Building a Metabolomic Bridge from Genotype to Phenotype
or
How *and* Why Do Things Fall Apart with Age?

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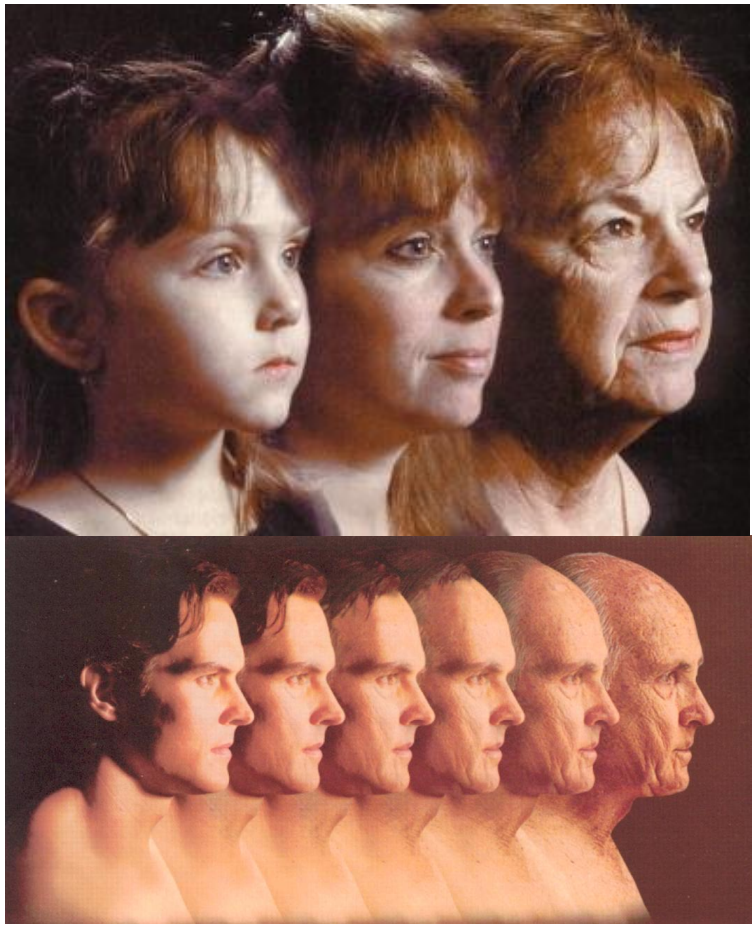
Two messages

1. By thinking about how cells (and organisms) fall apart, we can understand some things about how they are put together.
2. Analysis of the building blocks (the metabolome) can help us accomplish this.

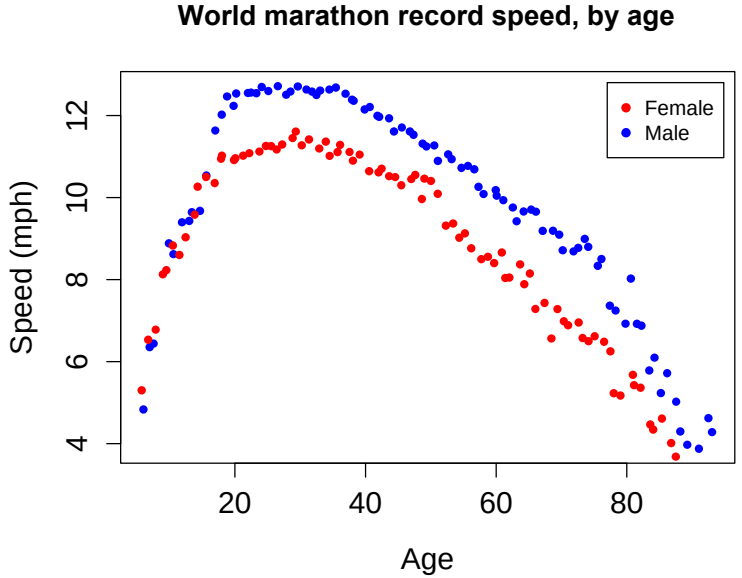
1. How (and why) do some species live longer than others?

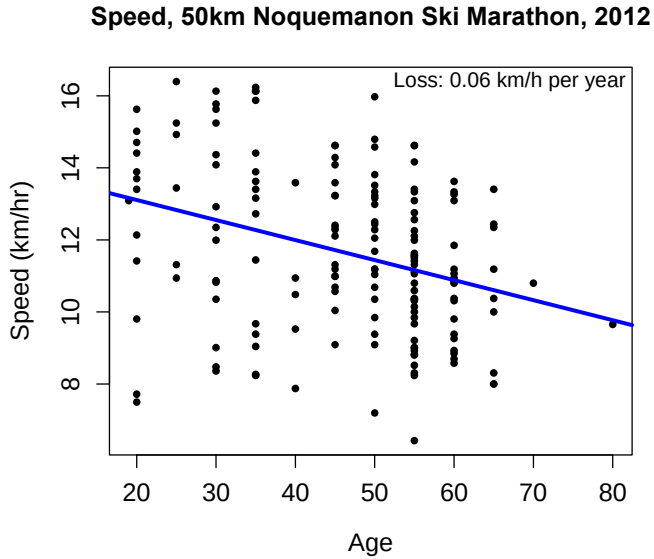
Short-lived **Long-lived**

2. How (and why) do some individuals age faster than others?

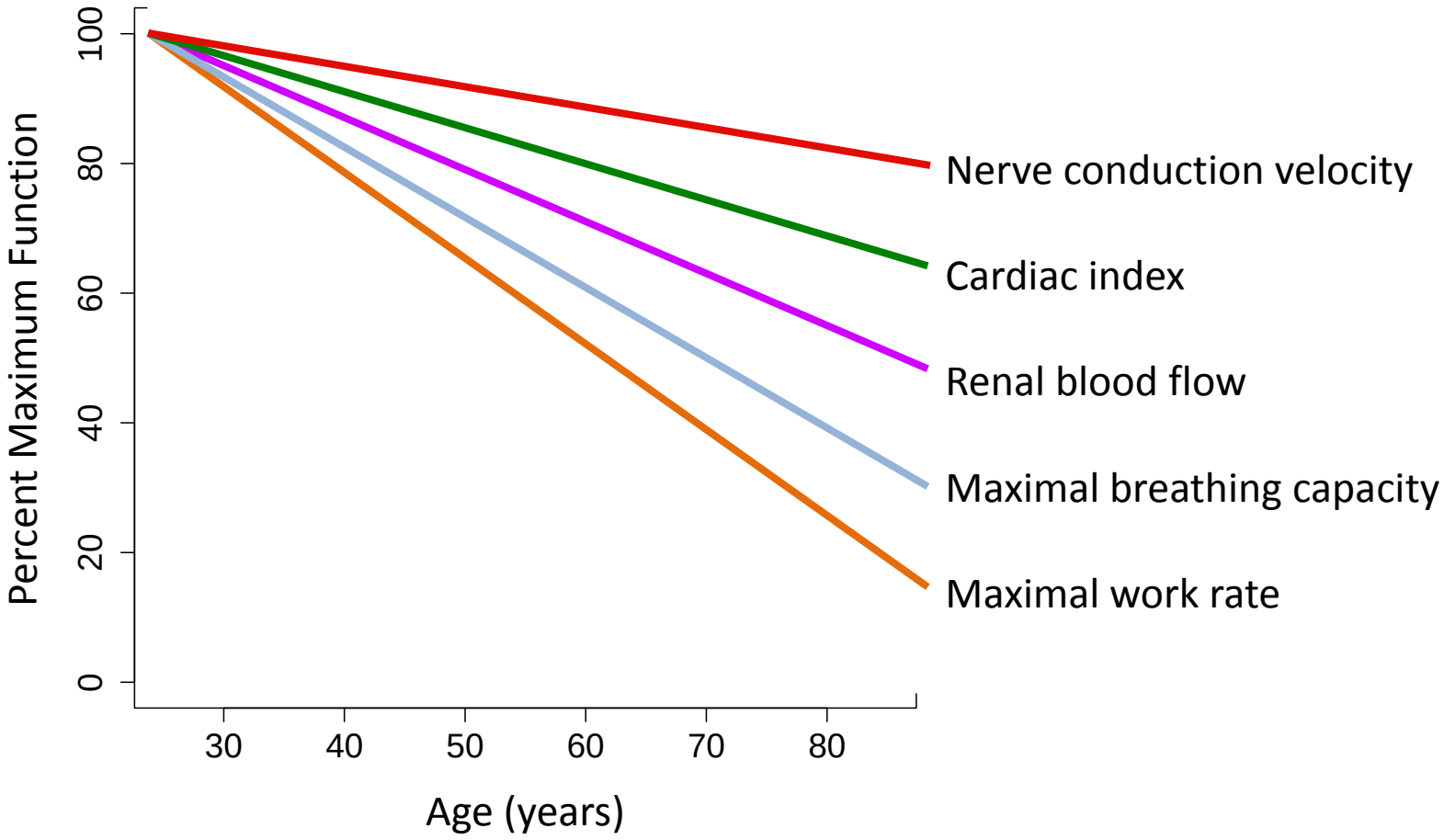


Data source: http://arrs.net/SA_Mara.htm



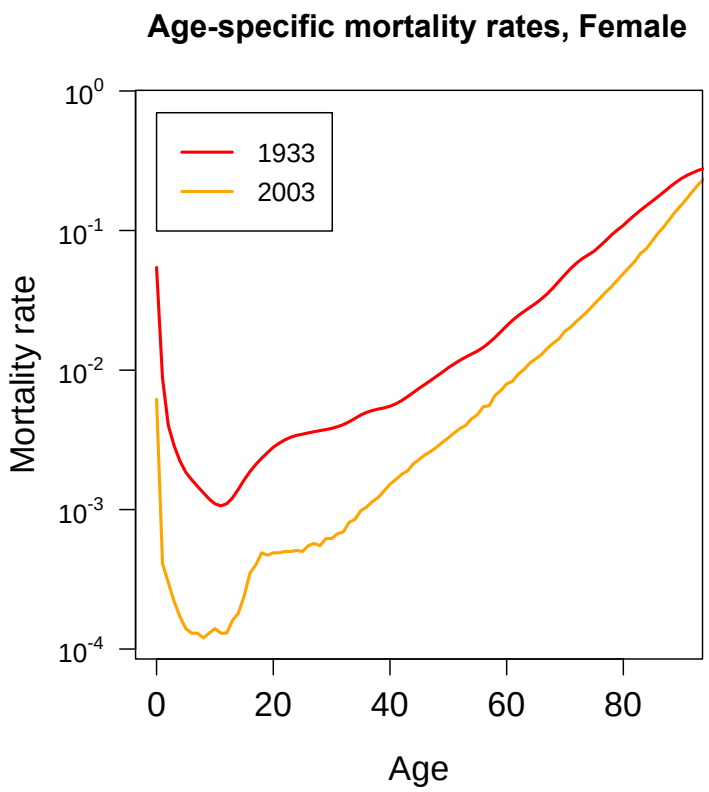


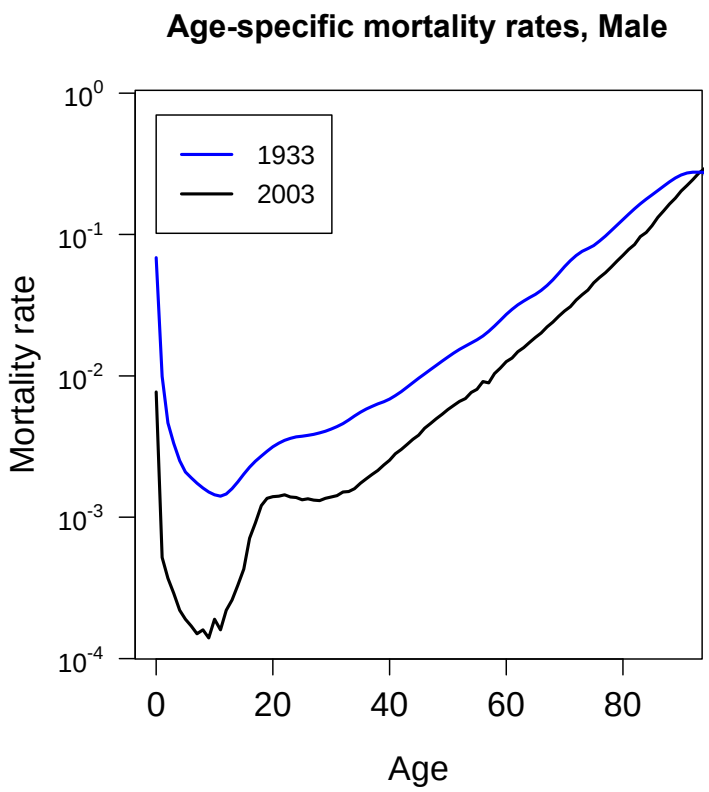
3. An *unasked* question: Why do some traits decline faster than others?



After Baker & Martin *in* Geriatric Medicine (ed. Cassel *et al.*, 1997)

Senescence: age-related decline in fitness components





The Human Mortality Database: http://www.mortality.org/

Julia Bell (1879-1979)

AT THE CAMBRIDGE UNIVERSITY PRESS

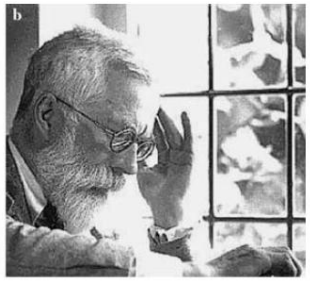
THE TREASURY OF HUMAN INHERITANCE.

Vol. II. Part II. (Nettleship Memorial Volume.) *Colour Blindness.* By Julia Bell, M.A., M.R.C.S., M.R.C.P. 143 pp. of Text, Chronological Bibliography of 425 titles, Figures of 235 pedigrees on 15 Plates and frontispiece portrait of John Dalton. *Price Forty-five shillings net.*

"The name of Edward Nettleship is among the great ones of ophthalmology. He was in the succession of Bowman, von Graefe and Donders, but his mind was of a philosophical cast, and he was particularly interested in hereditary defects, and comparatively early in life he retired from practice in order to devote himself entirely to the study of this subject. His pupils founded a medal in his honour, which, at his request, is awarded for the encouragement of scientific and ophthalmic work. His best memorial, however, has been erected by Professor Karl Pearson, who has devoted one of the fine volumes of *The Treasury of Human Inheritance* to the subject of hereditary diseases and anomalies of the eye, to stand as a Nettleship Memorial Volume and record of Nettleship's own work and that of his immediate students and friends. Part I, which was published in 1922...contained an account of the life of Nettleship and dealt with the subjects of retinitis pigmentosa and allied conditions, congenital stationary night-blindness, and glioma retinae. Part II, which is now published, is devoted to colour-blindness, and under the capable authorship of Dr Julia Bell exhibits to the full the high standard of workmanship that we are accustomed to expect of the Cambridge University Press. The volume, indeed, may be considered as an *édition de luxe*, which will give equal satisfaction to the bibliophile and the man of science. An excellent reproduction of C. Turner's engraving of the portrait of Dalton by Lonsdale forms an appropriate frontispiece, since Dalton was the first to give a scientific description of colour-blindness."—*British Medical Journal*

"Diese grosszügige, dem Gedächtnis Nettleships gewidmete Monographie bringt an der Hand einer 425 Nummern umfassenden Literatur und der Reproduktion von 235 Stammbäumen, die alle näher nach den Originalien erläutert werden, eine ausgezeichnete Darstellung der Erblichkeitsverhältnisse der angeborenen Farbenblindheit. Klar und schön ist auch die einleitende historische Darstellung der Lehre vom Farbensinn. Man kann die Verfasserin und den Verlag nur beglückwünschen zu dieser auch in der Form hervorragenden Leistung, der vollständigsten, die unser Fach besitzt und die dies für die Erblichkeitslehre so wichtige Kapitel in einer sehr erwünschten Weise zusammenfasst."

Prof. Axenfeld in *Klinische Monatsblätter f. Augenheilkunde*



Pearson, Fisher & Penrose

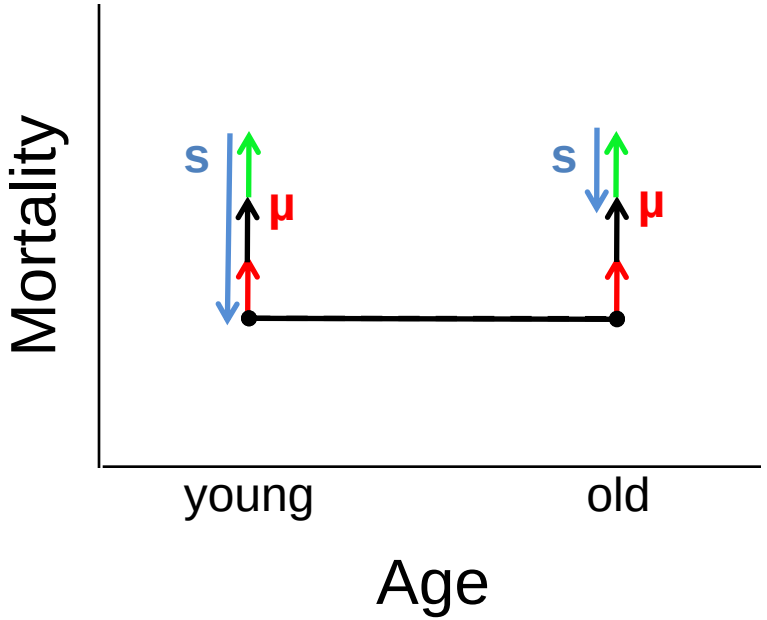
Haldane

Aging evolves due to maladaptation (Mutation Accumulation)

"… If a genetic disaster happens late enough in individual life, its consequences may be completely unimportant"

-An Unsolved Problem of Biology 1952

Peter Medawar 1946, 1952

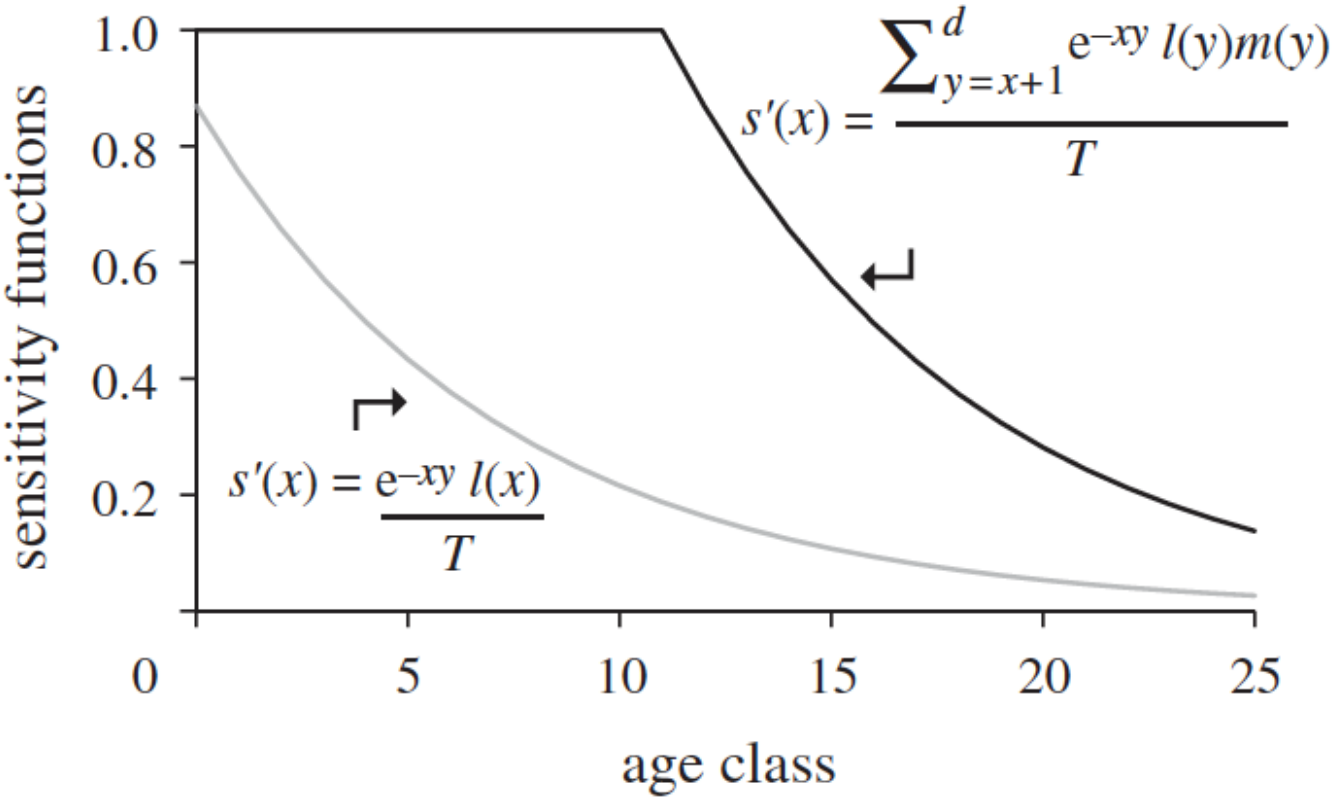


Mutations with uncorrelated or positively correlated effects across ages

J. Theoret. Biol. (1966) **12**, 12–45

The Moulding of Senescence by Natural Selection

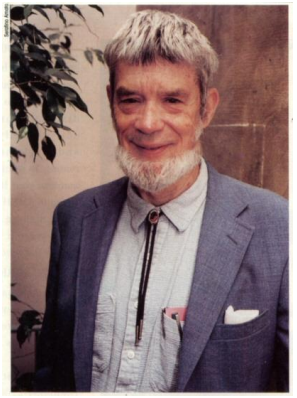
W. D. HAMILTON



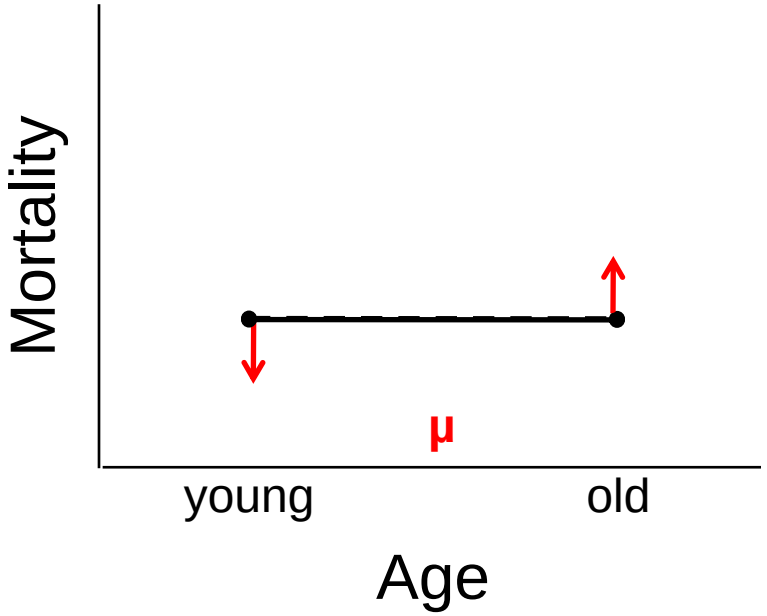
From K.A. Hughes 2010. Phil. Trans. Roy. Soc.

Aging evolves due to constraint (Antagonistic Pleiotropy)

Natural selection favors alleles that increase survival at early age at the expense of survival at late age



George C Williams 1957



Mutations with negatively correlated effects across ages

Outline

Evolution of cellular aging

1. Single-cell models of aging

Metabolomics and aging

2. Phylogenetic variation
3. Genetic variation
4. Diet Restriction and Networks

Single-celled organisms won't senesce

PLEIOTROPY, NATURAL SELECTION, AND THE EVOLUTION OF SENESCENCE [1]

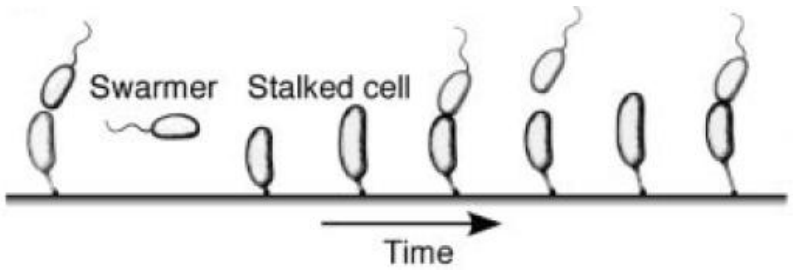
GEORGE C. WILLIAMS

Michigan State University

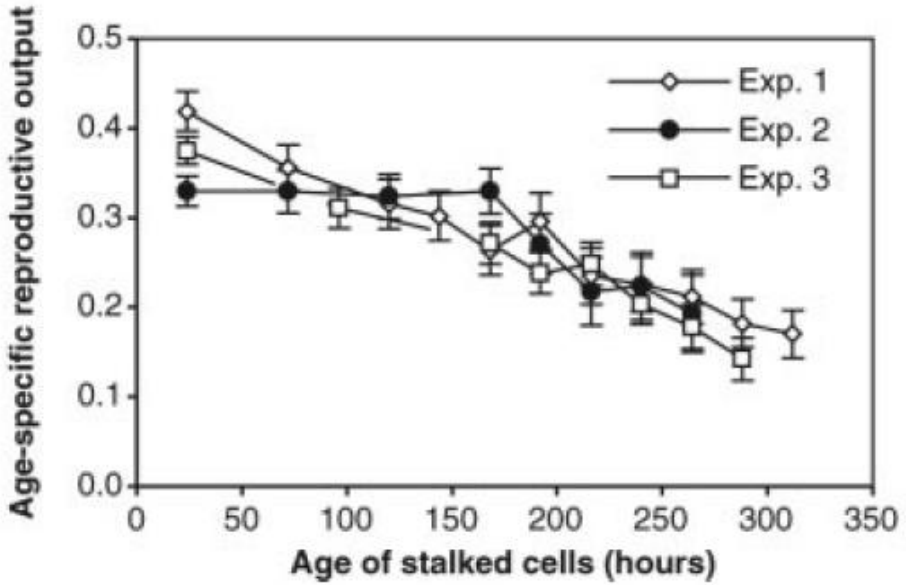
Received February 26, 1957

(1) *Senescence should be found wherever the conditions specified in the theory are met, and should not be found where these conditions are absent.* There are organisms in which the distinction between soma and germ-plasm may not exist, but the other assumptions of the theory would seem to be inevitable for any organism, at least for any that has a clear distinction between soma and germ-plasm. The theory regards senescence as an evolved characteristic of the soma. We should find it wherever a soma has been evolved, but not elsewhere.

Bacteria senesce I

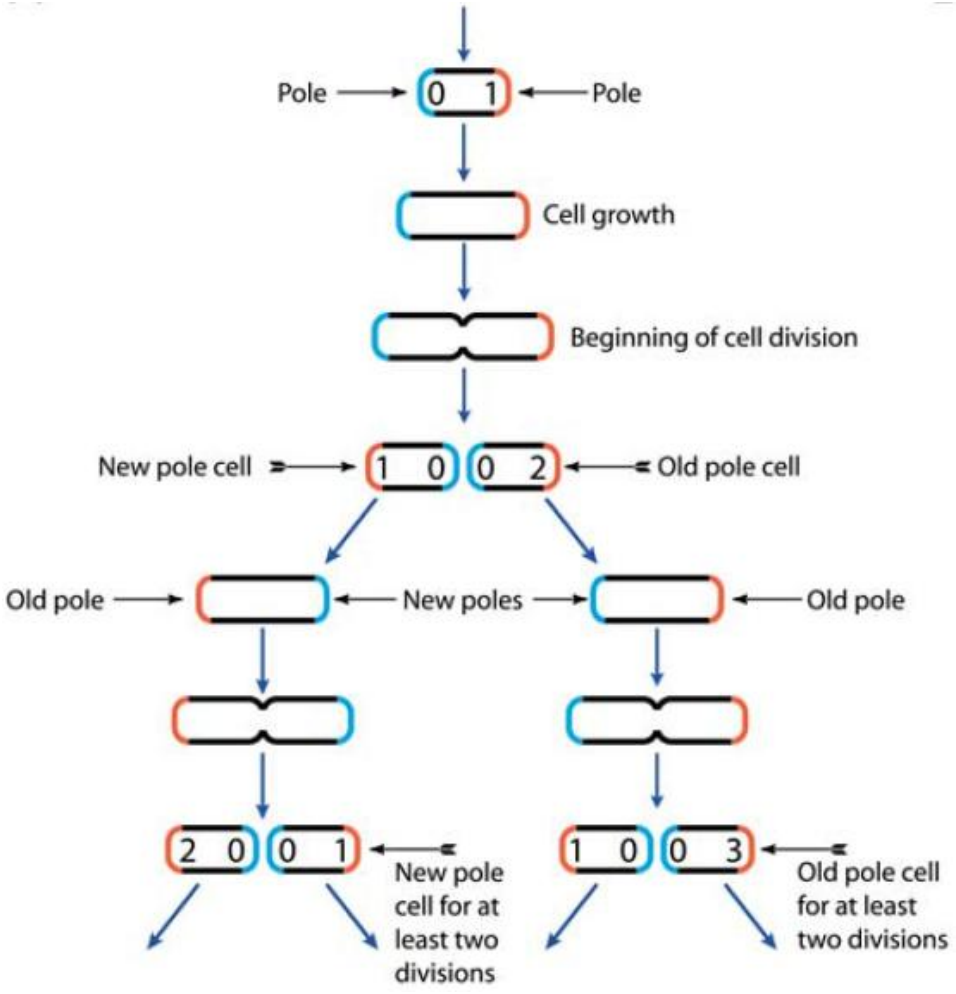


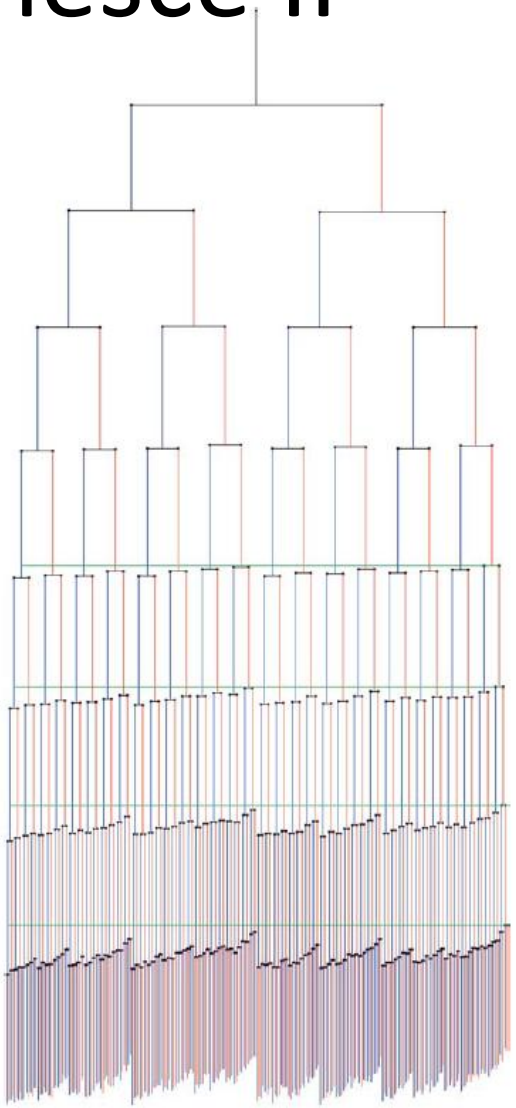
Life cycle of *Caulobacter crescentus*



Ackermann *et al*. 2003. Science

Bacteria senesce II

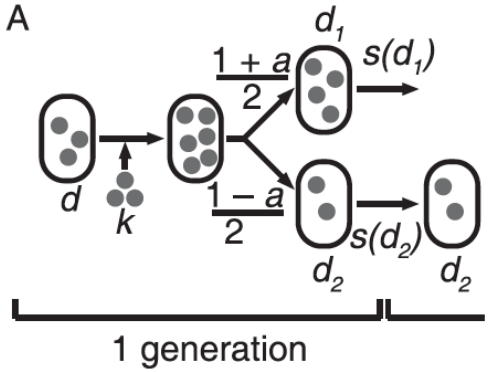


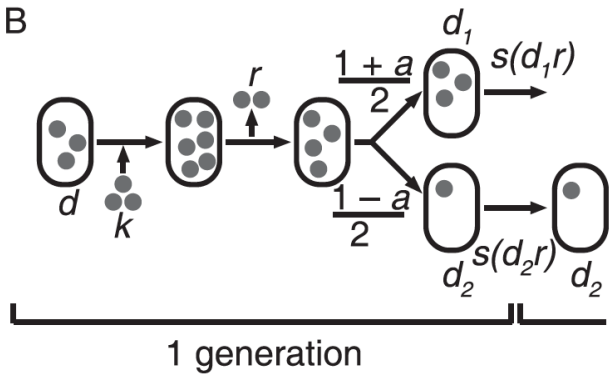


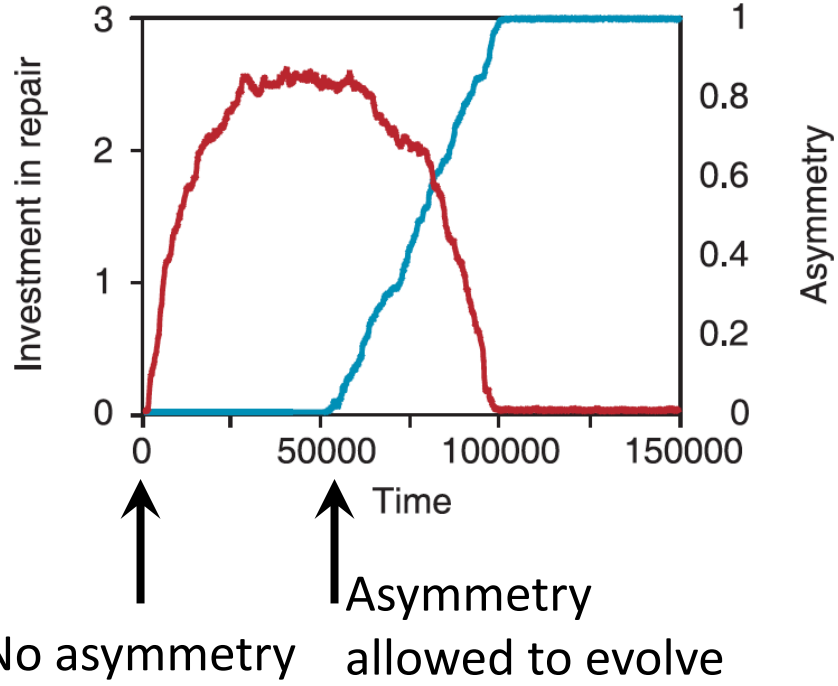
Old pole—red
New pole--blue

Stewart *et al*. 2005. PLoS Biology

Bacteria senesce—Theory

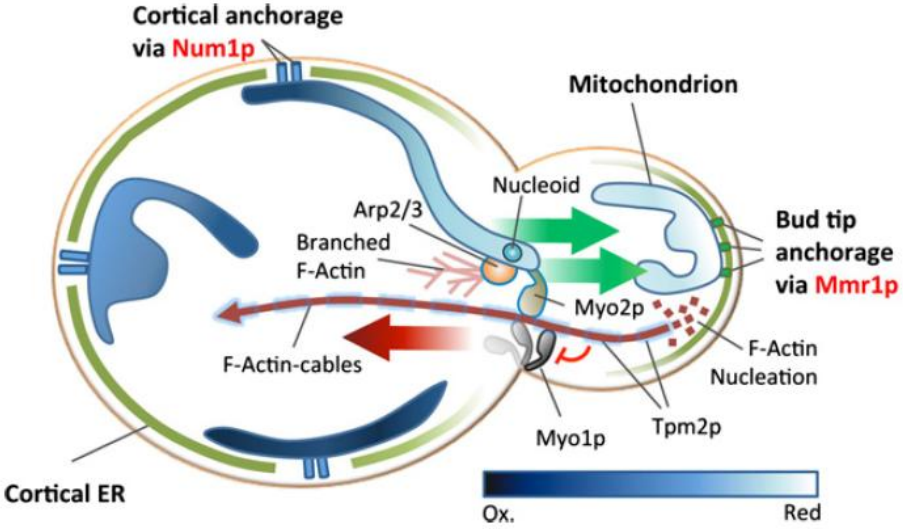


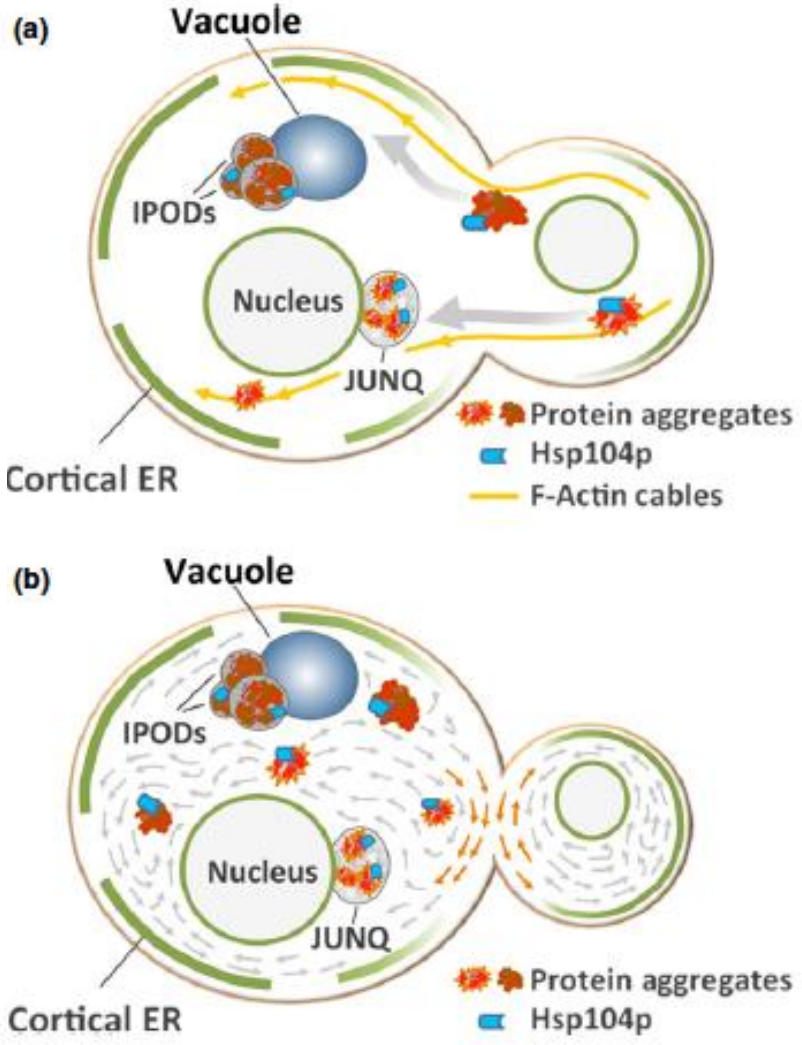




Ackermann *et al*. 2007. Aging Cell

Retrograde flow and selection





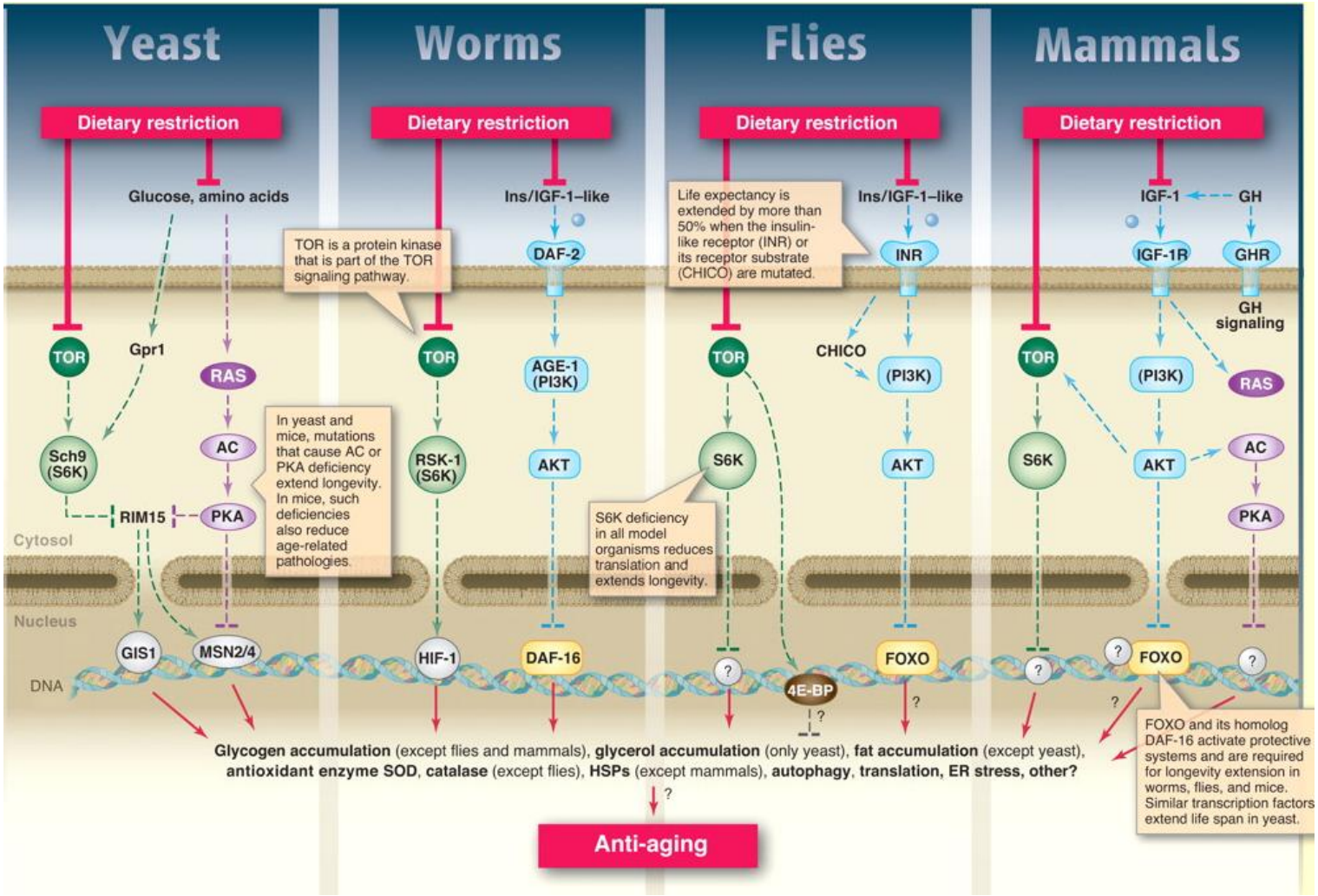
Higuchi-Sanabria *et al*. 2014. FEMS Yeast Res.

Cell

Leading Edge
Review

The Hallmarks of Aging

Carlos López-Otín,[1] Maria A. Blasco,[2] Linda Partridge,[3,4] Manuel Serrano,[5,*] and Guido Kroemer[6,7,8,9,10]



L Fontana et al. Science 2010;328:321-326

Historical Conclusions

Based on evolutionary and molecular studies:

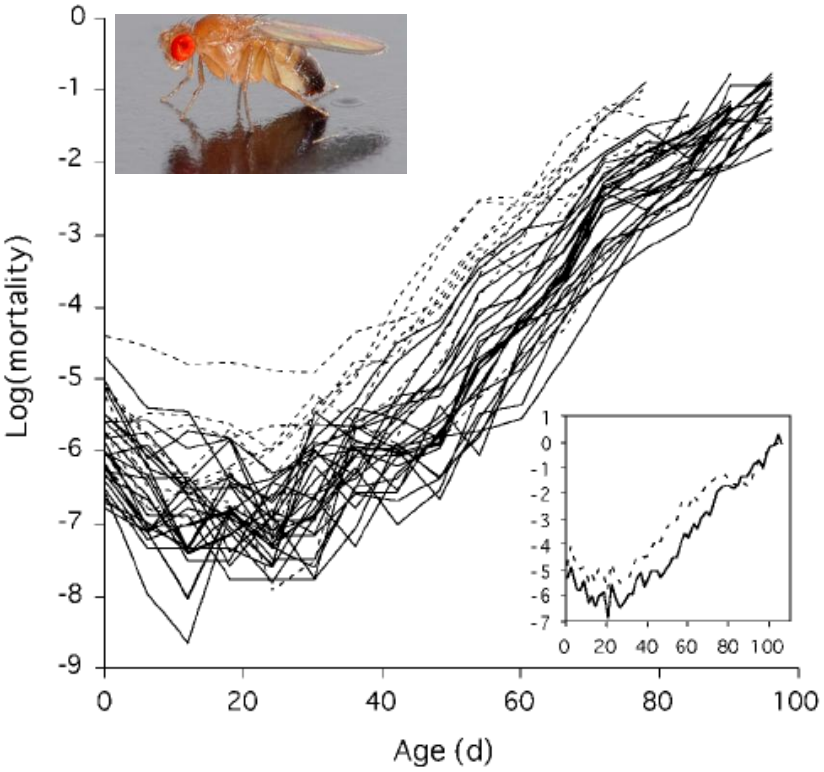
- Aging has a genetic basis
- Longevity influenced by genes with major effects
- Gene pathways and cellular targets of aging appear to be evolutionarily conserved

Challenge:

- Can we translate lab findings to the real world?

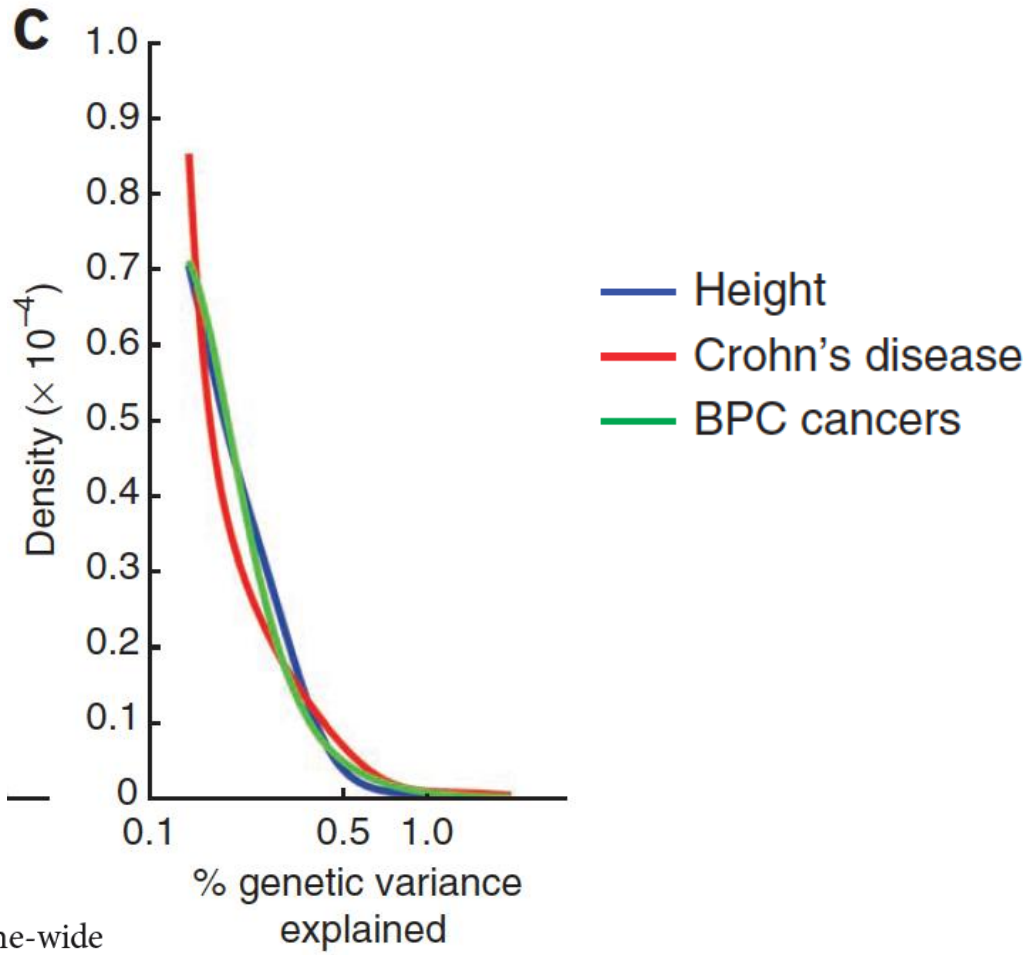
Natural genetic variation

Lifespan is variable, heritable and polygenic



Snoke & Promislow, 2003

Lab—genes of major effect
Nature—genes of minor effect

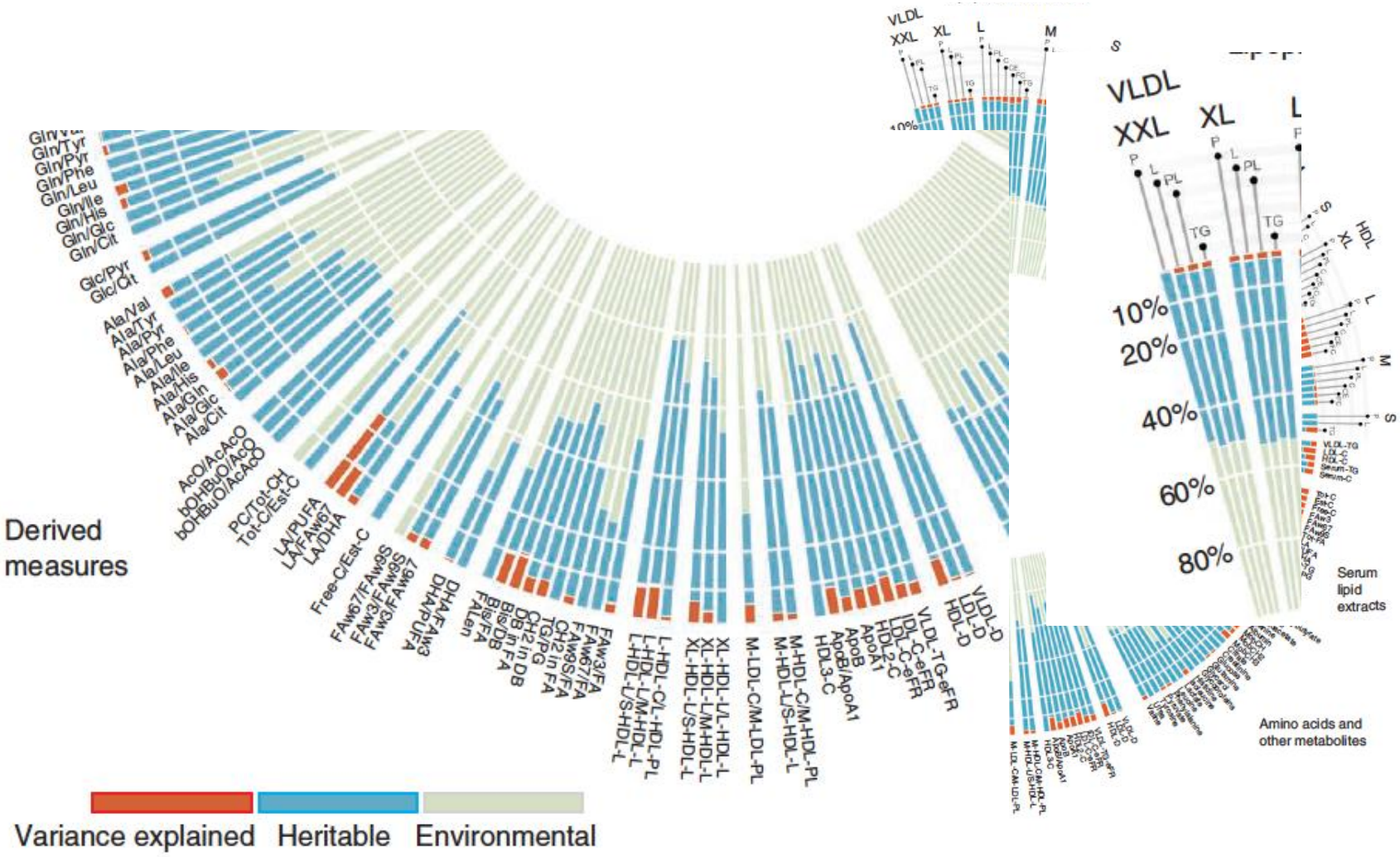


Estimation of effect size distribution from genome-wide association studies and implications for future discoveries

Ju-Hyun Park[1], Sholom Wacholder[1], Mitchell H Gail[1], Ulrike Peters[2], Kevin B Jacobs[3], Stephen J Chanock[1,3] & Nilanjan Chatterjee[1]

VOLUME 42 | NUMBER 7 | JULY 2010 NATURE GENETICS

Genome → Metabolome



Kettunen *et al*. Nature Genetics 2012

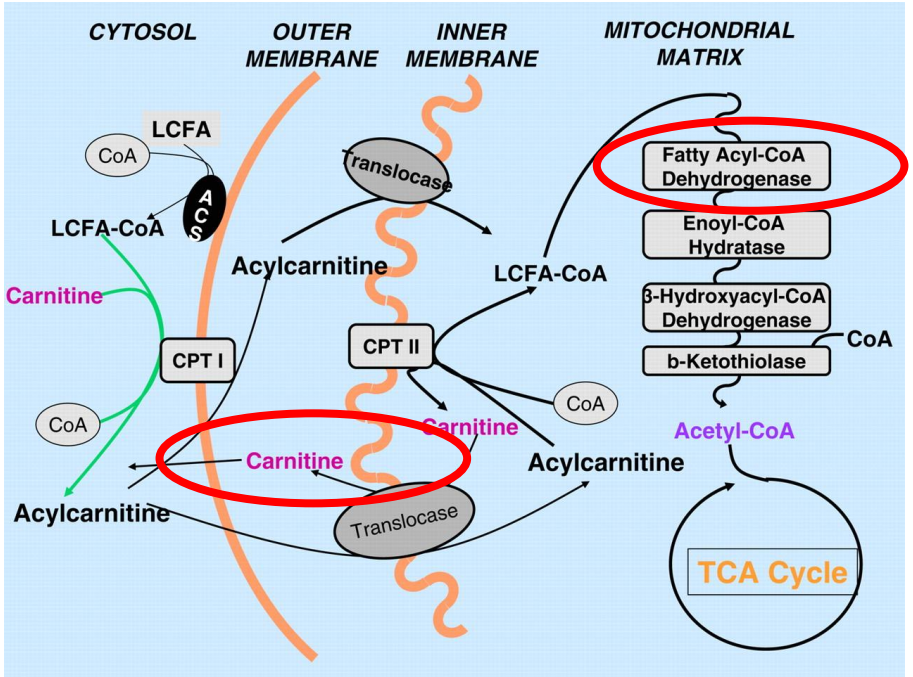
Human metabolic individuality in biomedical and pharmaceutical research

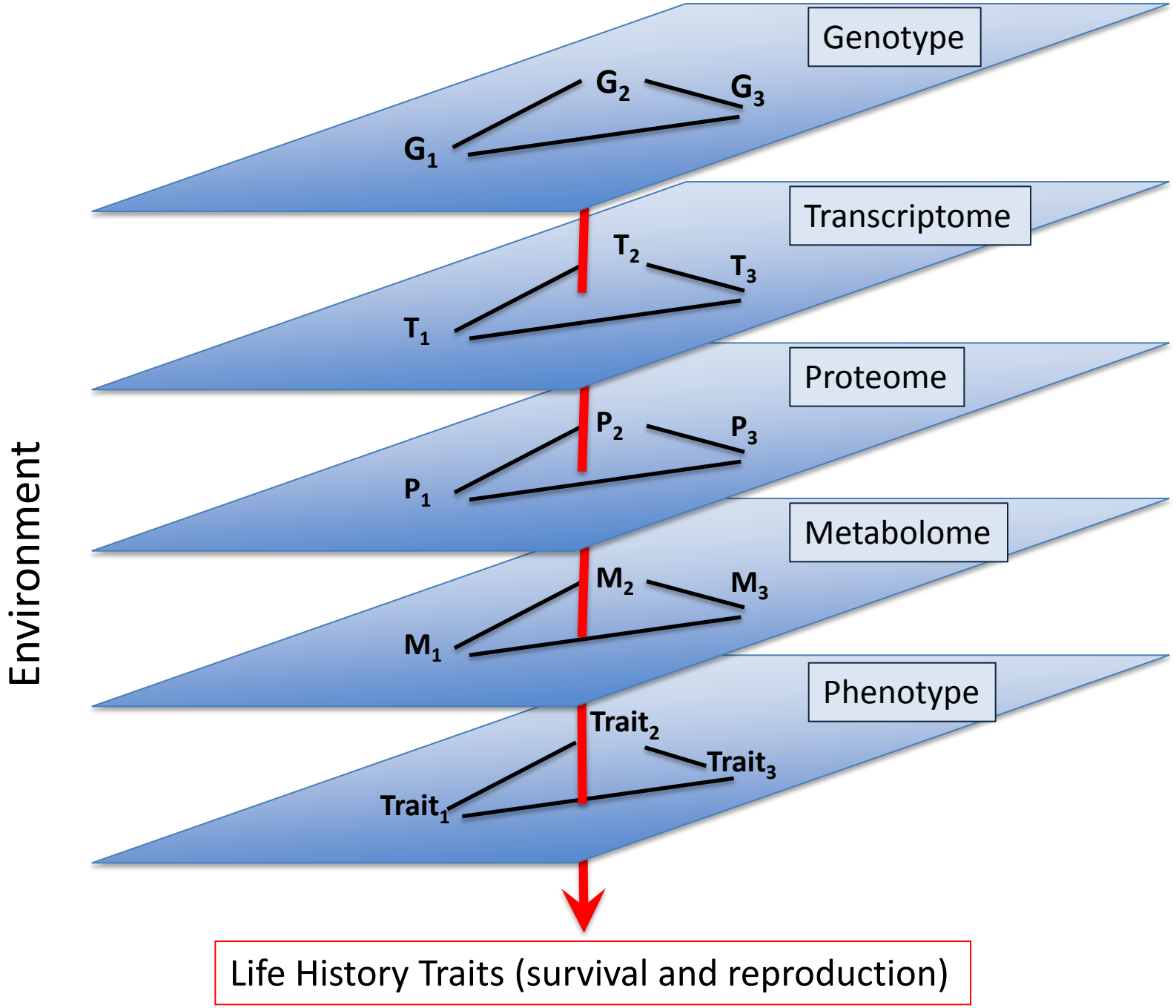
Karsten Suhre[1,2,3], So-Youn Shin[4]*, Ann-Kristin Petersen[5]*, Robert P. Mohney[6], David Meredith[7], Brigitte Wägele[1,8], Elisabeth Altmaier[1], CARDIoGRAM†, Panos Deloukas[4], Jeanette Erdmann[9], Elin Grundberg[4,10], Christopher J. Hammond[10], Martin Hrabé de Angelis[11,12], Gabi Kastenmüller[1], Anna Köttgen[13], Florian Kronenberg[14], Massimo Mangino[10], Christa Meisinger[15], Thomas Meitinger[16,17], Hans-Werner Mewes[1,8], Michael V. Milburn[6], Cornelia Prehn[11], Johannes Raffler[1,2], Janina S. Ried[5], Werner Römisch-Margl[1], Nilesh J. Samani[18], Kerrin S. Small[10], H.-Erich Wichmann[19,20,21], Guangju Zhai[10], Thomas Illig[22], Tim D. Spector[10], Jerzy Adamski[11,12], Nicole Soranzo[4]* & Christian Gieger[5]*

NATURE | VOL 477 | 1 SEPTEMBER 2011

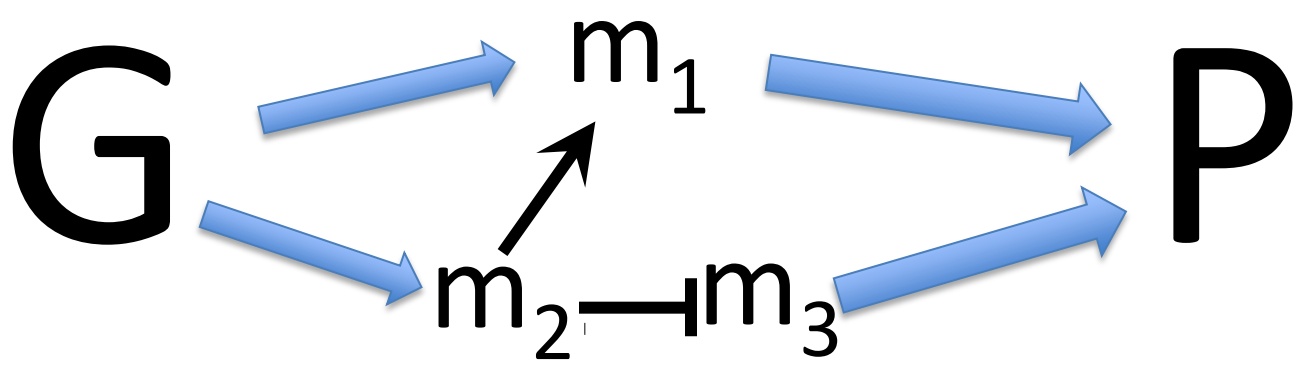
for diabetes and cardiovascular disease. In all except three loci, the SNPs are common, with minor allele frequencies greater than 10%. In 25 cases, the effect size per allele copy is larger than 10%, and up to 60% in the case of the acyl-CoA dehydrogenase (*ACADS*) locus.

ACADS rs2066938	Butyrylcarnitine/propionylcarnitine	$< 4.4 \times 10^{-305}$




Genotype
G2
G3
G1
Transcriptome
T2
T3
T1
Proteome
P2
P3
P1
Metabolome
M2
M3
M1
Phenotype
Trait2
Trait3
Trait1
Environment
Life History Traits (survival and reproduction)


G
P


G
m1
m2
m3
P

Metabolomics and Aging

1. Phylogenetic variation
   - evolution of the metabolome
2. Genetic variation
   - Genetics, metabolomics, and aging
3. Environmental variation
   - Diet restriction and metabolomic networks

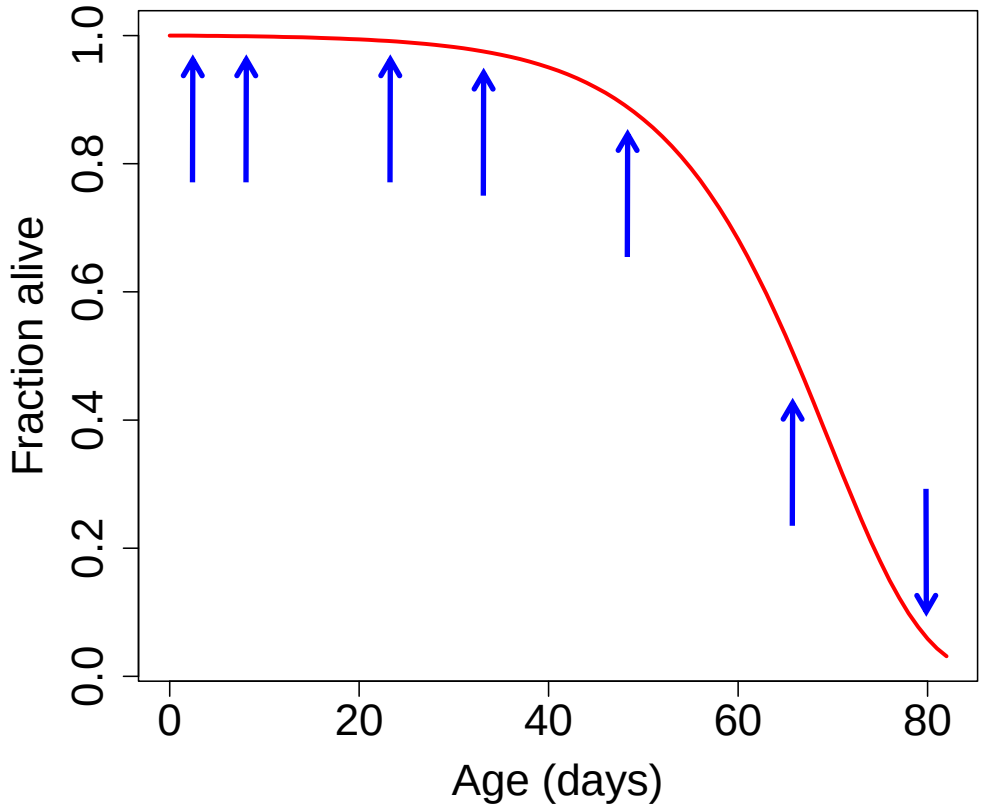


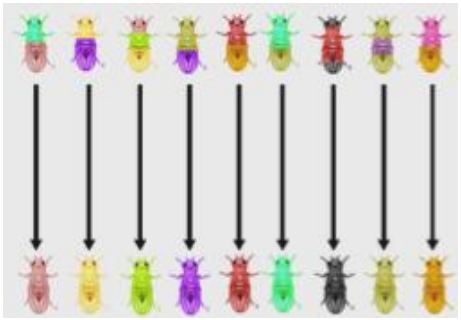

Metabolomics and Aging

1. Evolution of the metabolome
2. Genetics, metabolomics, and biomarkers of aging
3. Diet restriction and metabolomic networks




Effect of aging on the metabolome in the *Drosophila* Genome Reference Panel?



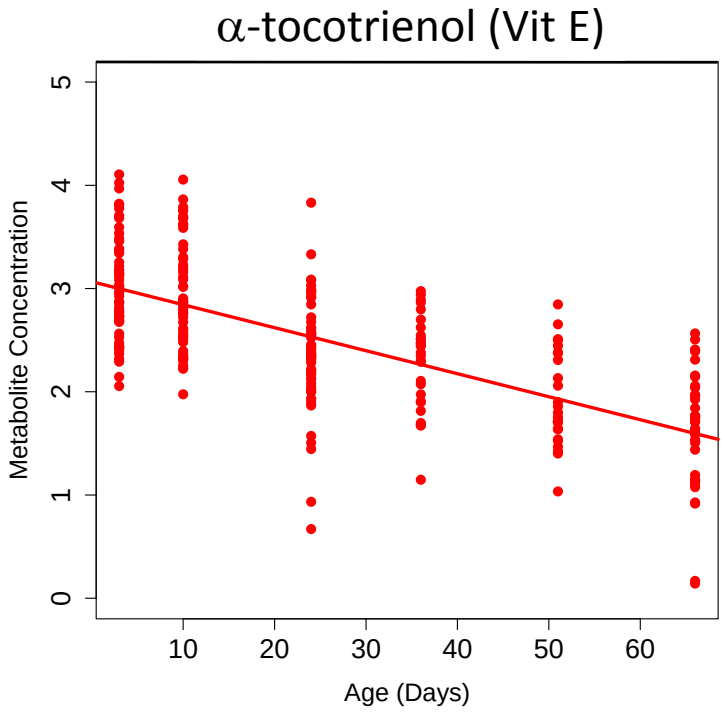


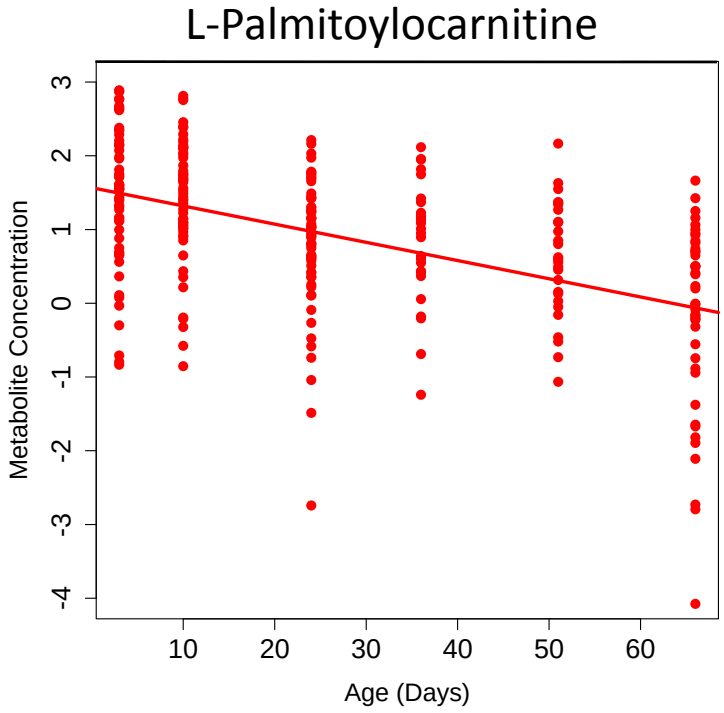
Assay three key factors that affect survival:

- Sex
- Age (7 ages)
- Genotype (15 inbred lines)
- Biological and technical replicates (~600 samples)

Hoffman *et al*. 2014. Aging Cell

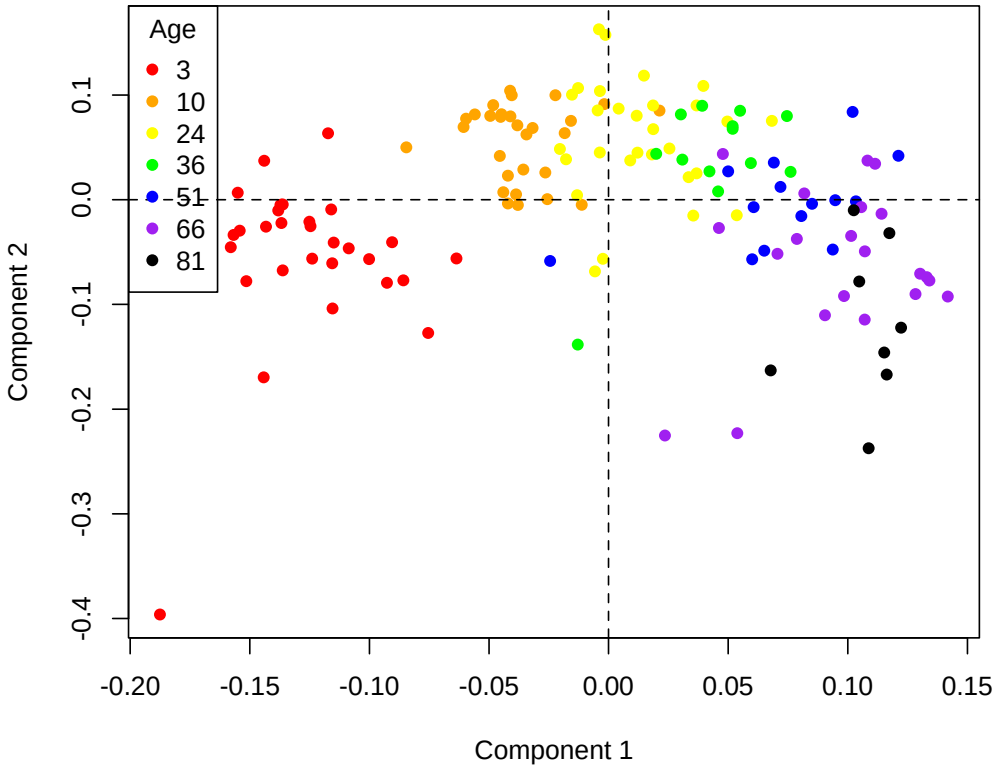
Effects of age

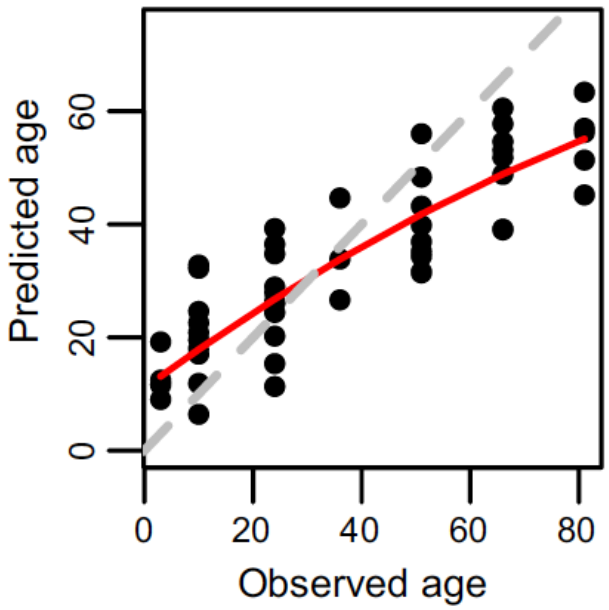




Hoffman *et al*. 2014. Aging Cell

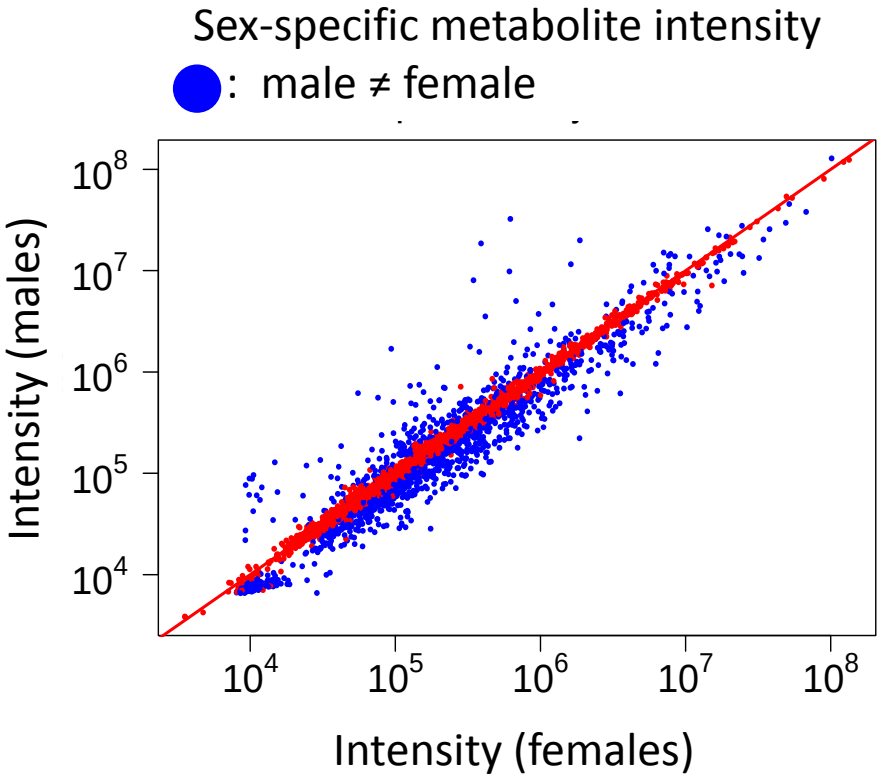
The *Drosophila* metabolome is age-specific

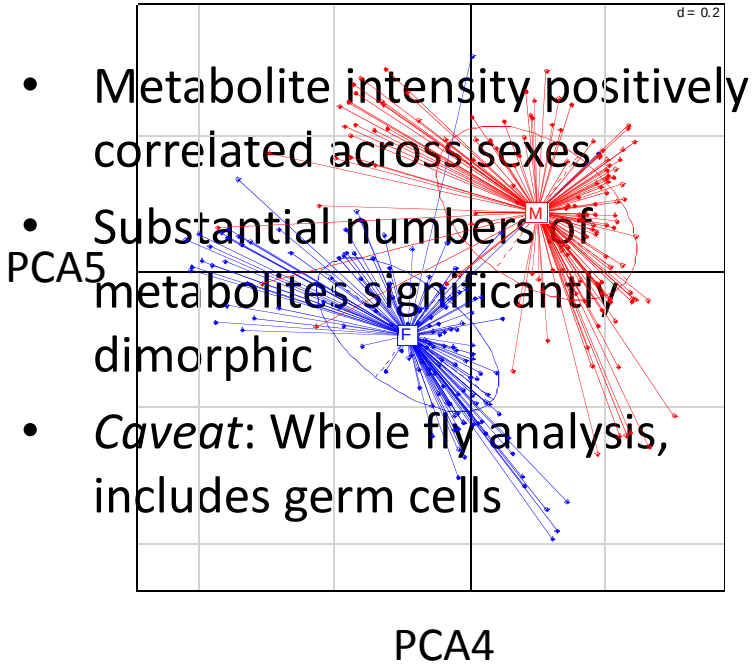




Hoffman *et al*. 2014. Aging Cell

The *Drosophila* metabolome is sexually dimorphic



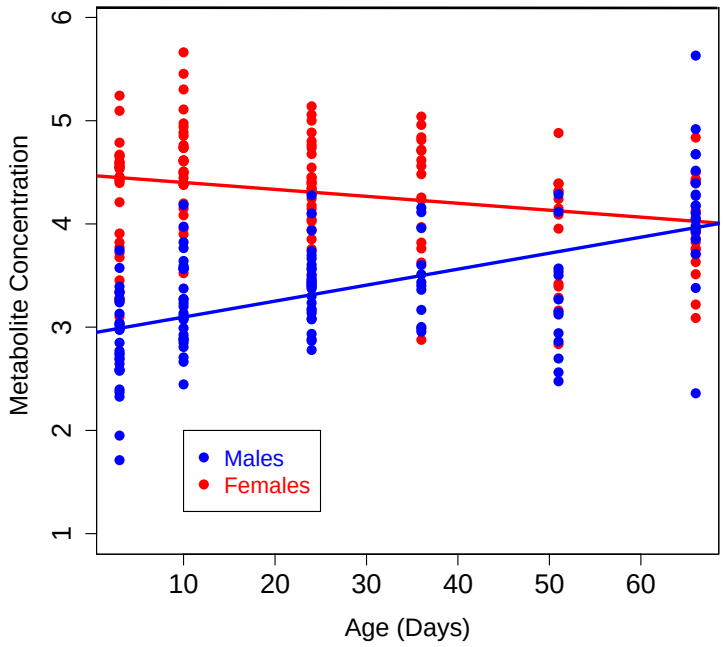


- Metabolite intensity positively correlated across sexes
- Substantial numbers of metabolites significantly dimorphic
- *Caveat*: Whole fly analysis, includes germ cells

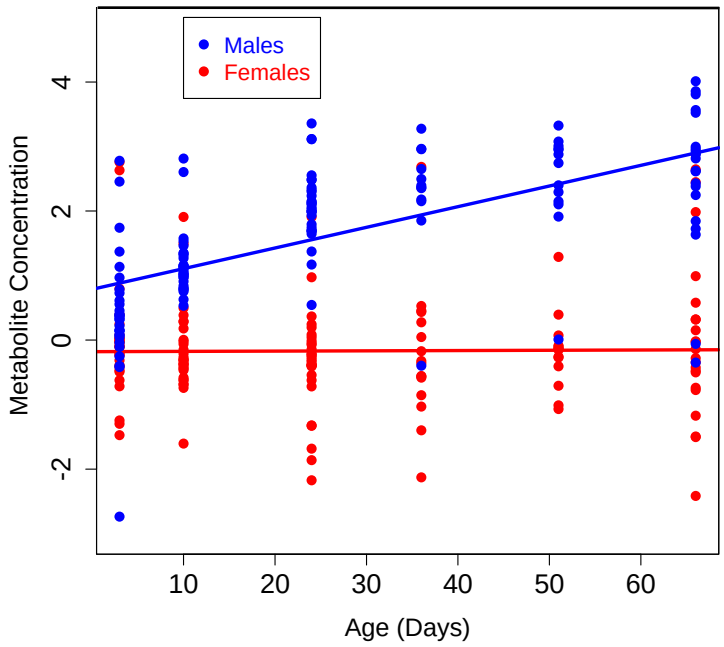
Hoffman *et al*. 2014. Aging Cell

Age-Sex interactions

5-dehydro avenasterol (plant sterol)

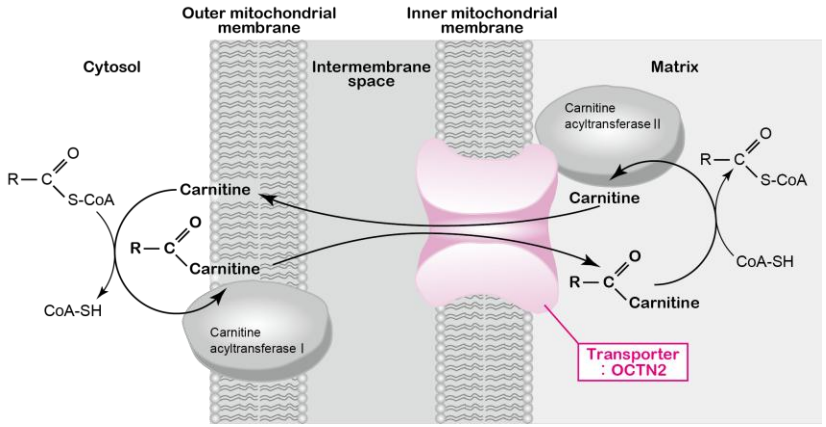


Pantothenate (CoA biosynthesis)

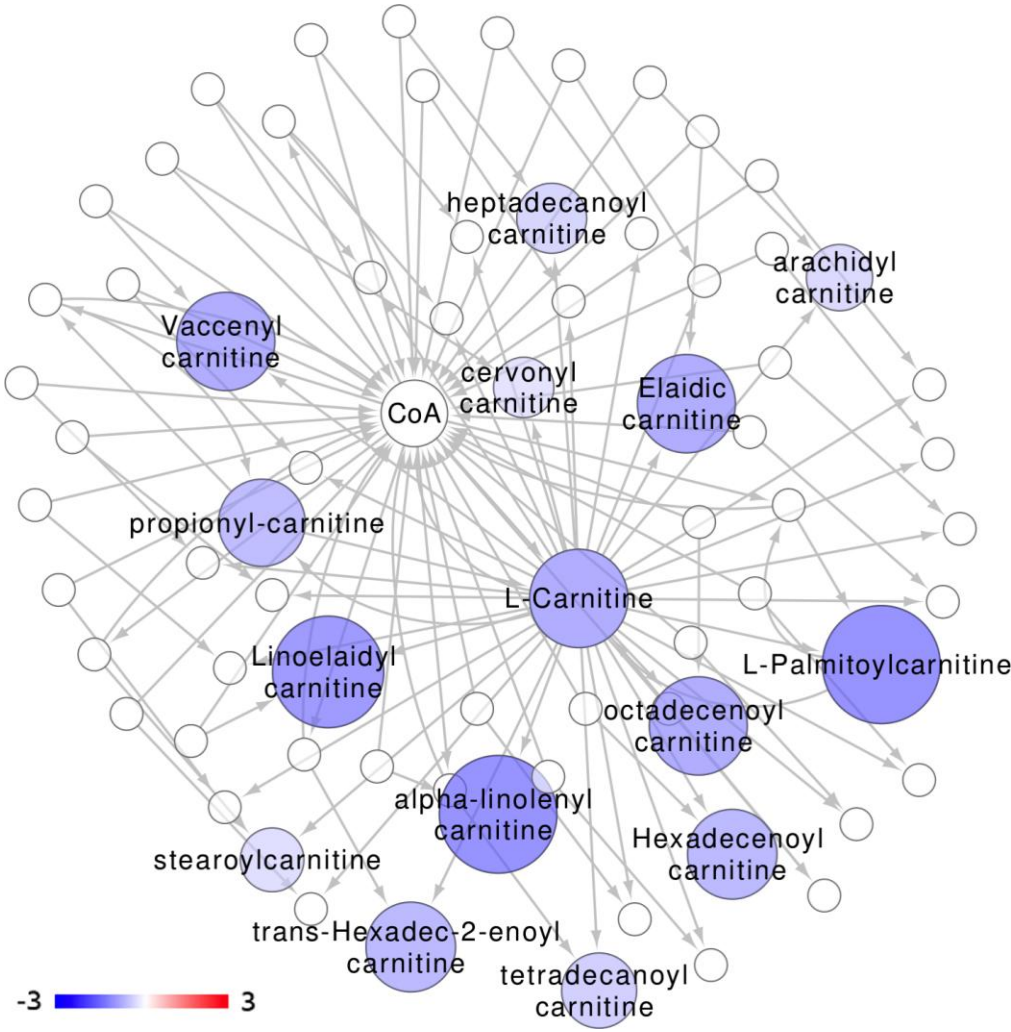


Hoffman *et al*. 2014. Aging Cell

Fatty acid metabolism



http://www.kanazawa-u.ac.jp/research_bulletin/feature201309F003.html

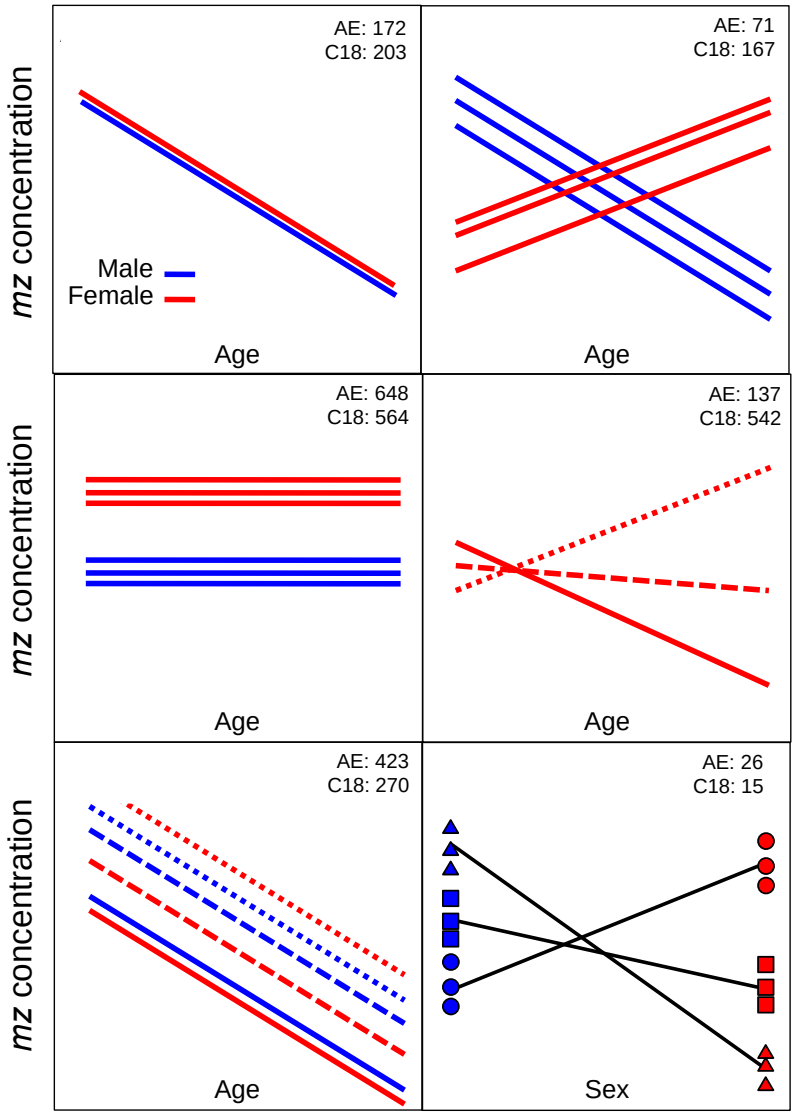


Hoffman *et al*. 2014. Aging Cell

Age

Sex

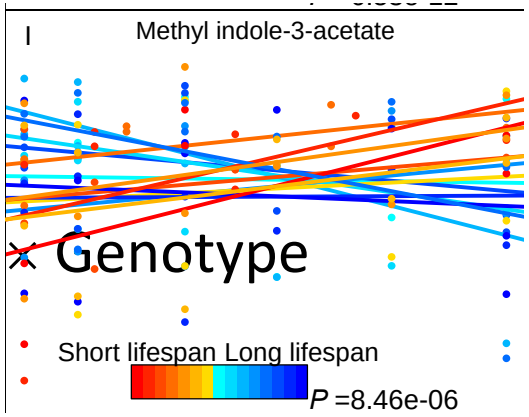
Genotype



Age × Sex

- Fatty acid metabolism
- Neurotransmitters
- Amino acids
- Glycerophospholipids

Age × Genotype



Sex × Genotype

Hoffman *et al*. 2014. Aging Cell

Metabolomics and Aging

1. Evolution of the metabolome
2. Genetics, metabolomics, and biomarkers of aging
3. Diet restriction and metabolomic networks




Metabolomics and Diet Restriction

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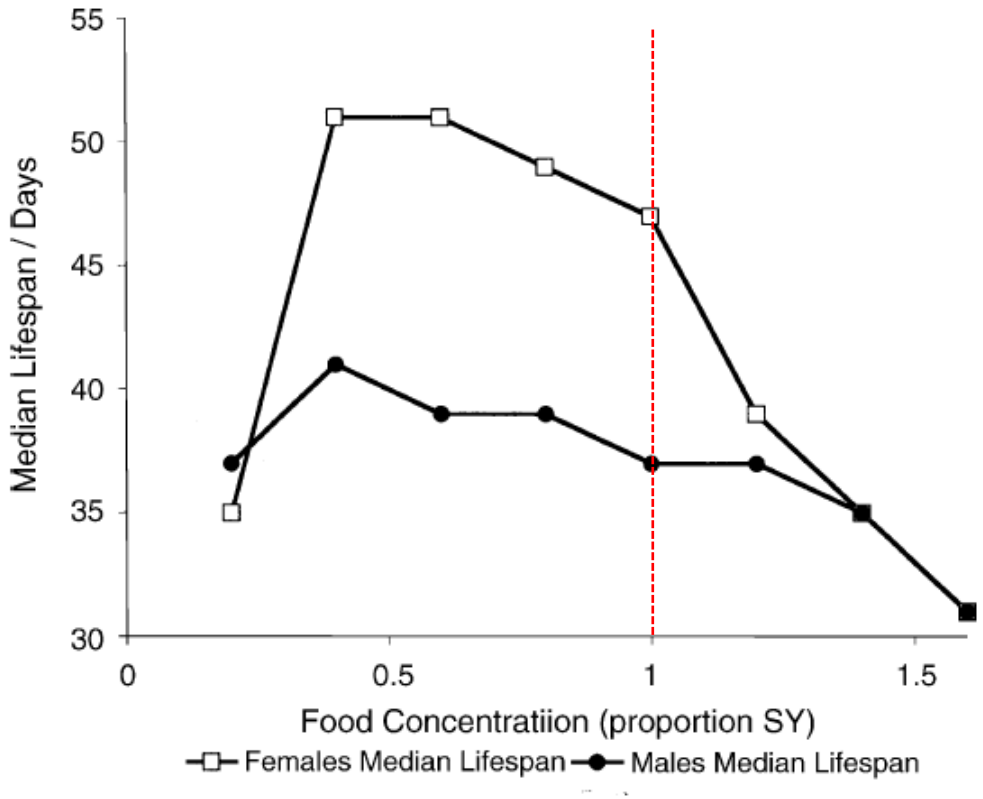
Dietary restriction in *Drosophila*

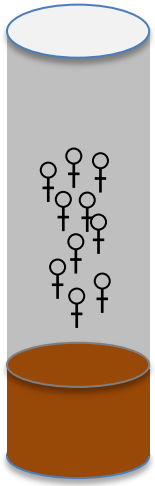
Linda Partridge *, Matthew D.W. Piper, William Mair



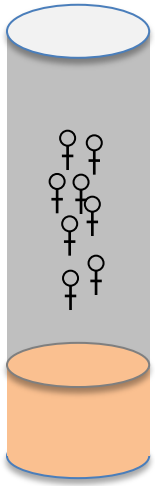
Metabolomics & Diet Restriction

1. Does DR work by slowing aging of the metabolome?
2. Can metabolome networks point to mechanism?

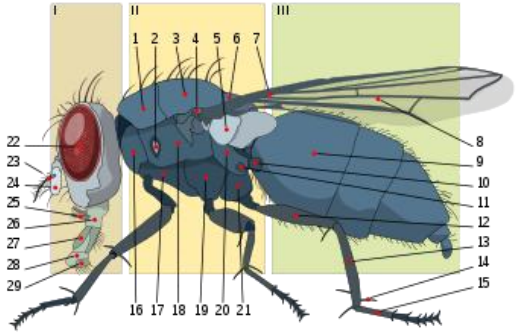
Metabolomics and Diet Restriction

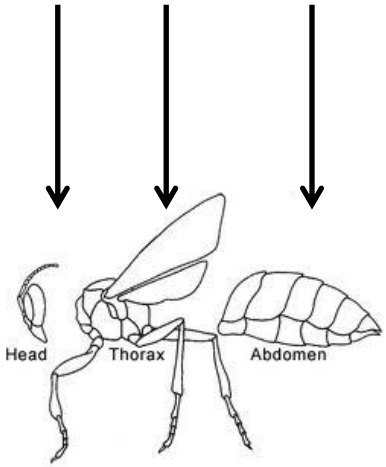


High yeast (AL) diet
Collect age 10, 20, 40 d



Low yeast (DR) diet
Collect age 10, 20, 40 d





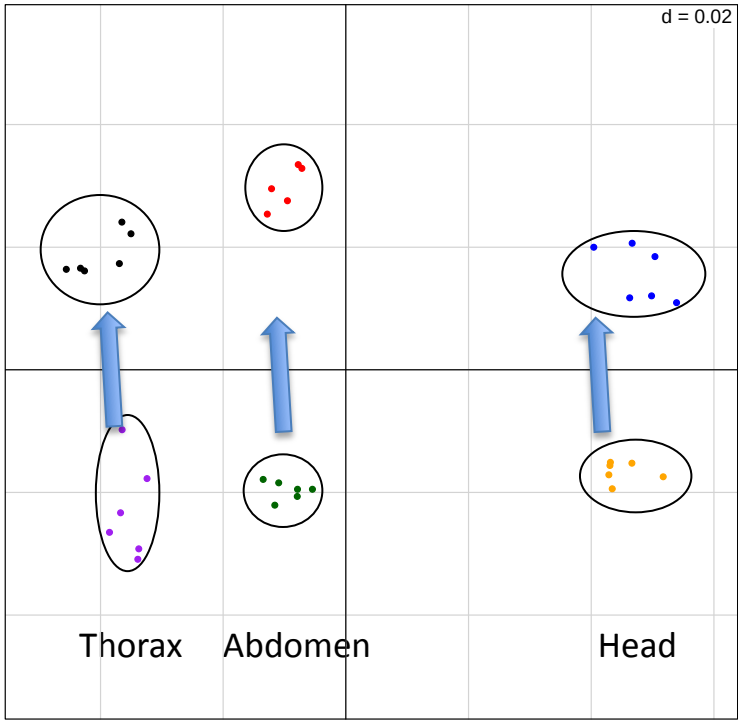
X 6 samples/tissue

Buck Institute

Pankaj Kapahi

Matt Laye

Principle Component Analysis
DR and body part

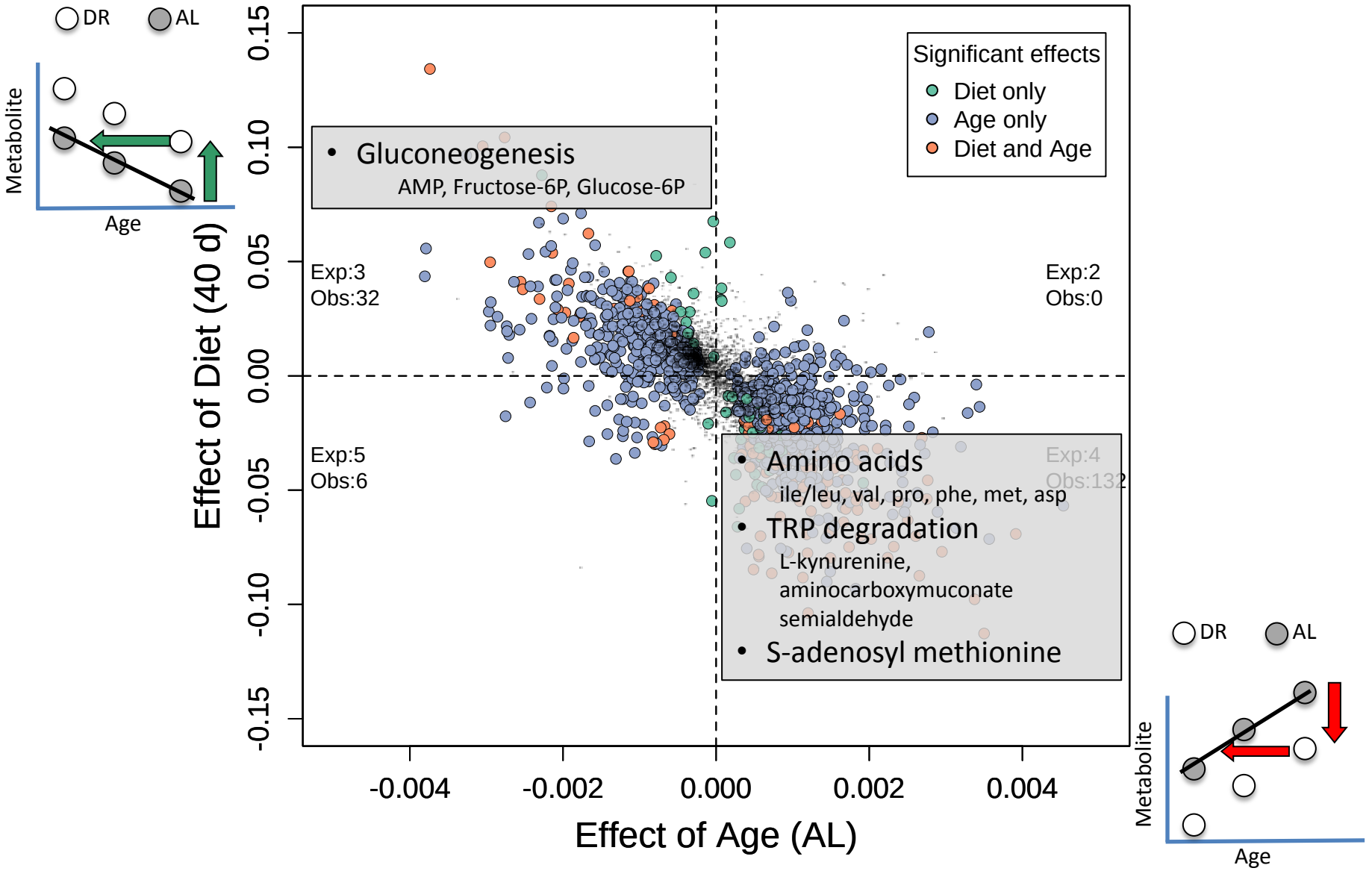


Axis 3

Axis 2

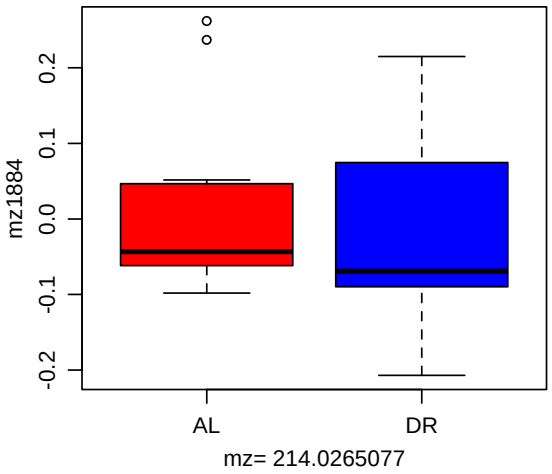
Diet restriction

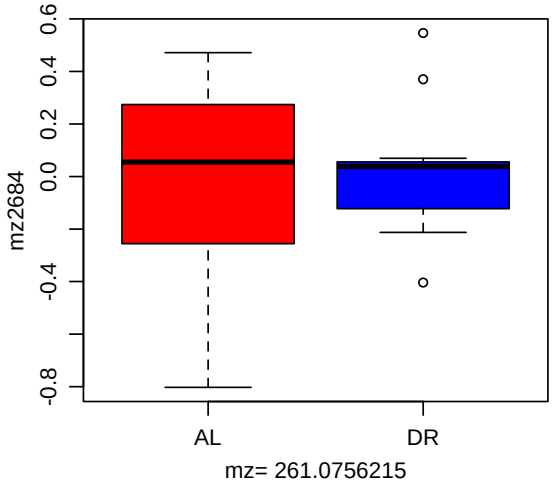
Ad libitum


DR
AL
Metabolite
Age
Effect of Diet (40 d)
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.004
-0.002
0.000
0.002
0.004
Effect of Age (AL)
Significant effects
Diet only
Age only
Diet and Age
• Gluconeogenesis
AMP, Fructose-6P, Glucose-6P
Exp:3
Obs:32
Exp:2
Obs:0
Exp:5
Obs:6
Exp:4
Obs:132
• Amino acids
ile/leu, val, pro, phe, met, asp
• TRP degradation
L-kynurenine,
aminocarboxymuconate
semialdehyde
• S-adenosyl methionine
DR
AL
Metabolite
Age

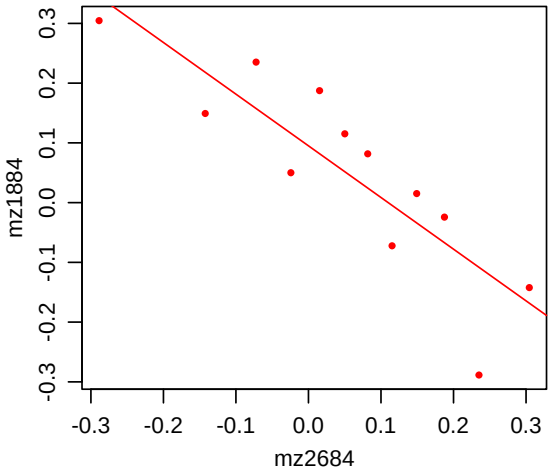
DR and
metabolome network structure

Correlations vs. Main Effects

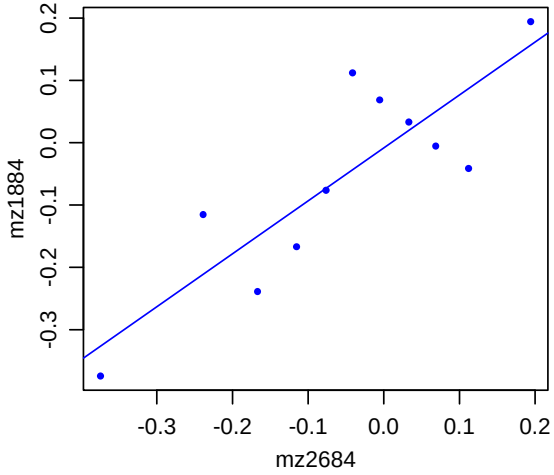




Ad Lib

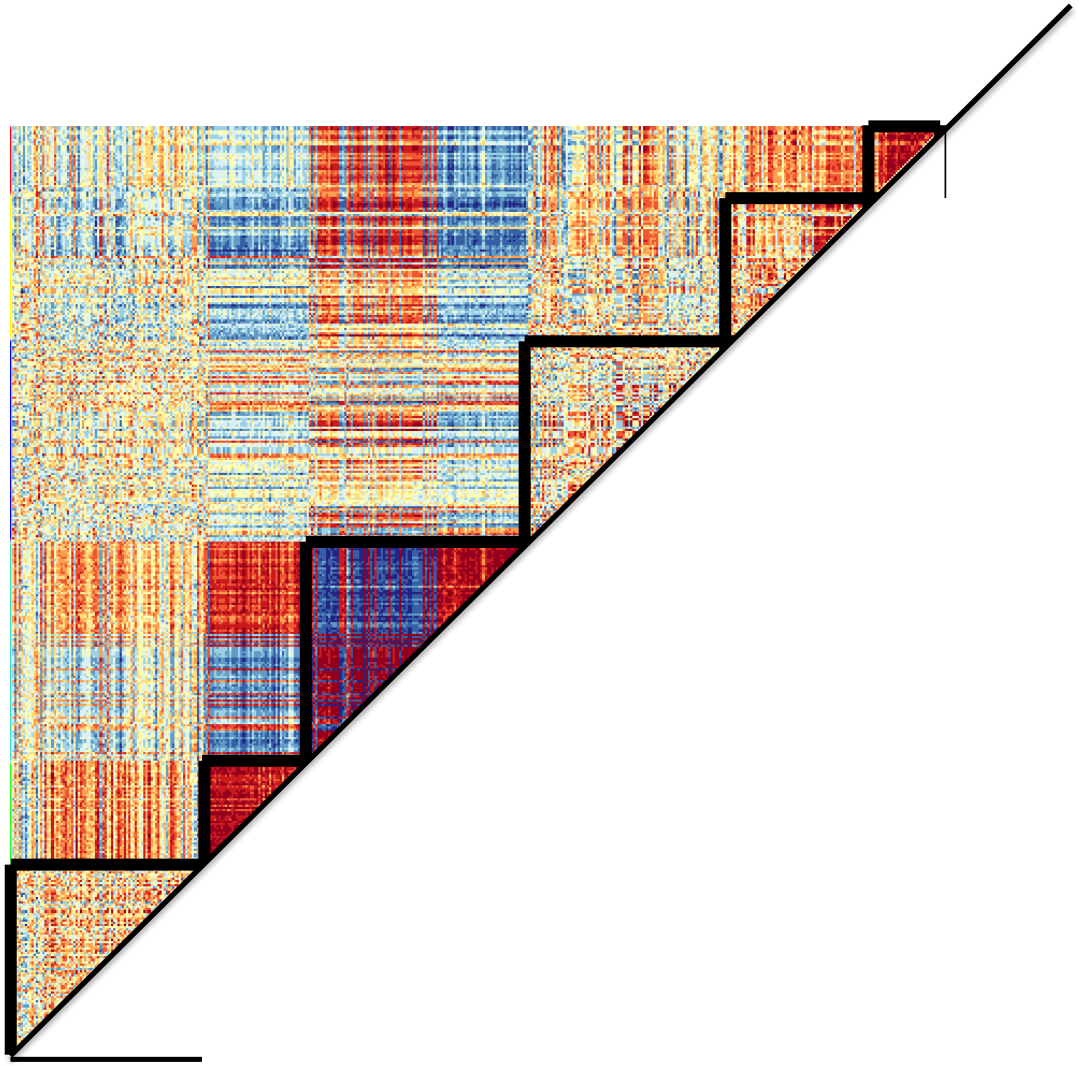


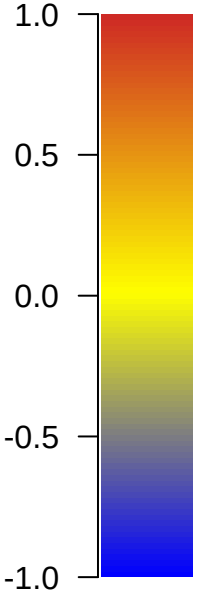
DR



WGCNA analysis of DR in flies

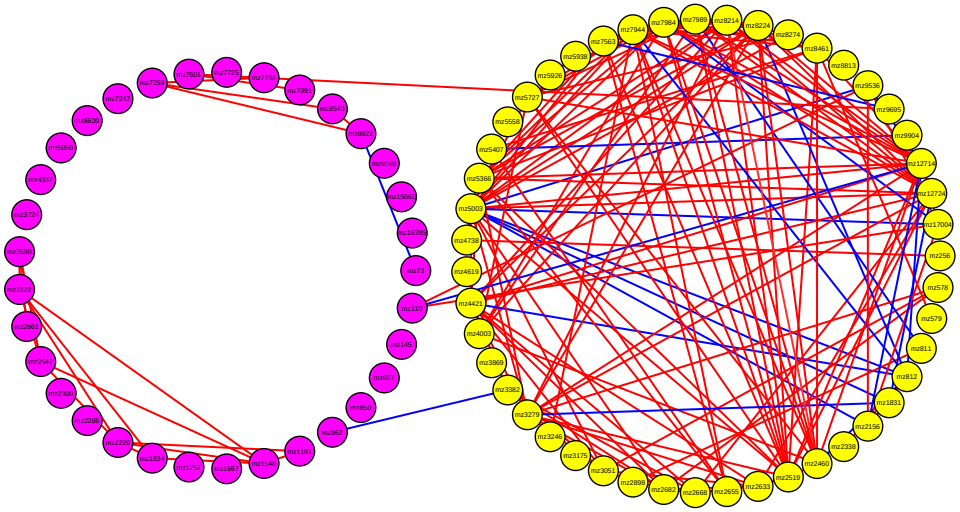
Ad Lib Thorax & Whole body, 40 d



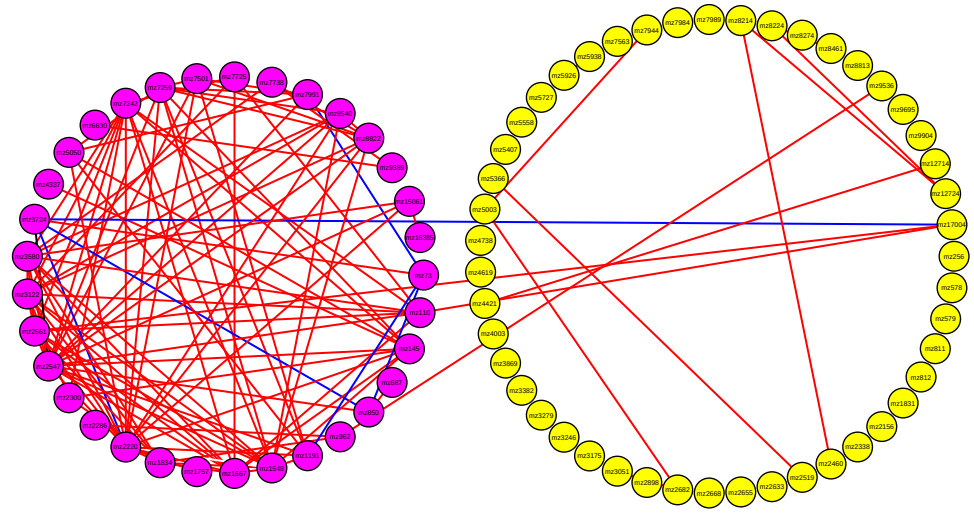


Diet Restricted Thorax & Whole body, 40 d

Some gain connections, some lose connections…

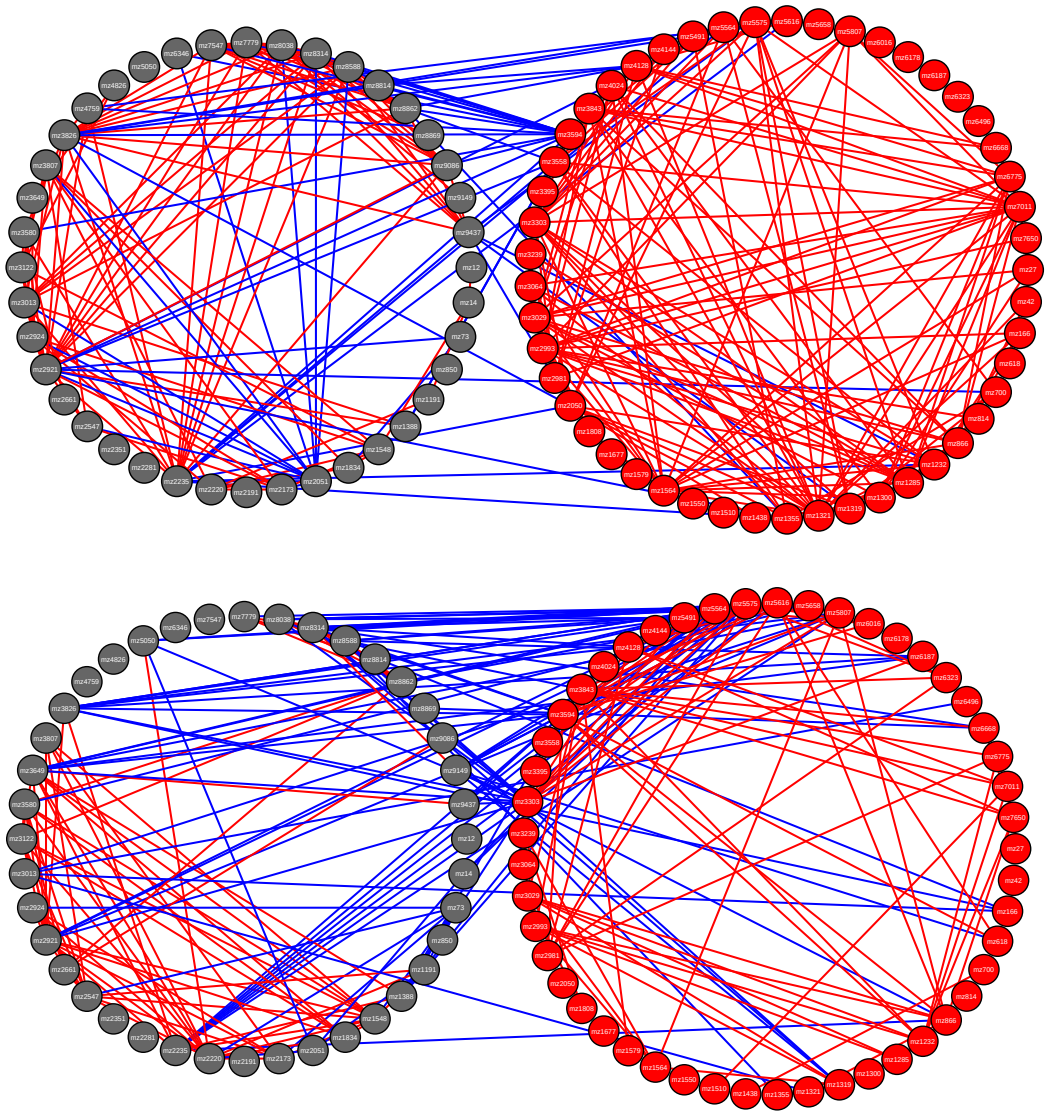


Thorax, Magenta/Yellow, AL



Thorax, Magenta/Yellow, DR

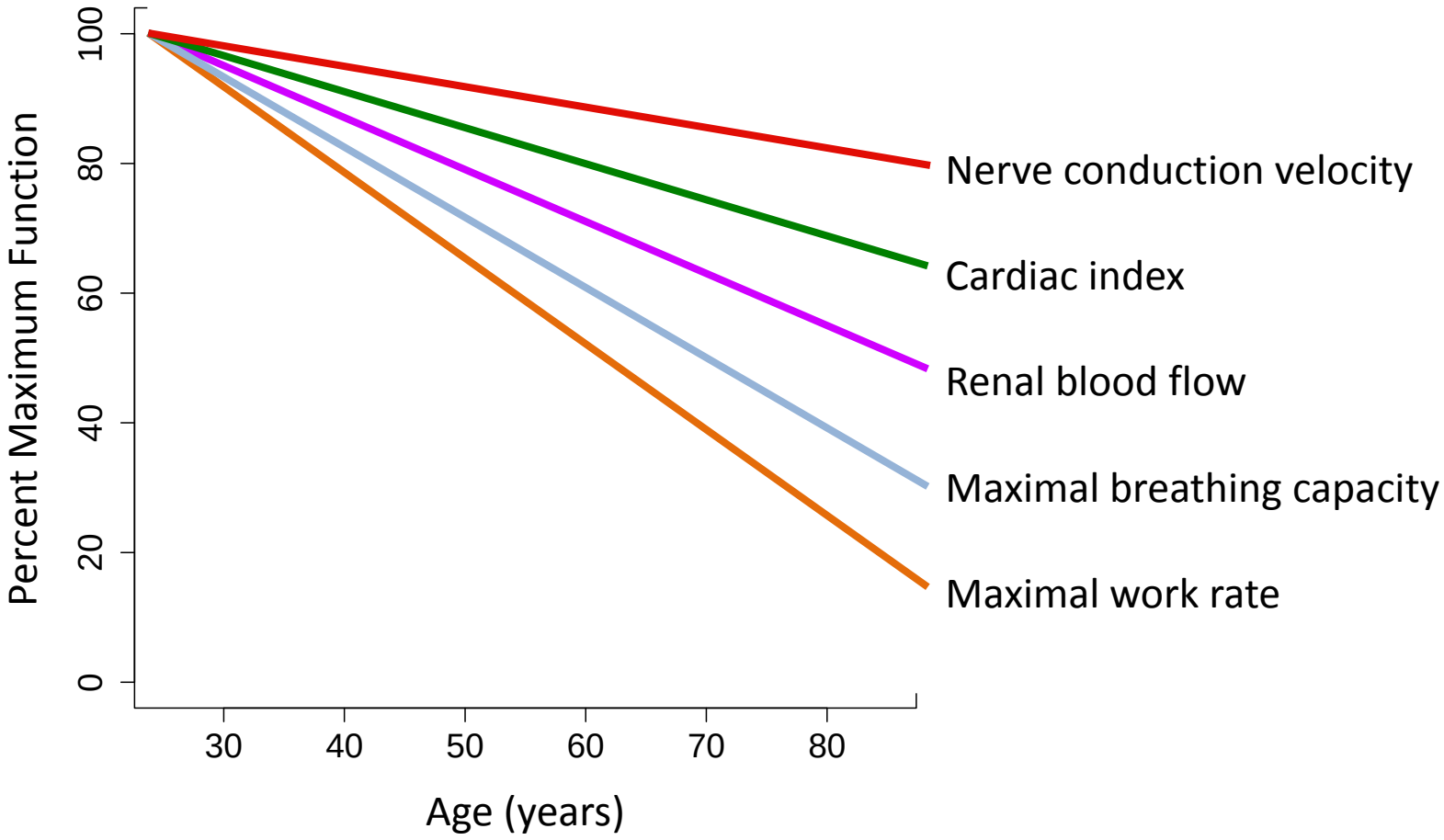
…and some stay the same.



Thorax Red/Black, AL

Thorax Red/Black, DR

Next step: Identify modules that age at different rates



After Baker & Martin *in* Geriatric Medicine (ed. Cassel *et al.*, 1997)

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