Genetic and environmental factors of fitness variation in yeast gene-deletion strains

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Population genetics seeks to characterize mutations in terms of:

- frequency distribution of fitness effects
- dominance / recessivity
- epistasis (interactions between loci)
- impact of environment (stress) on fitness effects

In this talk I will describe and compare these parametrs for:

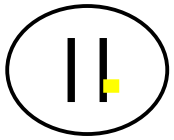
- random (point) mutations
- engineered gene deletions

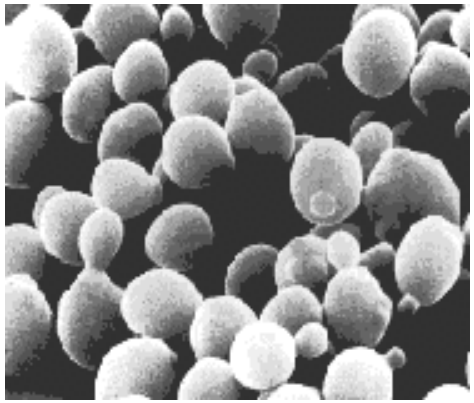
(in the budding yeast *Saccharomyces cerevisiae*)

Random mutations - fitness effects

A special feature of yeast is that basic genetic analyses can be done in one generation

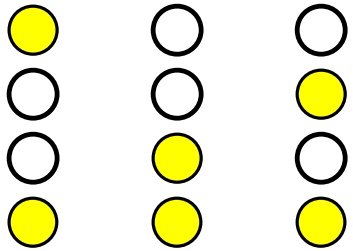
heterozygous locus





meiosis:

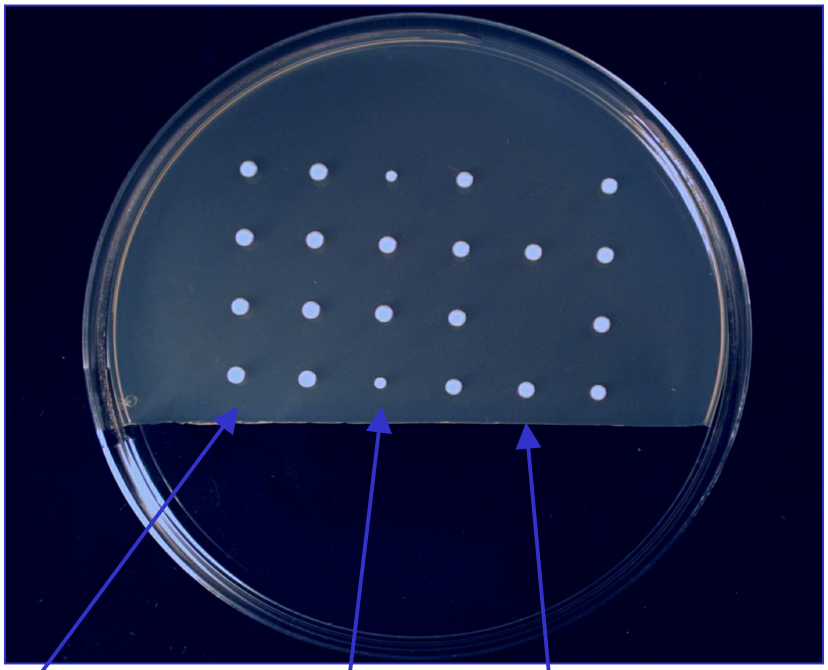
4 haploid spores



2:2 segregation in „tetrad analysis”

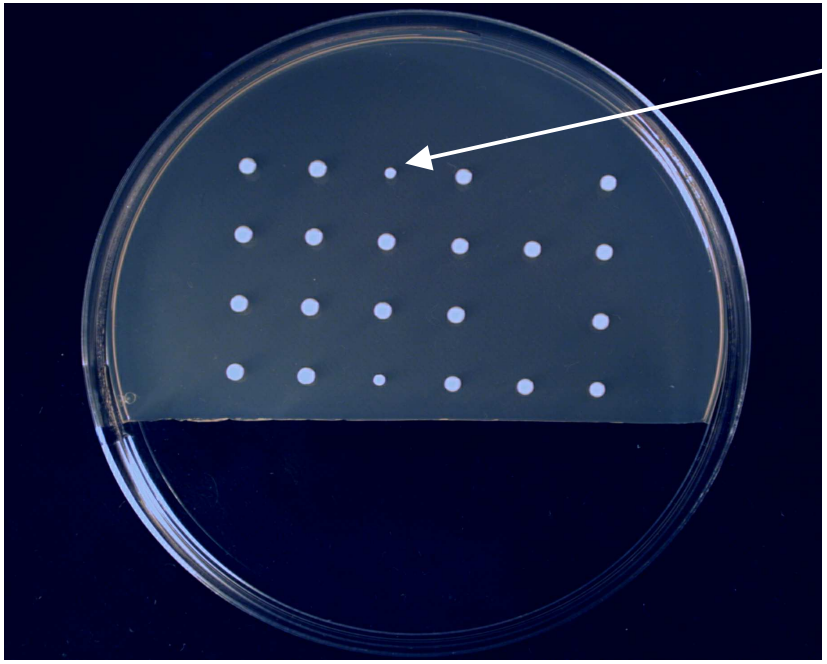
Random mutations - fitness effects

We used this approach to screen for mutations affecting growth:



Random mutations - fitness effects

... to detect and quantify fitness effects:



mutant colony, 8 times smaller than a wild-type

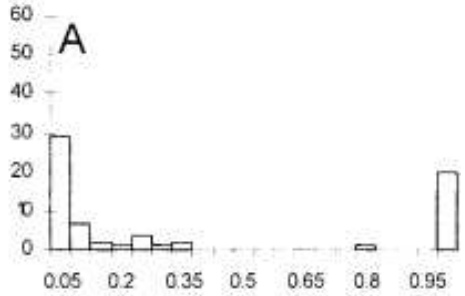
this means 22 instead of 25 cell divisions

relative growth (fitness)
$w = 22/25 = 0.88$

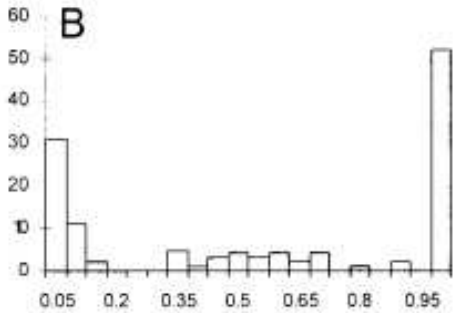
selection coefficient
$s = 1 - 0.88 = 0.12$

Random mutations - fitness effects

spontaneous

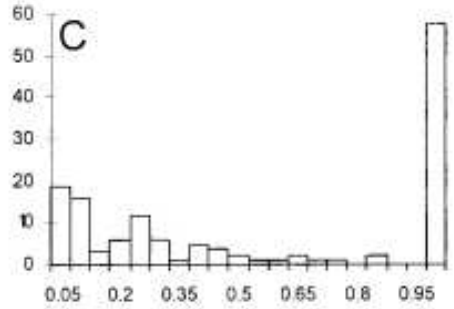


induced by EMS



strongly bimodal distribution

induced on mutator bkgd



slightly deleterious *s* lethals

Random mutations - fitness effects

Were there any beneficial effects?

Yes, 2 with $s \sim 0.01\text{-}0.02$

Compared to:

-130 lethals

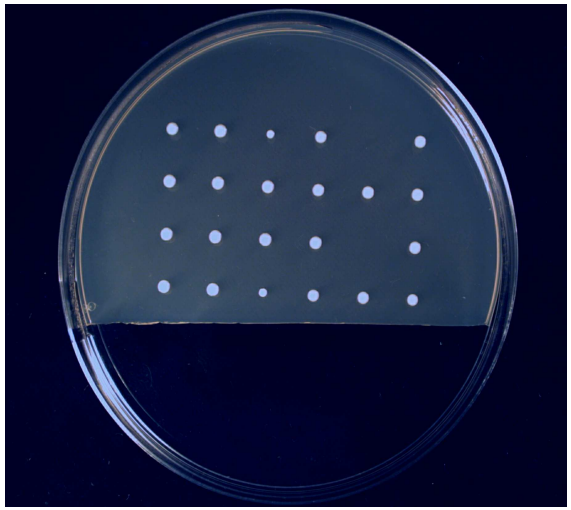
- 244 deleterious with average $s \sim 0.15$

Random mutations - fitness effects

Two conclusions:

- distribution of deleterious mutations is strongly bimodal with lethals comprising ~35% of detectable effects

- beneficial mutations are 100 times rarer and 10 times weaker

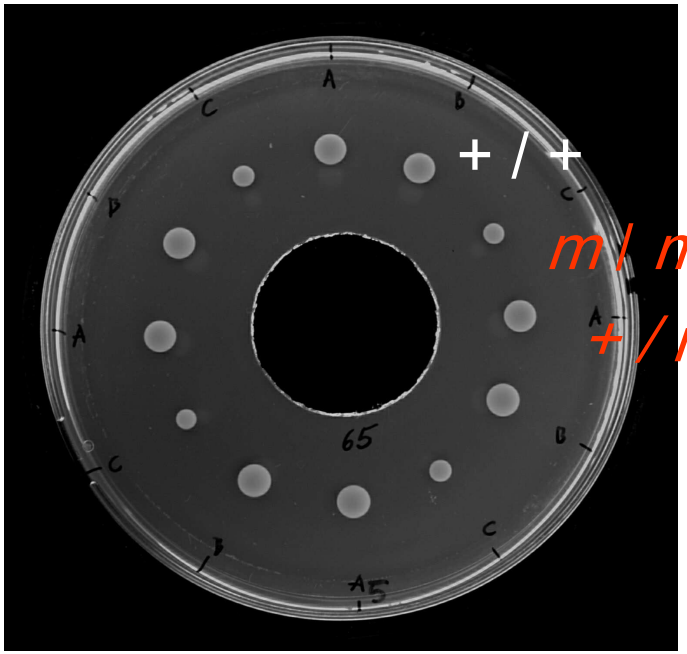


Wloch *et al*. (2001) *Genetics* 159:441

Random mutations - dominance

Wild-types, hetero- and homozytes were derived from strains known to carry *single* mutations:

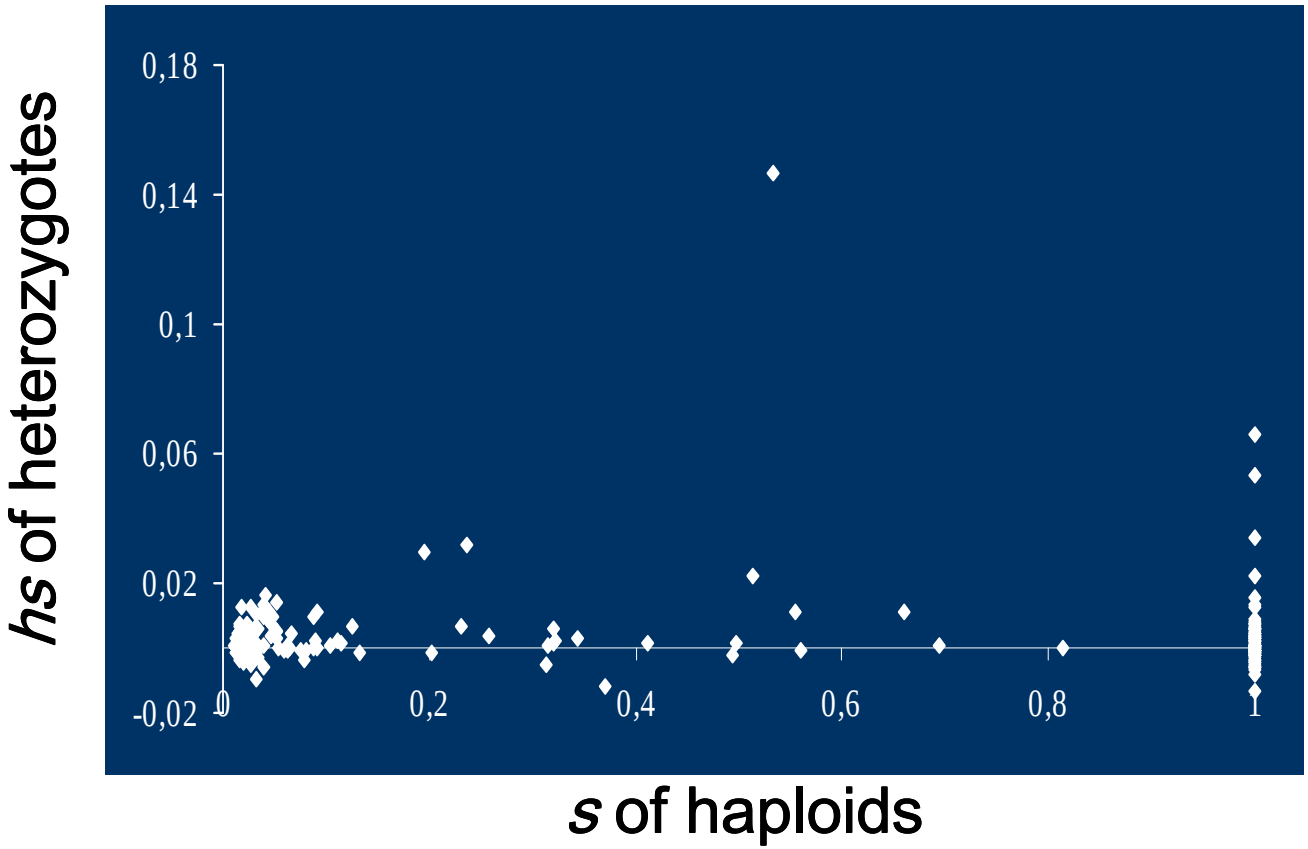
+ / +	$w = 1$
+ / *m*	$w = 1 - hs$
m / *m*	$w = 1 - s$



Fitness was calculated from the size of colony

Random mutations - dominance

Lethals and strongly harmful are much more recessive than modestly deleterious – **heterozygotes are uniform**

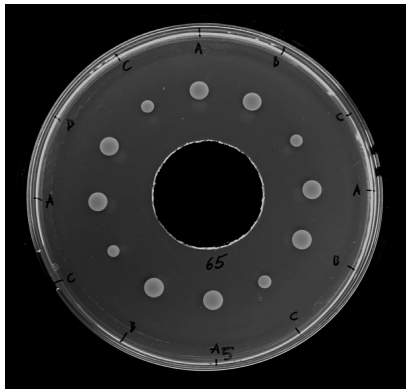


non-lethal:

mean *hs* = 0.0048

lethal:

mean *hs* = 0.0032

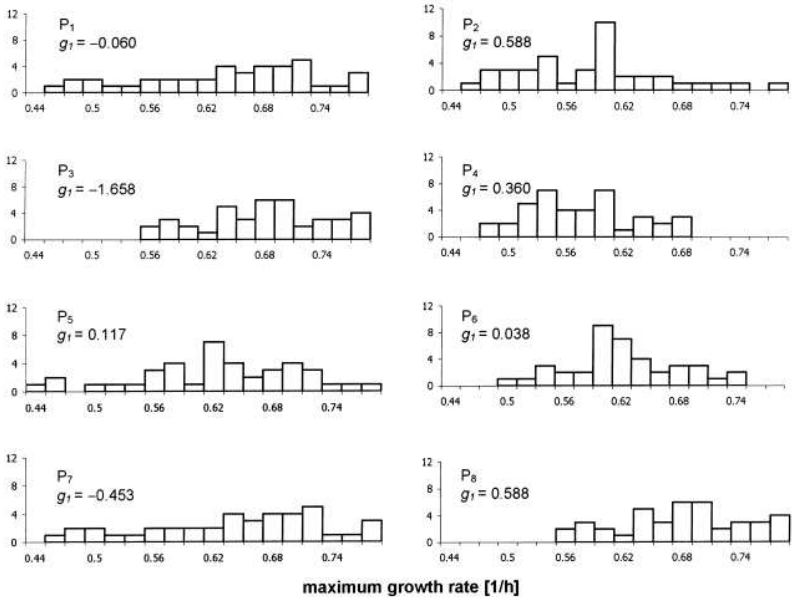


Szafraniec *et al*. (2003) *Genet Res* 82:19

Random mutations - epistasis

Interaction between multiple deleterious mutations would skew the distribution of fitness effects:

- to the left – if they reinforced one another
- to the right – if they alleviated one another



No clear overall tendency

Wloch et al. (2001) *J Evol Biol* 14:310

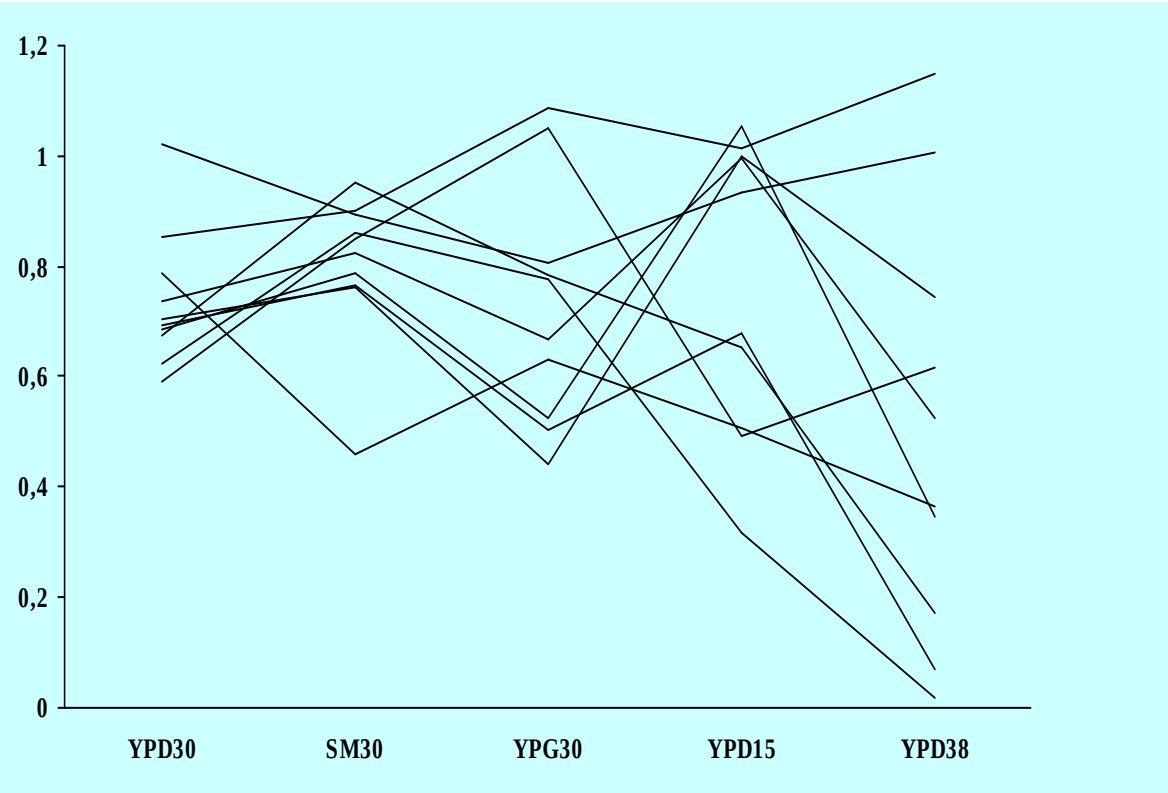
Random mutations - dominance and epistasis

Random mutations are recessive, recessivity incereases as selection coefficient increases.

Measurements of epistasis between multiple random mutations did not lead to conclusive results.

Random mutations – environmental stress

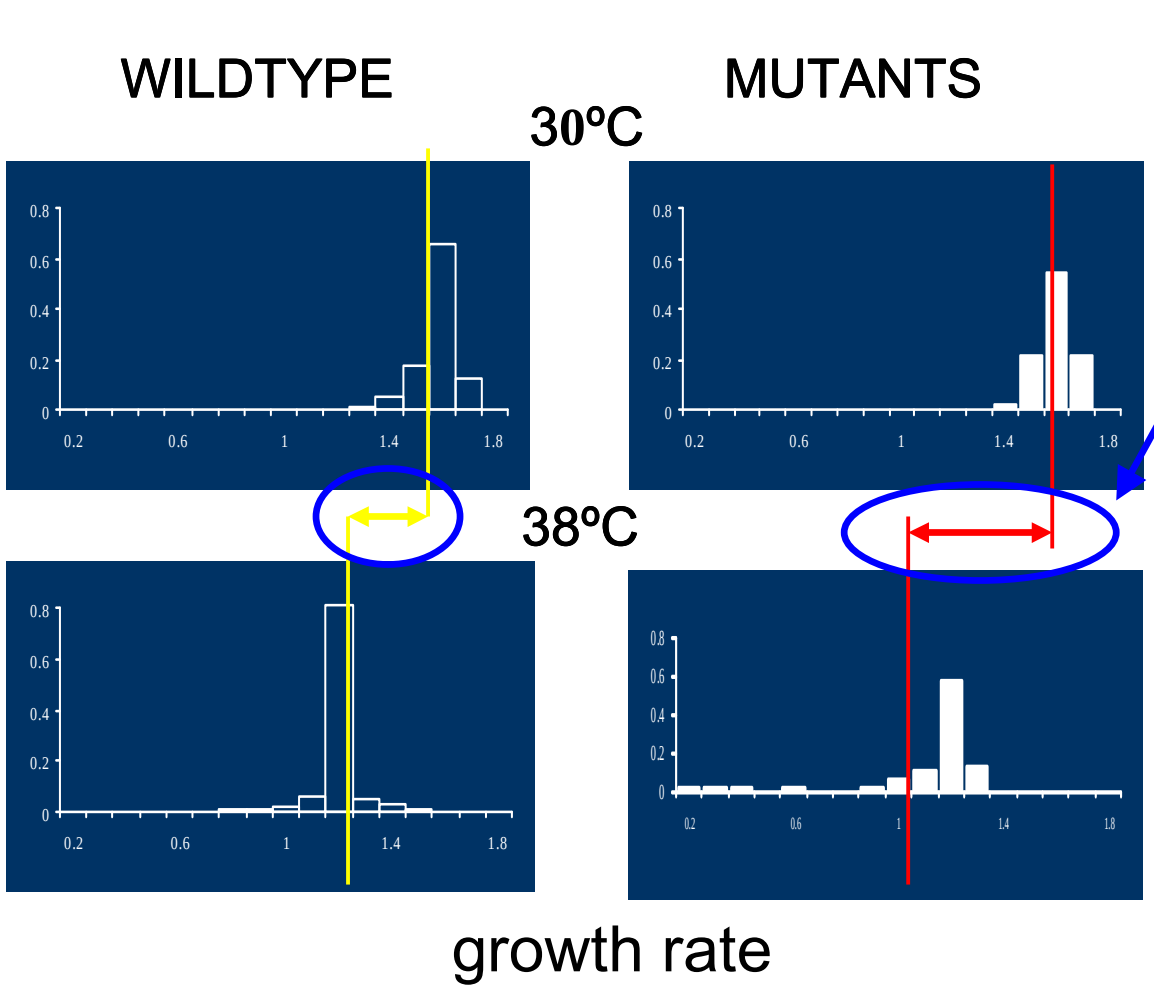
Multiple mutations accumulated in (haploid) mutator strains are increasingly visible as stress increases



Korona (1999)
Evolution 53:1966

Random mutations – environmental stress

Also multiple (heterozygous) mutations accumulated in diploid mutator strains are more harmful under thermal stress



Szafraniec *et al.* (2001)
PNAS USA 98:1107

Random mutations – environmental stress

Environmental stress enlarges loss of fitness observed in haploids and heterozygotes carrying random mutations

Caveat: these were multiple mutations (a few, dozens) with unknown location and molecular basis

Engineered gene deletions

Fitness effects of gene deletions

- frequency distribution
- dominance / recessivity
- epistasis (interactions between loci)
- impact of environment (stress)

Deletions - fitness effects

Yeast gene deletions under *normal conditions*

- 18% lethal
- 20 – 30% (mostly slightly) deleterious to growth
- 50% - no visible effects

(Giaever et al. 2002; Steinmetz et al.. 2002)

Much more effects were seen when *hundreds of different drugs* were tested (as specific environments)

(Hillenmeyer *et al*. 2008 Science 230:362)

Important general feature: deleterious effects of gene deletions are distributed *bimodally* (lethals and small effects), much like in case of point mutations

Deletions - deleterious fitness effects

We focused on the **bimodality of distribution** ...

... does it reflect a split between vital and unimportant functional modules of the cell ...

... or the the split is present in most functional subunits of the cell.

Deletions - deleterious fitness effects

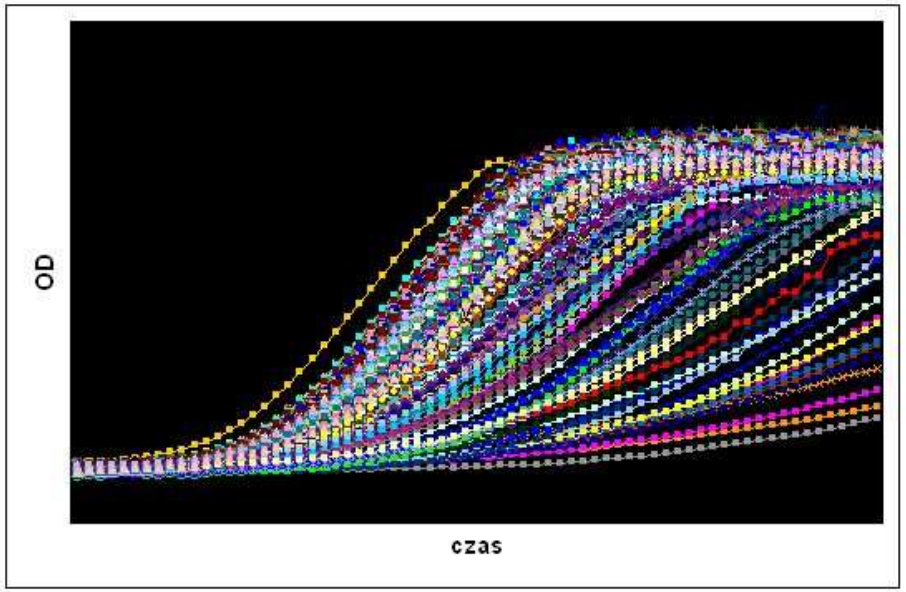
How to separate functional subunits ...

... a conservative (but safe) approach is to take large complexes of proteins as representation of functional subunits.

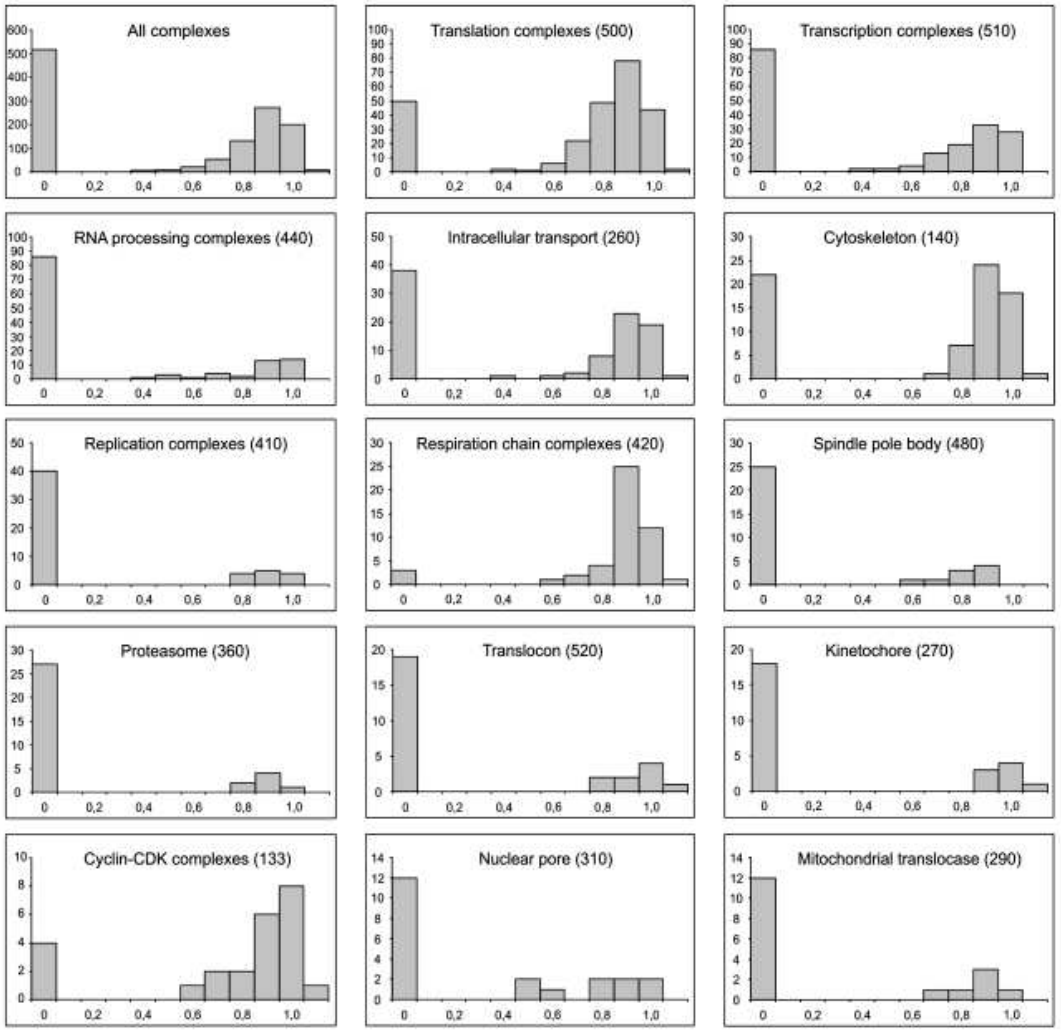
Deletions - deleterious fitness effects

We selected 38 largest protein complexes from MIPS database and assayed 701 non-essential gene deletions (fitness of 515 essentials was set to 0)

We estimated maximum growth rate by analysing individual growth curves.



Deletions - deleterious fitness effects



Bimodality was universal, present **within virtually all complexes** (functional subunits of the cell)

Fudala & Korona (2009) *Evolution* 63:2164

Deletions - beneficial fitness effects

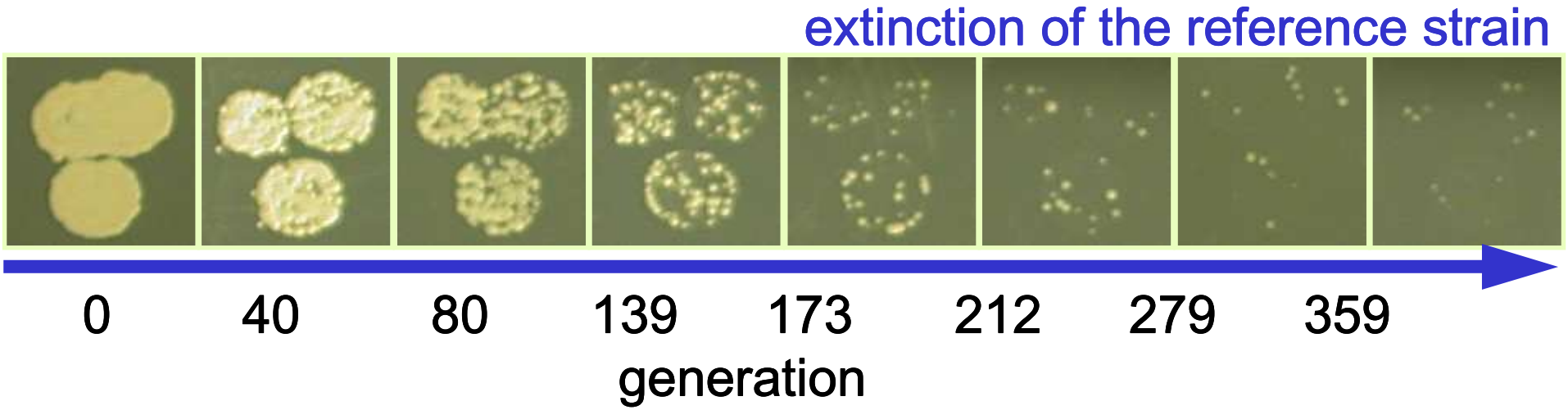
Are there any benefical effects of gene deletions? Should they be expected?

Yes, because in yeast grown under standard (luxury) conditions ...

- many genes appear „dispensable”
- thus, deletion of (some of) them could be advantageous

Deletions - beneficial fitness effects

Deletions were competed against a reference strain ....

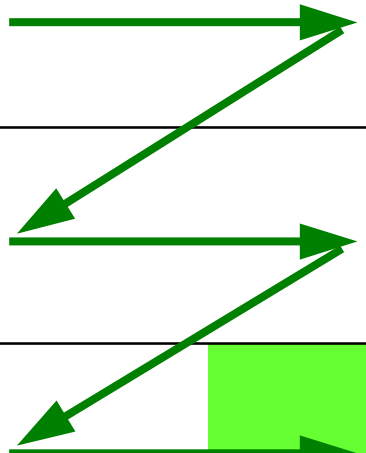


... extinction of the latter marked ascendance of a fitter strain (resolution no lower than 0.005 of relative fitness)

Deletions - beneficial fitness effects

Promising candidates were verified ...

Experiment	Number of deletions	Winners
first competition	4653	133
repeated competition	74 unique	24
de novo construction	24	12



Deletions - beneficial fitness effects

ORF/*gene*	no. of wins	Fitness advantage
YIL006W / *YIA6*	2	0.005
YJL215C / dubious	5	0.007
YLR104W / unchar.	4	0.008
YJL150W / dubious	1	0.009
YIL077C / unchar.	5	0.010
YHL014C / *YLF2*	1	0.010
YIL087C / unchar.	6	0.011
YGR035C / unchar.	6	0.011
YIL001W / unchar.	2	0.012
YLR207W / *HRD3*	3	0.012
YIL041W / *GVP36*	3	0.013
YNL027W / *CRZ1*	3	0.015

few and small adaptive effects (12 out of thousands)

only 5 real genes

no metabolic explanation

several non-coding

some specific for the s288c background adjustments of chromosomal elements ?

Deletions - beneficial fitness effects

Paucity (absence?) of advantageous deletions suggests that there is **no (little) selection pressure for genome downsizing** ...

... even if one lives in an extract of itself ...

... as does yeast in YPD (for several decades) ...

... much like an intracellular parasite.

Sliwa *et al*. (2005) *PNAS USA* 102:17670

Deletions - summary on fitness effects

Distribution of deleterious fitness effects of deletions:

- is strongly bimodal
- bimodality is present within cellular subsytems

Beneficial effects are (at best !!) 100x less frequent and 10x weaker

Very similar to random point mutations !

Deletions - dominance

We have not studied dominance of gene deletions ...

... because many studies showed that heterozygotes are are generally uniform (like out random mutations)

- it takes specific drugs to show altered growth of relatively few heterozygotes (haploinsufficiency)

Deletions - interactions

Not only our studies of random mutations could not establish whether their deleterious effects are generally reinforced or alleviated by interactions

- reinforcement: higher plants, fungi, viruses, algae

- alleviation: viruses, fungi, yeast, bacteria

- none or both: fruitflies, viruses, diploid yeast, bacteria

Reviewed: Kouyos et al. (2007) *TREE* 22:308
de Visser and Elena (2007) *Nat Genet Rev* 8:139

Deletions - interactions

We chose 750 deletions with growth defects

- marked half of them wtih *kan* the other half with *nat*
- crossed to derive wt, single, and double deletions
- estimated fitness as maximum growth rate *(m)*

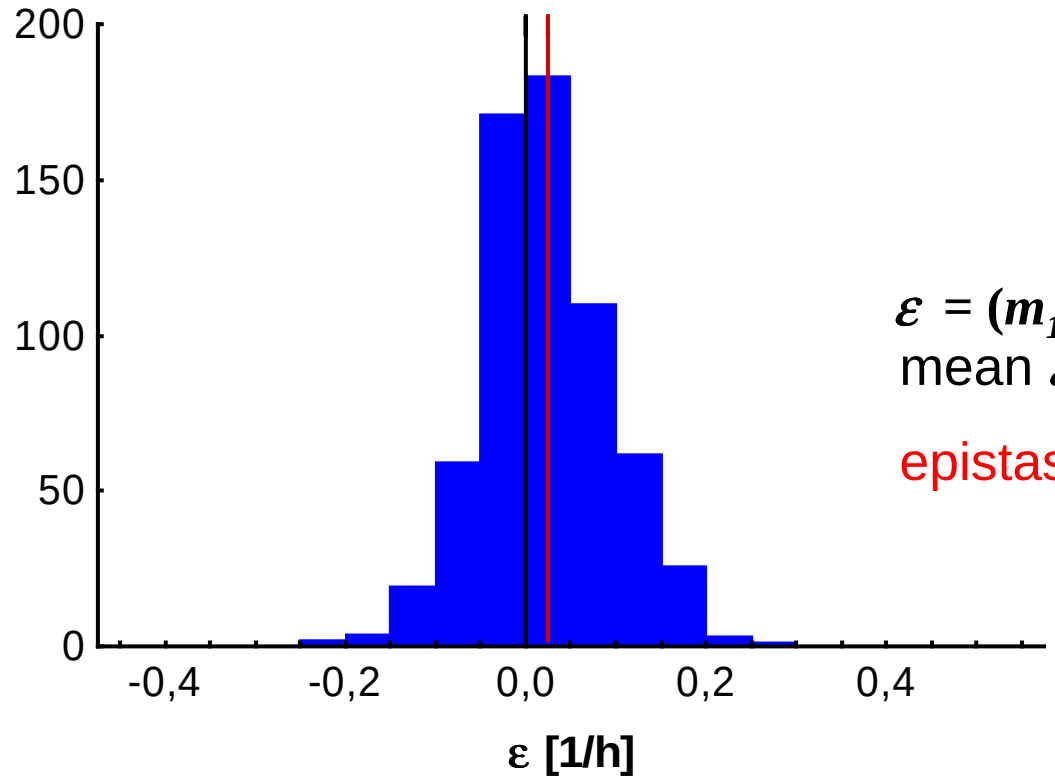
$$(m_{kn}+m) - (m_n+m_k) = \varepsilon$$

$\varepsilon = 0$ no interaction

$\varepsilon > 0$ alleviation

$\varepsilon < 0$ reinforcement

Deletions - interactions

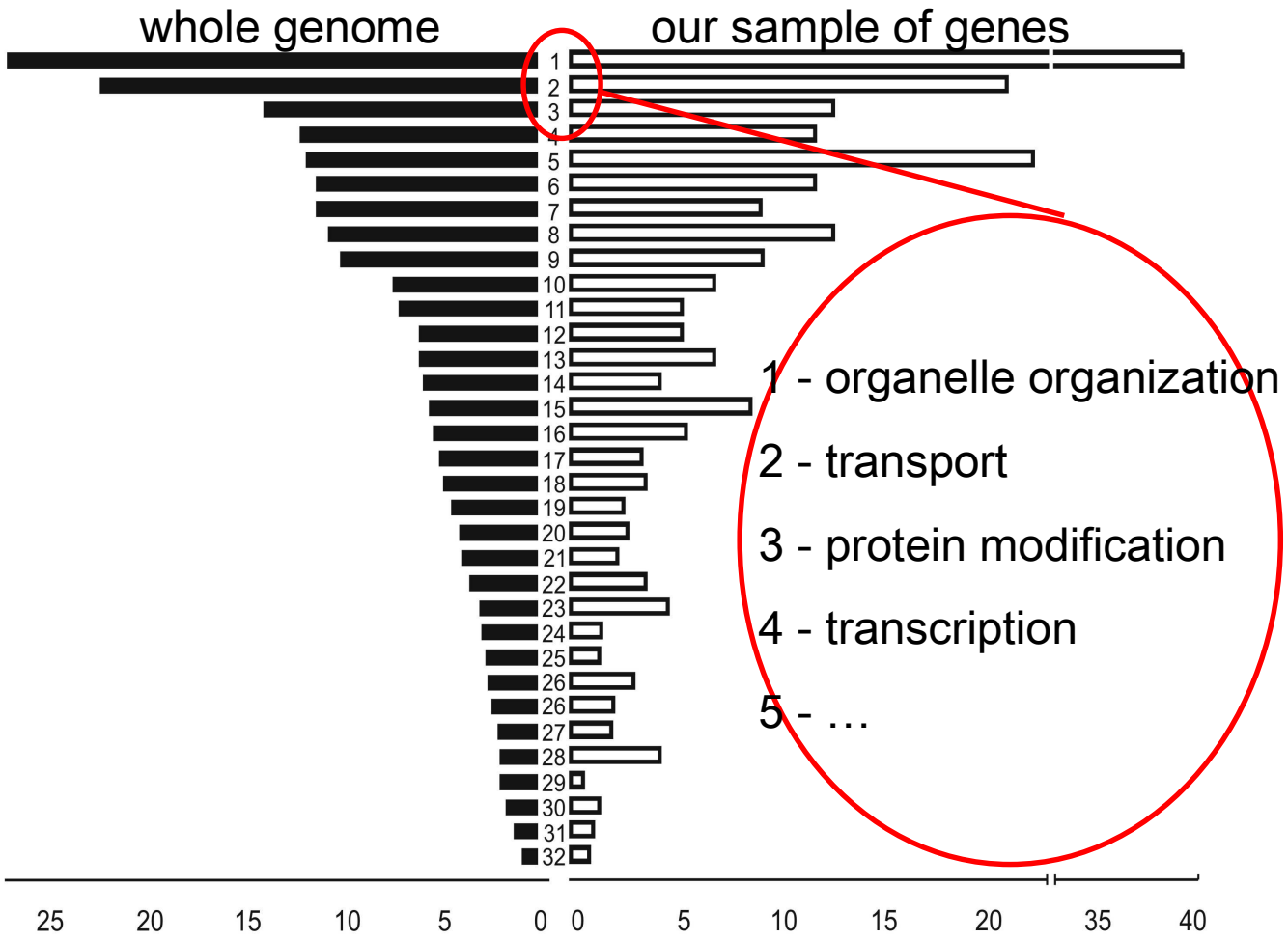


$\varepsilon = (m_{1,2}+m_{WT}) - (m_1+m_2)$

mean ε = 0.021, median = 0.015

epistasis is **positive** (alleviating)

Deletions - interactions



Our sample reflects the functional profile of the yeast cell

Deletions - interactions

There is consistent bias towards positive (alleviating) effect of epistasis between pairs of gene deletions

Studies of interaction between random mutations had much less statistical power and little (no) insight into functions

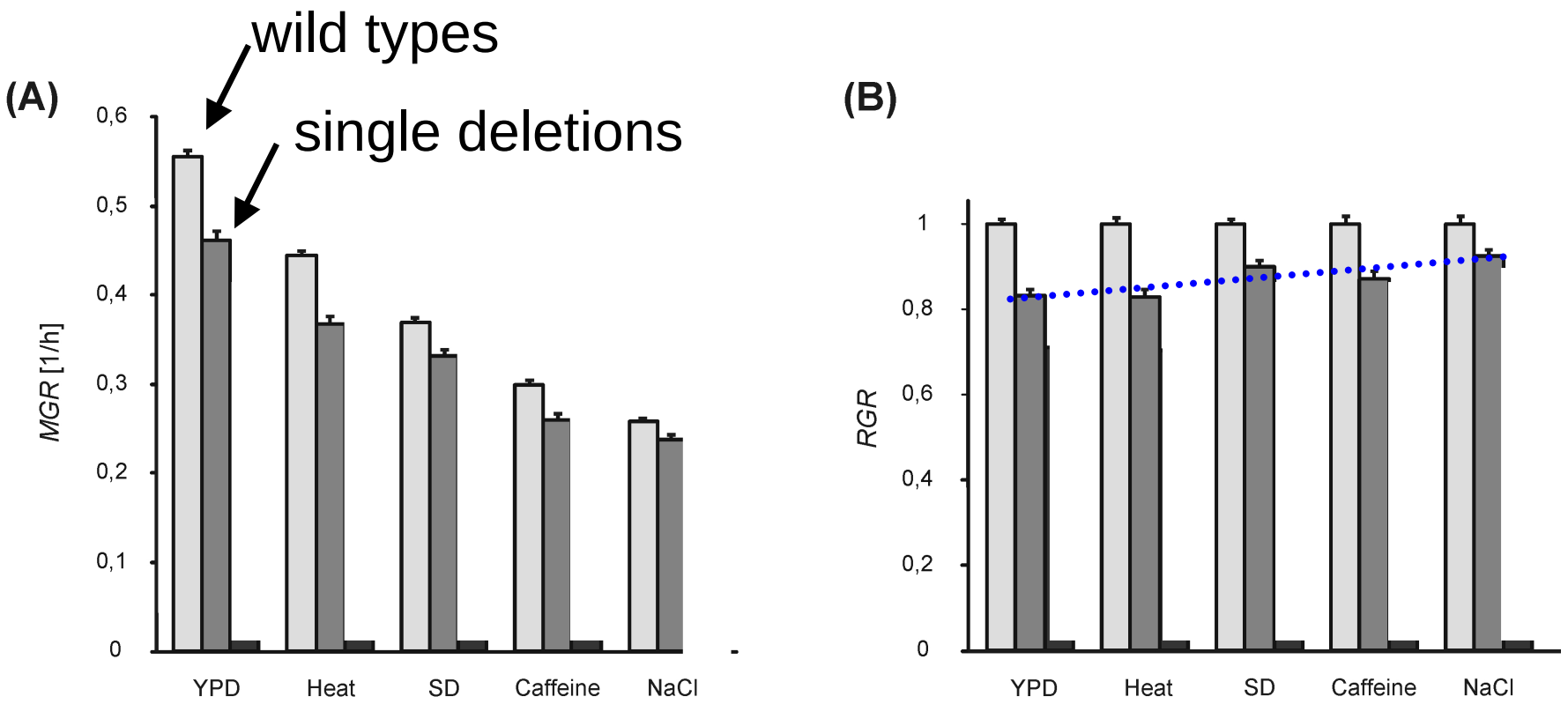
Jasnos & Korona (2007) *Nat Genet* 39:550

Deletions – environmental stress

Four stressful environments:

- **poor medium** (synthesis of aa and nucleotides)
- **high temperature** (protein destabilization)
- **saline** (osmotic and ionic stress)
- **caffeine** (deregulation of signalling pathways)

Deletions – environmental stress



Conversion of maximum growth rate (A) to relative growth rate (B) shows that deletions are ***less*** deleterious under stress !!

Jasnos et al. (2008) *Genetics* 178:2105-2111.

Deletions – environmental stress

Alleviation of deleterious mutations by stress was reported for bacteria (Kishony & Leibler 2003) but it apparently contradicts our results with random mutations.

Explanation:

- ***deletions*** mean complete loss of protein

- in contrast, ***point mutations*** cause destabilization of proteins and stress further further destabilizes structure enlarging functional damage

Conclusions

Several important characteristics of engineered deletions closely match those of random point mutations

Population genetics should use resuts obtained with systematic collections of mutants even though they are „not natural”

Differences are often easily interpretable in terms of complete versus incomplete loss of function

Most importantly, systematic study of mutants promises explanation instead of description ...

Conclusions

Some clarifications have already emerged:

- bimodality of deleterious effects is functionally universal

- loss-of-function mutations are rarely advantageous

- epistatic efects are predominantly positive/alleviating

- it can be the molecular basis of a mutation that determines whether stress strengthens or weakens its effect

References:

Fudala, A.and R. Korona. 2009. Low frequency of mutations with dtrongly deleterious but non-lethal fitness effects. *Evolution* 63:2164-2171.

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Szafraniec, K., D. M. Włoch, P. Sliwa, R. H. Borts and R. Korona. 2003. Small fitness effects and weak genetic interactions between deleterious mutations in heterozygous loci of the yeast *Saccharomyces cerevisiae*. *Genetical Research* 82: 19-31.

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Korona, R. 1999. Unpredictable fitness transitions between haploid and diploid strains of genetically loaded yeast *Saccharomyces cerevisiae. Genetics* 151:77-85.