Subset of SNPs that fulfil criteria 1 and 2	Subset of SNPs that fulfil criteria 1, 2 and 3	Genes at or near SNPs that fulfil all three criteria
chr1:166	CHB + JPT	LRH, iHS	0.4	92	39	30	2	*BLZF1, SLC19A2*
chr2:72.6	CHB + JPT	XP-EHH	0.8	732	250	0	0	
chr2:108.7	CHB + JPT	LRH, iHS, XP-EHH	1.0	972	265	7	1	*EDAR*
chr2:136.1	CEU	LRH, iHS, XP-EHH	2.4	1,213	282	24	3	*RAB3GAP1, R3HDM1, LCT*
chr2:177.9	CEU,CHB + JPT	LRH, iHS, XP-EHH	1.2	1,388	399	79	9	*PDE11A*
chr4:33.9	CEU,YRI, CHB + JPT	LRH, iHS	1.7	413	161	33	0	
chr4:42	CHB + JPT	LRH, iHS, XP-EHH	0.3	249	94	65	6	*SLC30A9*
chr4:159	CHB + JPT	LRH, iHS, XP-EHH	0.3	233	67	34	1	
chr10:3	CEU	LRH, iHS, XP-EHH	0.3	179	63	16	1	
chr10:22.7	CEU, CHB + JPT	XP-EHH	0.3	254	93	0	0	
chr10:55.7	CHB+JPT	LRH, iHS, XP-EHH	0.4	735	221	5	2	*PCDH15*
chr12:78.3	YRI	LRH, iHS	0.8	151	91	25	0	
chr15:46.4	CEU	XP-EHH	0.6	867	233	5	1	*SLC24A5*
chr15:61.8	CHB + JPT	XP-EHH	0.2	252	73	40	6	*HERC1*
chr16:64.3	CHB + JPT	XP-EHH	0.4	484	137	2	0	
chr16:74.3	CHB + JPT, YRI	LRH, iHS	0.6	55	35	28	3	*CHSTS, ADAT1, KARS*
chr17:53.3	CHB + JPT	XP-EHH	0.2	143	41	0	0	
chr17:56.4	CEU	XP-EHH	0.4	290	98	26	3	*BCAS3*
chr19:43.5	YRI	LRH, iHS, XP-EHH	0.3	83	30	0	0	
chr22:32.5	YRI	LRH	0.4	318	188	35	3	*LARGE*
chr23:35.1	YRI	LRH, iHS	0.6	50	35	25	0	
chr23:63.5	YRI	LRH, iHS	3.5	13	3	1	0	
	Total SNPs		16.74	9,166	2,898	480	41	

In the European population, involved in skin pigmentation

In the West African population, related to Lassa virus infection

Sabeti et al. (2007) Nature

Genomes Provide a Common Yardstick for Comparison



Fedorova et al. (2008) PLoS Genet.

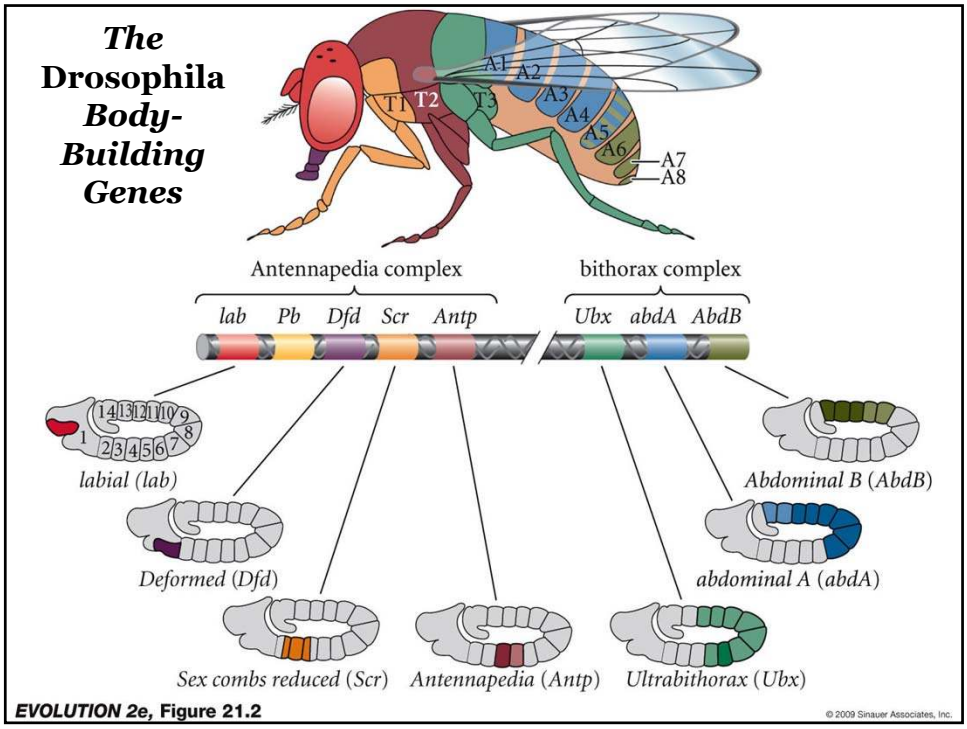
What Makes Us Sick Is the Stuff of Life

F	W	Y	Cancer
+			ABL1
+			Acute Myeloid Leukemia-DEK
+			Adenomat. Polyposis Coli-APC
+			AKT2
+			Ataxia Telangiectasia-ATM
-			BRCA1
-			BRCA2
+			Basal Cell Nevus-PTC
+			B-Cell Lymphoma 2-BCL2
-			B-Cell Lymphoma 3-BCL3
+			Bloom-BLM
+			Burkitt's Lymphoma-MYC
-			CDKN2C
-			CSF1R/C-Fms
+			Chk2 Protein Kinase
-			PDGFB
+			CML-BCR
+			Cyclin D1-CCND1
+			Cyclin Dep. Kinase 4-CDK4
+			EGFR
+			ERBB2
-			ETS
+			E-Cadherin-CDH1
+			Ewing Sarcoma-FLI-1
-			FGF3
-			Fanconi's Anemia A-FANCA
-			Fanconi's Anemia C-FANCC
-			Fanconi's Anemia G-FANCG
+			HNPCC*-MSH2
+			HNPCC*-MSH3
+			HNPCC*-MSH6
+			HNPCC*-MLH1
+			HNPCC*-PMS2
-			KIT

F	W	Y	Neurological
+			Adrenoleukodystrophy-ABCD1
+			Alzheimer-PS1
+			Alzheimer-APP
+			Amyotrophic Lat. Sclero.-SOD1
+			Angelman-UBE3A
+			Aniridia-PAX6
+			Best Macular Dystrophy-VMD2
+			Ceroid-Lipofuscinosis-PPT
+			Ceroid-Lipofuscinosis-CLN3
-			Ceroid-Lipofuscinosis-CLN2
-			Charcot-Marie-Tooth 1A-PMP22
-			Charcot-Marie-Tooth 1B-MPZ
+			Choroideremia-CHM
-			Creutzfeldt-Jakob-PRNP
+			Deafness, Hereditary-MYO15
+			Deafness, X-Linked-TIMM8A
+			Diaphanous 1-DIAPH1
+			Dementia, Multi-Infarct-NOTCH3
+			Duchenne MD*-DMD
-			Emery-Dreifuss MD*-EMD
+			Emery-Dreifuss MD*-LMNA
+			Familial Encephalopathy-PI12
+			Fragile-X -FRAXA
+			Friedreich Ataxia-FRDA
+			Frontotemporal Dement.-TAU
-			Fukuyama MD*-FCMD
+			Huntington-HD
+			Limb Girdle MD* 2A-CAPN3
+			Limb Girdle MD* 2B-YSF
-			Limb Girdle MD* 2E-BSG
+			Lissencephaly, X-Linked-DCX
+			Lowe Oculocerebroren.-OCRL
-			Machado-Joseph-MJD1
+			Miller-Dieker Lissen.-PAF

F	W	Y	Malformation Syndromes
-			Aarskog-Scott-FGD1
+			Achondroplasia-FGFR3
+			Alagille-JAG1
+			Barth-TAZ
-			Beckwith-Wiedemann-CDKN1C
-			Cerebral Cavern. Malf.-CCM1
+			Chondrodyspl. Punct. 1-ARSE
+			Cleidocranial Dysplasia-OFC1
-			Cockayne I-CKN1
+			Coffin-Lowry-RPS6KA3
+			Diastrophic Dyspl.-SLC26A2
+			EEC 3-Ket. P63
+			Greig Cephalopolysynd.-GLI3
-			Hand-Foot-Genital-HOXA13
+			Holoprosencephaly 3-SHH
+			Holoprosencephaly-SIX3
+			Holt-Oram-TBX5
-			ICF-DNMT3B
+			Kallman-KAL1
-			Laterality, X-Linked-ZIC3
+			Melnick-Fraser-EYA1
+			Nail Patella-LMX1B
-			Opitz-MID1
+			Renal Coloboma-PAX2
+			Rieger, Type 1-PITX2
-			Rubinstein-Taybi-CREBBP
+			Saethre-Chotzen-TWIST
-			Septooptic Dysplasia-HESX1
+			Simpson-Golabi-Behmel-GPC3
+			Townes-Brockes-SALL1
-			Treacher-Collins-TCOF1
-			VMCM-TEK
+			Wardenburg-PAX3
+			Zellweger-PEX1

Human disease-associated genes shared with flies (F), worms (W), and Yeast (Y); from Rubin et al. (2000) Science


The Drosophila Body-Building Genes
A1
A2
A3
A4
A5
A6
A7
A8
T1
T2
T3
Antennapedia complex
bithorax complex
lab
Pb
Dfd
Scr
Antp
Ubx
abdA
AbdB
labial (lab)
Deformed (Dfd)
Sex combs reduced (Scr)
Antennapedia (Antp)
Ultrabithorax (Ubx)
abdominal A (abdA)
Abdominal B (AbdB)
EVOLUTION 2e, Figure 21.2
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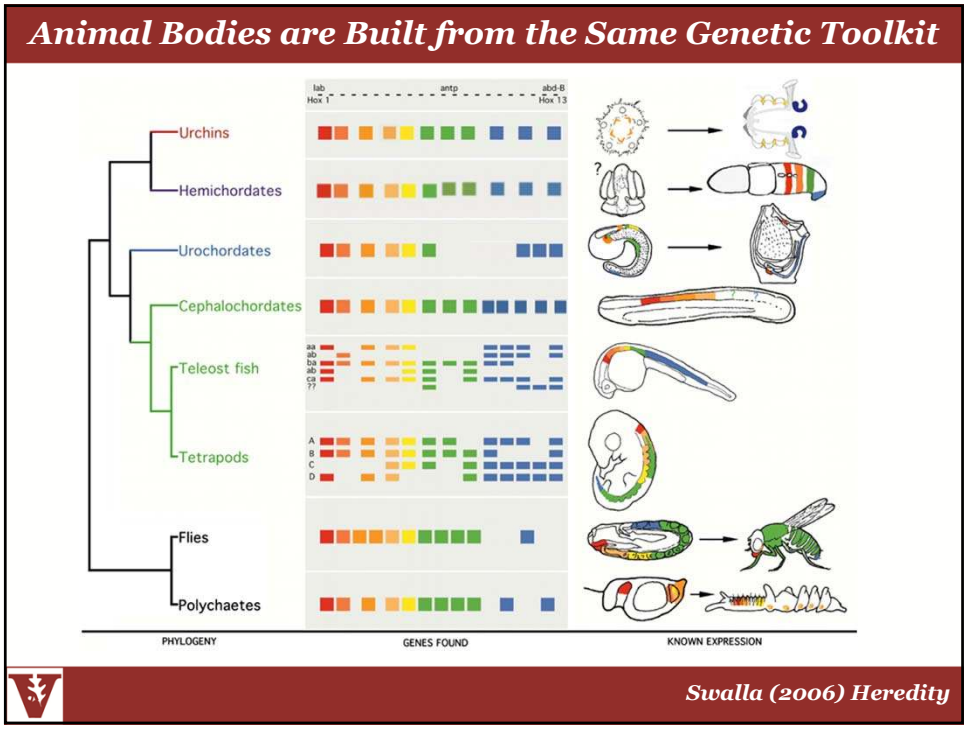
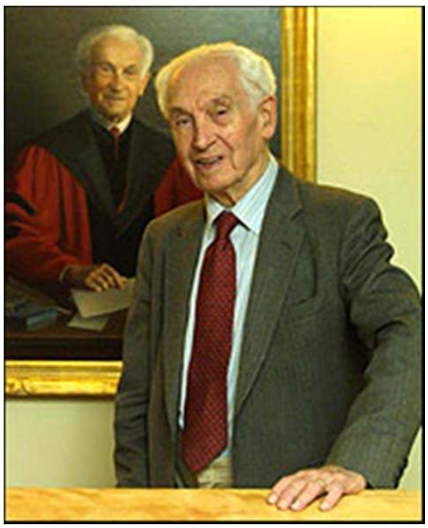
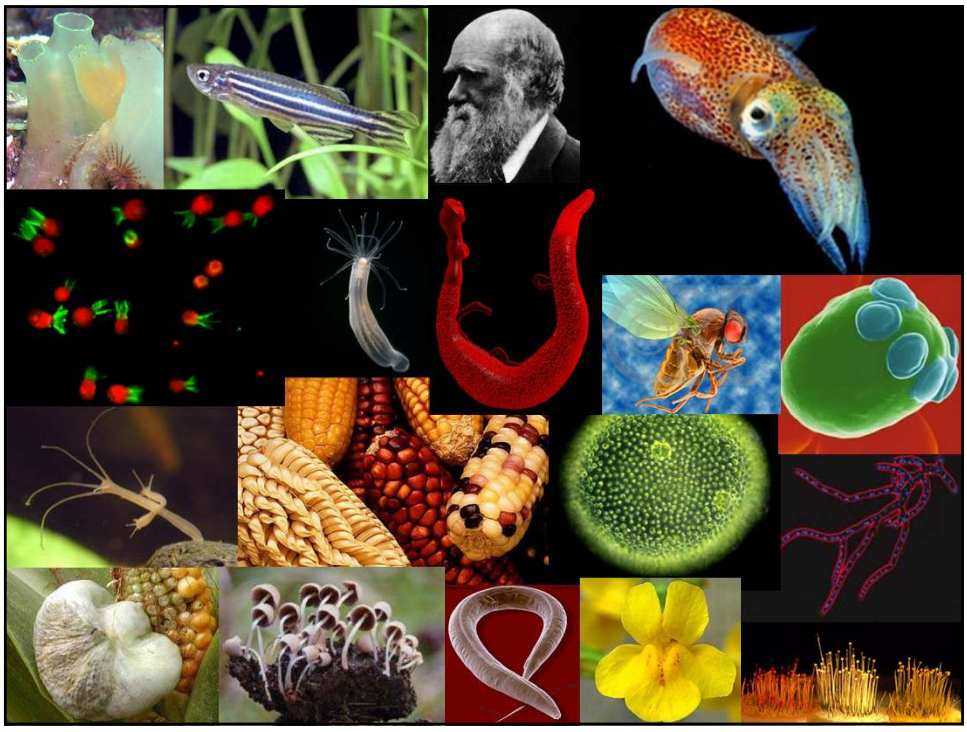

Animal Bodies are Built from the Same Genetic Toolkit
lab
antp
abd-B
Hox 1
Hox 13
Urchins
Hemichordates
Urochordates
Cephalochordates
Teleost fish
Tetrapods
Flies
Polychaetes
PHYLOGENY
GENES FOUND
KNOWN EXPRESSION
Swalla (2006) Heredity

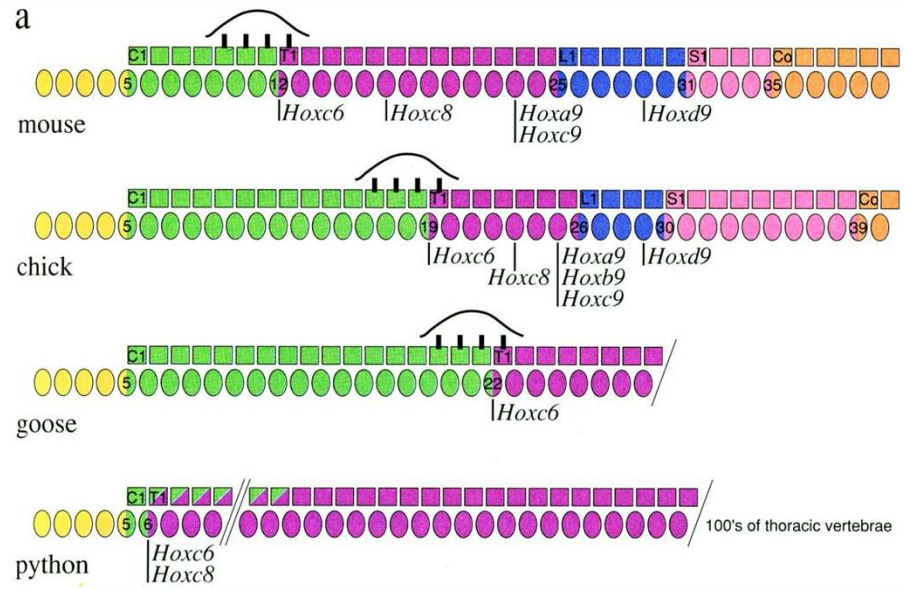
"...the search for homologous genes is quite futile except in very close relatives. If there is only one efficient solution for a certain functional demand, very different gene complexes will come up with the same solution, no matter how different the pathway by which it is achieved. The saying 'Many roads lead to Rome' is as true in evolution as in daily affairs"

Ernst Mayr, 1963



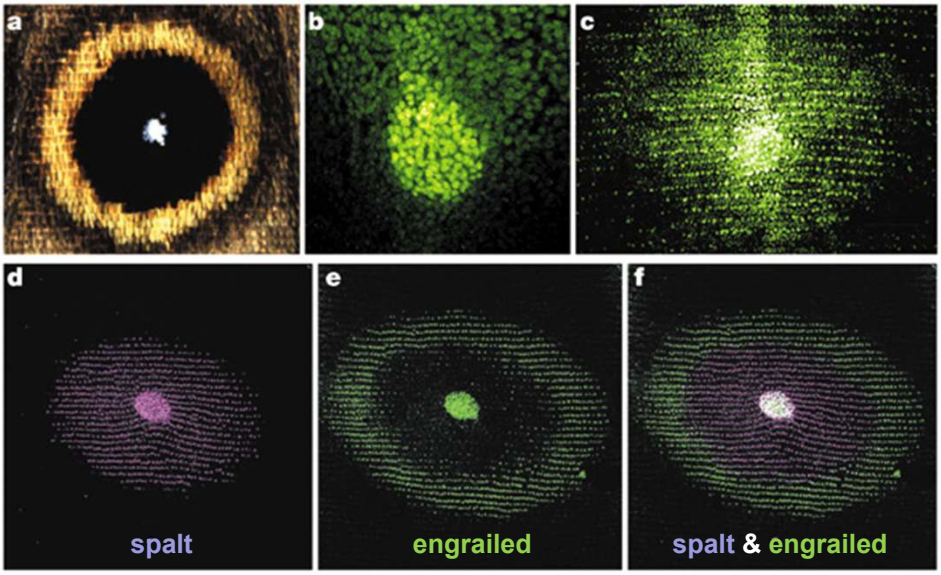


Altering the Expression of Old Genes



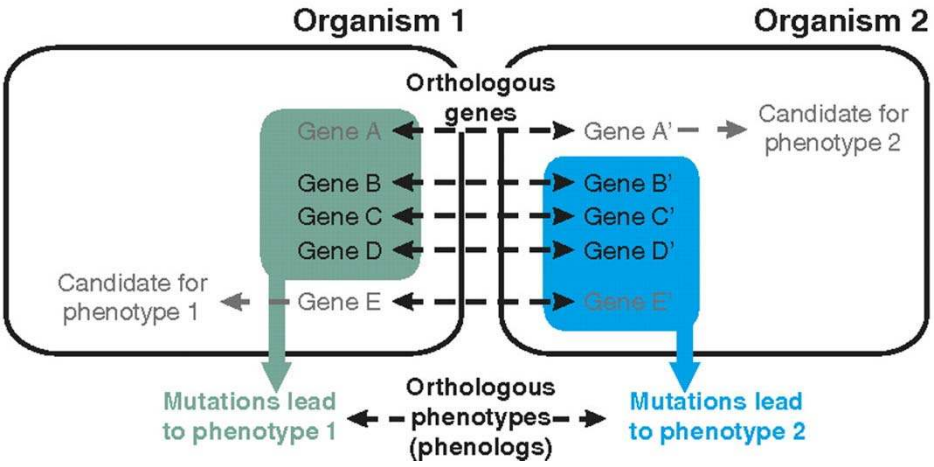
Carroll et al. (2005) From DNA to Diversity, 2nd ed., Fig. 5.6

Teaching Old Genes New Tricks (Gene Co-Option)



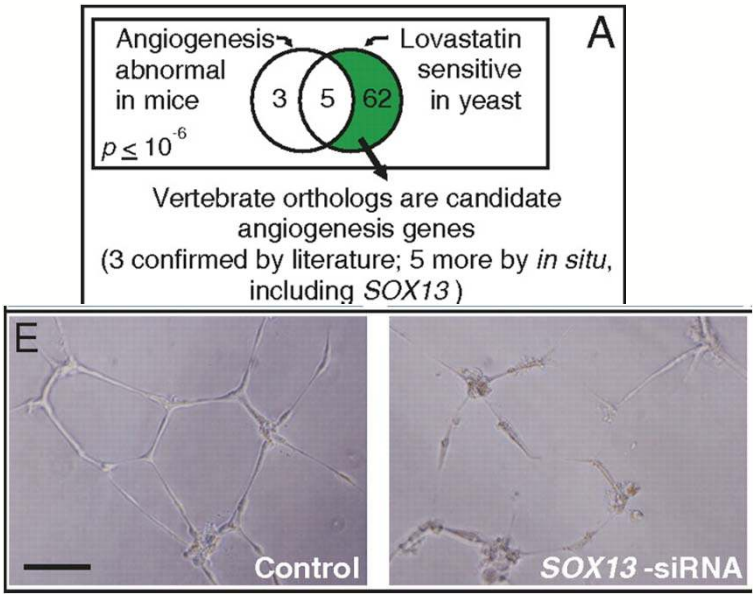
Beldade & Brakefield (2002) Nature Rev. Genet.

Deep Homology Extends To Gene Networks



McGary et al. (2010) PNAS

A Yeast Model for Angiogenesis



McGary et al. (2010) PNAS

"Our method suggests a yeast model for angiogenesis defects, a worm model for breast cancer, mouse models of autism, and a plant model for the neural crest defects […], among others"

McGary et al. (2010) PNAS

"Modelling neurodegeneration in *Saccharomyces cerevisiae*"

Khurana & Lindquist (2010) *Nat. Rev. Neurosci.*

Lecture Outline

- **Introduction to evolutionary genomics**
- **Brief summary of high-throughput DNA sequencing & applications**
- **Benchmarking *de novo* transcriptome sequencing for functional and evolutionary genomics**

----------Coffee Break----------

- **Phylogenomics**
- **Population Genomics**

----------Coffee Break----------

- **Comparative & Functional Genomics**

Genomics: "Big Science" Driven by a Few Centers

wellcome trust sanger institute

BCM Baylor College of Medicine

BROAD INSTITUTE

JGI DOE JOINT GENOME INSTITUTE US DEPARTMENT OF ENERGY OFFICE OF SCIENCE

J. Craig Venter INSTITUTE

THE Genome CENTER AT WASHINGTON UNIVERSITY

High-Throughput DNA Sequencing Technologies

Helicos		
2 x 55 bp	~8 days	35 Gb

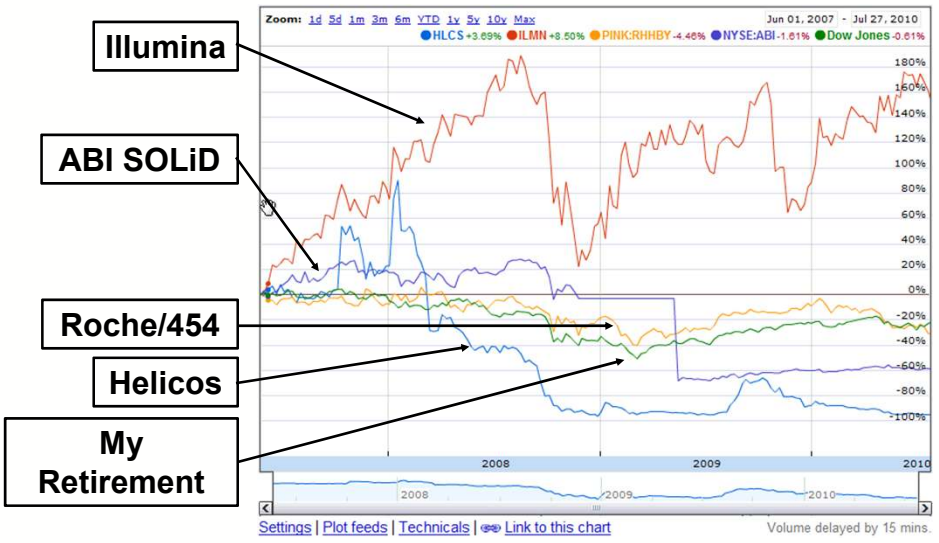
Illumina		
2 x 100 bp	~8 days	200 Gb

454 / Roche		
2 x 400 bp	~0.4 days	0.6 Gb

SOLiD ABI		
2 x 75 bp	~14 days	300 Gb

For the latest specs consult: http://ngsbuzz.blogspot.com/

The Speaker Declares No Competing Financial Interests



High-Throughput DNA Sequencing Technologies

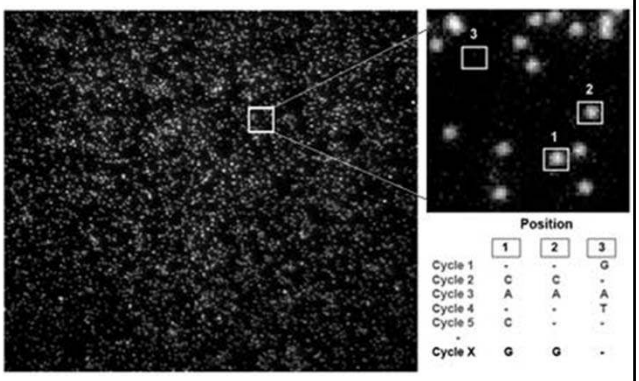
The principle:
Detecting base incorporation during DNA strand synthesis at a massively parallel scale. Base detection and strand synthesis are key differences between competing technologies

How it works:

1. Physically separate a large number of ssDNA fragments

2. Fix the fragments' location on a substrate (& amplify)

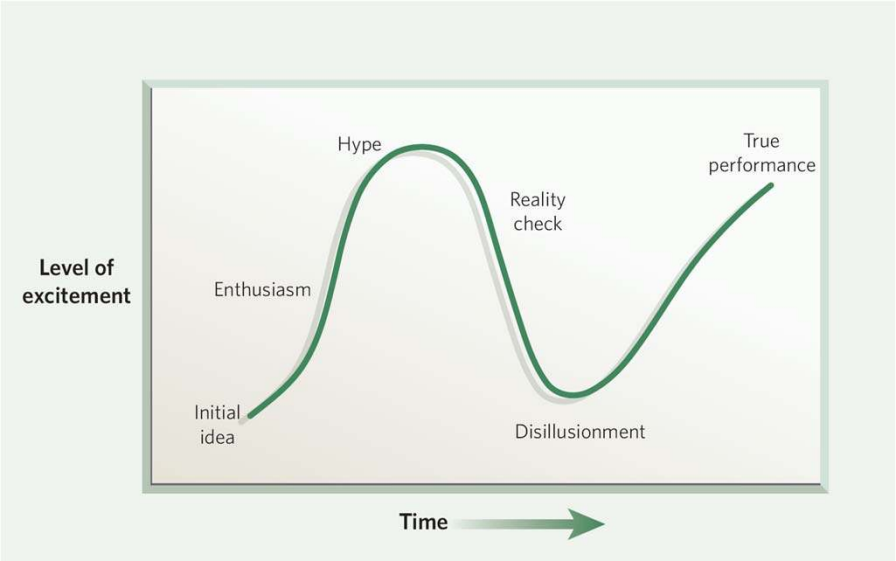
3. Detect the incorporation of each base during strand synthesis in each location through a light signal



4. Repeat nucleotide incorporation and detection cycles, thus permitting the simultaneous tracking of thousands to millions of sequencing reactions

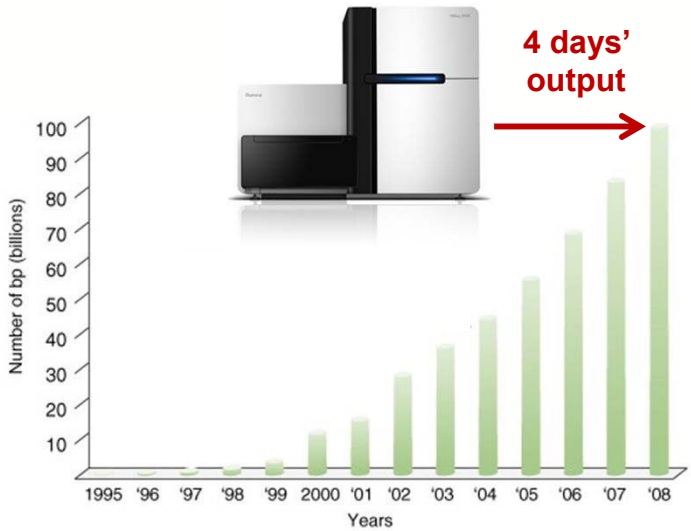
Rokas & Abbot (2009) Trends Ecol. Evol.

"New Technology Level of Enthusiasm" Curve



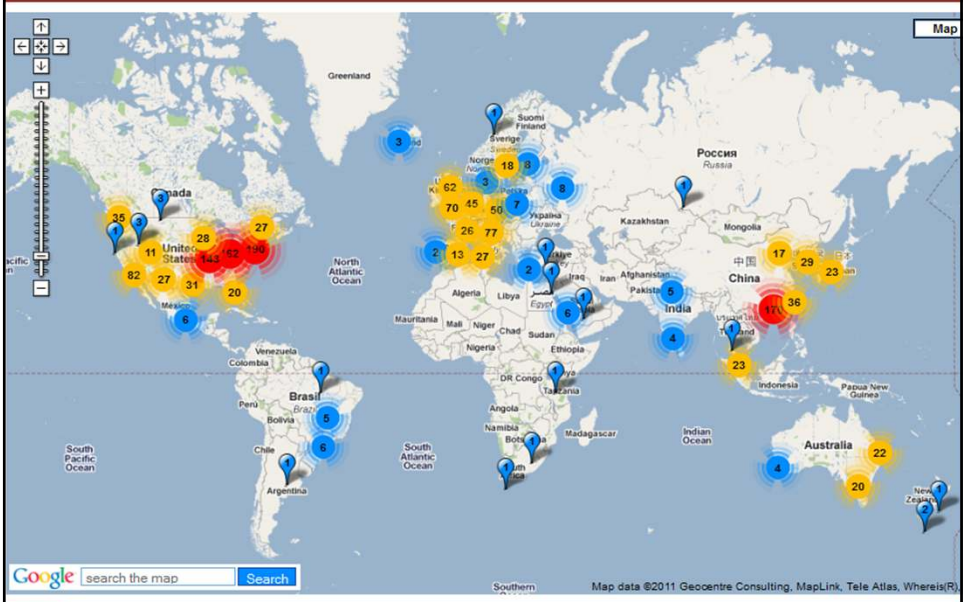
Collins (2006) Nature

Why Is Next-Generation DNA Sequencing So Exciting?



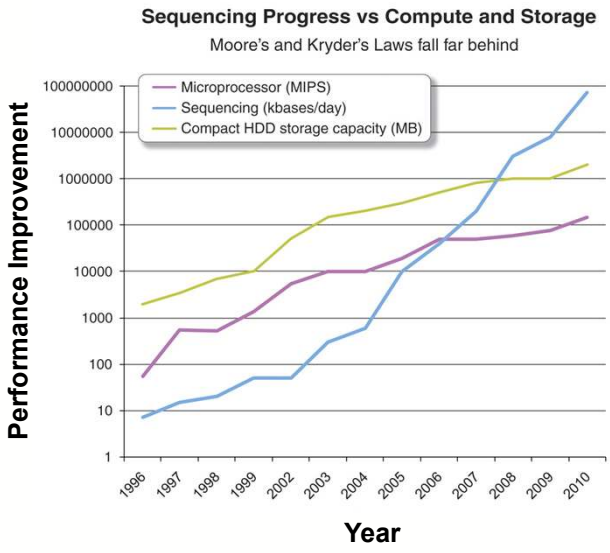
Gilad et al. (2009) Trends Genet.

World-Map of High-Throughput Sequencers



http://pathogenomics.bham.ac.uk/hts/

Sequencing is Outpacing Storage and Computation



Kahn (2011) Science

NCBI Sequence Read Archive

Sequence Read Archive (SRA) and Trace Archive repositories have been discontinued

Due to budget constraints, NCBI will be discontinuing its Sequence Read Archive (SRA) and Trace Archive repositories for high-throughput sequence data. Closure of the databases will occur in phases. SRA and Trace will stop accepting some types of submissions in the coming weeks, and all submissions within the next 12 months. Over the next several months, NCBI will be working with staff from NIH Institutes that fund large-scale sequencing efforts to develop an approach for future access to and storage of the existing data. NCBI will continue to support and develop information resources for biological data derived from next-generation sequencing such as genotypes, common variations, rare variations, sequence assemblies and gene expression data. We therefore encourage the research community to continue submissions of these data to the applicable databases, including:

1. RNA-Seq and epigenomic data to GEO
2. Variants, genotypes, phased haplotypes, and polymorphisms to dbVar, dbGaP and dbSNP
3. Genomic assemblies to GenBank/WGS
4. Transcript assemblies to GenBank/TSA
5. 16S ribosomal RNA and other targeted locus survey assemblies to GenBank

NCBI expects new applications will continue to emerge for next generation technology. We are excited to work with the community to develop strategies for archiving other summary experimental measures that are informative, efficient, and valuable to the biomedical research community.

For further information about submissions, contact NCBI's Help Desk.

Meeting report

Genome informatics: taming the avalanche of genomic data

Erik LL Sonnhammer

Address: Center for Genomics and Bioinformatics, Karolinska Institutet, 171 77 Stockholm, Sweden. E-mail: Erik.Sonnhammer@cgb.ki.se

Comment

Tsunami

Gregory A Petsko

Address: Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, MA 02454-9110, USA.
E-mail: petsko@brandeis.edu

PERSPECTIVES

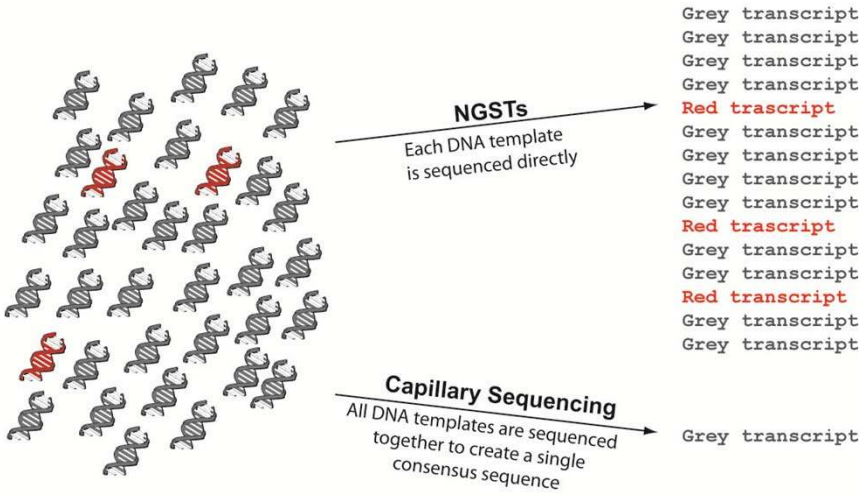
Preparing for the flood: evolutionary biology in the age of genomics

James R. Brown

Beyond the Tsunami: Developing the Infrastructure to Deal with Life Sciences Data

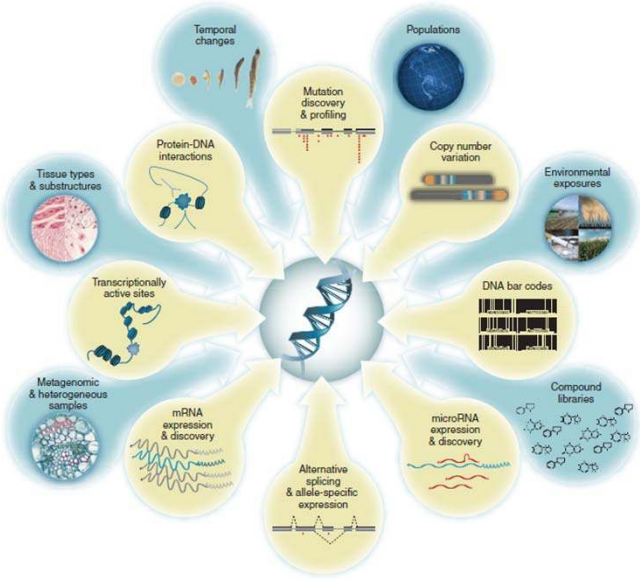
CHRISTOPHER SOUTHAN
GRAHAM CAMERON
EMBL-European Bioinformatics Institute

Next-Gen Sequencing is Qualitative and Quantitative



Rokas & Abbot (2009) Trends Ecol. Evol.

Sequence Census Assays



Kahvejian et al . (2008) Nature Biotech.

Lecture Outline

- Introduction to evolutionary genomics
- Brief summary of high-throughput DNA sequencing & applications
- Benchmarking *de novo* transcriptome sequencing for functional and evolutionary genomics

----------Coffee Break----------

- Phylogenomics
- Population Genomics

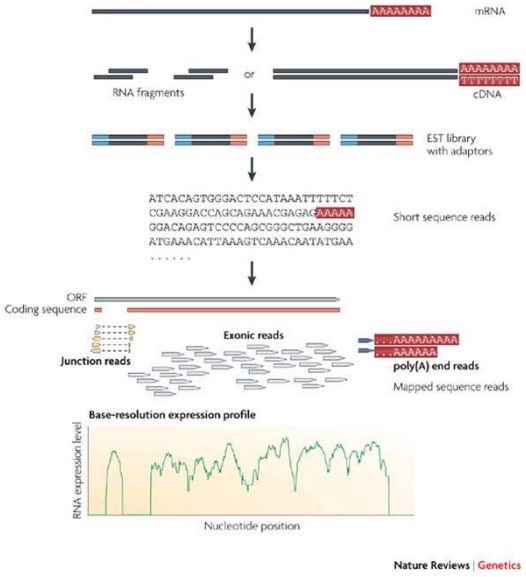
----------Coffee Break----------

- Comparative & Functional Genomics

RNA-Seq for Non-Model Organisms: What's in it for You?

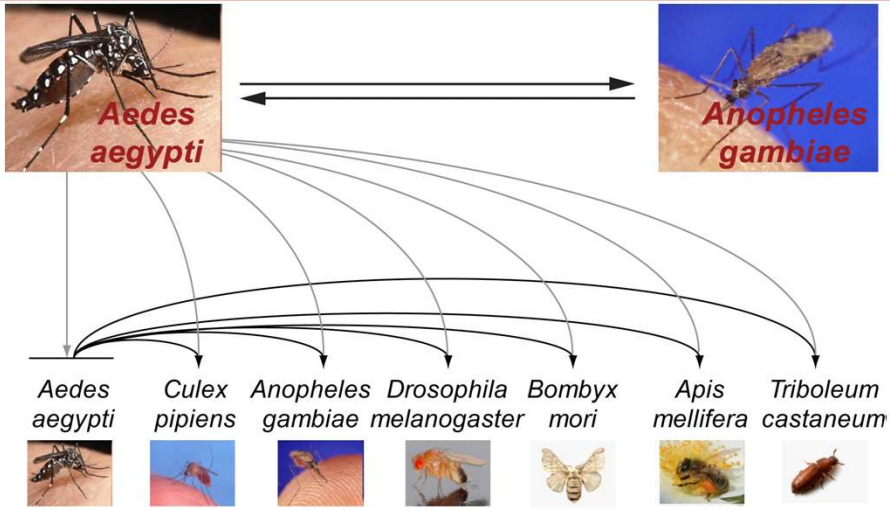
John Gibbons

A Typical RNA-Seq Experiment



Wang et al. (2009) Nature Rev. Genet.

The Next-Gen Revolution: What's in it for You?



How much? How good? How useful for functional studies? How useful for evolutionary studies?

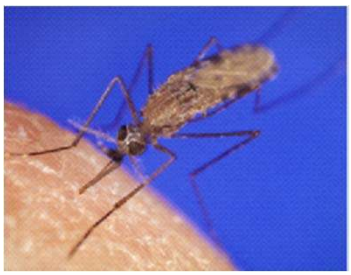
Gibbons et al. (2009) Mol. Biol. Evol.

How Much?

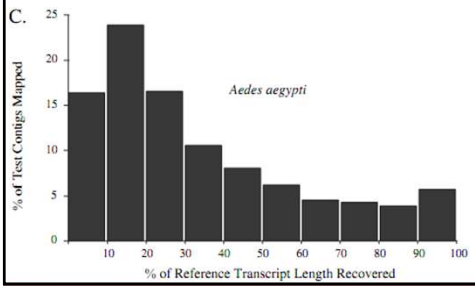
Aedes aegypti

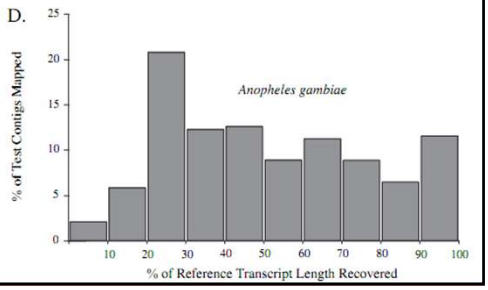
27% of the transcriptome
34% of each transcript

Anopheles gambiae



20% of the transcriptome
50% of each transcript





RNA-Seq Data is Qualitatively Accurate

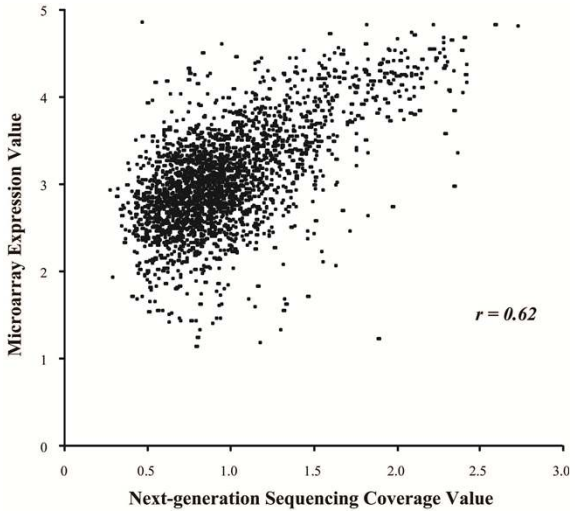
```
                Reference CAG CCA ATG AGG TCT CAG TCG
Substitutions             ||| ||| ||  ||| ||  ||| |||   3x10^-3
                     Test CAG CCA ATC AGG TCA CAG TCG

                Reference CAG CCA ATG --- --- CAG TCG
Indels                    ||| ||| |||         ||| |||   4x10^-5
                     Test CAG CCA ATG AGG TCA CAG TCG

                Reference CAG CCA ATG AGG TCT CAG TCG
Assembly Breaks             |       | ||| ||| ||| |||   2x10^-4
                     Test ATG GGG TAC AGG TCT CAG TCG
```

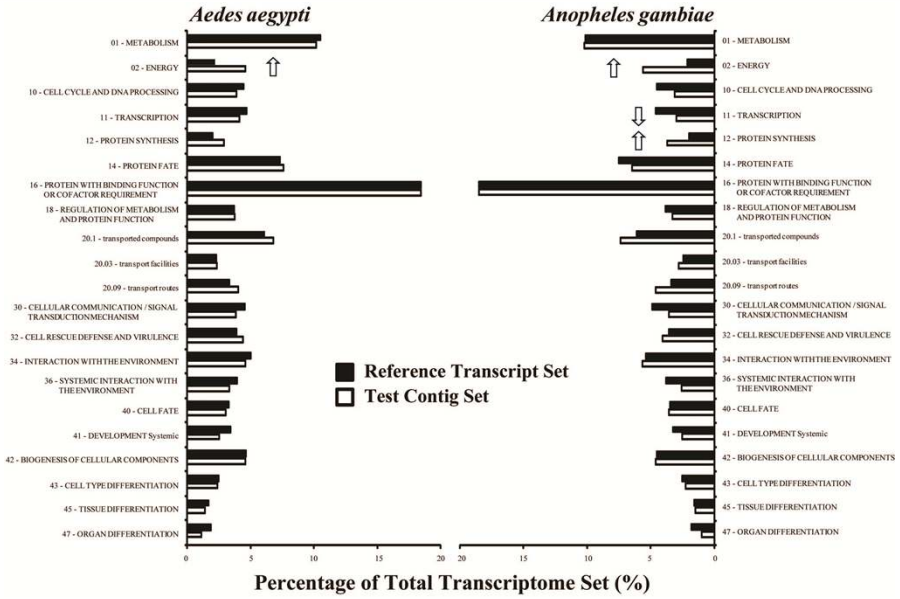
Gibbons et al. (2009) Mol. Biol. Evol.

RNA-Seq Data is Quantitatively Accurate



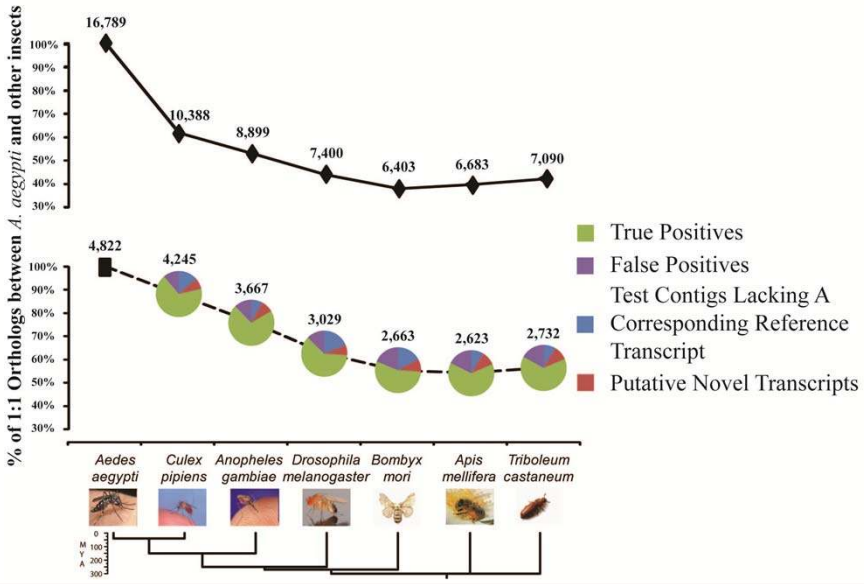
Gibbons et al. (2009) Mol. Biol. Evol.

RNA-Seq Data is Functionally Diverse



Gibbons et al. (2009) Mol. Biol. Evol.

Our RNA-Seq Data is a Goldmine for Molecular Markers



Gibbons et al. (2009) Mol. Biol. Evol.

What Can We Assemble de novo *with Current Data?*

Velvet & Oases *de novo* assembly of 10 million 100bp paired-end reads from *Anopheles albimanus*

	A. gambiae	*A. albimanus*
Transcriptome size	19.3 Mb	11.8 Mb
Gene number	13,683	11,802
Average gene length	4,542 bp	996 bp

Data from collaboration with Dinglasan lab (Johns Hopkins Univ.)

Coffee Break

Lecture Outline

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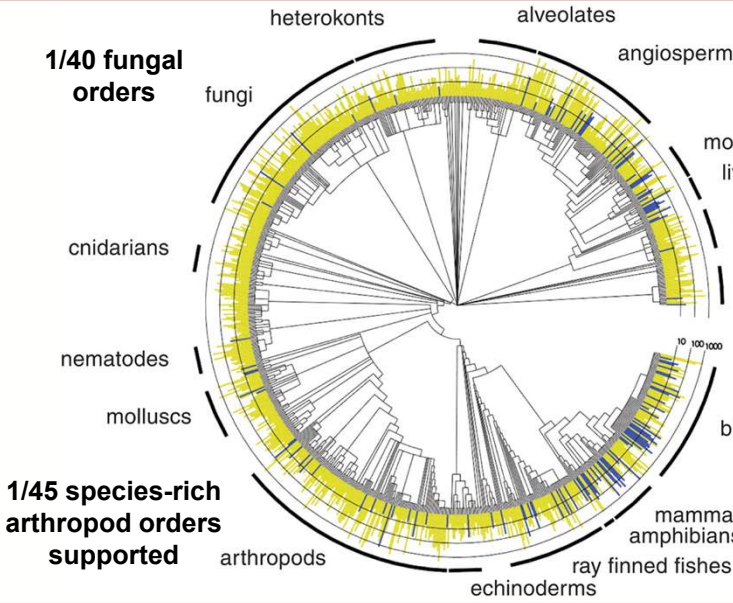
----------Coffee Break----------

- Phylogenomics
- Population Genomics

----------Coffee Break----------

- Comparative & Functional Genomics

Taxonomic Breadth versus Genomic Depth



1/40 fungal orders

3/45 angiosperm orders

1/45 species-rich arthropod orders supported

16/57 vertebrate orders

Sanderson (2008) Science

Can we Use RNA-Seq to Increase Genomic Depth?

Targeting the transcriptome is a good idea because:

1. **Smaller than genome (coding parts cover 7% of *Anopheles gambiae* genome)**
2. **Fewer repetitive and transposable elements**
3. **Unequal representation (> 5 orders of magnitude)**
   - **Enriched for housekeeping and energy genes (they tend to be conserved)**
4. **The overwhelming majority of sequence evolution models have been developed for and tested in coding sequences**

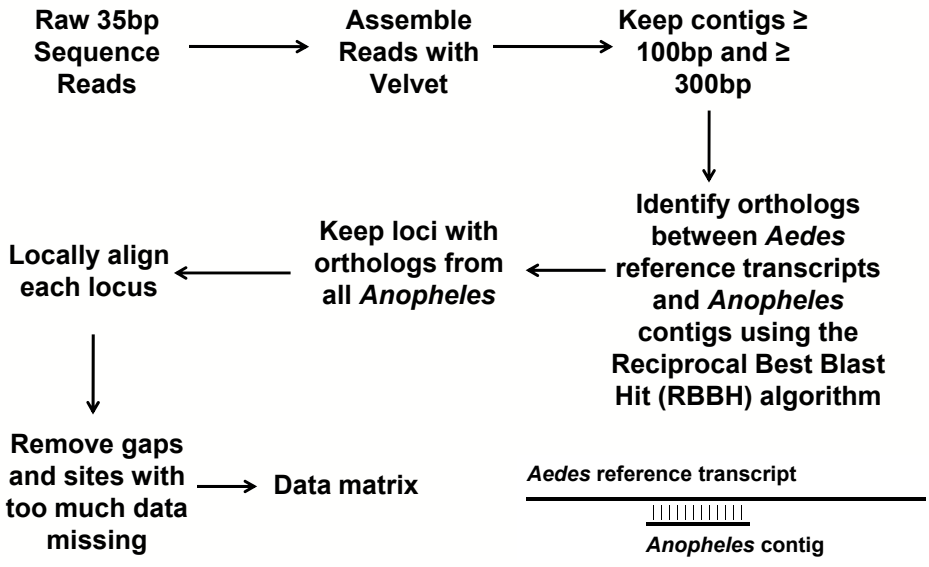
Hittinger et al. (2010) PNAS

Can we Use RNA-Seq to Increase Genomic Depth?

Species	Stock No.	Collection Location
Anopheles albimanus(Nyssorhynchus)	MRA-126	El Salvador
Anopheles arabiensis Cellia)	MRA-339	Zimbabwe
Anopheles dirus (Cellia)	MRA-700	Thailand
Anopheles farauti (Cellia)	MRA-489	Papua New Guinea
Anopheles freeborni (Anopheles)	MRA-130	USA
Anopheles gambiae (Cellia)	MRA-765	Liberia
Anopheles quadriannulatus (Cellia)	MRA-761	South Africa
Anopheles quadrimaculatus (Anopheles)	MRA-139	USA
Anopheles stephensi (Cellia)	MRA-128	India
Aedes aegypti (Stegomyia)	MRA-735	West Africa

Hittinger et al. (2010) PNAS

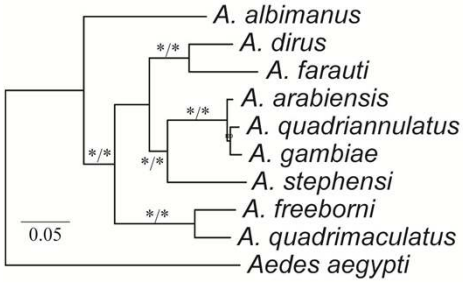
Data Matrix Construction: The "Singlecontig" Strategy



Hittinger et al. (2010) PNAS

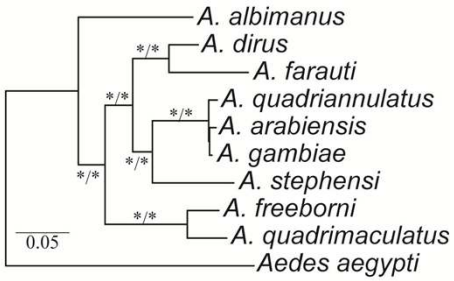
Robust Phylogenetic Inference from RNA-Seq Data

Using ≥ 100bp contigs



 # Loci = 553
Aln Length = ~390 Kb
% Missing data = 51

Using ≥ 300bp contigs

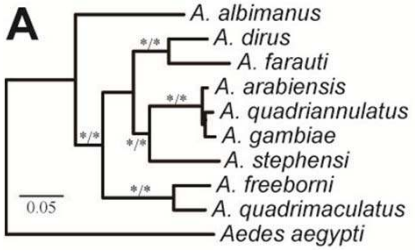


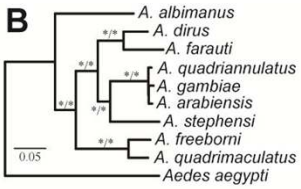
 # Loci = 69
Aln Length = ~73 Kb
% Missing data = 44

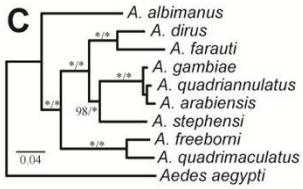
Hittinger et al. (2010) PNAS

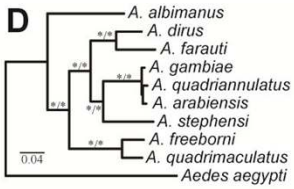
Accurate Phylogenetic Inference From our Data

553 loci
Aln L: ~390 Kb
Missing data: 51%









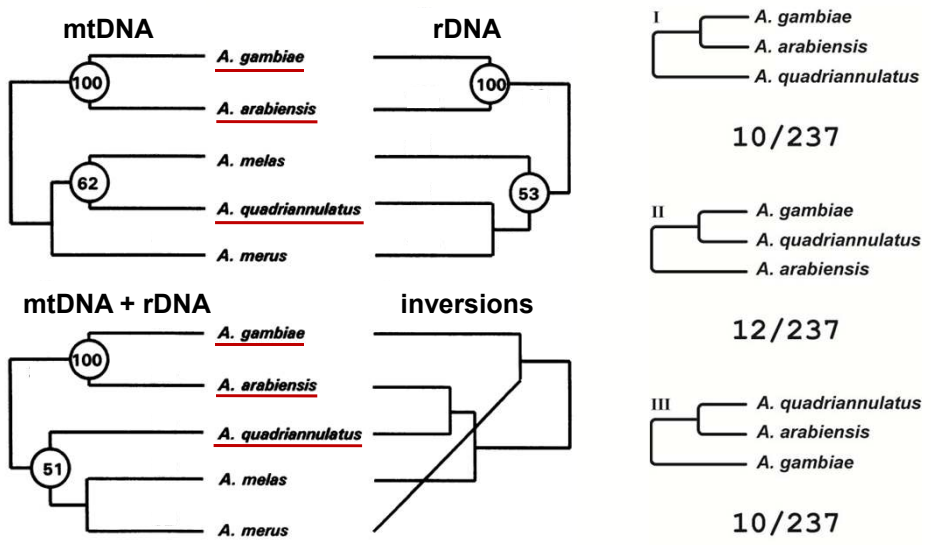
<u>Exclude erroneous loci</u>
491 loci
Aln L: ~329 Kb
Missing data: 50%

<u>Use only sites without data missing</u>
Aln L: ~15 Kb
Missing data: 0%

<u>Use *A. gambiae* as ref.</u>
634 loci
Aln L: ~472 Kb
Missing data: 50%

Hittinger et al. (2010) PNAS

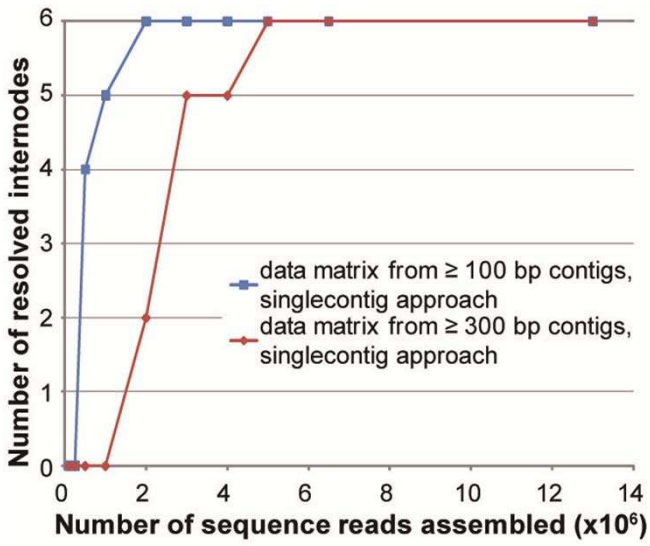
Our Data Matrices Can Detect Population-Level Events



Besansky et al. (1994) PNAS

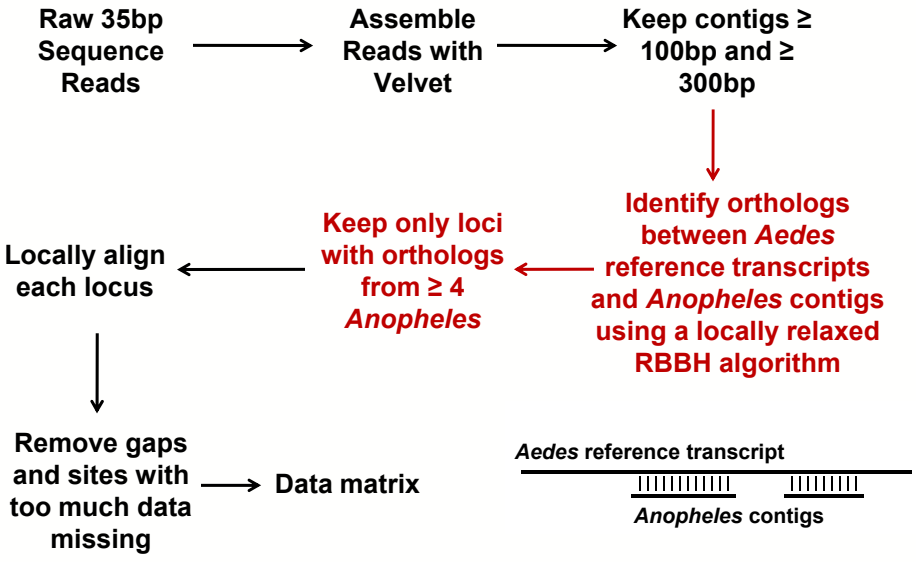
Hittinger et al. (2010) PNAS

Robust Phylogenetic Inference From Few Sequence Reads



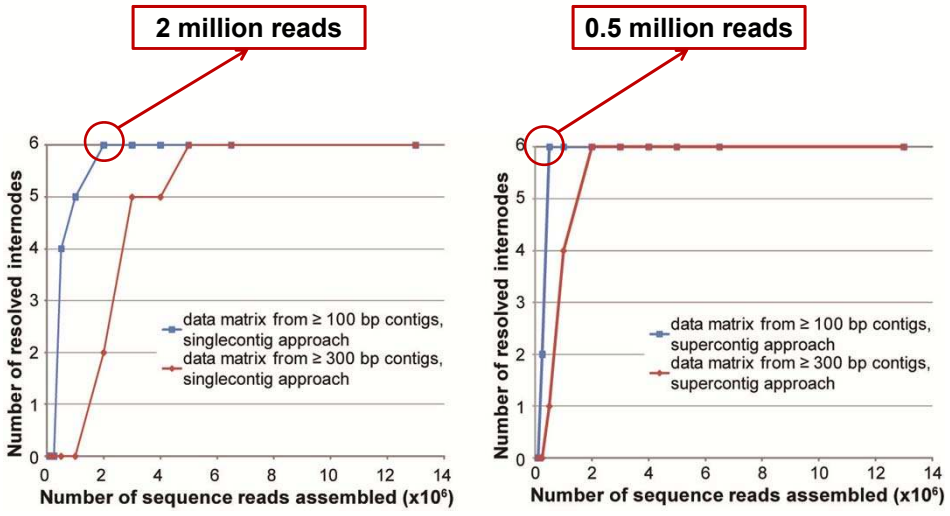
Hittinger et al. (2010) PNAS

Experimental Design: The "Supercontig" Strategy



Hittinger et al. (2010) PNAS

Robust Phylogenetic Inference From Very Few Reads



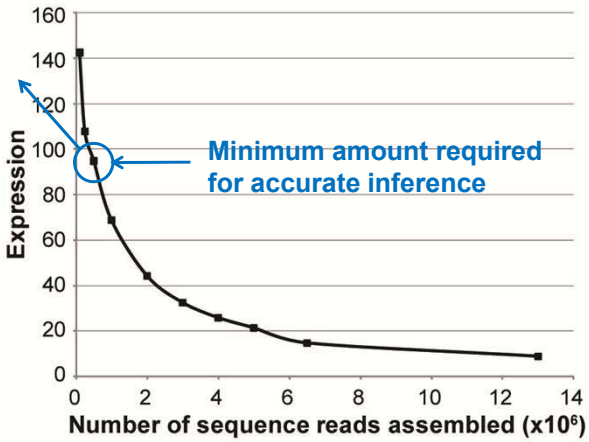
553 loci, AlnL: ~390Kb, %miss: 51

2,661 loci, AlnL: ~971Kb, %miss: 62

Hittinger et al. (2010) PNAS

Our Sequences are from Highly-Expressed Transcripts

2008 cost: ~$50
2011 cost: <$10



Hittinger et al. (2010) PNAS

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----------Coffee Break----------

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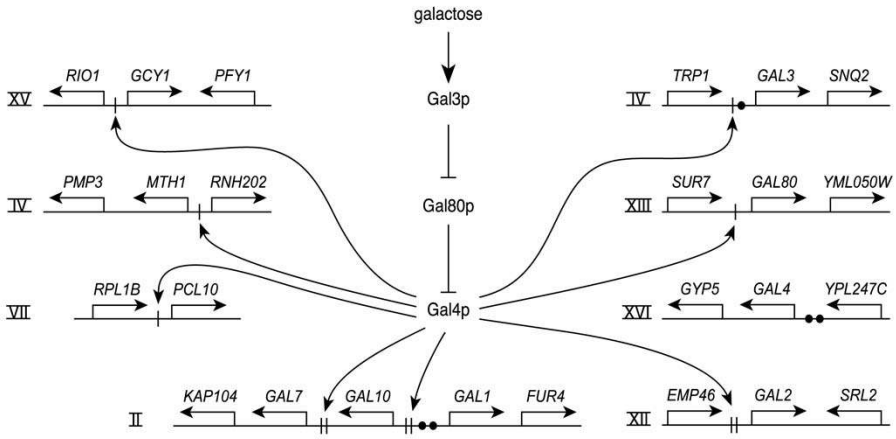
----------Coffee Break----------

- Comparative & Functional Genomics

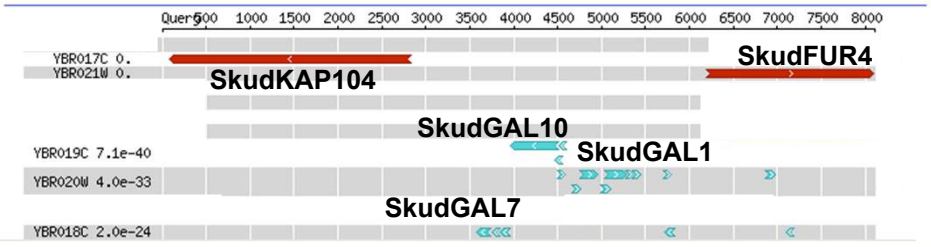
The GAL Pathway in the Baker's Yeast S. cerevisiae



S. kudriavzevii GAL Genes are Syntenic Pseudogenes

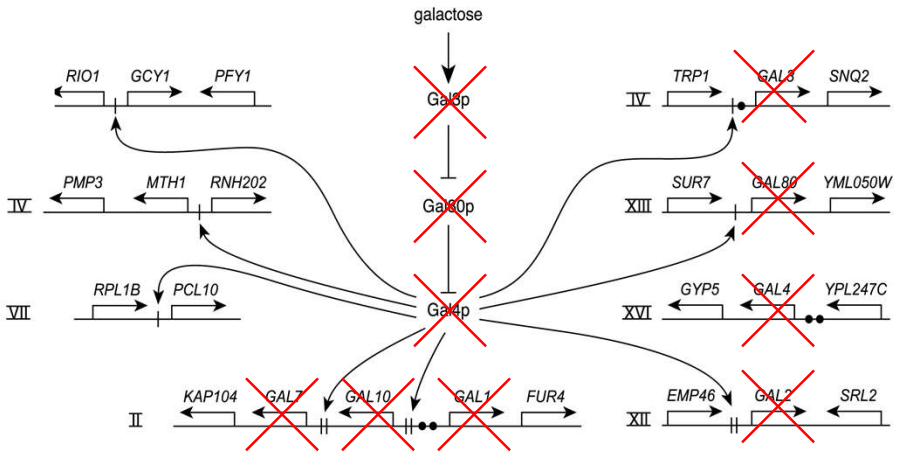
```
Skud:  5026 SPSNFK-L*SYTRDQNMPTQSLREF*NGL*MTFTVKFSNDEEFFEQFGALKNES*VSCDK 5202
            SP  F+ L  Y R +++ ++SLR     + +  T  F+ DE+FF+QFGAL NES  SCDK
Scer:   381 SPVRFQVLKLYQRAKHVYSESLRVL-KAVKLMTTASFTADEDFFKQFGALMNESQASCDK 439

Skud:  5203 LYGCSCAETDTVCSIALLSGSYNSRL 5280
            LY CSC E D +CSIAL +GSY SRL
Scer:   440 LYECSCPEIDKICSIALSNGSYGSRL 465
```



Hittinger, Rokas & Carroll (2004) PNAS

The Entire GAL Pathway is Degenerate



Hittinger, Rokas & Carroll (2004) PNAS

But S. kudriavzevii is More Diverse!

Strain	Nationality
IFO1802	Japan
IFO10990	Japan
IFO10991	Japan
IFO1803	Japan
ZP513	Portugal
ZP537	Portugal
ZP542	Portugal
ZP591	Portugal
ZP594	Portugal
ZP595	Portugal
ZP620	Portugal
ZP621	Portugal
ZP623	Portugal
ZP625	Portugal
ZP627	Portugal
ZP629	Portugal
ZP630	Portugal
ZP634	Portugal

2004: 4 Japanese GAL- strains

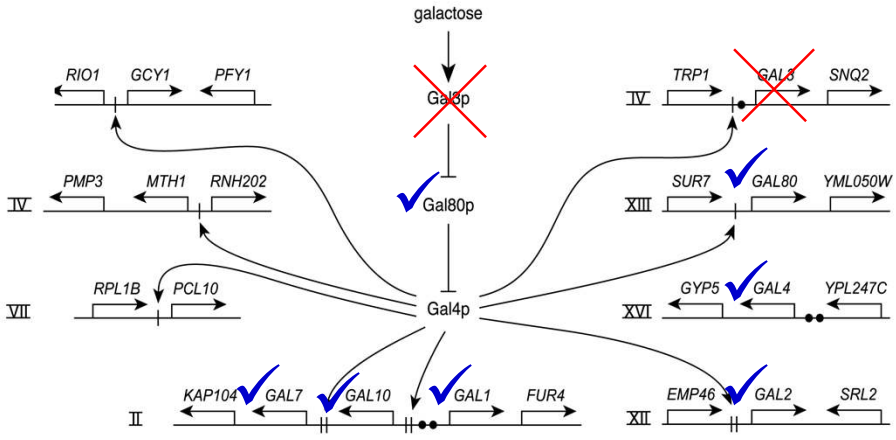
2007: 14 Portuguese GAL+ strains discovered

Illumina sequencing

80,000,000 reads
2,800,000,000 bp

Sampaio & Gonçalves (2008) Appl. Environ. Microbiol.

The Reduced GAL⁺ *Network of* S. kudriavzevii

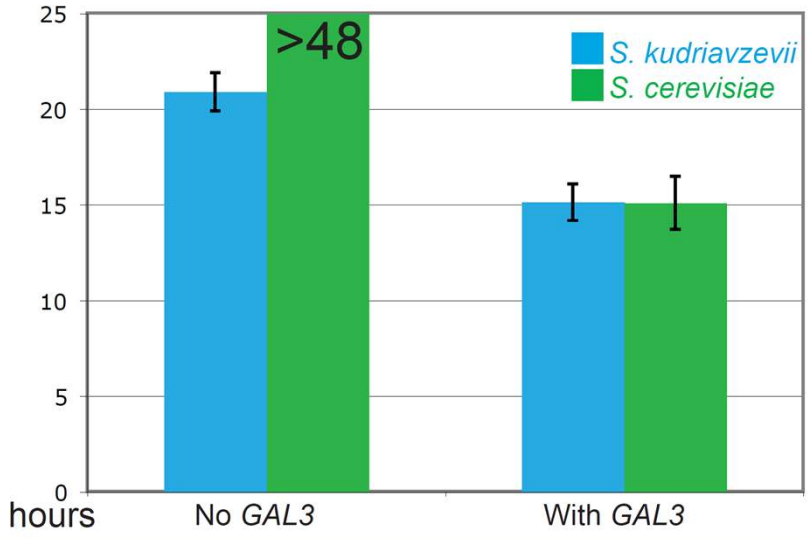


How can we explain the presence of such striking multi-locus gene network polymorphism within a species?

de novo assembly from ~30x Illumina of ZP591; confirmed by Sanger

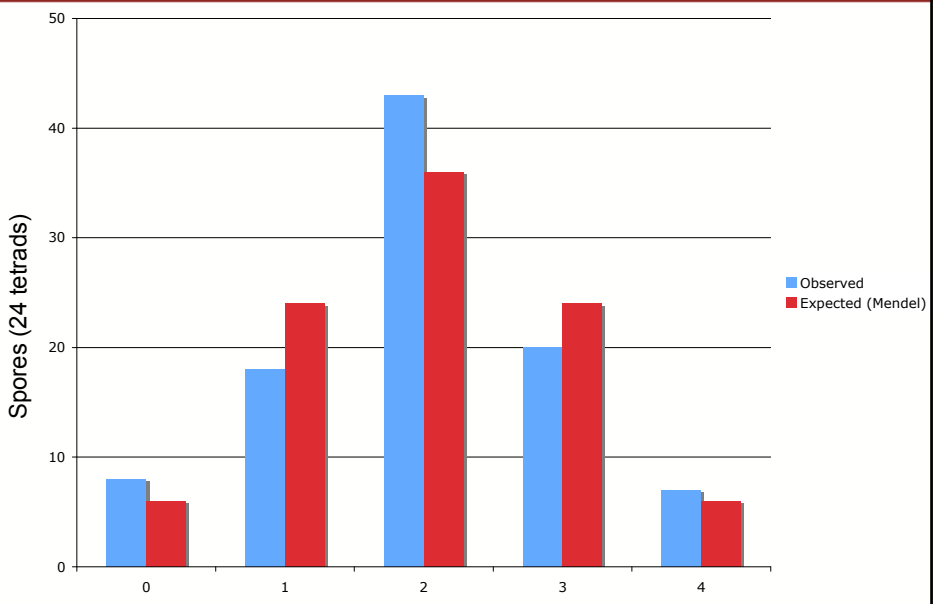
Hittinger et al. (2010) Nature

The S. kudriavzevii GAL⁺ *Network Can Function Without* GAL3



Hittinger et al. (2010) Nature

S. kudriavzevii GAL *Loci Segregate Independently*



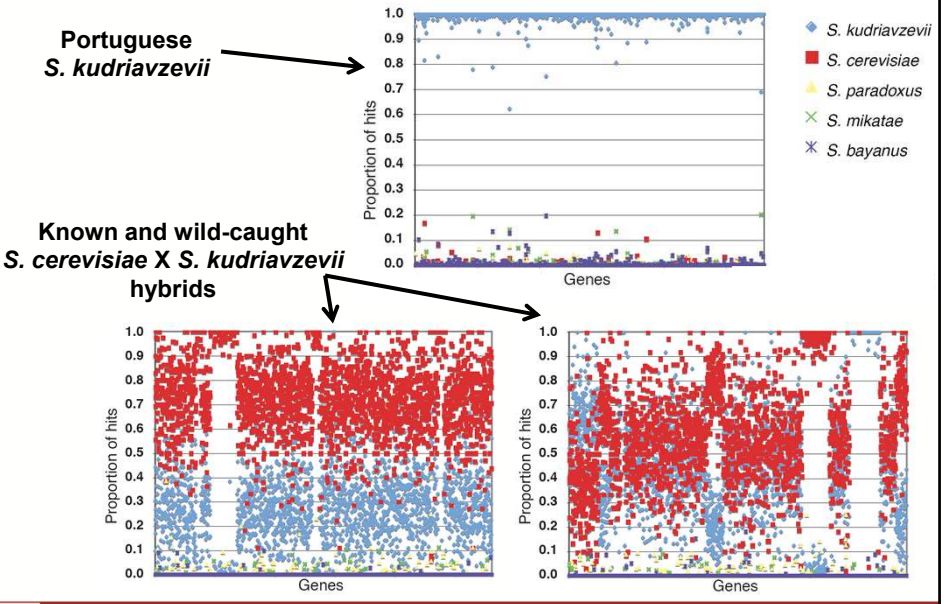
 # *GAL*⁺ alleles

Hittinger et al. (2010) Nature

The GAL⁺ *Isolates of* S. kudriavzevii *are Not Hybrids*

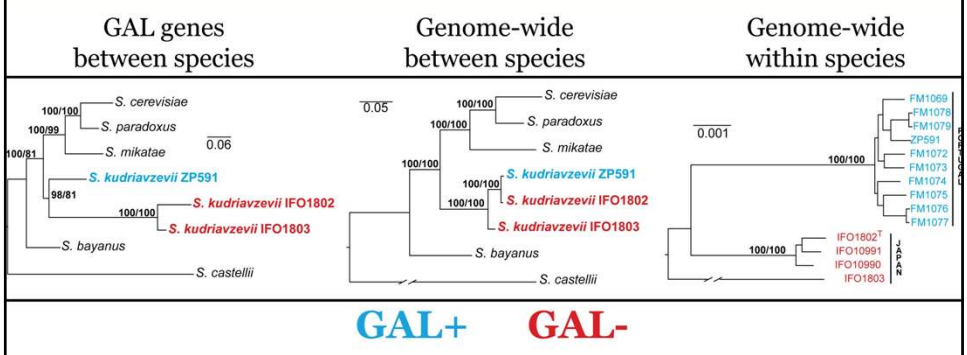
Portuguese *S. kudriavzevii*

Known and wild-caught *S. cerevisiae* X *S. kudriavzevii* hybrids



Hittinger et al. (2010) Nature

The Two GAL *Network States Evolved Within* S. kudriavzevii

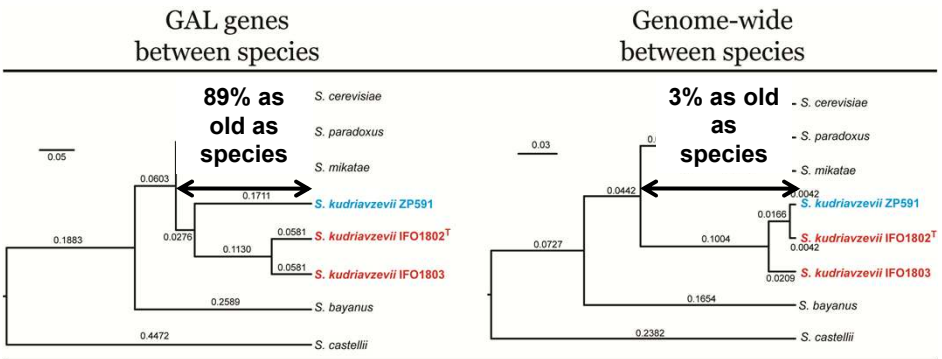


GAL+ **GAL-**

Only two non-*GAL* genes in the *S. kudriavzevii* genome significantly reject the lineage phylogeny in favor of the *GAL* gene phylogeny – both flank *GAL* loci

Clade Support Values: Posterior Probability / ML Bootstrap

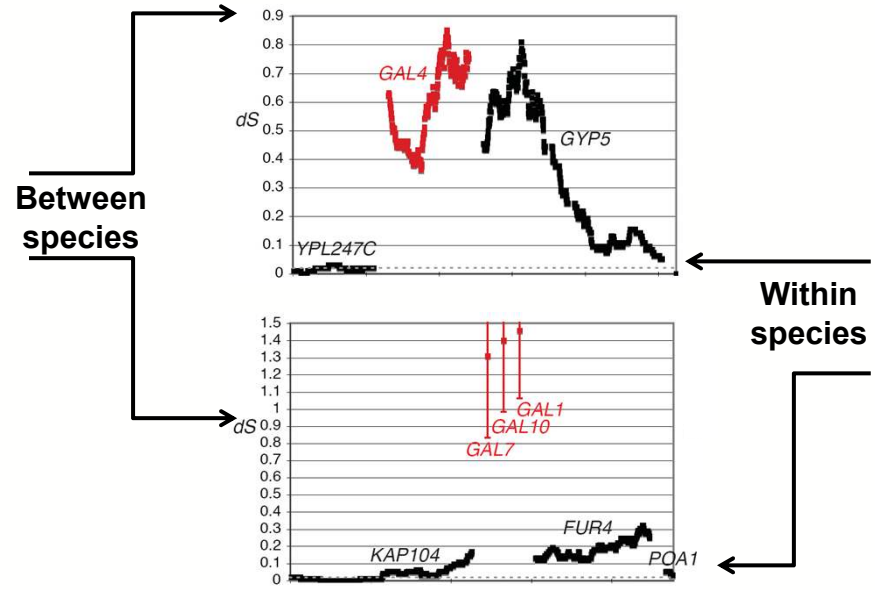
Ancient GAL *Divergence Despite Recent Genome Divergence*



GAL+ **GAL-**

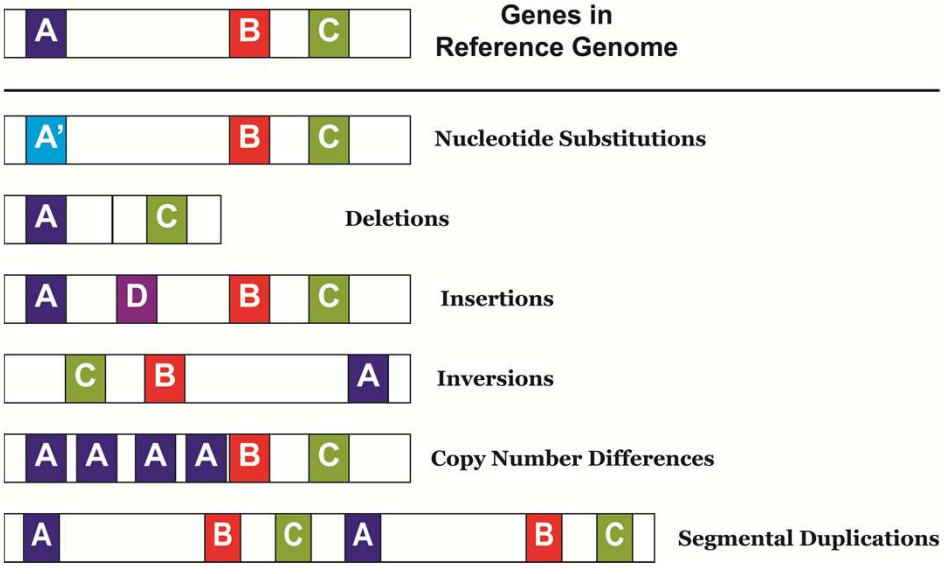
Ultrametric trees constructed using BEAST

The S. kudriavzevii GAL *Loci Are Remarkably Divergent*

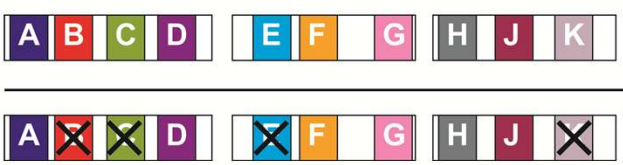


Hittinger et al. (2010) Nature

Types of Genetic Variation Within Species



Balanced unlinked Gene Network Polymorphisms (BuGNPs)



How can we explain such striking polymorphism in a multi-locus gene network?

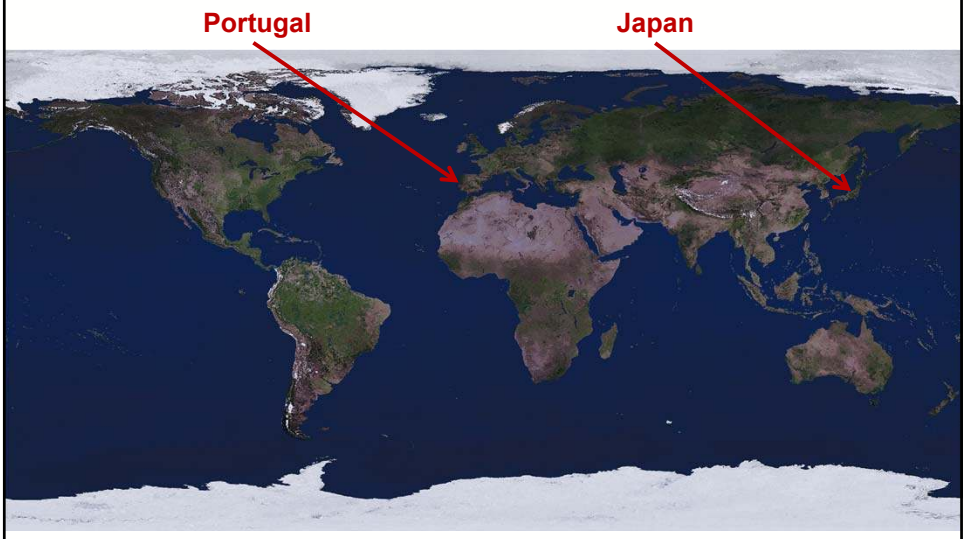
Hittinger et al. (2010) Nature

How Can We Explain BuGNPs?

"It is now generally understood that […] alleles at different loci may not be randomly associated with each other in a population. While this effect is generally regarded as a consequence of linkage, even *genes on different chromosomes* may be held temporarily or permanently out of random association by forces of *selection*, *drift* and *non-random mating*."

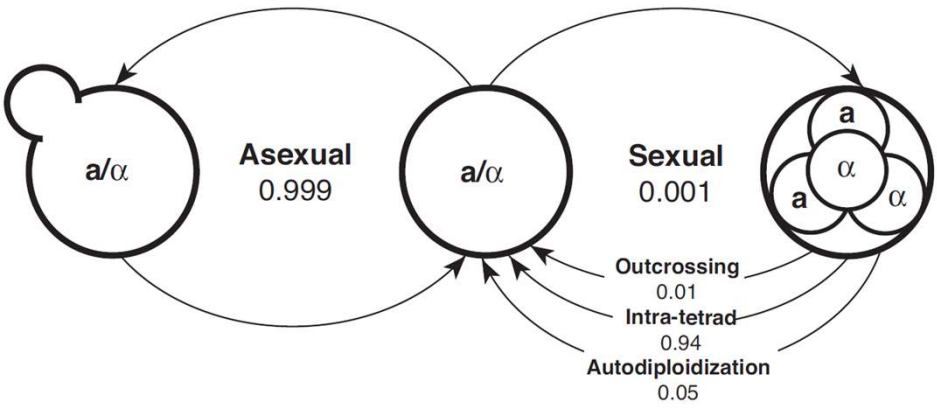
Lewontin (1998) Genetics

Maintaining BuGNPs Through Geographic Isolation



http://nssdc.gsfc.nasa.gov/planetary/image/earth_day.jpg

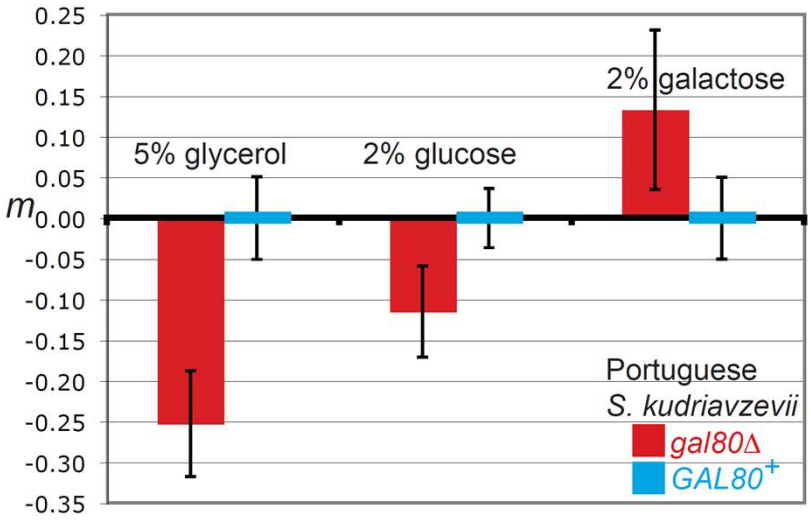
Maintaining BuGNPs by Selfing



The reproductive lifestyle of *Saccharomyces paradoxus*

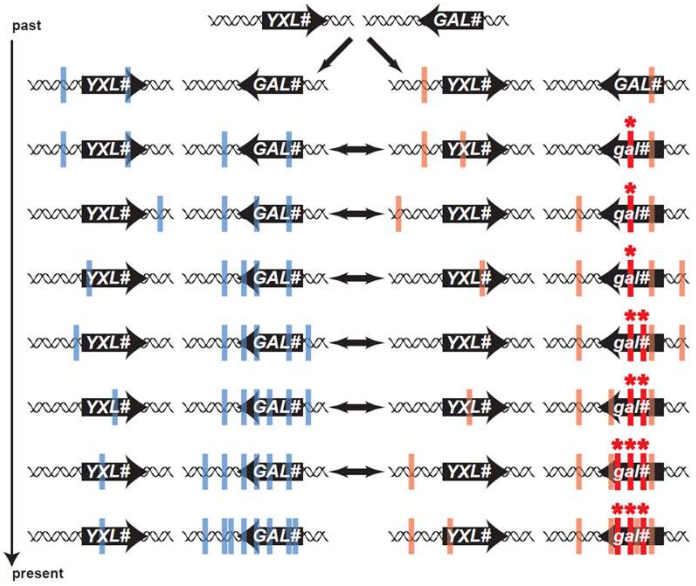
Tsai et al. (2008) PNAS

Maintaining BuGNPs by Selecting Against Intermediates



Hittinger et al. (2010) Nature

Origin and Maintenance of GAL Network Polymorphism



GAL+ **GAL-**

The Genomes of Non-Model Organisms are the New Frontiers

Epidemiology	What is the aetiology of emerging, uncharacterized infectious diseases? What are the core genomes of different microbes or pathogens occupying different niches or hosts?
Social Evolution	Are maternal care and sibling care behaviors regulated by similar patterns of gene expression?
Biodiversity	What is the extent of undocumented microbial diversity in different ecosystems? What is the microbial flora of the human gut?
Phylogenetics	What is the phylogeny of all vertebrate species or angiosperms using organelle or nuclear genome data?
Population Genetics	What is the variation in the mutation rate across organisms?
Experimental evolution	What is the genetic basis of phenotypes emerging during laboratory evolution?
Palaeontology	What are the evolutionary relationships of ancient organisms to extant taxa?
Evolution of development	How do regulatory networks evolve and rewire? What are the *cis*-regulatory targets of all transcription factors in a species and how do they evolve?

Rokas & Abbot (2009) Trends Ecol. Evol.

Personal High-Throughput Sequencers

Illumina MiSeq		$125K
2 x 150 bp	27 hours	1 Gb

454 / Roche GS Junior		$100K
2 x 400 bp	10 hours	35 Mb

Personal Genome Sequencer	$49K
1 x 100 bp	2 hours	10 Mb

Coffee Break

Lecture Outline

- Introduction to evolutionary genomics
- Brief summary of high-throughput DNA sequencing & applications
- Benchmarking *de novo* transcriptome sequencing for functional and evolutionary genomics

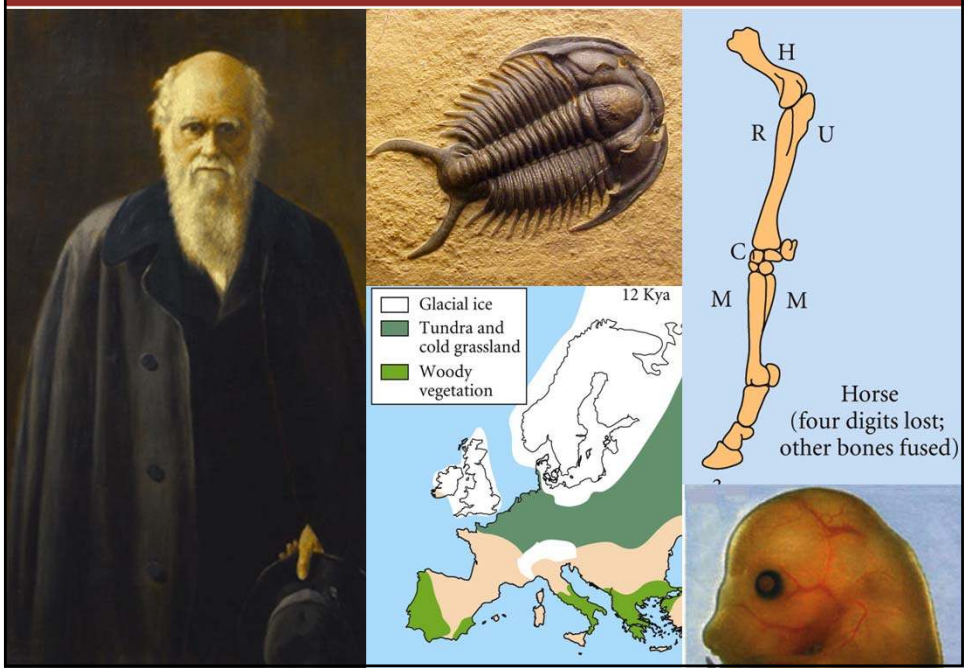
----------Coffee Break----------

- Phylogenomics
- Population Genomics

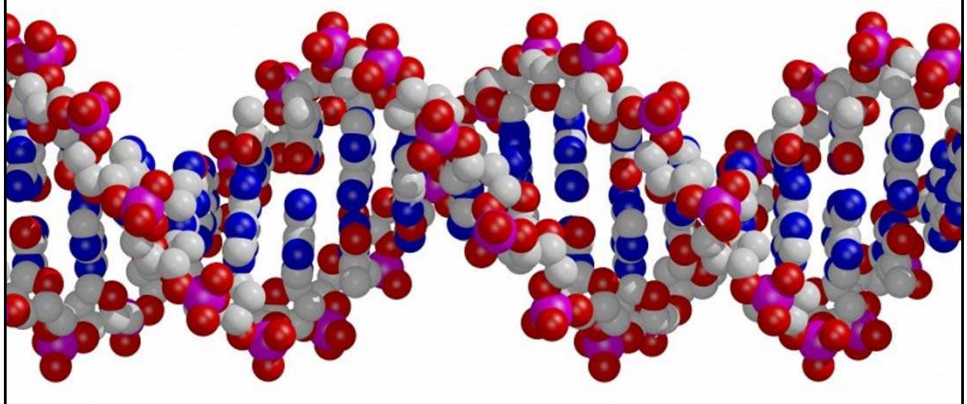
----------Coffee Break----------

- Comparative & Functional Genomics

Darwin's Data

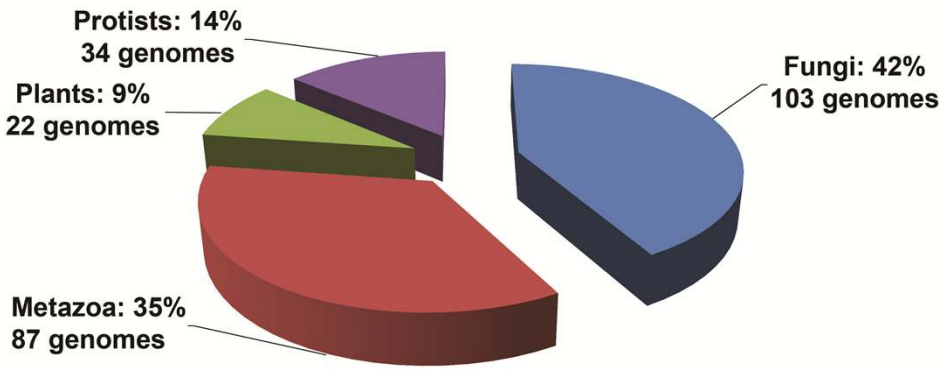


The DNA Record



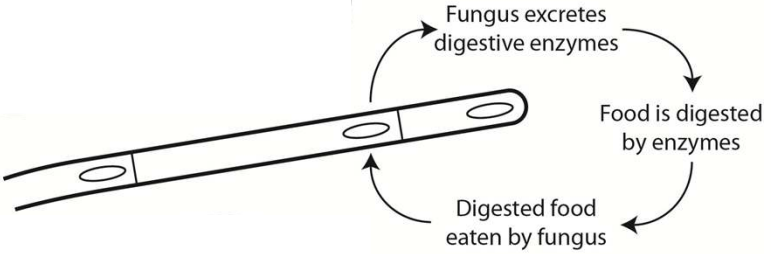
The DNA record contains important clues about organisms' biological past, and their history of change and adaptation

Fungi: the Most Sequenced Eukaryotic Lineage



Data from GOLD 3.0 (www.genomesonline.org) March 2011

Fungi Eat by Absorption

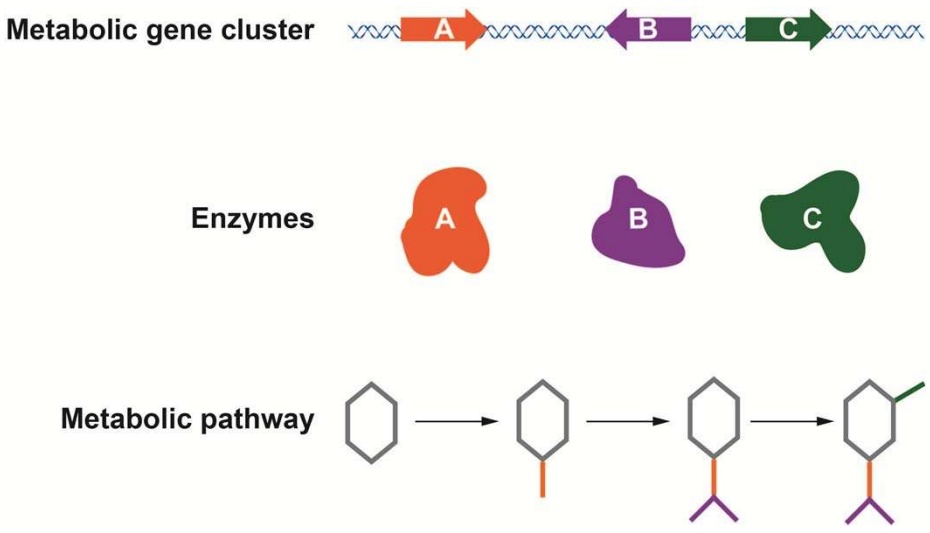


- **Fungi need to defend their food**
- **Different fungi "eat" different foods**
- **Collectively, fungi can "eat" almost everything: leather, cloth, wood, manure, animal carcasses, live hosts, ink, syrup, paint, glue, hair, etc.**

What Fungi Cannot Eat!

Diner fries McDonald's fries

Fungal Metabolic Genes Are Often Clustered



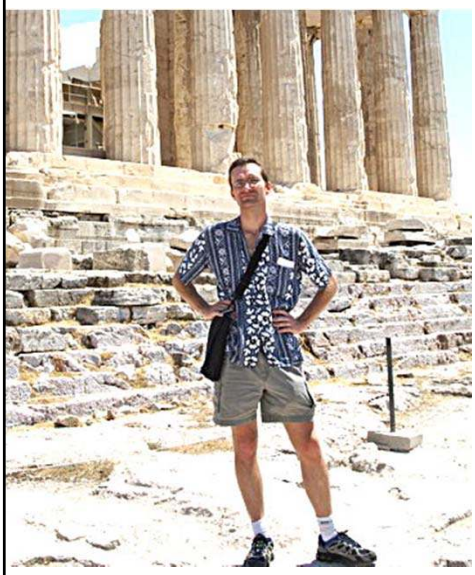
Major Questions

A typical fungal genome contains ~1,000 metabolic genes. ~6% of metabolic genes reside in clusters

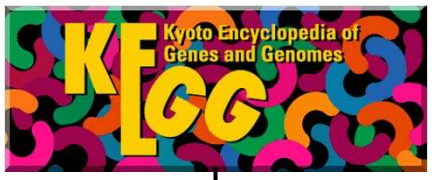
<u>Q1:</u> Why genes that participate in fungal metabolic pathways cluster?

<u>Q2:</u> What are the evolutionary and functional implications of this clustering?

The CRAP (Cluster Reconstruction And Phylogeny) Pipeline



Jason Slot



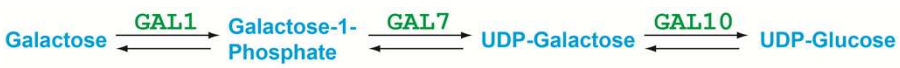
Retrieve genes from 173 pathways from 35 KEGG-annotated genomes (23 fungi)

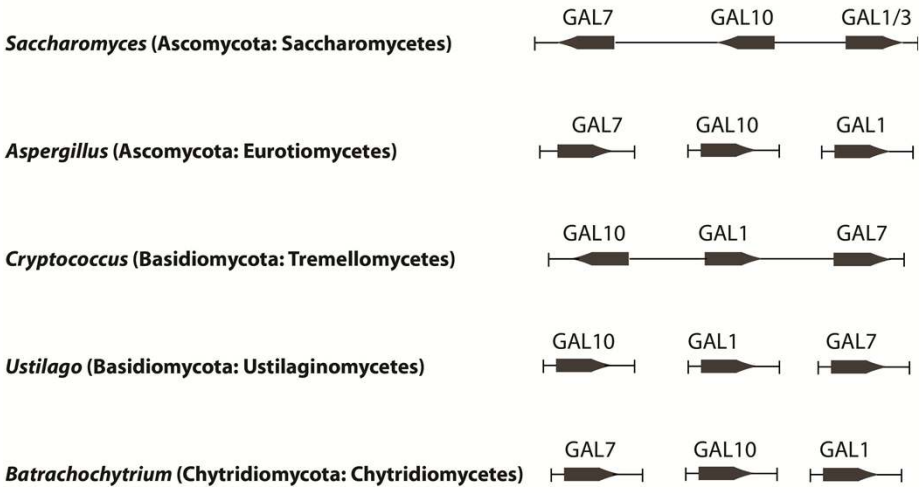
Search for presence of pathway homologs in ~130 genomes (105 fungi) by BLAST and OrthoMCL

Infer phylogenetic relationships of pathway homologs

Search for physical linkage (clustering) of pathway homologs

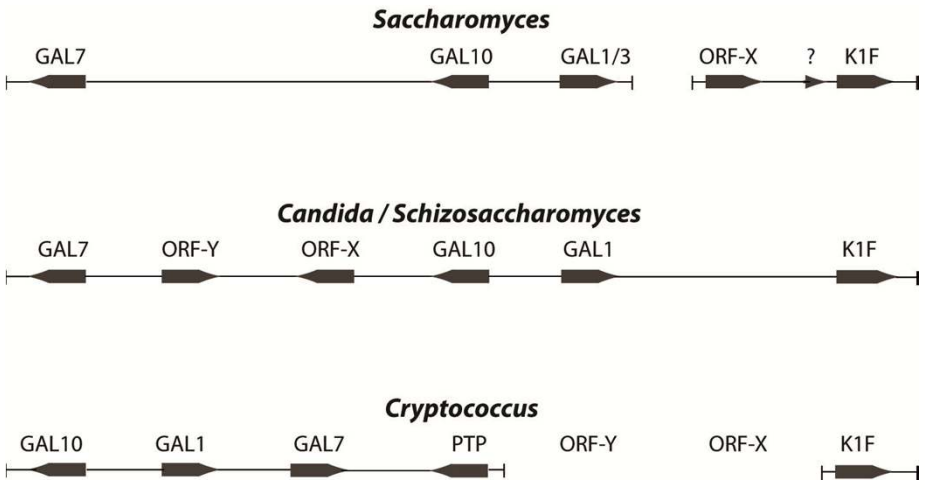
Variation in GAL Gene Clustering Across Fungi





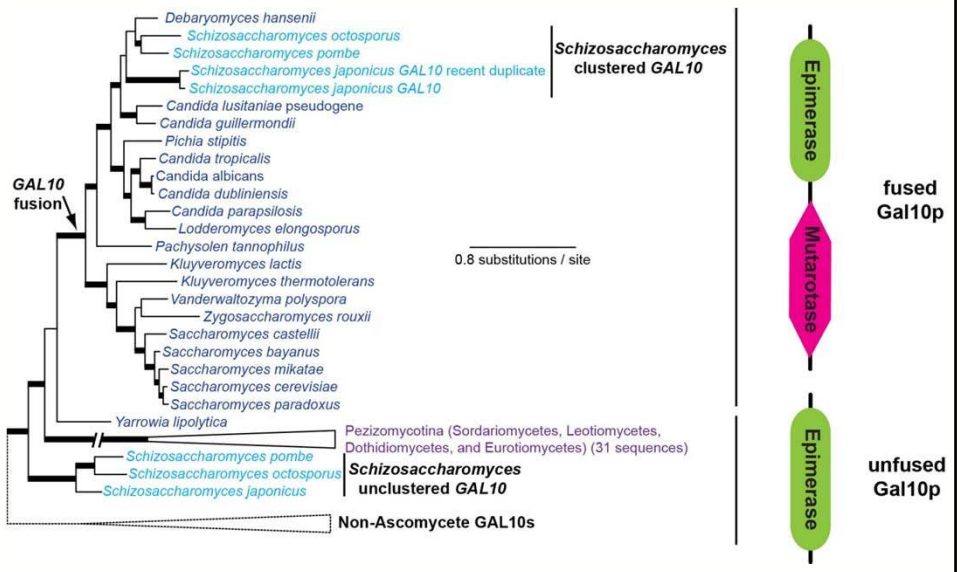
Slot & Rokas (2010) PNAS

Three Distinct Types of Fungal GAL *Gene Clusters*



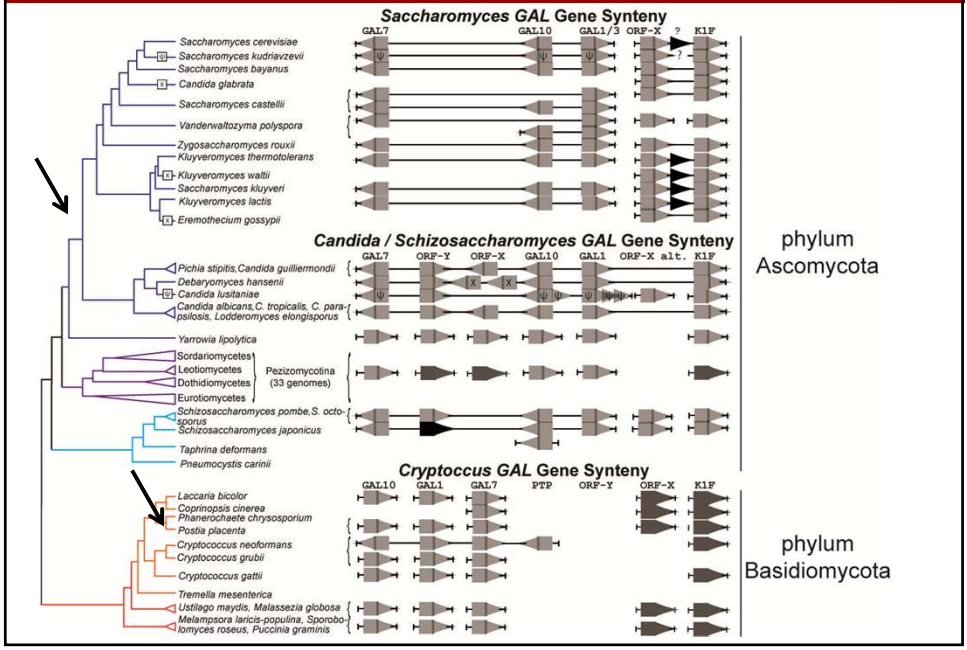
Slot & Rokas (2010) PNAS

Schizosaccharomyces *Gained Its* GAL *Cluster by HGT from* Candida

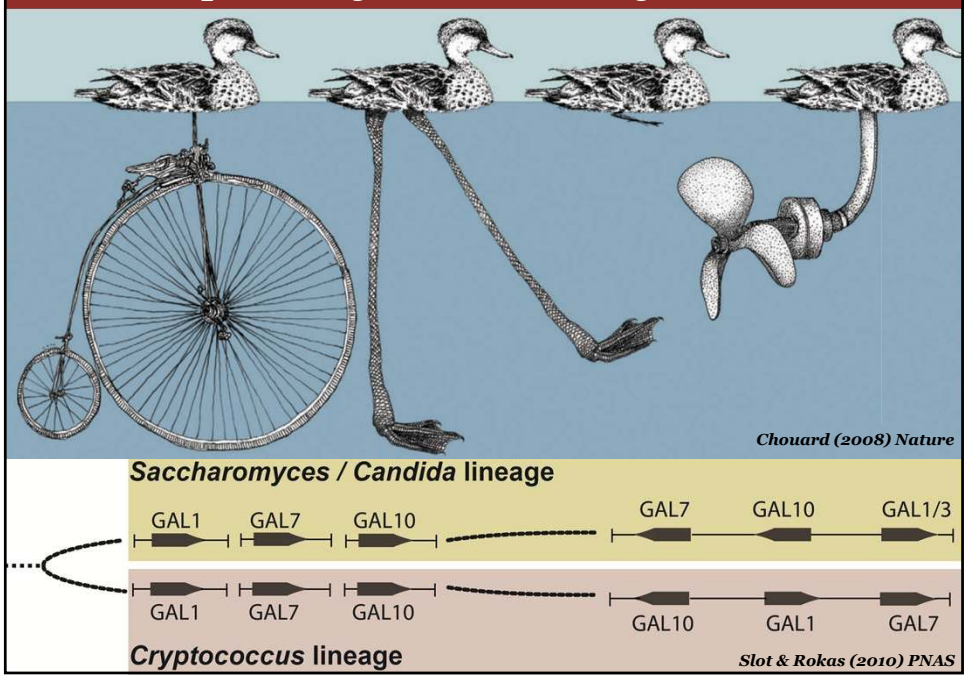


Slot & Rokas (2010) PNAS

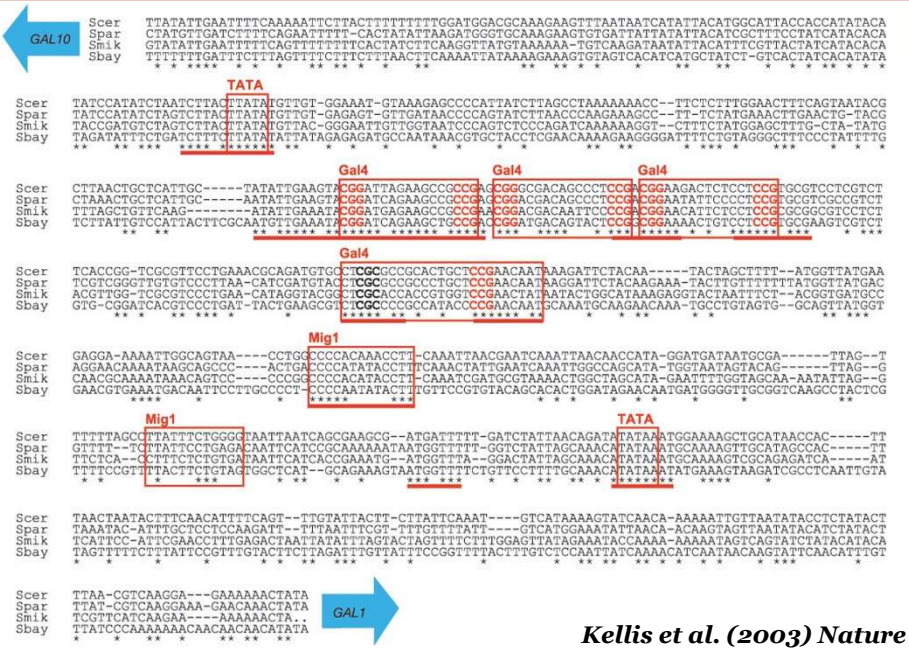
The Saccharomyces & Cryptococcus *Clusters Evolved Independently by Native Gene Relocation*



Independently Evolved (Analogous) Traits

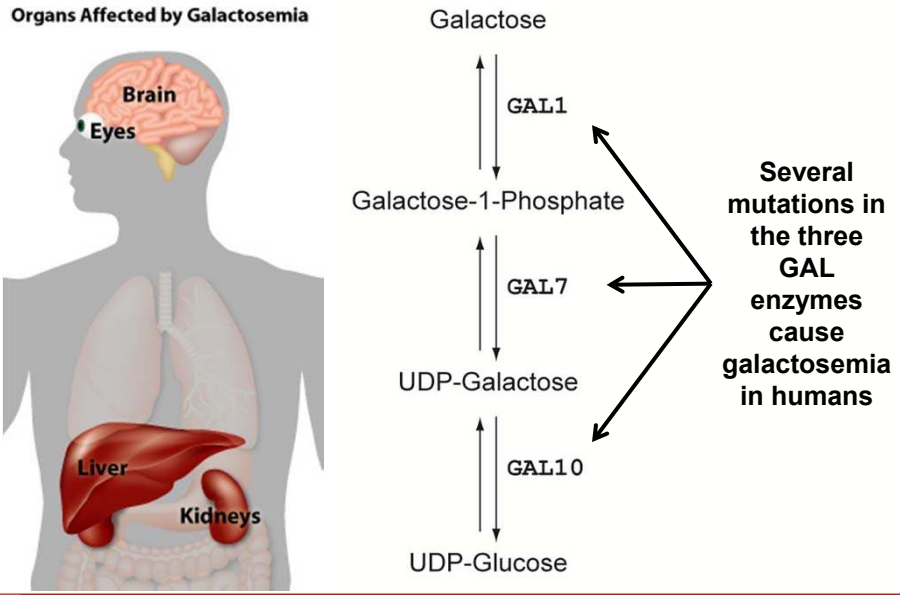


Why Cluster? Coordinating Pathway Regulation



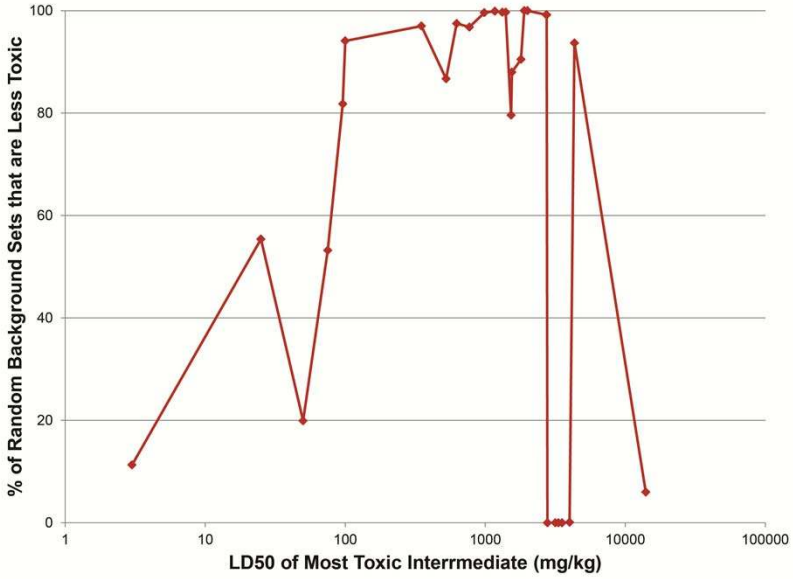
Kellis et al. (2003) Nature

Why Cluster? Minimizing Impact of Toxic Intermediates



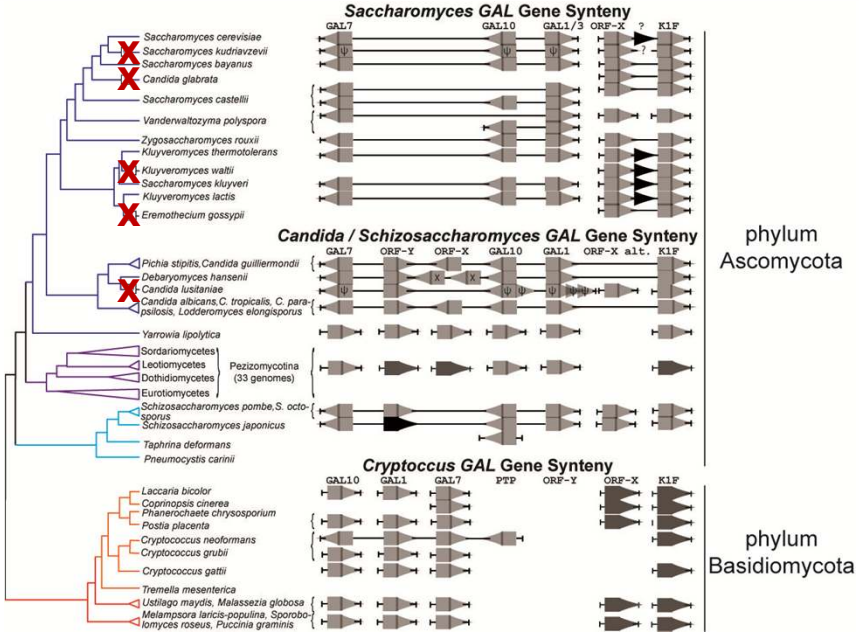
Fridovich-Keil (2006) J. Cell. Physiol.

Intermediates in Clustered Pathways Are More Toxic

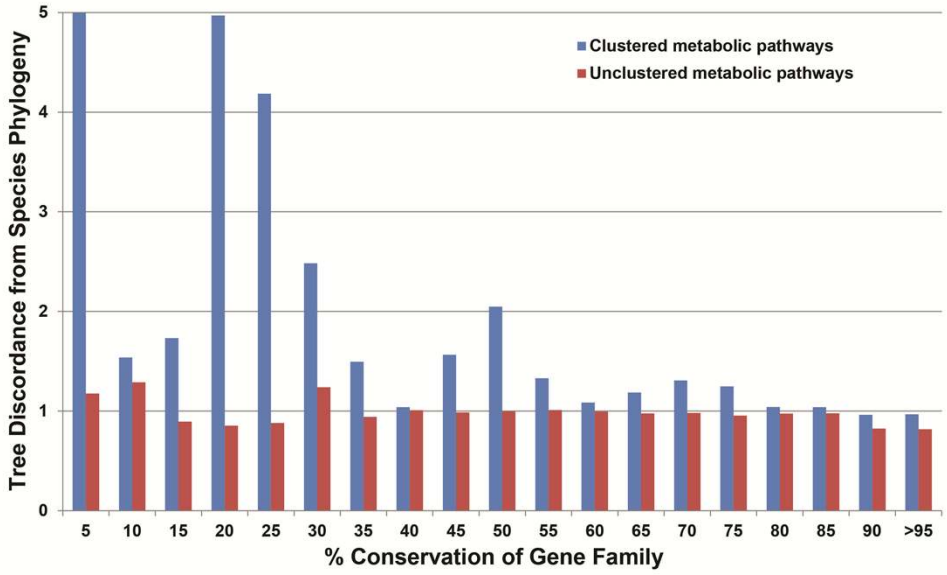


Kris McGary & Jason Slot

Why Cluster? Clustered Pathways Are Easier Lost

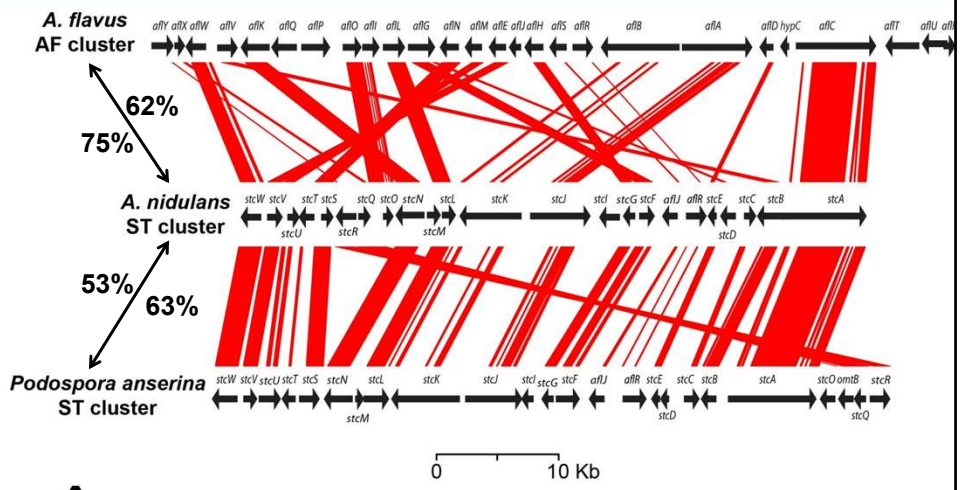


Clusters are Subject to Higher Rates of Both HGT & Loss



Jason Slot

The Aflatoxin & Sterigmatocystin Gene Clusters

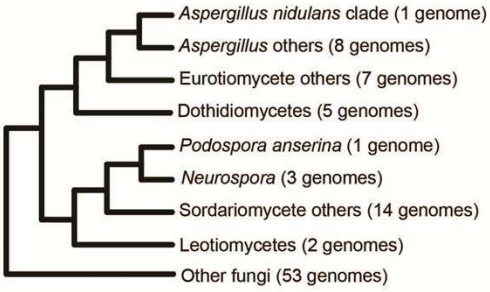


In the United States, agricultural economic losses due to aflatoxin contamination amount to $270 million per year

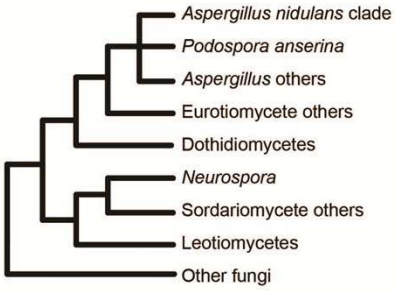
Slot & Rokas (2011) Curr. Biol.

Did P. anserina *Acquire the* ST *Cluster via HGT?*

Gene tree expected from species phylogeny



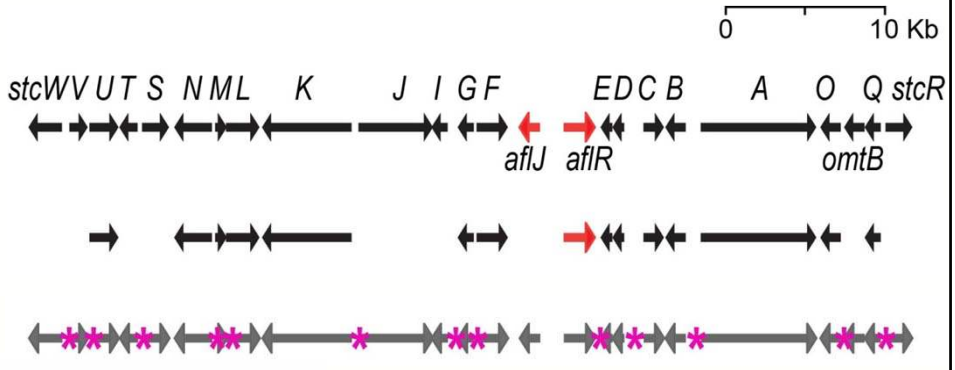
Gene tree expected from HGT

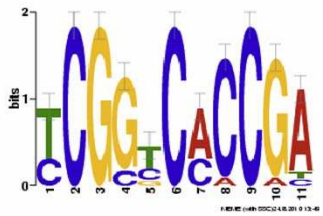


- **All gene phylogenies are consistent with the hypothesis that *P. anserina* acquired its ST cluster via HGT from *Aspergillus***
- **9 / 23 gene phylogenies rejected *Aspergillus* monophyly (the remaining 14 genes were uninformative)**

Slot & Rokas (2011) Curr. Biol.

Is the P. anserina ST *Cluster Functional?*





Expression for 14/24 genes in EST data set

Best hit in *de novo* search is the AflR-binding motif

Slot & Rokas (2011) Curr. Biol.

Parasitol Res
DOI 10.1007/s00436-010-2098-1

ORIGINAL PAPER

Larvicidal activity of metabolites from the endophytic *Podospora* sp. against the malaria vector *Anopheles gambiae*

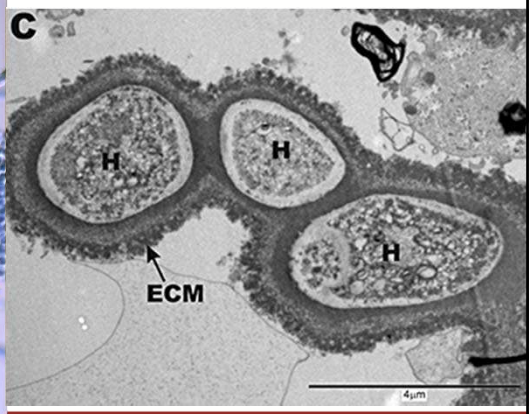
Josphat C. Matasyoh · Birger Dittrich
Anja Schueffler · Hartmut Laatsch

Abstract In a screening for natural products with mosquito larvicidal activities, the endophytic fungus *Podospora* sp. isolated from the plant *Laggera alata* (Asteraceae) was conspicuous. Two xanthones, sterigmatocystin (**1**) and secosterigmatocystin (**2**), and an anthraquinone derivative (**3**) 13-hydroxyversicolorin B were isolated after fermentation on M_2 medium. These compounds were characterised using spectroscopic and X-ray analysis and examined against third instar larvae of *Anopheles gambiae*. The results demonstrated that compound **1** was the most potent one with LC_{50} and LC_{90} values of 13.3 and 73.5 ppm, respectively. Over 95% mortality was observed at a concentration 100 ppm after 24 h. These results compared favourably with the commercial larvicide pylarvex® that showed 100% mortality at the same concentration. Com-

Aspergillus fumigatus, *the Deadliest Fungal Pathogen*

90% invasive aspergillosis, >50% mortality rate, multidrug resistant

***A. fumigatus* biofilms confer increased drug resistance**



Loussert et al. (2010) Cell. Microbiol.
Beauvais et al. (2007) Cell. Microbiol.

Which Genes are Differentially Expressed During Biofilm Growth?

A. fumigatus
ATCC46645

Liquid growth

- Shaken
- Liquid BRIAN media
- 37^{0}C
- 16 hours

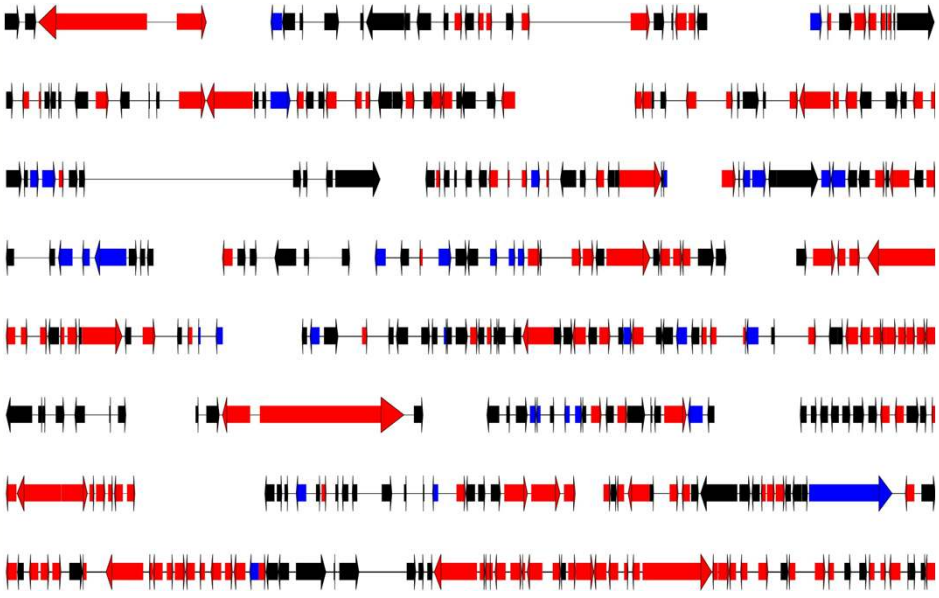
Biofilm growth

- Solid BRIAN agar
- 37^{0}C
- 16 hours

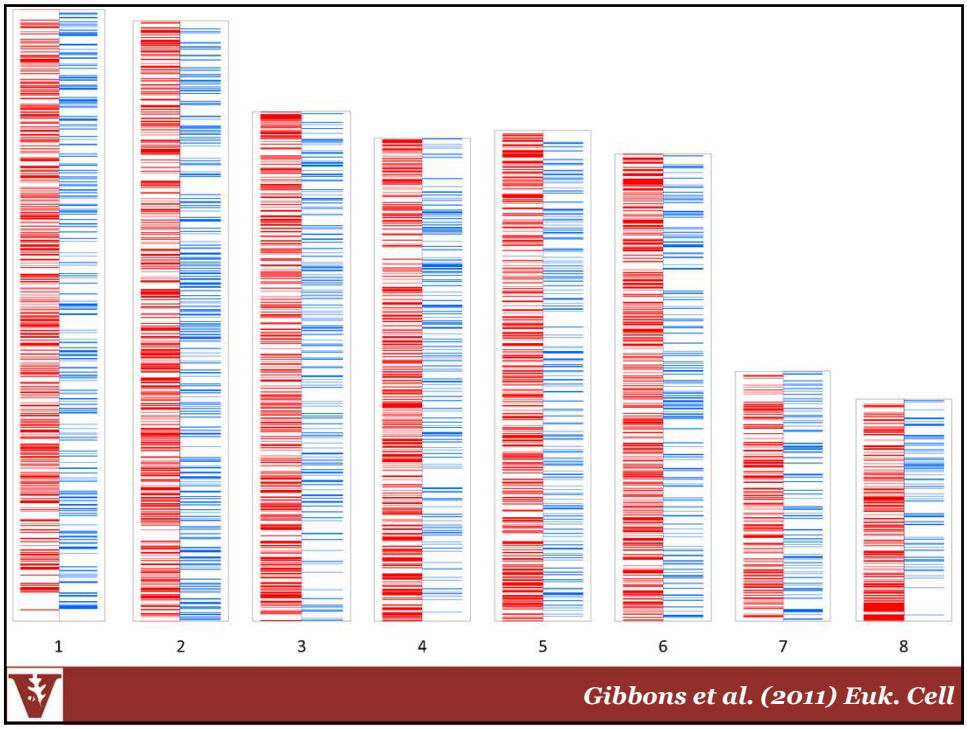
RNA-Seq using Illumina next-generation sequencing technology

John Gibbons & Latgé lab, Institut Pasteur

Up-regulated Sec. Met. Genes are Overrepresented in Biofilm

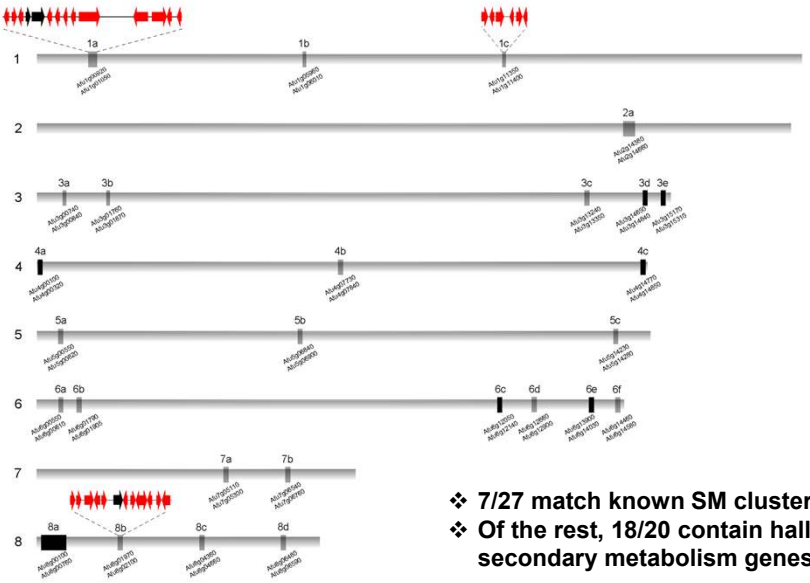


43% of genes up-regulated, 9% down-regulated (p-value< 10^{-17})



Gibbons et al. (2011) Euk. Cell

27 Up-regulated Gene Clusters in Biofilm



- 7/27 match known SM clusters
- Of the rest, 18/20 contain hallmark secondary metabolism genes

Gibbons et al. (2011) Euk. Cell

The Birth, Life and Death of Metabolic Gene Clusters

Birth

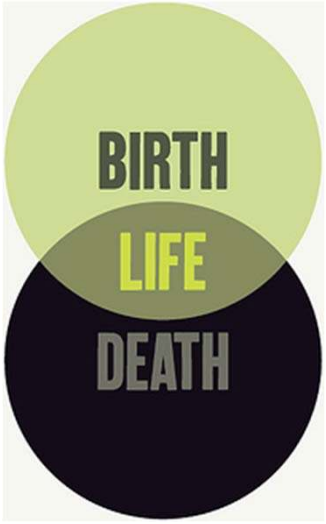
- Clusters originate by native gene relocation and by HGT from pre-existing clusters

Life

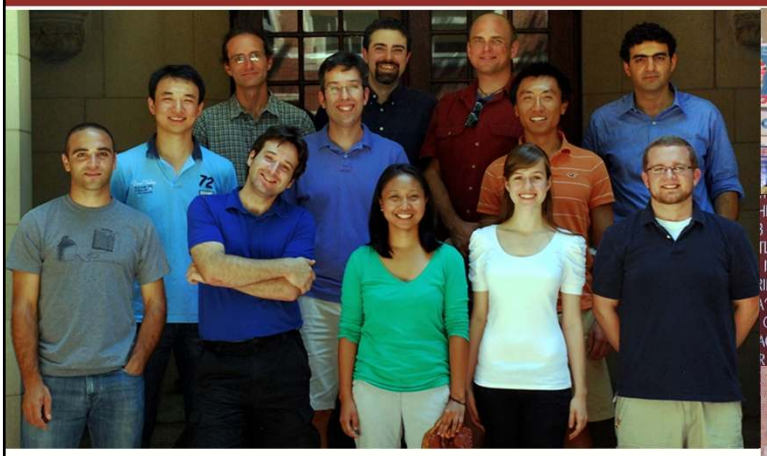
- Clustering ameliorates toxicity and allows coordination of regulation
- Clustering makes HGT easier
- Clustering facilitates fungal adaptation to diverse and changing nutritional environments, including the human one

Death

- Clustered pathways experience more frequent loss



Acknowledgements



SEARLE SCHOLARS PROGRAM

National Science Foundation
WHERE DISCOVERIES BEGIN

National Institutes of Health
The Nation's Medical Research Agency

http://as.vanderbilt.edu/rokaslab

Literature

1. Rokas & Abbot (2009) Harnessing genomics for evolutionary insights. *TREE 24:192-200.*
2. Hittinger et al. (2010) Leveraging skewed transcript abundance by RNA-Seq to increase the genomic depth of the tree of life. *PNAS107: 1476-1481.*
3. Hittinger et al. (2010) Remarkably ancient balanced polymorphisms in a multi-locus gene network. *Nature 464: 54-58.*
4. Slot & Rokas A (2010) Multiple *GAL* pathway gene clusters evolved independently and by different mechanisms in fungi. *PNAS 107: 10136-10141.*
5. Slot & Rokas (2011) Horizontal transfer of a large and highly toxic secondary metabolic gene cluster between fungi. *Curr. Biol. 21: 134-139.*
6. Gibbons et al. (2012) Global transcriptome changes underlying colony growth in the opportunistic human pathogen *Aspergillus fumigatus*. *Eukaryotic Cell: in press*.