7.27.97

I never engage in that kind of dating game myself.

Allen Campbell

Viral evolution

Historical

- Ultimate origins
  - Degenerate parasites (like organelles) - losing favor for viruses
  - Relics of precellular life - maybe only for RNA viruses
  - Cellular dropouts - (large DNA viruses)

- Change through time

Phylogenies

- Genes vs. Genomes (Chimeras)
  - RNA replicases
  - Tracing lineage backward

- Methods
  - Alignable sequences
  - Signatures

- Speciation (Does concept apply?) How much recombination is there within populations

Ongoing population biology

Origins (emerging viruses) → suggests most "new" viruses aren't really new, just changed a little.

Maintenance (detection proving) → suggests most are degenerate old viruses.

Maintenance

- viruses have been around a long time so suggests that there is some sort of steady state.

- maintained over LONG periods of time (not necessarily constant but little major change)

Recombination

Influenza -

- segmented genome
- get reassorted of different chromosomes
- sporadic massive epidemics in humans
- very narrow lines of descent (very narrow bottlenecks)
- occasional replacement from Avian viruses

Suggests occasional replacements are an adaptation to muller's Ratchet

Lin Chou grew ϕ6 phage ... Compared some grown with small bottlenecks vs. others w/o bottlenecks.

Lambda

λ + its relic recombine frequently in nature

Comparison of two phages

1. Special explanation for sharpness of transitions.

2. Models of recombination unlikely to be the same as "modes" for origin of virus

Signatures →

Homogenous w/in genomes } Despite variation w/in phages } in GC.

Roger Hendrix - phage λ

Sequencing phage genomes

phage L5 - infects Mycobacterium smegmatis
- looks like λ-like phages, but no detectable sequence similarity

λ-like
Hk97
P22
Hk022
other
} stretches w/ high % similarity interspersed w/ low % similarity.

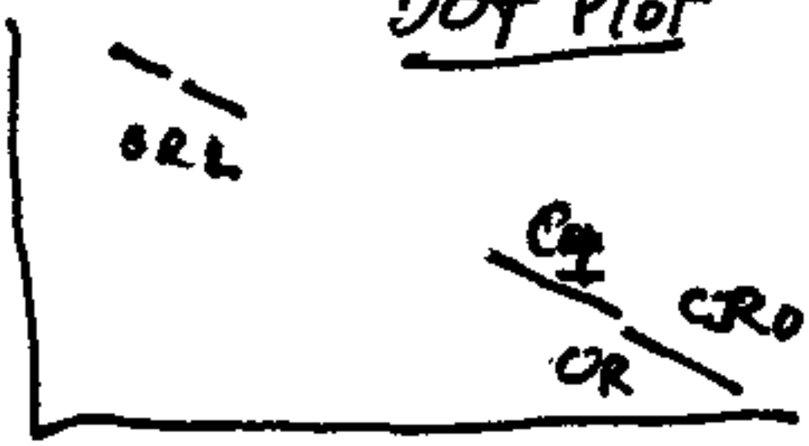
- boundaries are approximately at gene boundaries

- favors view that the recombination events are ~~not~~ random and that selection causes observed boundaries to be at gene boundaries

Examples of evidence

- Get some boundaries w/in genes -- but these are at *module* boundaries w/in genes

Dot Plot



HK97 clr

} Genes that f(x) together, segregate together (suggests selection did this)

- Also - head genes tend to stay together w/in a group and tail genes tend to stay together. Sometimes get recombination w/in FZ gene -- which they think ½ interacts w/ head + ½ w/ tail.

Despite tight packing of genes within λ-like phages head-tail genes .... there is a gap in ~~them~~ between two λ genes (L, K). However HKO22 shows genes here contiguous + therefore suggests λ had frameshift and selection hasn't removed the gap yet.

MORONS = new genes that are inserted into head-tail region. They have significant GC content difference from rest of genome.

Tail of λ

U G T H M

U translated by itself

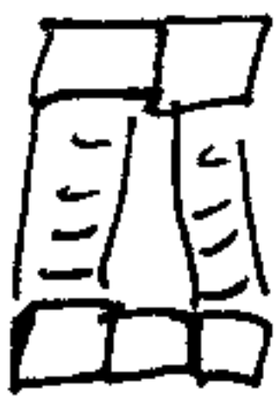
G only translated if -1 frameshift from G

many other λ phages have two overlapping genes here even though no sequence similarity

Mycobacterium phages

many seem to be mosaics

gene 10

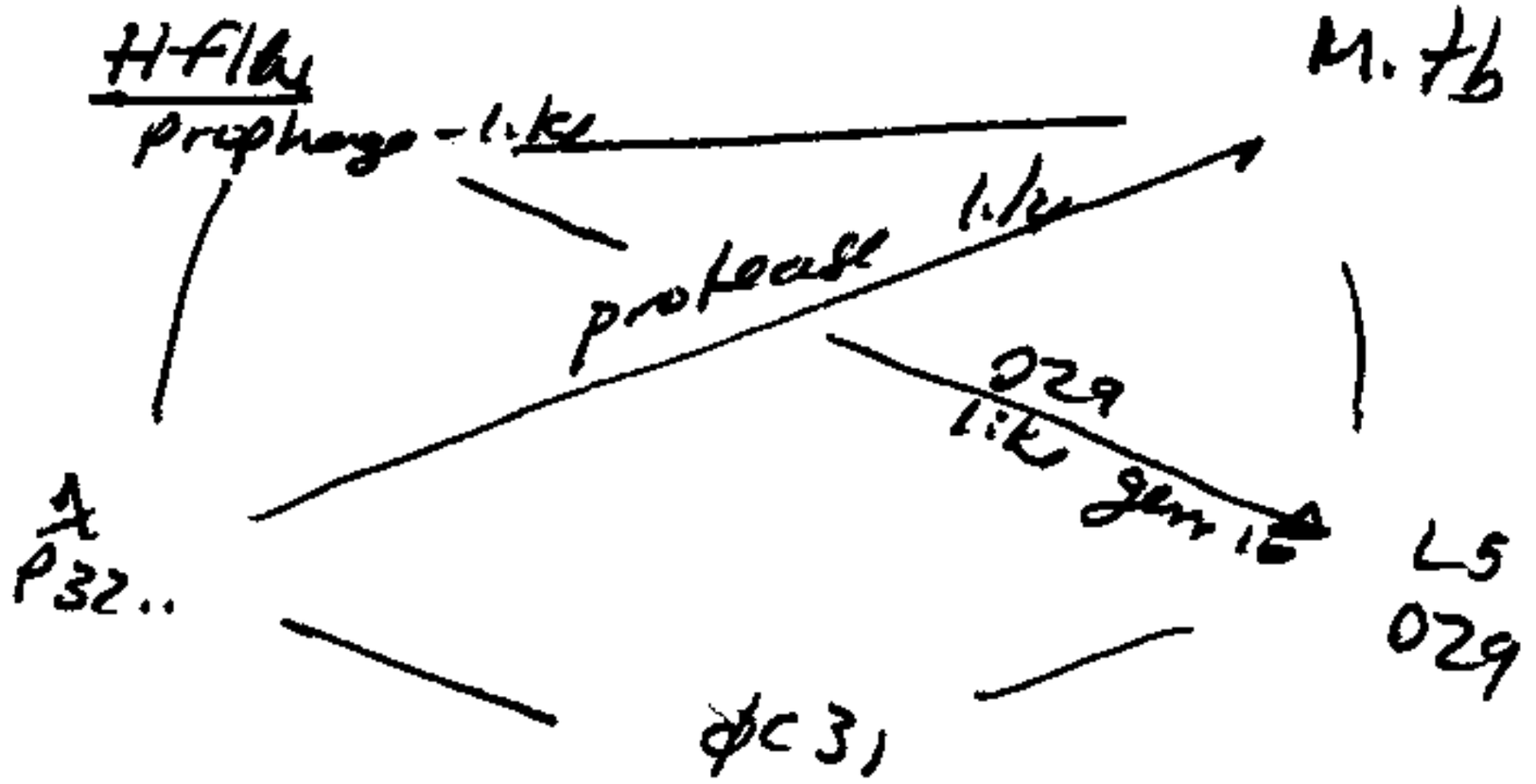


L5

D29 (has an insert)

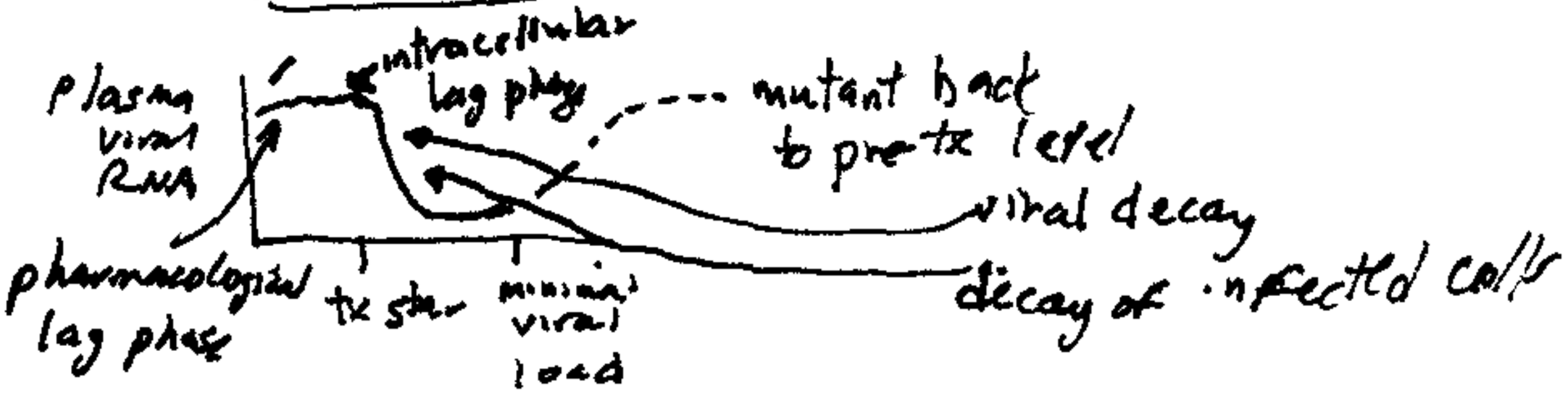
↓

- this region similar to a region in flu genome



- many connections found between variety of phage. Suggest lots of exchange over time.

HIV Infection



RT inhibitors, PRT inhibitors

Hep B virus

RT inhibitors block two steps of viral cycle.

Reproductive Ratio (R_0)

- use this to define drug resistance
- if $R_{0\ w/drug} > 1$ then resistant

Discussion

What about

Species concept

- Long history of viruses
- How is genomics going to help?
  - e.g phage T4 only ~1/3 show similarity to things in databases.

Recombination

- regd. for replication on many phages

Mutation v. high

What are the hosts?

- can ID a thing those grow on in labs but what about in field

The lightbulb was'nt invented by ~~Jno.~~ Thomas Edison but by another man of the same name.

Antibiotic Resistants

Two routes to resistance

① Mutation/altering of target

② Acquisition from another source

③ Acquisition from lineage

"The Earth is bathed in a dilute solution of tetracycline."
SFalkow

Where did they come from

① housekeeping genes
- β-lactamase (cell wall turnover)
- Aminoglycoside-acetyltransferase (peptidoglycan synthesis)

② antibiotic-producing organisms

③ natural resistance in soil communities

Aminoglycoside Resistance

- can be caused by modification in 750 ways of aminoglycosides

Antibiotic producing strains

- many Ab^R appear to have originated in Ab producing organisms

Acquisition of new genes

① get ORF
② fusion w/ expression signals
③ low level expression.
⋮
④ association w/ replicon
⑤ inc. expression

Antibiotics III

How different does the concentration of Ab have to be in order to constitute diff selective environments.

Experiments

Mark-release recapture

Compartments in ligand media

β-lactamases

Random mutagenesis of all positions
&
Selection of Amp plate

what about cases where lab mutants don't correlate w/ evolutionary

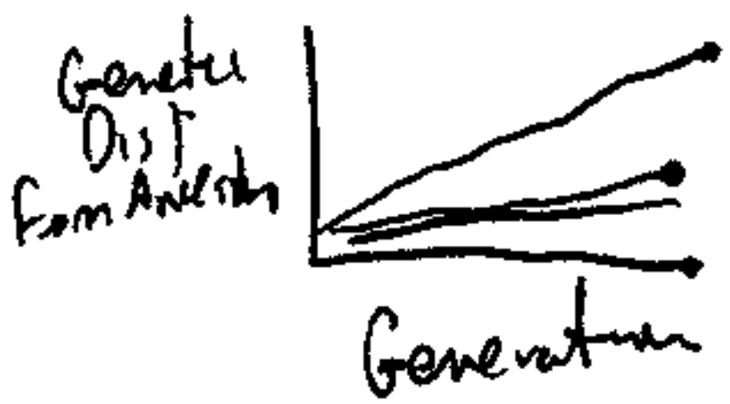
D-

Blevin

Michel Blot

Suggests IS elements are designed to make mutations

To maintain IS's, must have potential for beneficial mutations.

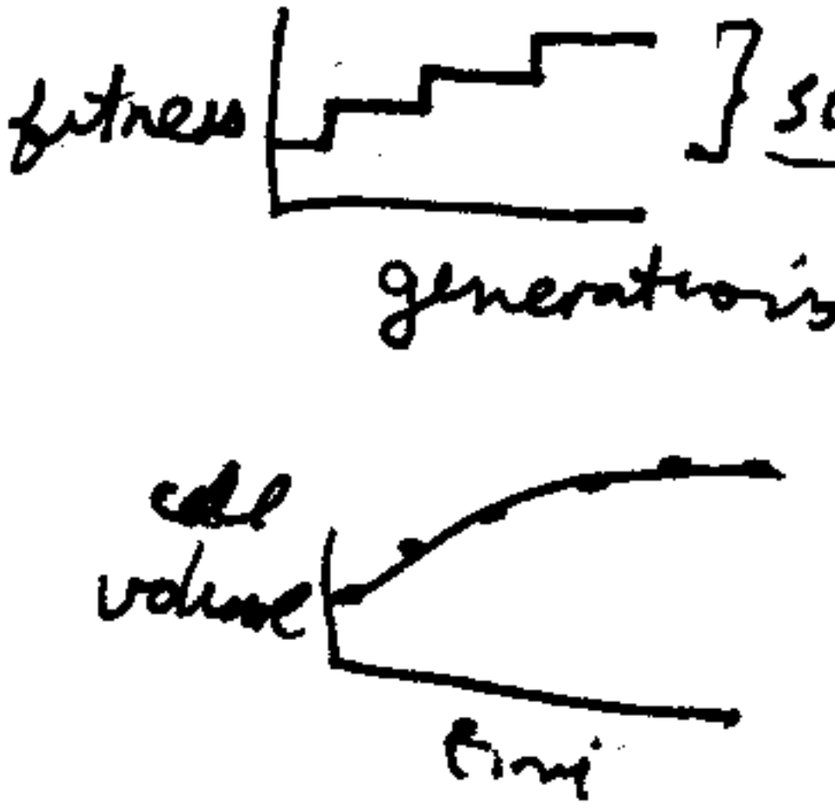


Lenski - Dynamics of Adaptation and Divergence During 10,000 Generations of Experimental Evolution

Escherichia Shrlenmerie

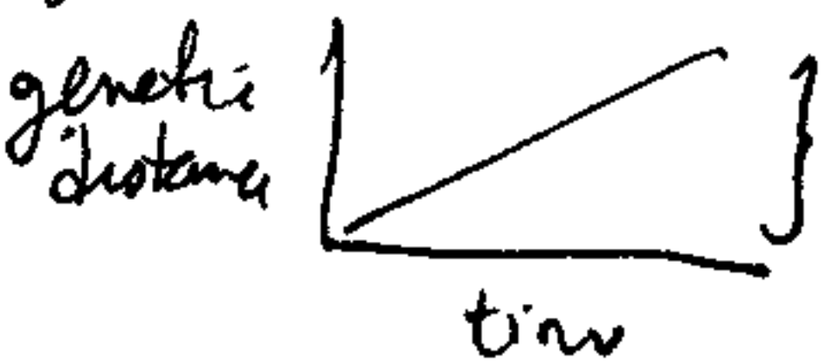
Tempo of evolution
- adaptation
- morphology
- molecular

Fitness = ratio of growth rates in head : head competition

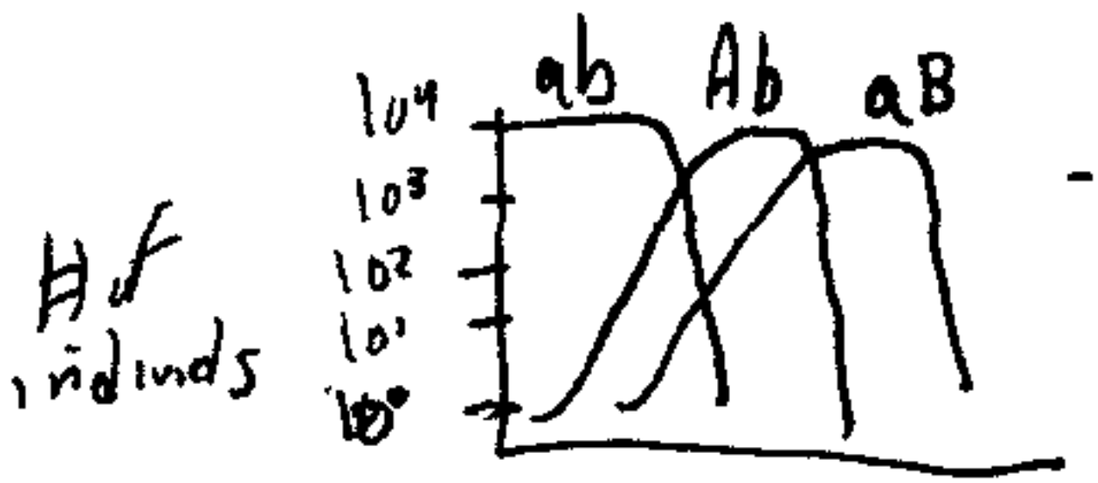


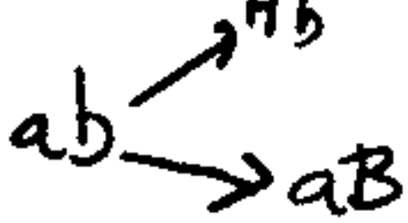
suggests that this is due to sweeps thru beneficial mutations
~ 100 generations between plateaus

Suggests IS elements are not causing large #'s of adaptive mutations b/c



} expect linear w/ neutral changes





- if ab is replaced by a beneficial mutation, then the next beneficial mutation will be most likely to come from ab because it will still have large #'s.

$N = 3 \times 10^7$

$G = 1 \times 10^4$ (generations)

$\mu = 4 \times 10^{-10}$

$\downarrow$

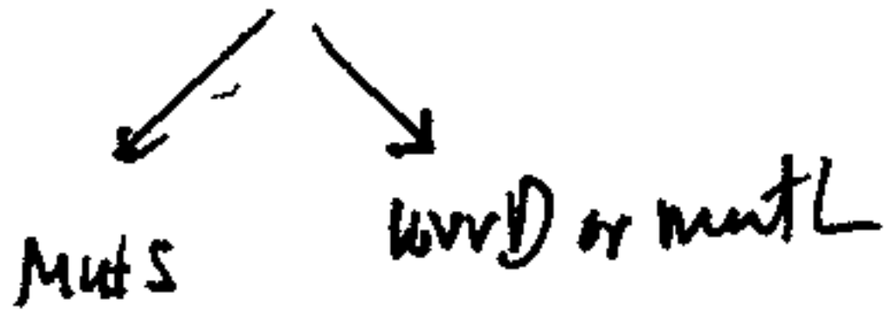
600,000,000 mutations over 4 years

$\downarrow$

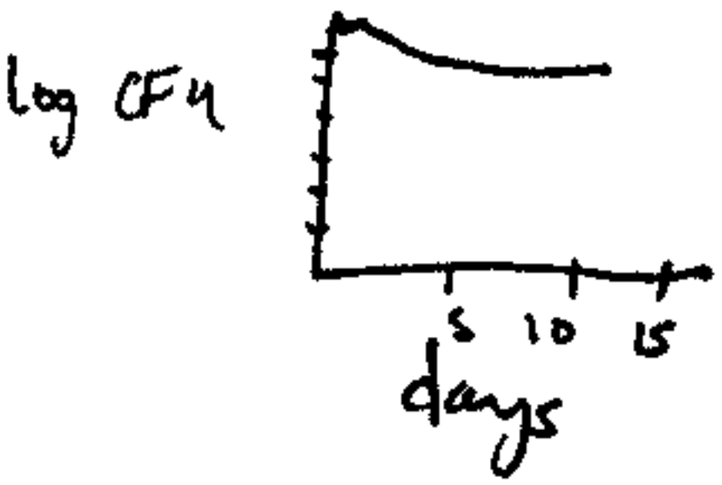
but small % of double mutations

Mutators

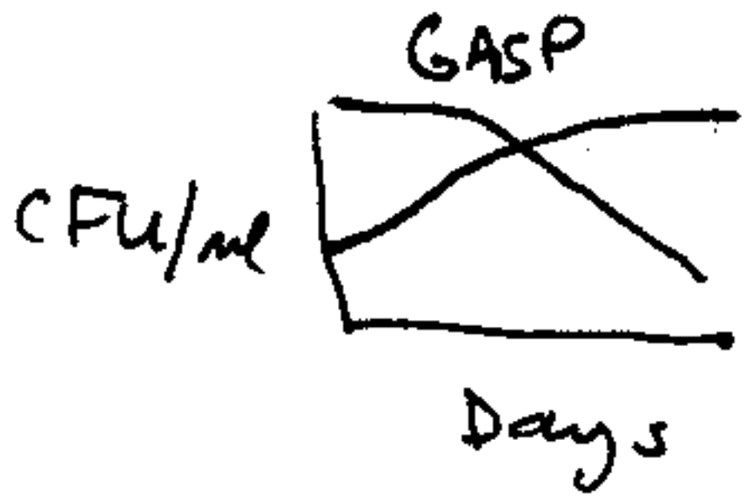
- 3 of lines have 2 orders of magnitude increase in μ



Roberto Kolter



take cells from late in SP, and ~~mix to~~ mix w/ early SP cells



Assay
E. cloacae
S. aureus
E. faecalis
B. globigii
S. cerevisiae

RpoS change
- get many diff. types of changes
- many diff. ways of affecting rpoS levels

Take rpoS mutants... do GASP again... GASPO

GASP2 = LRP

Get physiological adaptation 900.

Claire Cupples

- VSR up-regulated in Stat. phase

Ruben

- mutL limiting in SPM

Joel Torkleson EmboJ 16:3303

Lac+ revertants

look for other mutations in F' higher in lac+?

-10% of double-mutants are heritable mutators

PBR	much higher
chromosome	much higher
upp	"
Mal-	"
Xyl-	"
Fruc-	not

Reuben Harris

- mutL overproduction diminishes leads to ↓ in SPM but not Log-phase-M.

- overexpression of vsr may KO mismatch repair. Protein not made much during growth phase. } Claire Cupples
made a lot in SP.

- Barbara Wright — meth.
  - stringent response
  - Leucine starvation → Lrp mRNA↑
  - tx inc. mutation

- Barry Hall
  - mini-Tn10 insertion
  - how affect adaptive mutation at ebgr locus
    - 5 genes ... ↓ incr. at ebgr in SP
    - 6 genes - ↓ decr. at ebgr in SP

F. Taddei

- how do mutators do in competition?
- mutators do well in mouse competitions.
  But many have become very specialized, accumulating deleterious mutations.

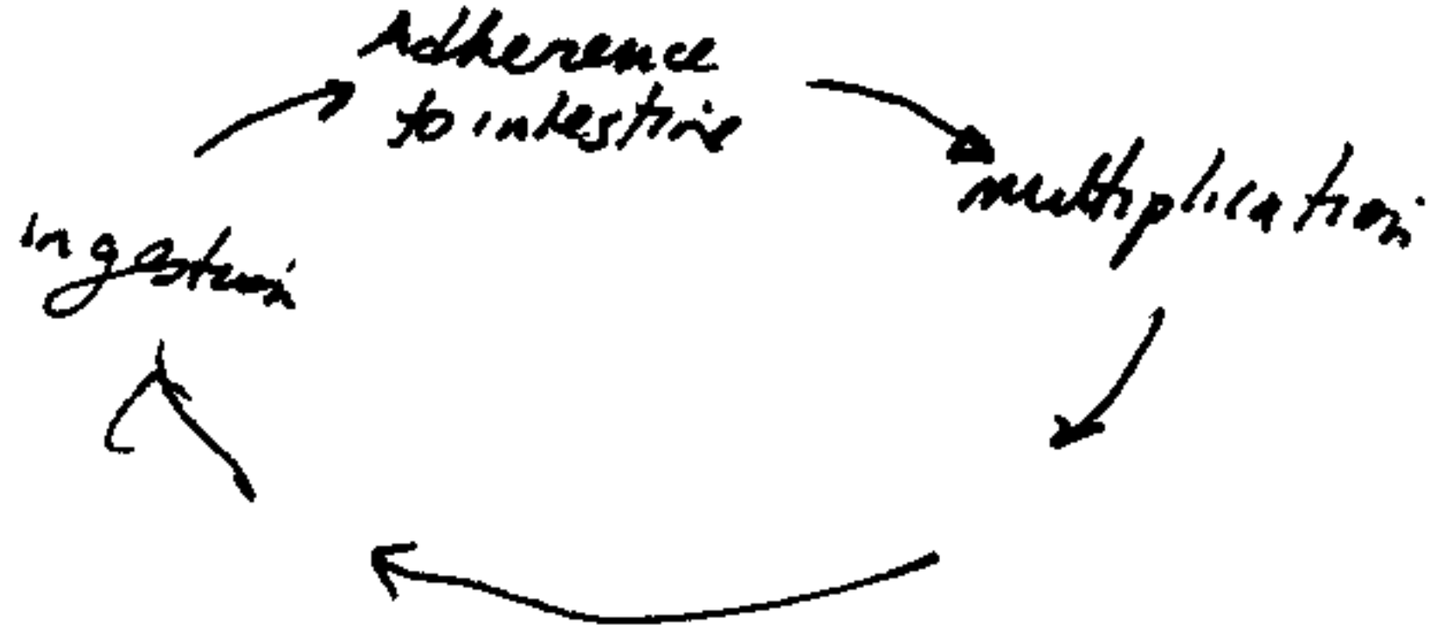
Peter Reeves

Environment for pathogens have changed a great deal recently.

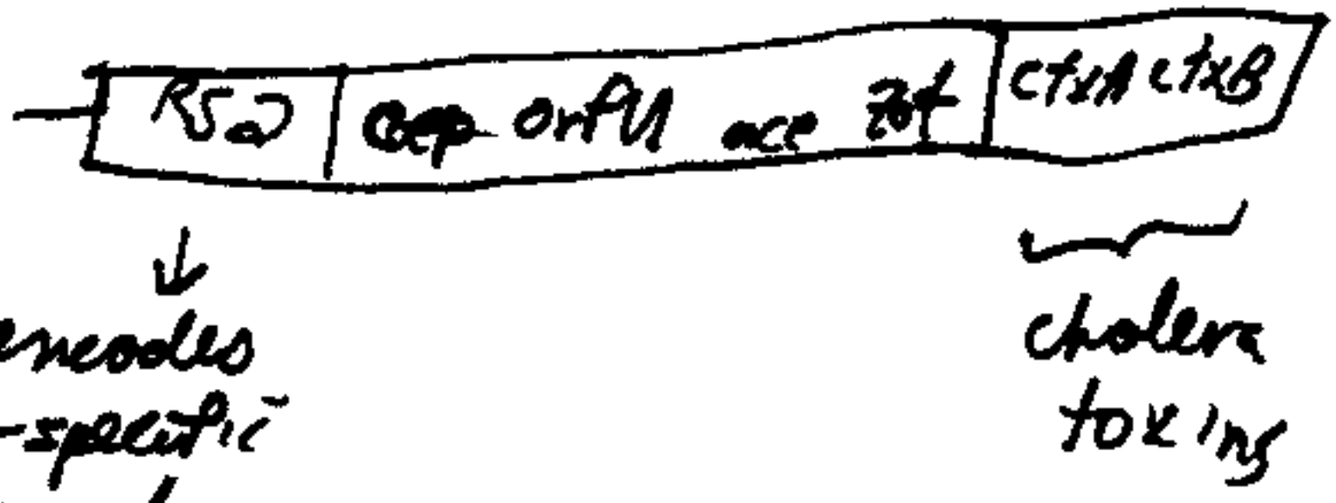
Clonality

- degree of clonality varies great deal
- e.g. Neisseria has lots of lateral transfer

Vi Cholerae "Promiscuity rules"



CTX Genetic element - found in all toxigenic strains



New strain of cholera involved in pandemic. VC0139.

Single-double recomb. event explains origin of VC0139.

_ Also carries genes for Sulf^R Trim^R on a self-transmissible element.

Bacteroides TPON →

- can mobilize DNA in trans

So... can this VC0139 self-transmissible element move VCDNA? No... actually... the CTX element can move on its own.

Can get transduction in vivo (in mouse) (depends on expression of receptor)

Do other species have TCP homologs

Jan Cebula

pathogenicity - says must remember that pathogenicity by some organisms is very specific and some genes involved in pathogenecity have other roles

Hypermutability, Horizontal transfer + homeologous recombination: ingredients for rapid emergency of pathogens.

What is freq. of mutators w/in natural populations of bacteria.

Ecoli	total analyzed	# put. mutators
0157:H7	120	5
Other types	20	3
ECOR collection	72	1
S. enterica		
S. enteritidis	15	1
Other serovars	106	14
SARS strains	69	1
SNEC	16	2

} 1 Pathogens

100-1000X incr in M compared to non-mutator

Frequency of mutators is much higher than you expect for a deleterious mutations

Complementation of mutators

- all mutators they isolated could be complemented by mismatch repair wt alleles.

MMR mutants are promiscuous

MutsL inhibit strand exchange

Advantages of mutator phenotype

- genetic variation
- opportunity to quickly change
- incr. in genetic exchange

Always pull out MMR mutants

Why always MMR mutants?

- why not other 50x↑ mutators
  - certain alleles may be more primed for mutator

- MOST of the mutators were mutS

fhlA mutS ← 6.9kb → rpoS

in some mutator strains, the 6.9kb intergenic region is messed up
- 12 bp direct repeat in Salmonella?
- all the E coli ones seemed to be deletions of part or all of mutS.

K12 vs O157 vs Shigella dysenteriae

5' K12, O157 v. similar

3' O157, Shigella v. similar

- this region highly variable in enterics

Yersinia enterocolitica → nuts ← rpoS Shigella sp.

- if you nutrient deprive strains rpoS leads to incr. in mutation

Suggests

- anti-nuts expressed (but not in K12)
- antisense might inhibit nuts and lead to a mutator

Dykhuizen · Lyme Disease

Lyme disease: suggests its been around a very long time

Borrelia hermsii - causing Relapsing fever
- replaces outer membrane proteins by recomb. mechanism

S. Levy

major antibiotic resistant bacteria

Hospital:

Community: Pneumococcus, MTB, N. gon, Staph, Ecoli.

Vancomycin resistance

Van^A Van^A gene -- spread
- Enterococcus
- Corynebacterium
- L. lactis

Van^A gene found in lots of animals.

} Animal use of antibiotics impact pool of resistant strains and plasmids.

Van^B gene -

New Van gene in Japan

Suggests using as little Ab as possible, to have as small an impact as poss. ble on non^R bacteria.

MAR locus

AbR
Microbial metabolism
NFO
SOX

} may be responsible for multidrug resistance

Antibacterials
Disinfectants

} Also lead to AbR

Posters

Rueben Harris

overexpression of MutL
- inhibits lac+ accumulation in SP
- fails to prevent decline of MutS and MutH proteins in SP

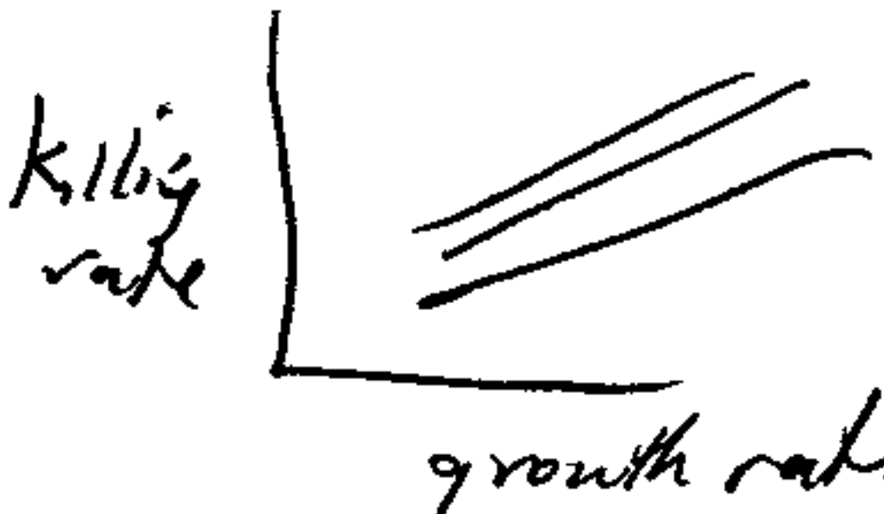
Claire Cupples + Gina MacIntyre

- overexpression of Vsr leads to ↑ mutation

- Vsr protein v. low in growing cells
- Vsr protein ↑ in SP
- ∴ when replicating Vsr is low.

Blanchard + Lynch

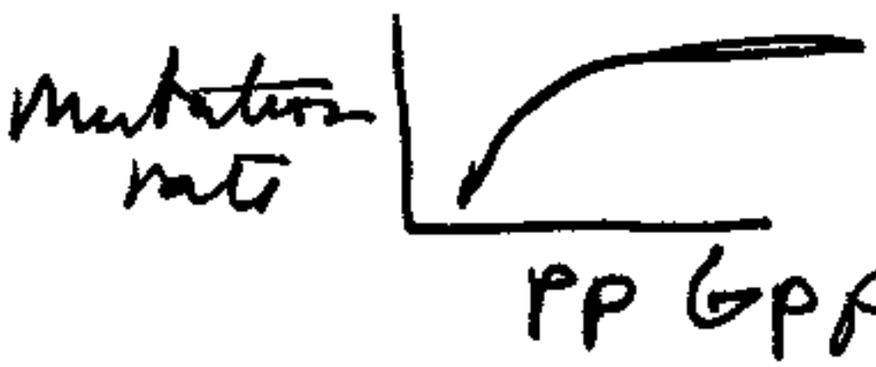
V. Perot - Kinetics of Ab Action in slowly growing cultures



Peter Young (& Susan Lloyd) → MCBI

RecA phylogeny in rhizobia

J Reimers + B. Wright



Rkolter SEFinkel

Ecoli remain viable for long pds of time
SP cultures are highly dynamic
cells acquire GASP phenotype during
prolonged SP
mutation freq. affects rates of
GASP appearance

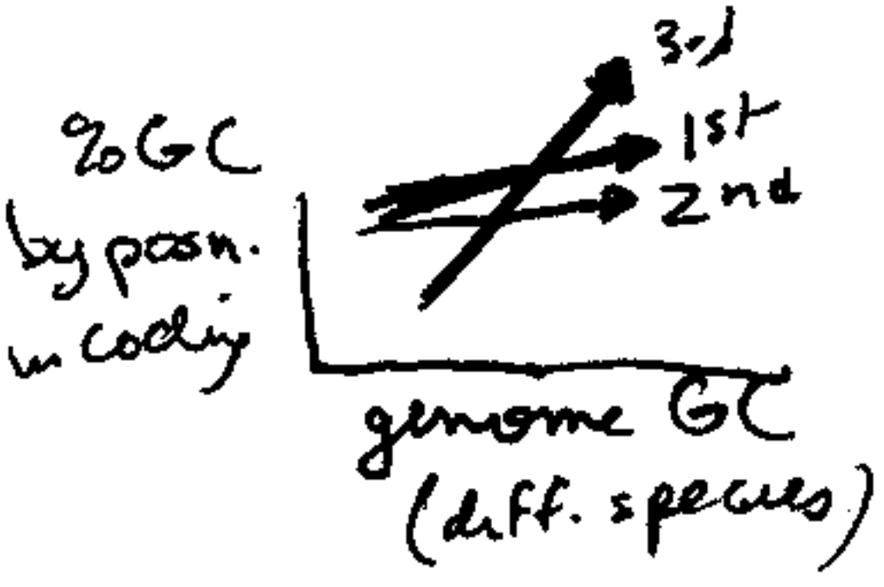
Cooper + Lenski

3 pops w/ incr. mutation rate

John Roth

How distinguish?

- lac+ (EC) vs. BL2(ST)
- Many of these things appear to be foreign



- can predict age of such sequences by how long it would take to change BC to fit curve.

- ~15% of E coli genes look weird } claims now are older than 90 mya... but how old could it detect.
- mean age = 26 million yrs

Estimate

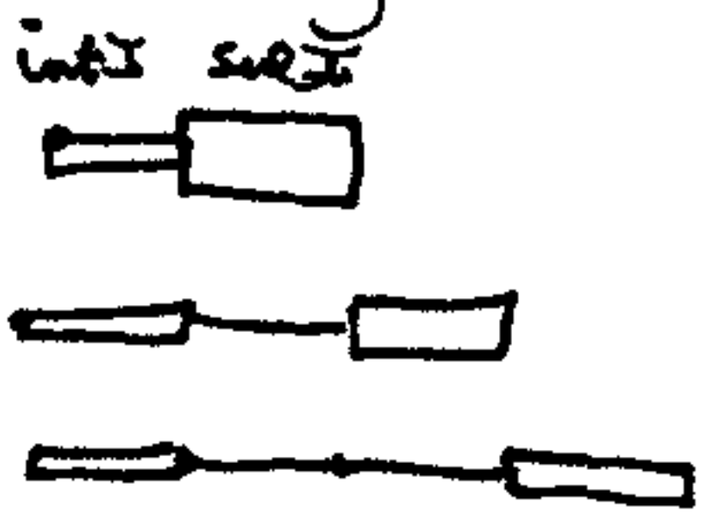
EC vs ST 75% = shared ancestral
25% = unique foreign + unique ancient

Who gets lost?

} essential
} important
} near neutral → small value always
→ important but rarely used

Genes responsible for species differences
unlikely to be essential for life.

Integrons - R. Hall CSIRO



- flanking sequences identical
- internal genes different
- 3 separate classes

Recombination Sites

- 57-141 bp
  - RYYYAAC...GTTRRRY
  - ~20 bases at each end conform to a consensus
  - imperfect inverted repeats

- Move by site-specific recombination
- Companion elements (integrons) code for int genes

3 families

class I :

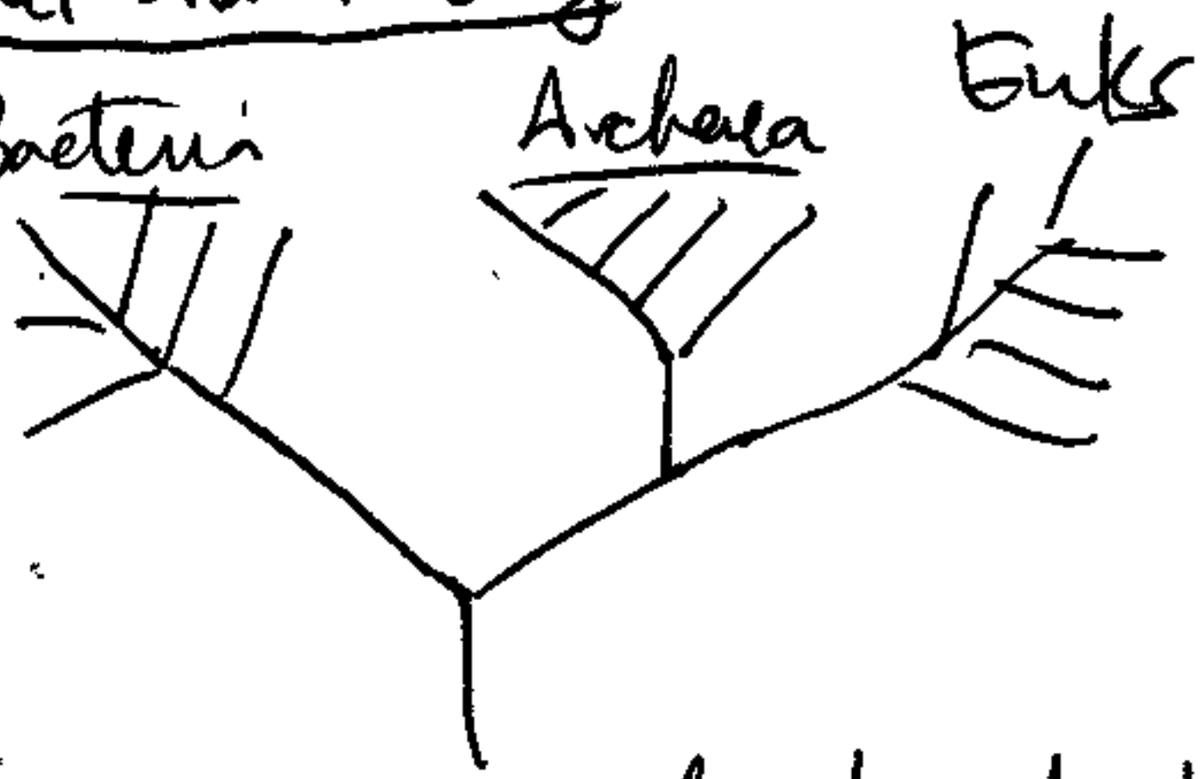
class II : Tn7

class III :

- can insert into 1° or 2° site
- most cassettes dont have promoters.
- integrons have promoter

Conjugative Transposons

Very broad host range



organism from v. distant taxa have the opportunity to interact + exchange.

The Colon

- complex environment
- is it a reservoirs for Ab^R genes

Ingested flora → Intestinal Microbiota → Bacteria pathogens

Kirsti is a bellydancer

Examples

Treponema denticola → ermF → Bacteroides

Enterococcus
Staph
Strep
Actin
B. fido

- tetM
- all virtually identical

Campylo
Fuso
Gardnerella
Haemop.
Neisseria
Veillonella

Conjugative Exchange

Plasmids
Chromosomal elements

Bacteroides

integrated → excision ○ → transfer to recipient

- can also transfer NBUIS (non-autonomous elements)

Donor -- ~~can be~~ Bacteriodes -- recipient can be many

Integrate at end of tRNAs

Howard Ochman -

- Evolutionary psychology
- Genome structure
  - Additions
  - Deletions
  - Inversion
  - Transpocations
- Size variation

- What role does genomics play
- How do genomes co-opt new genes

- Selection?
- Rate of change?
- Tolerable range of variation?
- Role of horizontal transfer in phenotypic variation w/in + among species
- Mechanisms?
- Location influence?

Microbial Evolution - Sharp

What can we get from genomes?

1. Phylogeny

2. Genome content
   a. protein coding genes
   b. RNA-coding genes
   c. repetitive elements

3. Genome structure
   a. ancient duplication
   b. GC variation

Steve Oliver

Genes w/ reported known f(x): 2600
Genes w/ f(x) predicted by sequence: 990
Genes w/ unknown f(x): 2200

5790

Lots of redundancy

Can you expunge most of it?
- See KWolfe paper

Repeated elements

T4 population biology

T2
T4
T6
} mostly highly similar, but many polymorphisms

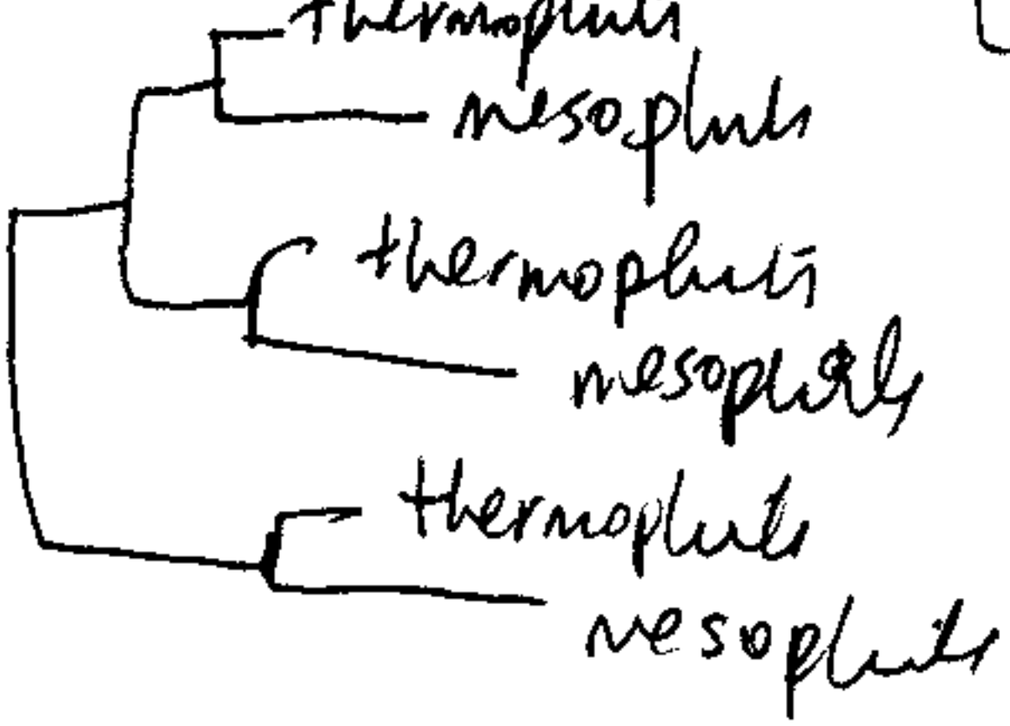
} much of the variation is in copies of repeats

- Some "pseudo-T-Evens"
  that look like T4
  but not alike in most DNA/genes

Gary Olsen

① use + abuse of molecular phylogenies
② inventions of genes
③ site specific rate of change

Need a model for evolution



① rates should correlate w/ # inferred by parsimony

② rates should correlate betw. groups

③ rates from one group should help study other groups

Physicists start with questions and look for answers.

Biologists start w/ answers and look for questions.

D. Hartl

Macroevolution: what is it good for?

"Lederberg - - it shows the difference between your luck and Lederberg.

- Cavalli- story. HFR-C.. don't know what ~~Cavalli~~ Cavalli means but thinks it might be a type of elimination

What is it?

- .
- .
- .

How does it apply to microbes?

- Suggests that microbes are not as prone to mass-extinctions as non-microbes

Plasmids

what is natural distribution of F. ↗ Now have IS in F in O

- ~15% of Ochman's Ecoli strains
- ~15% of Salmonella virulent strains
  - of these there are two groups:
    - some like Ecoli } all have SPV operon
    - some very different

Peggy Sakts -

aa + tRNA —(aa synthetase)→ tRNA-aa → translation

tRNA-aa
tRNA-aa
tRNA-aa

Evolution of code/tRNA isoacceptors

Does evolution of tRNA mirror evolution of synthetases?
(can imagine switching might be relatively easy)

Ecoli isoacceptors

(2) Replacement > complementation

(3) Isoacceptors may not share common ancestors-

EVOLUTION OF AMINOACYLATION SPECIFITY

<u>Mycoplasma</u>
lacks GlnRS

<u>methanococcus</u>
lacks GlnRS
AsnRS
CysRS - added by SerRS

GluRS

adds Glu to Glu-tRNA

adds Glu → Glu-tRNA

Glu → Gln

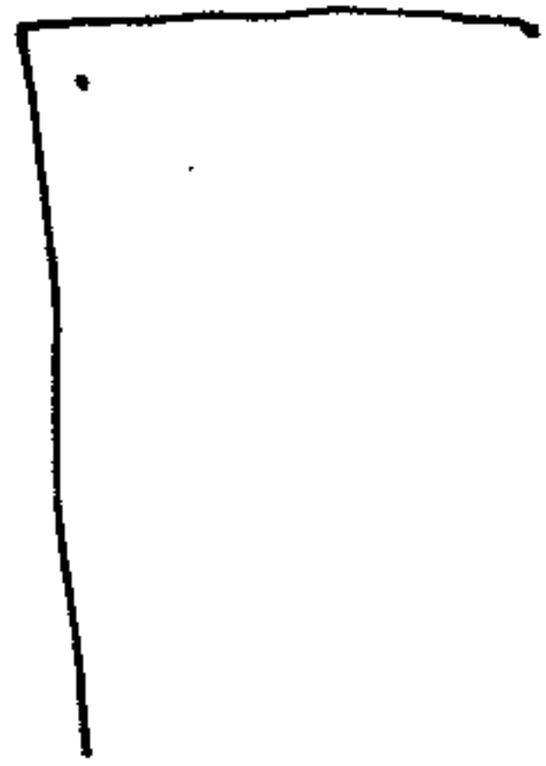
Gln

<u>Constraints</u>

Another constraint I can put on this model, or this verbal thing.

N. Moran - Endosymbionts

Buchnera
- passed on from ♀ → offspring
- enclosed w/in vacuoles
- what gain for aphids?
  - provides essential nutrients
  - produces ftsps
- phylogeny parallels host (w/in aphids)
- substitution rate looks ~2x > Ochman Wilson
- plasmids for (trpEG, leu ABCD)
- ~280 GC (used by host)
- 1 rRNA
- recF missing?
- Gene order similar to Ecoli?



- multiple infections w/ diff. endosymbionts

Pop Dynamics

Ne much lower than for free living

- long branches for endosymbionts

- d_S/d_N = lower than in other species — Stat. Phase

- suggests accumulation of slightly deleterious mutations

- rRNA structure analysis supports this

Restriction

↓ integration
restriction
exonuclease
recombination

Restriction Systems

Type I - ~~very~~ not widely distributed
Type II - very widely distributed
~~methylation sensitive~~ restriction dependent
modification dependent

} don "missing" its restriction gene

Why sex?
Repair
Eliminating mutations
Parasite selection

Restriction-Modification as Selfish Genes

cell w/ restriction genes

→ hard to lose restriction system (cells will die).

- Suggests this is similar to meiotic drive

- If two systems have same recognition, there is no advantage to maintaining both systems. Suggests that this explains diversity of recognition system.

Bacterial Sex

Chi sequences in other species

- Suggests RecBCD system is involved in selfish gene system

- if DNA from outside, then RecBCD will degrade completely
- but, if invading DNA has Chi, then it will be protected.

- DSBR protects agst genomic parasites

Miwo Radman

Genetic barriers between species come down to recombination barrier

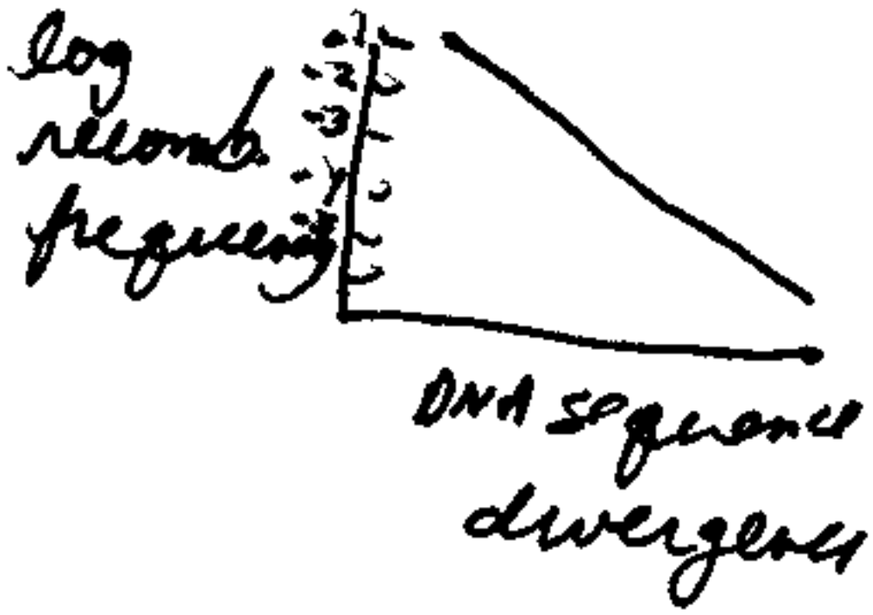
→ isolation

↓

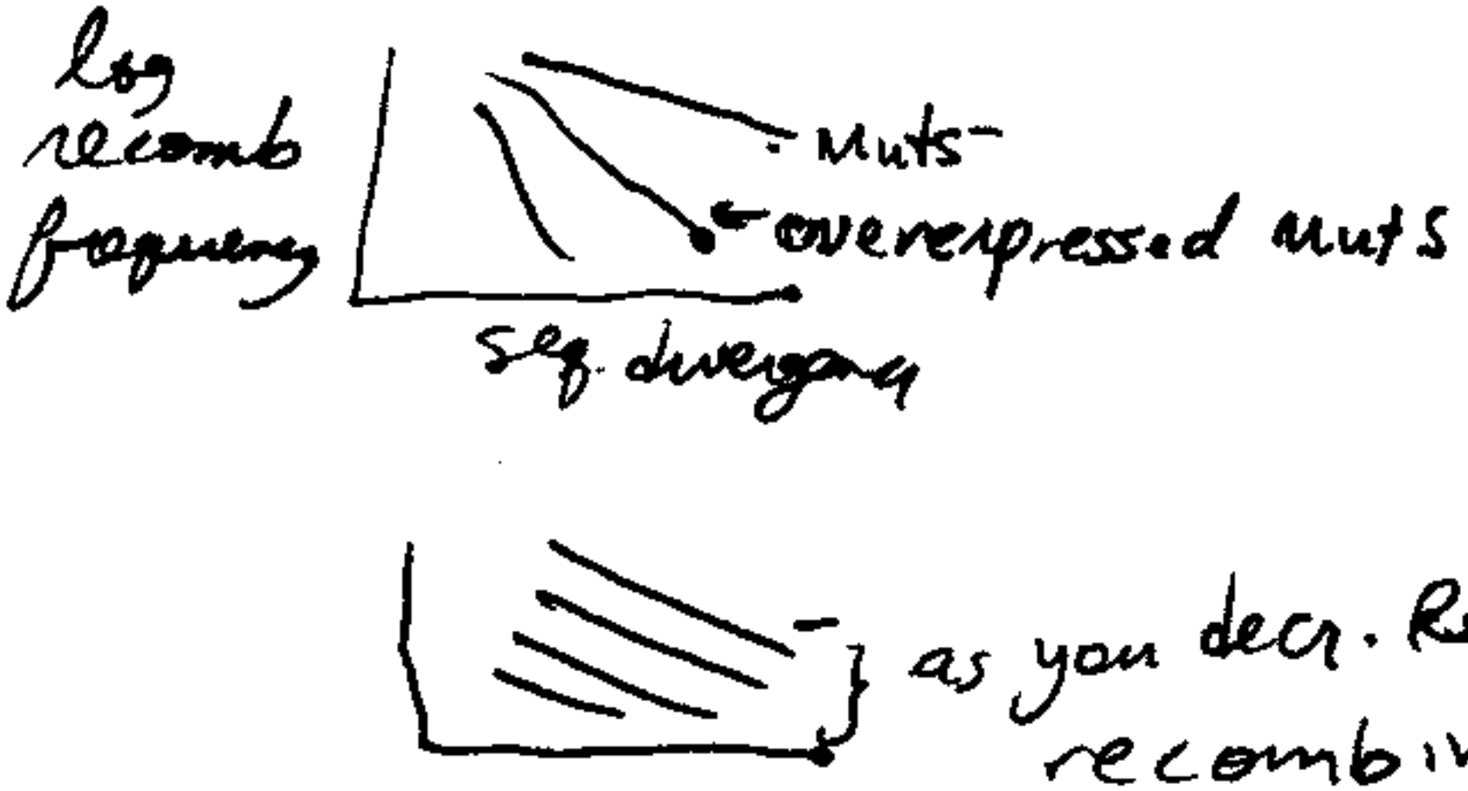
O ⇔ O
- MutS,L prevent exchanges
- SOS positively controls exchange

- MutSL controls accumulation of differences as well as whether exchange can occur

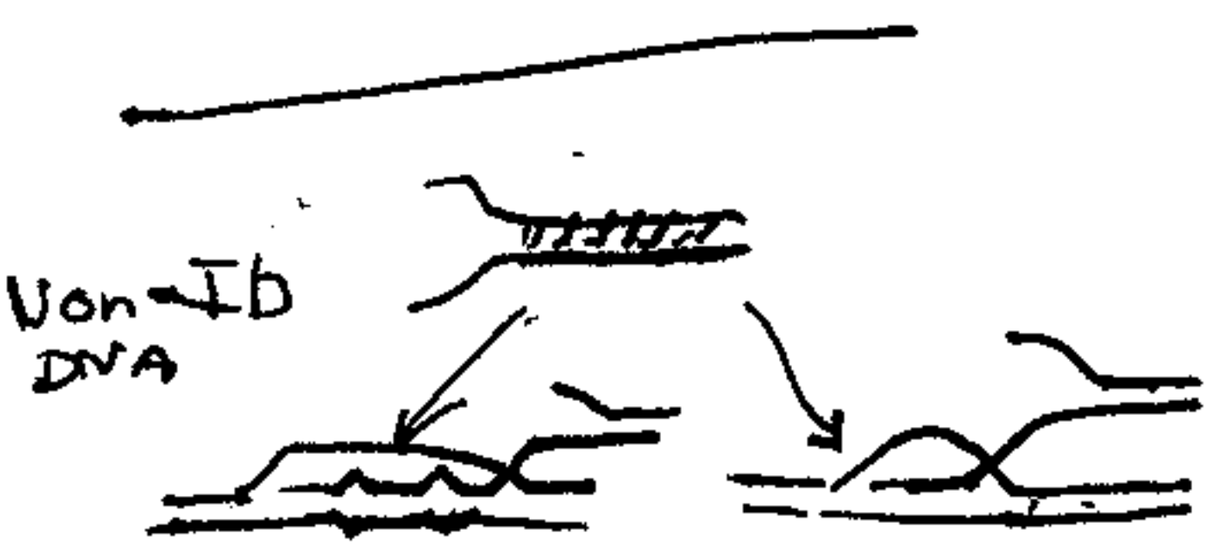
- RecA/SOS does same



$$R \sim N \approx L \cdot e^{-Hd}$$



as you decr. RecA, get less recombination



If sequences are identical, will align

↓

MMR corrects and reduces recombination

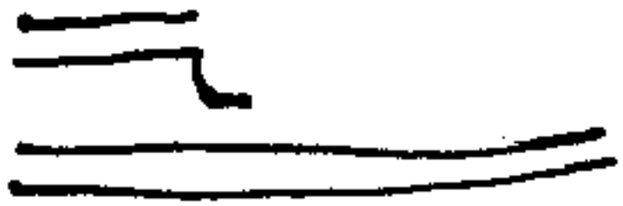
As RecA is searching for homology,
SOS is induced bec. of strand ends
↓
More RecA

Why this there?
- MutSL prevents recombination between repeats

stress → incr. mutation → stress relieved
via SOS
decr. mutSL

↓
variation w/in population

RecBCD
RecFOR
ExoVII (RecE)



RecA ↓↑ Muts, MutL can reverse, but only in RecBCD pathway

DNA Synthesis ↓ PriA, RecF

Vsr en

MutH
MutH

cut unmethylated GATC's

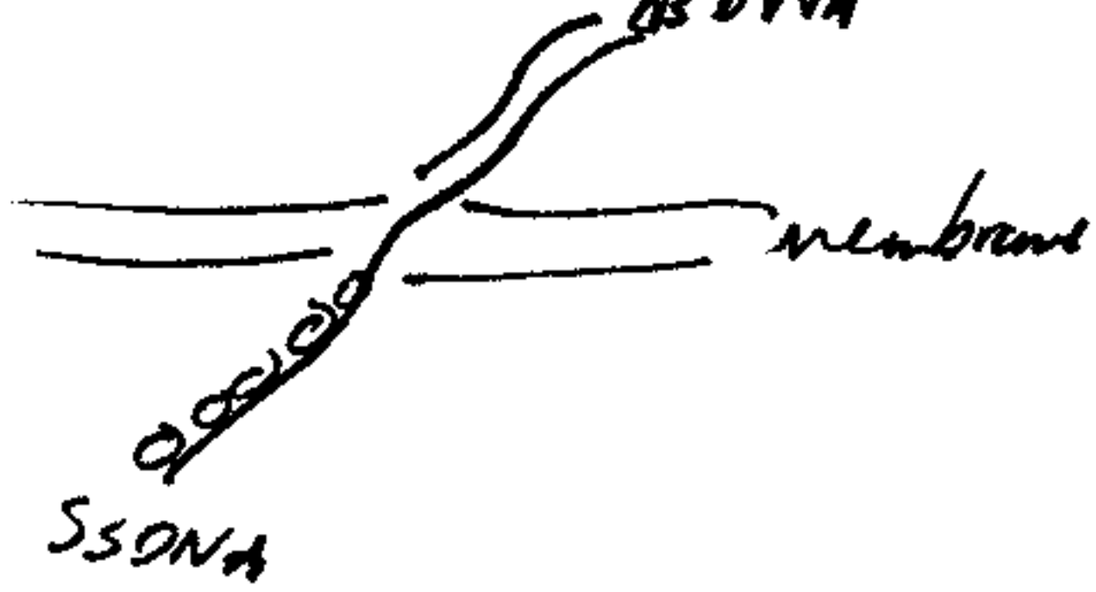
No RecA

Mike Lorenz

~~Sequence transform~~

Transformation

- in B. subtilis
- ~20 genes involved



If mismatches prevent transfer then more highly constrained genes should be more prone to transfer.

Function for natural transformation

Natural transformation

Nutrients

Repair of DNA

Gene regulation