The complex genetics of energetics

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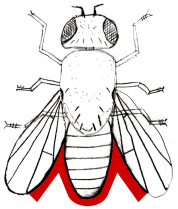
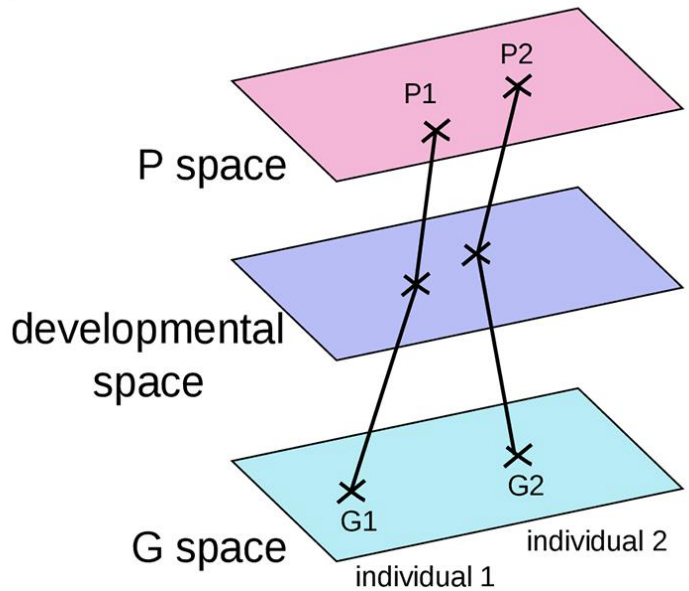


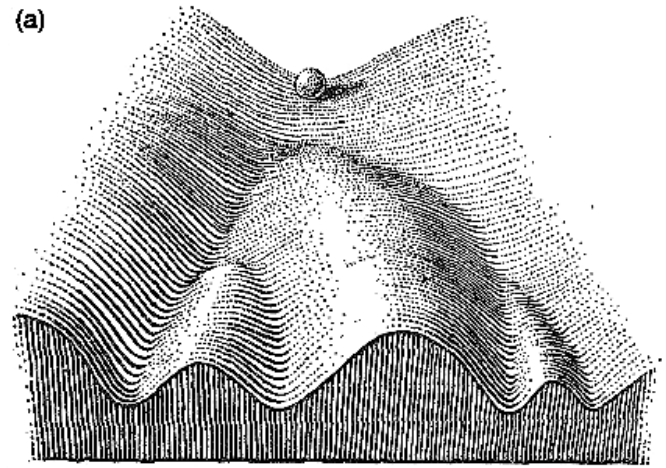
montoothlab.unl.edu
Studying how physiologies & genomes evolve

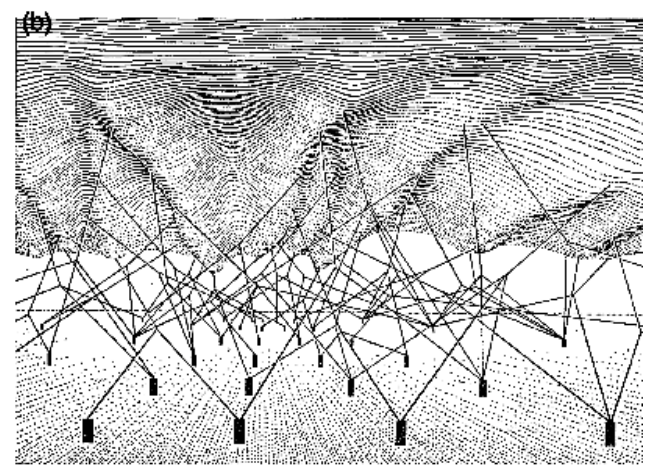
UNVEIL

University of Nebraska Lincoln

A genotype-phenotype-fitness map underlies trait evolution

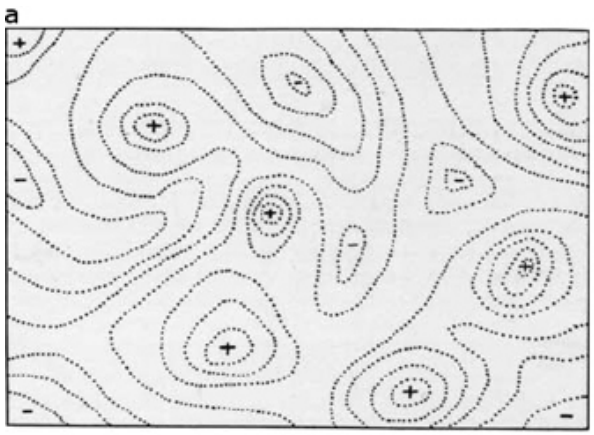


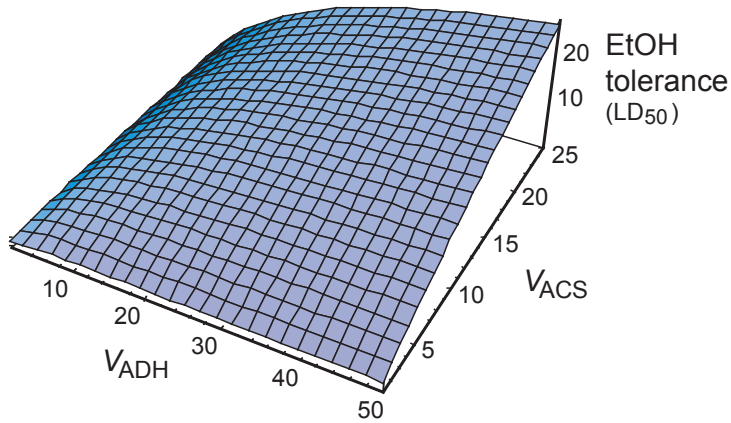


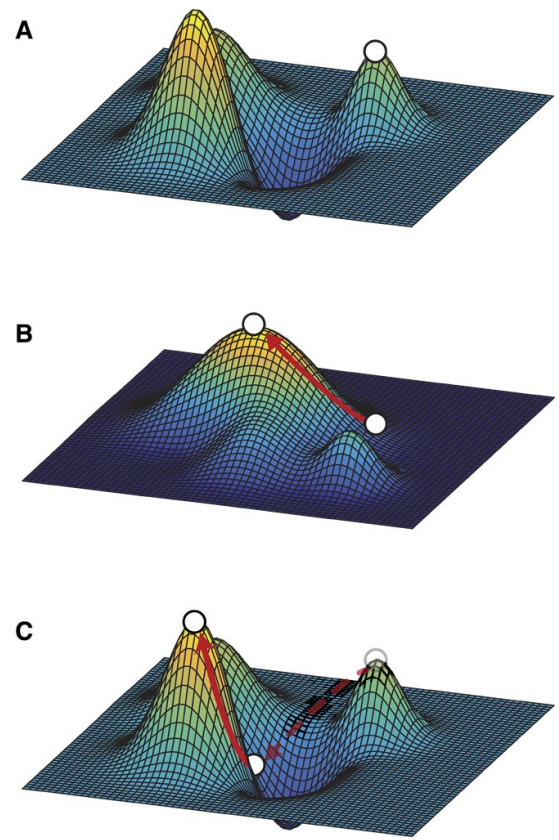


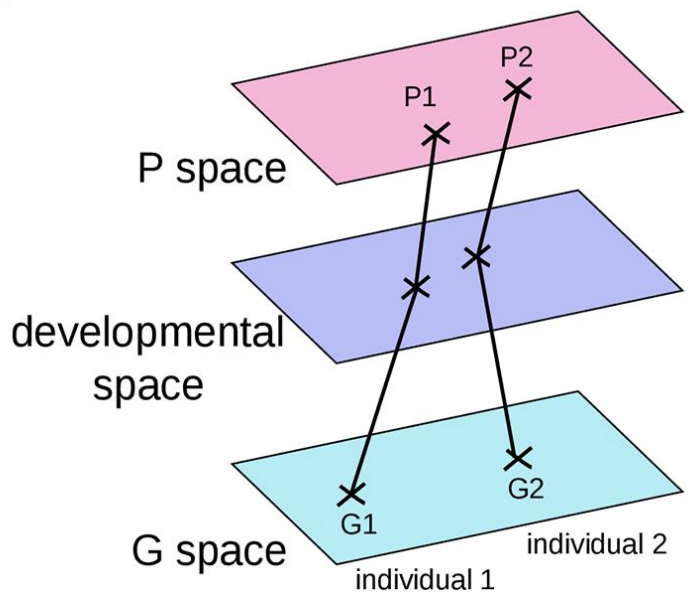
Lewontin (1974) The Genetic Basis of Evolutionary Change
Waddington (1957) The Strategy of the Genes
Orgogozo et al. (2015) Front Genet

A genotype-phenotype-fitness map underlies trait evolution



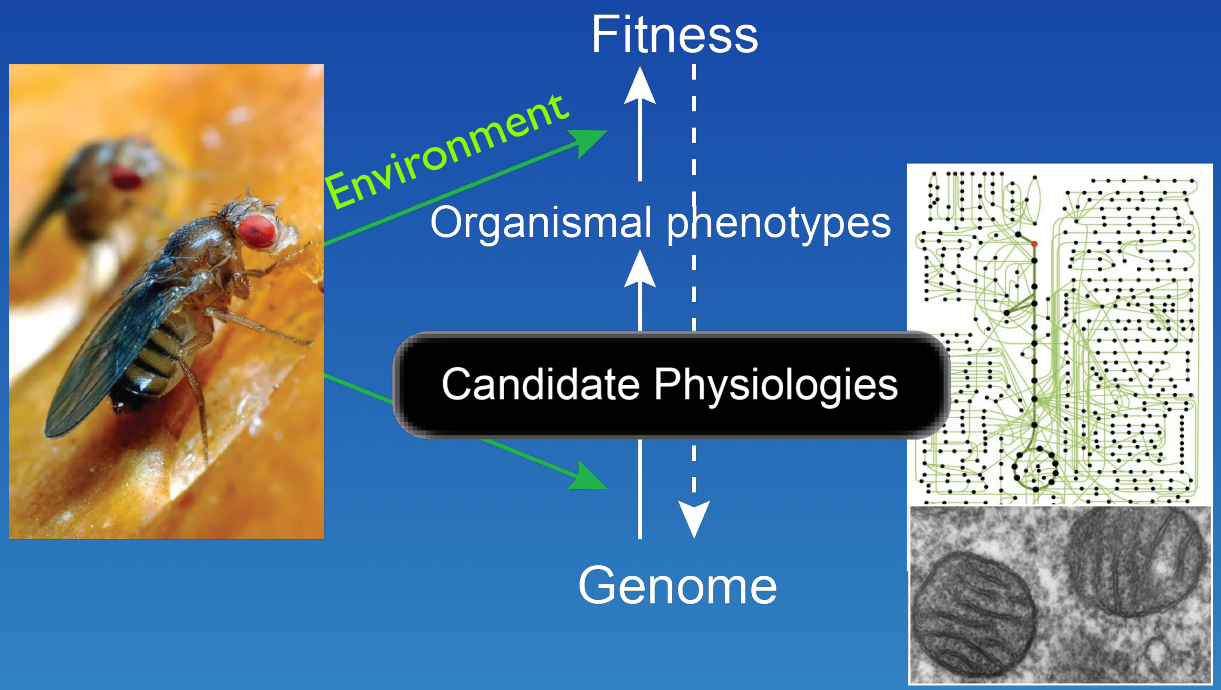






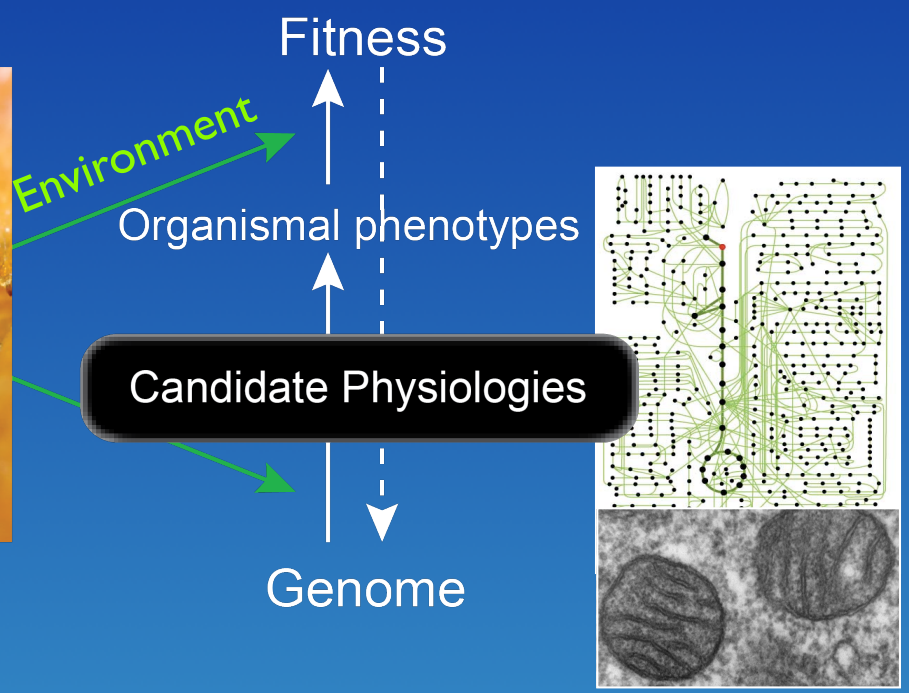
Wright (1931) Genetics 16, 97–159
Montooth et al. (2006) J Exp Biol
Orgogozo et al. (2015) Front Genet
Steinberg & Ostermeier (2016) Sci Advances

Energetic processes shape the genotype-phenotype-fitness map



How does climate change impact survival of overwintering insects?

How does sexual reproduction shape genome evolution?



What governs metabolic scaling relationships?

How does thermal performance evolve?

Take homes

- An energetic framework provides mechanistic insight on why the genotype-phenotype-fitness is complex
- Patterns exist that suggest molecular energetic processes and costs matter at organismal levels and evolutionary scales
- Scaling up from molecular energetics to tissue, organismal and population levels is an important step in connecting energetic theory of molecular dynamics to evolution

Outline

- The genotype-phenotype-fitness relationship is complex
  - Metabolism is polygenic and underlain by non-linear gene interactions
  - Case study of mitochondrial-nuclear epistasis
  - Genetic effects of metabolic mutations are condition-dependent and this may be governed by energy demand-supply

- Patterns exist that suggest energetic processes and molecular energetic costs matter at organismal levels and evolutionary scales
  - Evidence that mito-nuclear coevolutionary dynamics shape genome evolution in mammals
  - Energetic costs of adaptive gene expression can be manifest at the level of organismal metabolic rate

- An experiment to scale up from biochemistry to population growth rate reveals a lot of complexity

Genetic variation for metabolic enzyme activities does not always exist at the genes that encode the enzymes

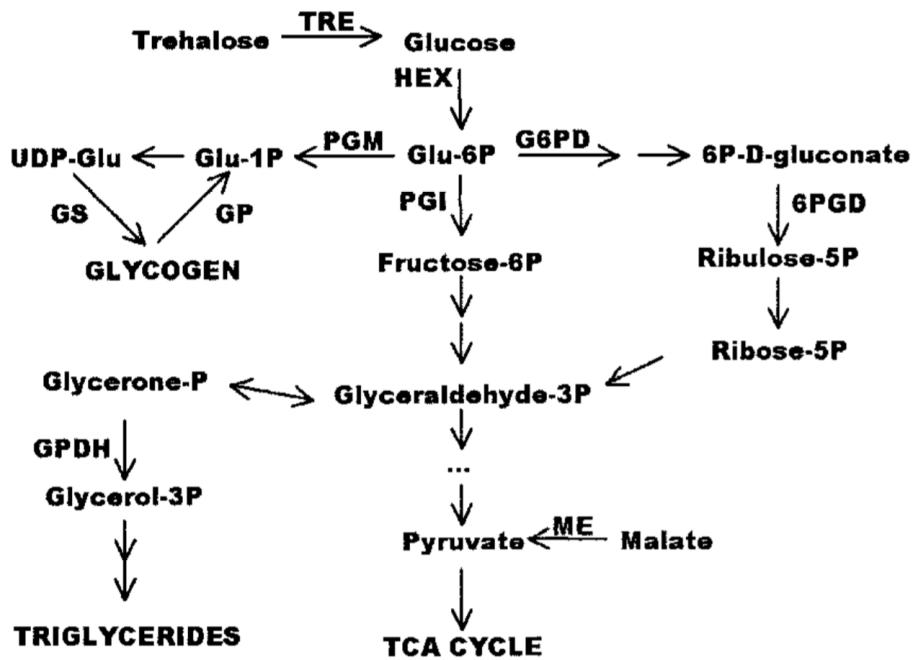
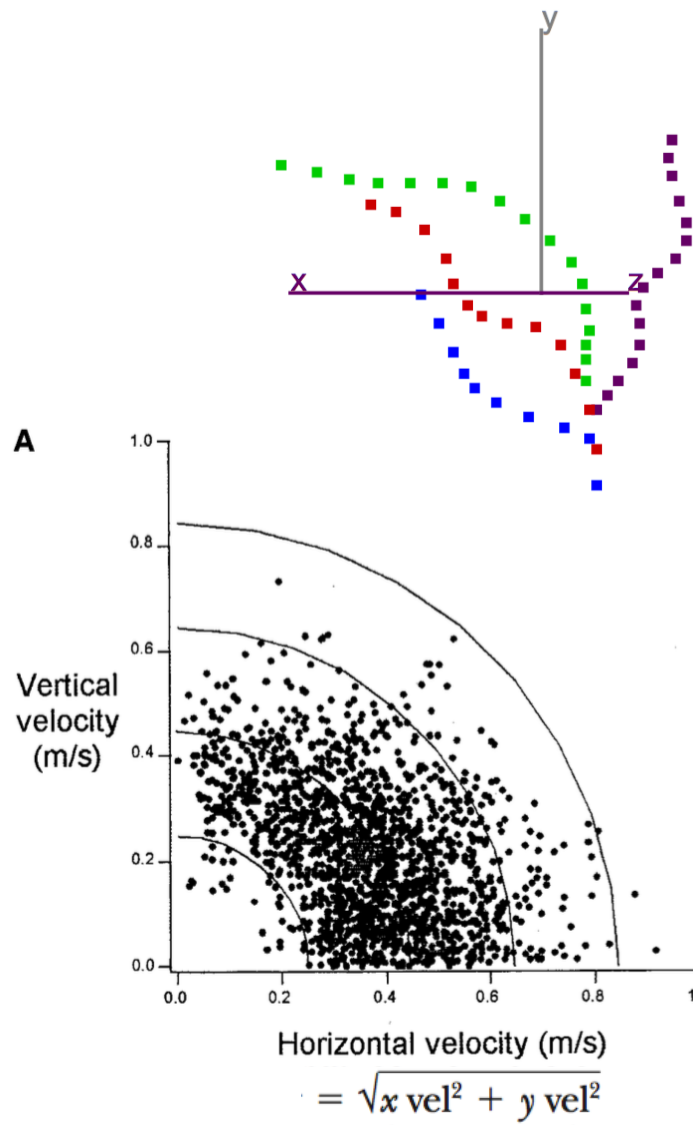


FIGURE 1.—The relationship among the components of energy metabolism assayed. Glu, glucose.



Montooth et al. (2003) Genetics

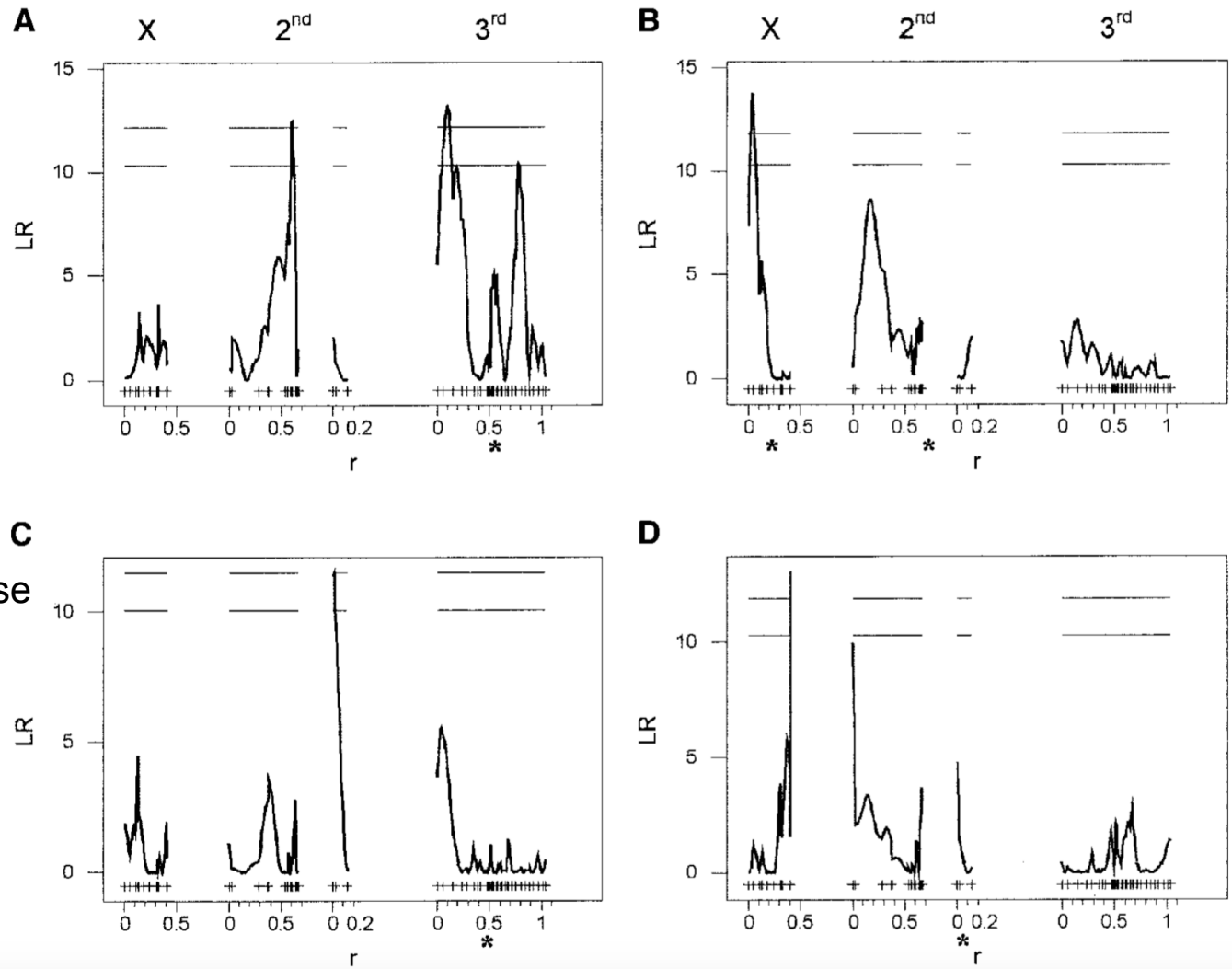
Genetic variation for metabolic enzyme activities does not always exist at the genes that encode the enzymes

Glycogen synthase

Hexokinase

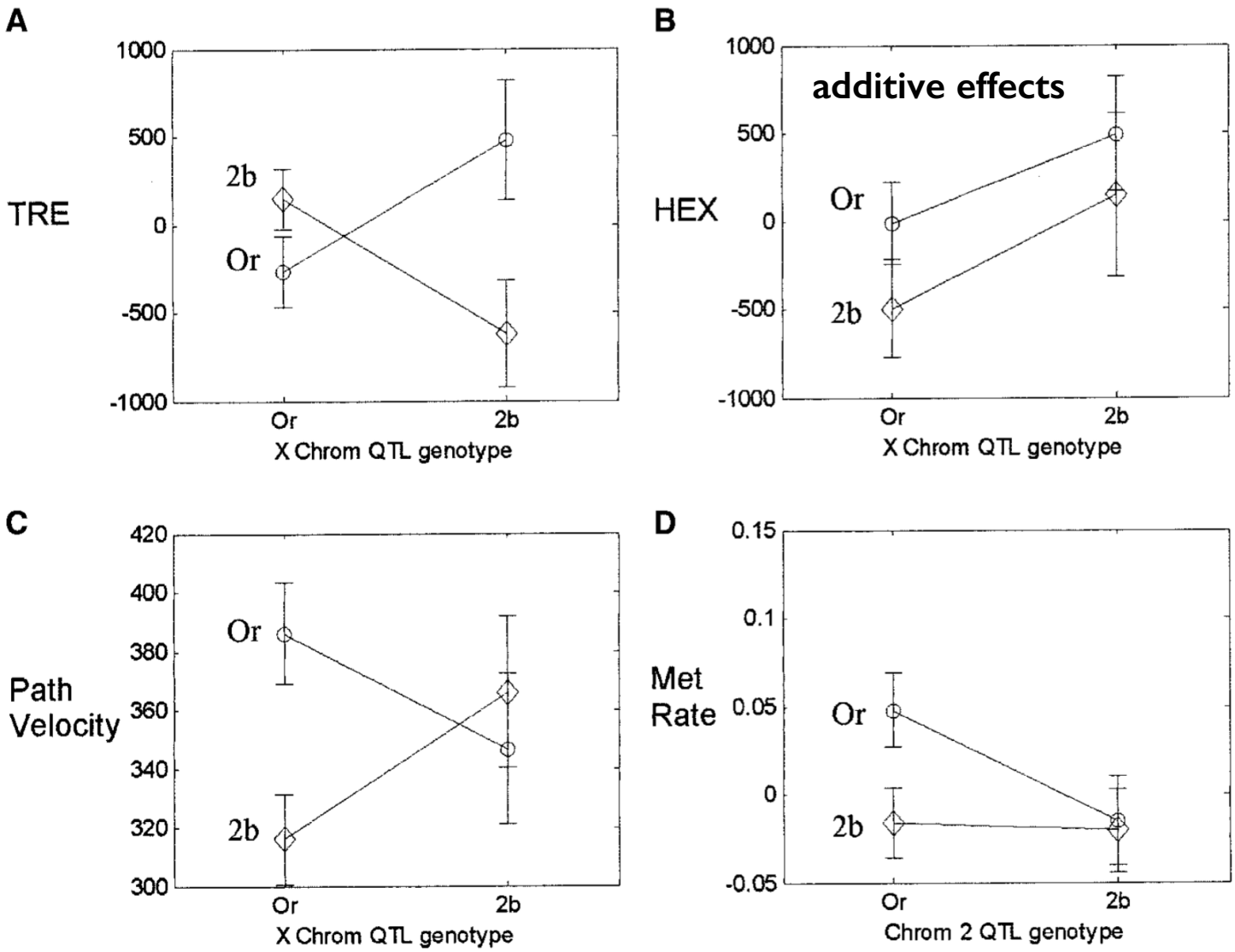
Phosphoglucomutase

Trehalase

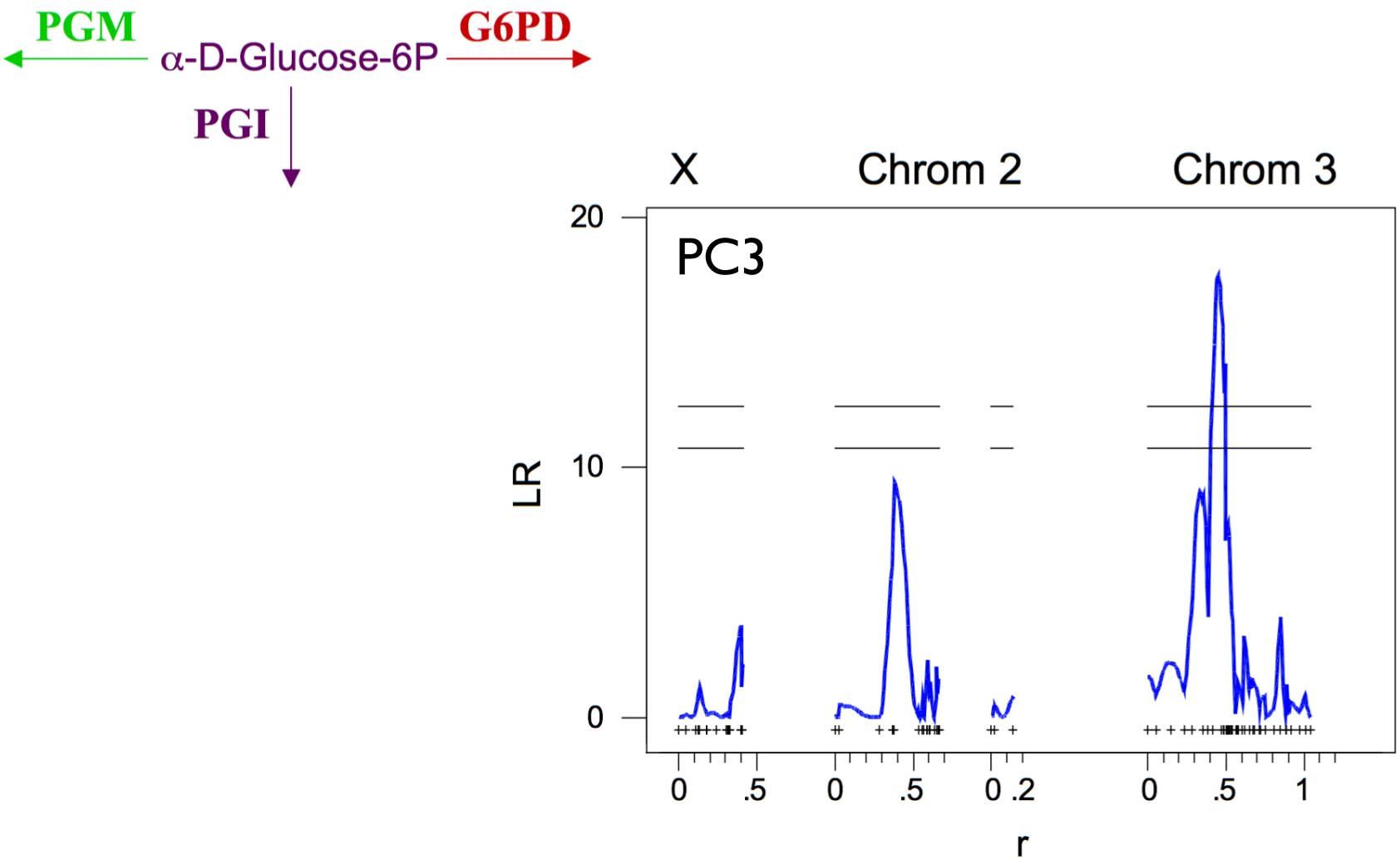


Genetic variation interacts across loci to shape metabolic enzyme activity & performance traits

GxG (non-additive, epistatic)



There is not a simple mapping of metabolic enzyme genetic variation onto higher-level physiological performance variation



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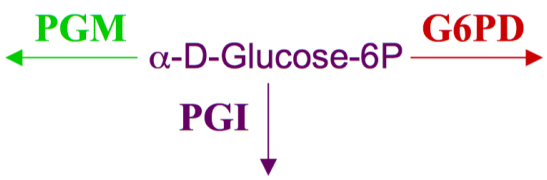
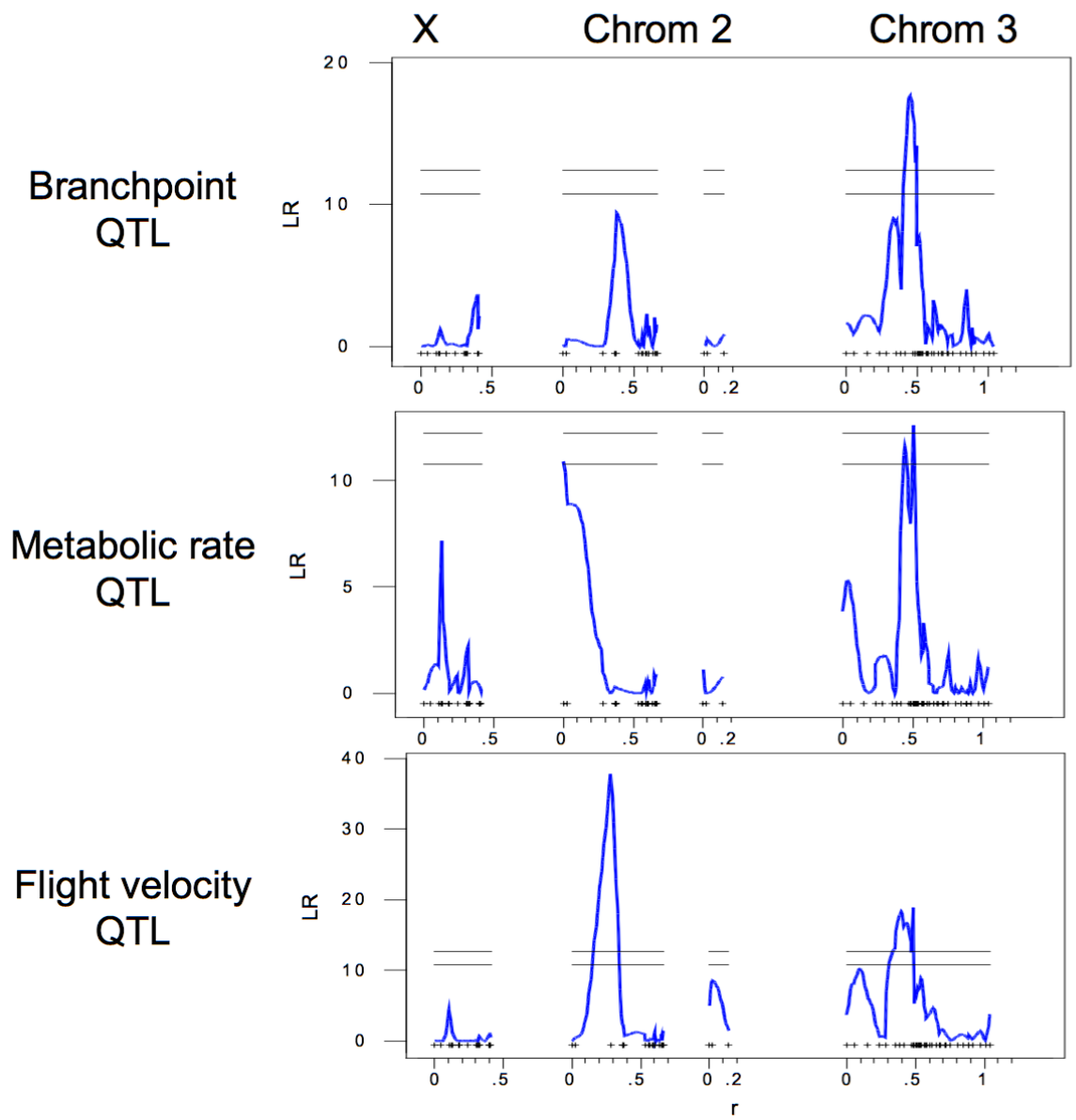
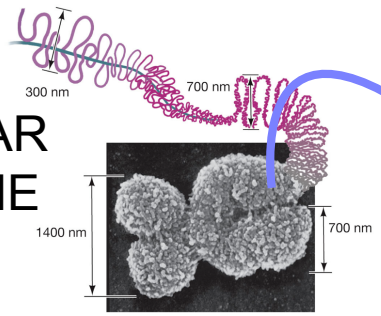


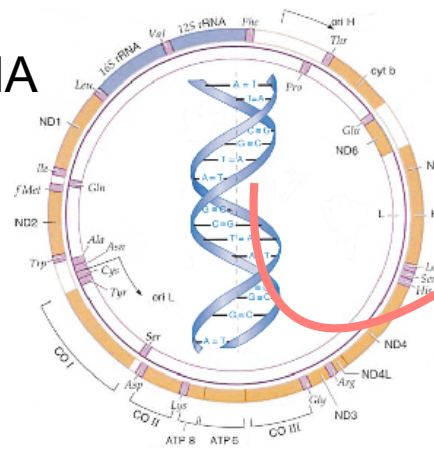


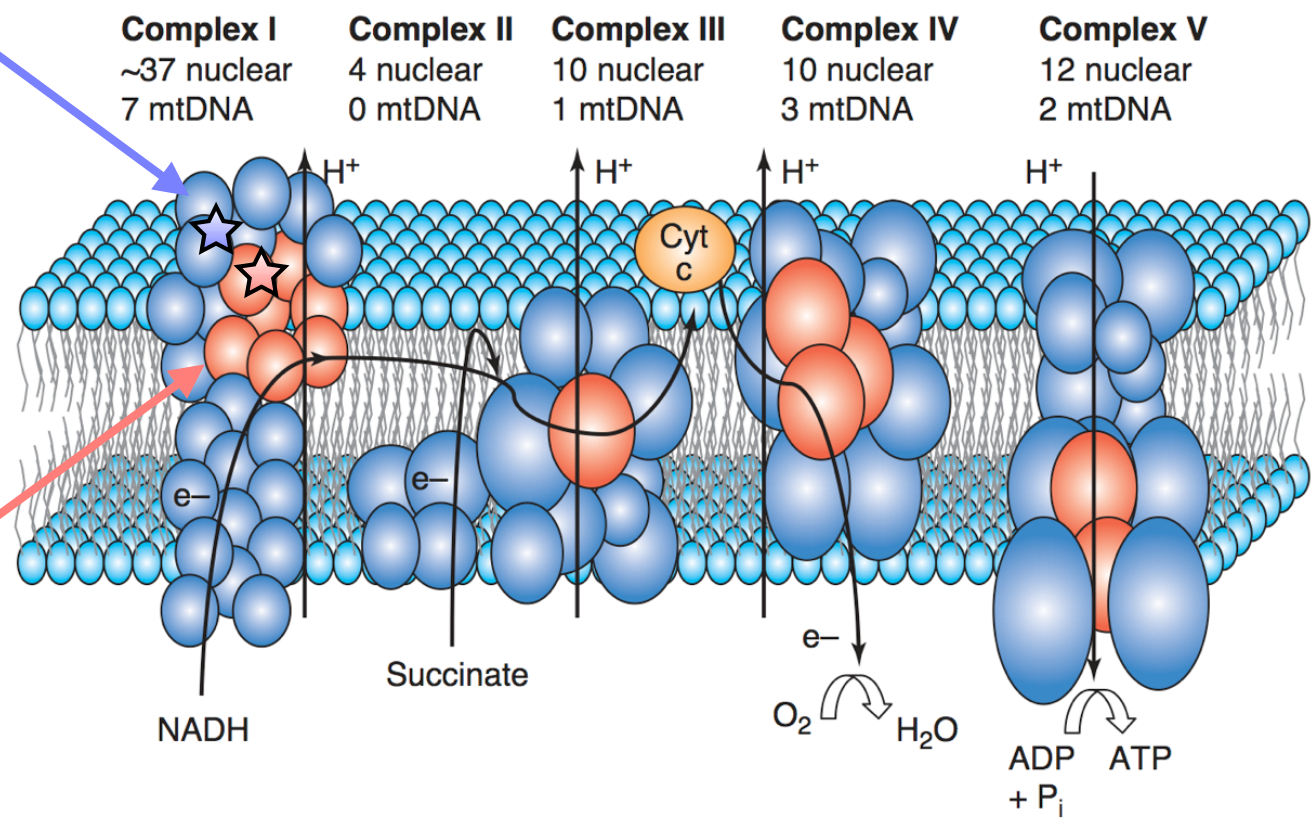
The core machine of oxygen-dependent ATP production requires functional (and coevolutionary) interaction between two genomes

NUCLEAR GENOME

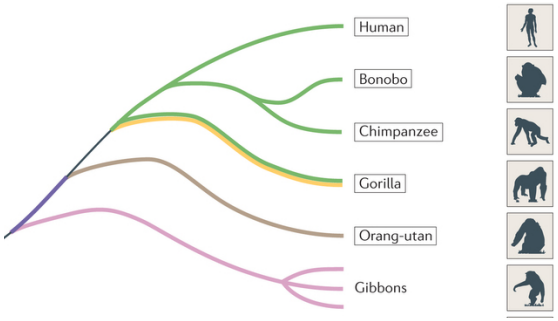


mtDNA



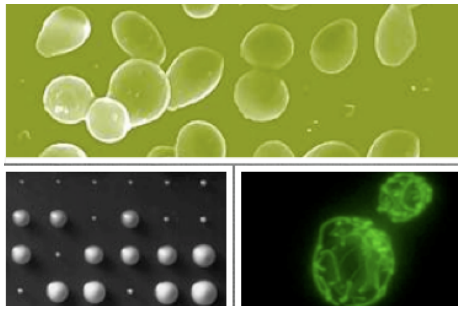


Evidence for mito-nuclear coevolution



Primate molecular evolution
Osada and Akashi 2012 MBE
Image from Rogers & Gibbs 2014 Nat Rev Genet

- Across diverged lineages
- Patterns indicative of correlated molecular evolution between mitochondrial and nuclear products



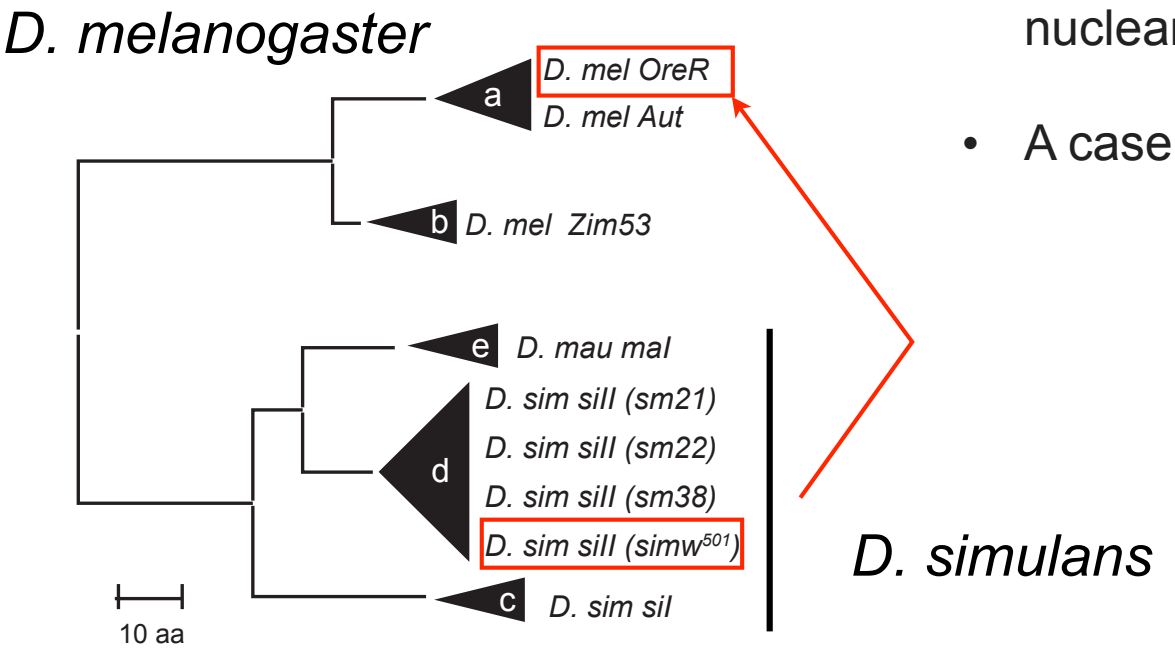
S. cerevisiae and *S. bayanus*
Lee et al. 2008; Groth et al. 2000

- Between closely related species
- Genetic incompatibility affects gene regulation

Marine copepod *Tigriopus*
Ron Burton and colleagues

- Between divergent populations, within species
- Multiple incompatibilities affect replication, transcription, and OXPHOS

Evolutionary genetic screen reveals mito-nuclear epistatic genetic interactions in *Drosophila*



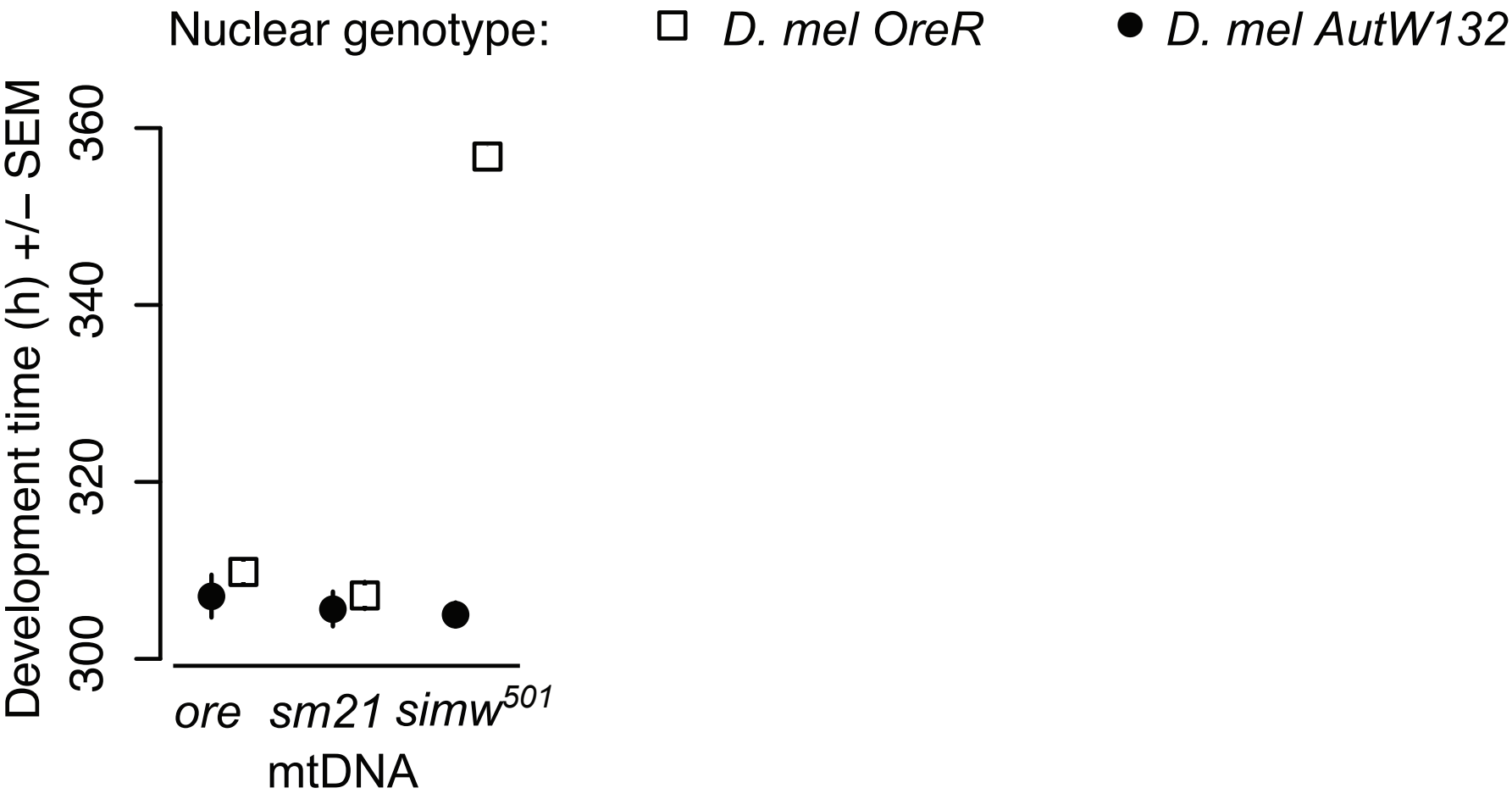
- 8 divergent mtDNAs in 2 *D. melanogaster* nuclear backgrounds
- A case study mito-nuclear incompatibility

David Rand

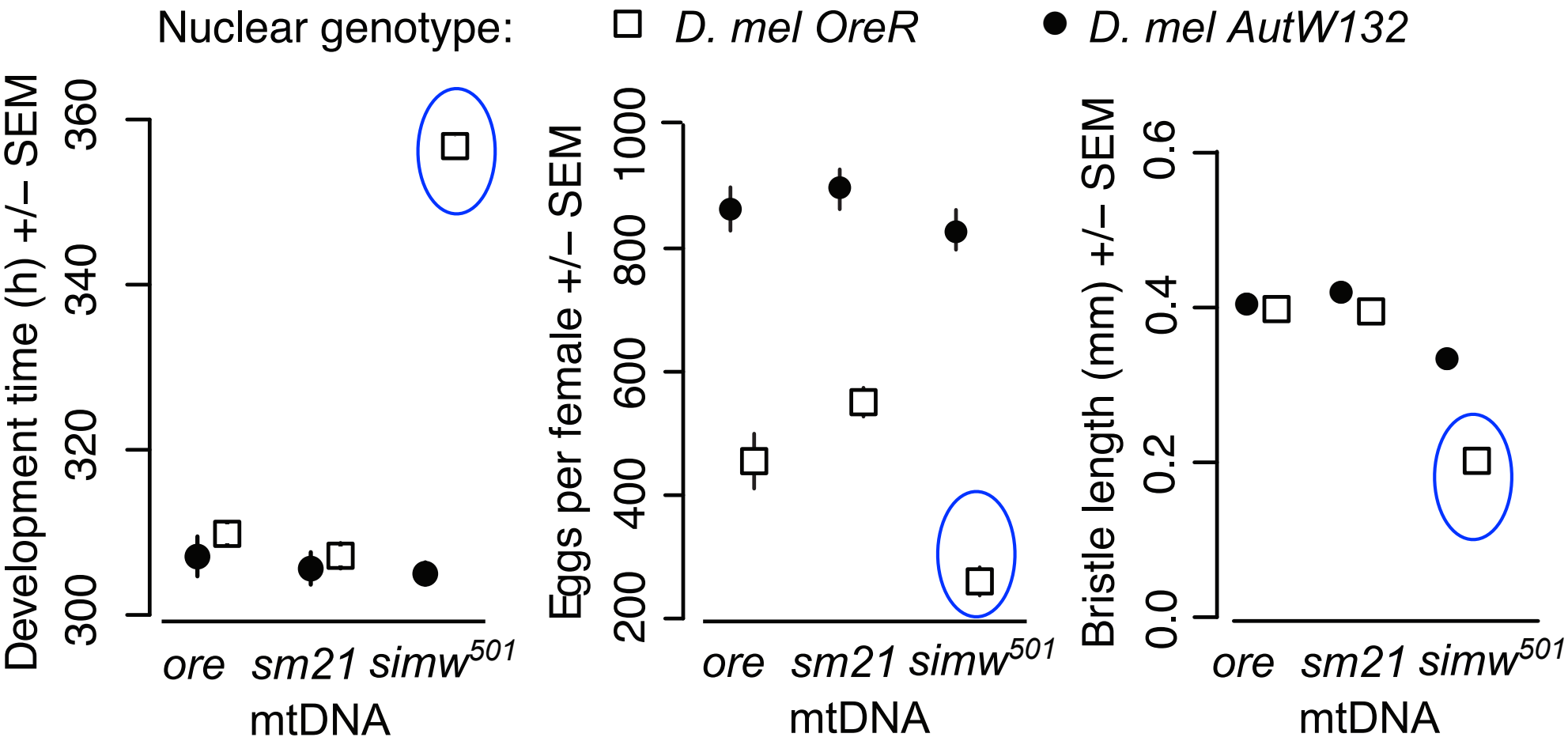
Colin Meiklejohn

Montooth *et al.* 2010 *Evolution*
Meiklejohn *et al.* (2013) PLoS Genetics

An incompatibility between mitochondrial and nuclear polymorphisms compromises development & reproduction



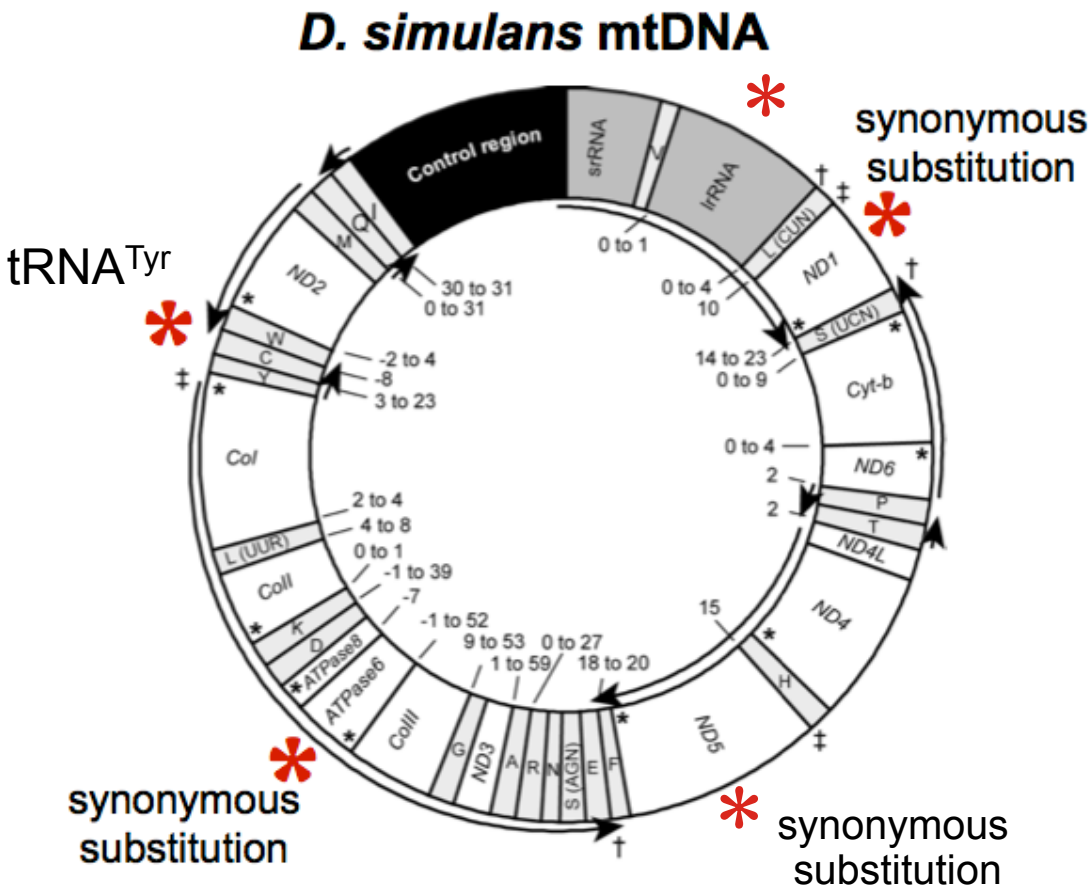
An incompatibility between mitochondrial and nuclear polymorphisms compromises development & reproduction

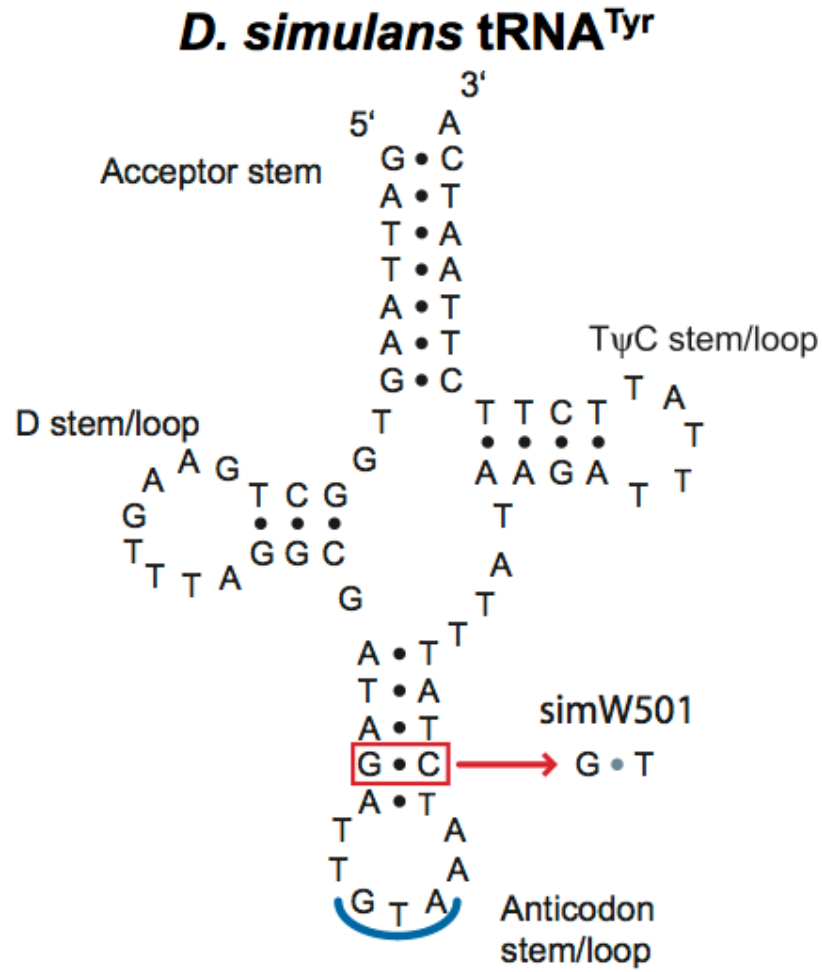


GxG (non-additive, epistatic)

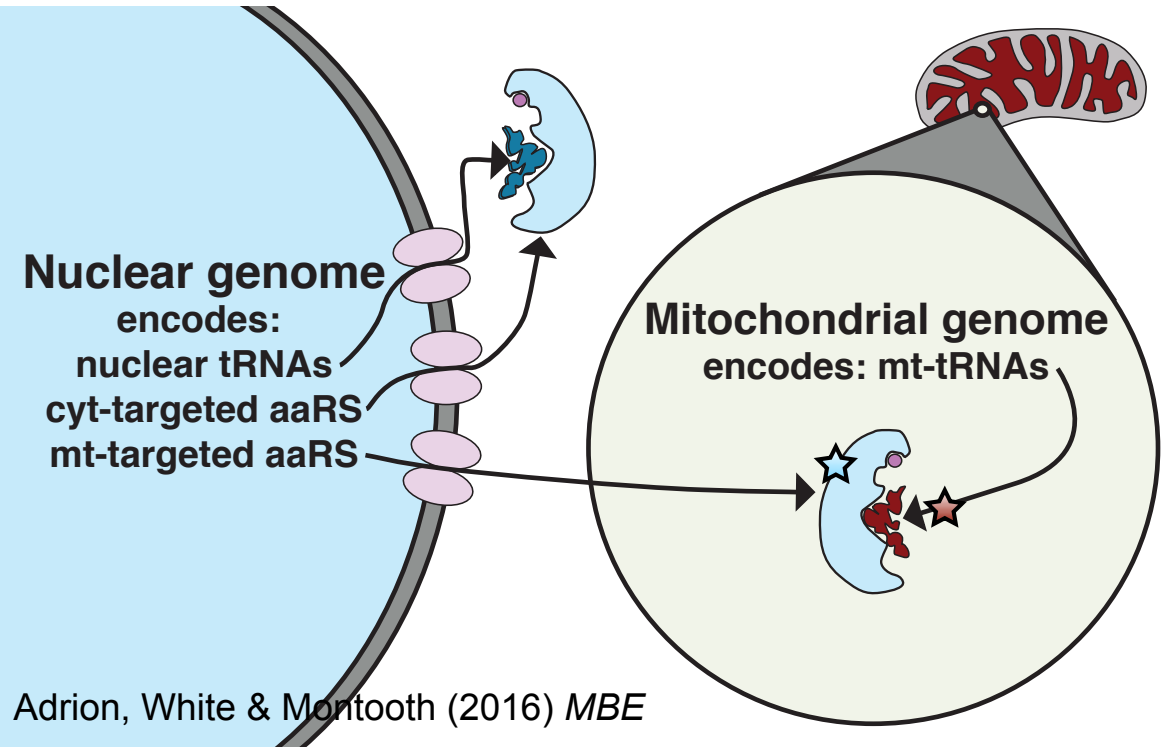
mt x nuc, P≤0.001, all traits

A small number of polymorphisms distinguish compatible and incompatible *D. simulans* mtDNAs

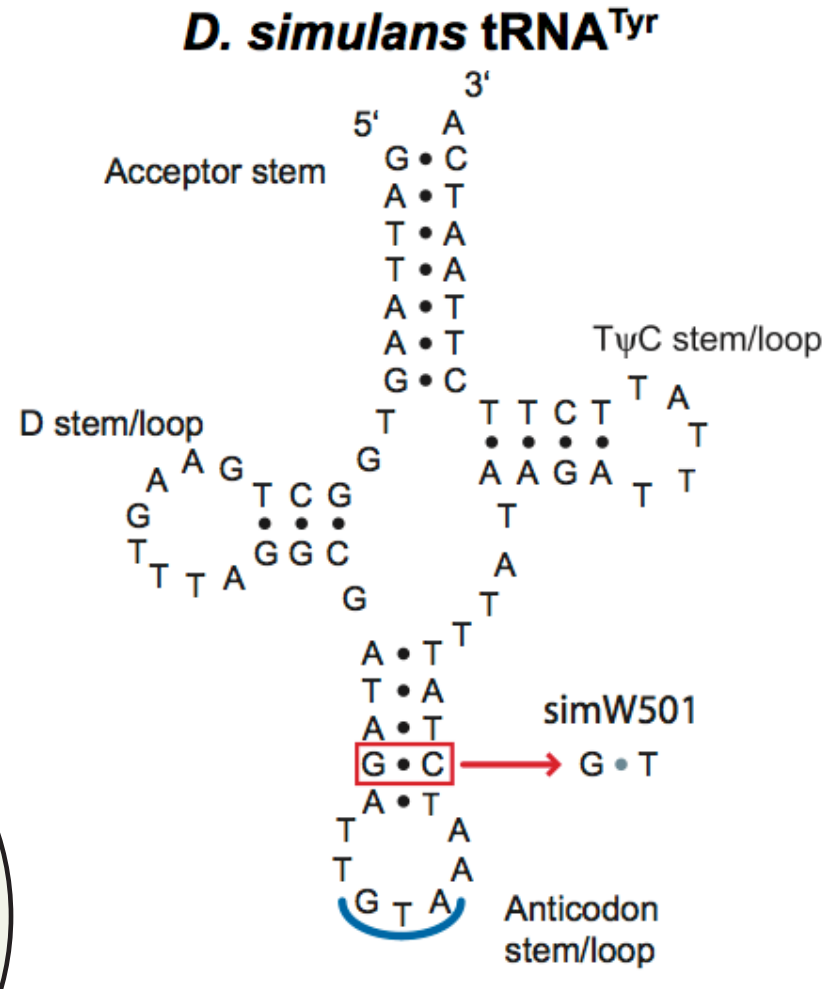




An incompatible interaction between a mt-tRNA and its nuclear partner (a mt-aaRS)

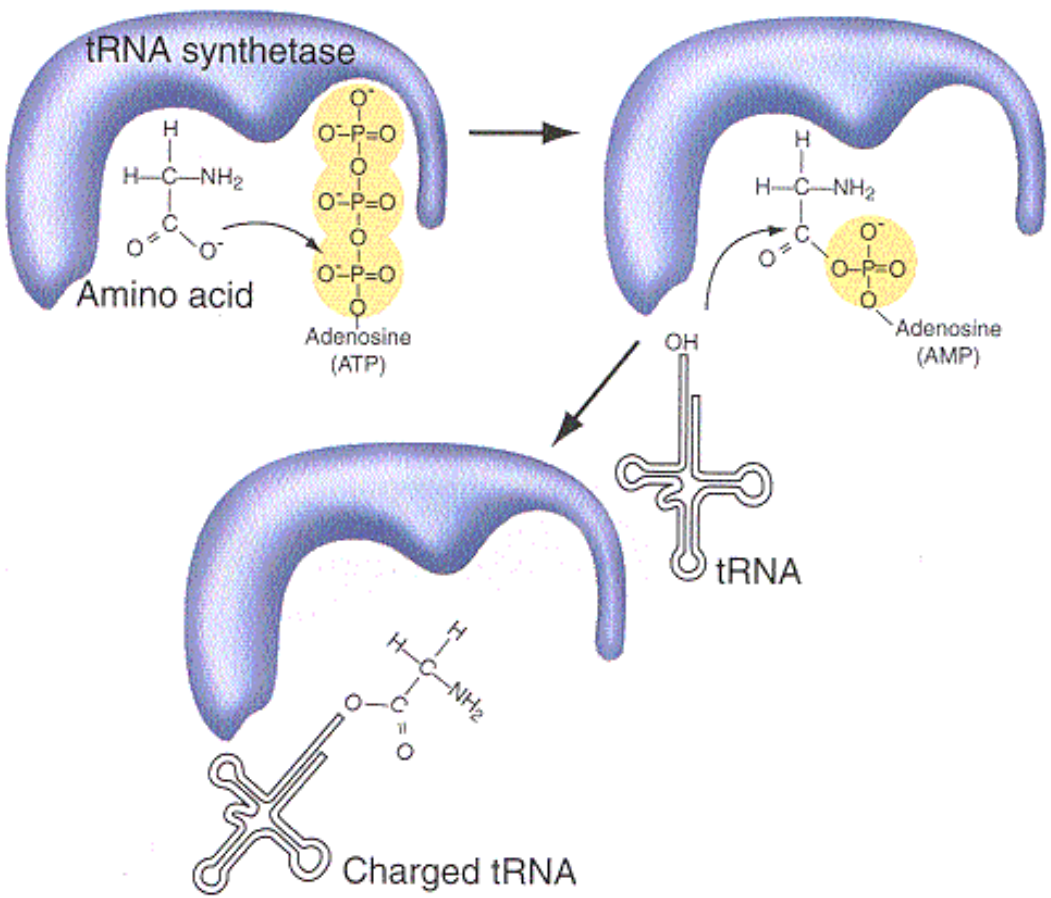


Adrion, White & Montooth (2016) *MBE*



An incompatible interaction between a mt-tRNA and its nuclear partner (a mt-aaRS)

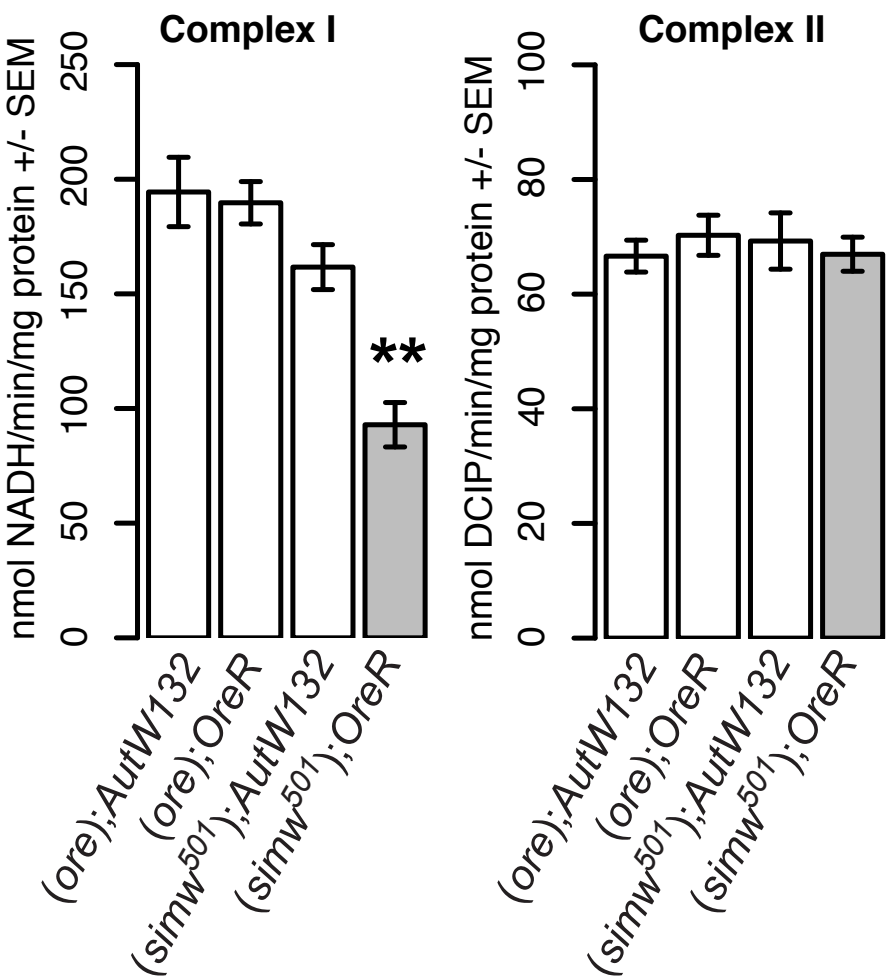
- *Aatm*: catalyzes attachment of tyrosine to the <u>mitochondrial</u> $tRNA^{Tyr}$

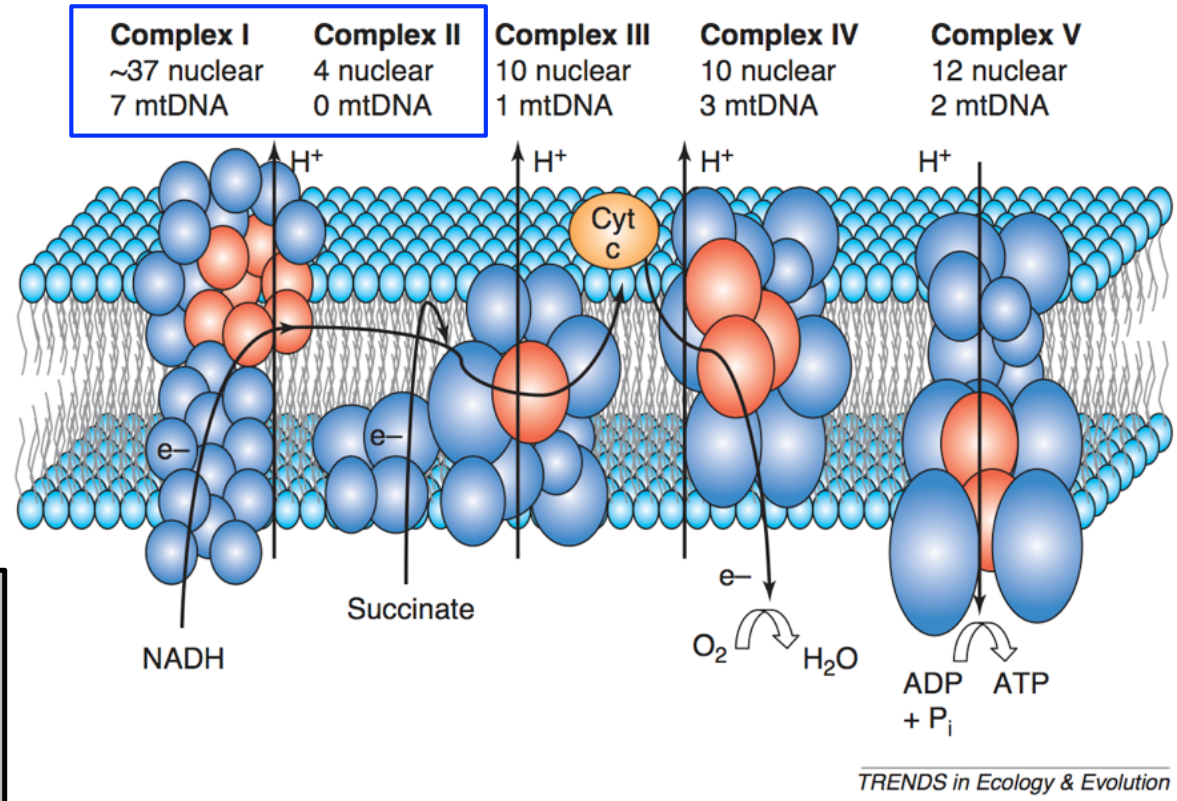


	catalytic domain	"KMSKS" sequence	ACB domain
Dmel	PLVTTEEGD	KFGKS	AGNAVWLDGNKTS
Dsim	PLVTTEEGD	KFGKS	AGNAVWLDCNKTS
Dyak	PLVTTEEGD	KFGKS	AGNAVWLDGNKTS
Dwil	PIVTNEEGD	KFGKS	AGNAVWLDPEKTS
Dana	PLVTNEEGD	KFGKS	AGNAVWLDANKTS
Dpse	PIVTNEEGD	KFGKS	AGNAVWLDENKTS
Dmoj	PIVTNEEGD	KFGKS	AGNAVWLDESKTS
Dvir	PIVTNEEGD	KFGKS	AGNAVWLDENKTS
Dgri	PIVTNEEGD	KFGKS	AGNAVWLDEKKTS
Agam	PLVTNEEGD	KFGKS	AGNAVWLSDDRTS
Drer	PLVTTSMGD	KLGKT	AGNAVWLNRDKTS
Mmus	PLITSTTGA	KLGKS	AGNAVWLNREKTS
Hsap	PLITSTTGA	KLGKS	AGNAVWLNRDKTS
OreR	PLVTTEEGD	KFGKS	VGNAVWLDGNKTS

Mo Siddiq

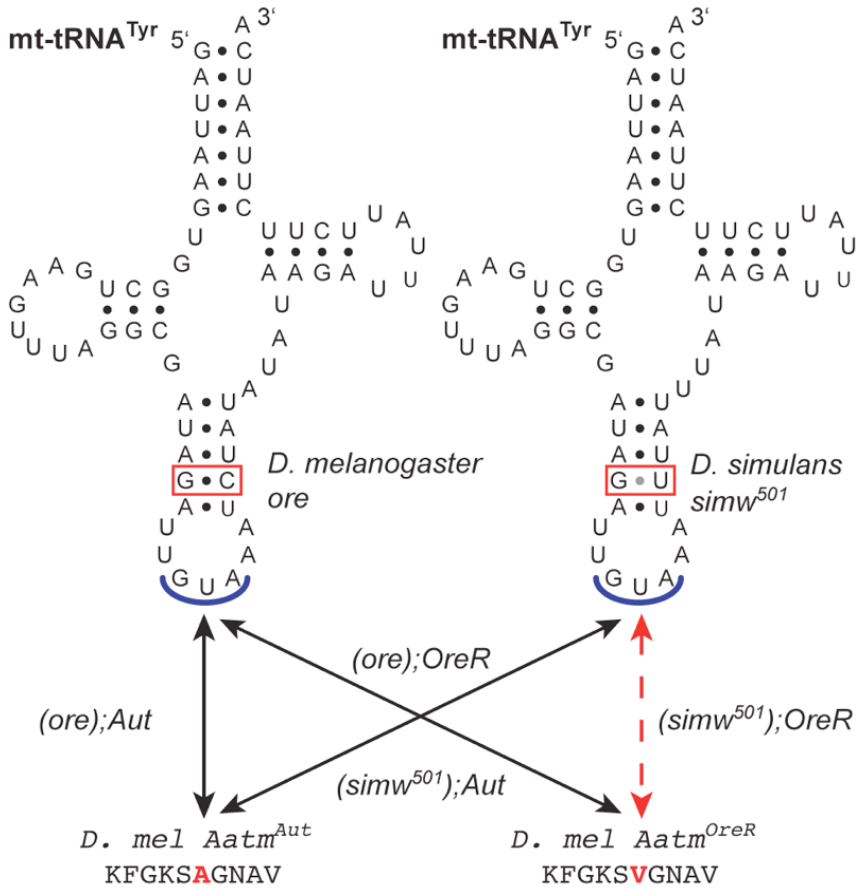
The mito-nuclear incompatibility compromises mitochondrial function





- Consistent pattern across complexes
- Decreased mitochondrial protein synthesis leads to decreased OXPHOS

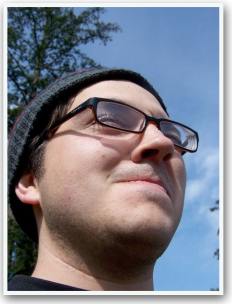
Effects of this mito-nuclear incompatibility are highly condition dependent



- Abiotic conditions
- Lifestage
- Sex

- G x G x E (xE)
- Vary with energy demand

Hoekstra, Siddiq & Montooth 2013 Genetics
Hoekstra *et al.* 2018 Evolution Letters



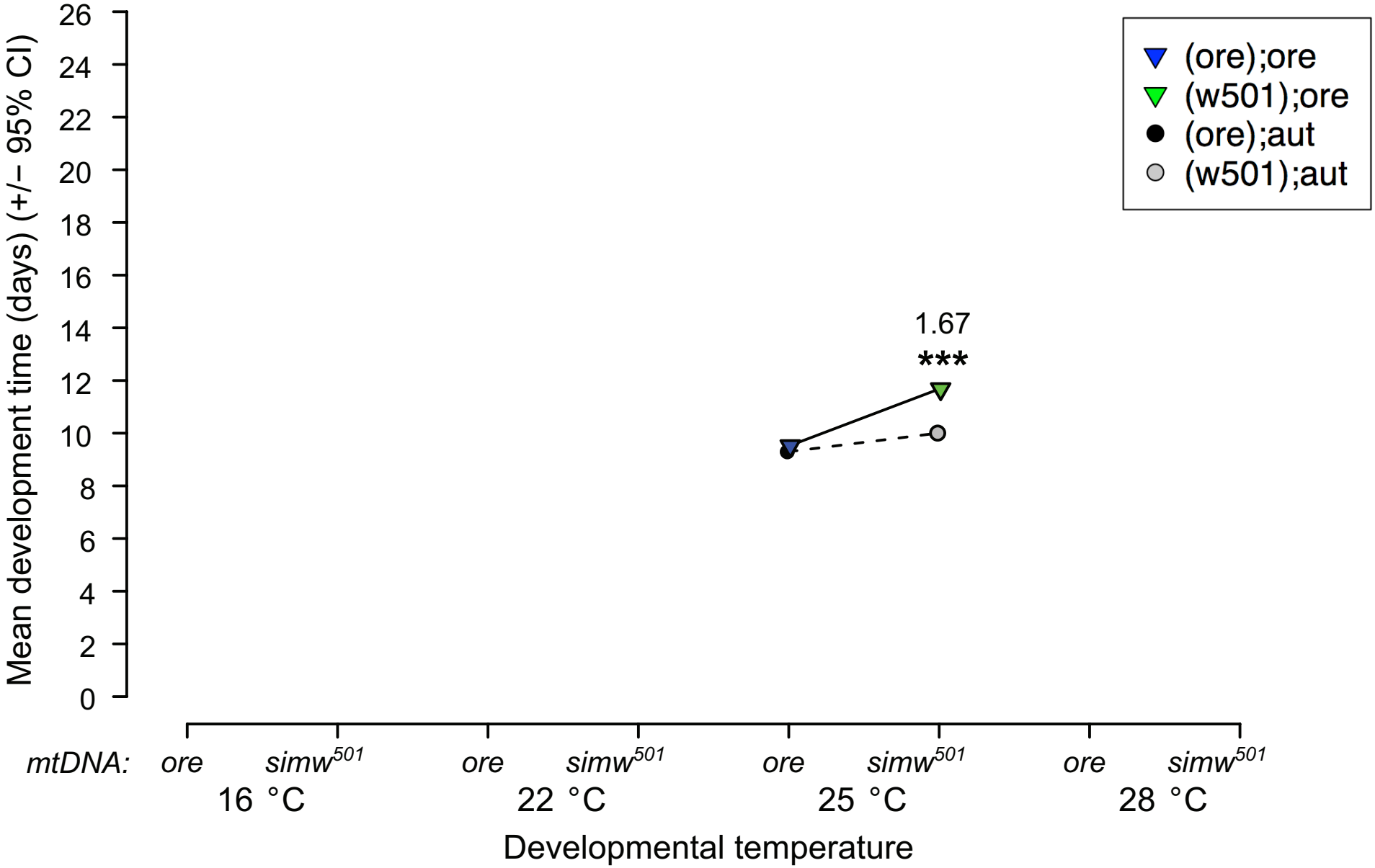
Luke Hoekstra

Mo Siddiq

Cole Julick

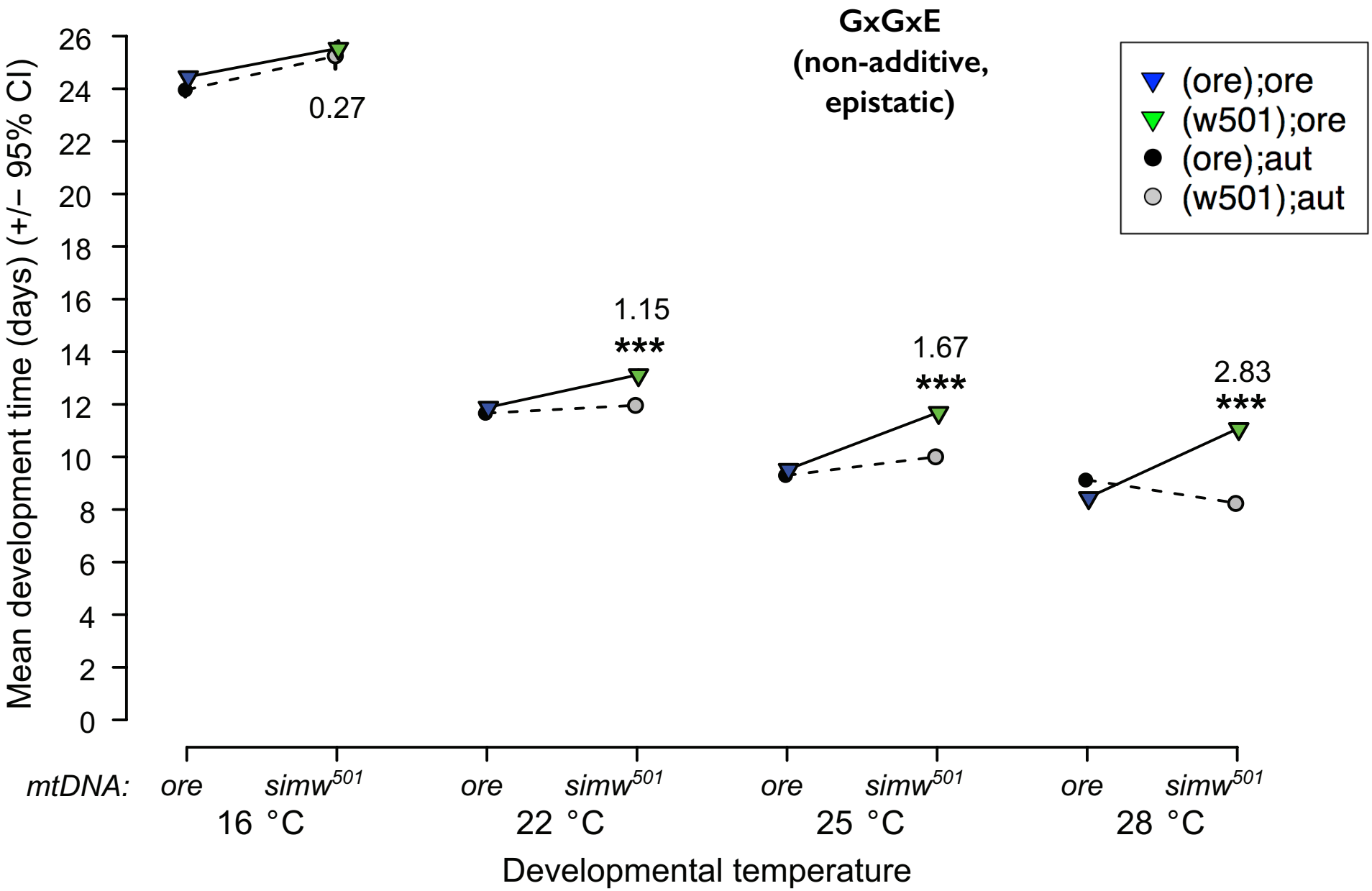
Katelyn Mika

The mito-nuclear incompatibility is strongly temperature dependent



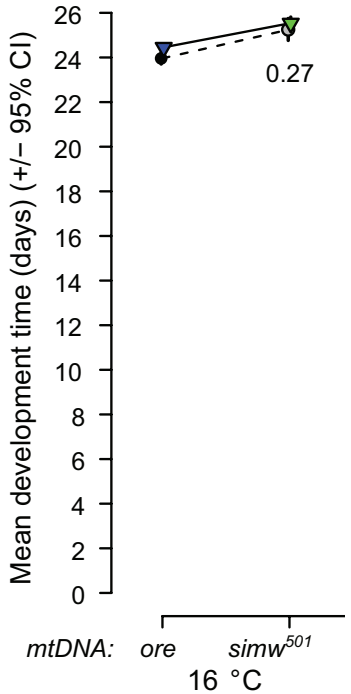
*** mtDNA x nuclear P<0.0001

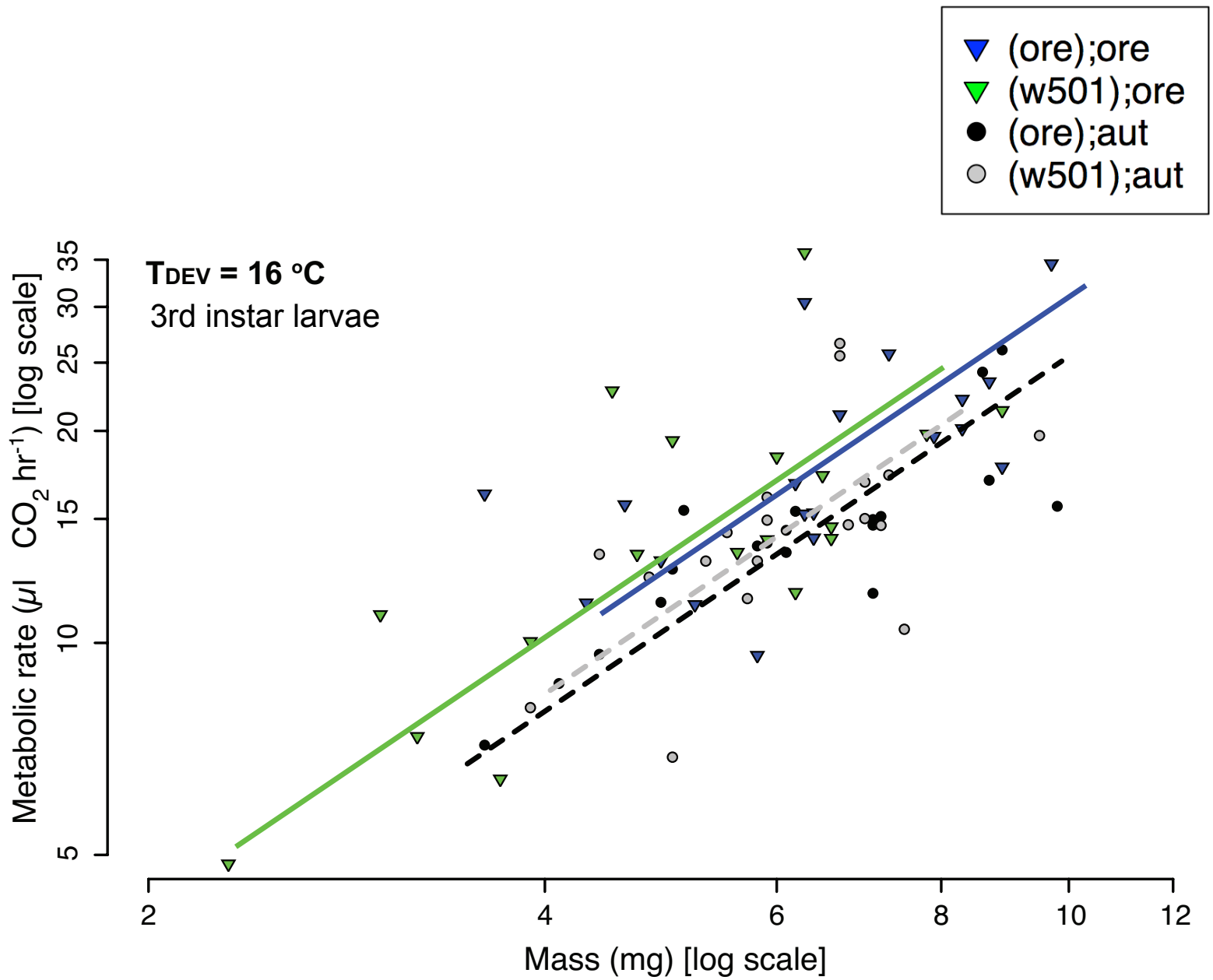
The mito-nuclear incompatibility is strongly temperature dependent



*** mtDNA x nuclear P<0.0001

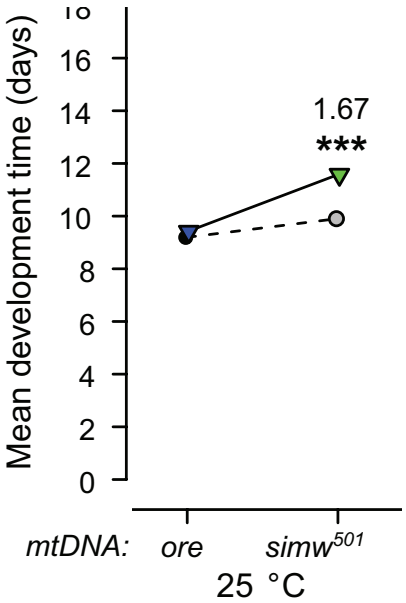
At 16C, larval development and metabolic rates are normal

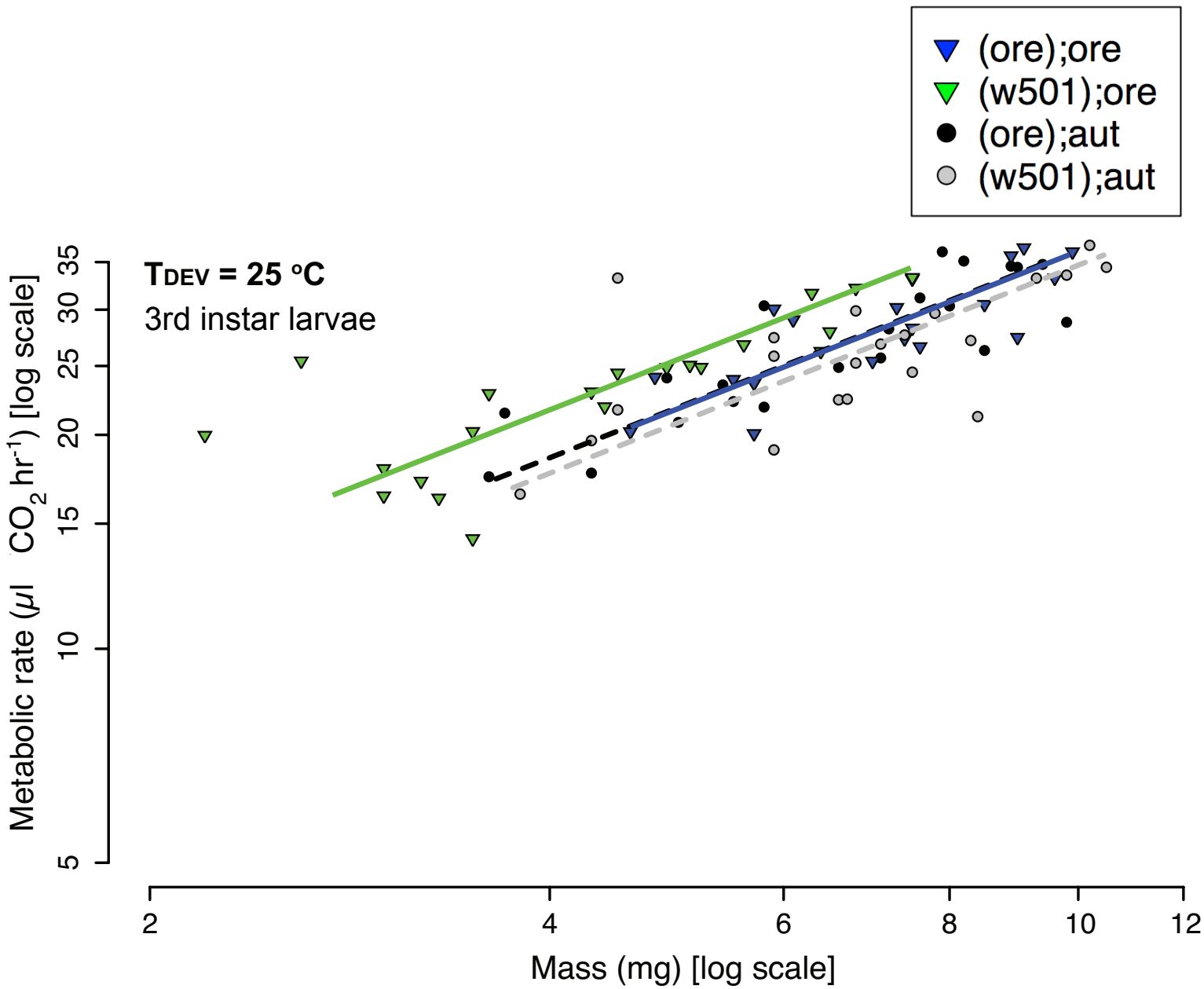




Hoekstra, Siddiq & Montooth 2013 Genetics

At 25C, larval metabolic rates are elevated and development is delayed

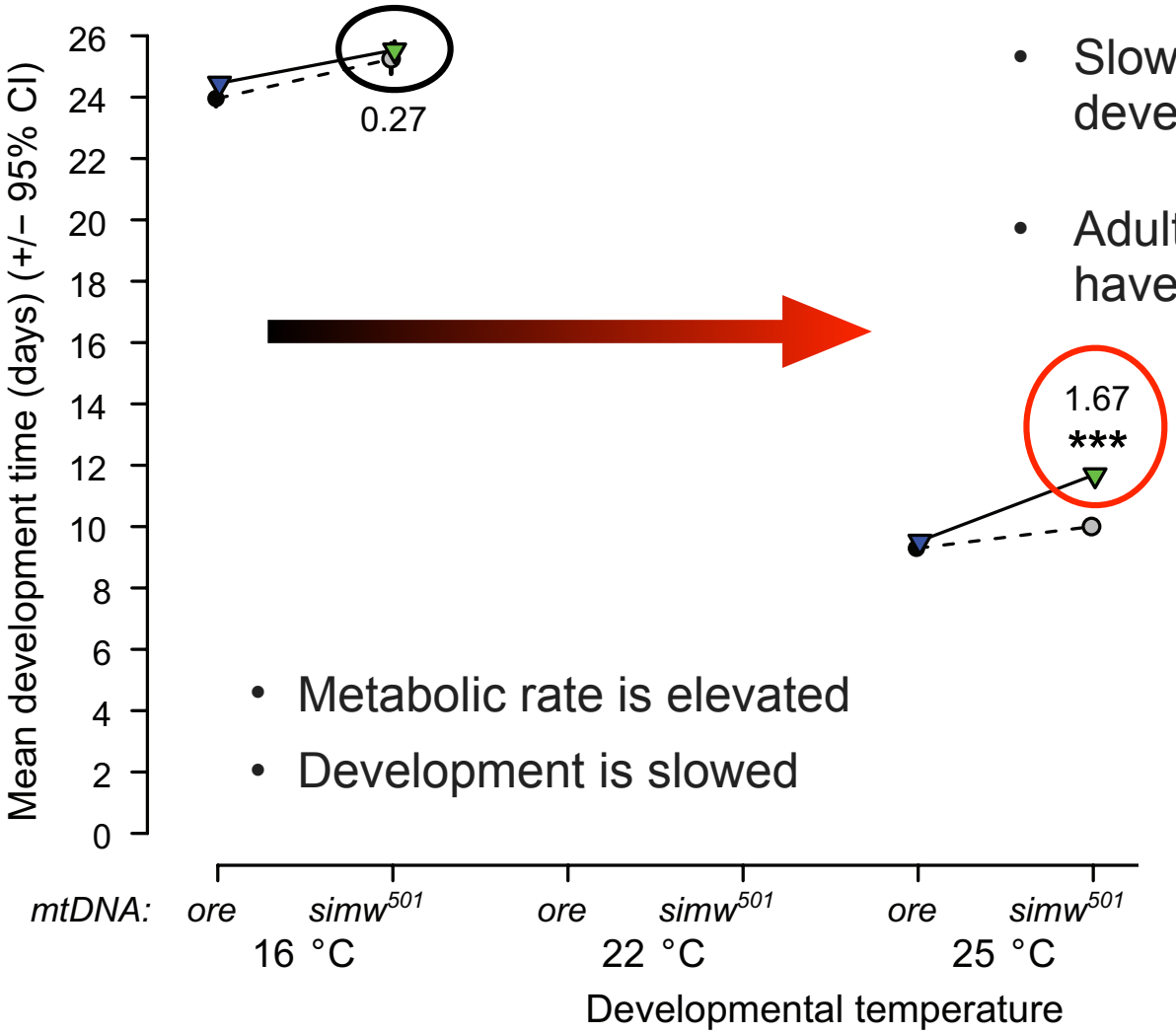




Hoekstra, Siddiq & Montooth 2013 Genetics

Genotype P < 0.05

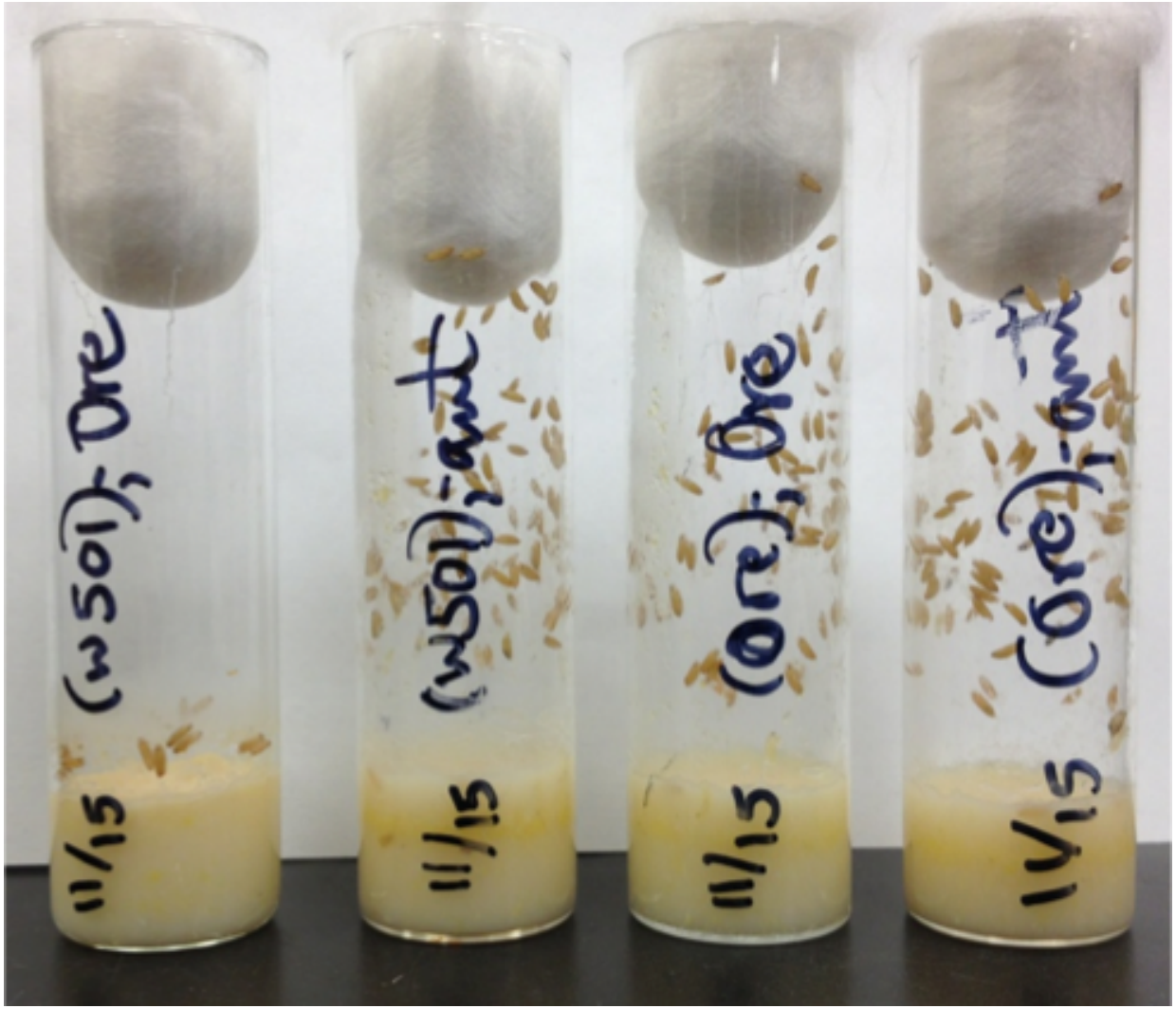
At higher temperatures, incompatible larvae are less energetically efficient



- Metabolic rate is elevated
- Development is slowed

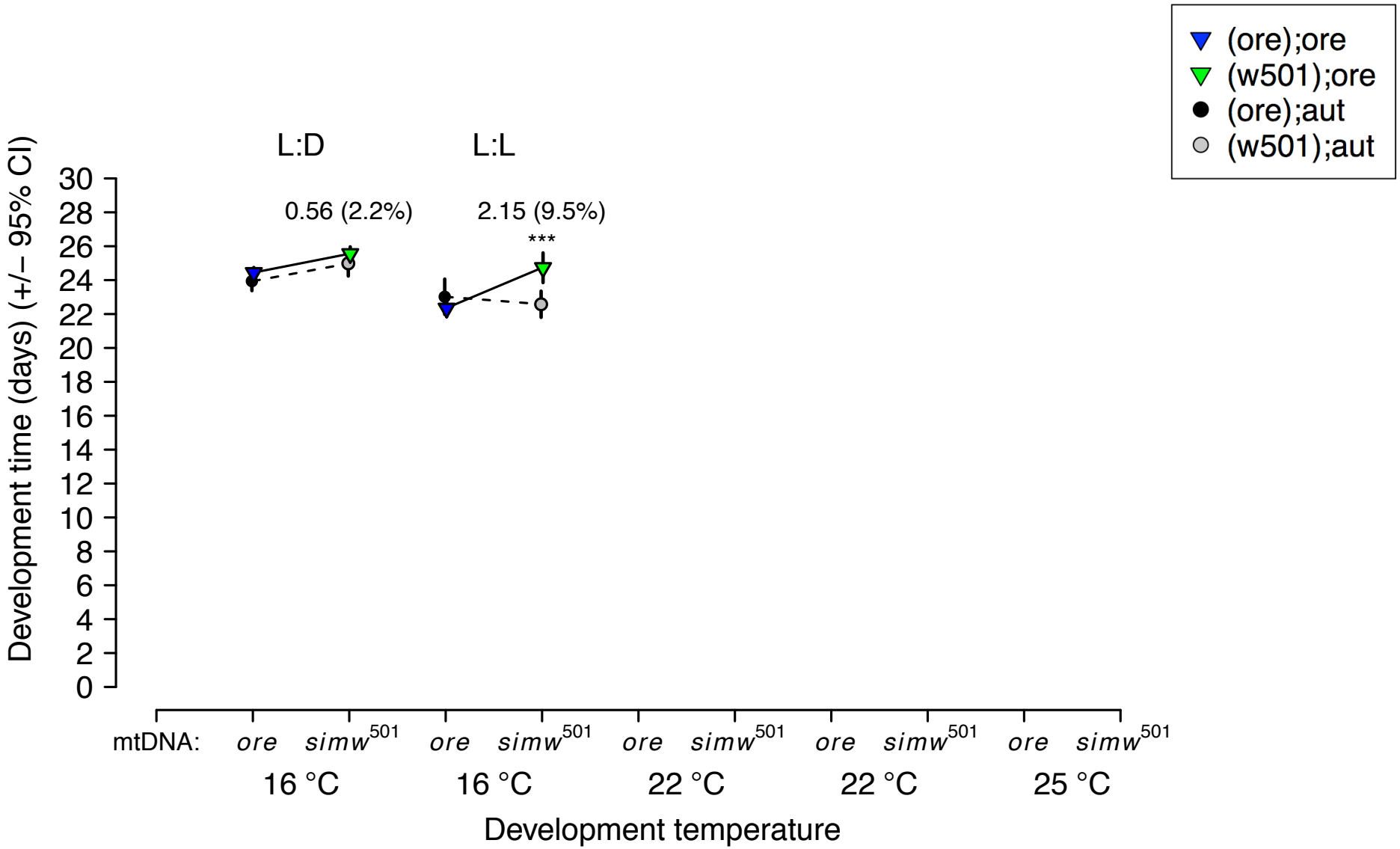
- Maintaining ATP homeostasis with less efficient OXPHOS requires the utilization of more energy stores
- Slows the accumulation of mass during development
- Adults that survive attain normal mass and have normal metabolic rates

Pupation height also supports that larvae are less energetically efficient



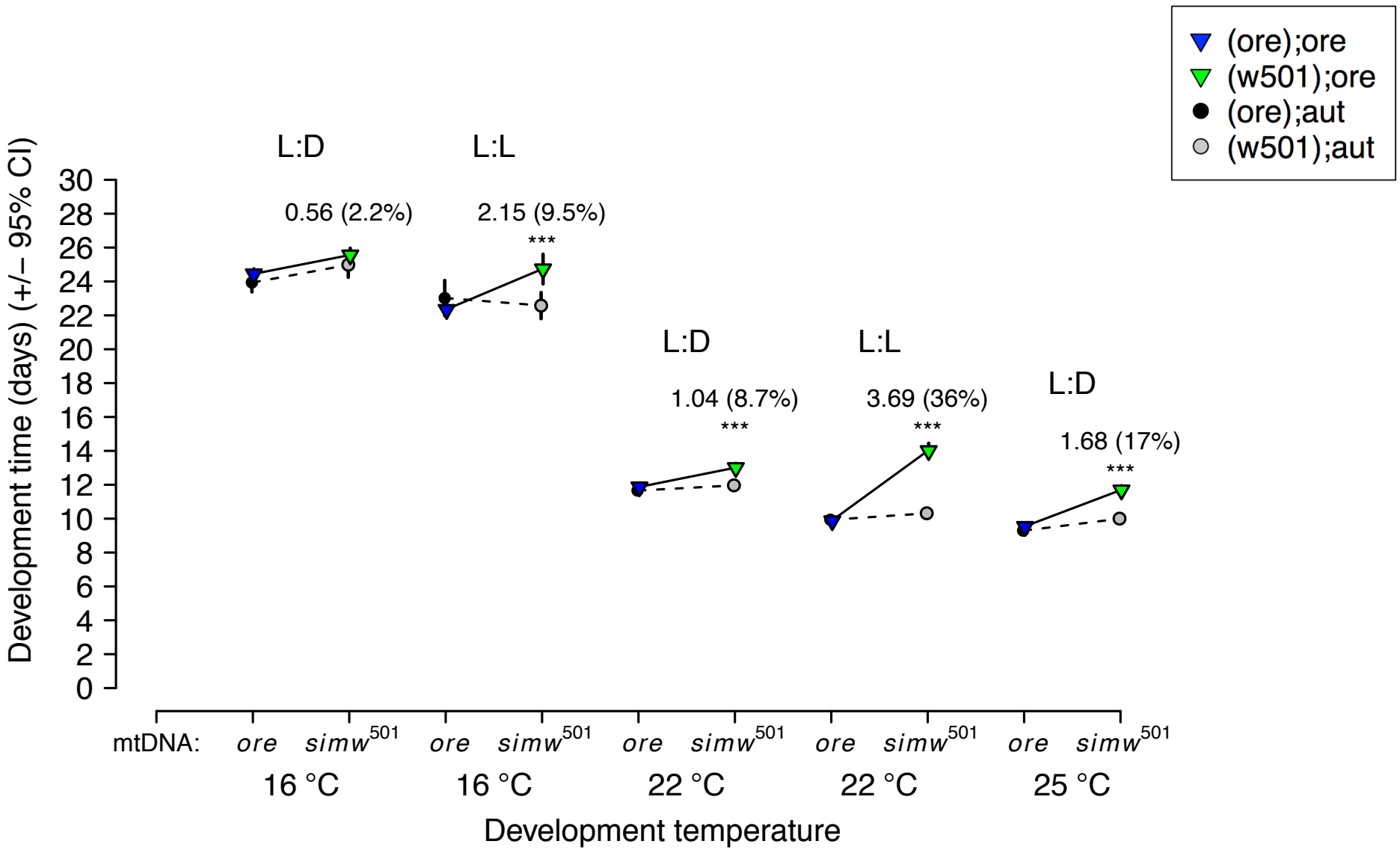
mito x nuc x temp $p<0.001$
(GxGxE)

Development in constant light mimics the temperature effect



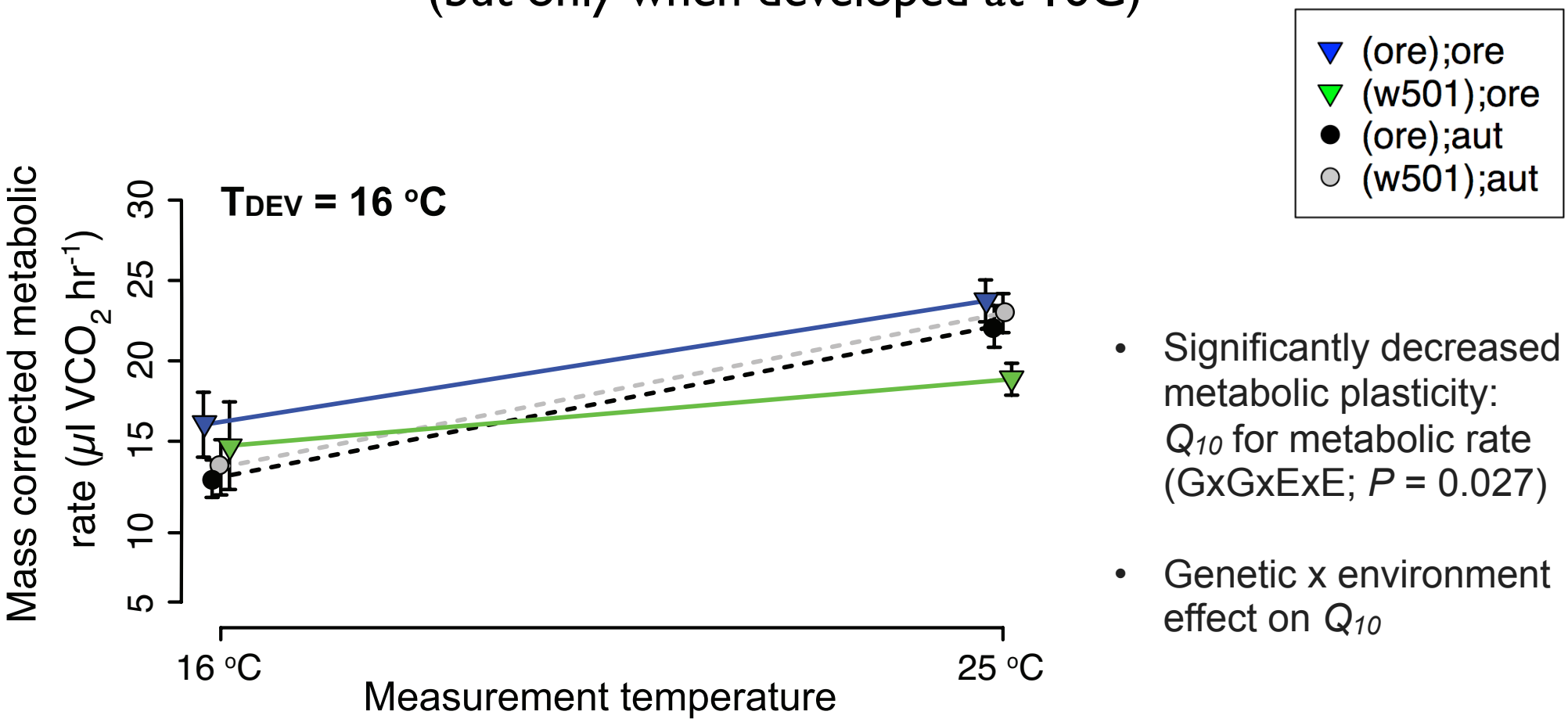
*** mtDNA x nuclear P<0.0001

Development in constant light mimics the temperature effect



*** mtDNA x nuclear P<0.0001

This is one of my all-time favorite results: Metabolic rate is less thermally plastic in incompatible larvae (but only when developed at 16C)

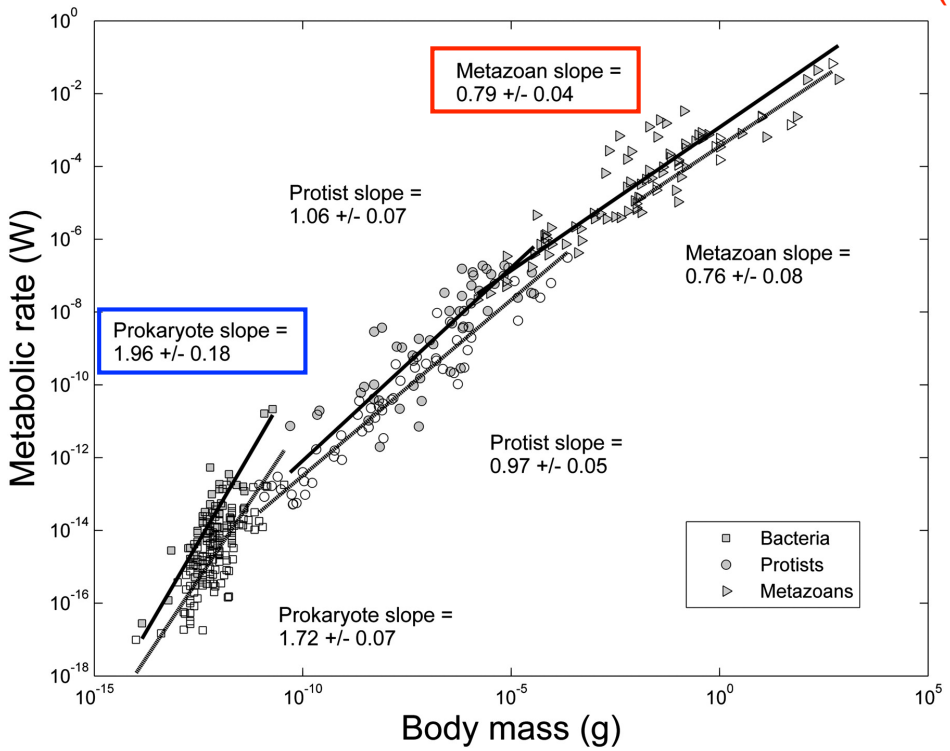


- Significantly decreased metabolic plasticity: Q_{10} for metabolic rate (GxGxExE; P = 0.027)
- Genetic x environment effect on Q_{10}

Hoekstra, Siddiq & Montooth 2013 Genetics

Temperature affects how metabolic rate scales with mass in larvae

Mass-specific metabolic rate decreases with mass (b<1)

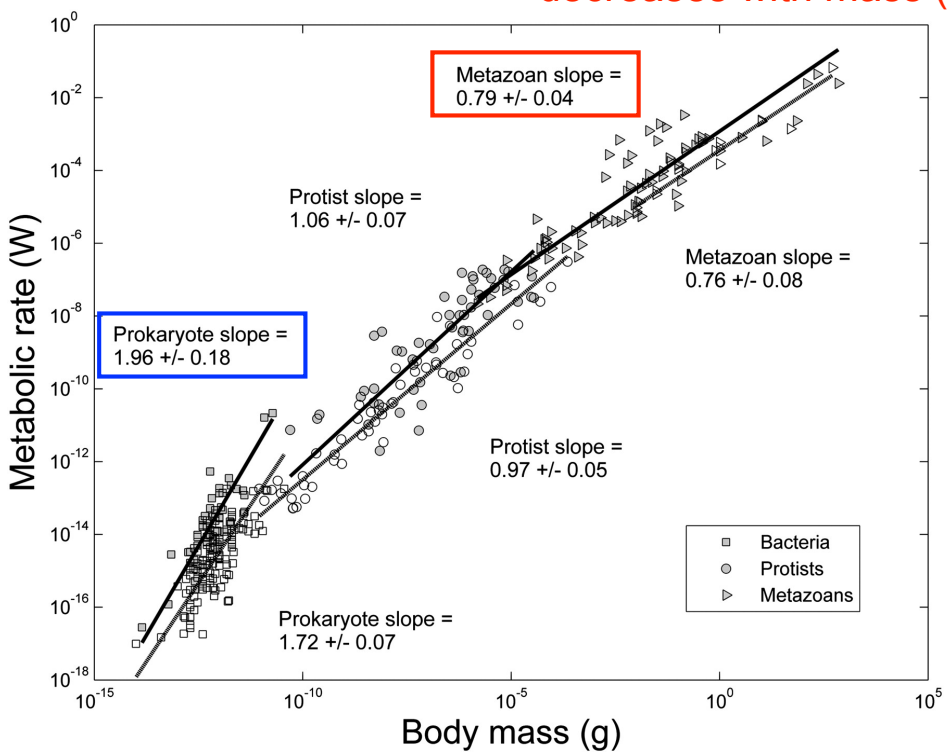


DeLong *et al.* 2010 PNAS

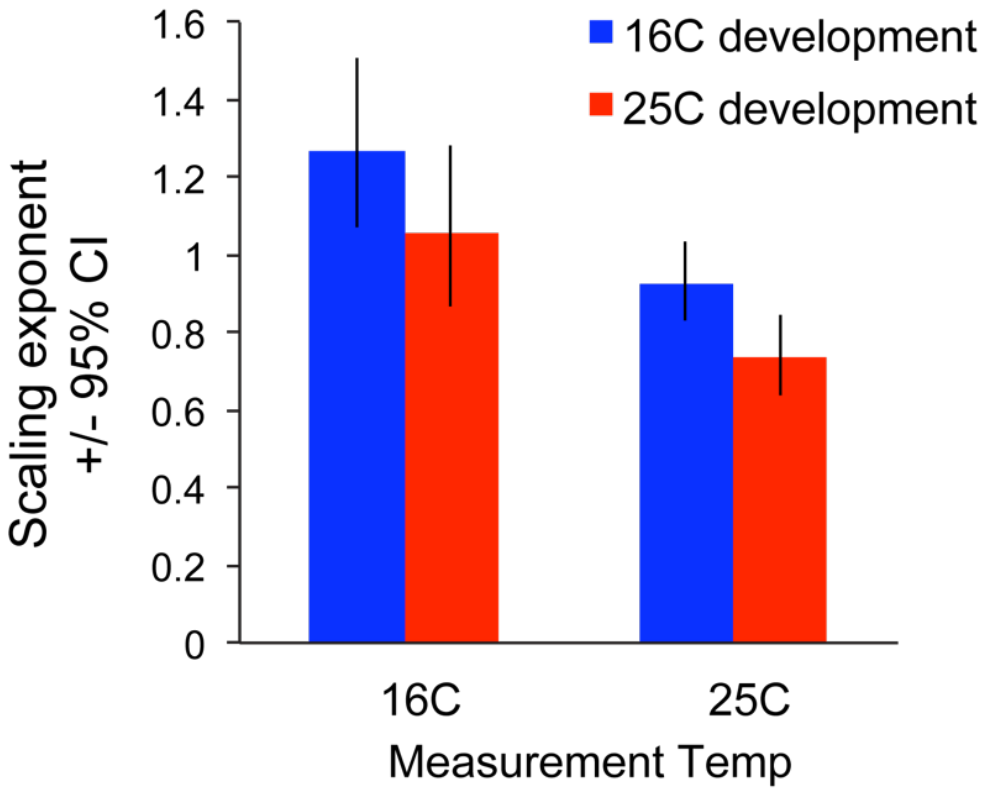
Mass-specific metabolic rate increases with mass (b>1)

Temperature affects how metabolic rate scales with mass in larvae

Mass-specific metabolic rate decreases with mass (b<1)



DeLong *et al.* 2010 PNAS

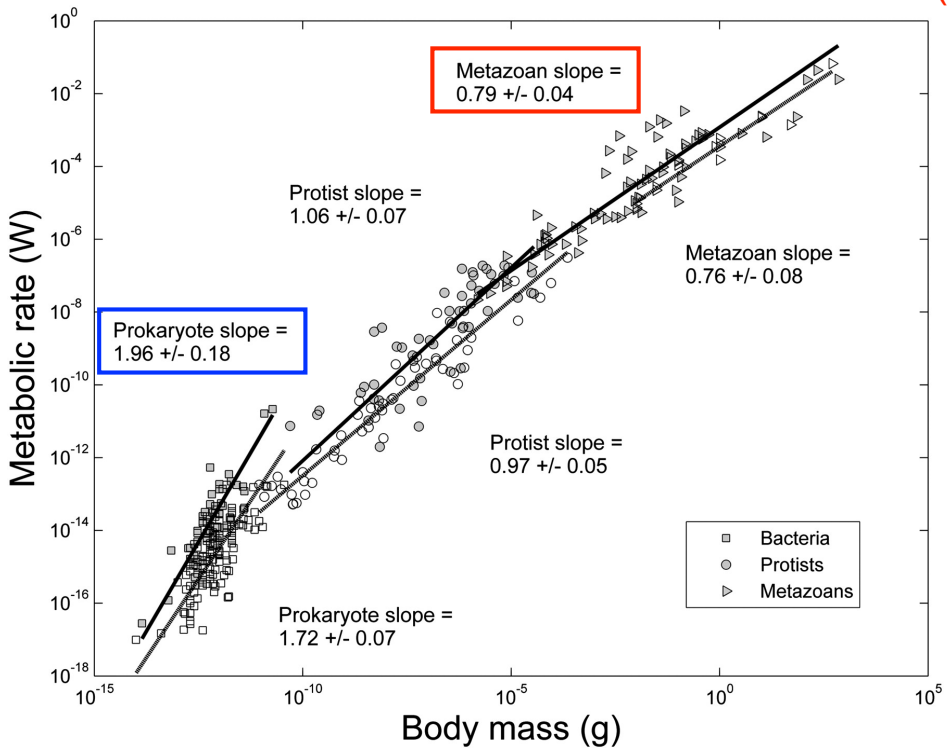


Mass-specific metabolic rate increases with mass (b>1)

Hoekstra, Siddiq & Montooth 2013 Genetics
Greenlee, Helm & Montooth 2014 ICB

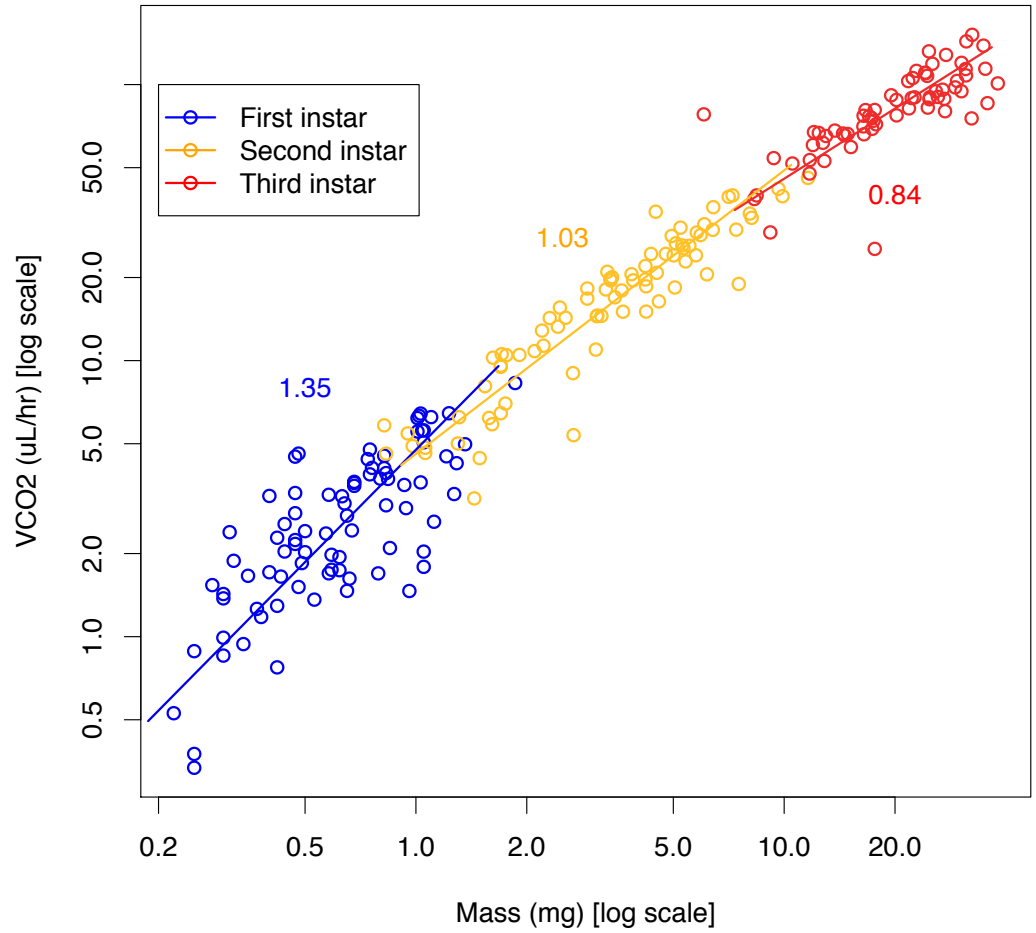
Ontogeny of metabolic scaling in insect larvae

Mass-specific metabolic rate decreases with mass (b<1)



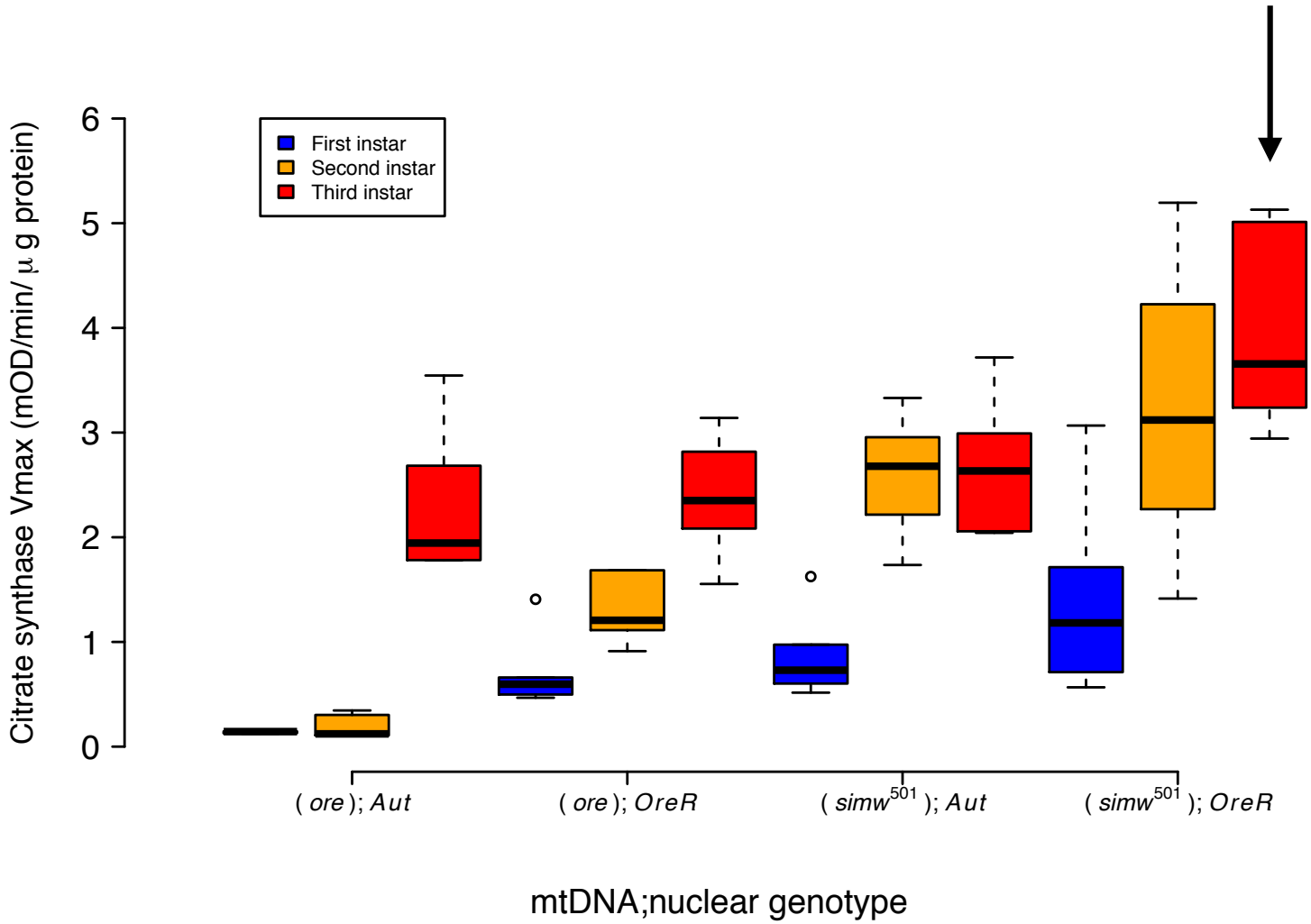
DeLong *et al.* 2010 PNAS

Mass-specific metabolic rate increases with mass (b>1)



Drosophila: Matoo et al. (2019) Genetics
Manduca: Callier & Nijhout (2012) PLoS One

Physiological compensation in mito-nuclear incompatible larvae

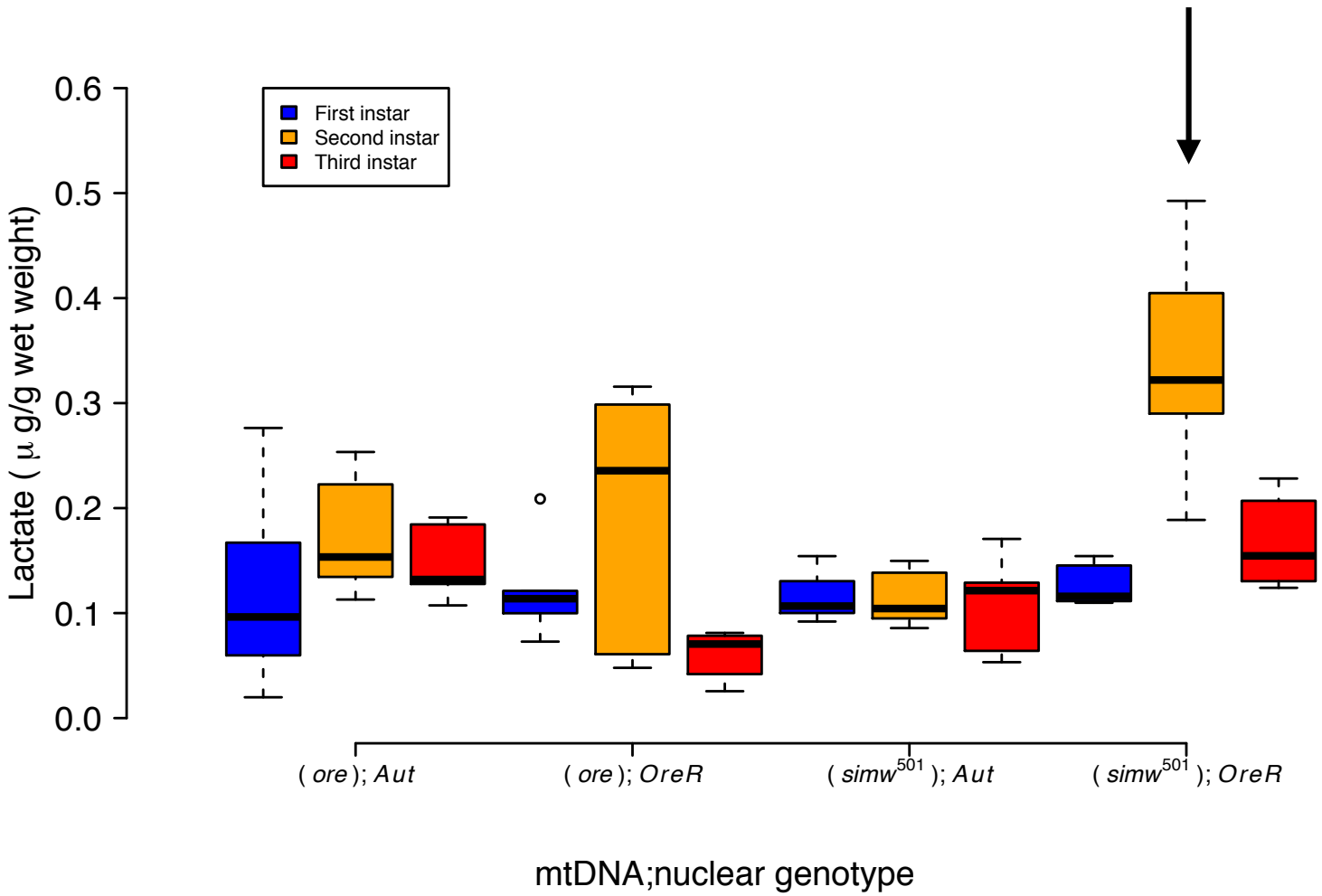



```
instar           P < 2.2e-16 ***
genotype         P = 2.13e-13 ***
instar:genotype  P = 0.001531 **
```

Omera Matoo

Matoo et al. (2019) Genetics

Mito-nuclear incompatible larvae have elevated lactate

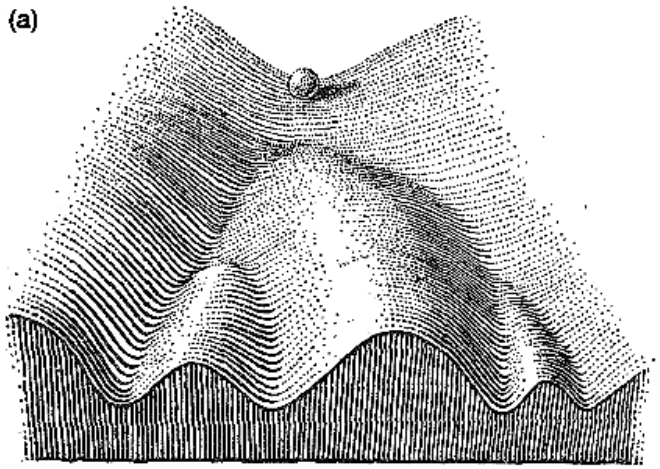


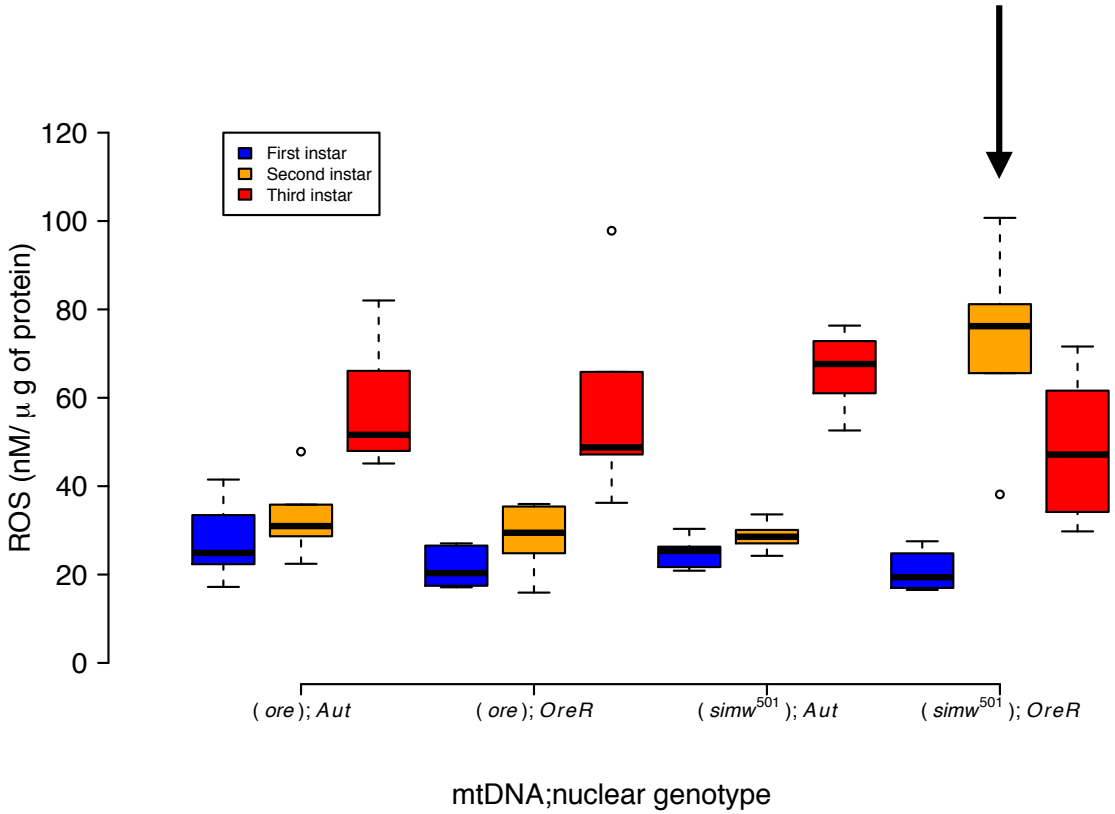

```
instar           P = 4.583e-07 ***
genotype         P = 1.142e-05 ***
instar:genotype  P = 0.0003799 ***
```

Matoo et al. (2019) Genetics

Mito-nuclear incompatible larvae have elevated lactate & reactive oxygen species (and they appear to uncouple their mitochondria)

- Physiological compensation
- Rely on anaerobic ATP production and drive more TCA/OXPHOS

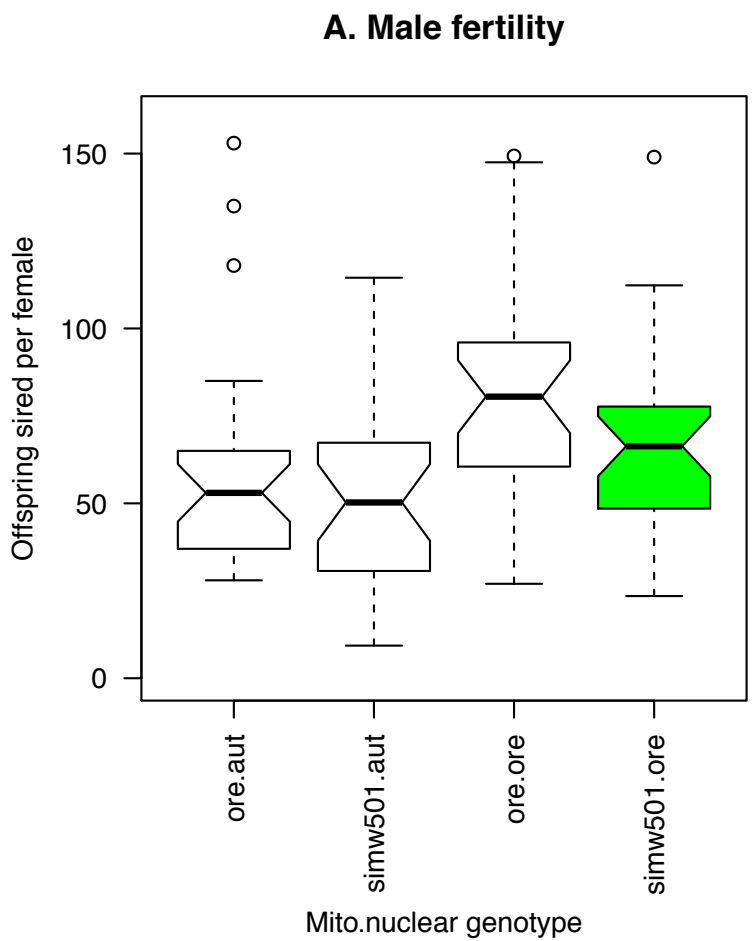


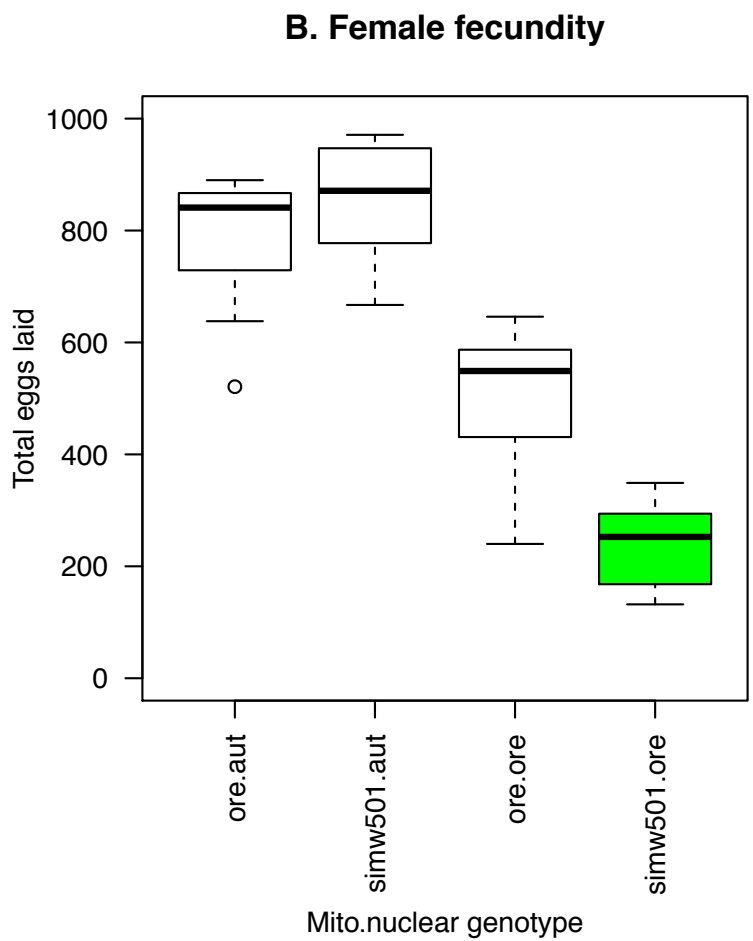



```
instar           P = 2.347e-12 ***
instar:mito:nuc  P = 5.166e-05 ***
```

Matoo et al. (2019) Genetics

GxGxSex: Things seem worse for females

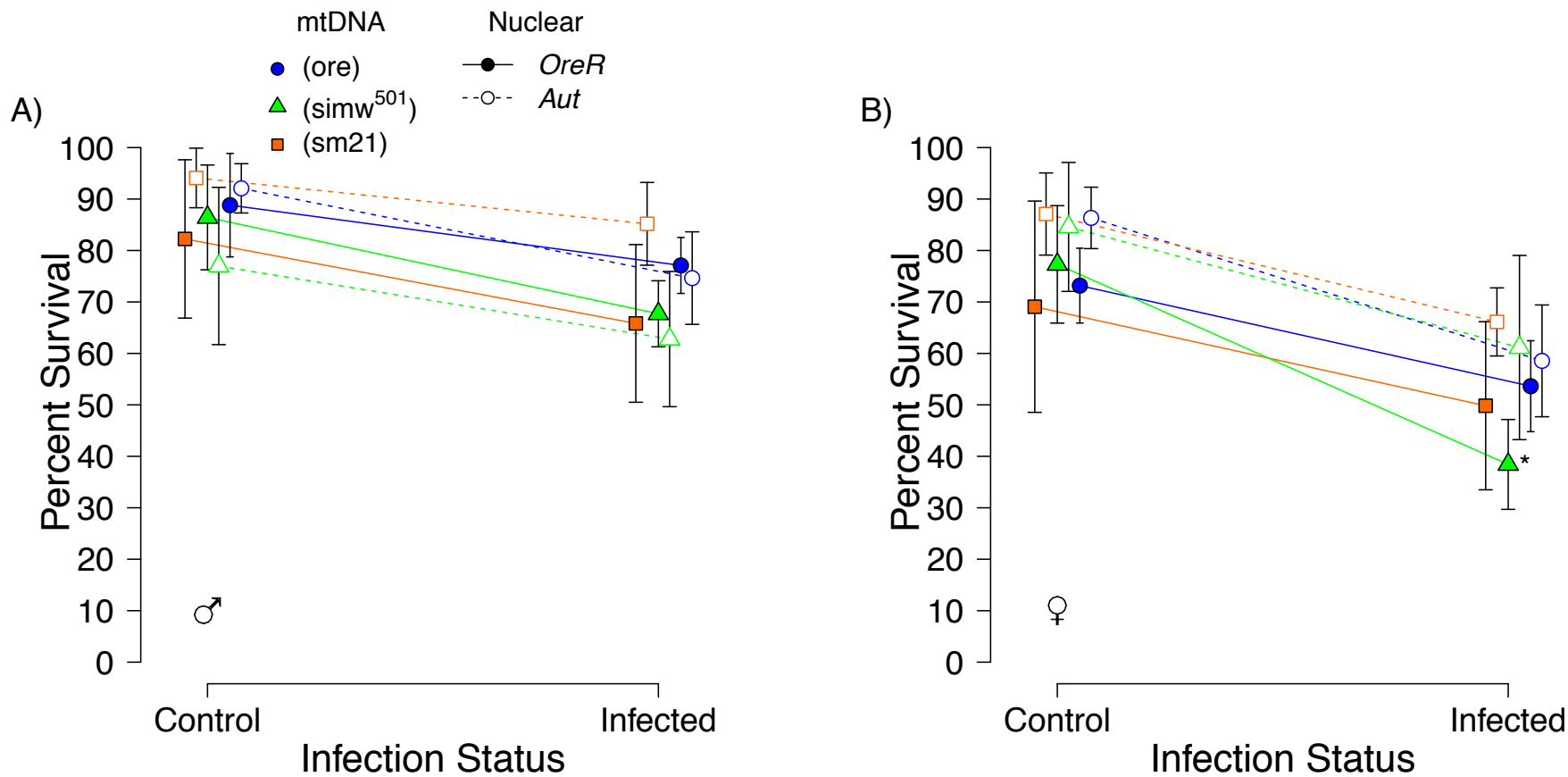




`mtDNA x nuclear NS`

`mtDNA x nuclear P=0.0003`

The mito-nuclear incompatibility compromises immune function



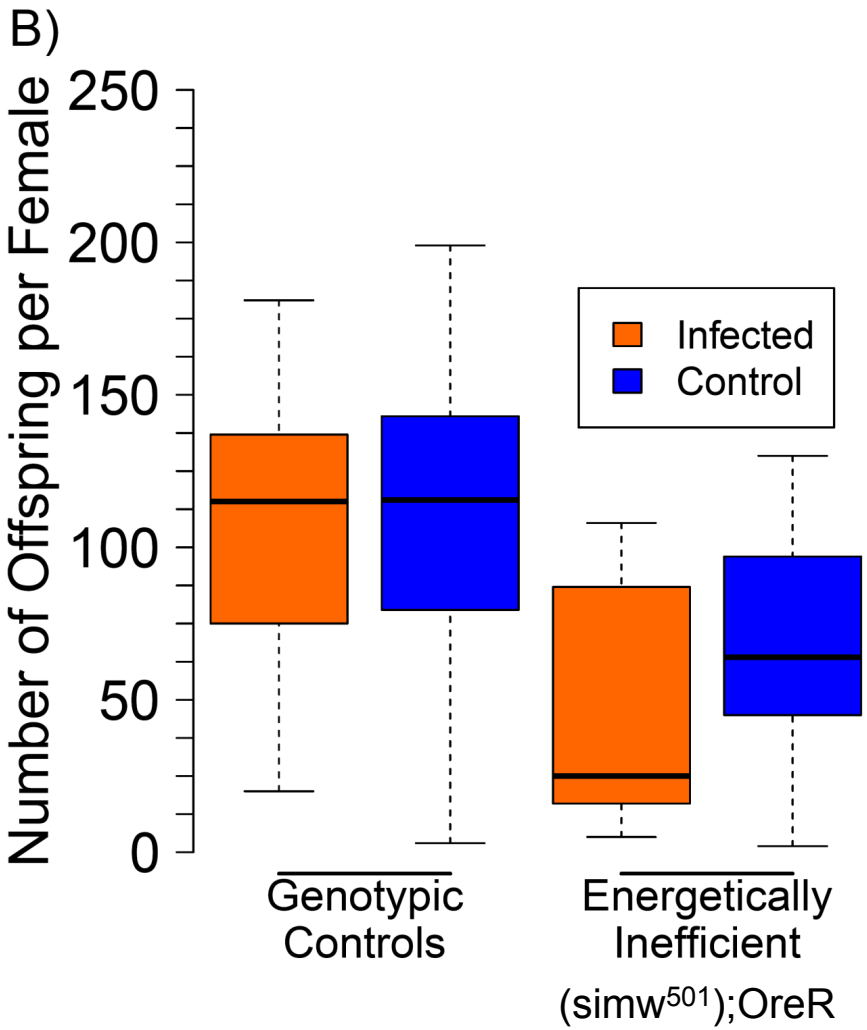
mtDNA*nuclear*infection: $\chi^2_{2,\,104} = 8.51$, $P = 0.014$

Infection with gram-negative extracellular natural pathogen *Providencia rettgeri*

Buchanan, Meiklejohn & Montooth (2018) ICB

Justin Buchanan

The mito-nuclear incompatibility reveals an immunity-fecundity tradeoff in females



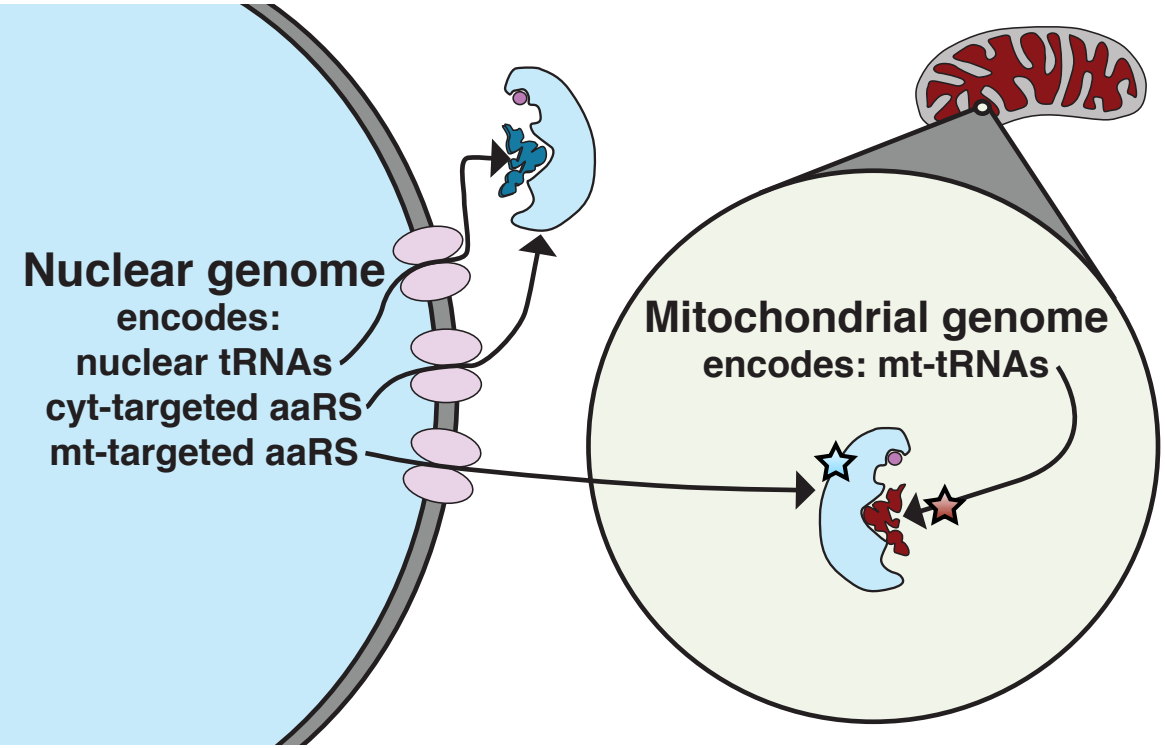
Buchanan, Meiklejohn & Montooth (2018) ICB

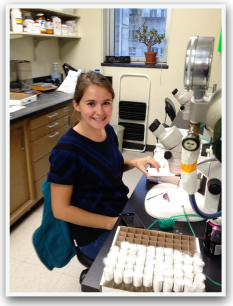
`mtDNA x nuclear x infection P=0.011`

Patterns exist that suggest molecular energetic costs and processes matter at organismal levels and evolutionary scales

mt-tRNA - aaRS interactions: Implications for genome evolution

- The nuclear genome has two sets of tRNA synthetases (aaRS)
  - cyt-aaRS interact with nuclear tRNAs in the cytoplasm
  - mt-aaRS interact with mt-tRNAs in the mitochondria
- Given faster rates of mtDNA substitution, mt-aaRS proteins should evolve faster



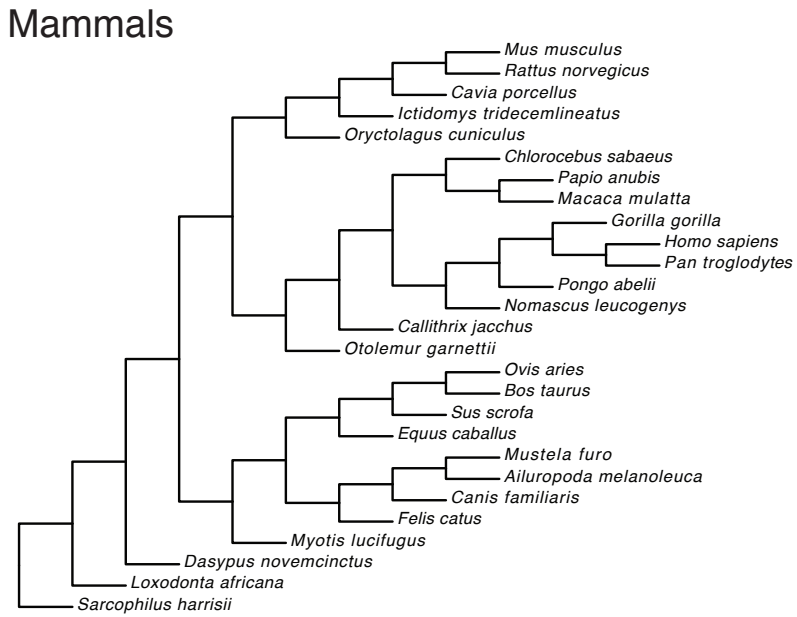


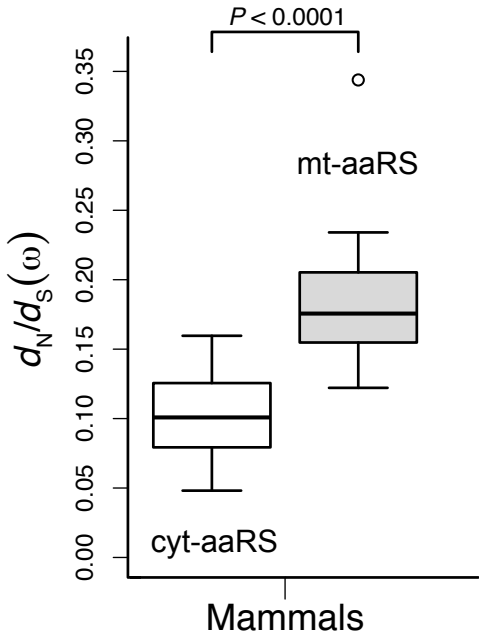
Signe White

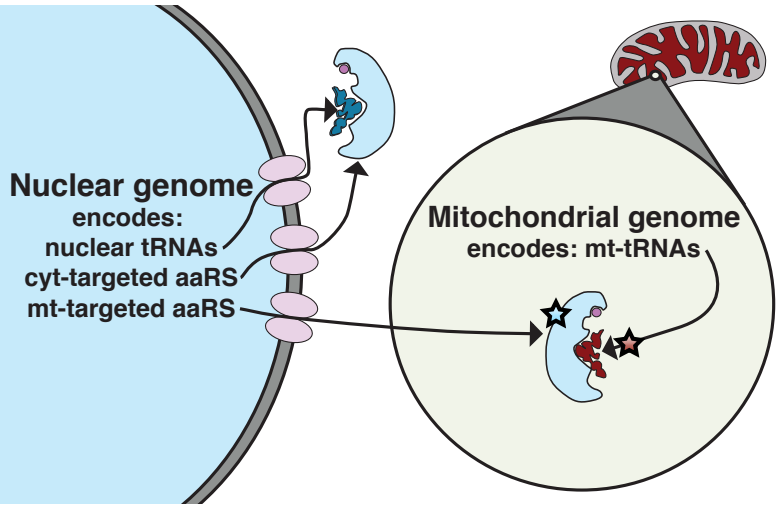
Jeff Adrion

mt-tRNA - aaRS interactions: Implications for genome evolution

- Given faster rates of mtDNA substitution, mt-aaRS proteins should evolve faster
- Evidence for this in mammals

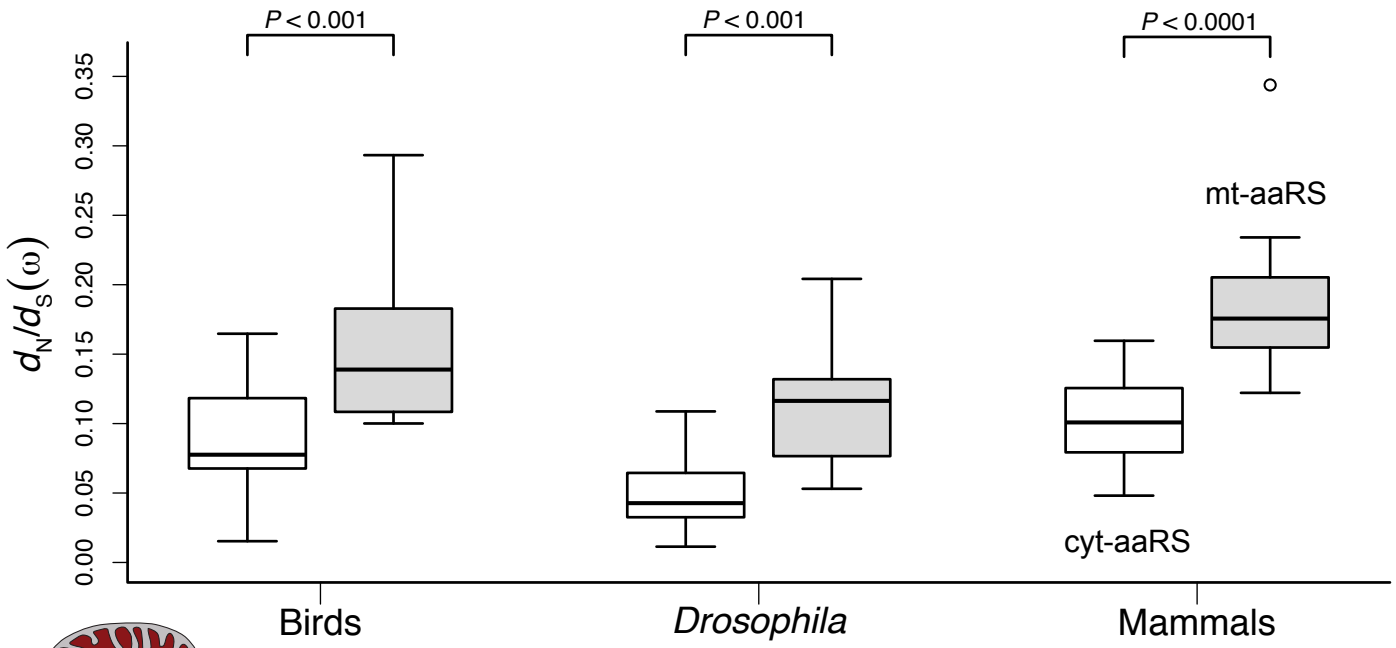


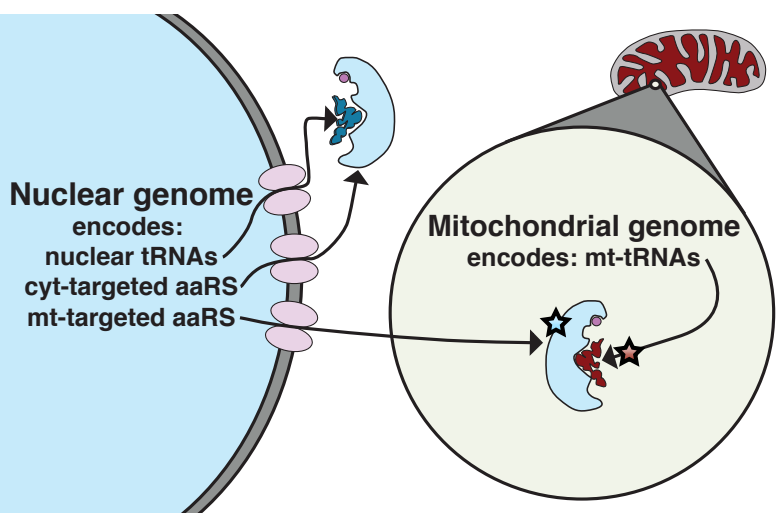




mt-tRNA - aaRS interactions: Implications for genome evolution

- Given faster rates of mtDNA substitution, mt-aaRS proteins should evolve faster
- Evidence for this in mammals, birds and Drosophila





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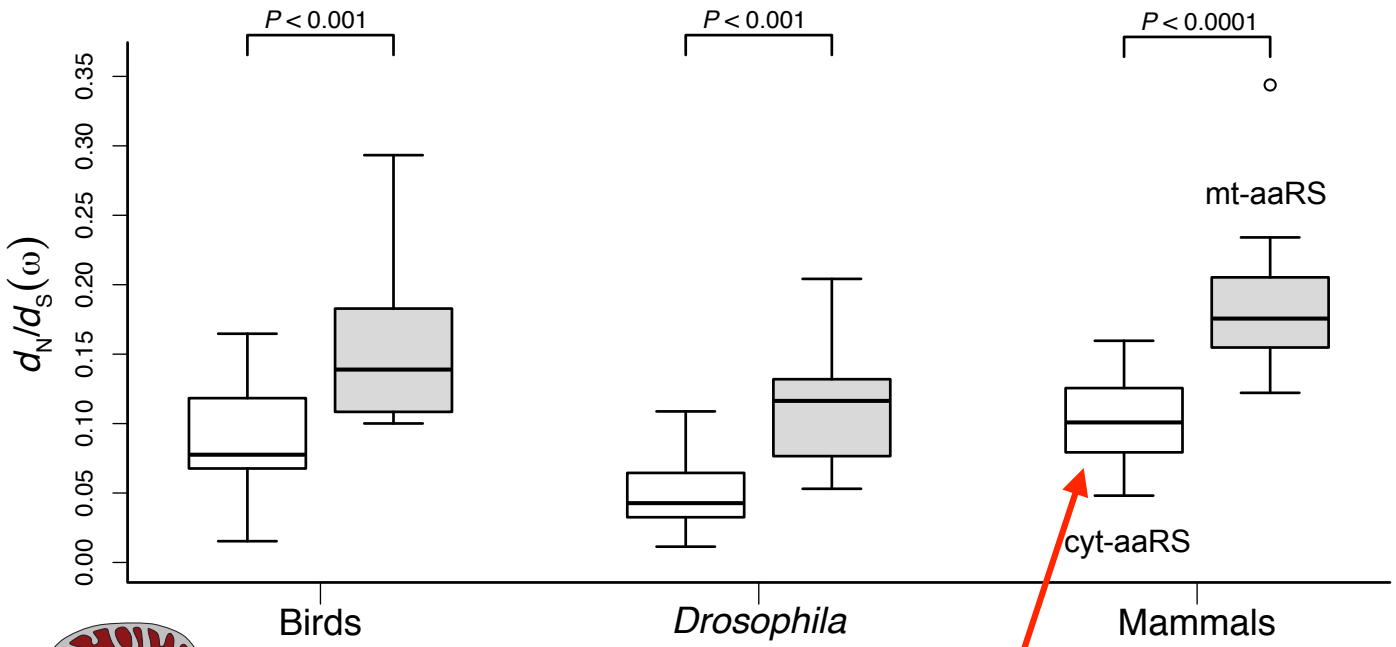
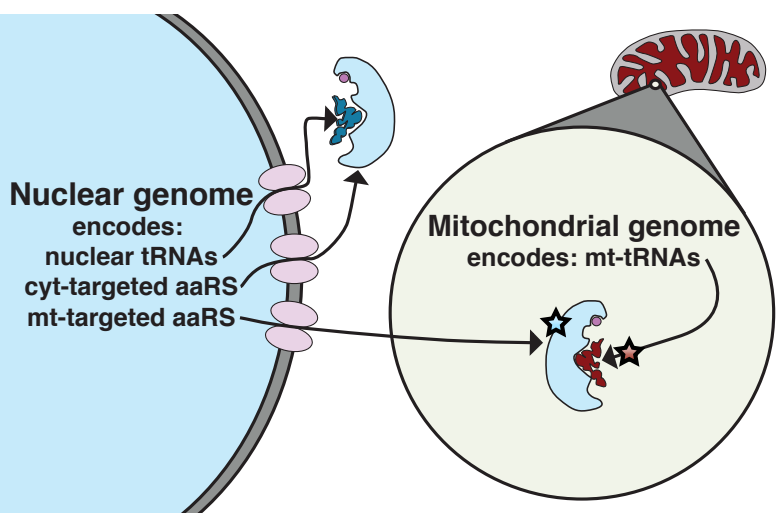


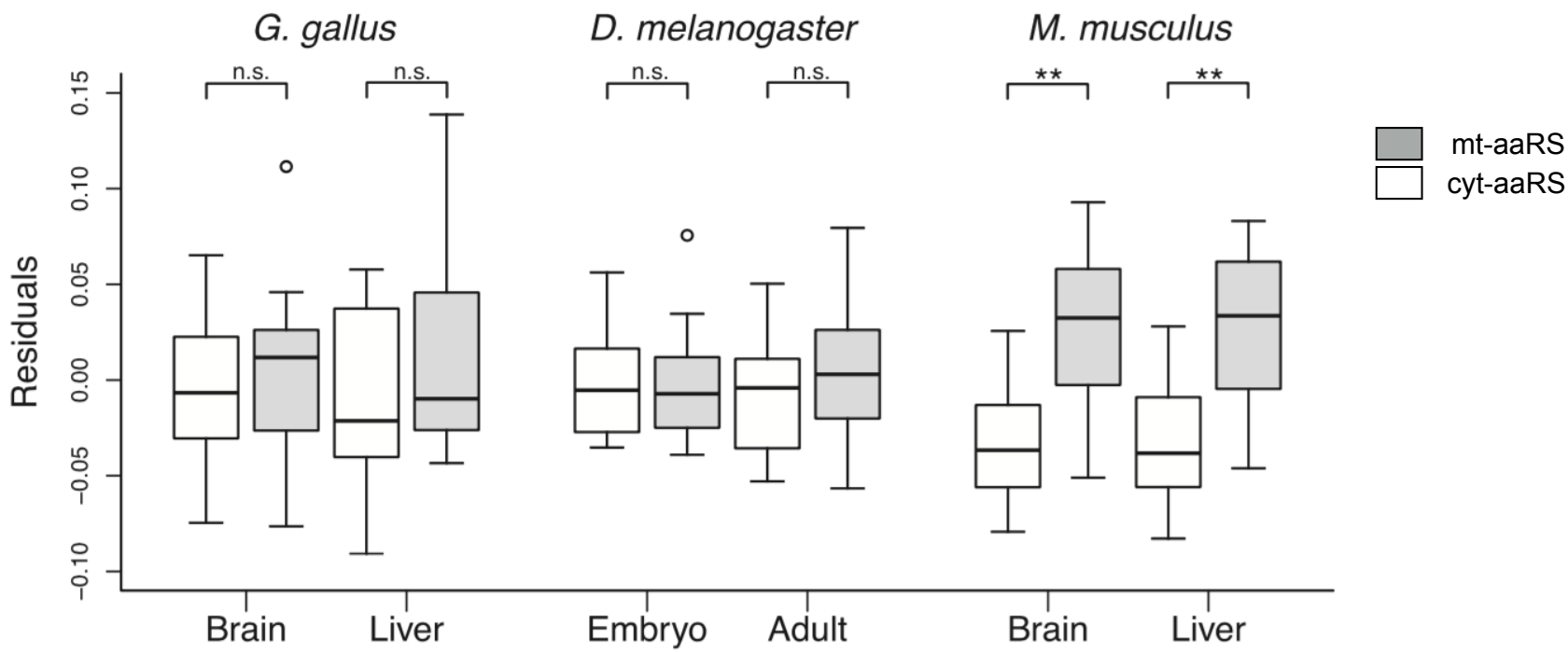


- Cyt-aaRS are more highly expressed than mt-aaRS
- More highly expressed proteins are more conserved

mt-tRNA - aaRS interactions: Implications for genome evolution

- Evidence for faster mt-aaRS co-evolution in mammals, but not in birds or Drosophila after you control for the effects of gene expression (i.e., selective constraint)

$d_N/d_S(\omega)$
corrected for gene expression

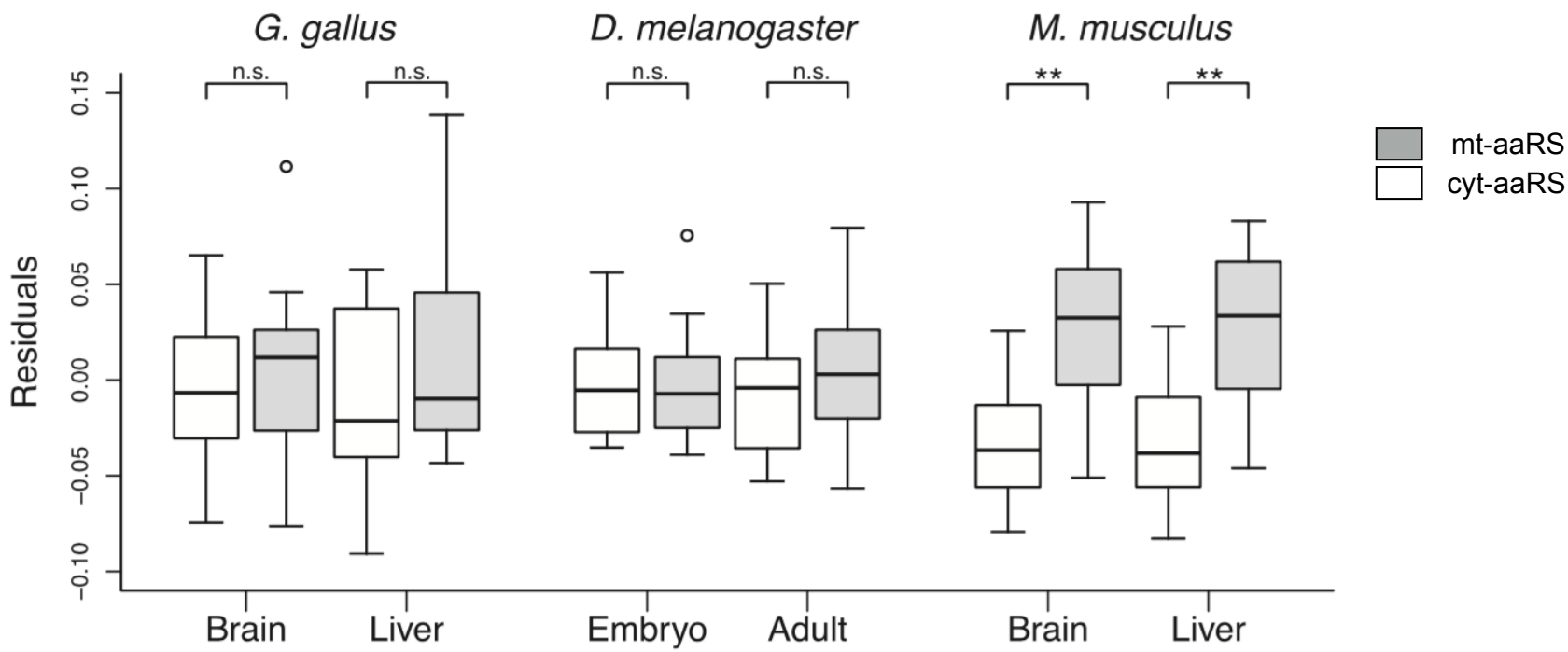


mt-tRNA - aaRS interactions: Implications for genome evolution

- Evidence for faster mt-aaRS co-evolution in mammals, but not in birds or Drosophila after you control for the effects of gene expression (i.e., selective constraint)

WHY?
Mammals have high ratio of mt:nuclear substitution rates

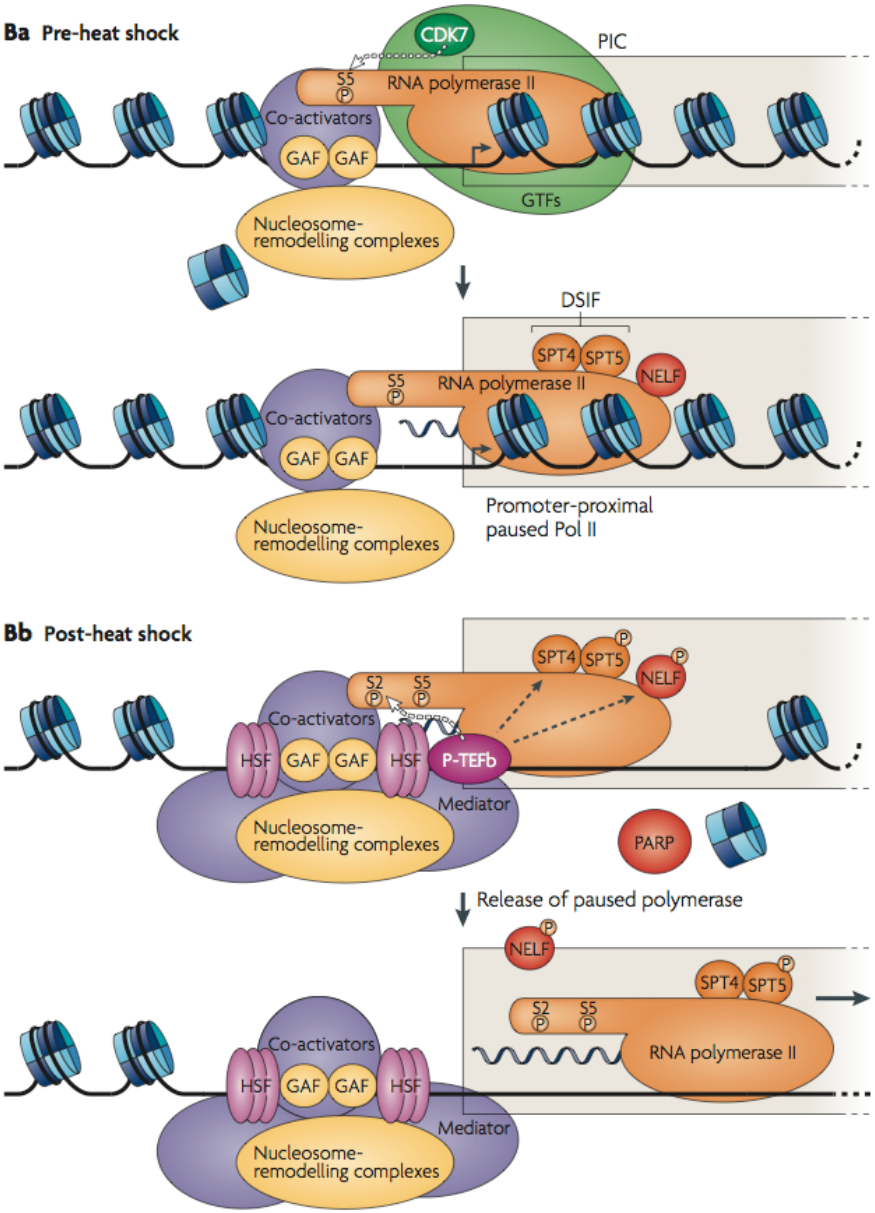
$d_N/d_S(\omega)$
corrected for gene expression



Energetic costs of cellular responses to stress

The *Drosophila* inducible heat shock response

- Protective response triggered at a sub-lethal stresses (36°C)
- Rapid induction of molecular chaperones (*Hsp70*) confers tolerance to otherwise lethal stress exposures
- Nucleosome remodeling, gene expression, protein synthesis and function are all ATP-dependent
- *Hsp70* gene copy number varies in nature

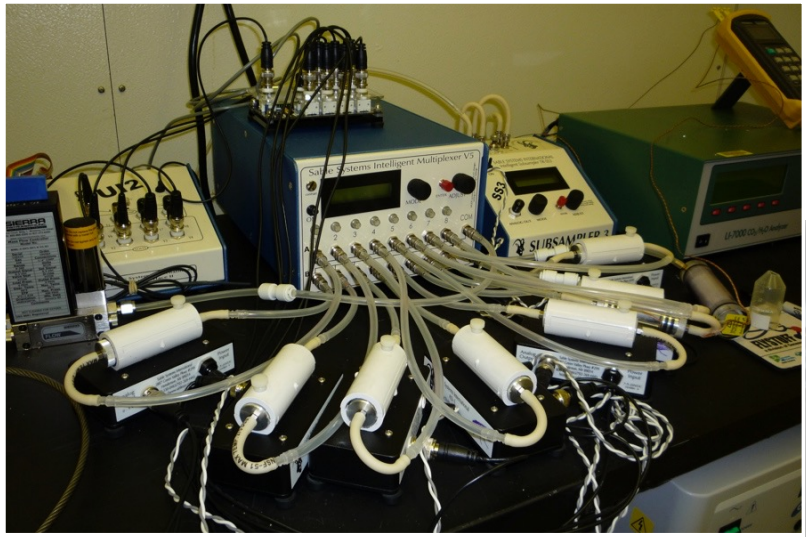


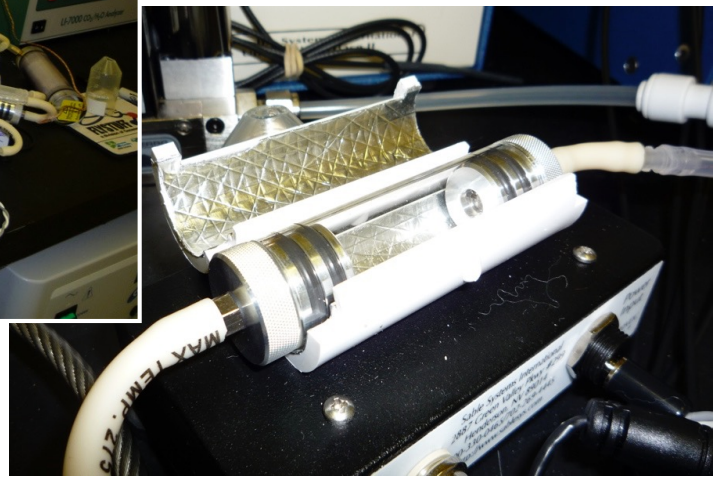
Weake & Workman 2010 *Nat Rev Genet*

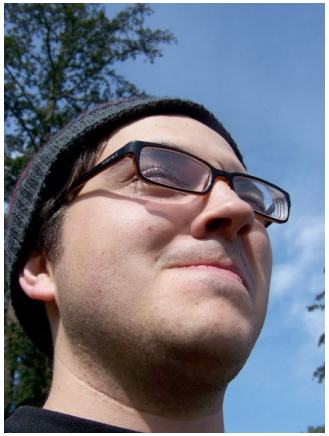
Can we detect an organismal level signature of this cellular-level cost?

- Approach: *D. melanogaster* with different *Hsp*70 gene copy numbers (3,6,12)
- Flow-through respirometry to dynamically measure metabolic rates during larval induction

$$6\ O_2 + C_6H_{12}O_6 \longrightarrow \boxed{6\ CO_2} + 6\ H_2O + 32\ ATP$$



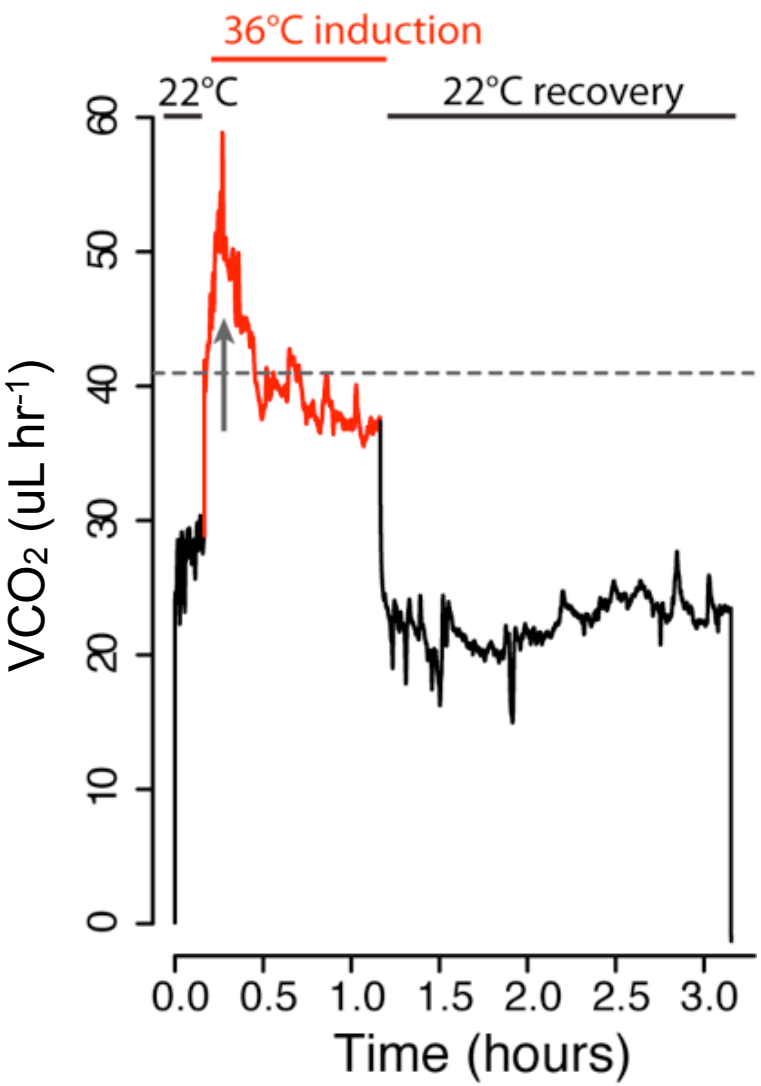




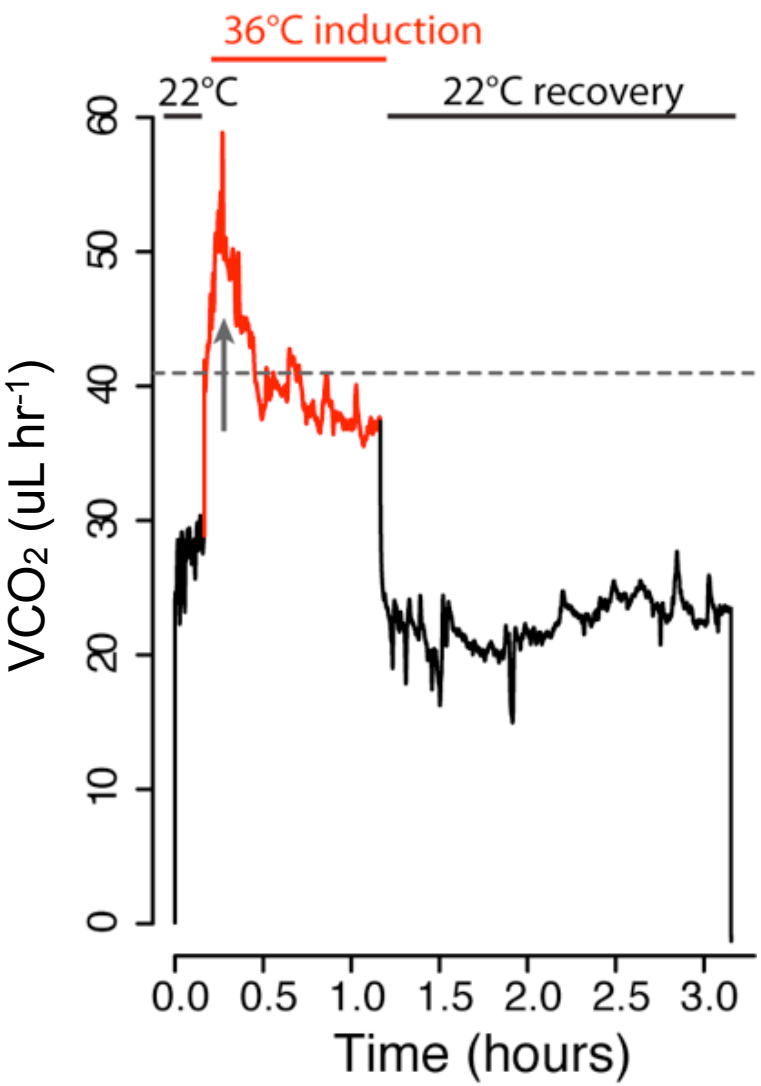
Luke Hoekstra

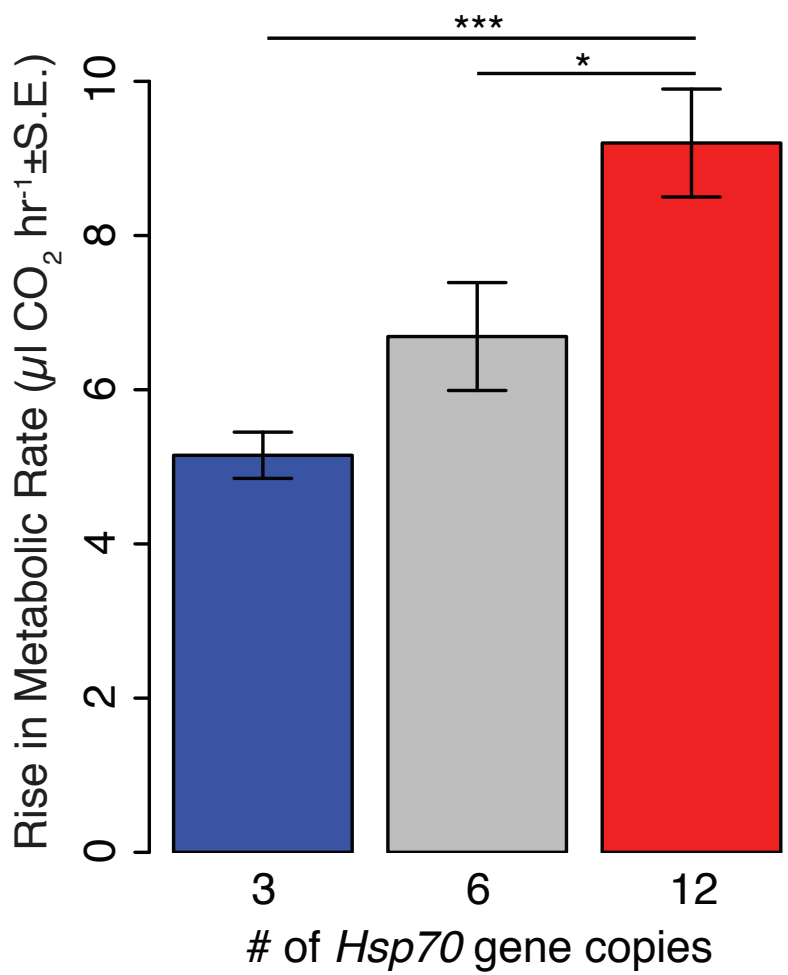
Inducing extra copies of the *Hsp70* gene increases energy expenditure

D. melanogaster larvae expressing
12 copies of *Hsp70*



Inducing extra copies of the *Hsp70* gene increases energy expenditure

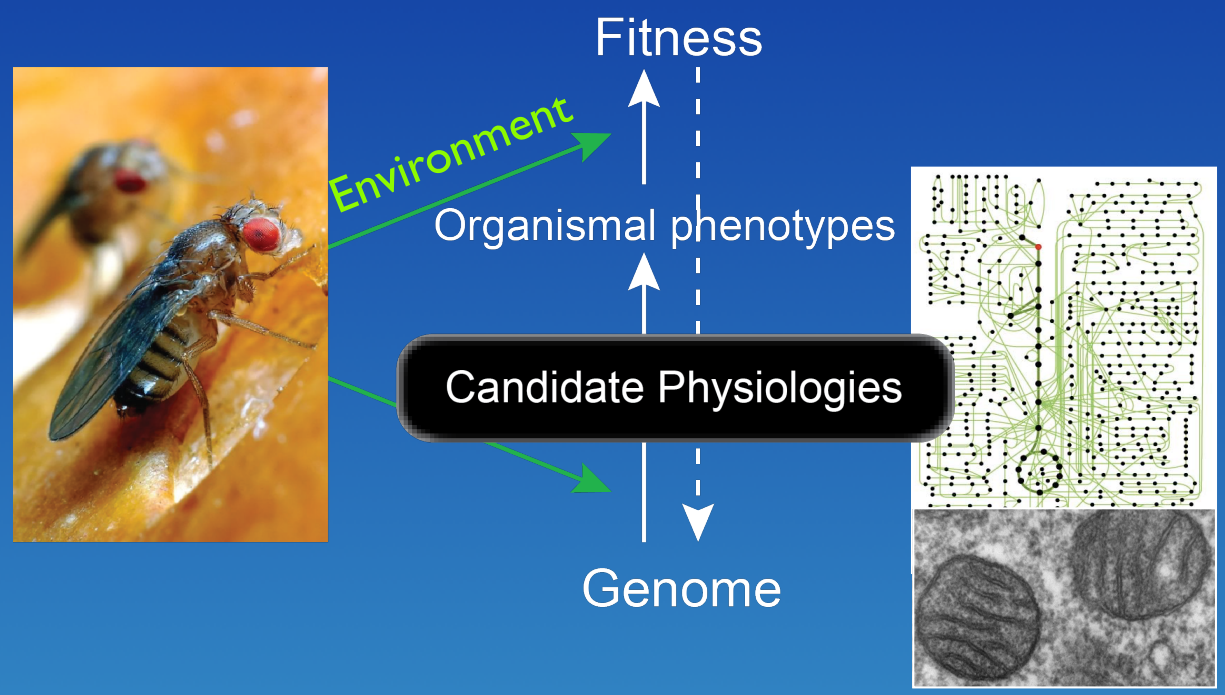




n=23 pools of 5 larvae per genotype

Hoekstra & Montooth (2013) *BMC Evol Biol*

Scaling up from molecular energetic to tissue, organismal and population levels is an important step in connecting theory of molecular dynamics to evolution



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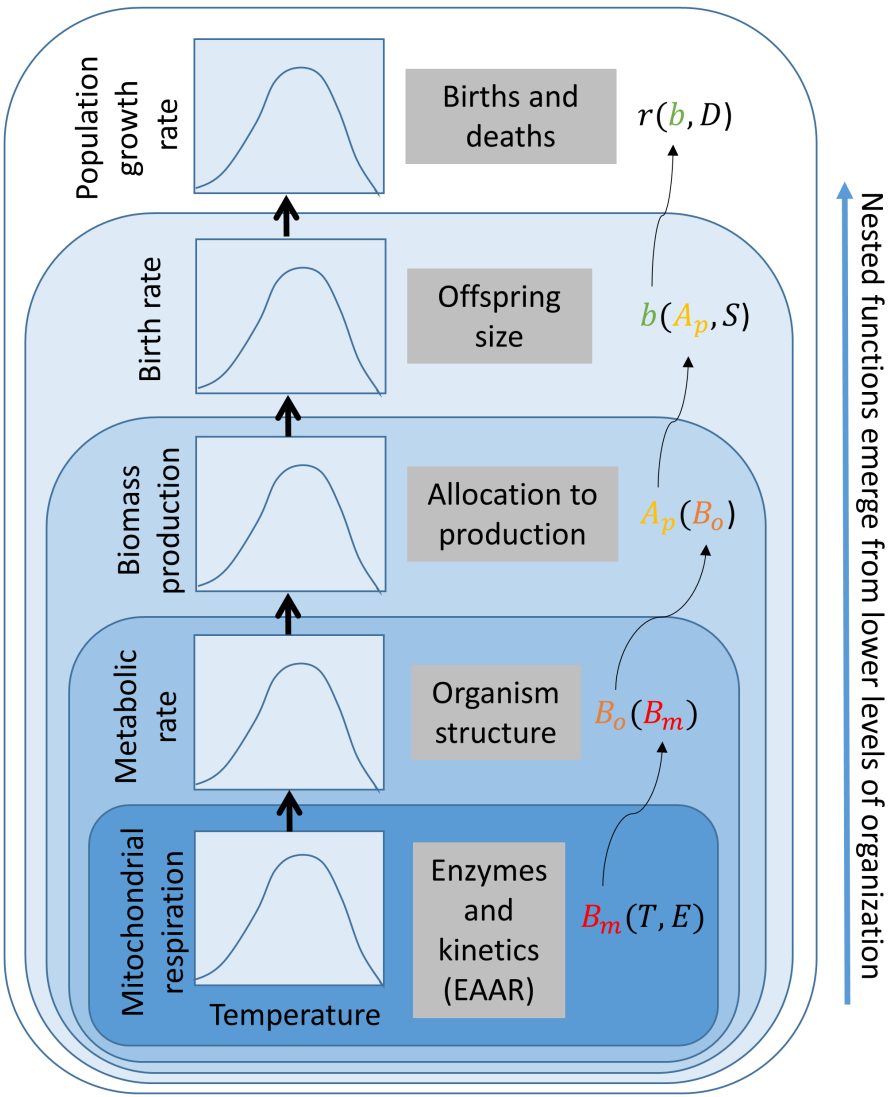
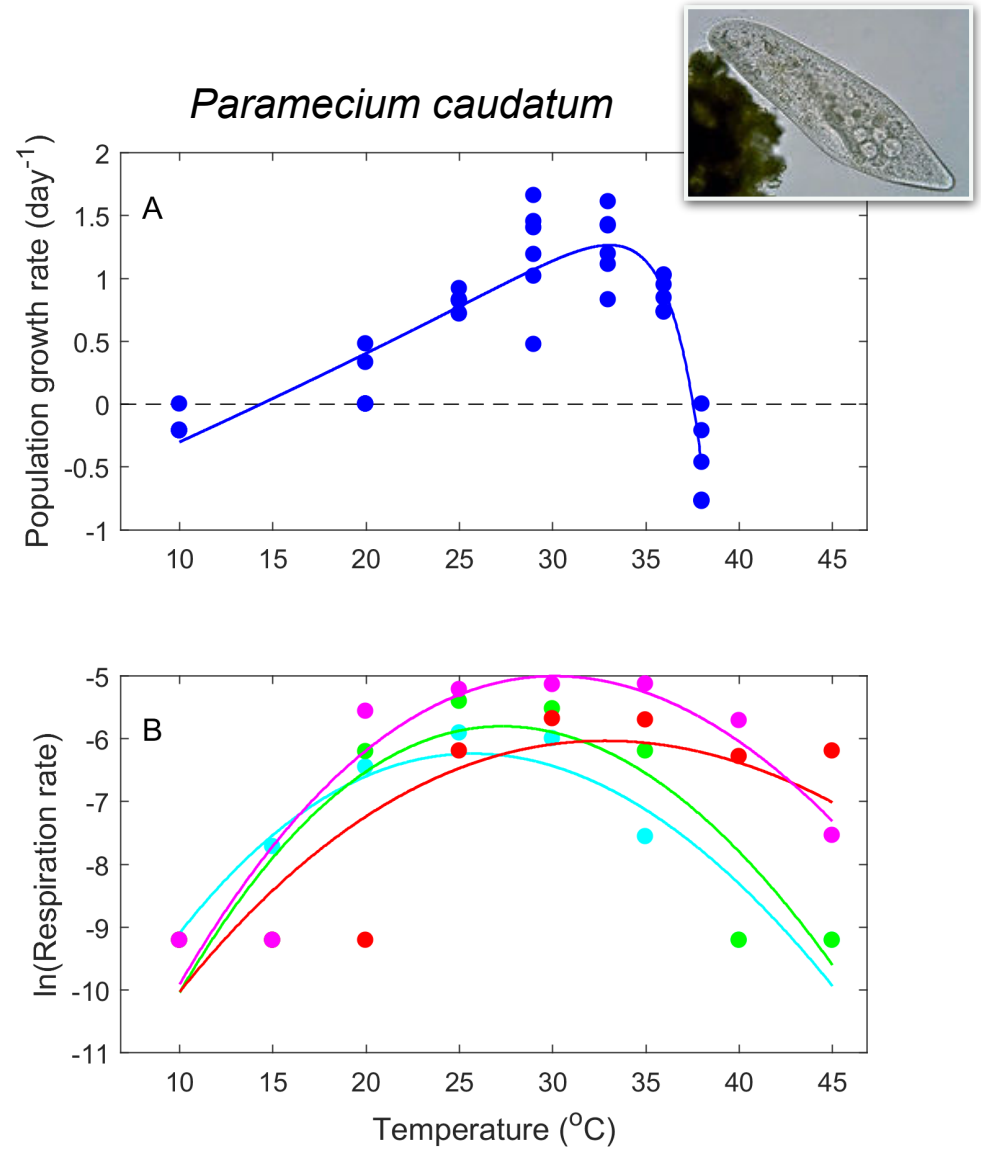
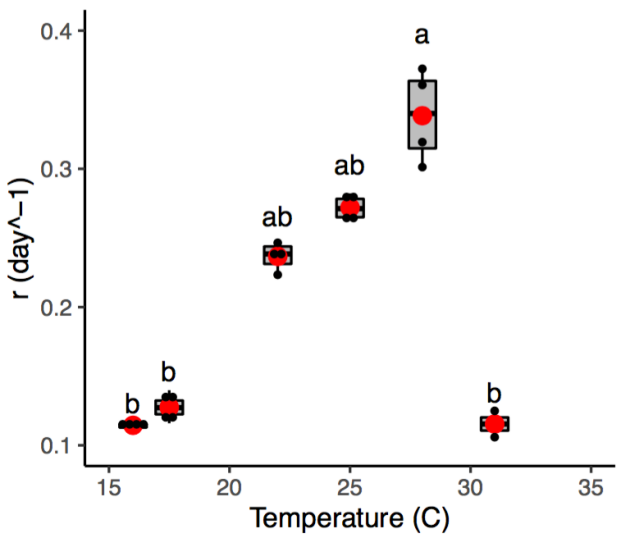
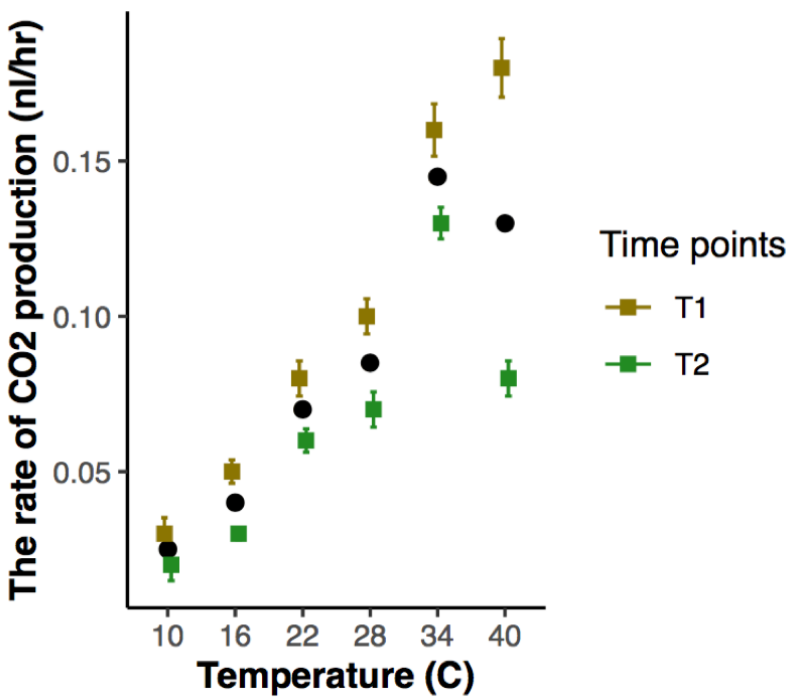




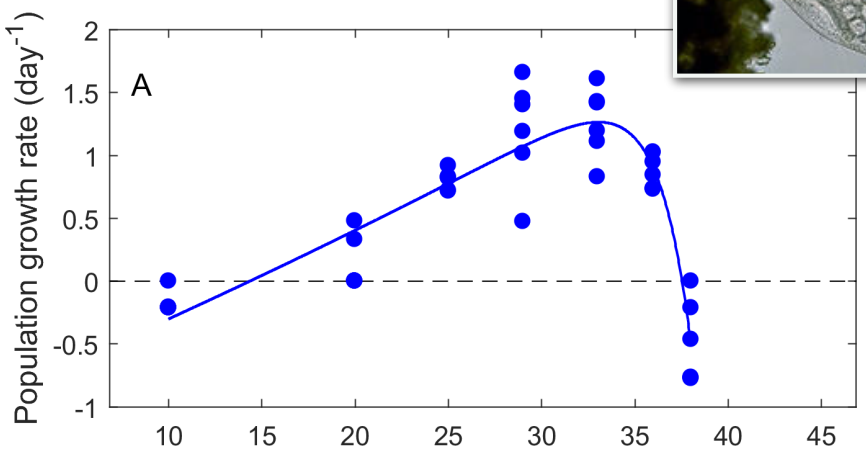
Scaling up from molecular energetic to tissue, organismal and population levels is an important step in connecting theory of molecular dynamics to evolution

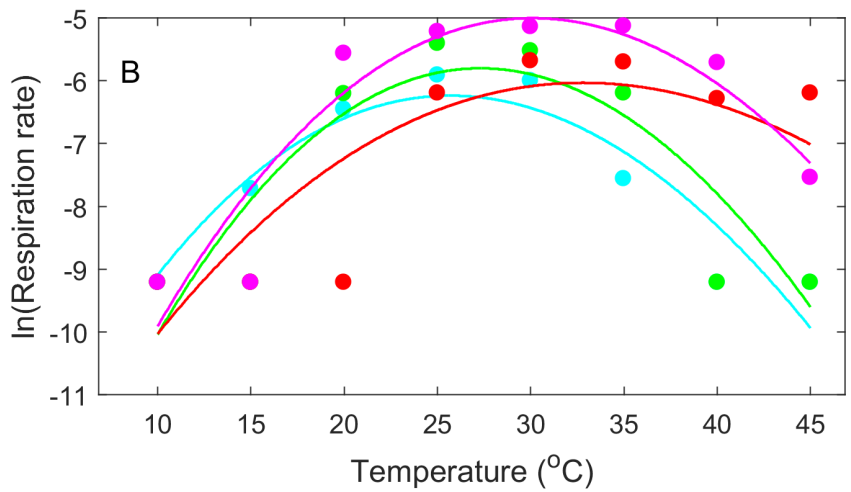
Drosophila melanogaster





Paramecium caudatum





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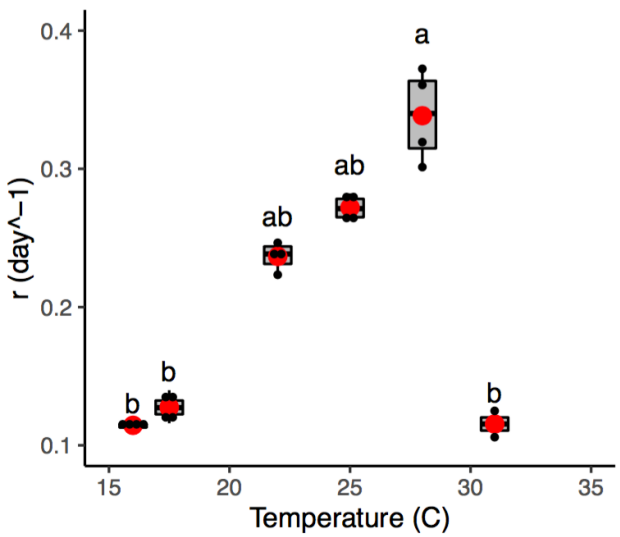

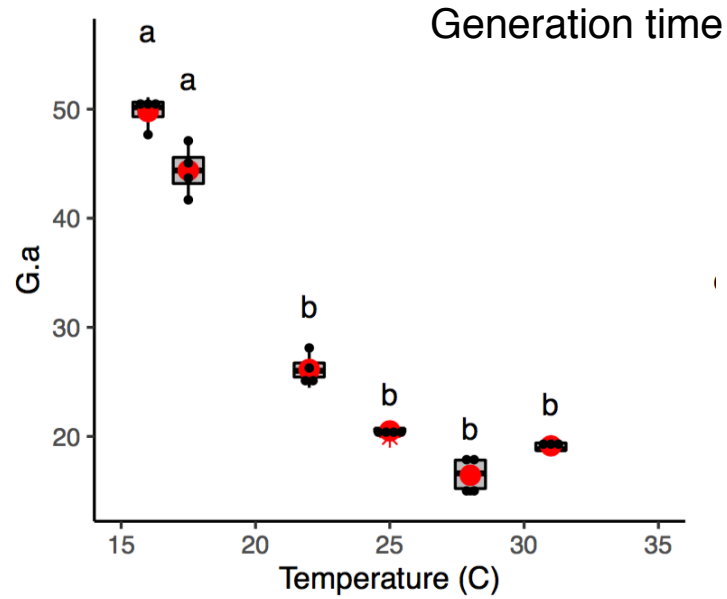

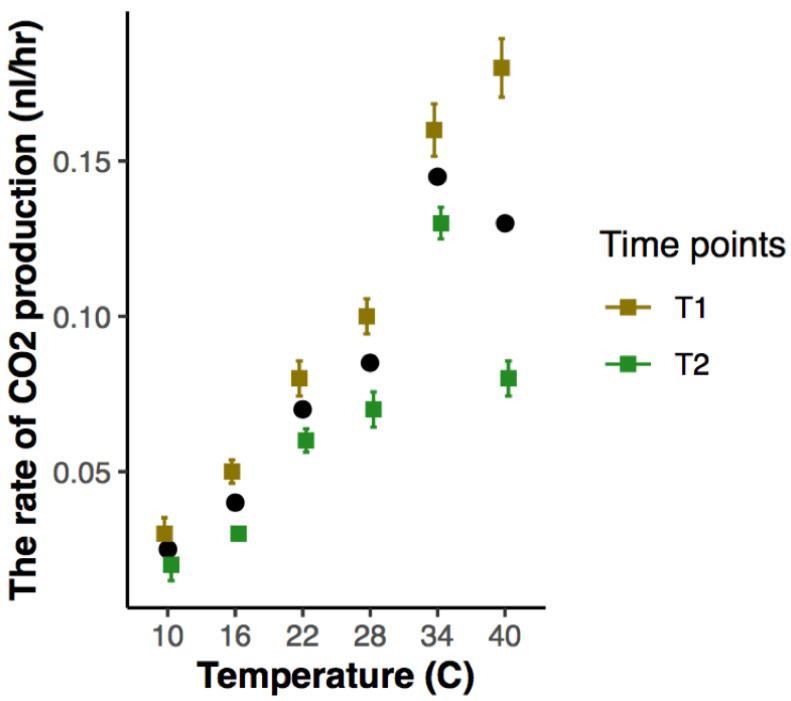

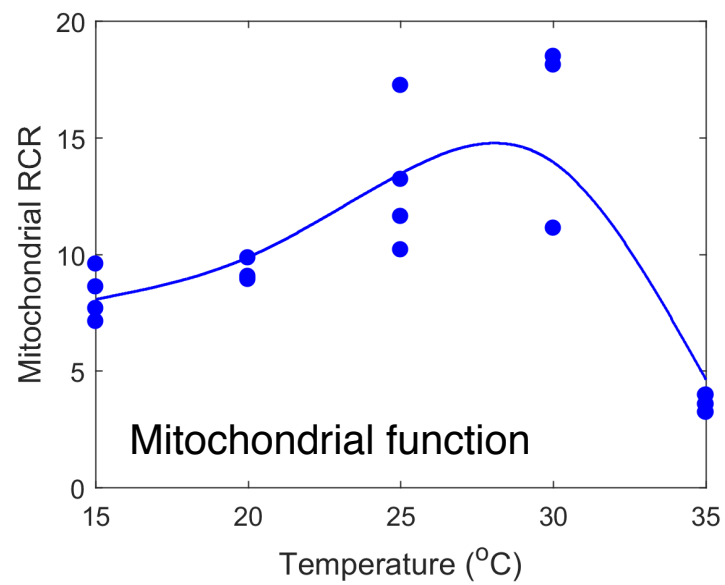

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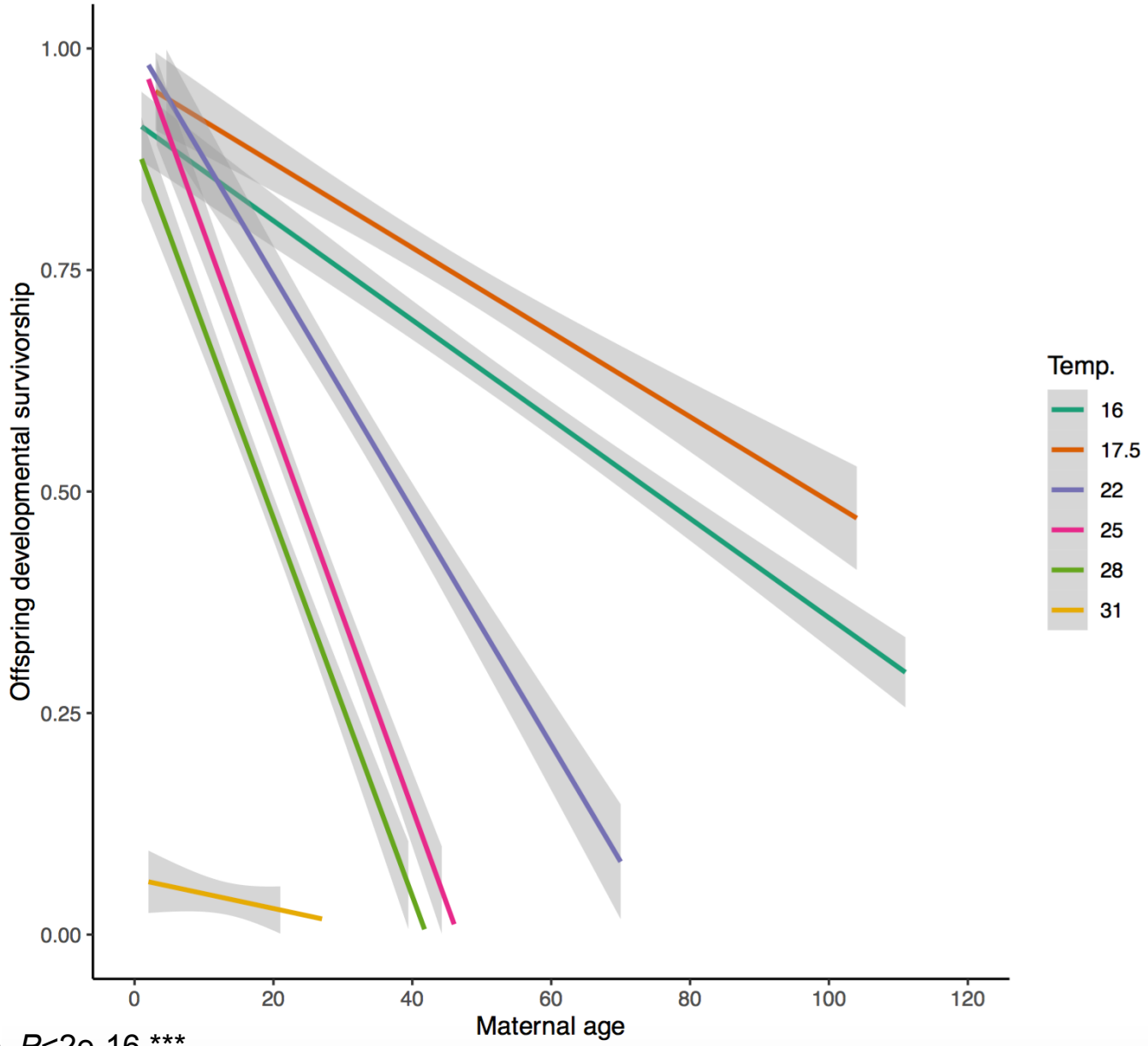

Temp x maternal age, *P*<2e-16 ***

Acknowledgments

Collaborators

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David Rand, Brown
John DeLong, UNL

Montooth Lab

Omera Matoo
Ibrahim El-Shesheny
Abhilesh Dhawanjewar
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Jenny Libov
Lauren Reiman
Hayley Dewit
Gina Marcuzzo
Kathryn Whittaker
Nicole Valentina Acosta Sandoval
Joevy Sum

montoothlab.unl.edu

Lab alumni whose work contributed to this talk

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Jeff Adrion (U Oregon)
Signe White (Emory)
Luke Hoekstra (Iowa State)
Katelyn Mika (U Chicago)
Mo Siddiq (U Michigan)

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