searching for functional divergence in genomes and metagenomes

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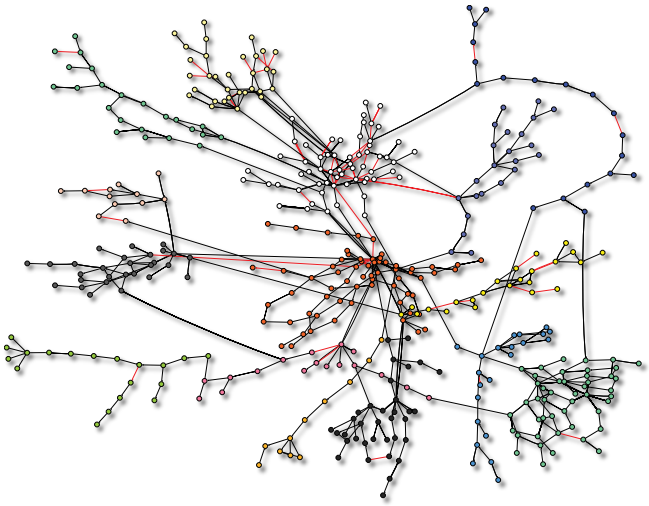
morphology

introduction

metabolic networks



introduction

molecules

gene sequences

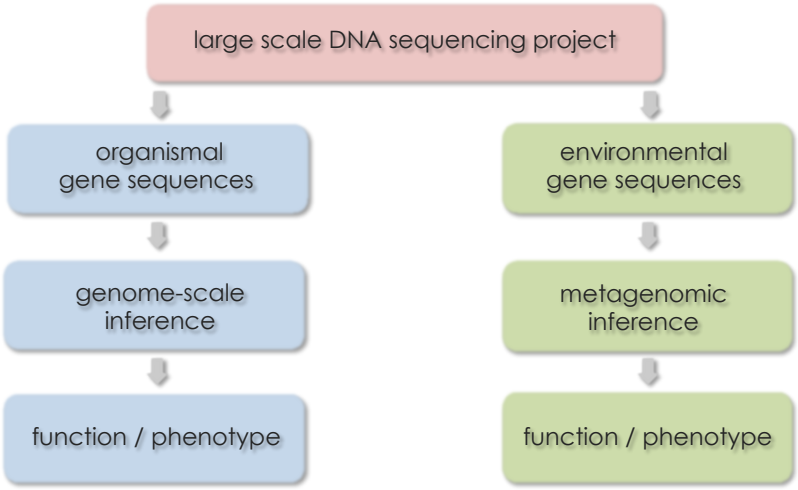
```
human
cow
rabbit
rat
opossum

GTG CTG TCT CCT GCC GAC AAG ACC AAC GTC AAG GCC GCC TGG GGC AAG GTT GGC GCG CAC
... ... ... G.C ... ... ... T.. ..T ... ... ... ... ... ... ... ... ... .GC A..
... ... ... ..C ..T ... ... ... ... A.. ... A.T ... ... .AA ... A.C ... AGC ...
... ..C ... G.A .AT ... ..A ... ... A.. ... AA. TG. ... ..G ... A.. ..T .GC ..T
... ..C ..G GA. ..T ... ... ..T C.. ..G ..A ... AT. ... ..T ... ..G ..A .GC ...

GCT GGC GAG TAT GGT GCG GAG GCC CTG GAG AGG ATG TTC CTG TCC TTC CCC ACC ACC AAG
... ..A .CT ... ..C ..A ... ..T ... ... ... ... ... ... AG. ... ... ... ... ...
.G. ... ... ... ..C ..C ... ... G.. ... ... ... ... T.. GG. ... ... ... ... ...
.G. ..T ..A ... ..C .A. ... ... ..A C.. ... ... ... GCT G.. ... ... ... ... ...
..C ..T .CC ..C .CA ..T ..A ..T ..T .CC ..A .CC ... ..C ... ... ... ..T ... ..A

ACC TAC TTC CCG CAC TTC GAC CTG AGC CAC GGC TCT GCC CAG GTT AAG GGC CAC GGC AAG
... ... ... ..C ... ... ... ... ... ... ... ..G ... ... ..C ... ... ... ... G..
... ... ... ..C ... ... ... T.C .C. ... ... ... .AG ... A.C ..A .C. ... ... ...
... ... ... T.T ... A.T ..T G.A ... .C. ... ... ... ... ..C ... .CT ... ... ...
..T ... ... ..C ... ... ... ... TC. .C. ... ..C ... ... A.C C.. ..T ..T ..T ...
```


gene sequences

1. How do we efficiently extract information from large and complex datasets?

2. How to identify information (signals) most relevant to function?

gene sequences

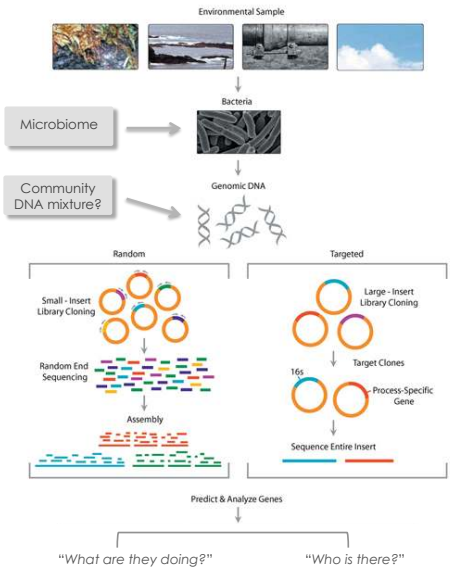


(part 2 of this talk)

(part 1 of this talk)

part 1: Searching for functional divergence metagenomics

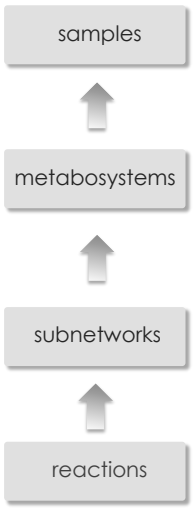
metagenomics

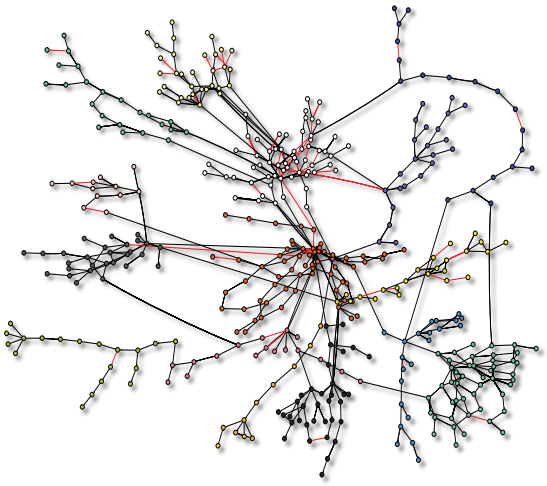


Adapted from: http://legacy.camera.calit2.net/education/what-is-metagenomics

network model

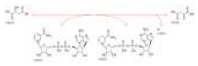
statistical modeling metabolism as a hierarchical mixture





network model

metabolic reactions



samples

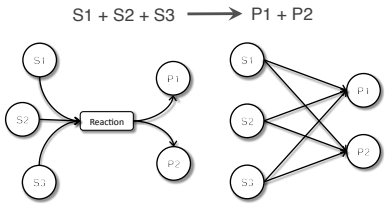
metabosystems

subnetworks

reactions

metagenomics:

- sequence ➔ E.C. ➔ reaction ➔ substrate-product pairs
- intermediates are both substrate & product
- sequence reads ➔ weighted abundance



network model

metabolic subsystems

samples

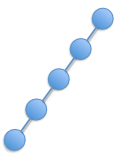
metabosystems

subnetworks

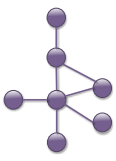
reactions

subnetworks = subsets of reactions (S & P pairs)

- conditional dependence of reactions
- similar relative abundance (weights)
- could be functionally related or a pathway



subsytem 1



subsytem 2

network model

subnetworks: mixtures of reactions

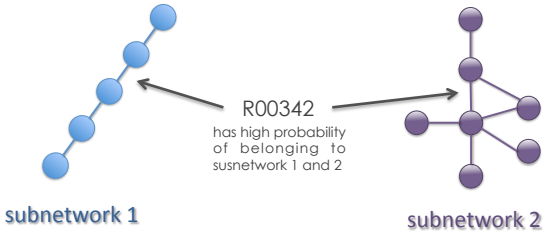
samples

metabosystems

subnetworks

reactions

- reaction membership: ≥ one subnetwork
- reactions have mixing (posterior) probabilities for different subnetworks
- "**soft**" boundaries



network model

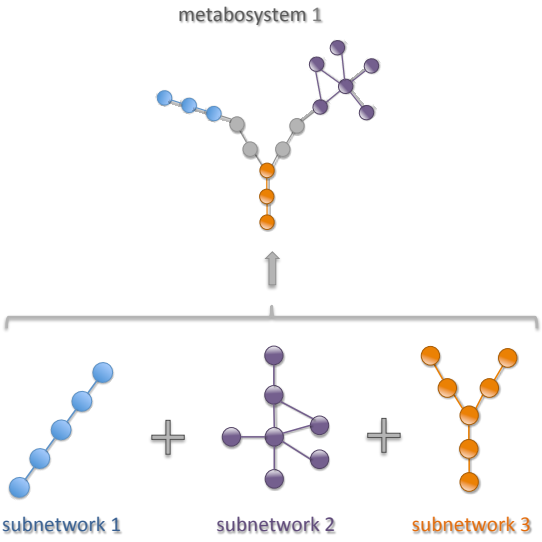
metabosystems

samples

metabosystems

subnetworks

reactions



network model

metabosystems: mixtures of subnetworks

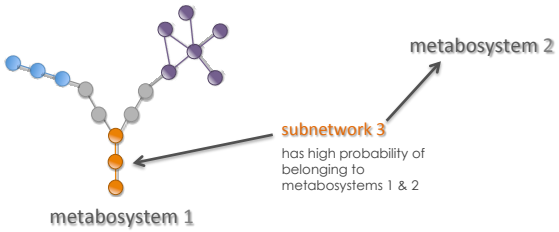
samples

metabosystems

subnetworks

reactions

- subnetwork membership: ≥ one metabosystem
- subnetworks have mixing probabilities
- "**soft**" boundaries



network model

microbiome samples: mixtures of metabosystems

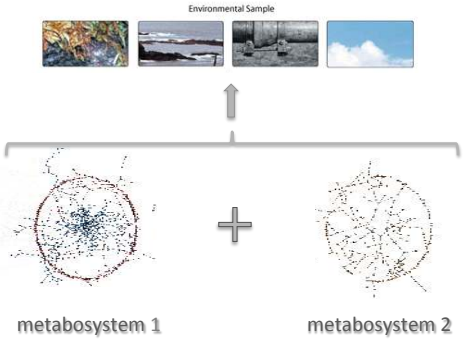
samples

metabosystems

subnetworks

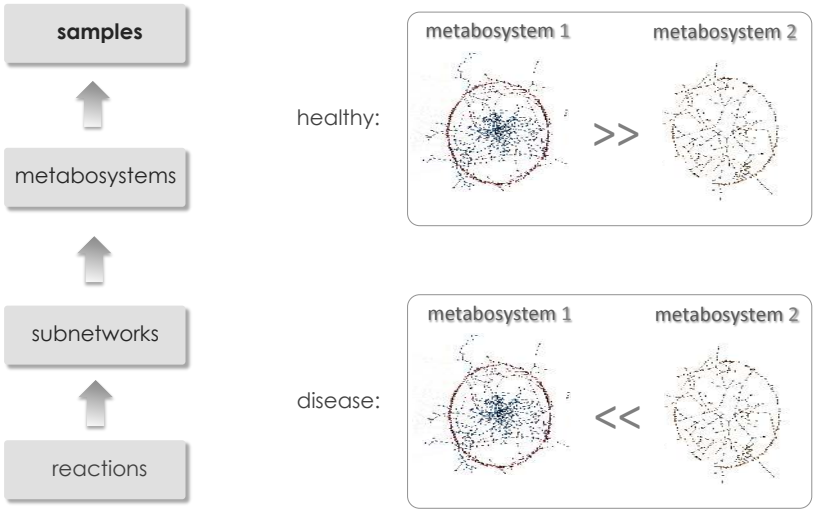
reactions

- metabosystem membership: ≥ one sample
- metabossystems have mixing probabilities



network model

microbiome samples: mixtures of metabosystems

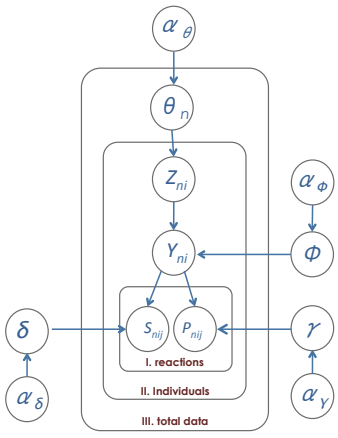


network model

analysis goals

1. Learn composition of metabosystems within samples

2. Discover discriminatory and core subnetworks



hypothetical mixture for ***K* = 3**

Θ_1 = (0.2, <0.01, 0.8)

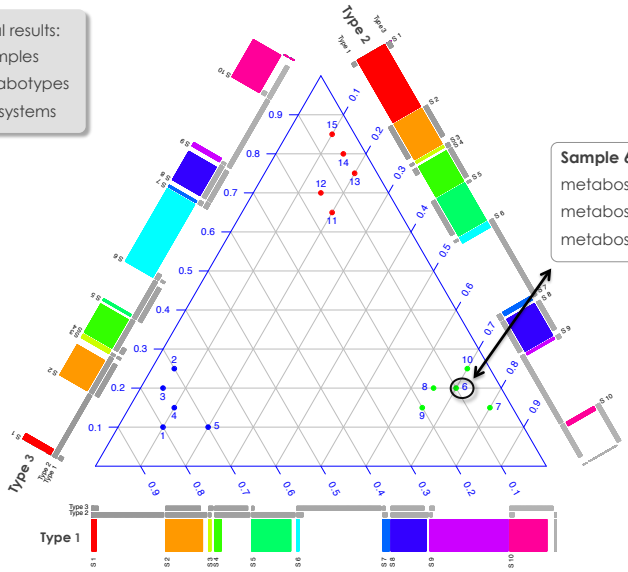
hypothetical mixture for ***L* = 10**

Φ_3 = (<0.01, <0.01, 0.2, <0.01, <0.01, 0.1, <0.01, <0.01, 0.7, <0.01)

network model

interpreting output

Hypothetical results:
N = 15 samples
K = 3 metabotypes
L = 10 subsystems



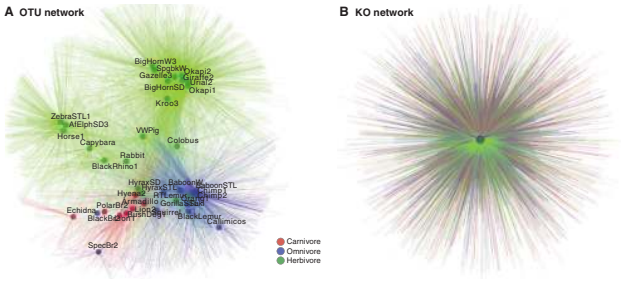
Sample 6:
metabosystem 1: 0.1
metabosystem 2: 0.7
metabosystem 3: 0.2

dataset 1

mammalian gut microbiomes

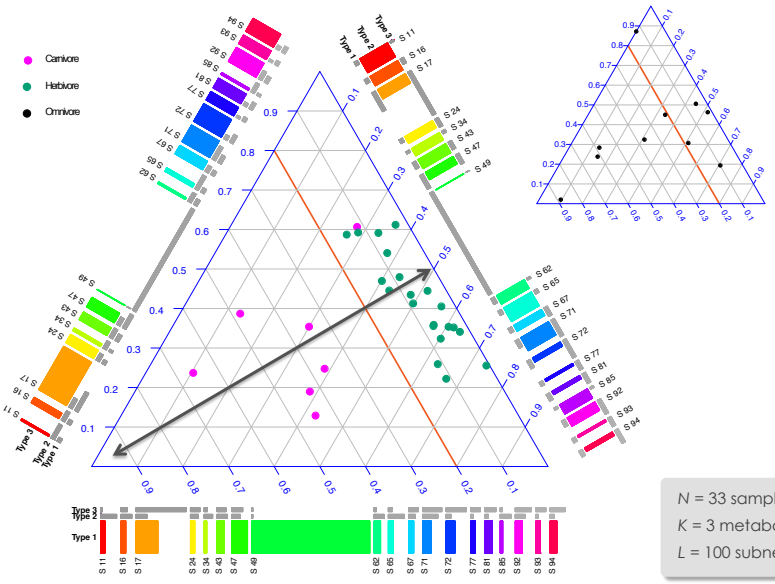
Muegge et al. (2011)

- *fecal samples: 33 mammals*
- *carnivore, herbivores, omnivores* (*K* = 3)
- *we obtained: 2,824 reactions*
  - *abundance: 11,744 to 161,626 counts / sample*



dataset 1

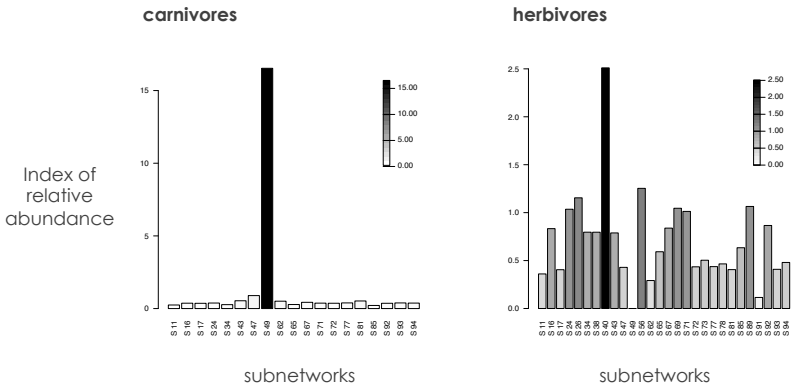
carnivores: high membership of metabosystem 1



N = 33 samples
K = 3 metabosystems
L = 100 subnetworkss

dataset 1

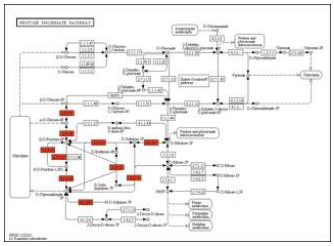
discriminatory subnetworks

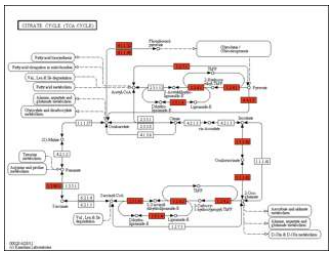


dataset 1

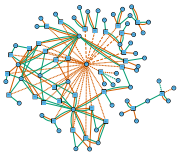
mammalian subnetworks

Core: correspond KEGG pathways

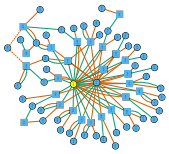




Discriminatory: do not correspond to KEGG pathways



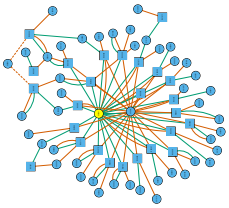
herbivore: S40



carnivore: S49

dataset 1

discriminatory for carnivores



subnetwork 49

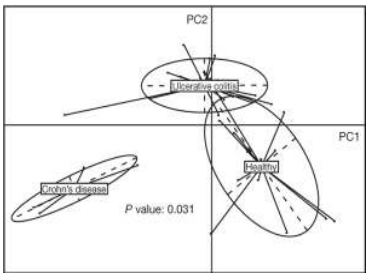
- importation of certain extracellular saccharides
  - N-acetylmuramic acid
  - N-acetylglucosamine
  - fucose
  - glucose
  - mannose
- good for exploiting alternate carbohydrate sources
  - bacterial cell walls: N-acetylmuramic acid and N-acetylglucosamine
  - hosts secretions: fucosylated mucins

dataset 2

Human gut microbiomes

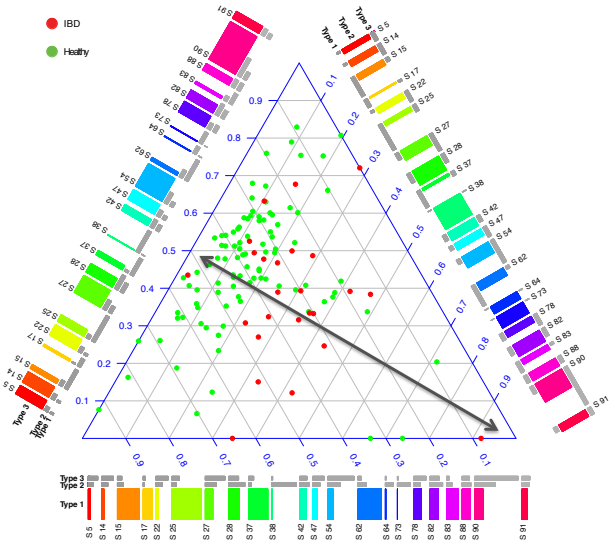
Qin et al. (2010)

- *124 adult humans*
  - *576.7 Gb of sequence data (4.5 Gb/sample)*
- *healthy, Crohn's, ulcerative colitis (K = 3)*
- *we obtained: 3,433 reactions*
  - *abundance: 5,968 to 1,270,409 counts / sample*



dataset 2

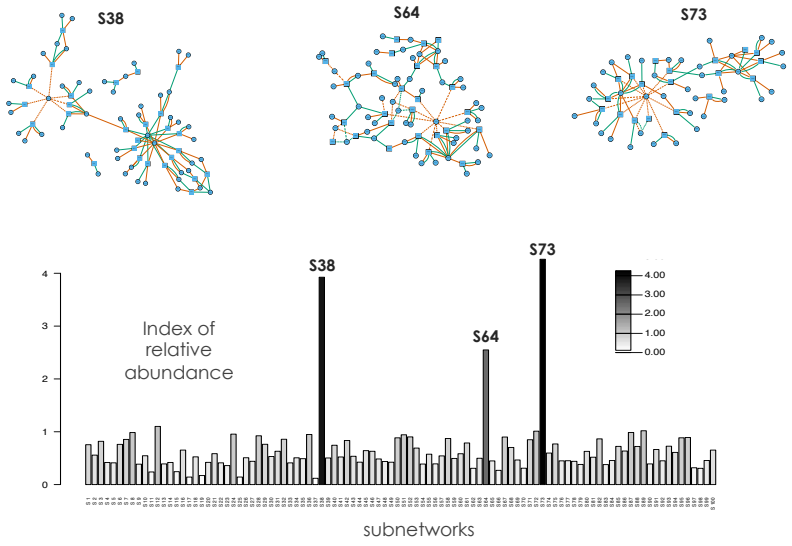
Human gut microbiomes



N = 124 samples
K = 3 metabosystems
L = 100 subnetworks

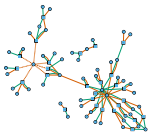
dataset 2

discriminatory subnetworks for IBD



dataset 2

discriminatory for IBD

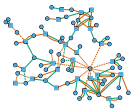


subnetwork 38

- Enriched for *some* PTS genes
  - suggests greater dependence on host-derived glycans for energy
    - mucin
    - shed-epithelial cells
  - suggests resistance to dietary changes
  - suggests close proximity to host cells
    - metabotype 2: greater impact on state of intestinal health
- no apparent connection to cause of gut inflammation of IBD
- other studies find enrichment of PTS genes in IBD patients

dataset 2

discriminatory for IBD



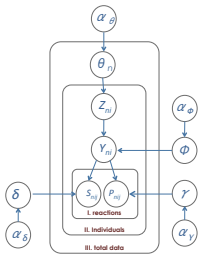
subnetwork 64

- enriched in ascorbate metabolism reactions
- metabolic profiling: IBD patients deficient in ascorbate
- ascorbate is an antioxidant
  - chronic IBD pathology related to excess reactive compounds
    - leads to oxidative stress within mucosa
    - IBD is associated with antioxidant levels and oxidative stress markers
  - absorption in gut depends on local gradient
  - metabotype 2 could interfere with human absorption

network model

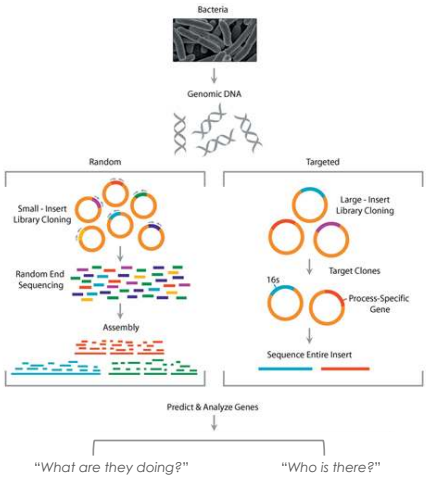
investigating community metabolic function

- any environment (soil, ocean, biofilm reactors, ...) or scale (distance, time, …)
- "soft" pathways, systems & metabosystems
- metatranscriptomes of un-cultivatable microbes
- "supervision" should improve power
- number of reactions >> number of samples
- depends on data quality & processing



metagenomic inference

joint use of both sources of info …



Adapted from: http://legacy.camera.calit2.net/education/what-is-metagenomics

let's take a break: ~20 mins?

part 2: searching for functional divergence in genes and genomes

overview

1. introduction (to ω ratio and codon models)
2. selected codon model
3. model based inference
4. gene-level example
5. genome-scale analyses

index of natural selection pressure: ω ratio

	U	C	A	G	
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

http://www.langara.bc.ca/biology/mario/Assets/Geneticode.jpg

Kimura (1968)

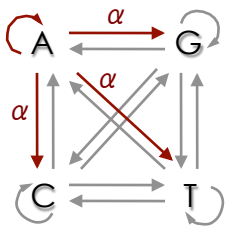
- d_S: number of synonymous substitutions per synonymous site (K_S)
- d_N: number of nonsynonymous substitutions per nonsynonymous site (K_A)
- ω: the ratio d_N/d_S; it measures selection at the protein level

index of natural selection pressure: ω ratio

rate ratio	mode	example
$\omega < 1$	**purifying (negative) selection**	**histones**
$\omega = 1$	**Neutral Evolution**	**pseudogenes**
$\omega > 1$	**Diversifying (positive) selection**	**MHC, Lysin**

a model of sequence evolution: DNA

DNA state space



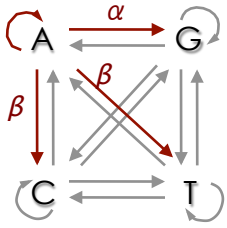
DNA transition matrix

$$\text{from:}\begin{matrix}\\ A\\ C\\ G\\ T\end{matrix}\begin{matrix}\text{to:}\\ \begin{matrix}A & C & G & T\end{matrix}\end{matrix}\begin{pmatrix} -3\alpha & \alpha & \alpha & \alpha \\ \alpha & -3\alpha & \alpha & \alpha \\ \alpha & \alpha & -3\alpha & \alpha \\ \alpha & \alpha & \alpha & -3\alpha \end{pmatrix}$$

* This is equivalent to most simple model for DNA (Jukes and Cantor, 1969).

a model of sequence evolution: DNA

DNA state space



DNA transition matrix

$$\text{from:}\begin{matrix}\\ A\\ C\\ G\\ T\end{matrix}\begin{matrix}\text{to:}\\ \begin{matrix}A & C & G & T\end{matrix}\end{matrix}\begin{pmatrix} * & \beta & \alpha & \beta \\ \beta & * & \beta & \alpha \\ \alpha & \beta & * & \beta \\ \beta & \alpha & \beta & * \end{pmatrix}$$

the CODON matrix is huge

universal code: 61 sense codons

	U		C		A		G	
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys
	UUC	Phe	UCC	Ser	UAC	Tyr	UGC	Cys
	UUA	Leu	UCA	Ser	UAA	Stop	UGA	Stop
	UUG	Leu	UCG	Ser	UAG	Stop	UGG	Trp
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg
	CUC	Leu	CCC	Pro	CAC	His	CGC	Arg
	CUA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
	CUG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser
	AUC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
	AUA	Ile	ACA	Thr	AAA	Lys	AGA	Arg
	AUG	Met	ACG	Thr	AAG	Lys	AGG	Arg
G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly
	GUC	Val	GCC	Ala	GAC	Asp	GGC	Gly
	GUA	Val	GCA	Ala	GAA	Glu	GGA	Gly
	GUG	Val	GCG	Ala	GAG	Glu	GGG	Gly

http://www.langara.bc.ca/biology/mario/Assets/Geneticode.jpg

codon matrix:

$61 \times 61 = 3{,}721$ matrix elements

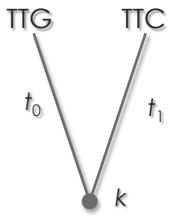
a model of sequence evolution: CODON

From codon below:	TTT (Phe)	TTC (Phe)	TTA (Leu)	TTG (Leu)	CTT (Leu)	CTC (Leu)	⋯▸	GGG (Gly)
	to codon below:							
TTT (Phe)	—	$\kappa\pi_{TTC}$	$\omega\pi_{TTA}$	$\omega\pi_{TTG}$	$\omega\kappa\pi_{TTT}$	0	⋯▸	0
TTC (Phe)	$\kappa\pi_{TTT}$	—	$\omega\pi_{TTA}$	$\omega\pi_{TTG}$	0	$\omega\kappa\pi_{CTC}$	⋯▸	0
TTA (Leu)	$\omega\pi_{TTT}$	$\omega\pi_{TTC}$	—		0	0	⋯▸	0
TTG (Leu)	$\omega\pi_{TTT}$	$\omega\pi_{TTC}$	$\kappa\pi_{TTA}$	—	0	0	⋯▸	0
CTT (Leu)	$\omega\kappa\pi_{TTT}$	0	0	0	—	$\kappa\pi_{CTC}$	⋯▸	0
CTC (Leu)	0	$\omega\kappa\pi_{TTC}$	0	0	$\kappa\pi_{TTT}$	—	⋯▸	0
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋱	
GGG (Gly)	0	0	0	0	0	0	0	—

* This is equivalent to the codon model of Goldman and Yang (1994). Parameter $\boldsymbol{\omega}$ is the ratio d_N/d_S, $\boldsymbol{\kappa}$ is the transition/transversion rate ratio, and $\boldsymbol{\pi_i}$ is the equilibrium frequency of the target codon (i).

$$P(t) = \{p_{ij}(t)\} = e^{Qt}$$

compute the probability of codons



$$L_h(TTG,TTC) = \sum_k \pi_k p_{kTTG}(t_0)\, p_{kTTC}(t_1)$$

Note: analysis is typically done by using an unrooted tree

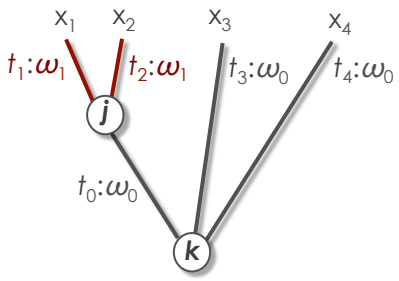
codon models

there are MANY different models

we will look at **three types of models**:

1. selection varies over time
2. selection varies among sites
3. selection varies among sites AND over time

1. branch models: ω varies over time

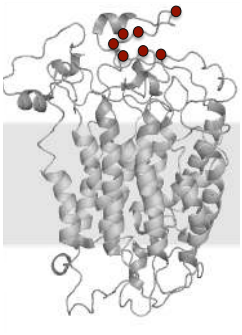


codon models

2. site models: ω varies among sites

Positive selection sites here

ω_3



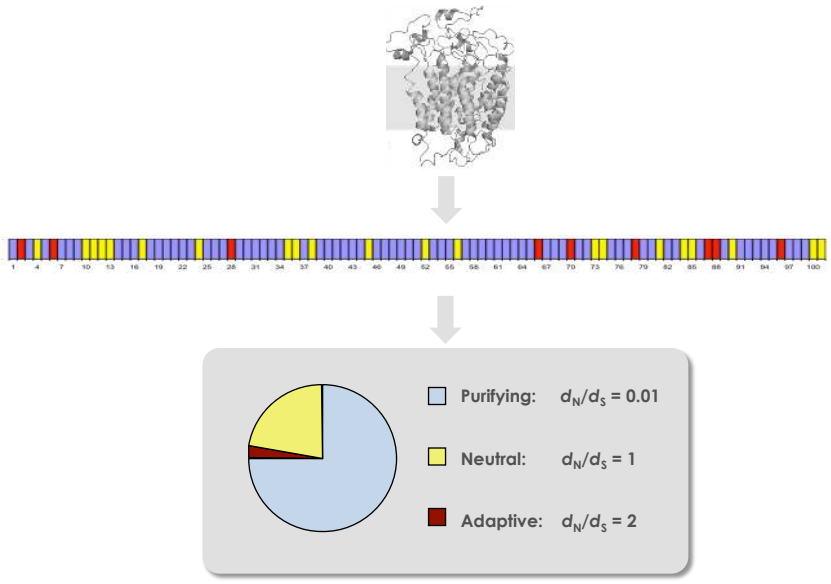
Loop structures extend into extra-cellular space: **Hydrophilic amino acids here** — ω_0

Cell membrane in grey; helix structures span the membrane: **Hydrophobic amino acids here** — ω_1

Loop structures extend into cytoplasm: **Hydrophilic amino acids here** — ω_0

codon models

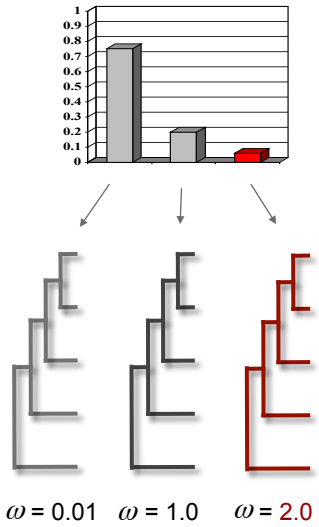
2. site models: ω varies among sites



codon models

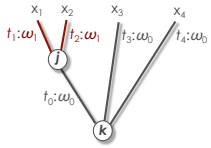
2. site models: ω varies among sites

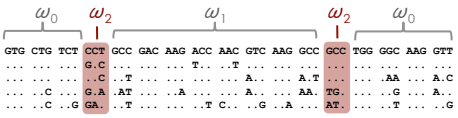
$$P(\mathbf{x}_h) = \sum_{i=0}^{K-1} p_i P(\mathbf{x}_h \mid \omega_i)$$



codon models

we made some progress ….





1. branch models
(ω varies among branches)

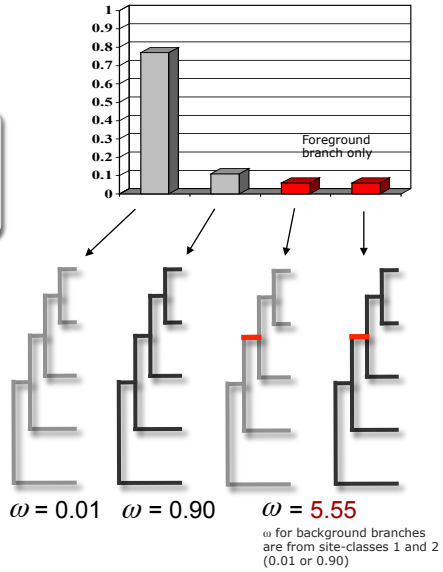
2. site models
(ω varies among sites)

3. branch-site models
(combines the features of above models)

codon models

3. branch-site model: ω varies among sites & branches

$$P(\mathbf{x}_h) = \sum_{i=0}^{K-1} p_i P(\mathbf{x}_h \mid \omega_i)$$



model based inference

analytical tasks

Task 1. parameter estimation (e.g., ω)

Task 2. hypothesis testing

Task 3. make predictions (e.g., sites having $\omega > 1$)

- we let the data tell us what the value of a parameter (e.g., ω) should be

- the optimal value for a parameter is the value that maximizes the probability of observing the data

Task 1

maximize likelihood and probability are inverted

Probabilities		Data	
		Data-1: 1H & 1T	Data-2: 2H
Hypotheses	H_1: P(H) = 1/4	0.375	0.0625
	H_2: P(H) = 1/2	0.5	0.25

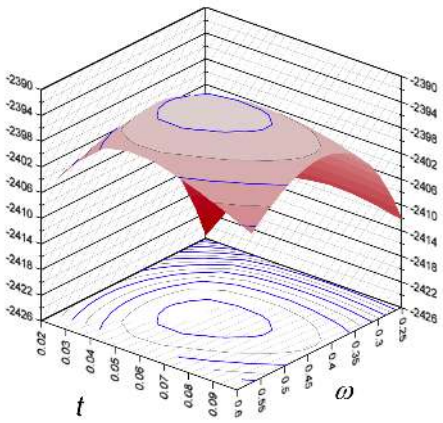
$$P(H \mid D) = \alpha P(D \mid H)$$

proportionality constant

Likelihoods		Data	
		Data-1: 1H & 1T	Data-2: 2H
Hypotheses	H_1: P(H) = 1/4	$\alpha_1 \times 0.375$	$\alpha_2 \times 0.0625$
	H_2: P(H) = 1/2	$\alpha_1 \times 0.5$	$\alpha_2 \times 0.25$

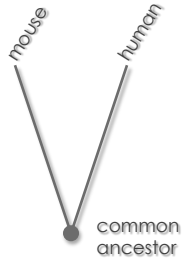
Task 1

maximum likelihood



Parameters: t and ω

Gene: acetylcholine α receptor



lnL = -2399

Task 2

How do we know that the estimate is significant?

Task 1. parameter estimation (e.g., ω) ✔

Task 2. hypothesis testing ⬅ **LRT**

Task 3. prediction / site identification

Task 2

testing nested hypotheses by using the LRT

ℓ_0 is the maximum log likelihood under $\mathbf{H_0}$ with parameters $\boldsymbol{\theta_0}$

and

ℓ_A is the maximum log likelihood under $\mathbf{H_A}$ with parameters $\boldsymbol{\theta_A}$

Test statistic $= 2\Delta\ell = 2(\ell_0(\theta_0) - \ell_A(\theta_A))$

Degrees of freedom = difference in the number of parameters between the two models

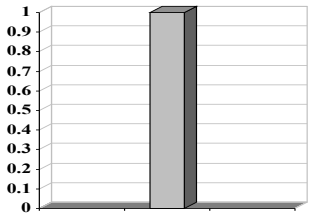
Task 2

LRT: Does selection pressure vary among sites?

H_0: uniform selective pressure among sites (M0)
H_A: variable selective pressure among sites (M3)

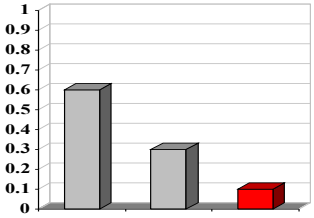
Compare $\mathbf{2\Delta l} = 2(l_1 - l_0)$ with a χ^2 distribution

H_0: Model 0



$\hat{\omega}$ = 0.65

H_A: Model 3



$\hat{\omega}$ = 0.01 $\hat{\omega}$ = 0.90 $\hat{\omega}$ = 5.55

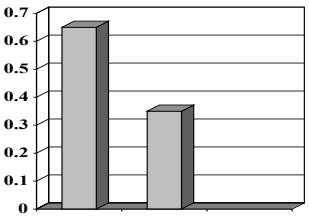
Task 2

LRT: Have some sites evolved under positive selection?

H_0: variable selective pressure but NO positive selection (M1)
H_A: variable selective pressure with positive selection (M2)

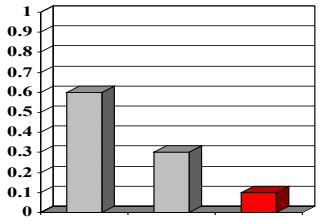
Compare $\mathbf{2\Delta l} = 2(l_1 - l_A)$ with a χ^2 distribution

H_0: Model 1a



$\hat{\omega}$ = 0.5 (ω = 1)

H_A: Model 2a



$\hat{\omega}$ = 0.5 (ω = 1) $\hat{\omega}$ = 3.25

Task 3

How do we identify the selected sites?

Task 1. parameter estimation (e.g., ω) ✔

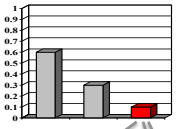
Task 2. hypothesis testing ✔

Task 3. prediction / site identification ← **Bayes' rule**

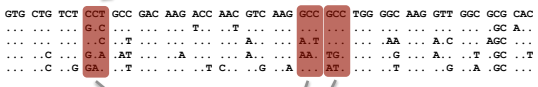
Task 3

Which sites have $\omega > 1$?

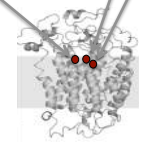
model:
5% have $\omega > 1$



Bayes' rule:
site 4, 12 & 13



structure:
sites are in contact



Task 3

Bayes' rule for identifying selected sites

site class 0: ω_0 = .03, 85% of codon sites

site class 1: ω_1 = .40, 10% of codon sites

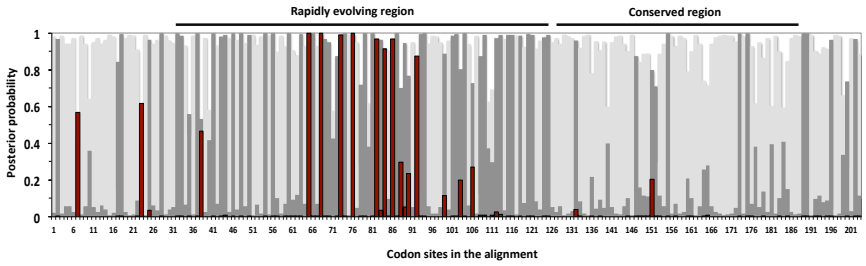
? → **site class 2: ω_2 = 14, 05% of codon sites**

Bayes' rule:

$$P(\omega_2 \mid \mathbf{x}_h) = \frac{p_2 P(x_h \mid \omega_2)}{P(x_h)}$$

Task 3

Bayes' rule for identifying selected sites



Site class 0: ω_0 = .03 (strong purifying selection)

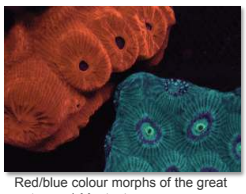
Site class 1: ω_1 = .40 (weak purifying selection)

Site class 2: ω_2 = 5 (positive selection)

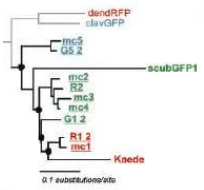
NOTE: The posterior probability should NOT be interpreted as a "*P*-value"; it can be interpreted as a measure of relative support

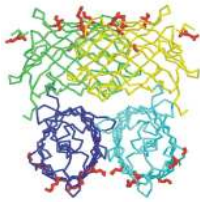
gene-level example

colour diversity of coral pigments



Red/blue colour morphs of the great star coal *Montastraea cavernosa*

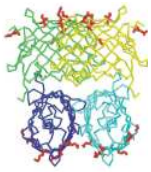




- Is color diversity tuned by natural selection?
- Is there a relationship between colour and endosymbiotic algae?

gene-level example

green fluorescent proteins (GFPs) in corals

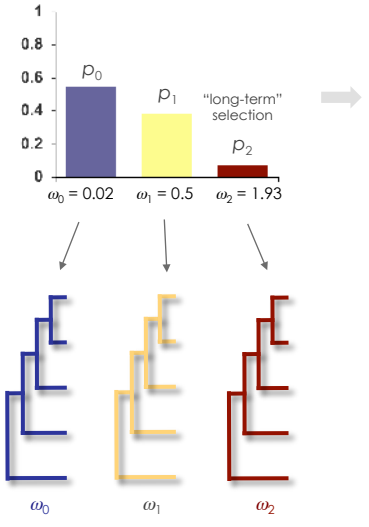


questions we have:

1. What is the intensity of selection on coral GFPs
2. Have there been episodes of positive selection during the evolution of colour diversity?
3. Are some sites in GFPs positively selected?
4. Which sites?
5. What happens to the colour when the amino acid is changes at these sites?

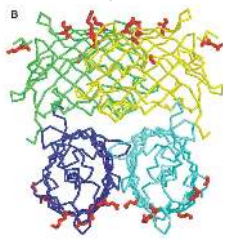
gene-level example

signal 1: long term (diversifying) selection



Bayes' rule:

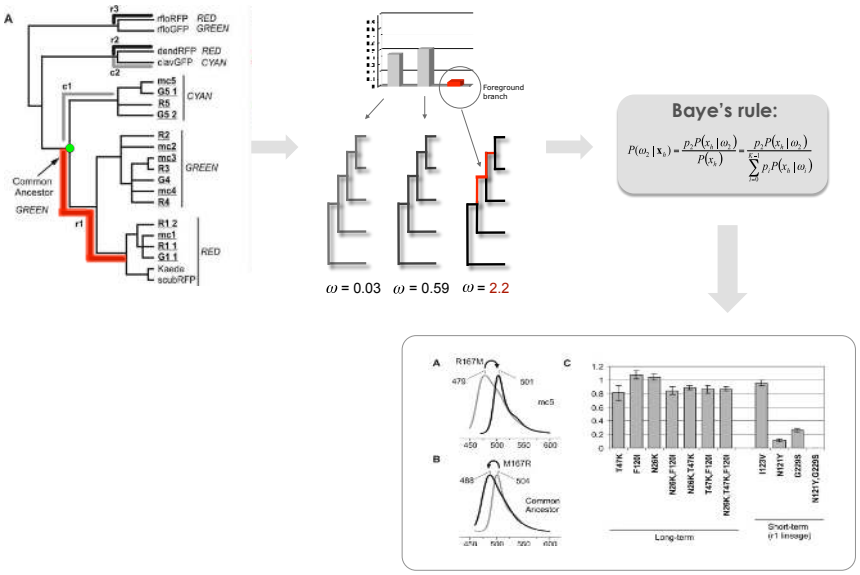
$$P(\omega_2 \mid \mathbf{x}_h) = \frac{p_2 P(x_h \mid \omega_2)}{P(x_h)} = \frac{p_2 P(x_h \mid \omega_2)}{\sum_{i=0}^{K-1} p_i P(x_h \mid \omega_i)}$$



sites in red correspond to the protein-binding region of ***non-colored*** *homologs of these GFP proteins*

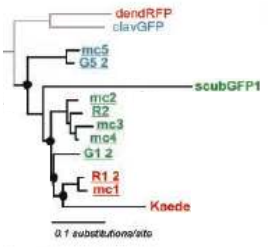
gene-level example

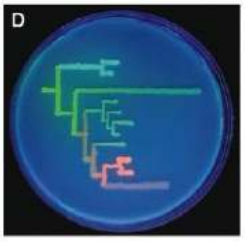
signal 2: episodic selection



gene-level example

just for fun ….





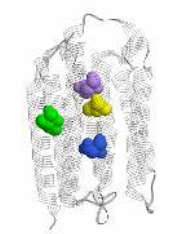
Bacteria were engineered to express the extant and ancestral GFP-like proteins. These bacteria were then cultured in a pattern that corresponded to the GFP-LIKE gene tree

genome-scale analysis

genome-scale analyses

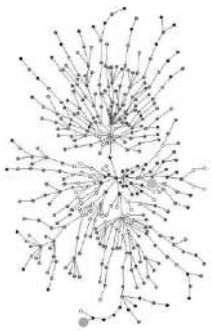
still about genotype-phenotype

gene analysis



3D protein cartography

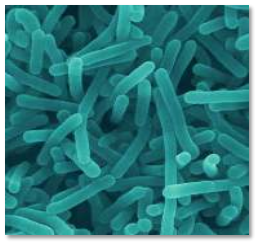
genomic analysis



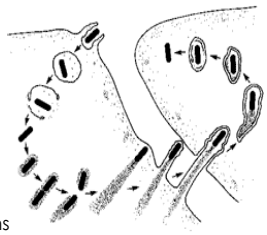
cartography of metabolic network

genome-scale analyses

bacterial genus *Listeria*



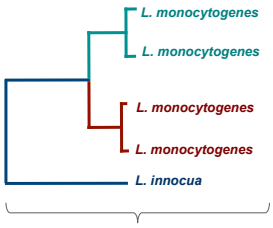
- gram positive
- no capsule
- non-spore forming
- low G+C%
- facultative anaerobes
- rod shaped
- opportunistic pathogens



- genus contains pathogenic and non-pathogenic species
- lineages of *L. monocytogenes* differ in (i) population structure, (ii) ability to respond to stress, and (iii) pathogenicity
- *L. monocytogenes* is serious food-borne pathogen; hence, several genomes

genome-scale analyses

core genome analysis



"core genome phylogeny"

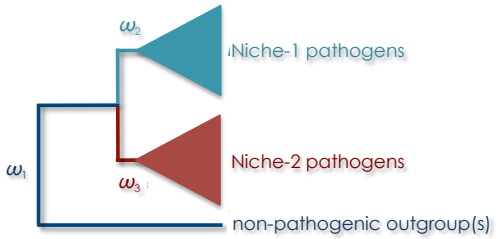
Lineage	Serotype	Pathogenicity	Envir. S.R.	%GC3	ORFs
Lm-L2	1/2a	lower	higher	38%	2973
Lm-L2	1/2a	lower	higher	37%	2971
Lm-L1	4b	higher	lower	38%	3024
Lm-L1	4b	higher	lower	37%	3199
Li	None	None	higher	37%	3065

- 1905 "core" genes
- concatenated genes
- 82% of single core genes

mosaic genome:
- > 18% recombination (HR)
- loci w/ "extended gene pools"

genome-scale analyses

branch models: divergent selection pressure



null (1 parameter):

$H_0: \omega_1 = \omega_2 = \omega_3$

2 parameter alternatives:

$H_1: \omega_1 \neq \omega_2 = \omega_3$

$H_2: \omega_1 = \omega_2 \neq \omega_3$

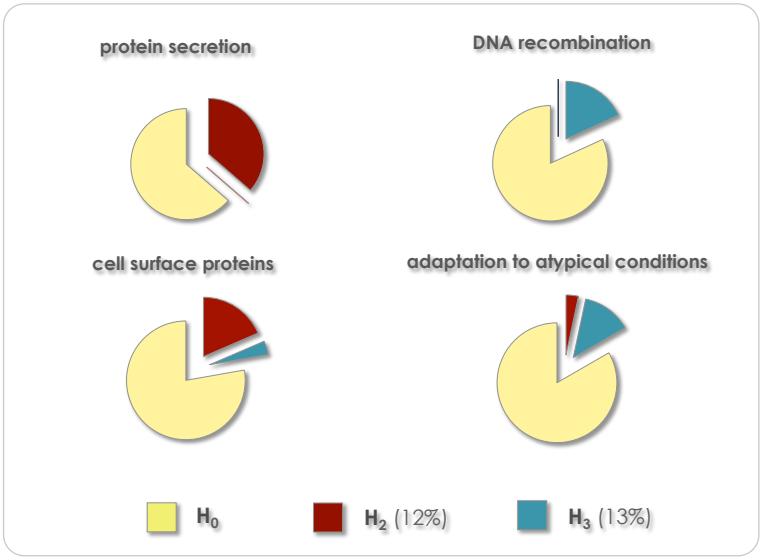
$H_3: \omega_1 = \omega_3 \neq \omega_2$

3 parameter alternative:

$H_4: \omega_1 \neq \omega_2 \neq \omega_3$

genome-scale analyses

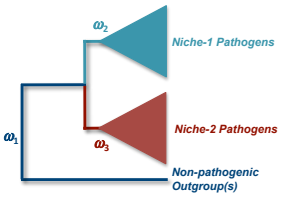
patterns of divergent selection



genome-scale analyses

patterns of divergent selection

A: Gene-level data analysis



Null model (1 parameter):

$H_0: \omega_1 = \omega_2 = \omega_3$

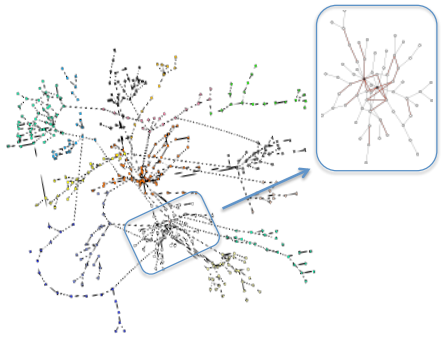
Alternatives (2 parameters):

$H_1: \omega_1 \neq \omega_2 = \omega_3$

$H_2: \omega_1 = \omega_2 \neq \omega_3$

$H_3: \omega_1 = \omega_3 \neq \omega_2$

B: Phenotype-level data analysis



3 modules, and 3 KEGG pathways, were significantly associated with a particular pattern of selection pressure (H_1, H_2, or H_3).

genome-scale analyses

divergent selection & gene expression

Odds of divergent selection when gene expression is altered during invasive disease

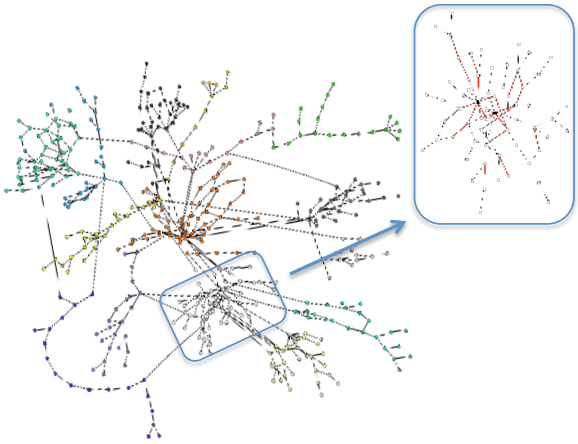
	odds of divergent selection		altered vs. unaltered expression	
genes	altered expression	unaltered expression	odds ratio	*P*-value
growth & survival*	0.506	0.293	1.72	0.017
other	0.320	0.392	0.82	0.1098

* genes in ListiList categories considered critical to intracellular growth & survival

For genes critical to intracellular growth:

The odds of divergent selection pressure was significantly higher if a gene had altered expression during intracellular growth.

genotype - phenotype



adaptive phenotypes are a function of **networks of genes**

"*We are drowning in information and starving for knowledge.*"

— Rutherford D. Roger