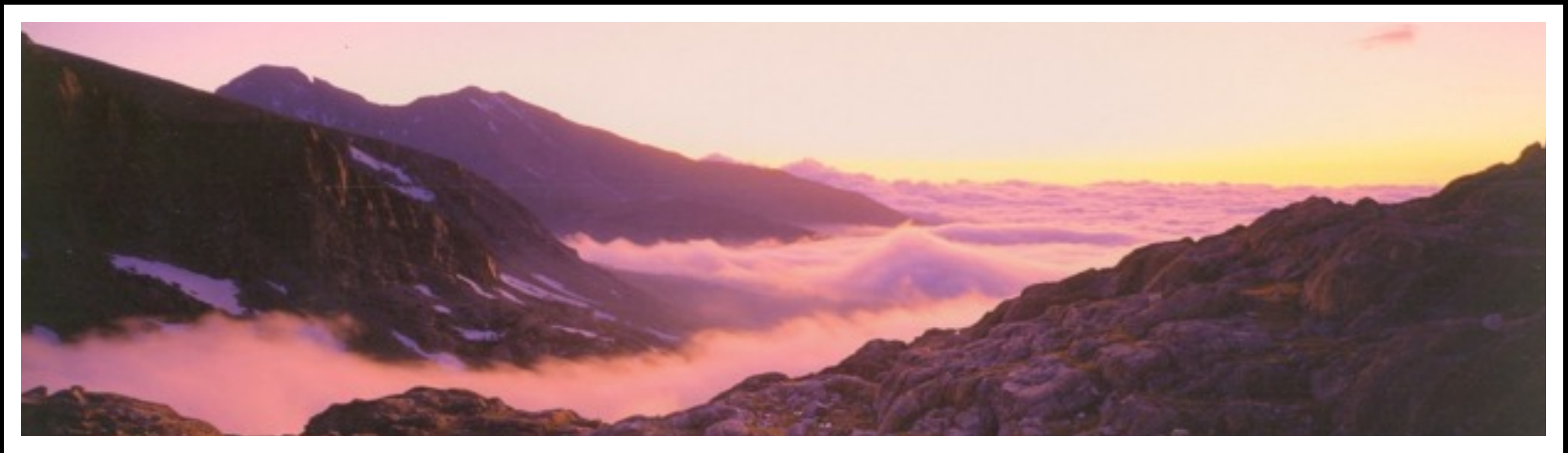
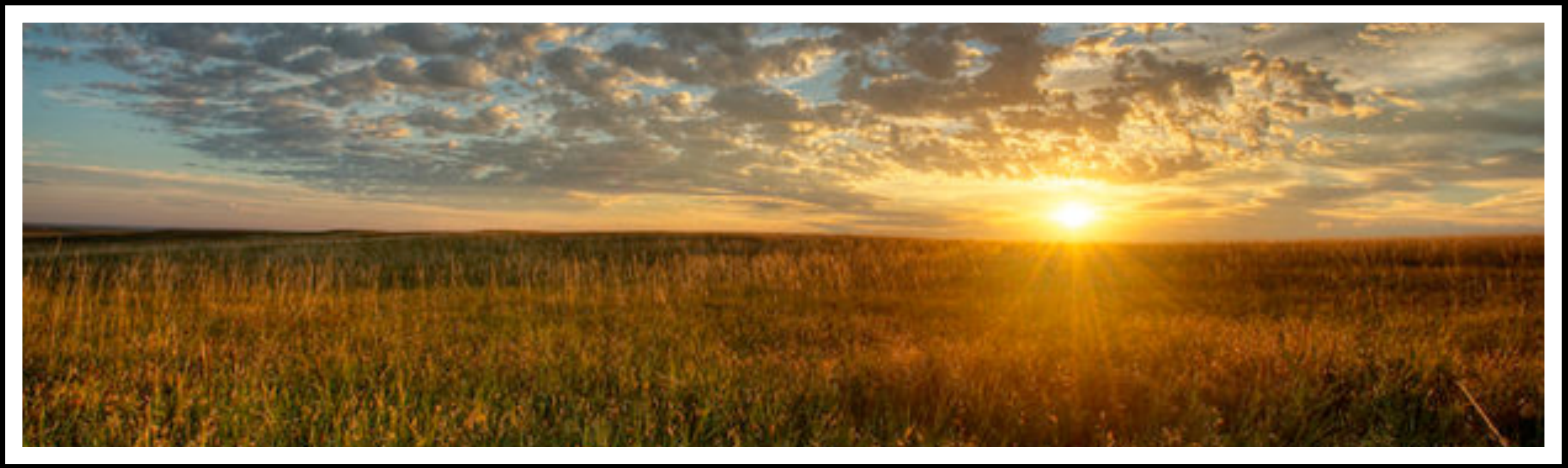
Evolutionary systems biology of adaptation to environmental stress: insights from high-altitude deer mice.





Zachary A. Cheviron[1], Jon Velotta[1], Kevin Tate[2] & Graham Scott[2]

[1]Division of Biological Sciences, University of Montana

[2]Department of Biology, McMaster University

Environmental heterogeneity is a universal problem

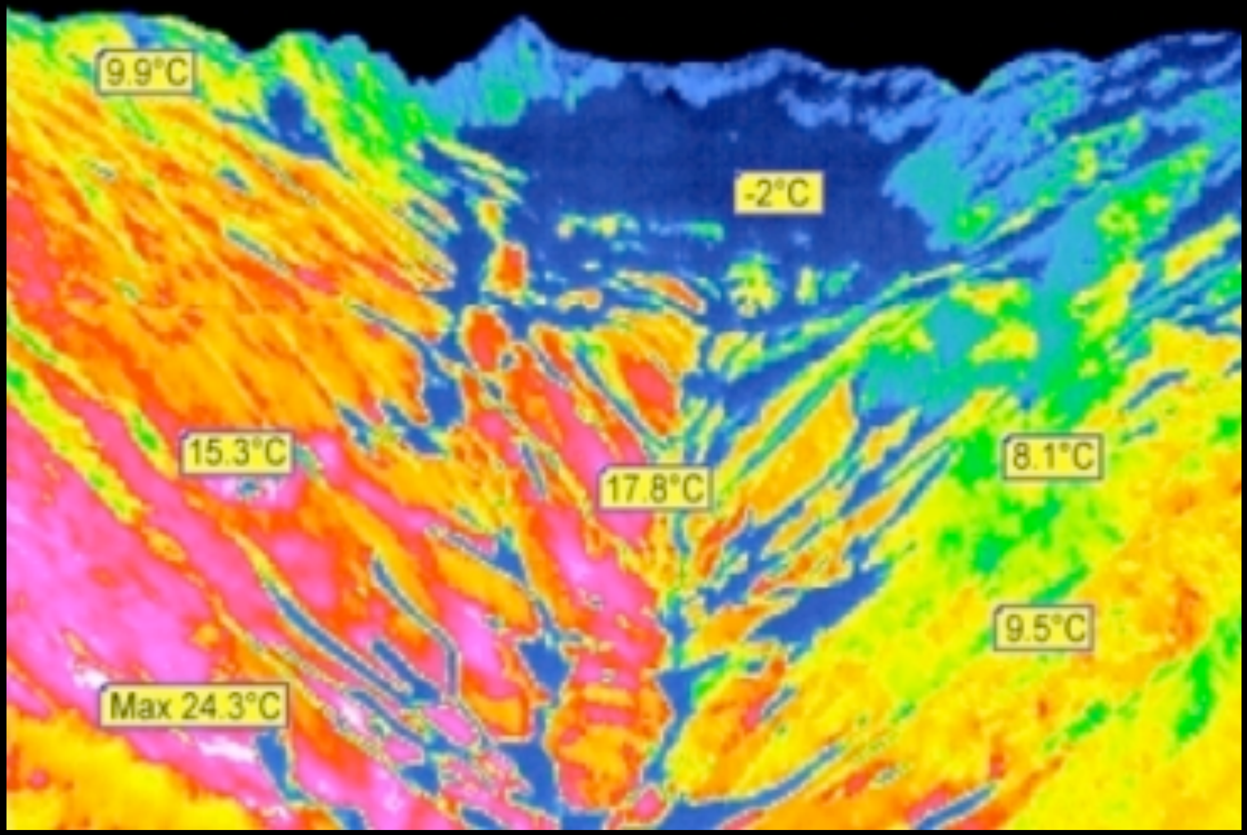
How do species cope with environmental heterogeneity?

- Behavioral responses

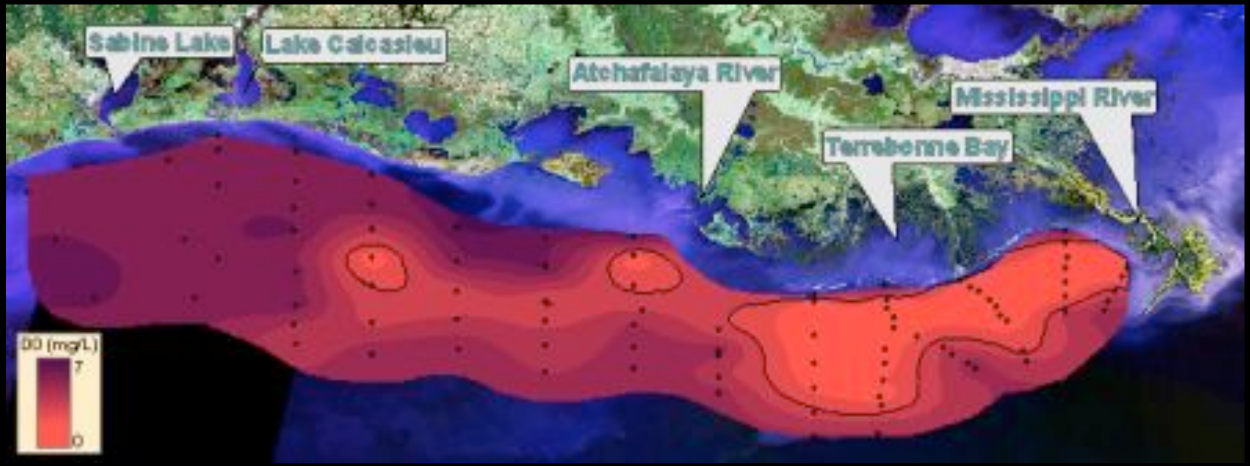
- Physiological responses

- Genetic adaptation

Thermal heterogeneity in the Alps



Dissolved oxygen in the Gulf of Mexico



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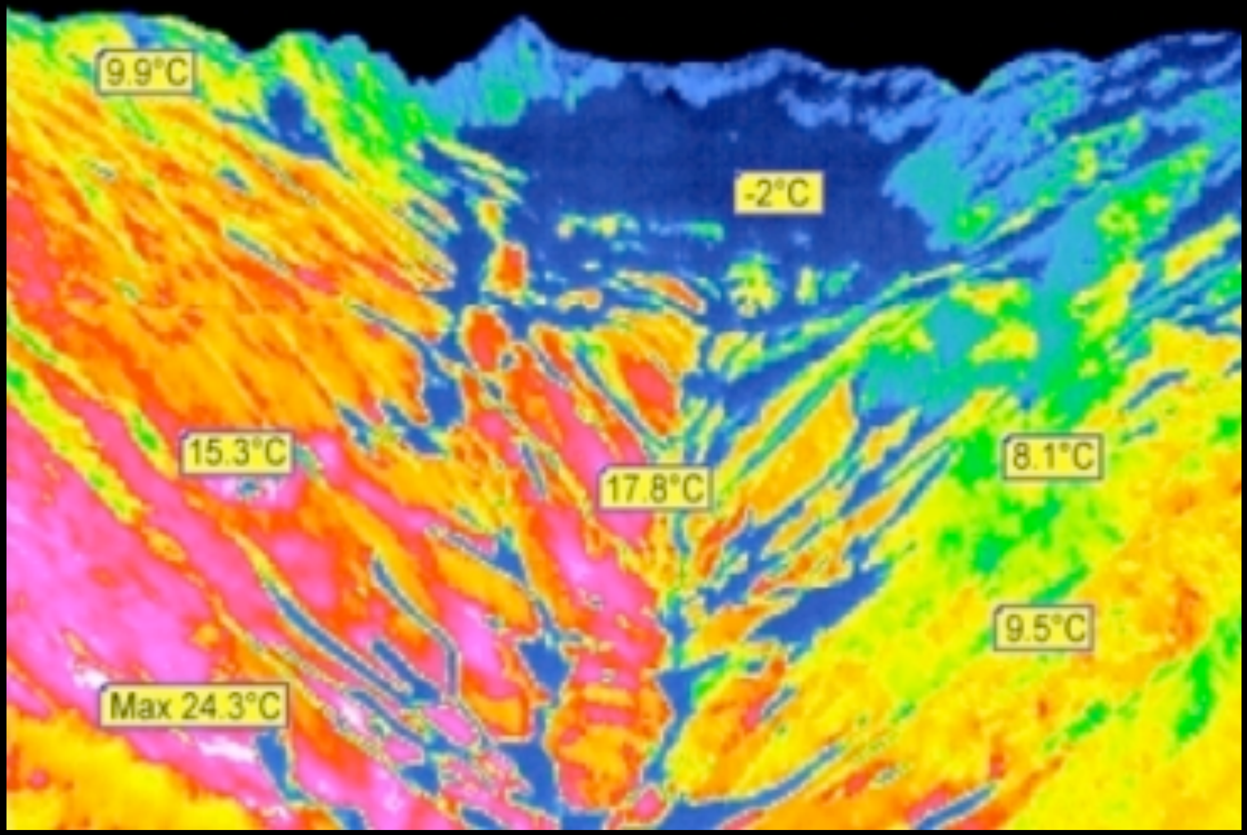

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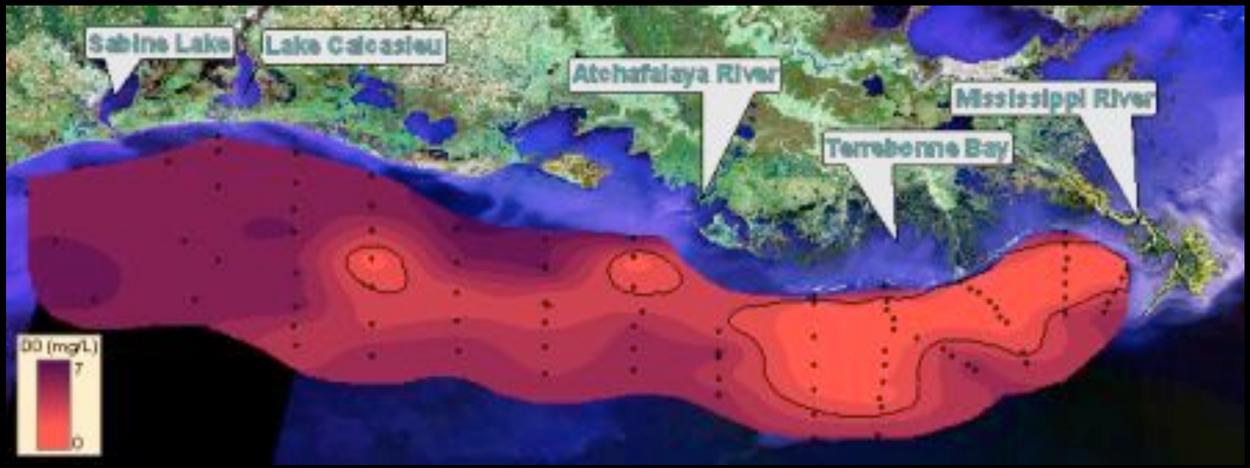


Variable responses to environmental stress

<u>Degree of canalization</u>

Flexible

Fixed

Phenotypic flexibility

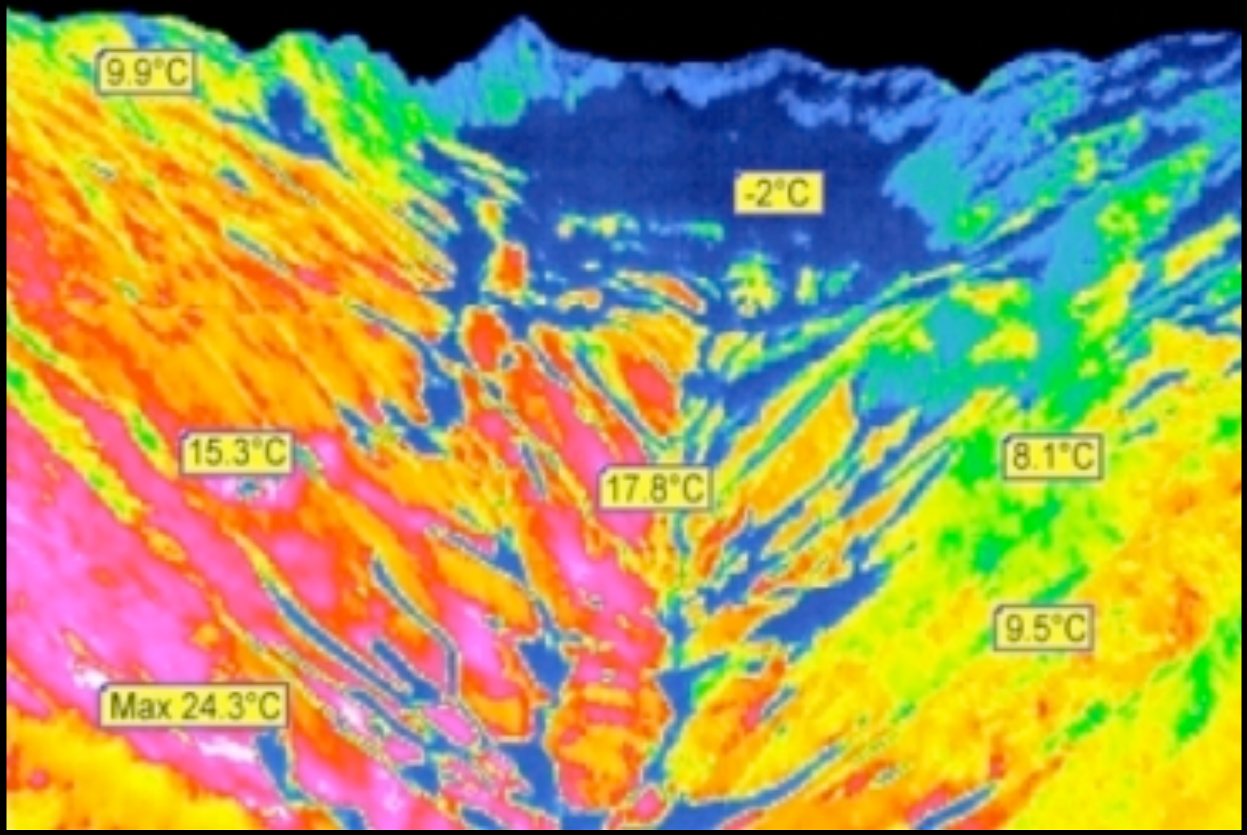
Genotypic specialization

<u>Time</u>

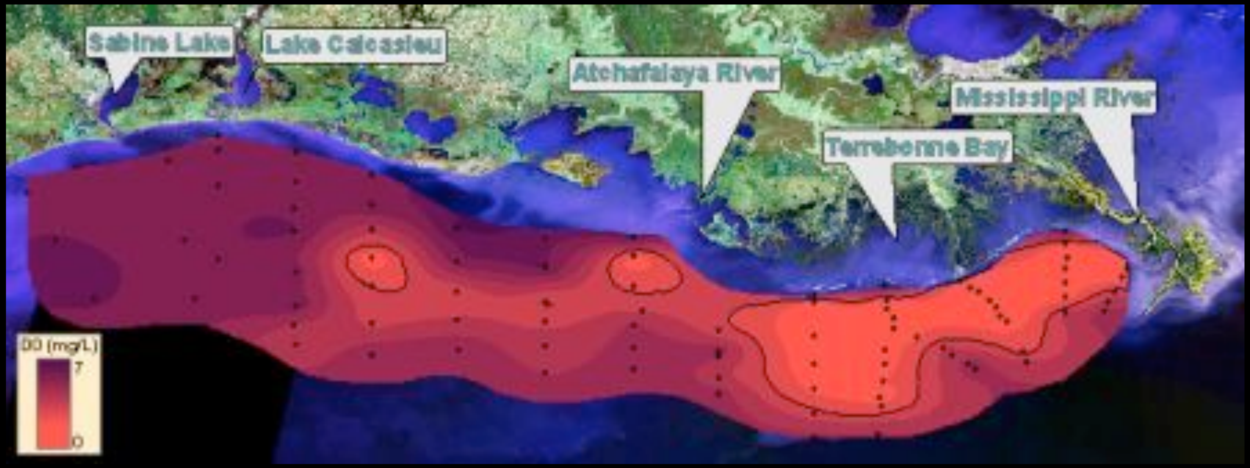
Seconds

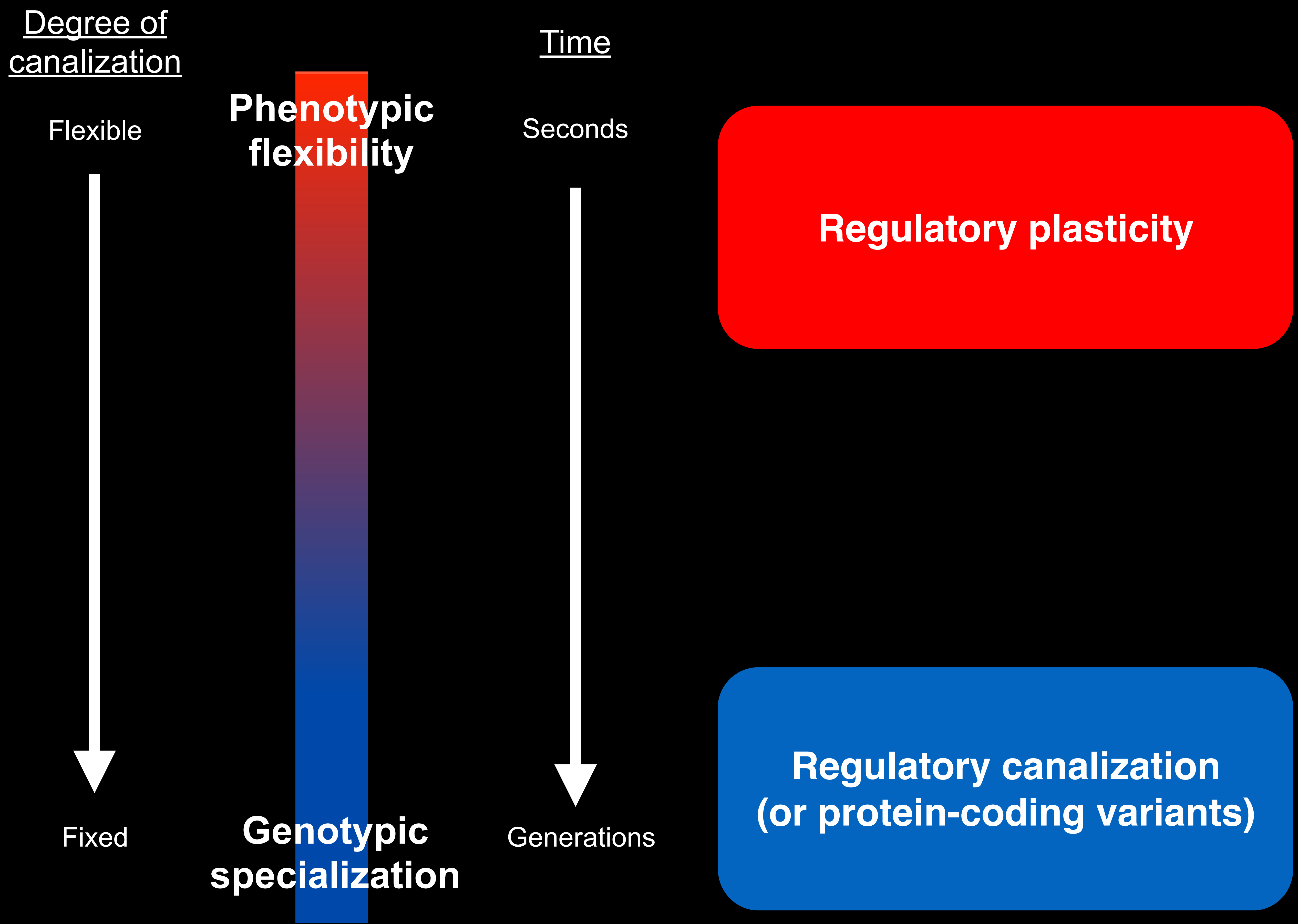
Generations

Thermal heterogeneity in the Alps

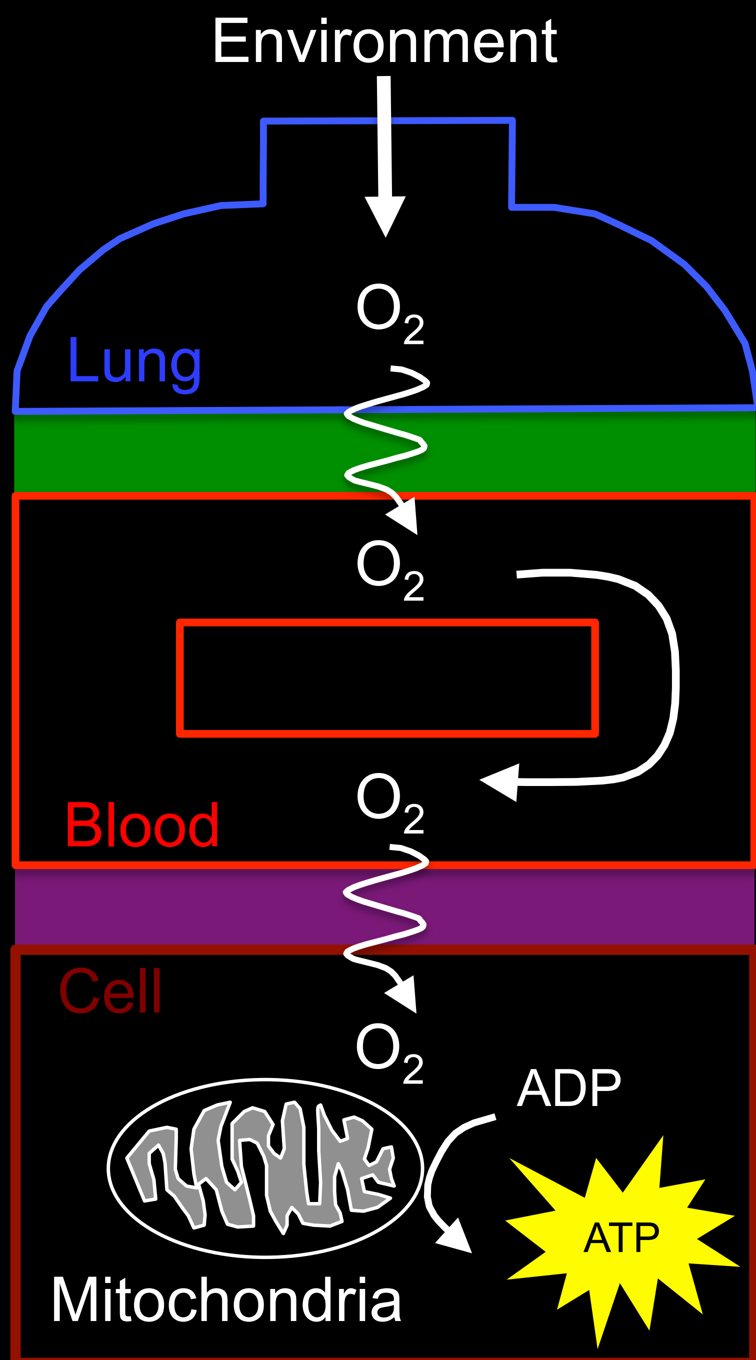


Dissolved oxygen in the Gulf of Mexico




Degree of canalization
Flexible
Fixed
Phenotypic flexibility
Genotypic specialization
Time
Seconds
Generations
Regulatory plasticity
Regulatory canalization
(or protein-coding variants)

Oxygen transport cascade

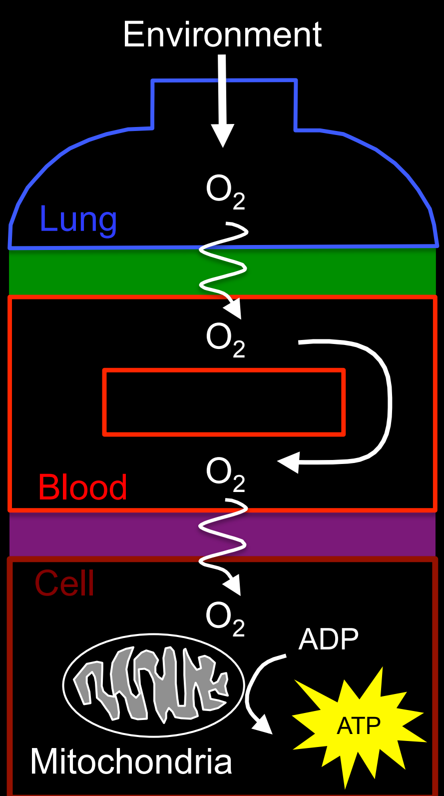


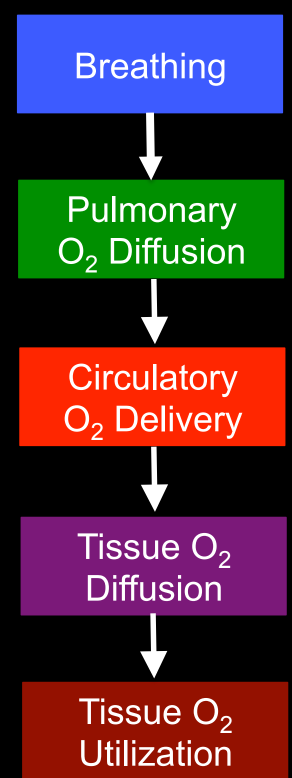
Step	Example Traits
Breathing	• Breath rate • Tidal volume
Pulmonary O_2 Diffusion	• Lung surface area • Thickness of pulmonary wall
Circulatory O_2 Delivery	• Heart Rate • Red blood cell concentration
Tissue O_2 Diffusion	• Capillary density • Muscle fiber composition
Tissue O_2 Utilization	• Cellular mitochondrial density • Cellular aerobic capacity

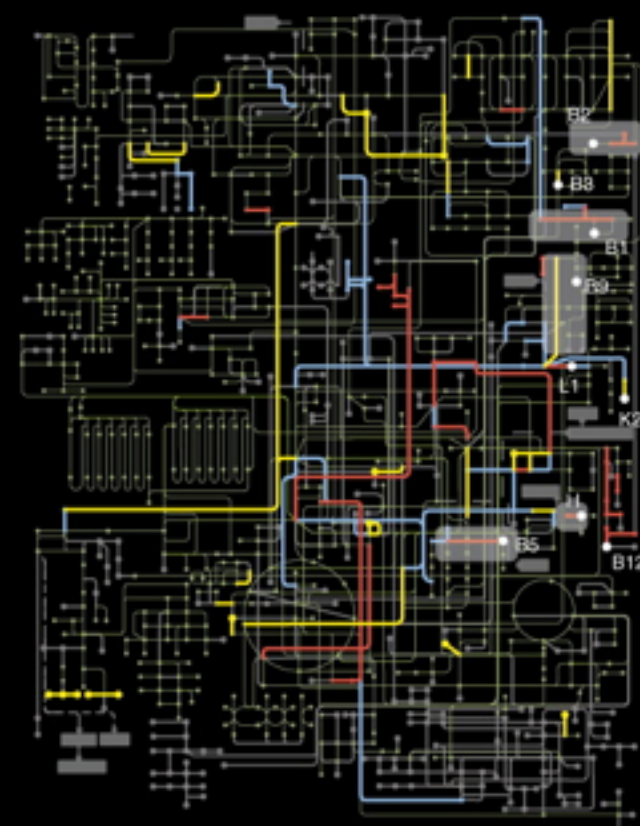
Aerobic Performance
($VO_{2\ max}$)

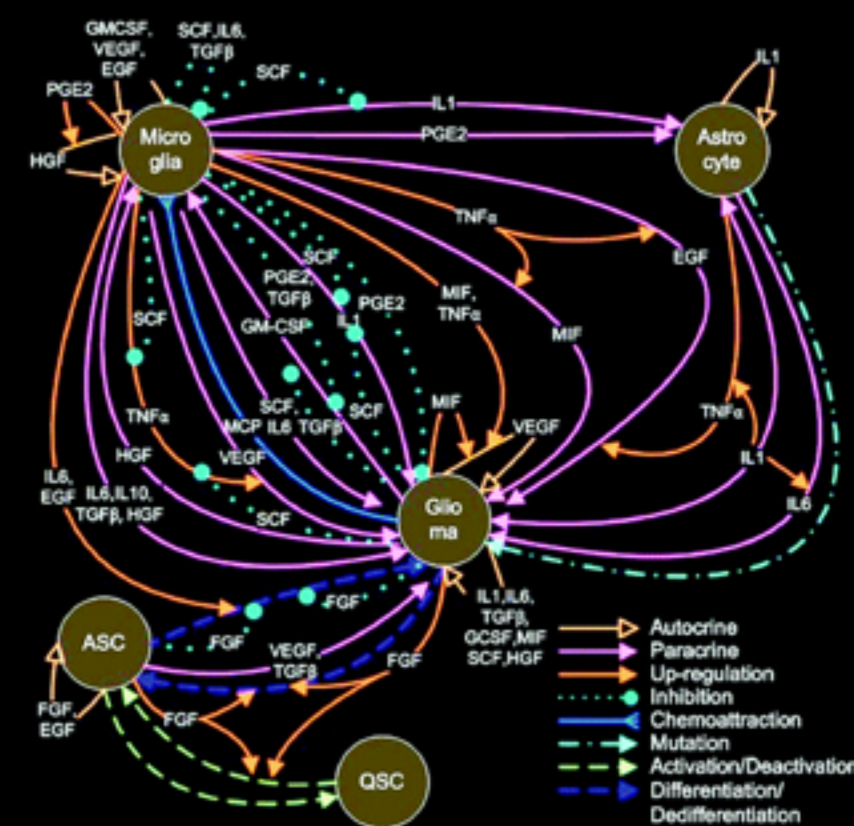
Storz, Scott & Cheviron. 2010. J. Exp. Biol.

Evolutionary adaptation and physiological acclimatization often requires coordinated changes in independent, but interacting, systems.









Evolutionary systems biology of adaptation

How is phenotypic control distributed among steps in hierarchical physiological, biochemical and regulatory pathways?

Can aspects of pathway architecture and control distribution be used to predict evolutionary outcomes?

Do the relative contributions of regulatory plasticity and canalization vary among steps in integrated physiological pathways?

Study systems





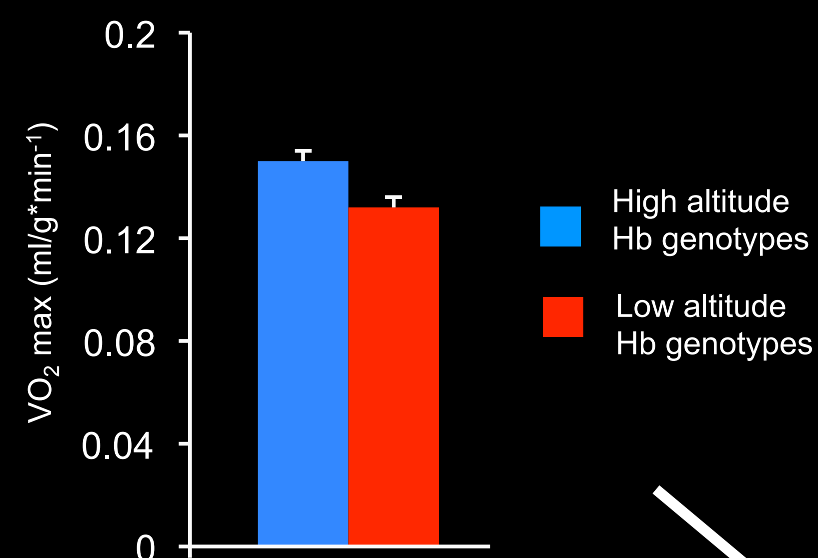

Deer mice (*Peromyscus maniculatus*)

Deer mice as a model system for adaptation to high-altitude

Variation in hemoglobin function

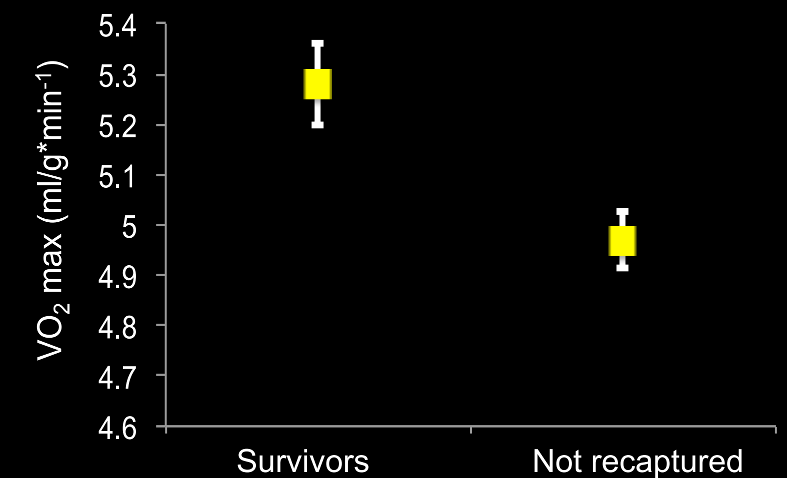
Snyder 1978 Genetics
Storz et al. 2007 PLoS Genetics
Stroz et al. 2009 PNAS
Storz et al. 2010 JEB

Enhanced thermogenic performance at high altitude



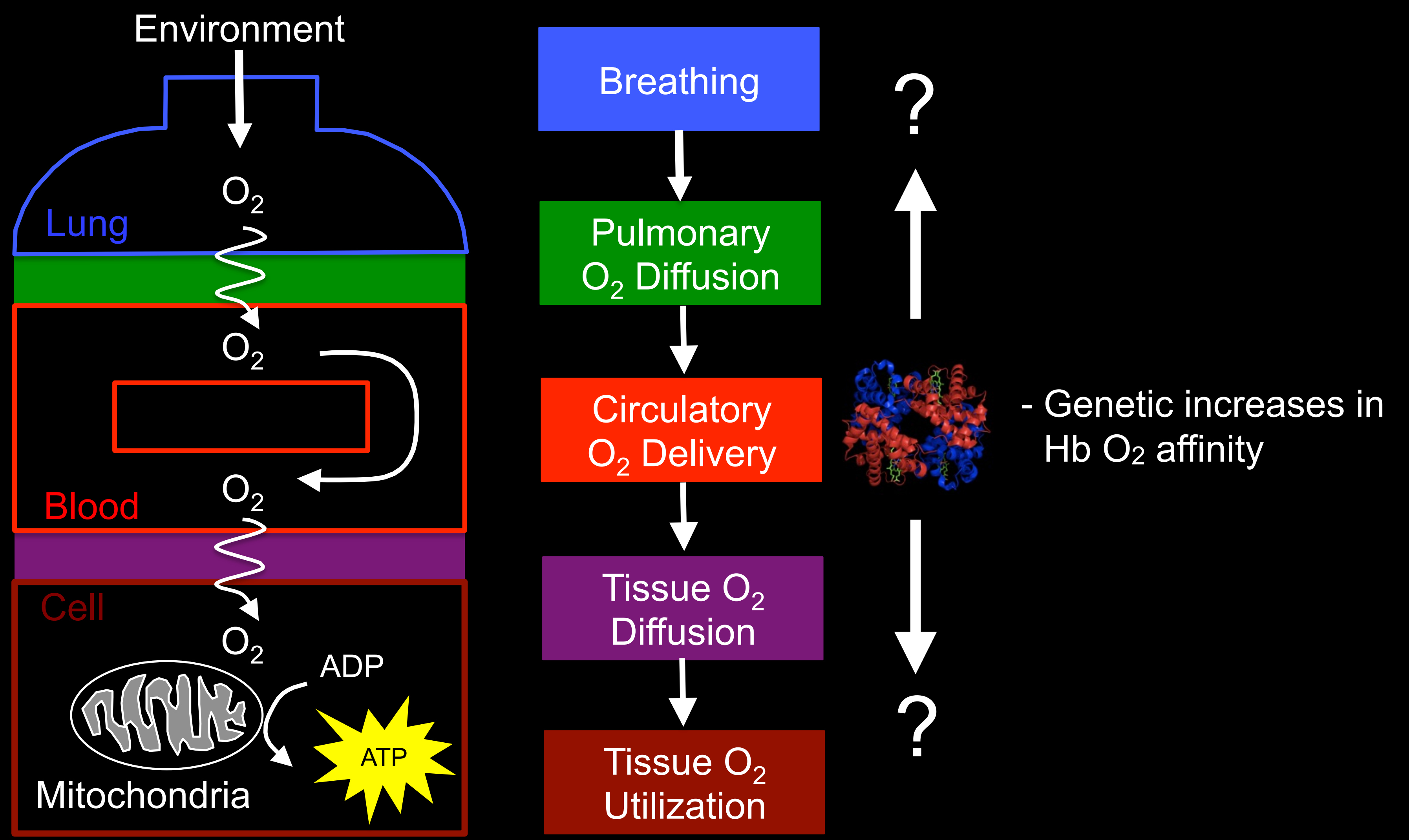
Chappell & Snyder 1984 PNAS

Thermogenic performance affects survival at high altitude

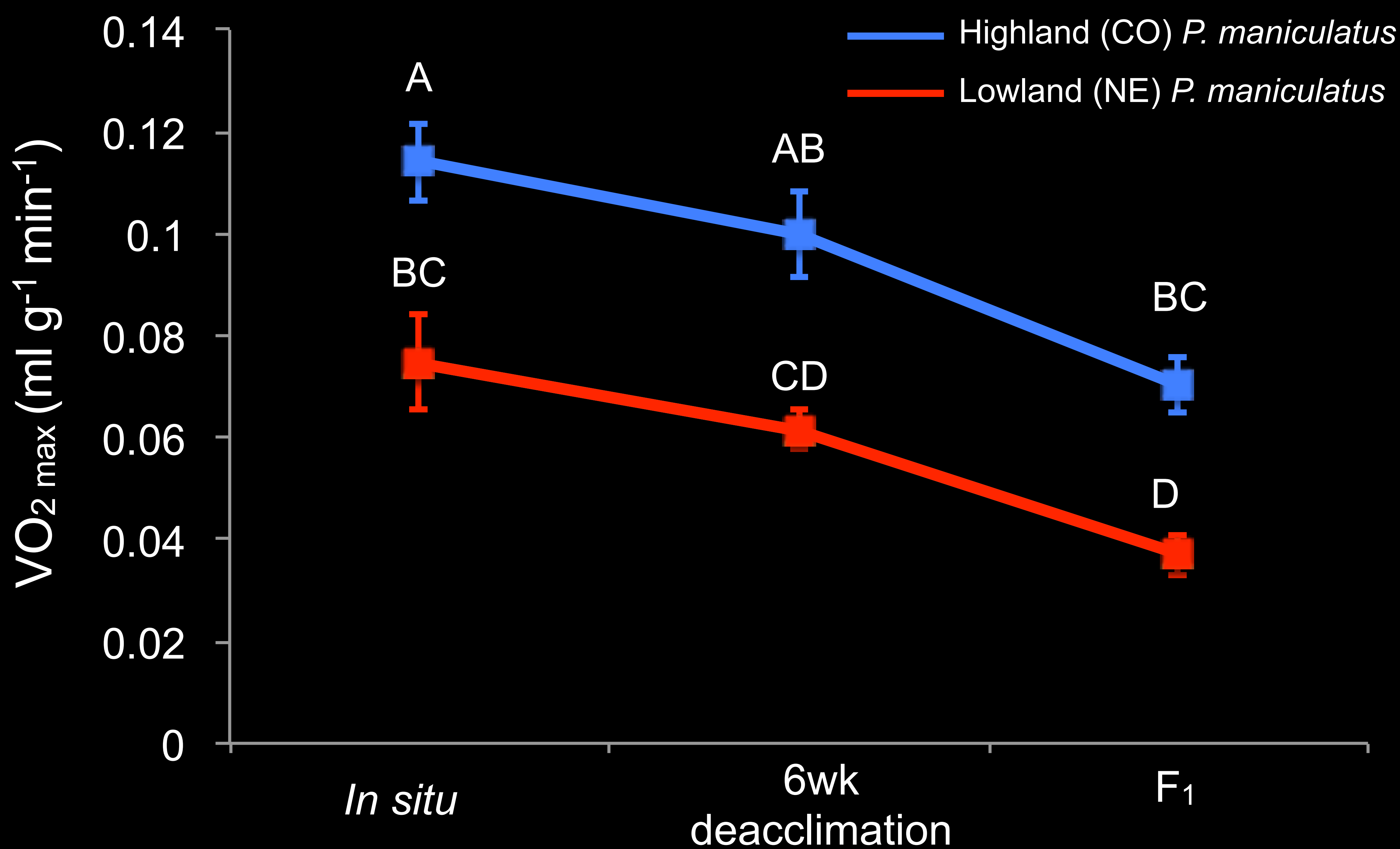


Hayes and O'Connor 1999 Evolution

Systems biology of high altitude adaptation in deer mice



Plastic and constituitive variation in thermogenic capacity under hypoxia

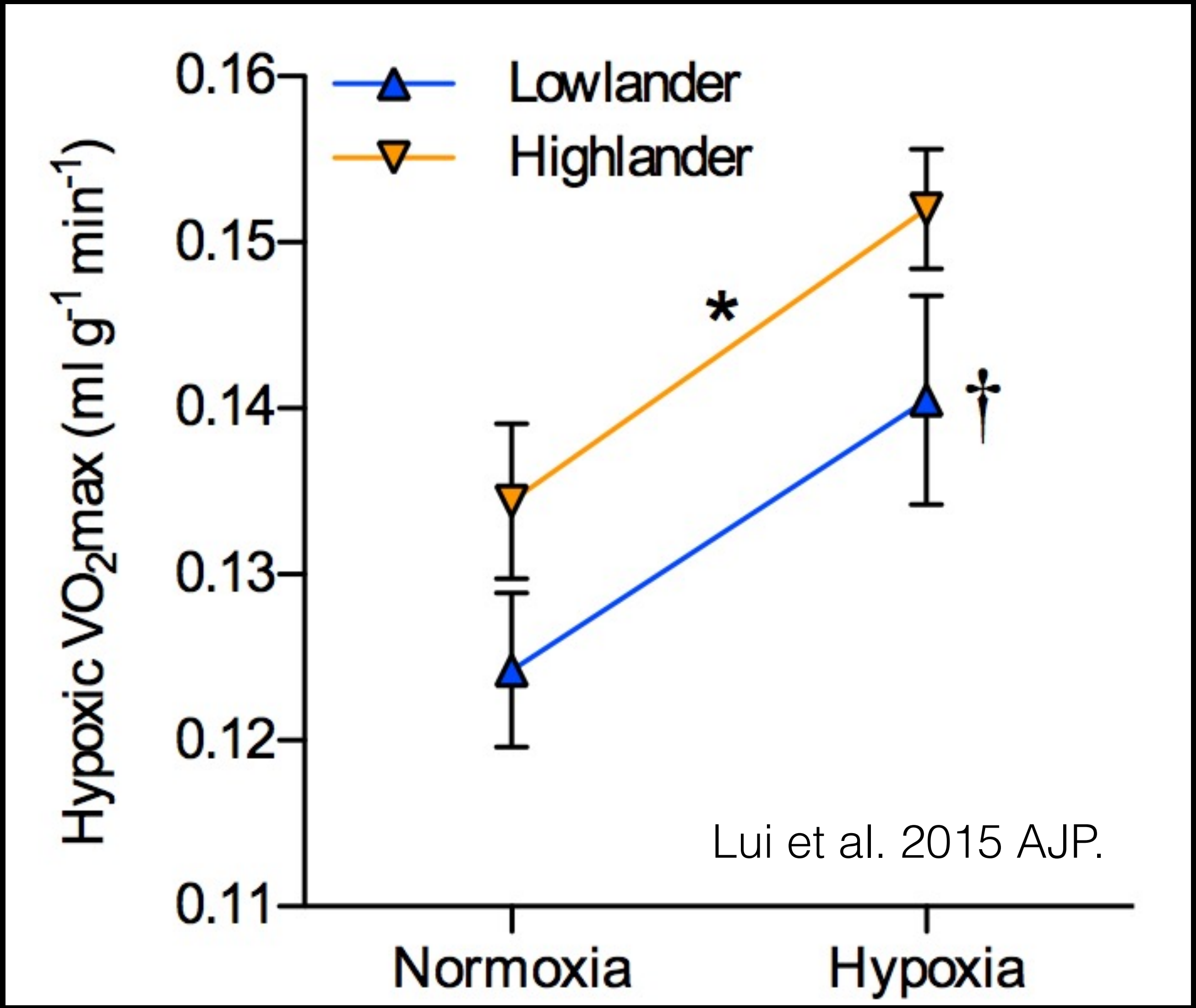


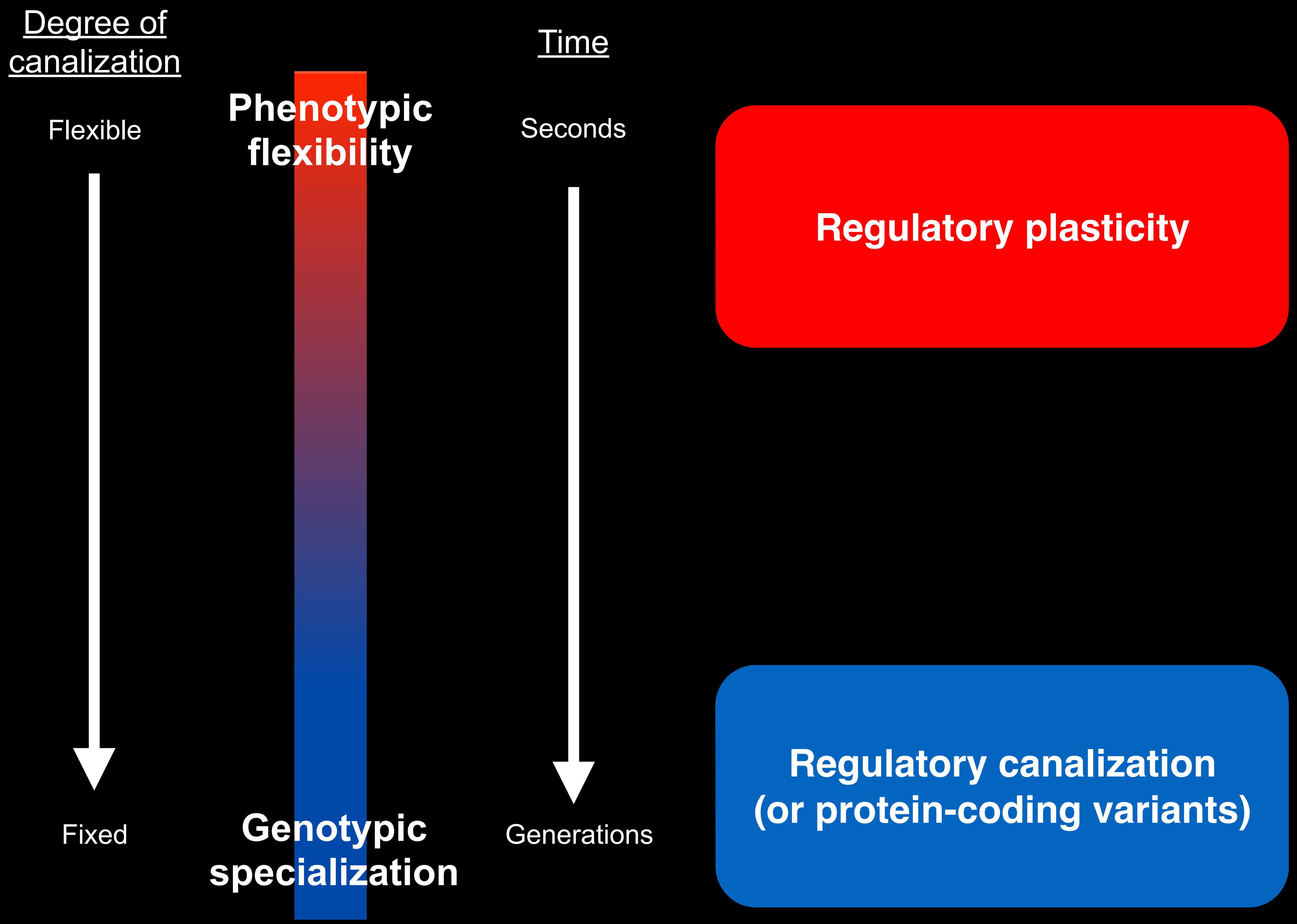
Cheviron et al. 2013 J. Exp. Biol.

Mean ± 1 SE

Plastic and constituitive variation in thermogenic capacity under hypoxia

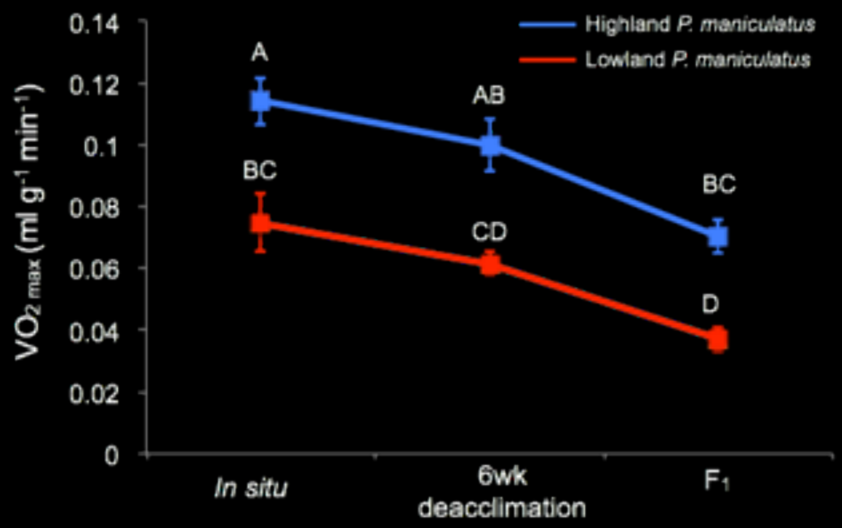
Offspring of wild-derived highland & lowland acclimated to low and high elevation for 6 wks




Degree of canalization
Flexible
Fixed
Phenotypic flexibility
Genotypic specialization
Time
Seconds
Generations
Regulatory plasticity
Regulatory canalization
(or protein-coding variants)

Exploring plastic and constitutive differences in thermogenic performance

Whole-organism performance



Exploring plastic and constitutive differences in thermogenic performance

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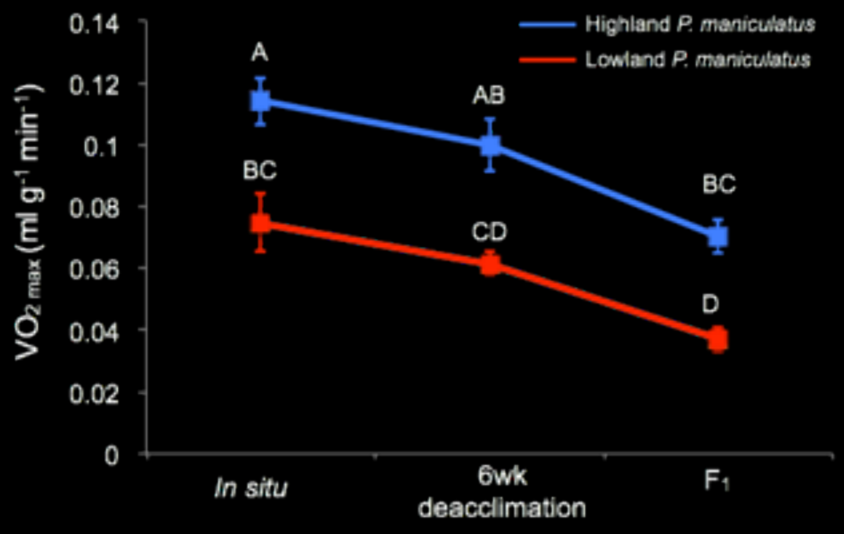
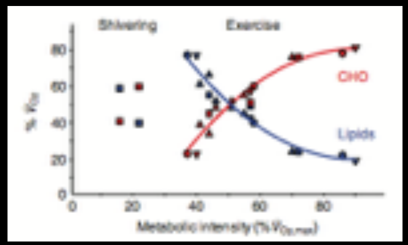
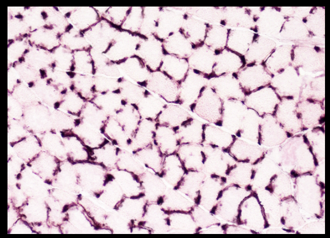


Subordinate physiological traits

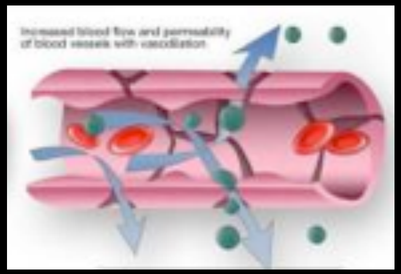
metabolic fuel use



vasculature



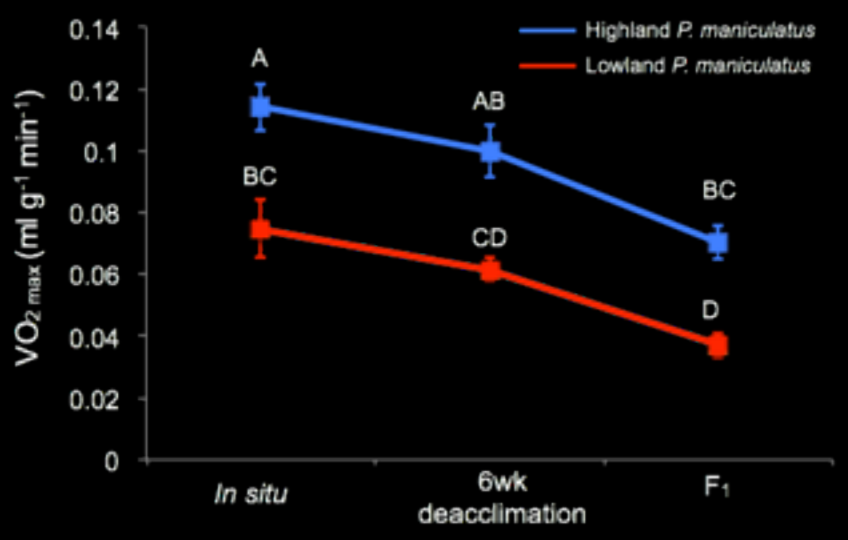
blood O_2 carrying capacity



O_2 diffusion capacity

Exploring plastic and constitutive differences in thermogenic performance

Whole-organism performance



Subordinate physiological traits

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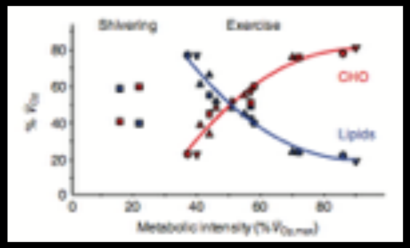

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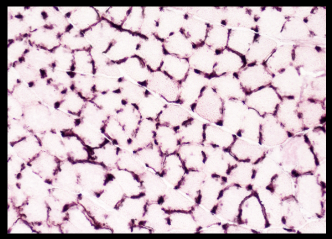
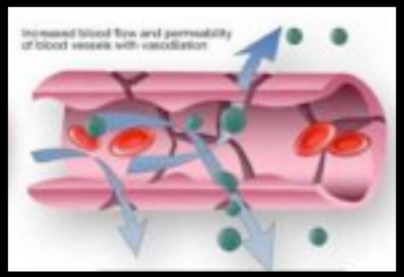
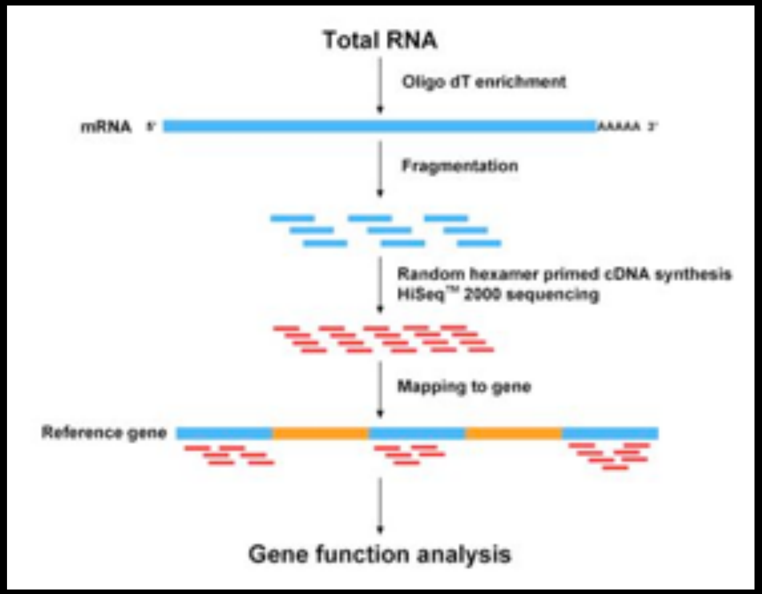


blood O_2 carrying capacity

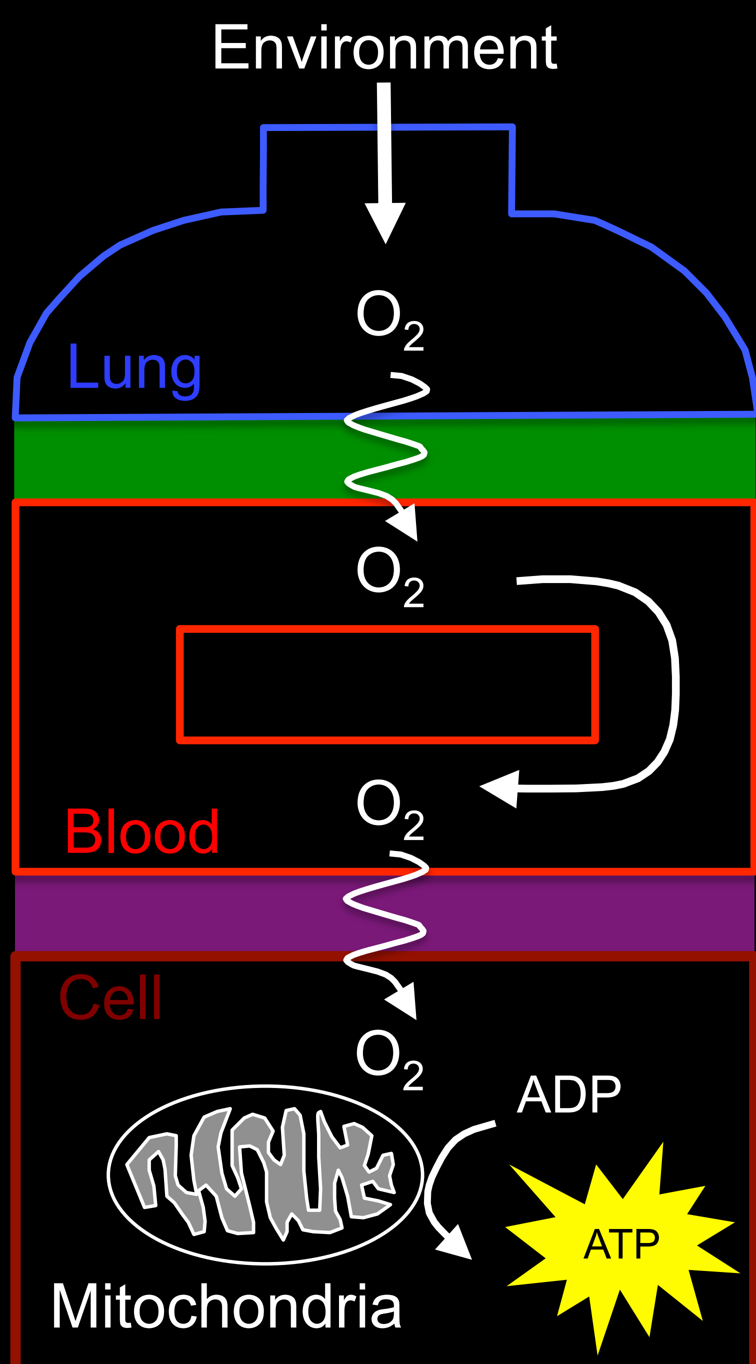
O_2 diffusion capacity



Functional and Evolutionary Genomics (RNA-seq & Sequence Capture)



How is pathway control distributed?



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Tissue O_2 Utilization	• Cellular mitochondrial density • Cellular aerobic capacity

↓

Aerobic Performance
($VO_{2\ max}$)

Storz, Scott & Cheviron. 2010. J. Exp. Biol.

How is pathway control distributed?

Breathing

P_{IO_2} = 75 torr
Breath rate (V) = 60 ml/min

Pulmonary O_2 Diffusion

$$\frac{dC_{cap_{O_2}}}{dt} = \frac{D_{L_{O_2}}}{t_{cap}\dot{Q}_{cap}}(P_{para_{O_2}} - P_{cap_{O_2}})$$

$$\frac{\dot{V}(P_{para_{O_2,1}} - P_{para_{O_2,2}})}{RT_b P_B} = \dot{Q}_{cap}(Ca_{cap_{O_2}} - C\bar{v}_{O_2})$$

Circulatory O_2 Delivery

$$C_{O_2} = 4C_{Hb}\frac{P^n_{O_2}}{P^n_{O_2} + P^n_{50}}$$

$$P_{50} = P_{50i} \times 10^{\phi\,\Delta pH(a-v)}$$

Tissue O_2 Diffusion

$$\frac{dC_{cap_{O_2}}}{dt} = \frac{-D_{T_{O_2}}}{t_{cap}\dot{Q}}(P_{cap_{O_2}} - P_{mito_{O_2}})$$

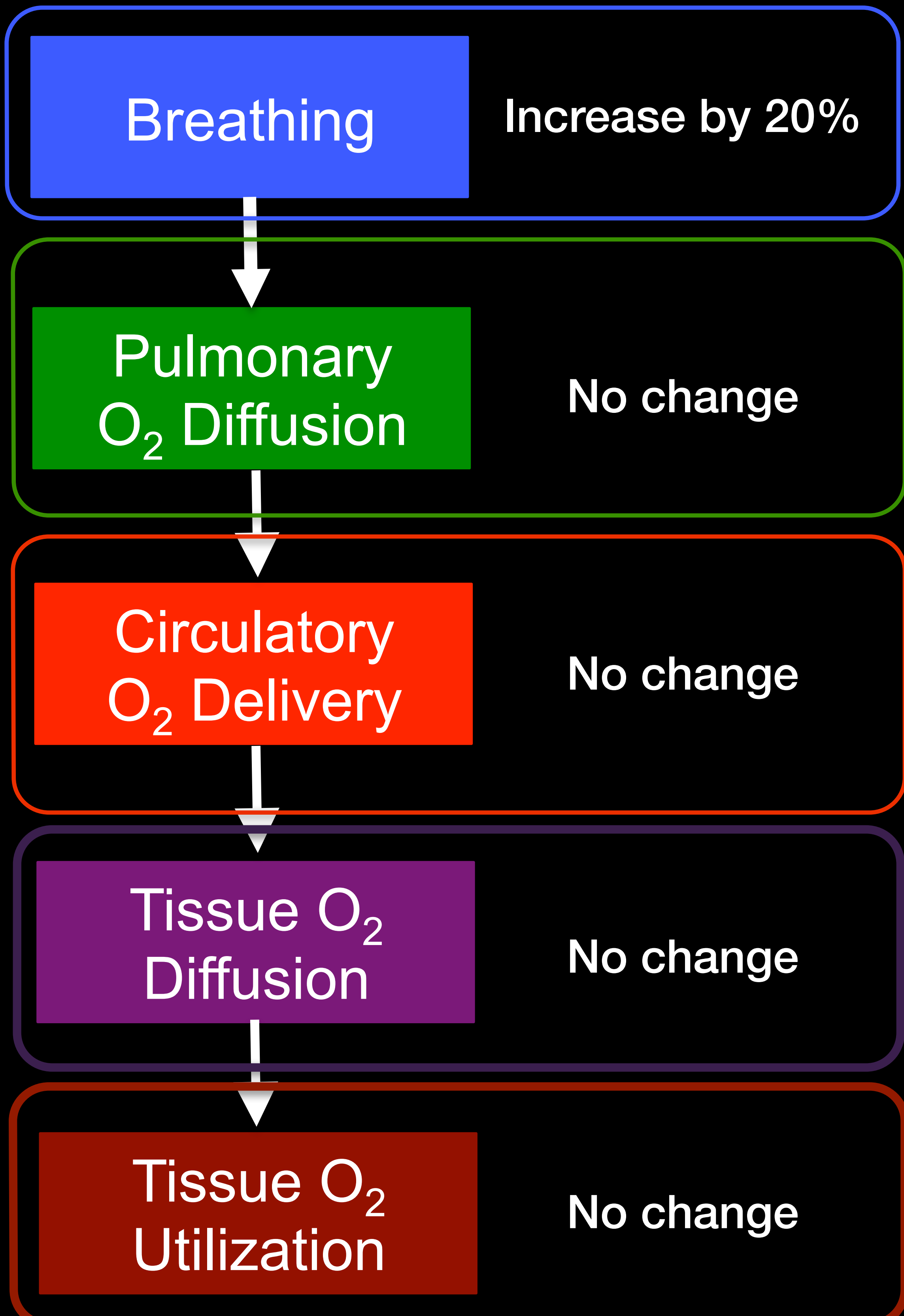
Tissue O_2 Utilization

$$\dot{V}_{O_2} = \frac{\dot{V}(P_{I_{O_2}} - P_{E_{O_2}})}{RT_b P_B}$$

$$\dot{V}_{O_2} = \dot{Q}(Ca_{O_2} - C\bar{v}_{O_2})$$

Respiratory System Model - Wagner 1996 Respir. Phys.; Scott & Milsom 2006

How is pathway control distributed?



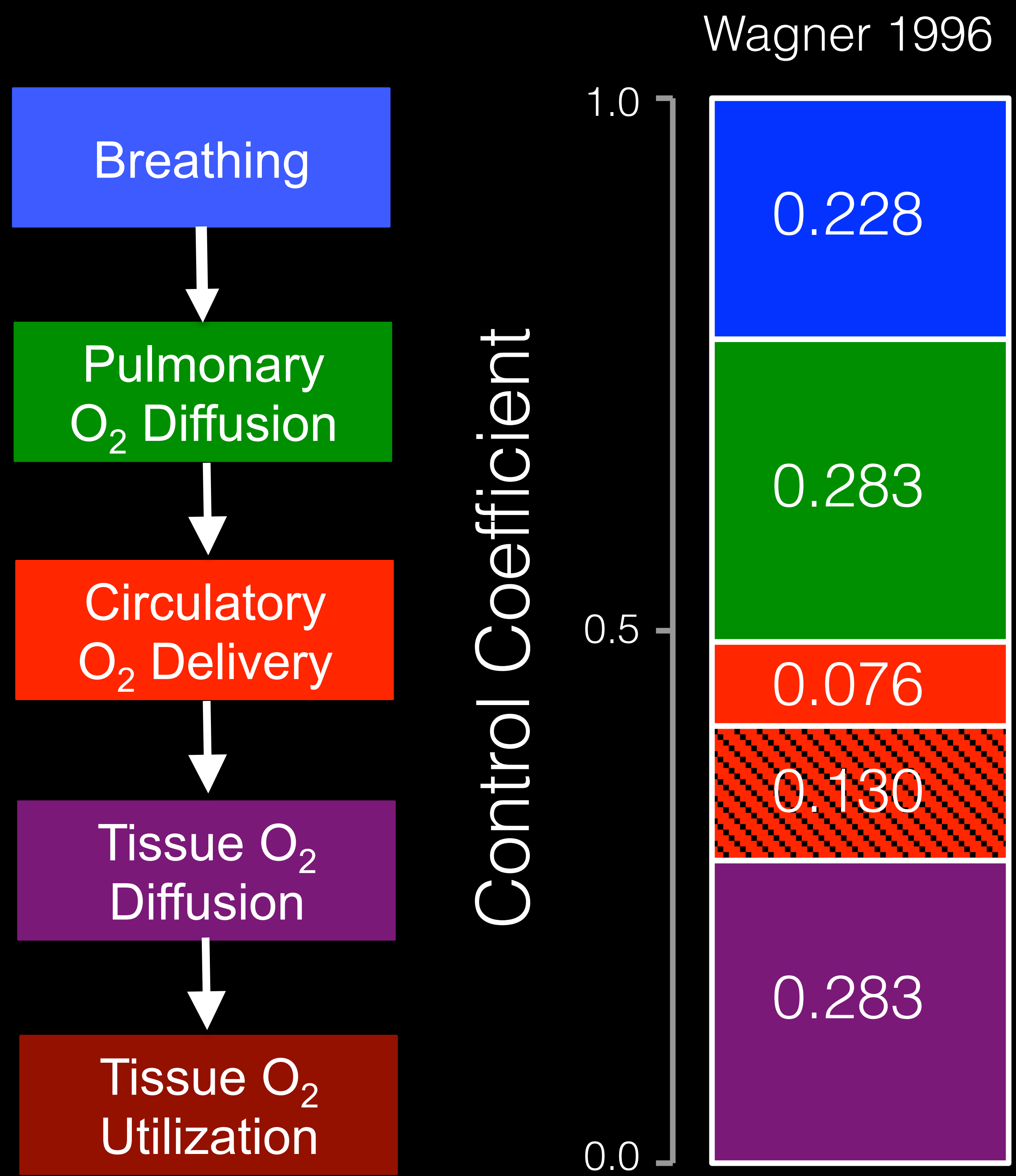
Calculate proportional increase in oxygen consumption (VO2)

If VO2 increases by 20%, then the control coefficient =1

If VO2 increases by 0%, then the control coefficient = 0

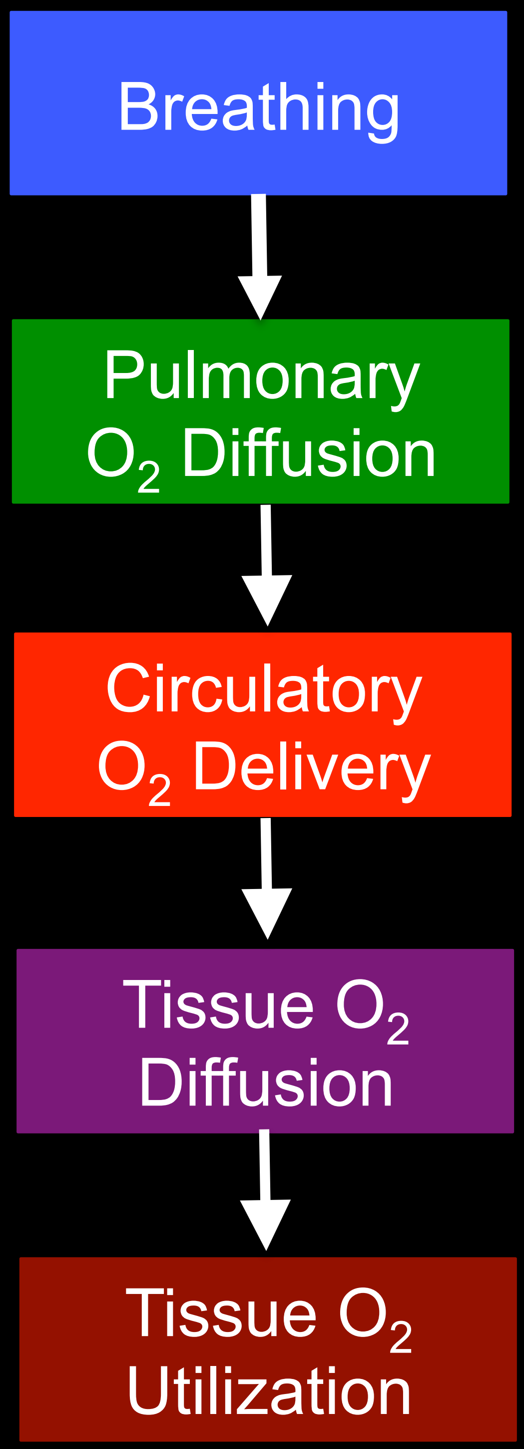
Respiratory System Model - Wagner 1996 Respir. Phys.; Scott & Milsom 2006

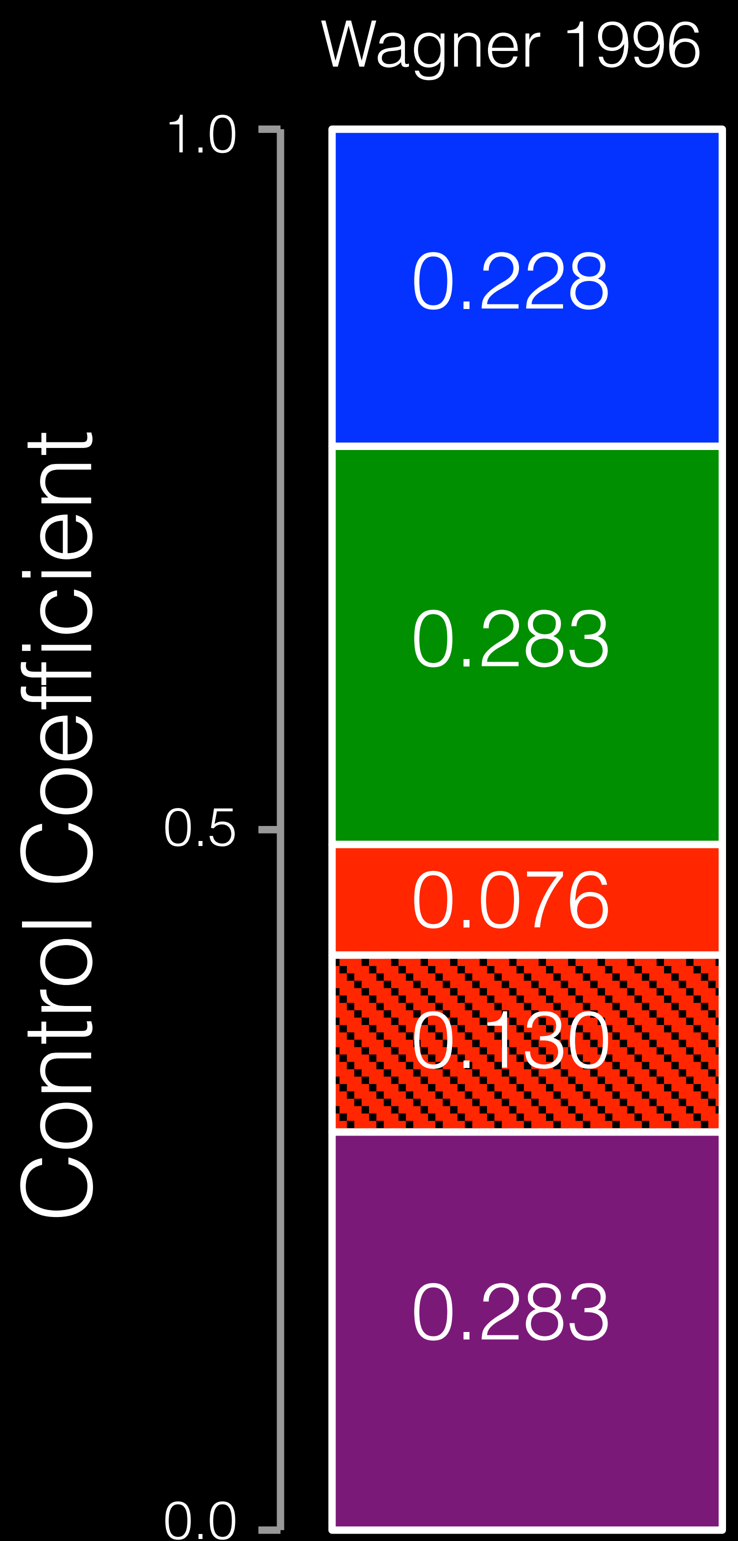
How is pathway control distributed?



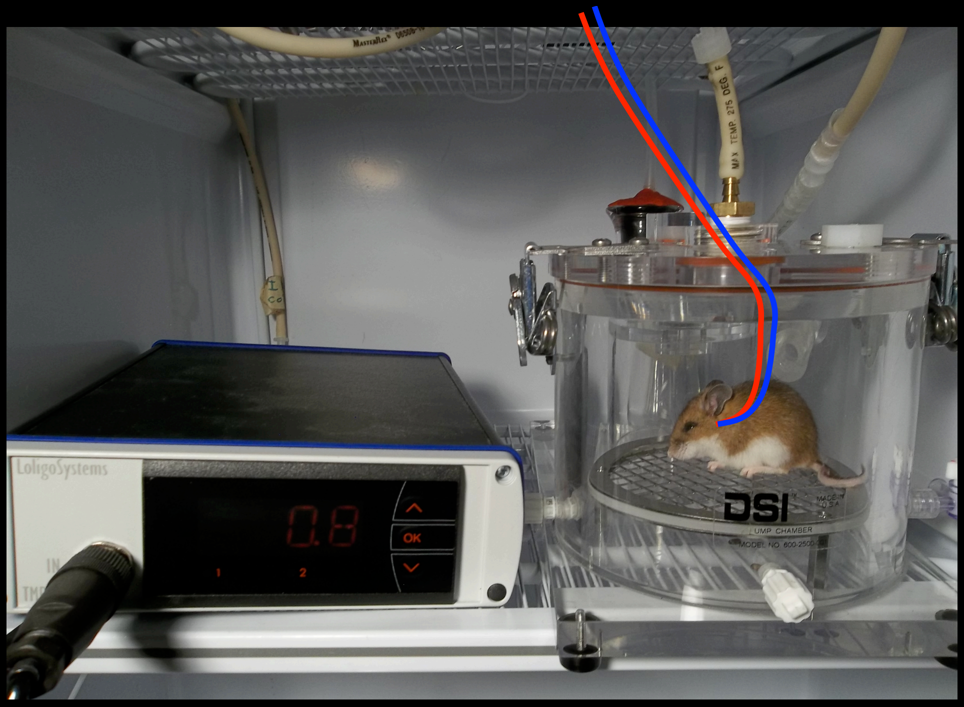
Wagner 1996 Respir. Phys.

How is pathway control distributed?



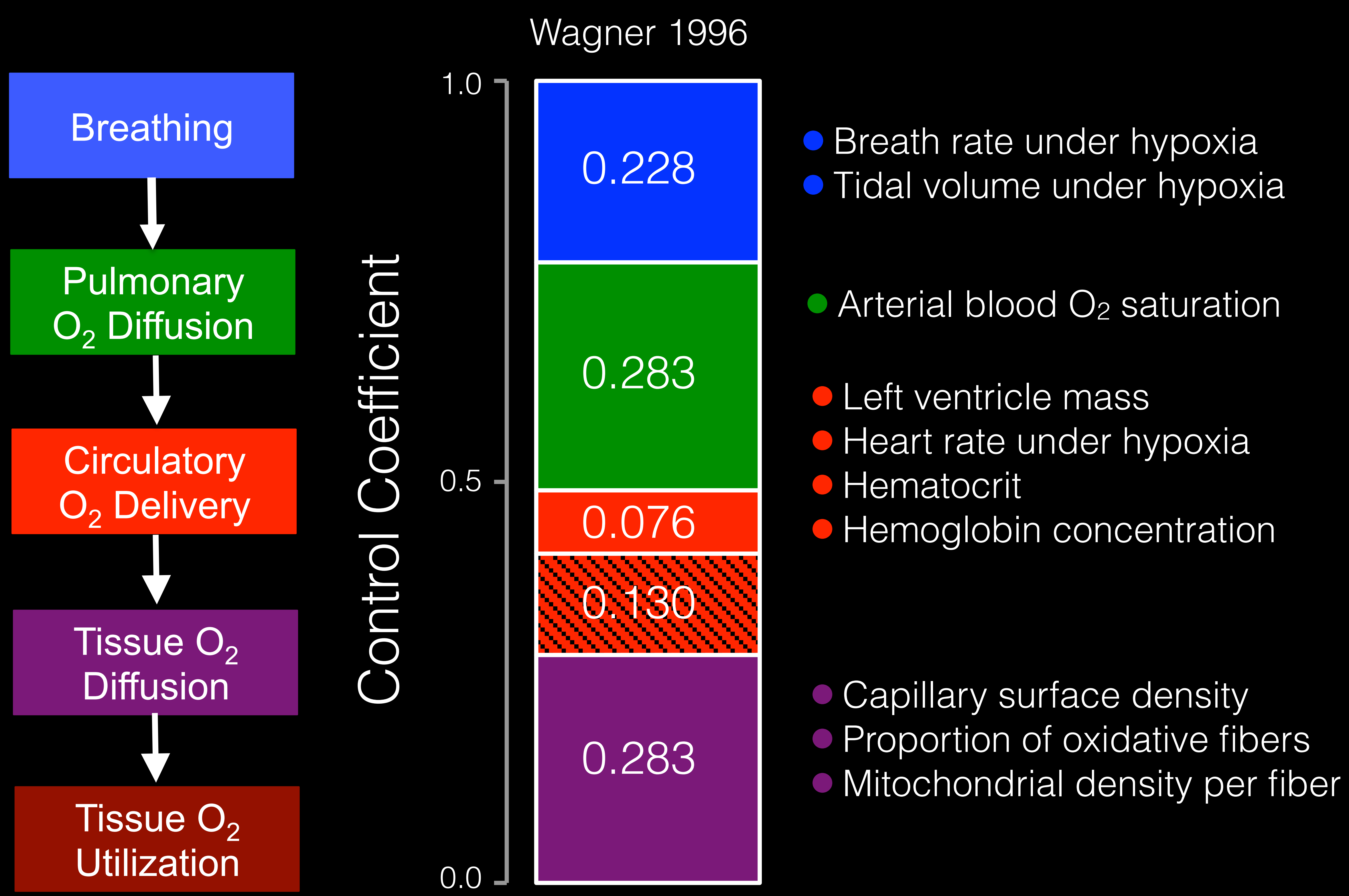


Kevin Tate
Postdoc
McMaster
University

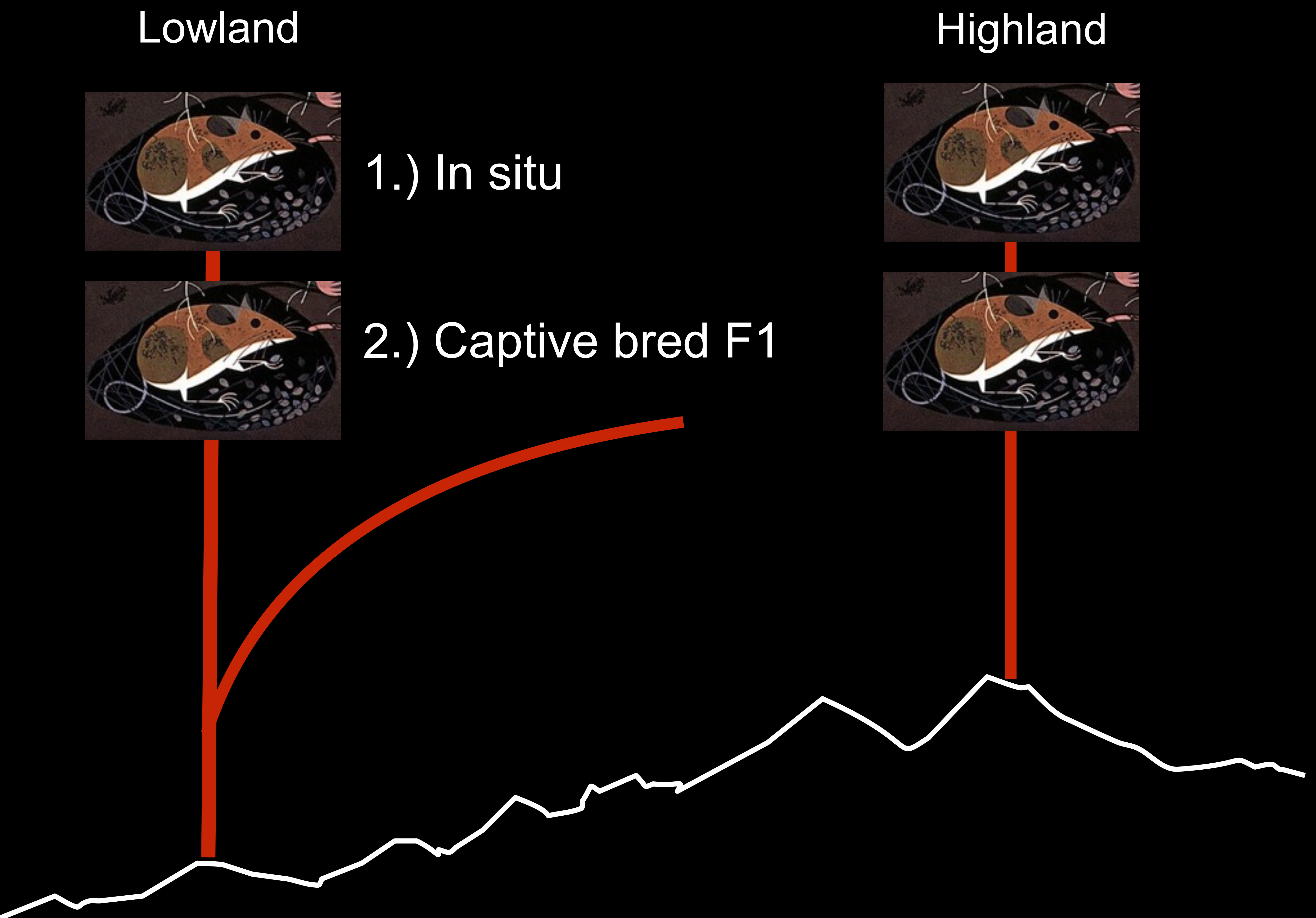


Wagner 1996 Respir. Phys.

Do control coeffecients predict population divergence?



Data from Lui et al. 2015 AJP; Scott et al. 2015 MBE; Tate et al. unpub; Velotta et al. unpub.; Ivy et al. unpub.; Malingham et al. unpub.


Lowland
Highland
1.) In situ
2.) Captive bred F1

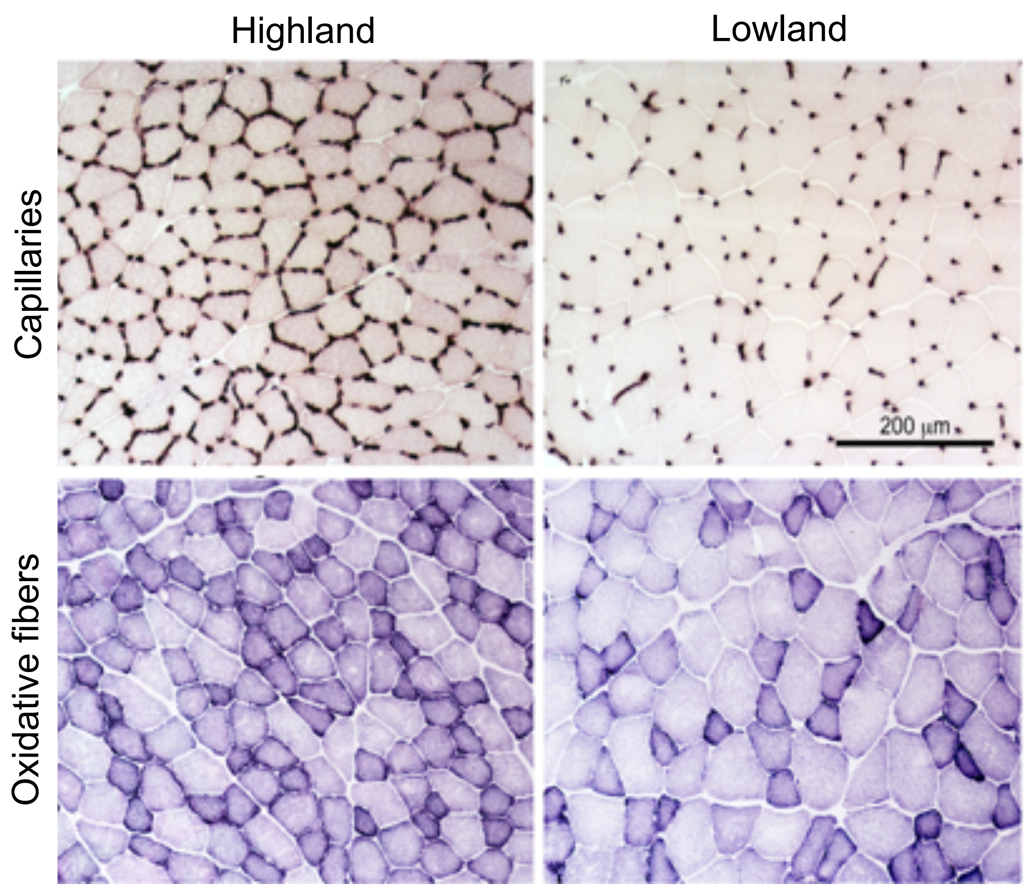
Lowland

Highland

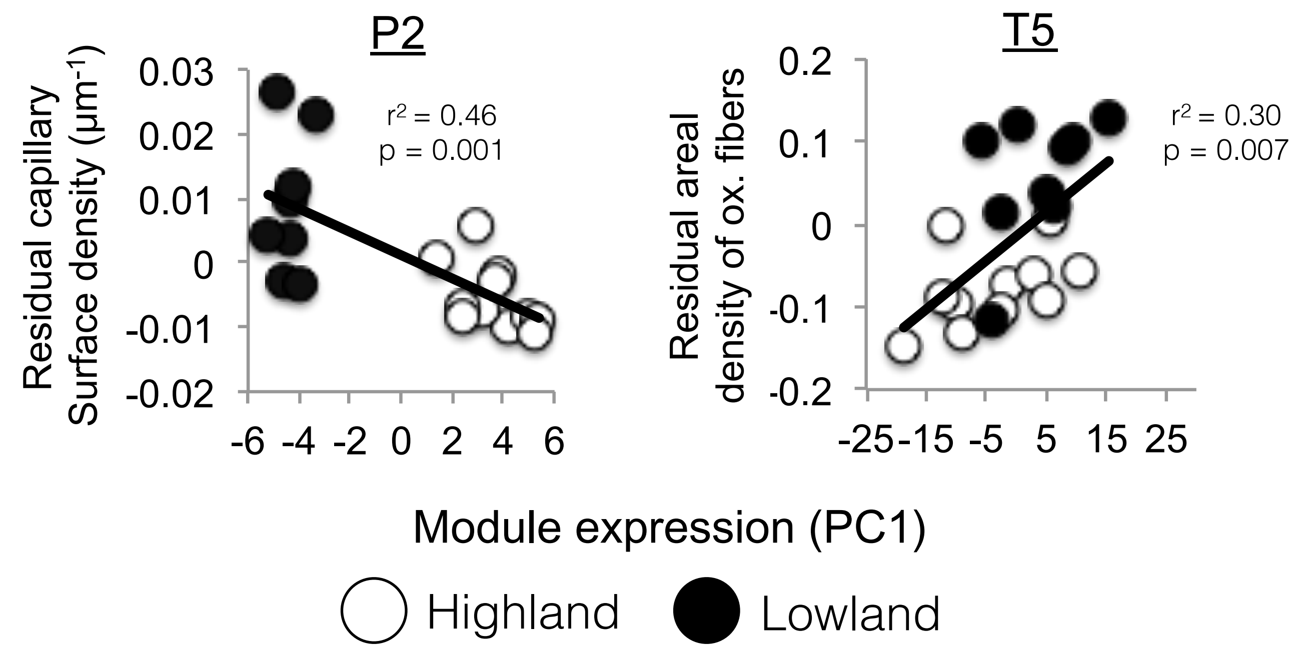
Offspring of wild-derived highland & lowland acclimated to low and high elevation for 6 wks

Altitudinal differences in muscle phenotype are associated with differences in the regulation of hypoxia sensitive genes

A.)



C.)



Module P2 Hub gene = *Notch4*
Module T5 Hub gene = *Ppargc1a*

Scott, Liu, Storz, & Cheviron 2015. *Mol. Biol. Evol.*

Transcriptome scan revealed candidate genes for hypoxia adaptation

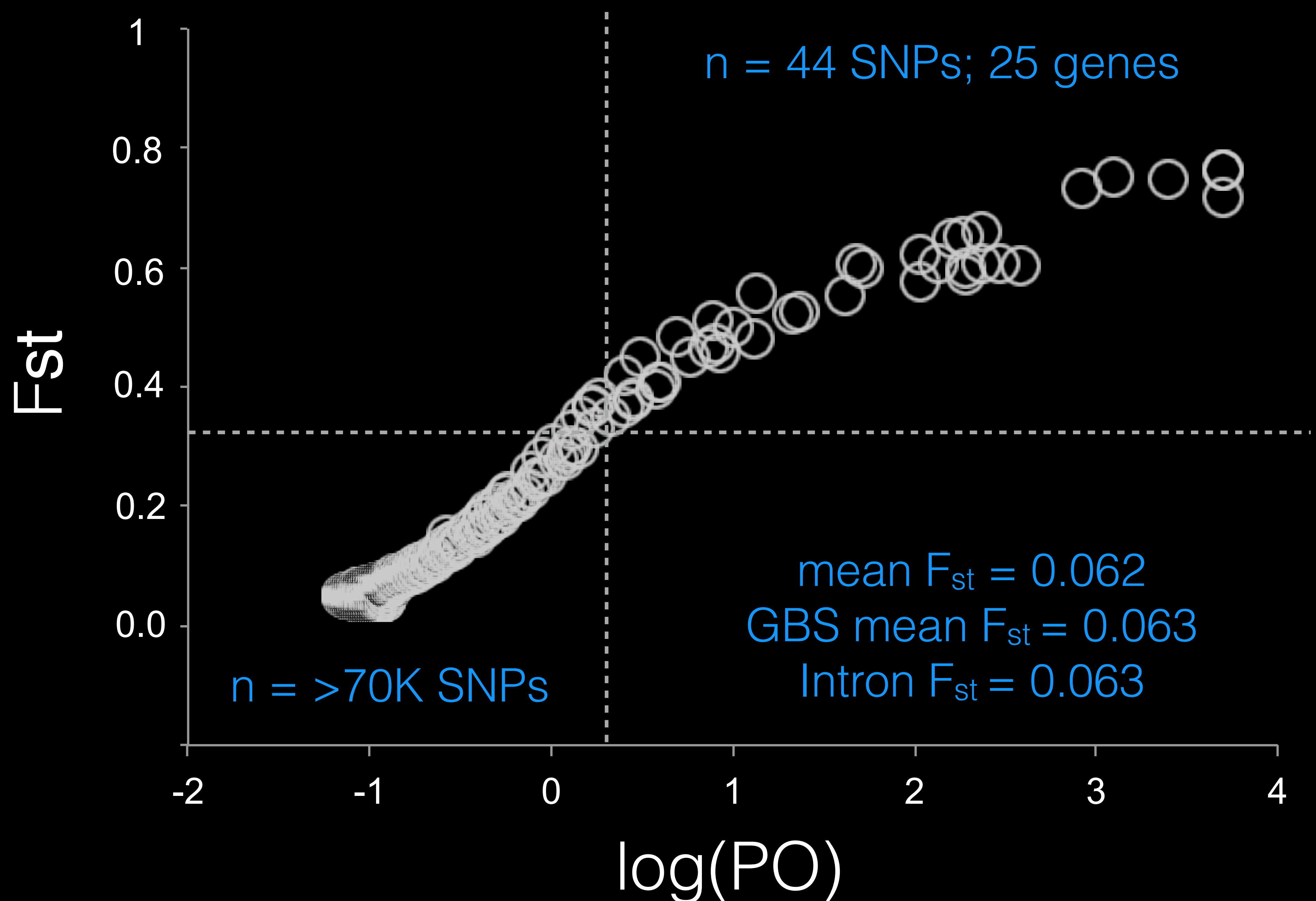
Lowland
(n = 30)

Highland
(n = 30)

RNAseq Skeletal Muscle
>70 K SNPs

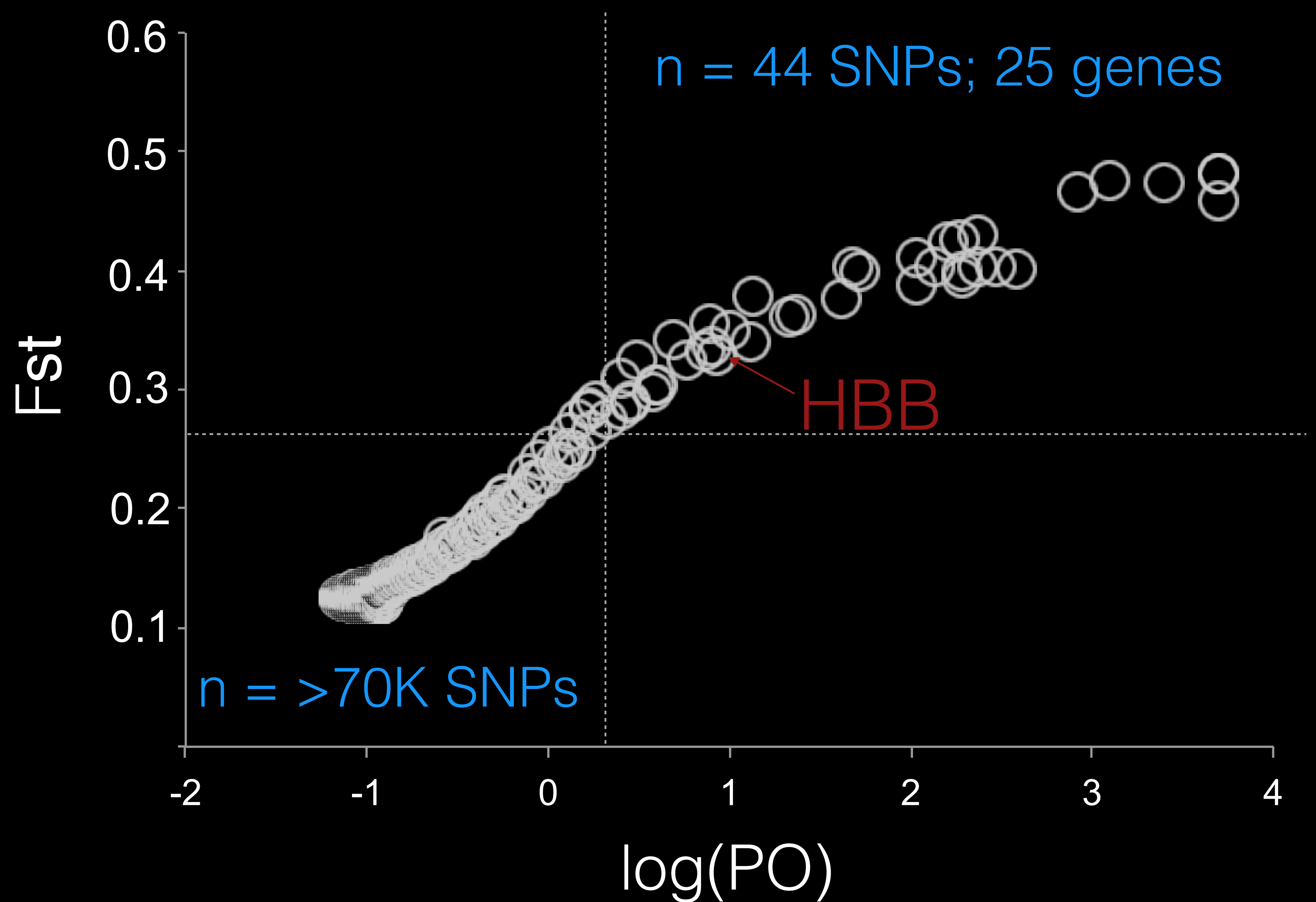
Data from Cheviron et al. 2012, 2014

Transcriptome scan revealed candidate genes for hypoxia adaptation



70,016 SNPs genotyped in 60 individuals (30/population)
RNAseq data from Cheviron et al. 2012, 2014)

Elevational patterns of allelic variation in expressed transcripts suggest targets of spatial varying selection



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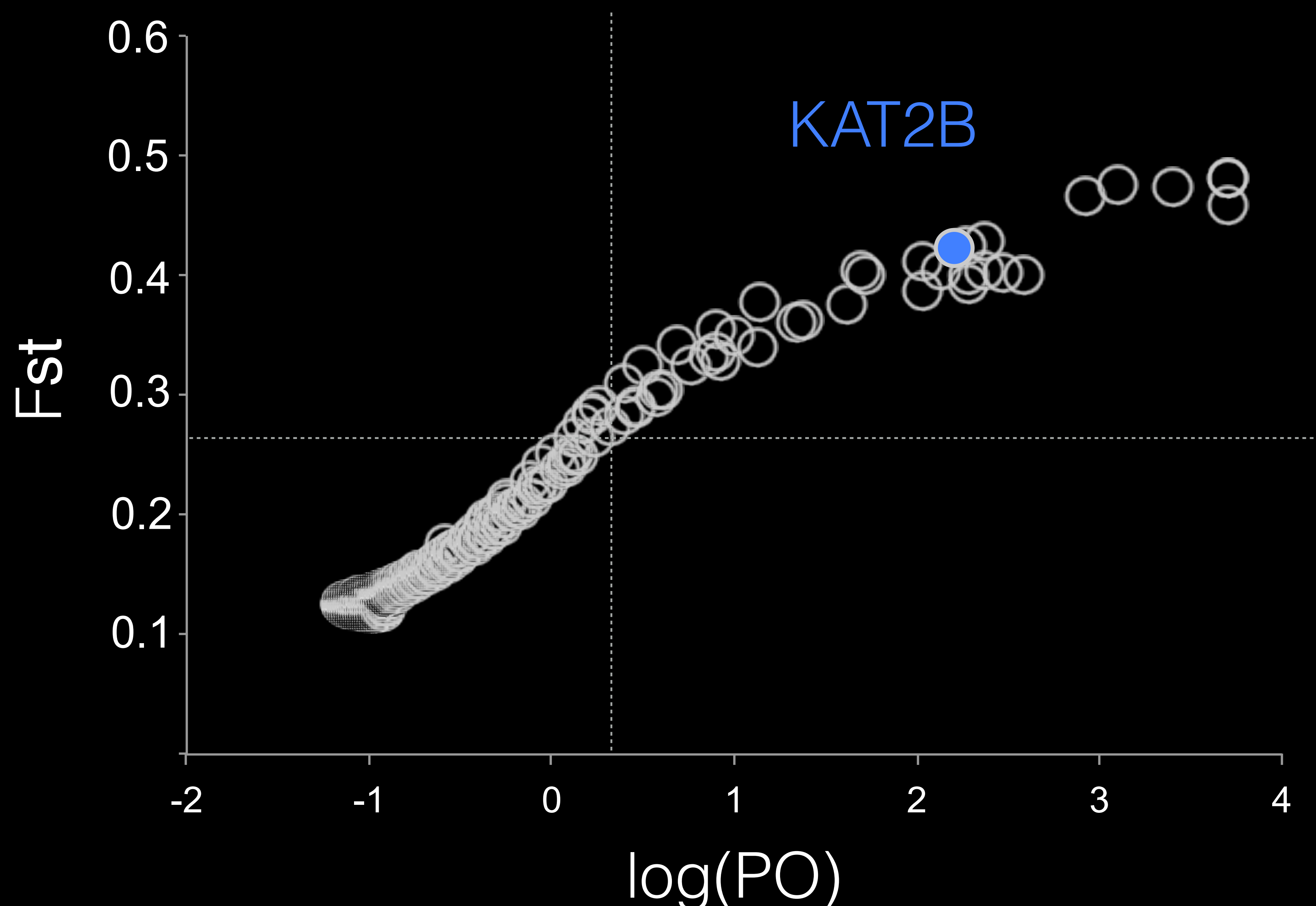
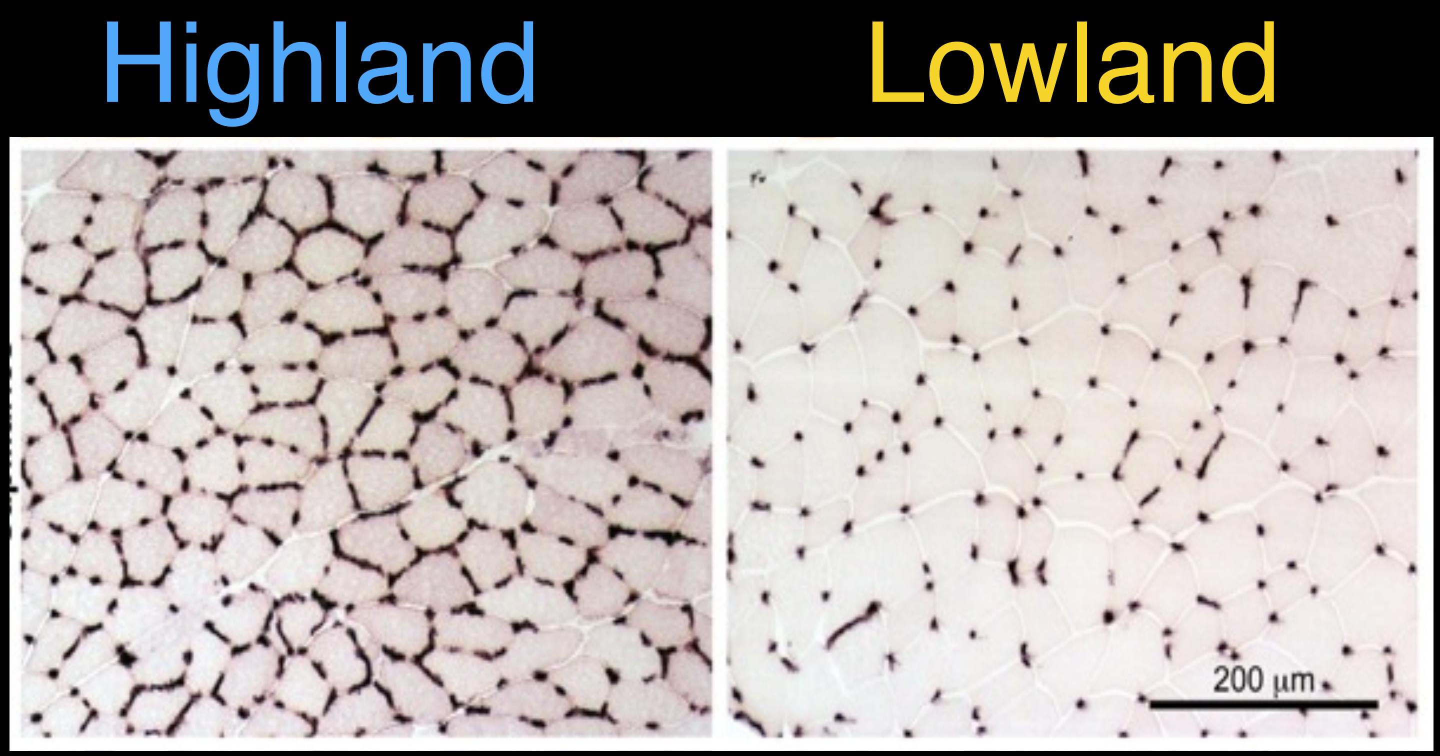
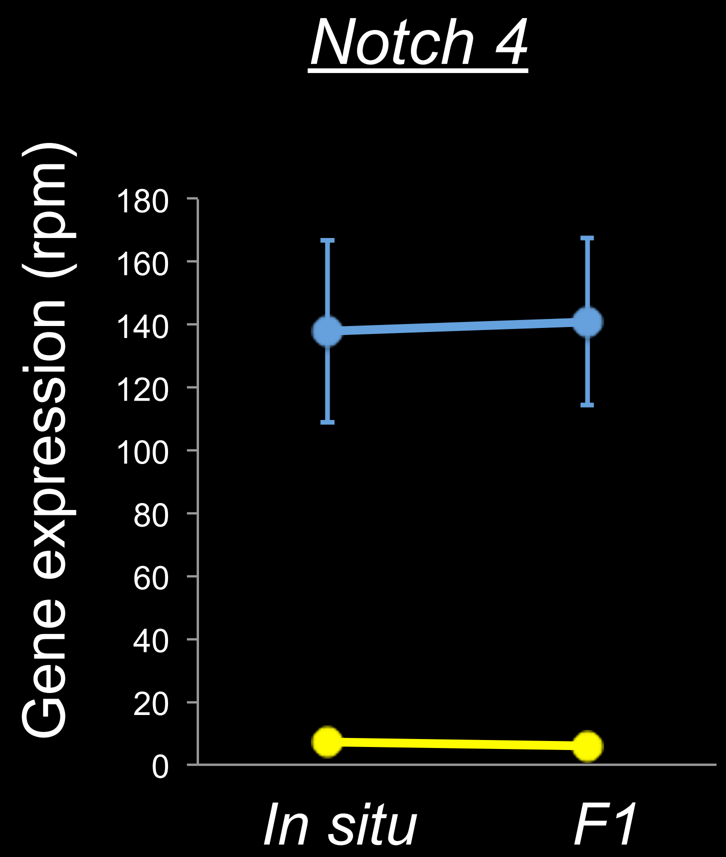


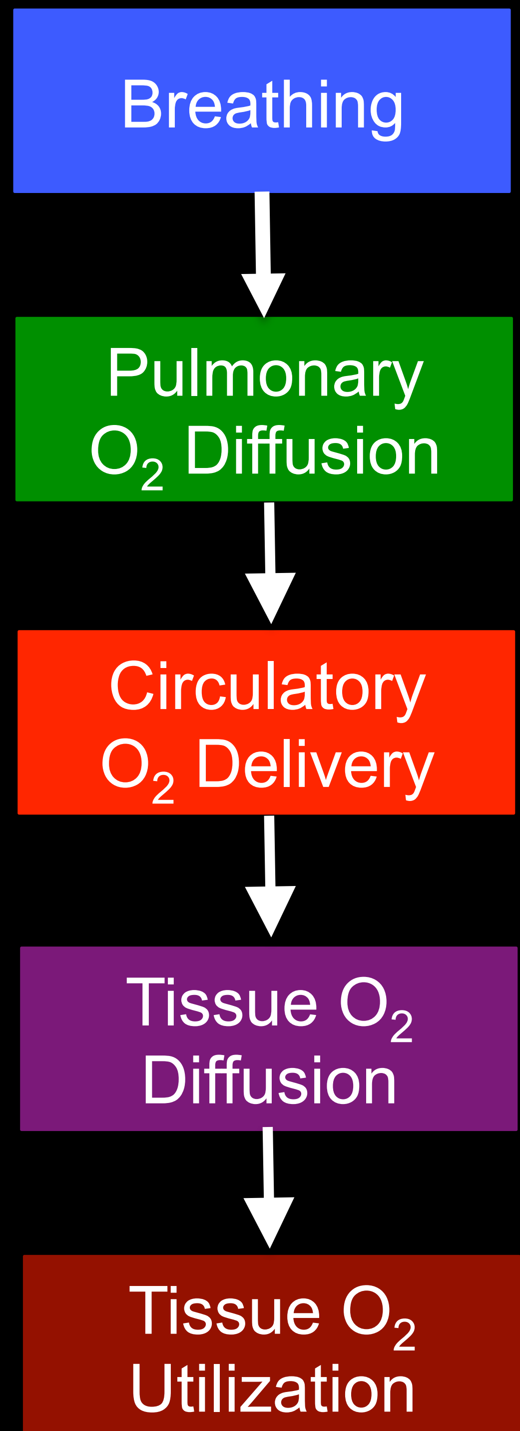
Persistent differences in muscle capillarity are associated with constituitive differences in *Notch 4* expression.





Notch 4 and *Kat2b* are components of the NICD/CSL coactivator complex in the Notch signaling pathway.

Phng & Gerhardt 2009 Dev. Cell



- Genetic increases in Hb O_2 affinity

- Increased in capillary destiny
- Canalized differences *Notch 4* expression
- Allelic differentiation at angiogenesis genes
- Increases in oxidative fiber density
- Elevated *Ppargc1a* expression.

Cheviron et al. 2012. PNAS; Cheviron et al. 2013 JEB; Cheviron et al. 2014. Evolution; Lui et al. 2015 AJP; Scott et al. 2015 MBE; Velotta et al, 2016 Mol. Ecol.

Breathing → Pulmonary O_2 Diffusion → Circulatory O_2 Delivery → Tissue O_2 Diffusion → Tissue O_2 Utilization

Breathing

- Elevated hypoxic ventilatory response
- Elevated tidal volume

Pulmonary O_2 Diffusion

- Reduced pulmonary hypertension under hypoxia
- Increased arterial blood O_2 saturation

Circulatory O_2 Delivery

- Increased left ventricle size
- Genetic increases in Hb O_2 affinity
- Blunted erythropoietic response

Tissue O_2 Diffusion

- Increased in capillary destiny
- Canalized differences *Notch 4* expression
- Allelic differentiation at angiogenesis genes
- Increases in oxidative fiber density
- Elevated *Ppargc1a* expression.

Tissue O_2 Utilization

Cheviron et al. 2012. PNAS; Cheviron et al. 2013 JEB; Cheviron et al. 2014. Evolution; Lui et al. 2015 AJP; Scott et al. 2015 MBE; Velotta et al, 2016 Mol. Ecol.

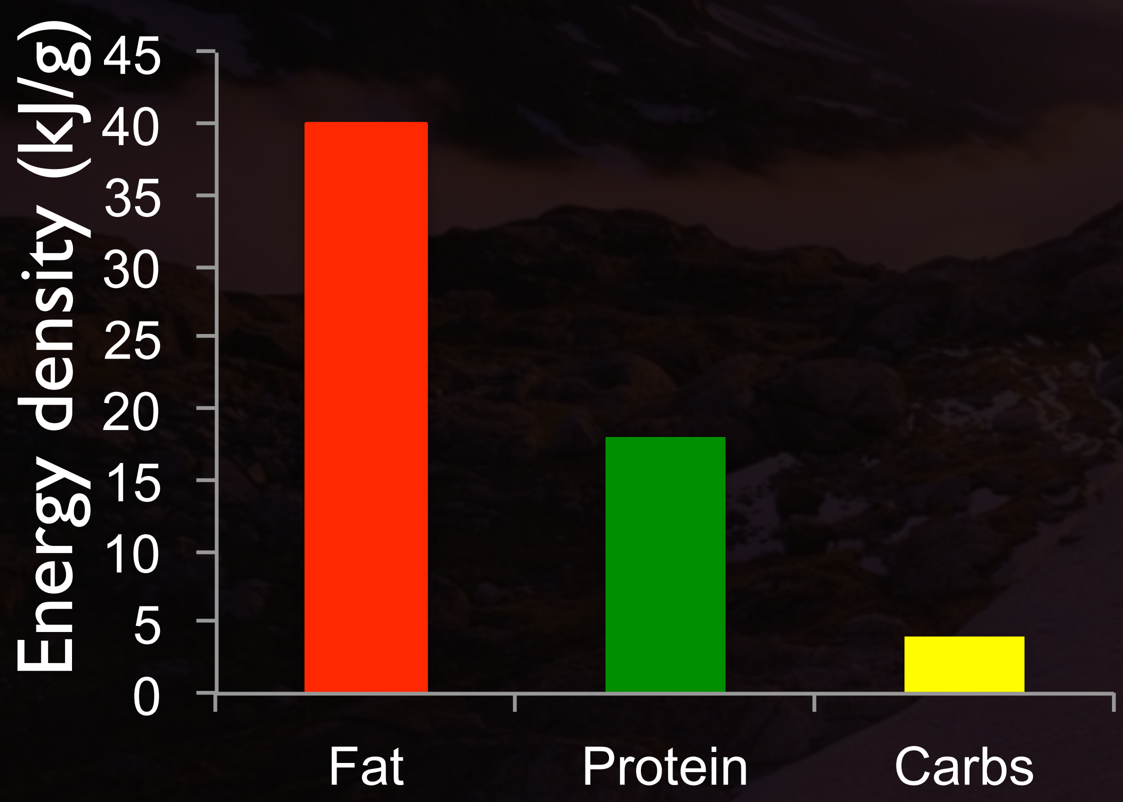
Metabolic fuel use at high altitude

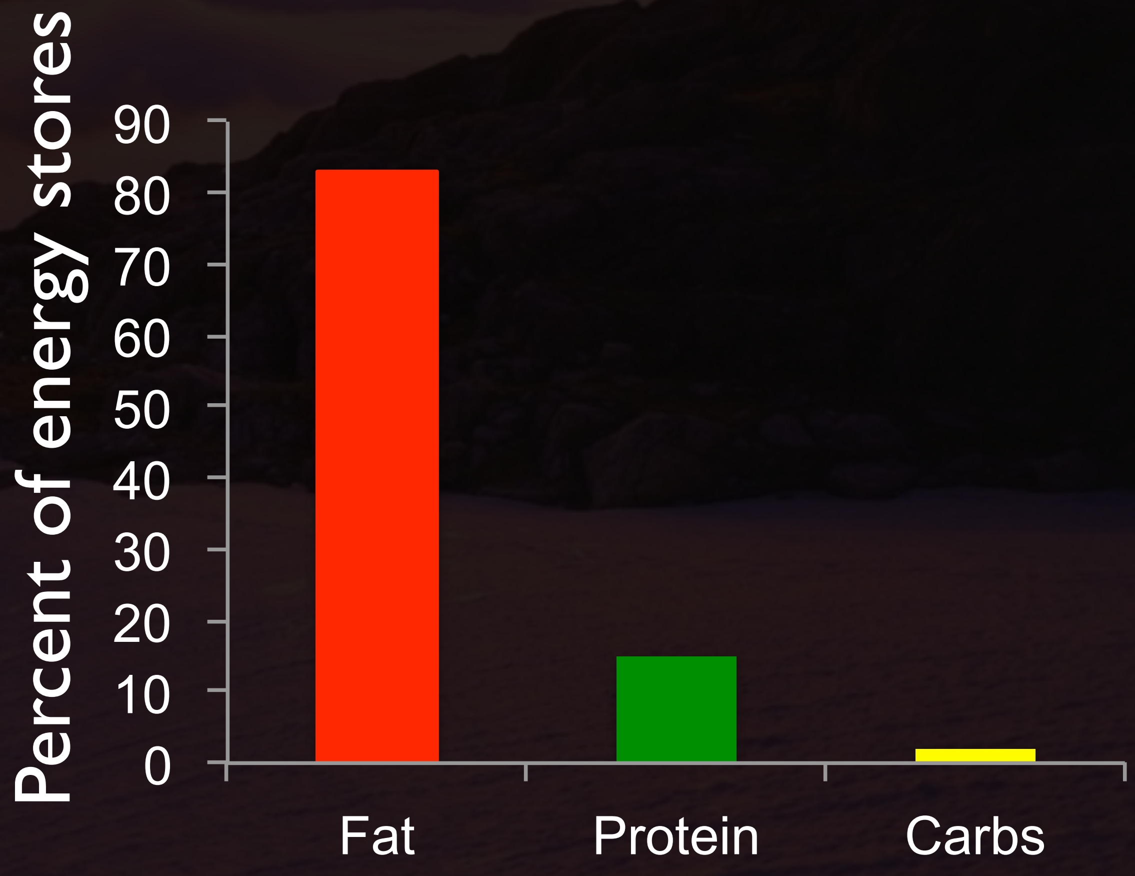
Quechua - Andes

Sherpa - Himalayas

Tradeoffs associated with increased carbohydrate utilization

But..... fats are a rich and abundant fuel source





Weber 2011 J. Exp. Biol.

Small questions

Can the evolved increases in hemoglobin O2 affinity and muscle capillarity in highland mice support enhanced lipid oxidation rates under hypoxic cold stress?

If so, what are the mechanistic underpinnings of this enhanced capacity for lipid oxidation?

Respiratory Exchange Ratio = VCO_2/VO_2

Respiratory Exchange Ratio = VCO_2/VO_2

Carbohydrates

$$6\ O_2 + C_6H_{12}O_6 \longrightarrow 6\ CO_2 + 6\ H_2O + 38\ ATP$$

RER = VCO2/VO2 = 6 CO2/6 O2 = 1.0

Fats

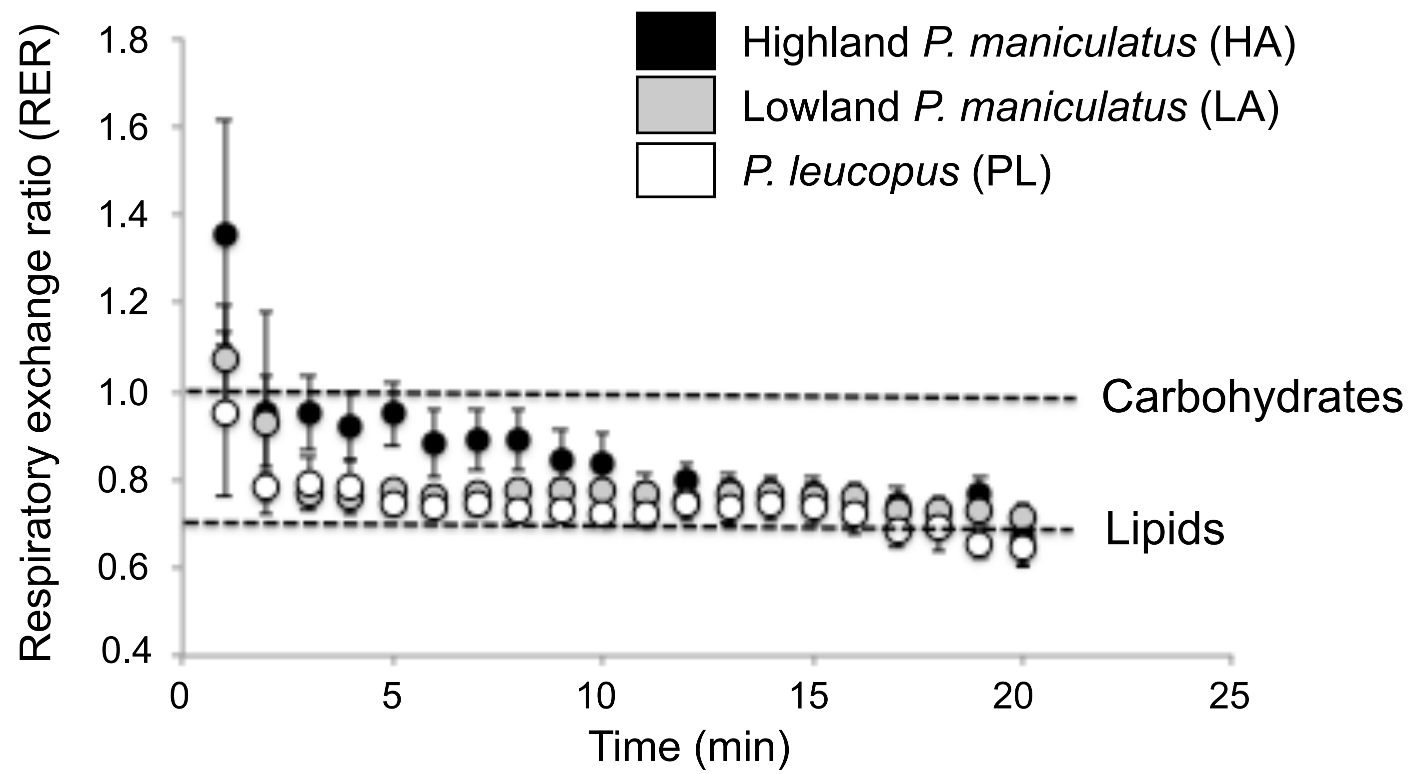
$$23\ O_2 + C_{16}H_{32}O_2 \longrightarrow 16\ CO_2 + 16\ H_2O + 129\ ATP$$

RER = VCO2/VO2 = 16 CO2/23 O2 = 0.7

Predictions:

I. Fats are an important fuel source during thermogenesis
II. High altitude mice will have whole-organism higher lipid oxidation rates

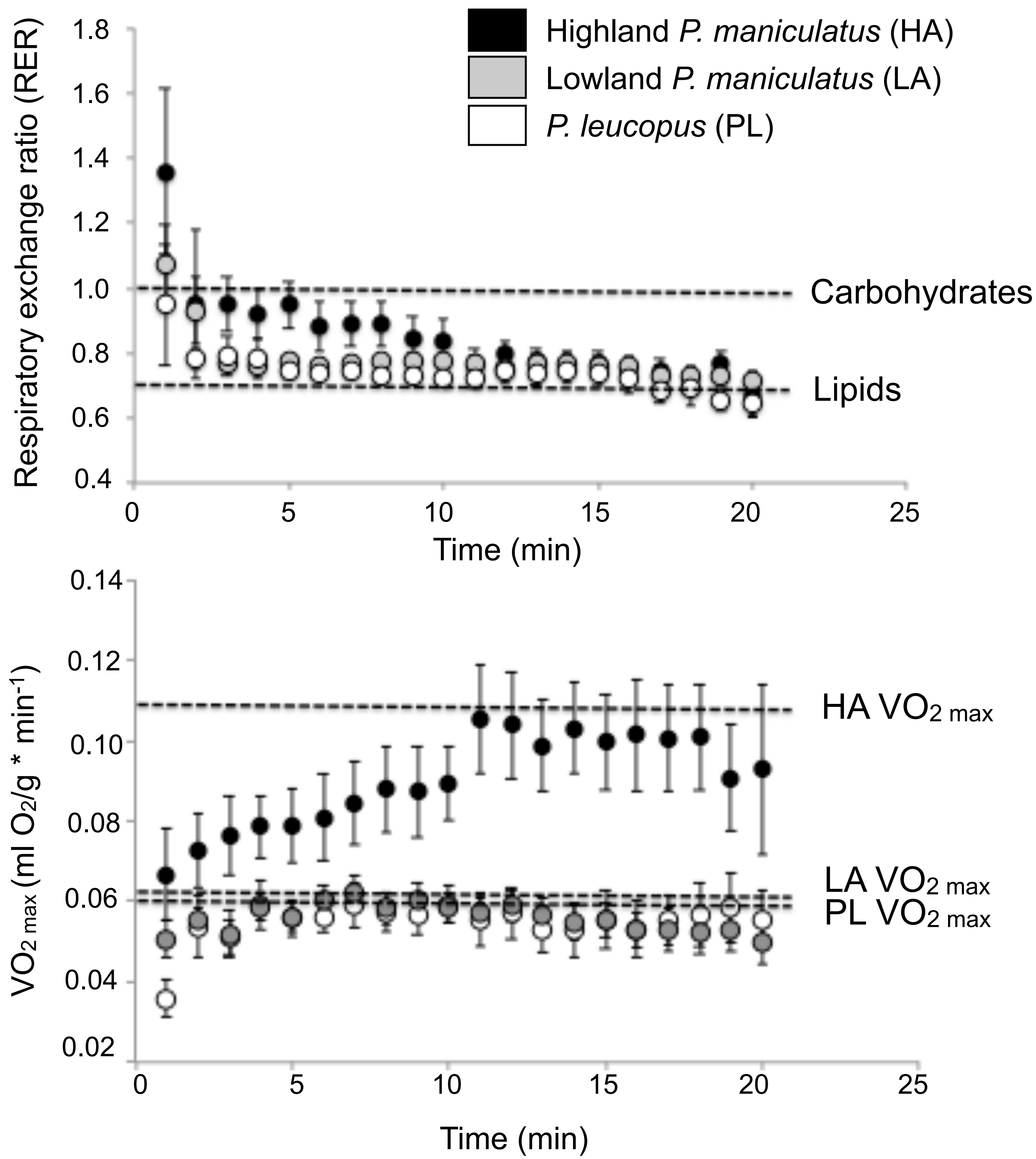
Fats power maximal thermogenesis in deer mice, and high-altitude mice have higher lipid oxidation rates.



n = 10/population
all mice acclimated to low elevation for 6 weeks

Cheviron et al. PNAS 2012

Fats power maximal thermogenesis in deer mice, and high-altitude mice have higher lipid oxidation rates.



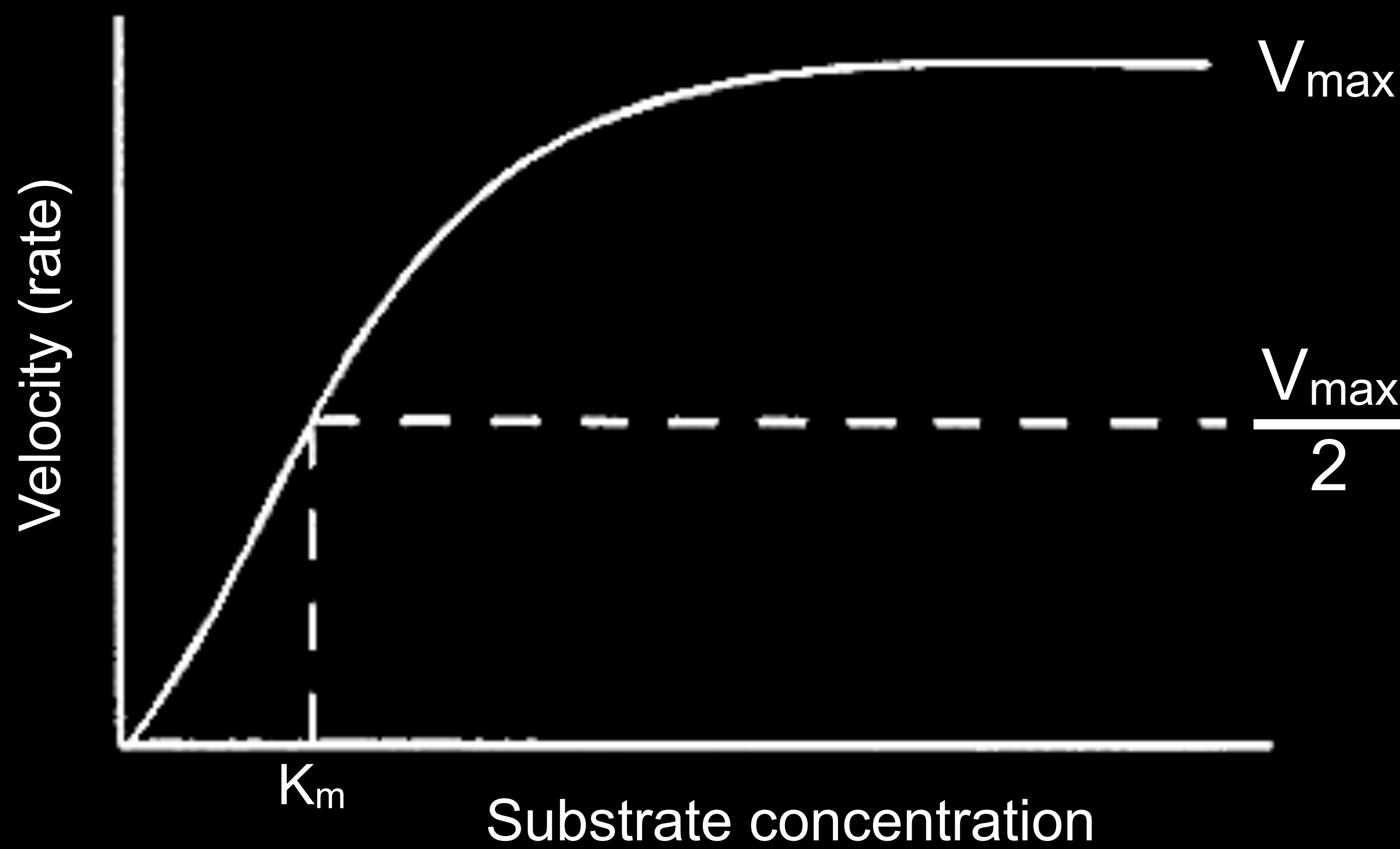
n = 10/population

all mice acclimated to low elevation for 6 weeks

Cheviron et al. PNAS 2012

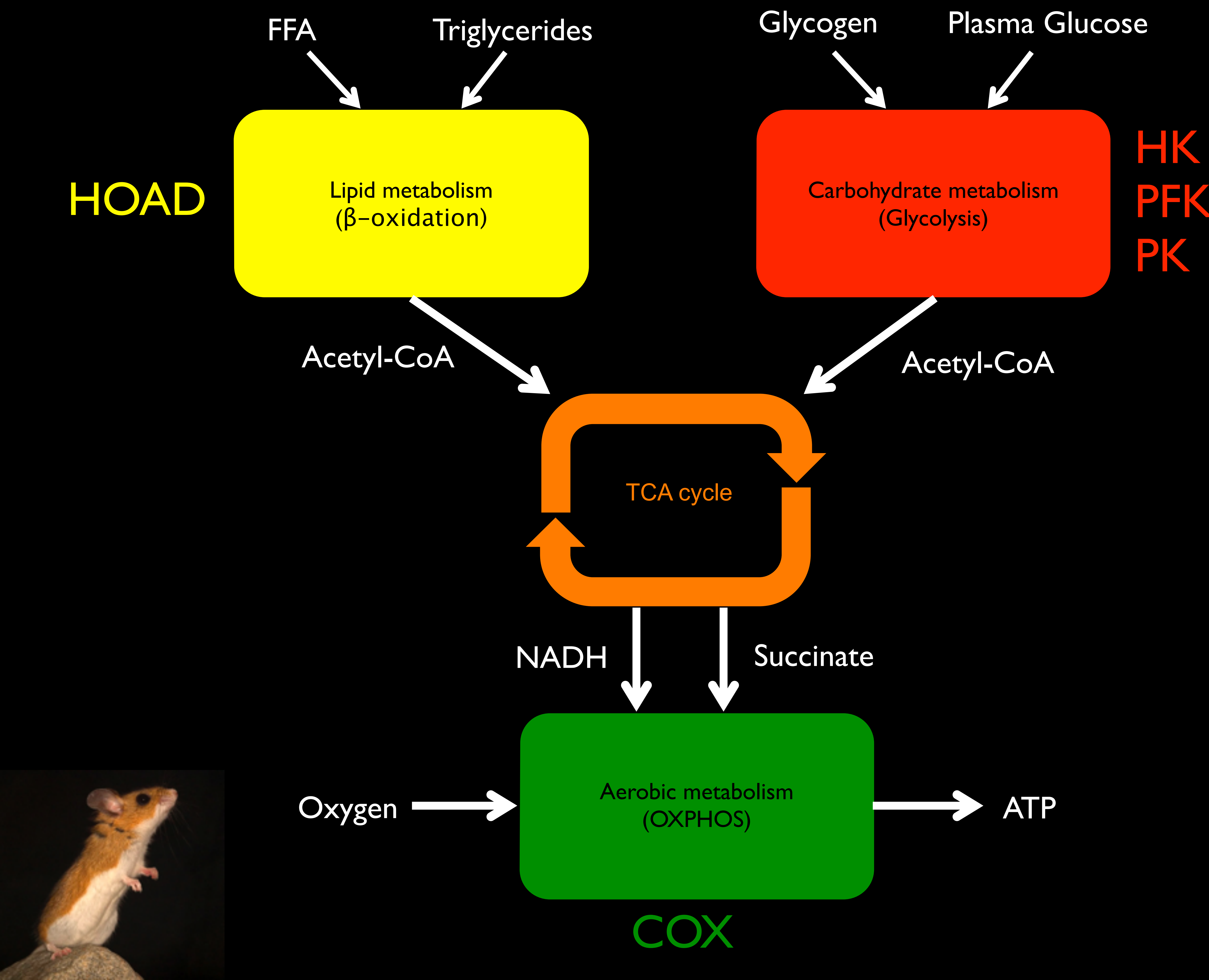
Examining fuel use at the biochemical level

$$\text{Enzyme } V_{max} = K_{cat} * [E]$$



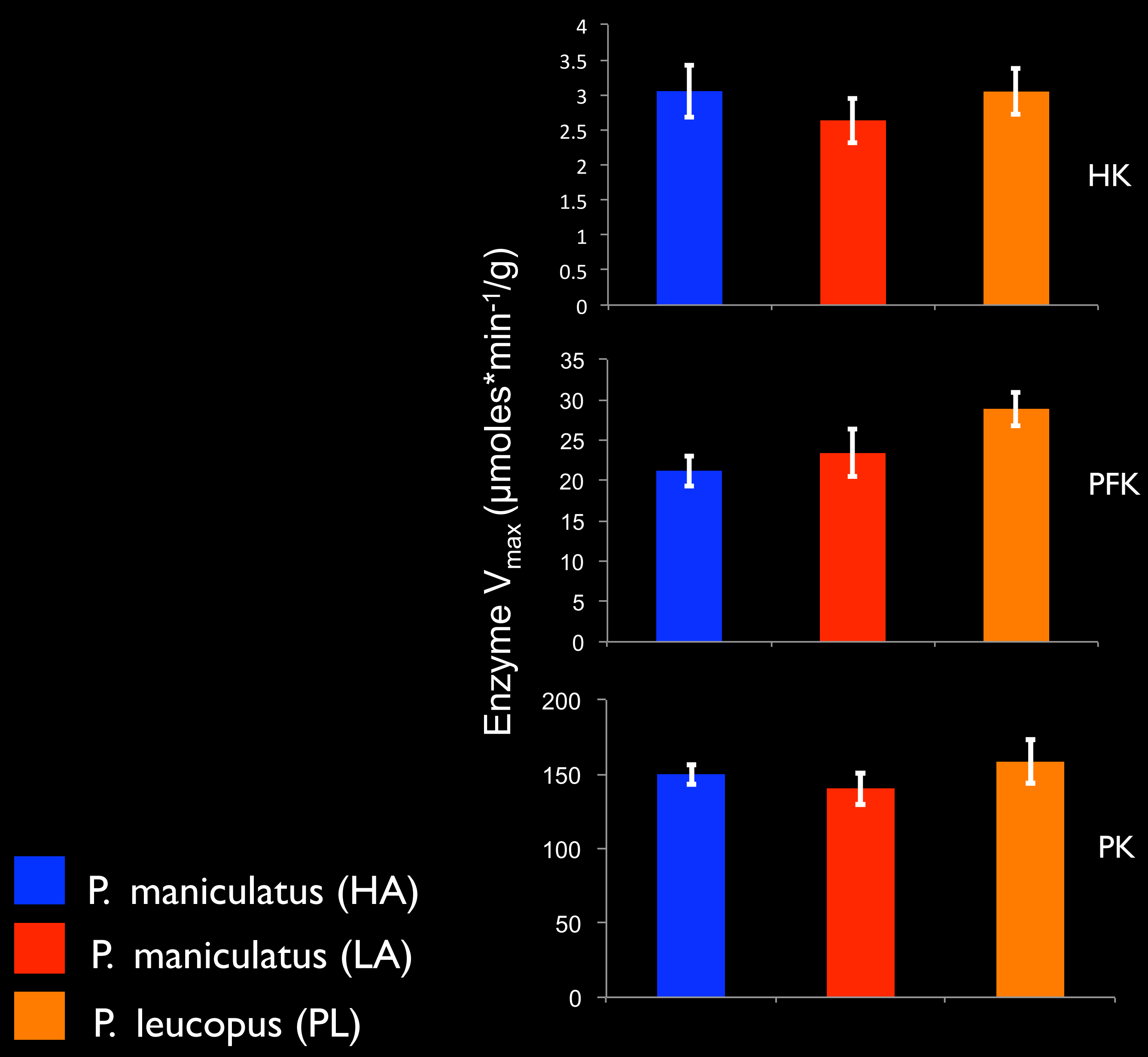
Predictions:

I. Enhanced activity (V_{max}) for fatty acid oxidation enzymes in high altitude deer mice.
II. No difference in the activities of glycolytic enzymes


FFA
Triglycerides
Glycogen
Plasma Glucose
HOAD
Lipid metabolism
(β–oxidation)
Carbohydrate metabolism
(Glycolysis)
HK
PFK
PK
Acetyl-CoA
Acetyl-CoA
TCA cycle
NADH
Succinate
Oxygen
Aerobic metabolism
(OXPHOS)
ATP
COX

Whole-organism patterns are mirrored by changes in enzyme activity.

Glycolytic enzymes

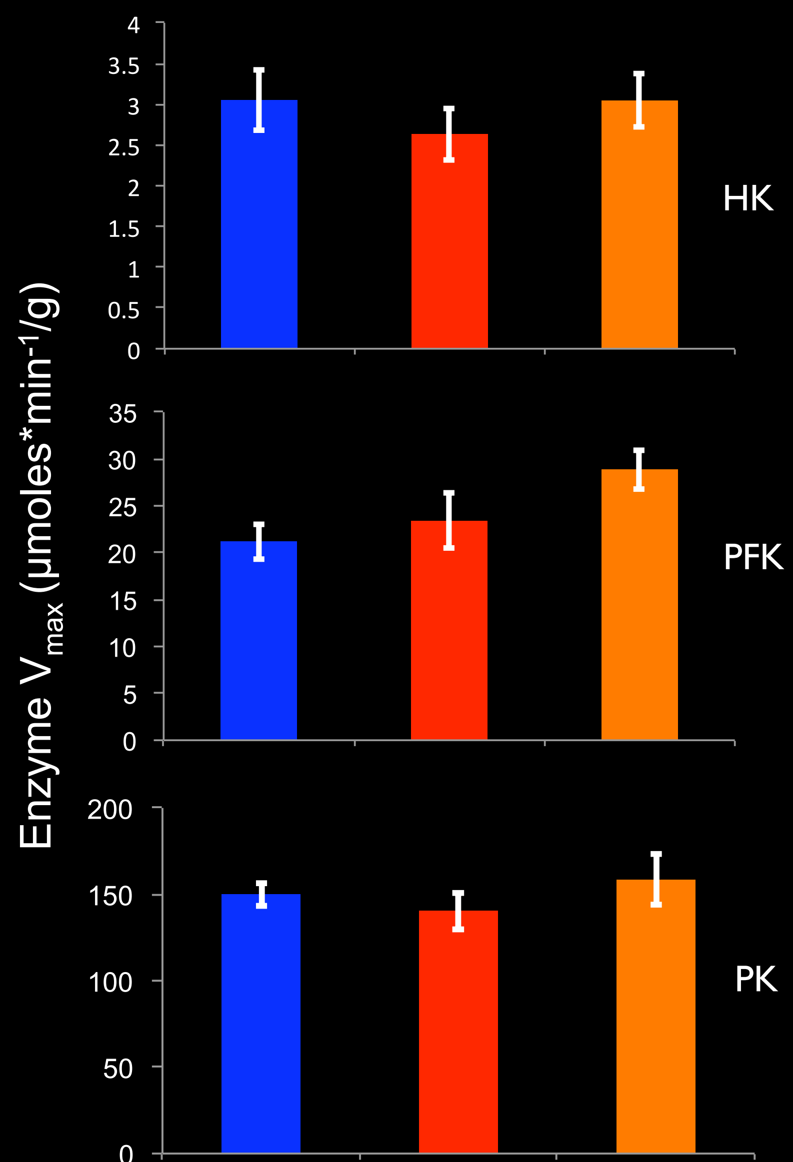


n = 10/population
all mice acclimated to low elevation for 6 weeks

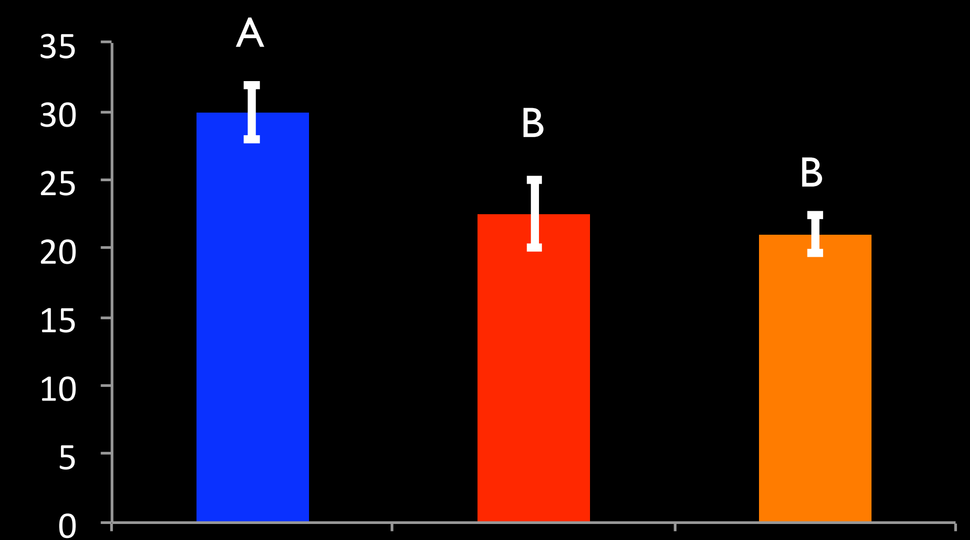
Cheviron et al. PNAS 2012

Whole-organism patterns are mirrored by changes in enzyme activity.

Glycolytic enzymes



Lipid oxidation (HOAD)



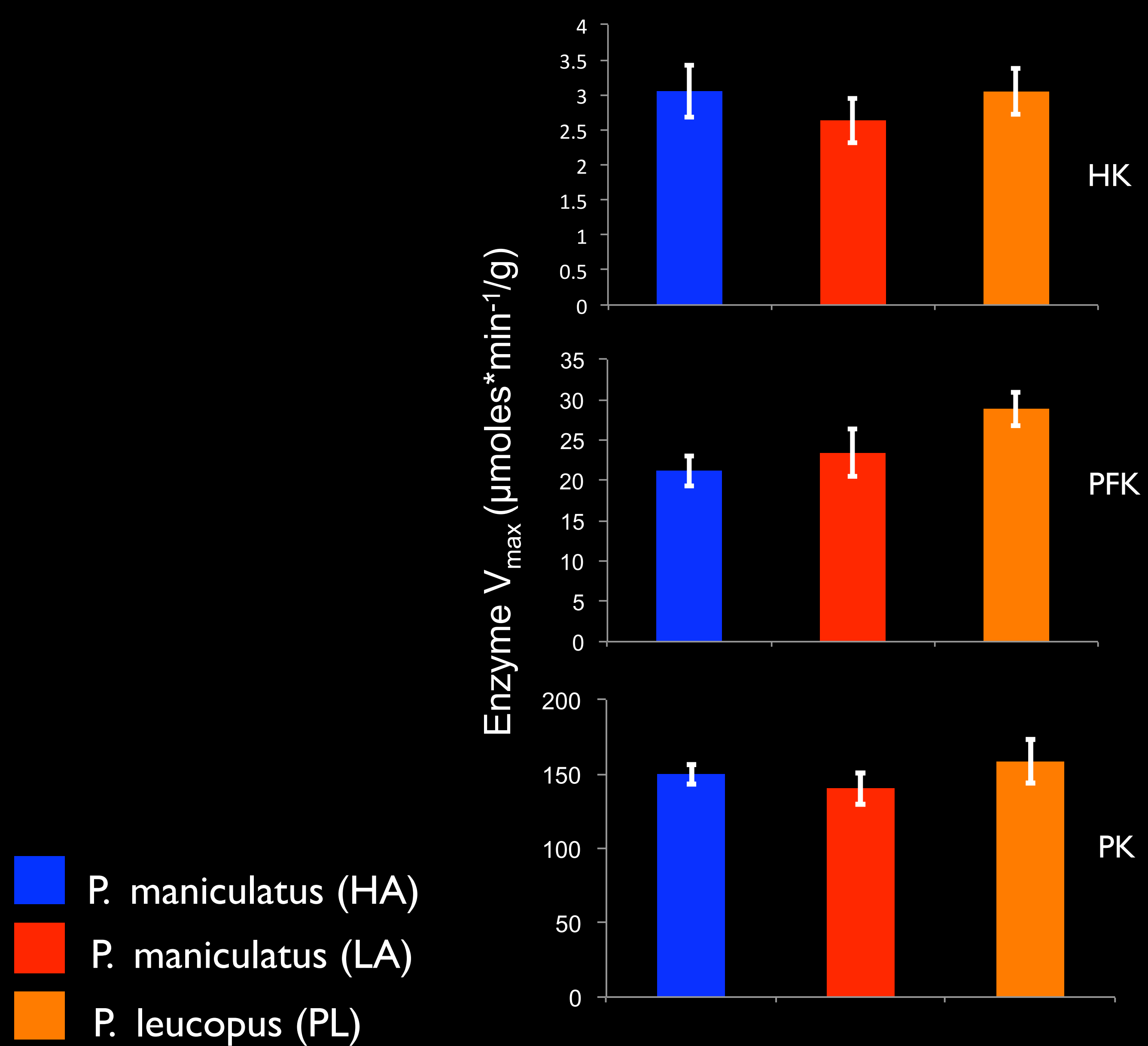
P. maniculatus (HA)
P. maniculatus (LA)
P. leucopus (PL)

n = 10/population
all mice acclimated to low elevation for 6 weeks

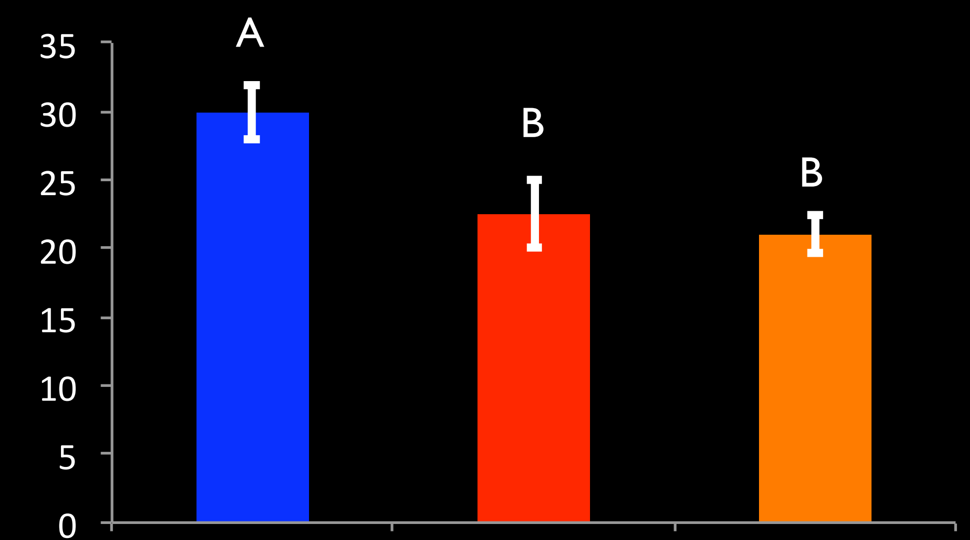
Cheviron et al. PNAS 2012

Whole-organism patterns are mirrored by changes in enzyme activity.

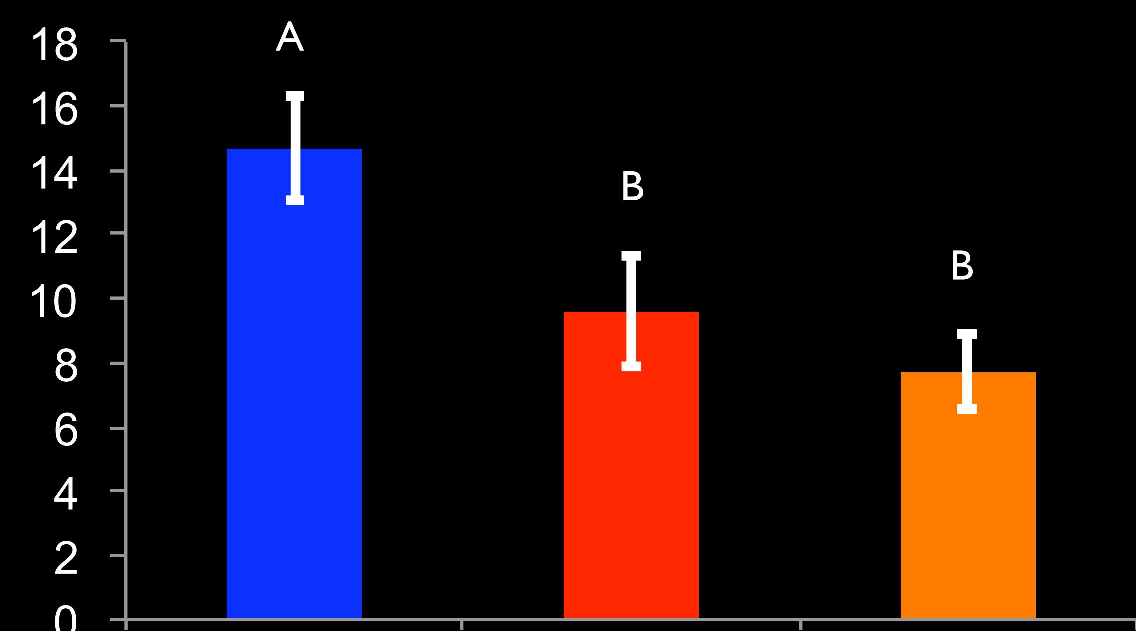
Glycolytic enzymes



Lipid oxidation (HOAD)



Aerobic capacity (COX)

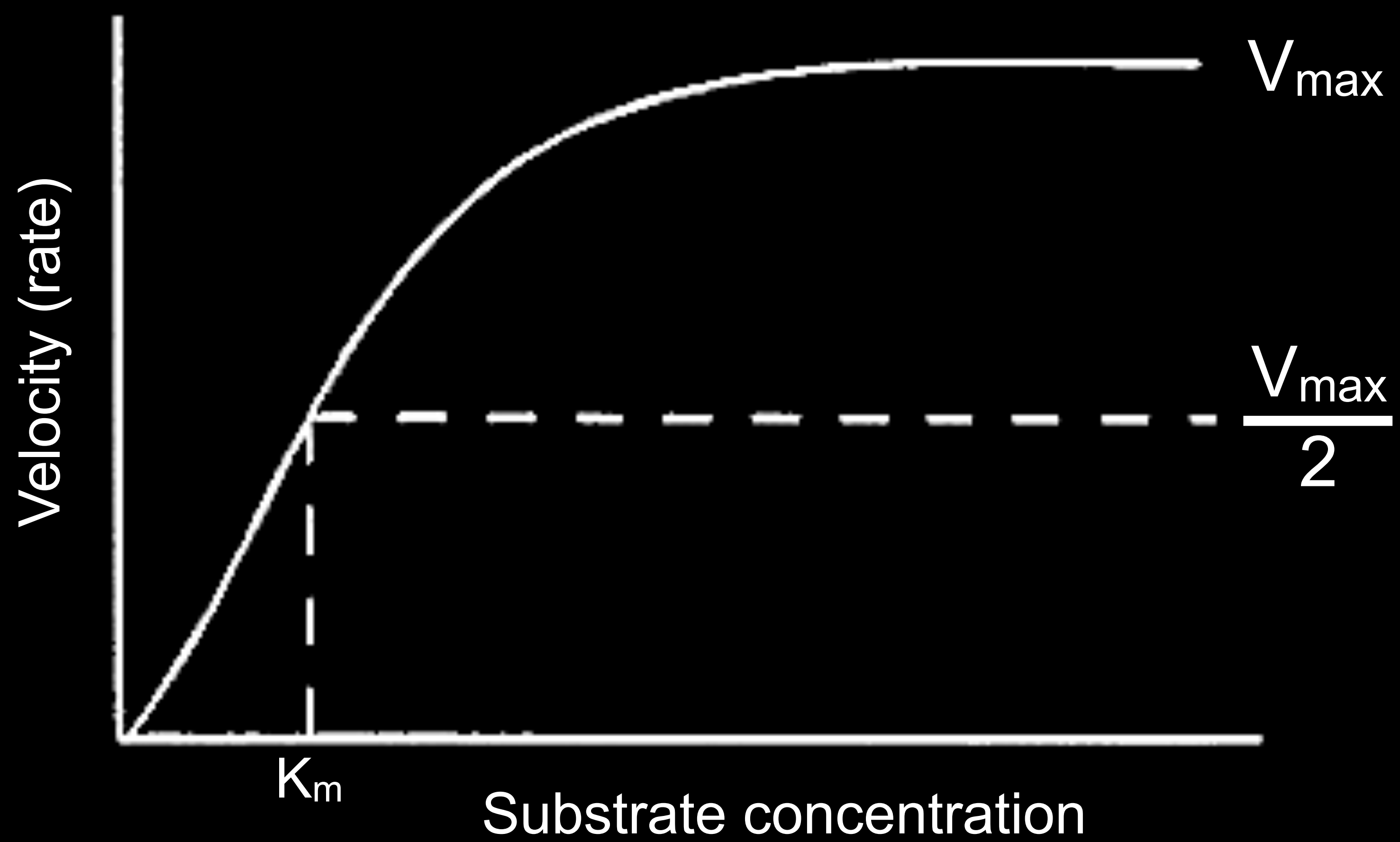


n = 10/population
all mice acclimated to low elevation for 6 weeks

Cheviron et al. PNAS 2012

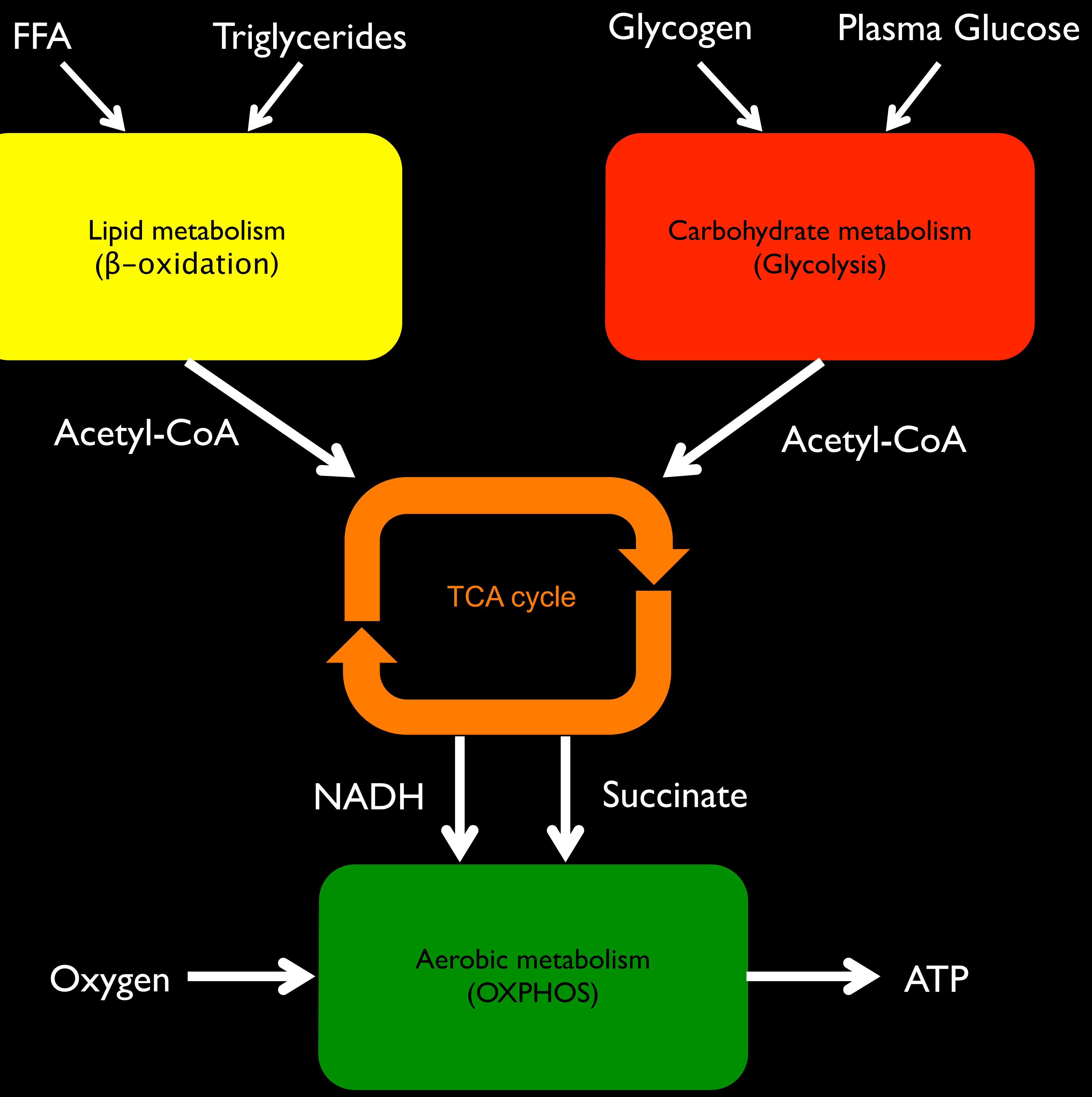
Connecting regulatory variation to enzyme activities

$$\text{Enzyme } V_{max} = K_{cat} * [E]$$



Prediction:

Concerted changes in gene expression across fatty acid oxidation and OXPHOS pathways


FFA
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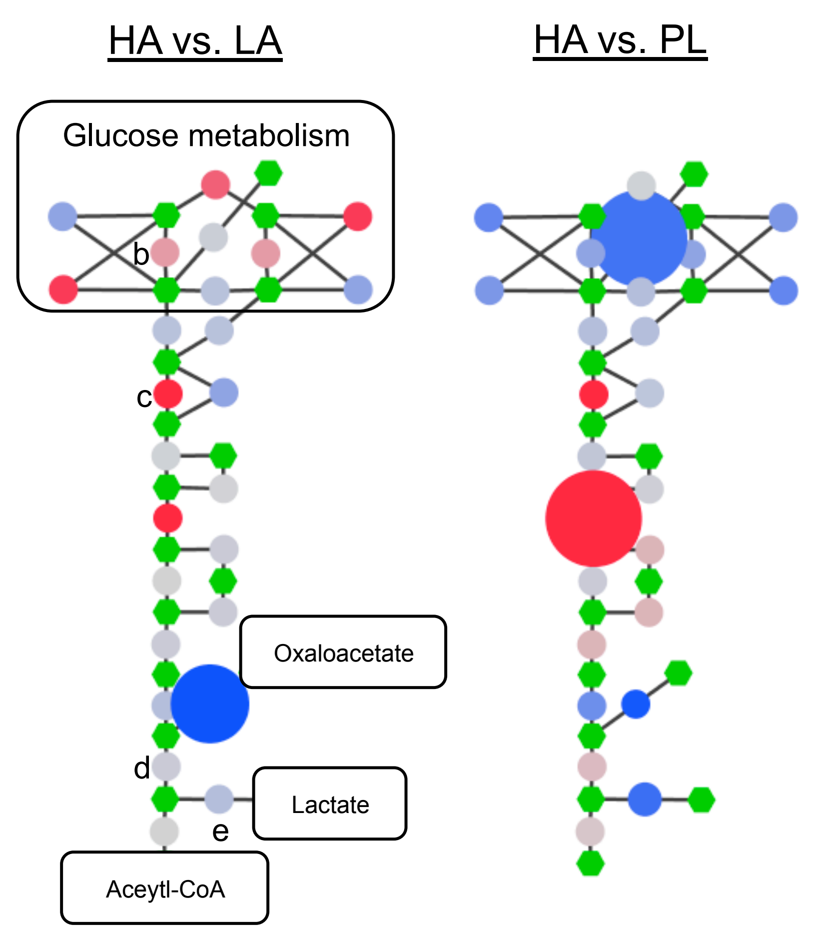
Carbohydrate metabolism
(Glycolysis)
Acetyl-CoA
Acetyl-CoA
TCA cycle
NADH
Succinate
Oxygen
Aerobic metabolism
(OXPHOS)
ATP

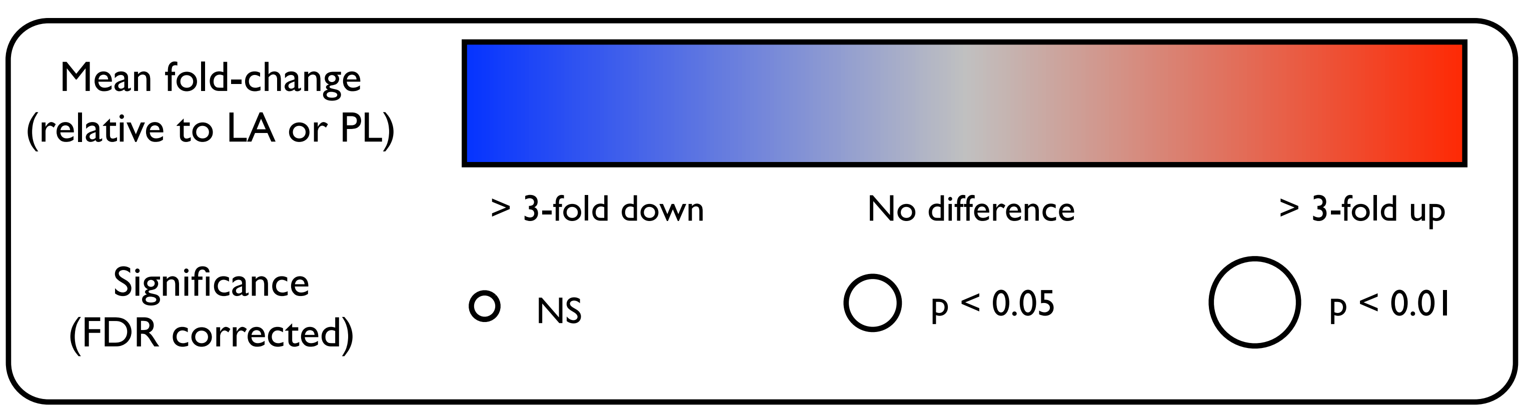
No difference in glycolytic gene expression

Glycolysis (carbs)

B.) Glycolysis



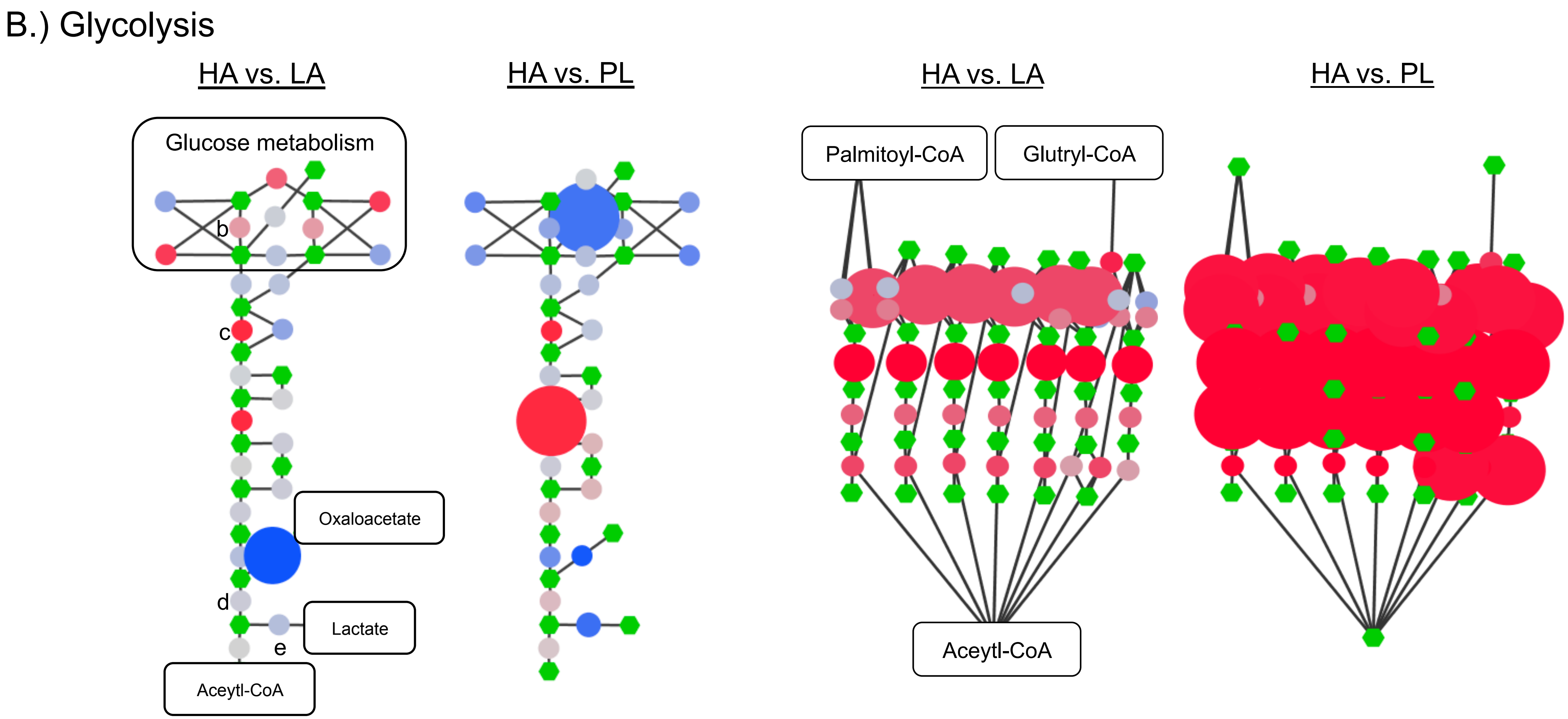
Two-sample binomial test	p = 0.98	p = 0.31



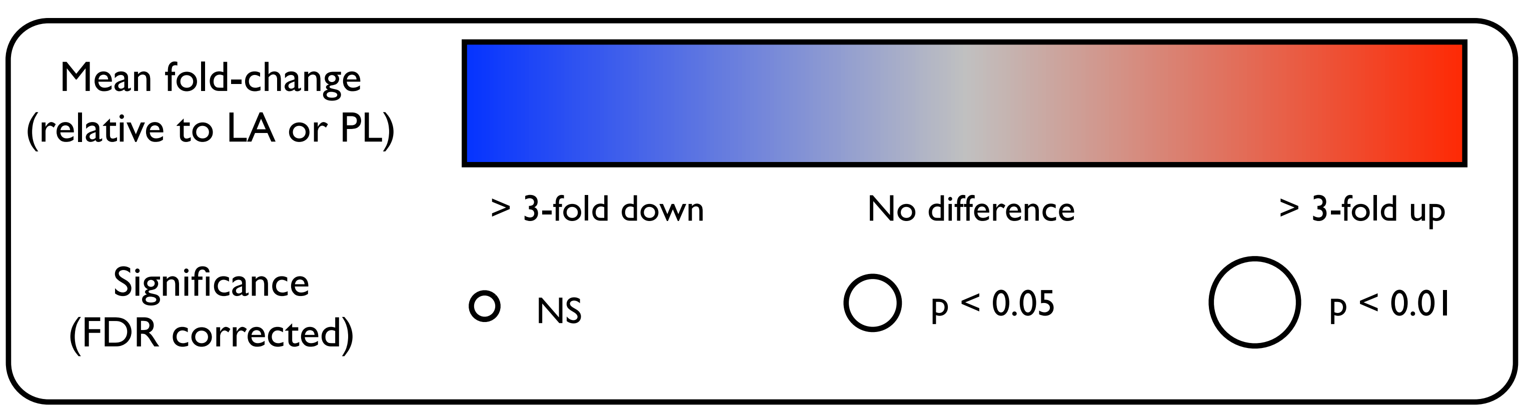
Cheviron et al. PNAS 2012

Upregulation of lipid oxidation gene expression in highland mice

Glycolysis (carbs) | Lipid oxidation (fats)

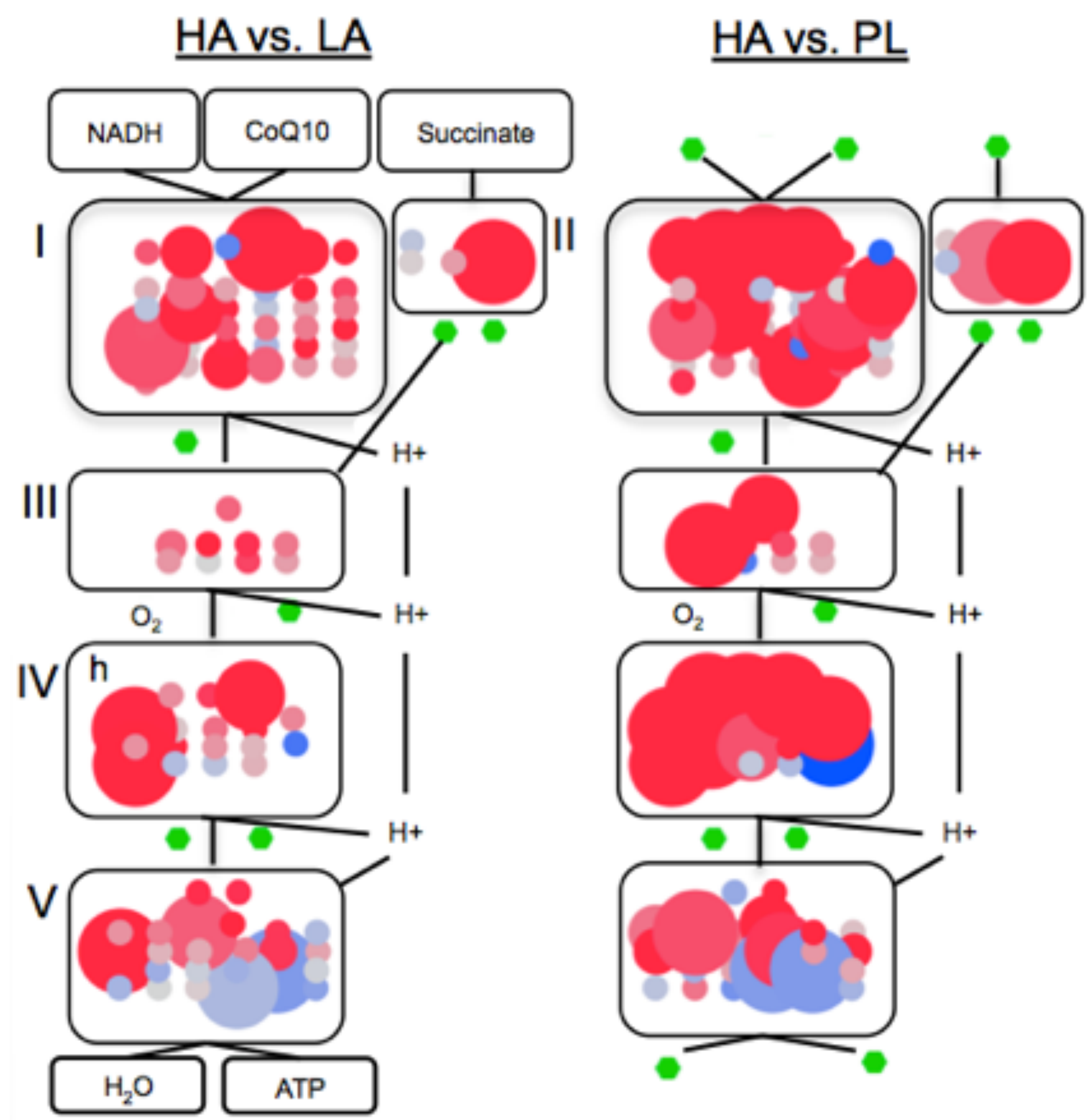


Two-sample binomial test	$p = 0.98$	$p = 0.31$	$p = 0.022$	$p = 0.002$



Cheviron et al. PNAS 2012

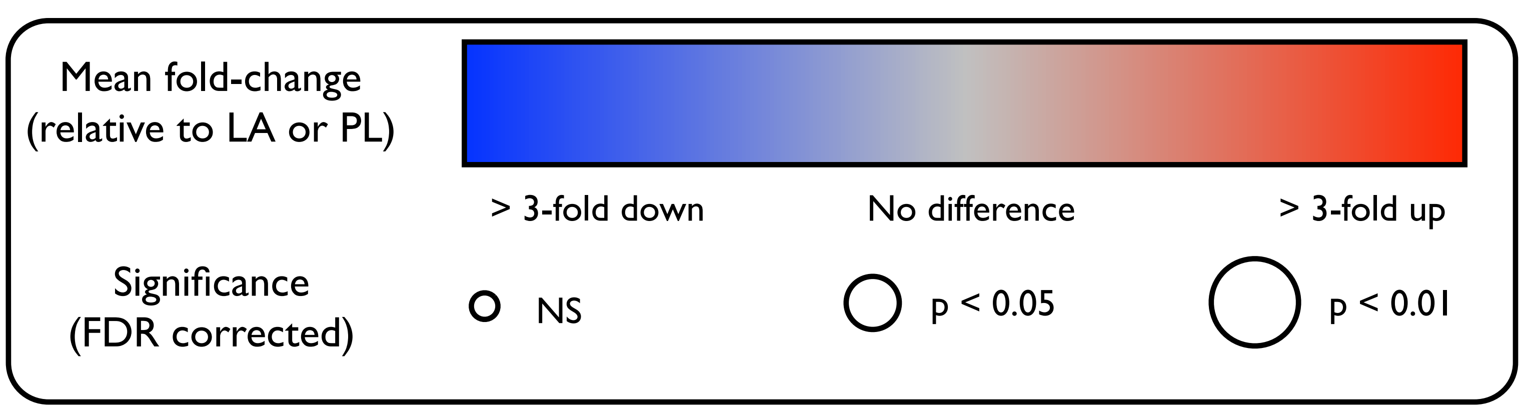
Upregulation across the OXPHOS pathway in high altitude mice



Two-sample binomial test

$p < 0.001$

$p < 0.001$



Variation in fitness-related physiological performance

Whole-organism lipid oxidation rates

Biochemical measures of cellular capacities for lipid oxidation

Differential regulation of relevant pathways

Cheviron et al. PNAS 2012

Breathing

- Elevated hypoxic ventilatory response
- Elevated tidal volume

↓

Pulmonary O_2 Diffusion

- Reduced pulmonary hypertension under hypoxia
- Increased arterial blood O_2 saturation

↓

Circulatory O_2 Delivery

- Increased left ventricle size
- Genetic increases in Hb O_2 affinity
- Blunted erythropoietic response

↓

Tissue O_2 Diffusion

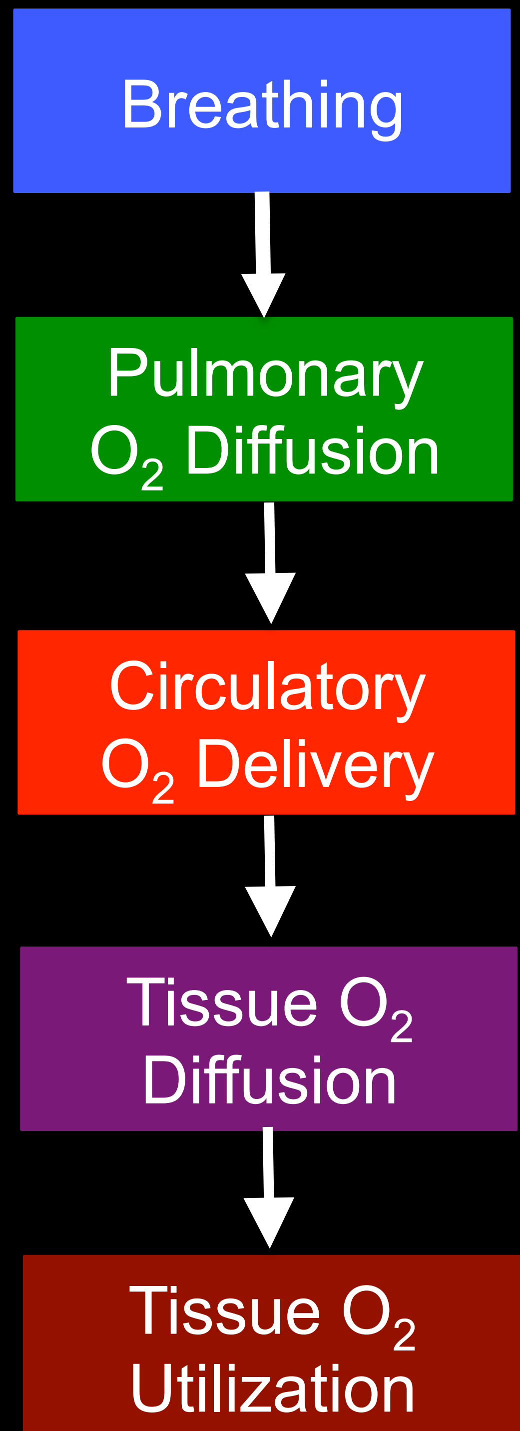
- Increased in capillary destiny
- Canalized differences *Notch 4* expression
- Allelic differentiation at angiogenesis genes
- Increases in oxidative fiber density
- Elevated *Ppargc1a* expression.

↓

Tissue O_2 Utilization

- Elevated lipid oxidation rates under hypoxia.

Cheviron et al. 2012. PNAS; Cheviron et al. 2013 JEB; Cheviron et al. 2014. Evolution; Lui et al. 2015 AJP; Scott et al. 2015 MBE; Velotta et al, 2016 Mol. Ecol.



- Elevated hypoxic ventilatory response
- Elevated tidal volume

- Reduced pulmonary hypertension under hypoxia
- Increased arterial blood O_2 saturation

- Increased left ventricle size
- Genetic increases in Hb O_2 affinity
- Blunted erythropoietic response

- Increased in capillary destiny
- Canalized differences *Notch 4* expression
- Allelic differentiation at angiogenesis genes
- Increases in oxidative fiber density
- Elevated *Ppargc1a* expression.

- Elevated lipid oxidation rates under hypoxia.

Highland and lowland deer mice diverged ~116,000 ybp
(Storz et al. 2012 Genetics)

Breathing

- Elevated hypoxic ventilatory response
- Elevated tidal volume

↓

Pulmonary O_2 Diffusion

- Reduced pulmonary hypertension under hypoxia
- Increased arterial blood O_2 saturation

↓

Circulatory O_2 Delivery

- Increased left ventricle size
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Tissue O_2 Diffusion

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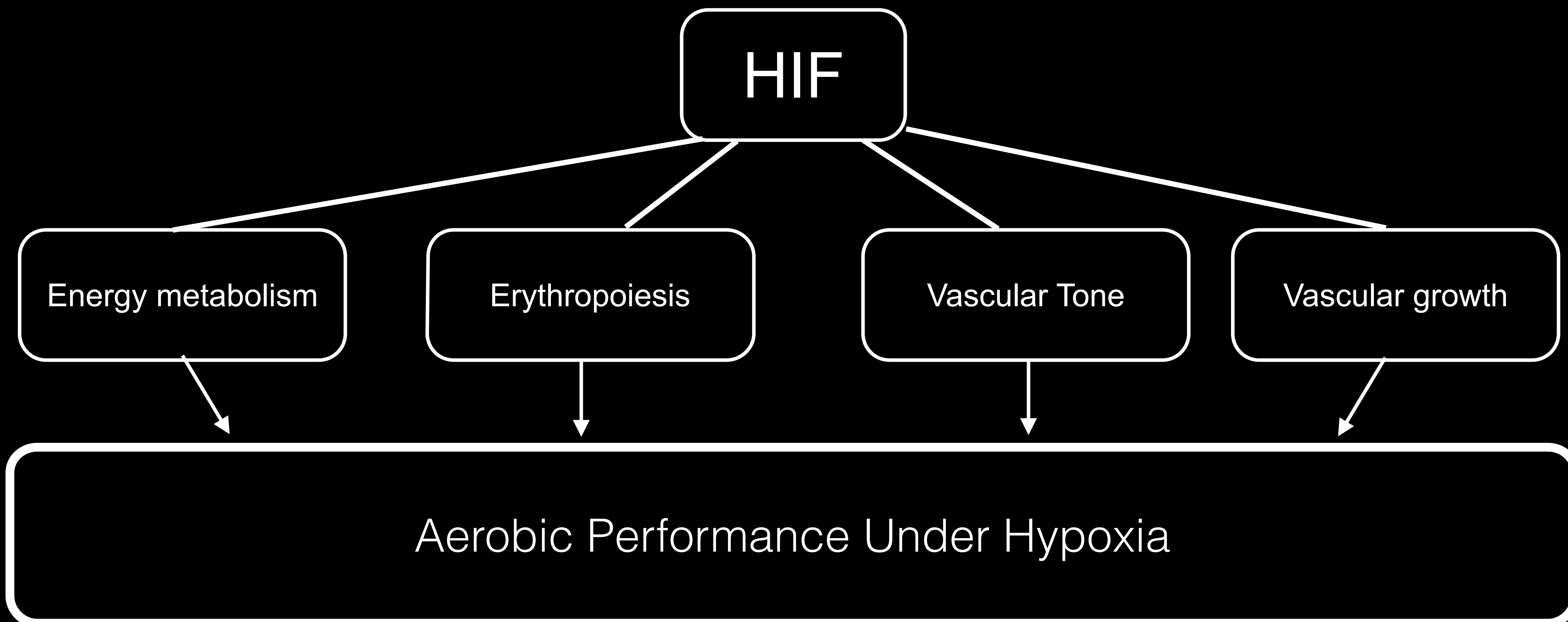
↓

Tissue O_2 Utilization

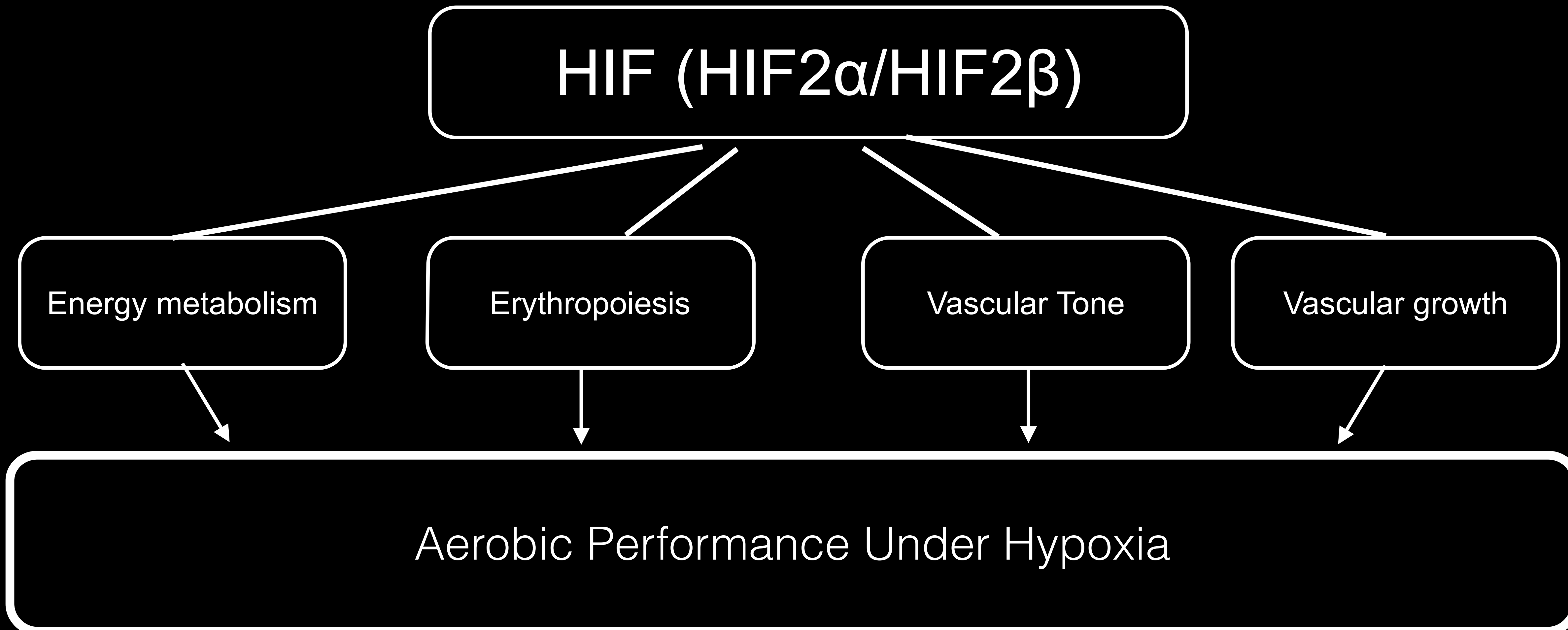
- Elevated lipid oxidation rates under hypoxia.

How are these changes coordinated?

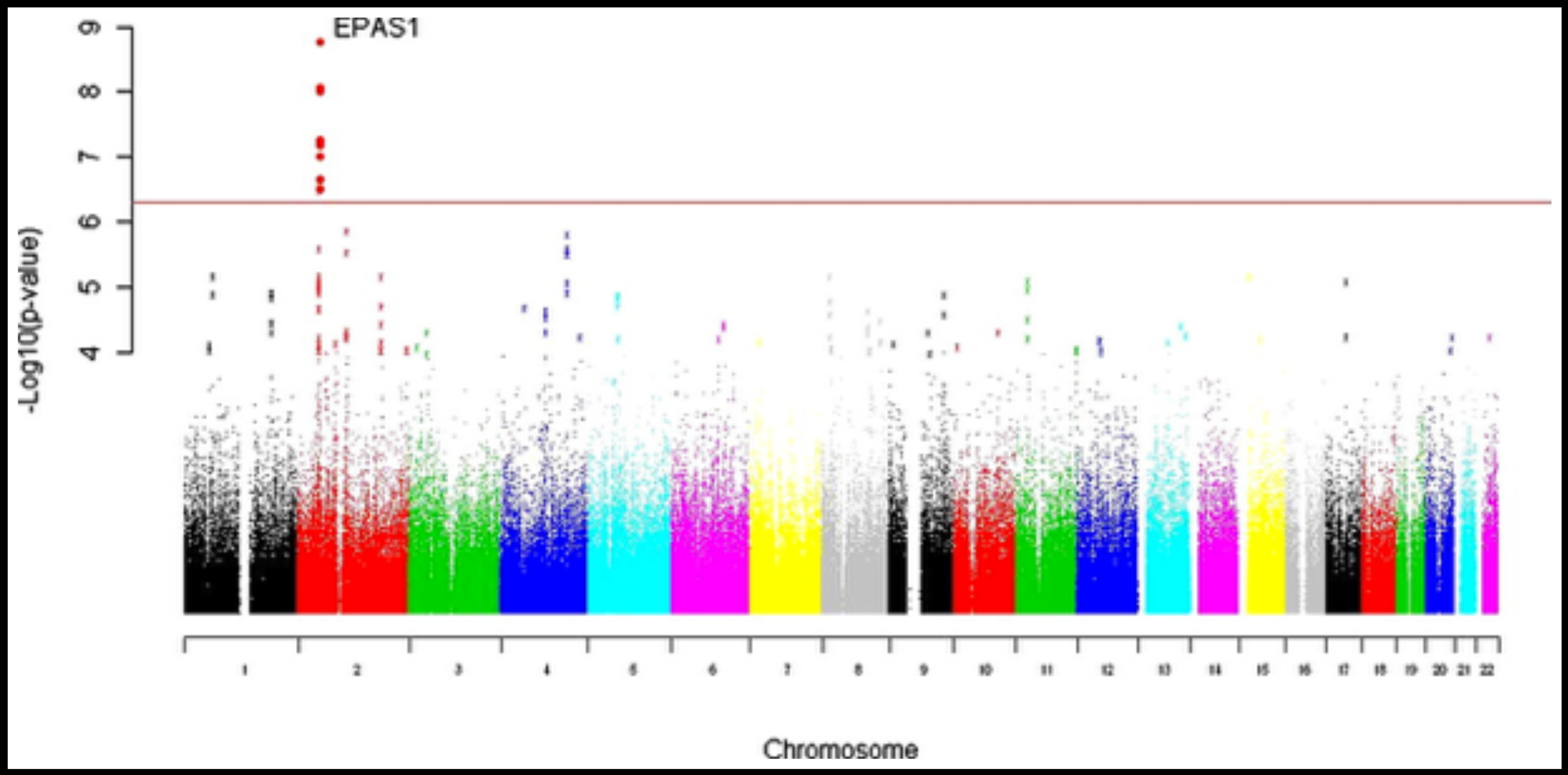
Hypoxia Inducible Factor (HIF) is a master regulator of O2 homeostasis



Hypoxia Inducible Factor (HIF) is a master regulator of O2 homeostasis

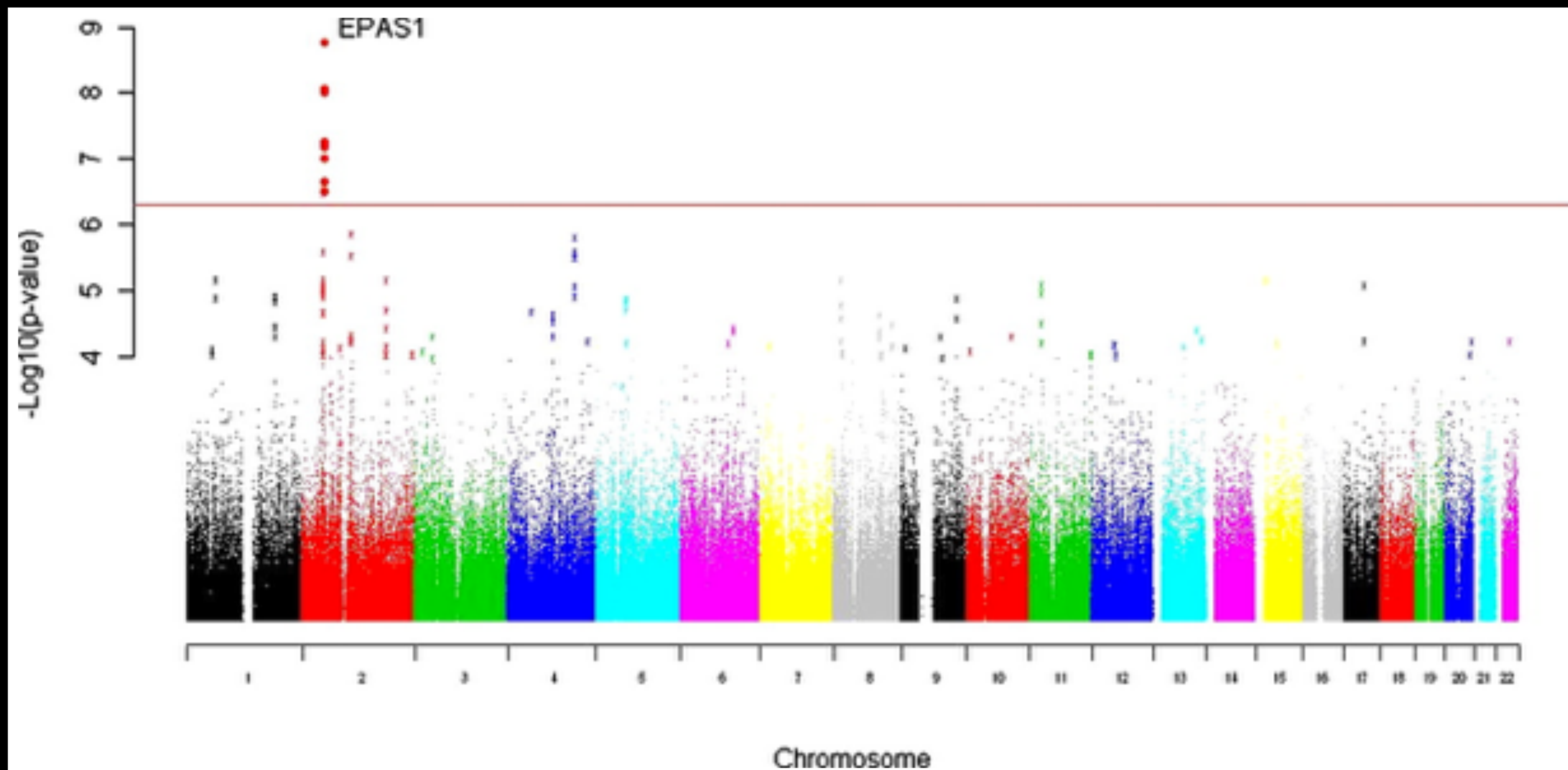


HIFα (=*EPAS1*) is a frequent target of natural selection at high elevation



Beall et al. 2010 PNAS; Simonson et al. 2010 Science; Yi et al. 2010 Science

HIFα (=*EPAS1*) is a frequent target of natural selection at high elevation



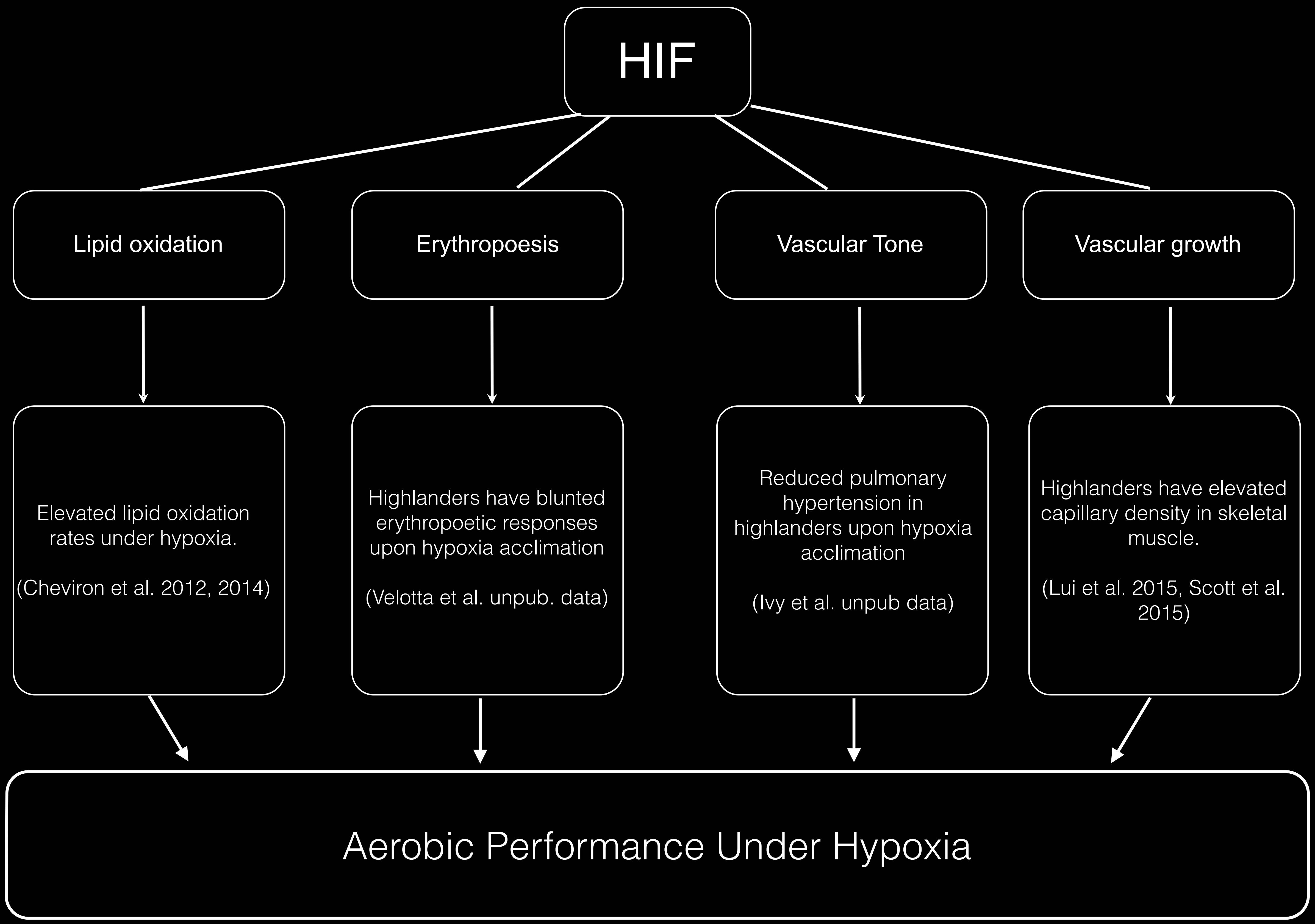
Beall et al. 2010 PNAS; Simonson et al. 2010 Science; Yi et al. 2010 Science

Tibetan Mastiff
Le et al. 2014 MBE

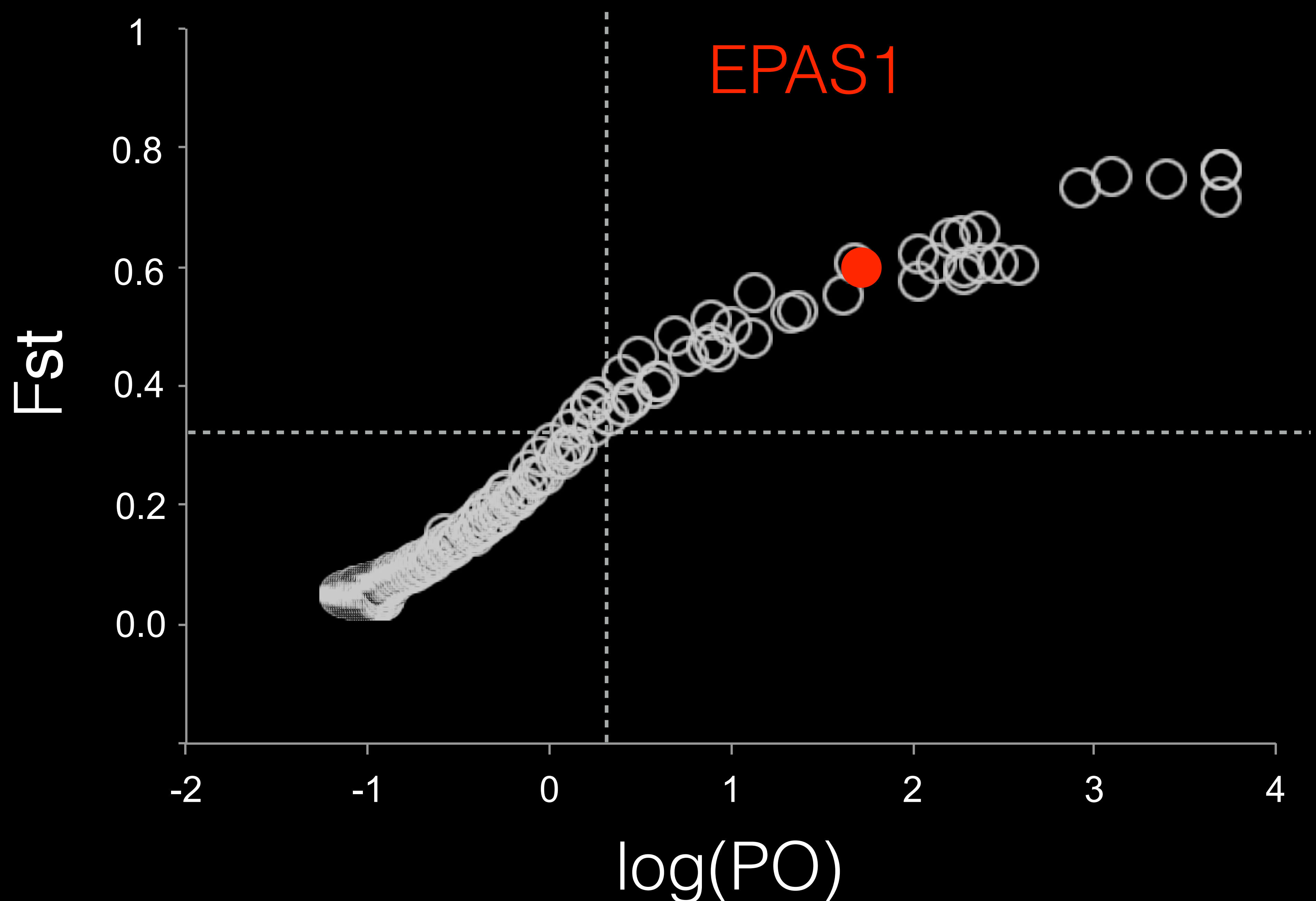
Tibetan Gray Wolf
Zhang et al. 2014 PLoS Genetics

Black Angus
Newman et al. 2015 Nature Comm.

Highland and lowland mice differ in HIF mediated traits

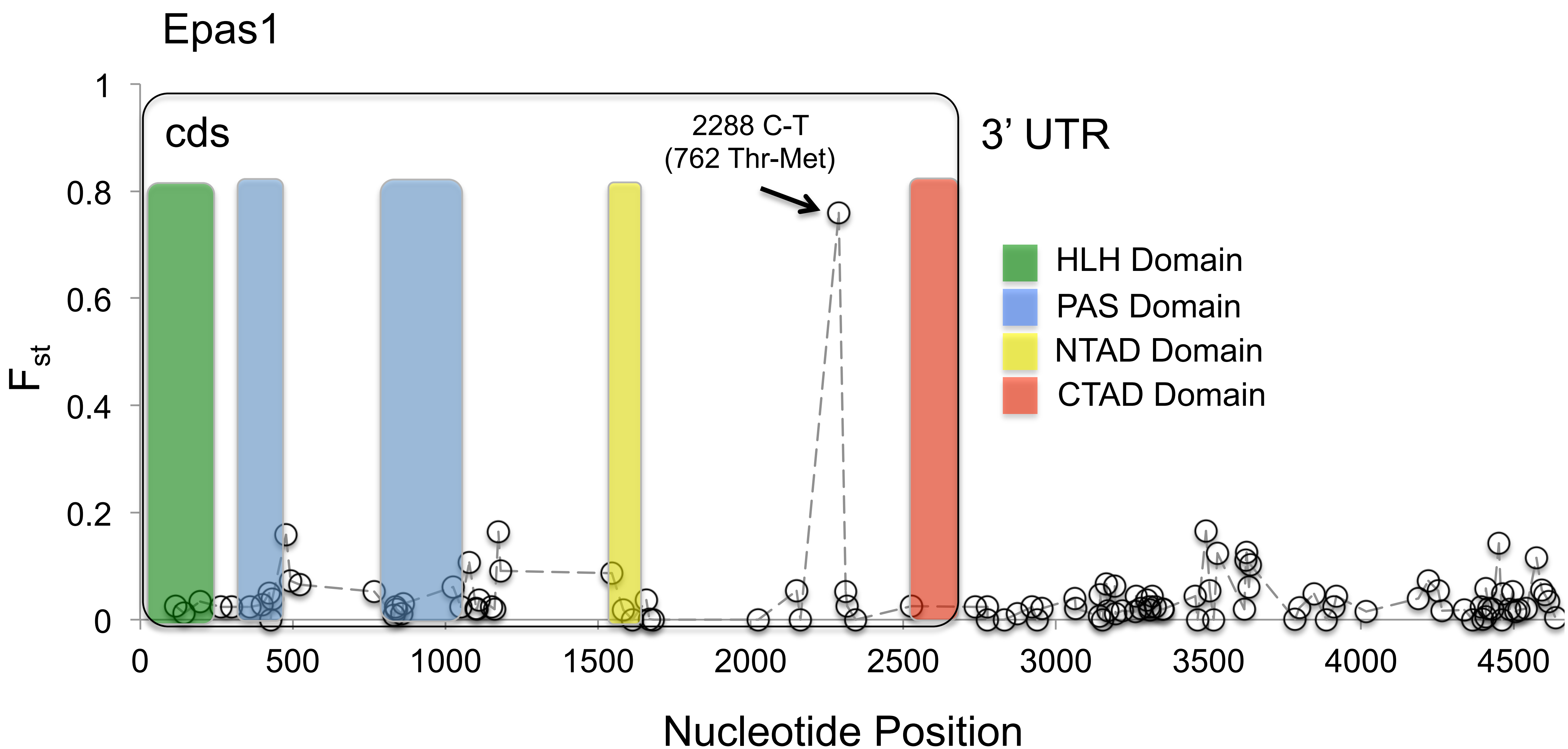


Evidence for natural selection on EPAS1



70,016 SNPs genotyped in 60 individuals (30/population)
RNAseq data from Cheviron et al. 2012, 2014)

Only a single, derived non-synonymous SNP in *EPAS1* is an outlier



The $762^{Thr}/762^{Met}$ substitution occurs at a nucleotide position that is invariant across 95 mammalian species

Do EPAS1 variants differ in the regulation of HIF target genes?

Experimental design

Environment	EPAS1 genotype	
	Thr^{762}/Thr^{762}	Met^{762}/Met^{762}
Hypoxia	450 mmHg (4350 m)	450 mmHg (4350 m)
Normoxia	720 mmHg (430 m)	720 mmHg (430 M)

- n = 5/treatment/genotype
- Acclimations lasted 6 weeks
- RNAseq of heart (left ventricle)
- Focused analysis of known *Mus* HIF targets

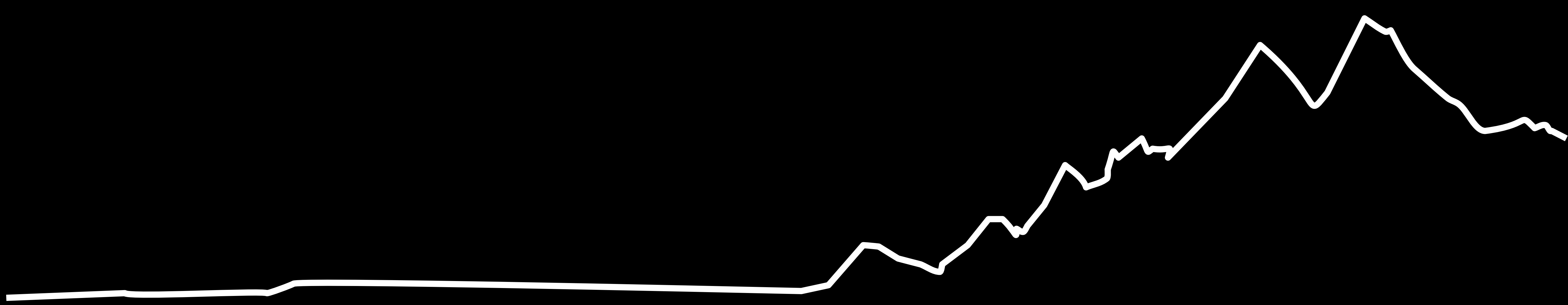
Experimental design

Environment	EPAS1 genotype: Thr^{762}/Thr^{762}	EPAS1 genotype: Met^{762}/Met^{762}
Hypoxia	450 mmHg (4350 m)	450 mmHg (4350 m)
Normoxia	720 mmHg (430 m)	720 mmHg (430 M)

Genotype only effects = persistent expression differences of HIF targets across environments

Environment only effects = Genotype does not effect of HIF target responses to hypoxia

Genotype by environment interactions = Different regulatory responses to hypoxia by HIF variants.



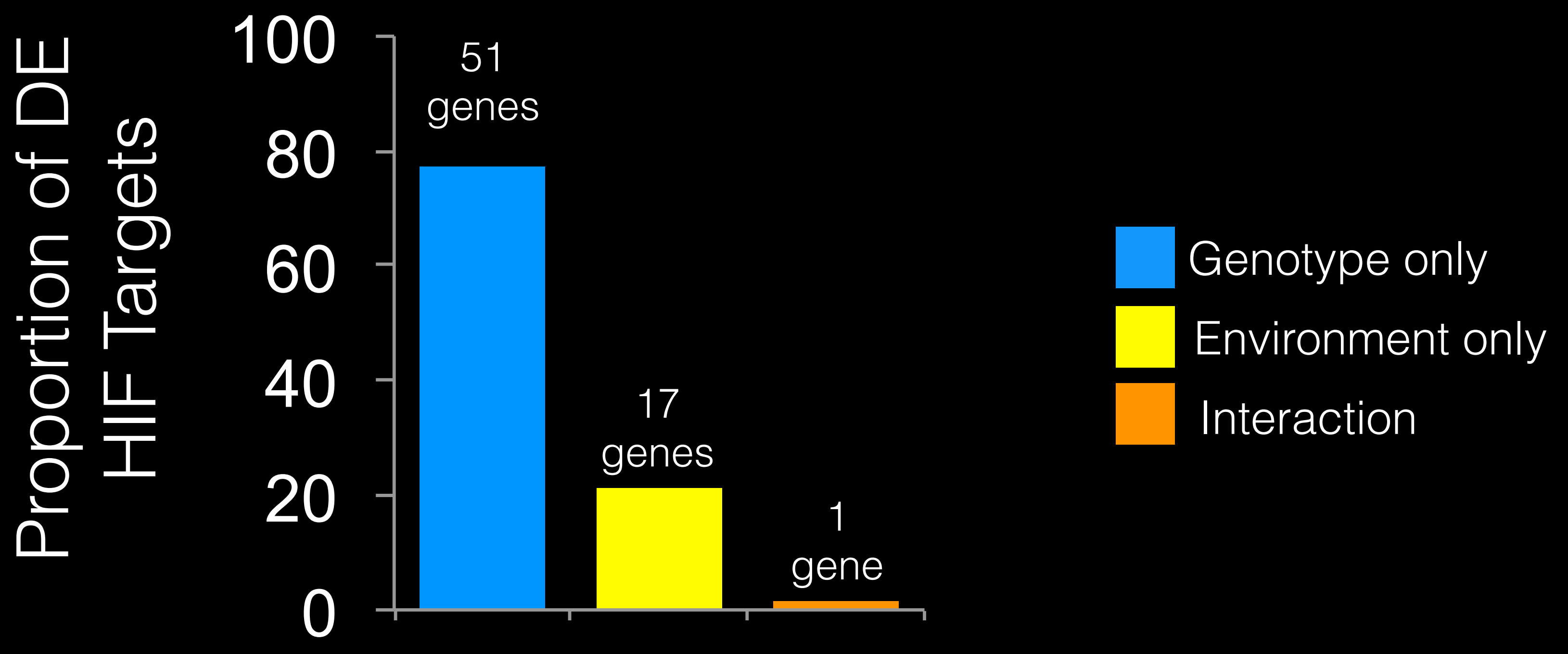
EPAS1 genotype influences HIF regulatory patterns under common conditions

Results

1.) Detected expression of 76% (175 of 231) known HIF targets.

2.) 40% of detected HIF target genes were differentially expressed.

3.) Most differentially expressed HIF targets showed genotype effects without an interaction.

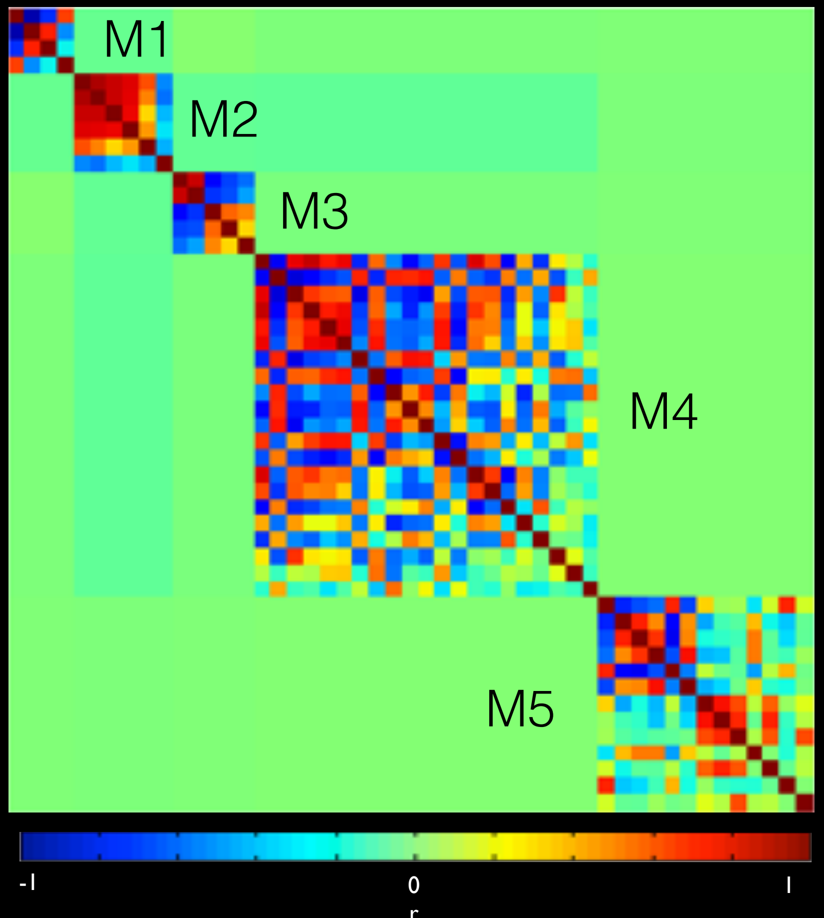


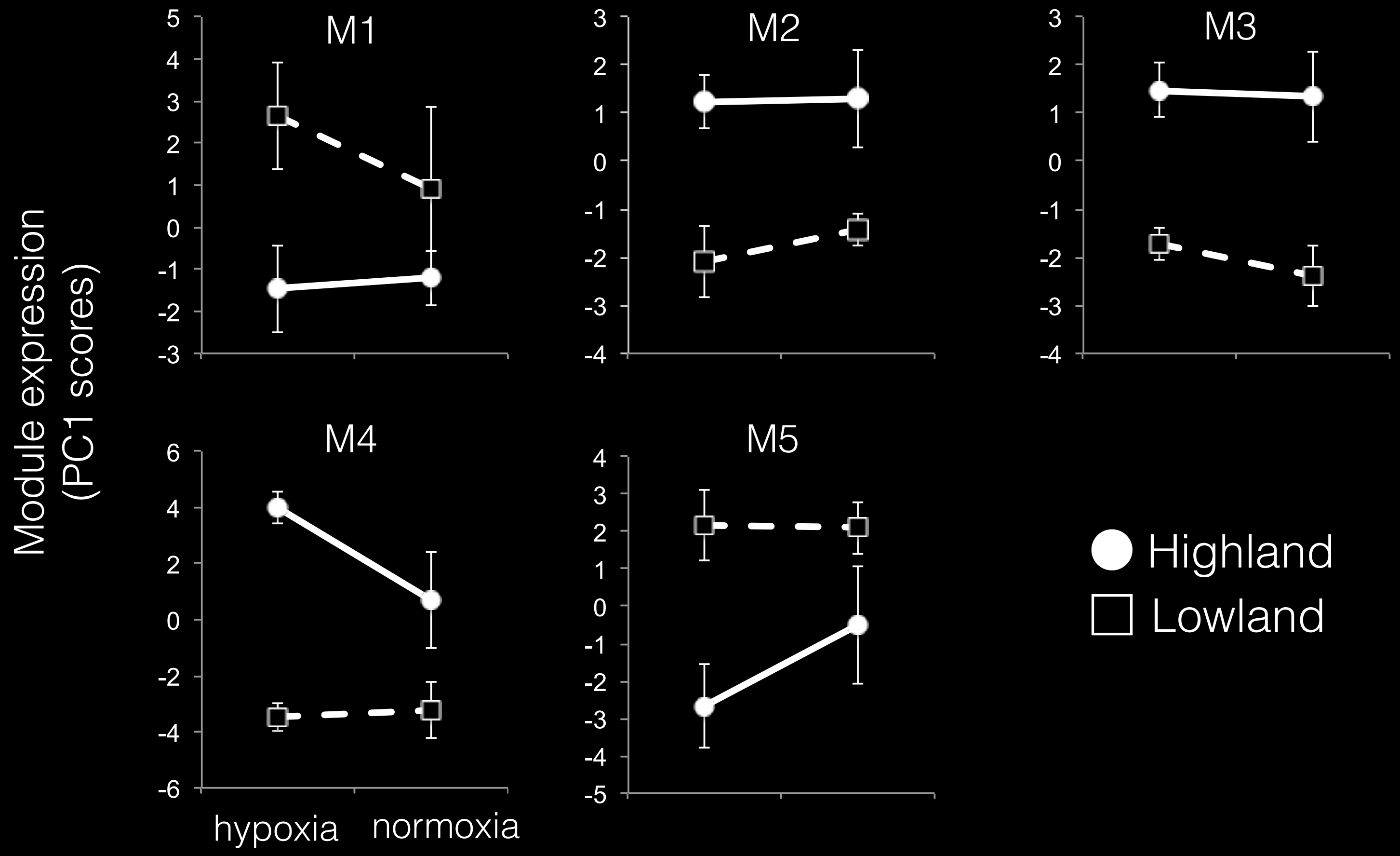
EPAS1 genotype influences HIF regulatory patterns under common conditions

Results

4. HIF target genes with genotype effects clustered into five modules of putatively co-regulated genes.

5. Expression plasticity varied among modules

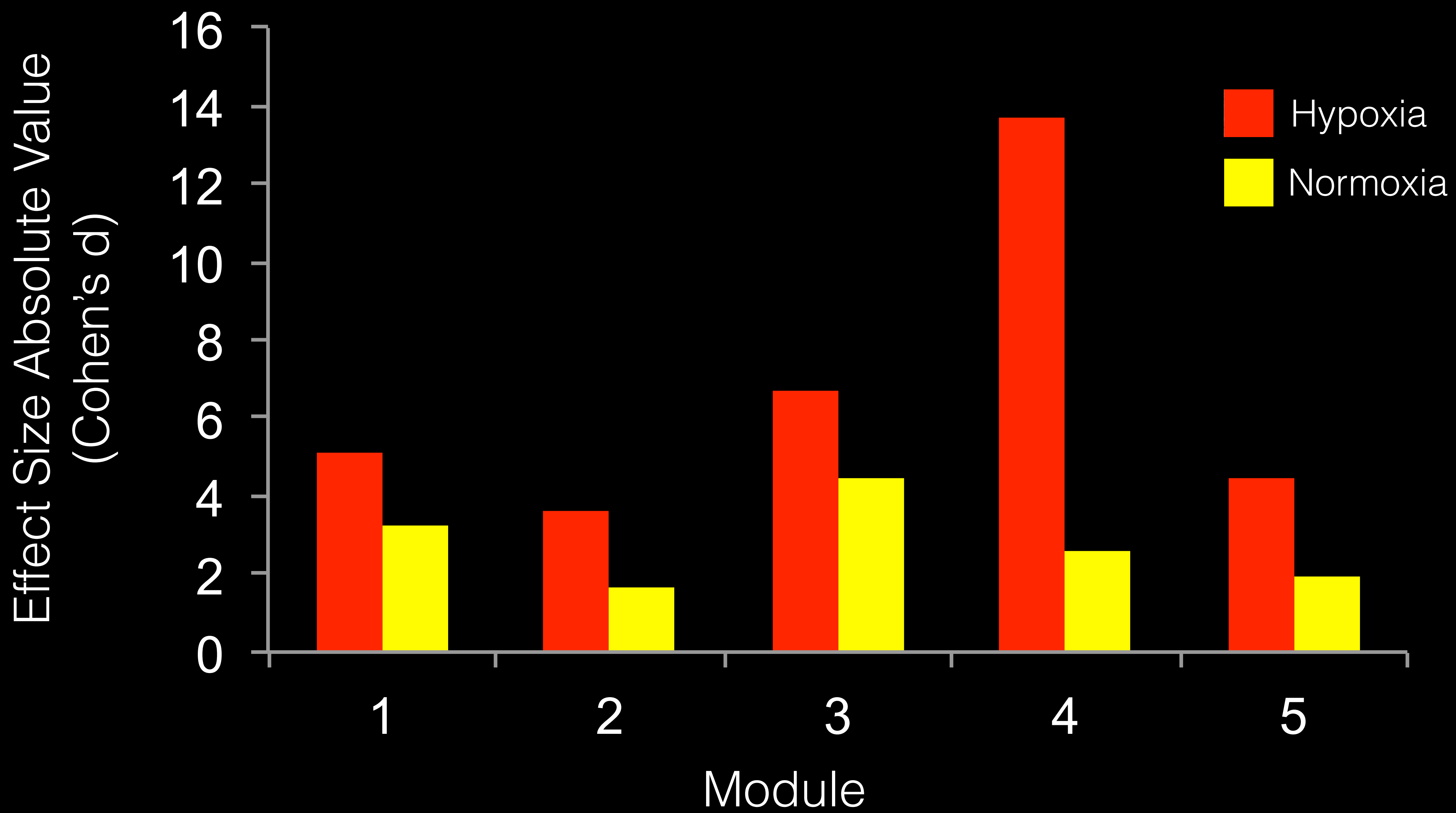




Clustering performed using MMC

Mean ± 1 SD

Genotypic differences in HIF target gene regulation are enhanced under hypoxia.



Summary

1.) Allelic variation at *EPAS1* appears to be under spatially varying selection.

2.) Highland and lowland mice differ in HIF-mediated traits.

3.) *EPAS1* genotype effects regulation of ~40% of HIF target genes, and these transcriptional differences tend to be enhanced under hypoxia.

Multifaceted modification of the O_2 transport cascade in highland mice is associated with allelic variation in a master regulator of physiological responses to hypoxia.

Future directions:

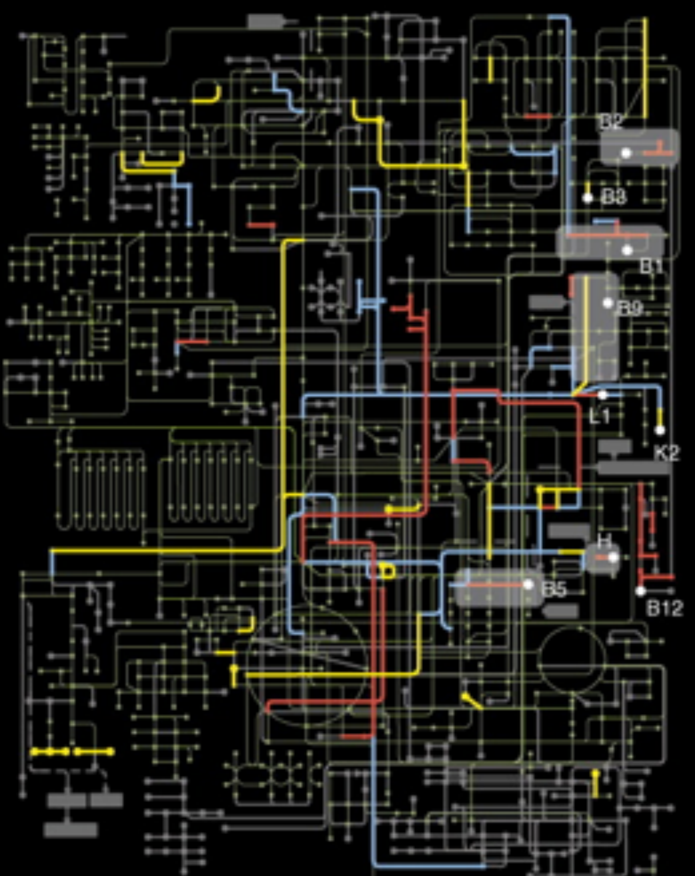
1. More acclimations
2. QTL mapping to test for co-localization with *EPAS1*

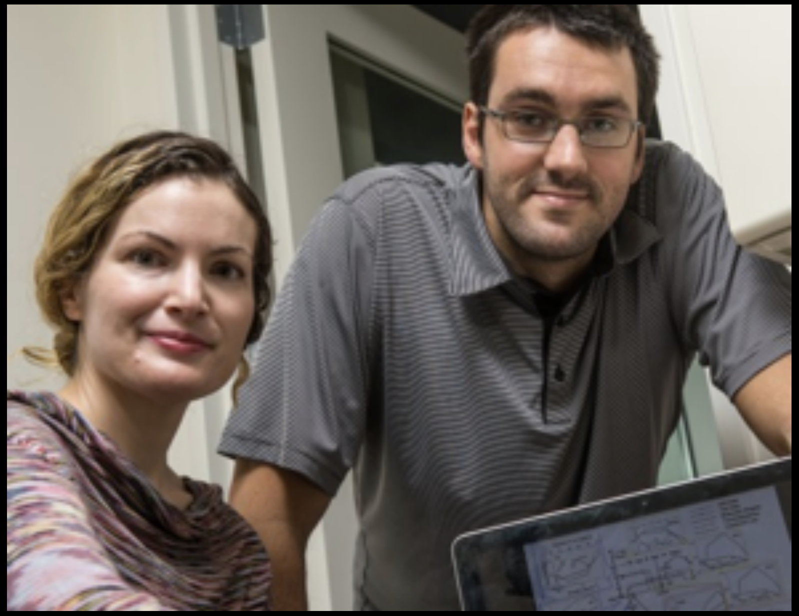
Overall conclusions

1. Understanding the evolution of integrated systems is one of the frontiers of the study of adaptive evolution and concepts from systems physiology can be useful in this effort.

2. The concept of control coefficient can be extended to other levels of organization.

Genome-wide metabolic map
(Mus musculus)





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University of Montana

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McMaster University

Jay Storz

University of Nebraska

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NSERC to GRS

NSERC CRSNG

Questions?