

Many ways to study the genomic
basis of adaptation
... Or all you can think about to
study adaptation with genomic
tools...

Claire Mérot & Anna Tigano
Physalia Courses

Analytical approaches

GWAS

Comparative genomics

Transcriptomics

Experimental evolution

QTL mapping

Epigenetics

Population genomics

Plenty of approaches

- Local adaptation / population genomics
 - Other statistics
- Comparative genomics
- Trait-focused genetics/genomics
 - GWAS
 - QTL
- Multi-omics
 - Transcriptomics
 - Epigenomics
 - Proteomics
- Functions and experiments
 - Experimental evolution / experimental selection
 - Candidate genes and pathways
 - Common garden
 - Gene-editing

Local adaptation / population genomics

Site frequency spectrum:

- Tajima's D: tests for excess of low or intermediate frequency alleles
- Distribution of fitness effects (DFE)

Signatures of selective sweeps:

- SweepFinder
- Extended haplotype homozygosity (iHS)

Inference of Distribution of Fitness Effects and Proportion of Adaptive Substitutions from Polymorphism Data

Tataru et al

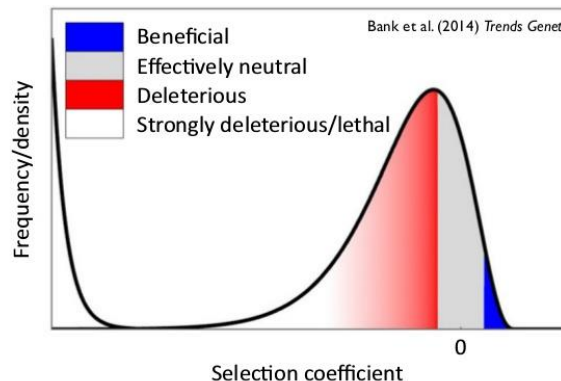
GENETICS November 1, 2017 vol. 207 no.

3 11031119;

<https://doi.org/10.1534/genetics.117.300323>

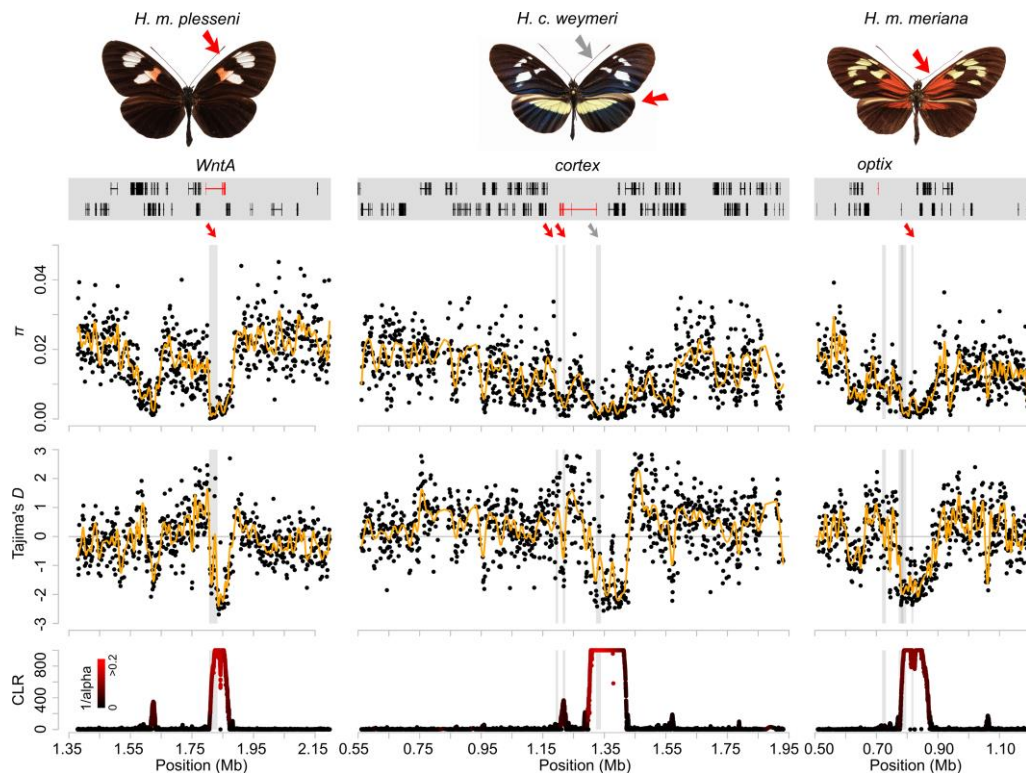
polyDFEv2.0

Distribution of fitness effects (DFE) of new mutations



Local adaptation / population genomics

Sweep finder, Tajima's D



Targeted sequence capture
towards colour loci

-> sweepFinder

-> low π (diversity),

-> Low Tajima's D

Moest M, Van Belleghem SM, James JE, Salazar C, Martin SH, Barker SL, et al. (2020) Selective sweeps on novel and introgressed variation shape mimicry loci in a butterfly adaptive radiation. PLoS Biol 18(2): e3000597. <https://doi.org/10.1371/journal.pbio.3000597>

Local adaptation / population genomics

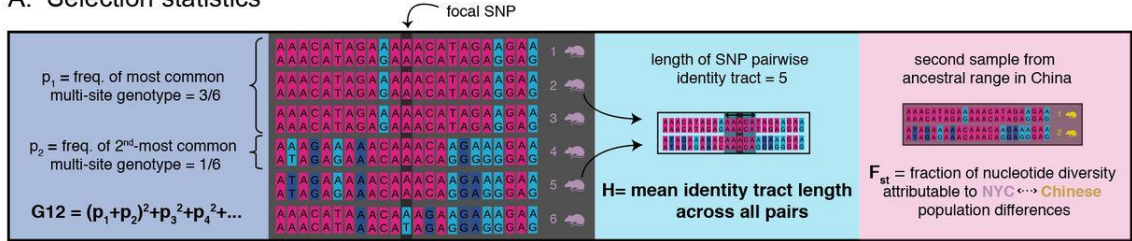
Haplotypes



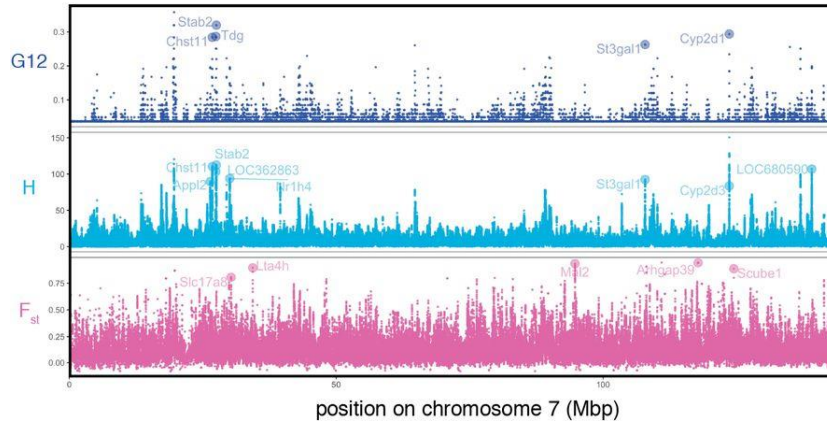
A signal of
adaptation in
New York
City rats?

Harpak, A., Garud, N., Rosenberg, N. A.,
Petrov, D. A., Combs, M., Pennings, P. S., &
Munshi-South, J. (2020). Genetic Adaptation
in New York City Rats. *GBE*
<https://doi.org/10.1093/gbe/evaa247>

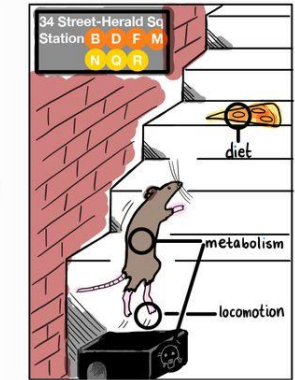
A. Selection statistics



B. Scans to identify candidate loci



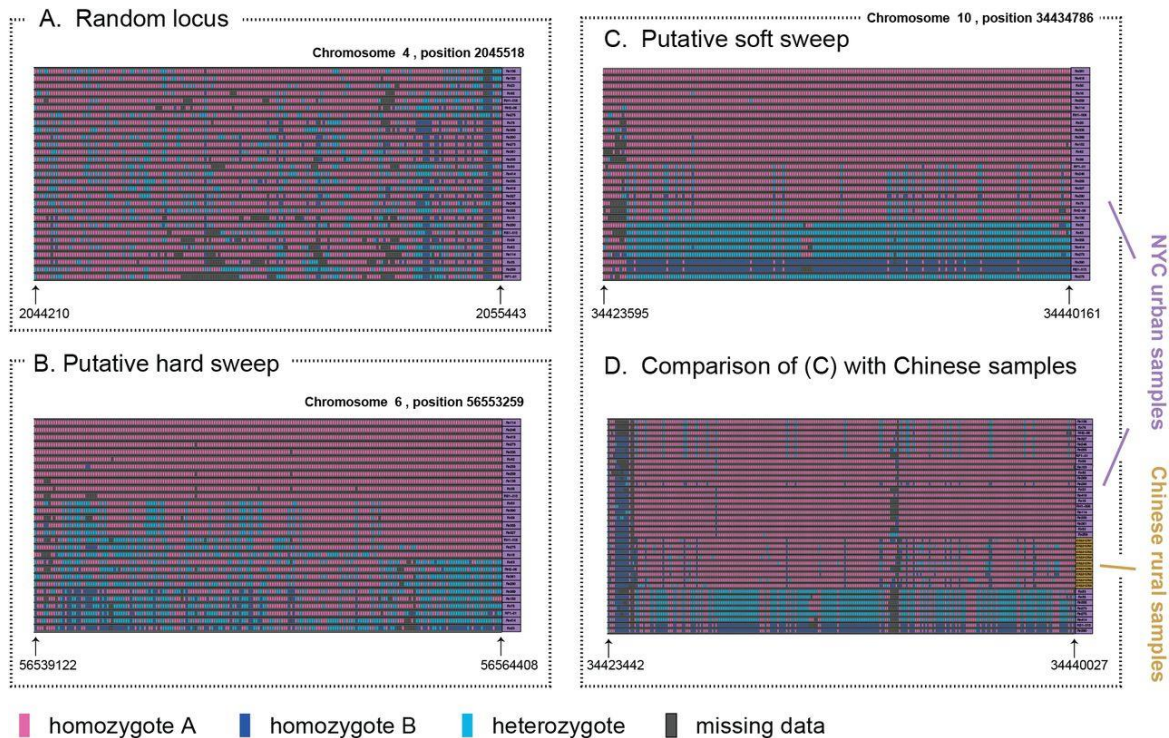
C. Hypotheses



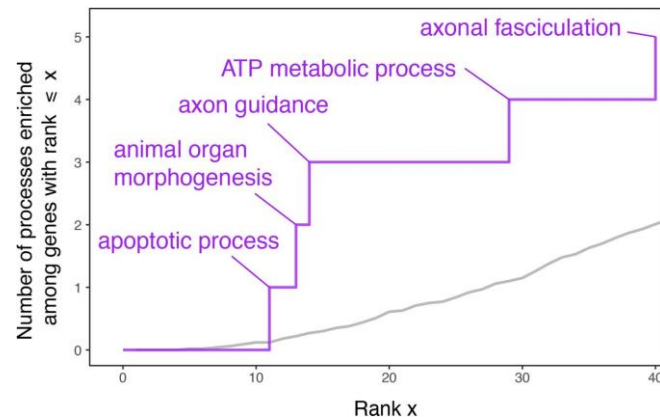
Methods to find hard sweep and soft sweeps based on haplotype + asymptotic MK test

Local adaptation / population genomics

Sweep signal in haplotypes



Biological processes in outlier loci



Harpak, A., Garud, N., Rosenberg, N. A., Petrov, D. A., Combs, M., Pennings, P. S., & Munshi-South, J. (2020). Genetic Adaptation in New York City Rats. *GBE*

<https://doi.org/10.1093/gbe/evaa247>

Population genomics/ comparative genomics

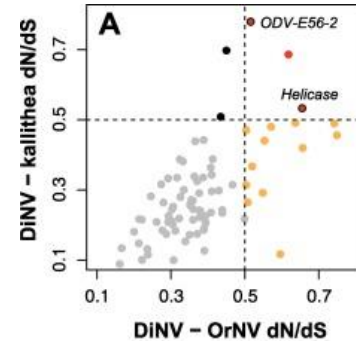
Comparison to outgroup:

- ω = dN/dS ratio of the number of non-synonymous to synonymous sites between target species and an outgroup
- McDonald Kreitman test: variation in rate of evolution across the genome and fixed vs. polymorphic sites
- Fay and Wu's H: Test of neutrality by comparing frequency of derived vs. ancestral alleles

asymptoticMK: A Web-Based Tool for the Asymptotic McDonald–Kreitman Test

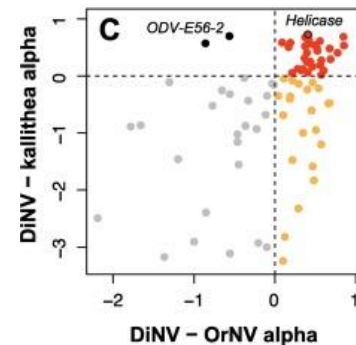
Benjamin C. Haller and Philipp W. Messer

G3: GENES, GENOMES, GENETICS May 1, 2017 vol. 7 no. 5 1569-1575; <https://doi.org/10.1534/g3.117.039693>



A virus (DiNV) compared to two outgroups (OrNV and DiNV)

⇒ Genes with high dN/dS ratio



⇒ Genes with high alpha value following McD-K test

⇒ Indicate rapid evolution under selection

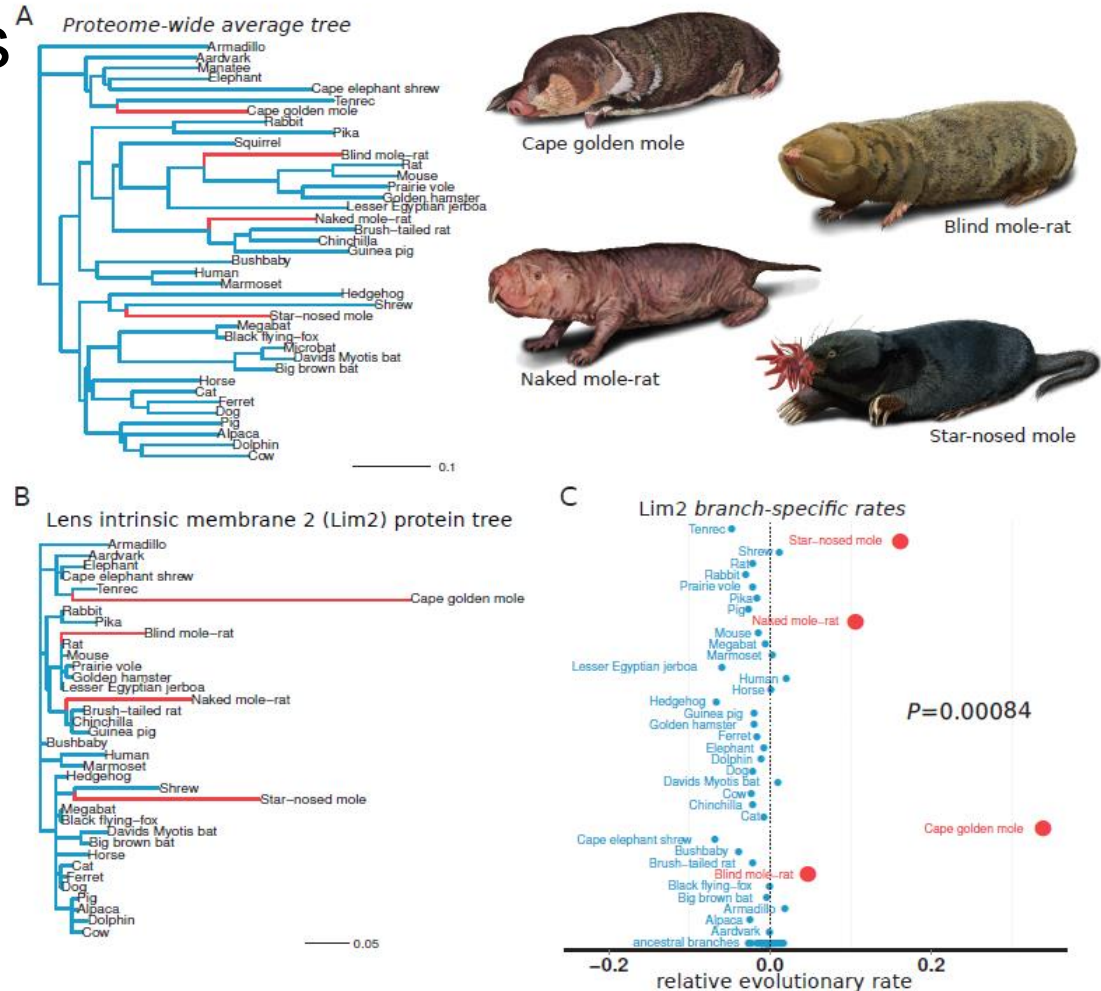
Hill & Unckless, 2018

<https://doi.org/10.1016/j.meegid.2017.11.013>

Comparative genomics

Study adaptive convergence across species

- What are the genes that evolved at a more rapid rate in organisms adapted to similar ecological conditions? That evolved comparable traits?



Partha, R.,... & Clark, N. L. (2017). Subterranean mammals show convergent regression in ocular genes and enhancers, along with adaptation to tunneling. *Elife*, 6, e25884.
<https://doi.org/10.7554/eLife.25884.001>

Trait-based approaches

Some local-adaptation/pop genomics approaches are also based on traits when contrasting populations with different phenotypes, morphotypes within a species or ecotypes...

- ⇒ Need to characterize phenotypes and/or adaptative traits
- ⇒ Quantifying phenotypes can be complex (physiology, morphology, etc)

Trait-based approaches

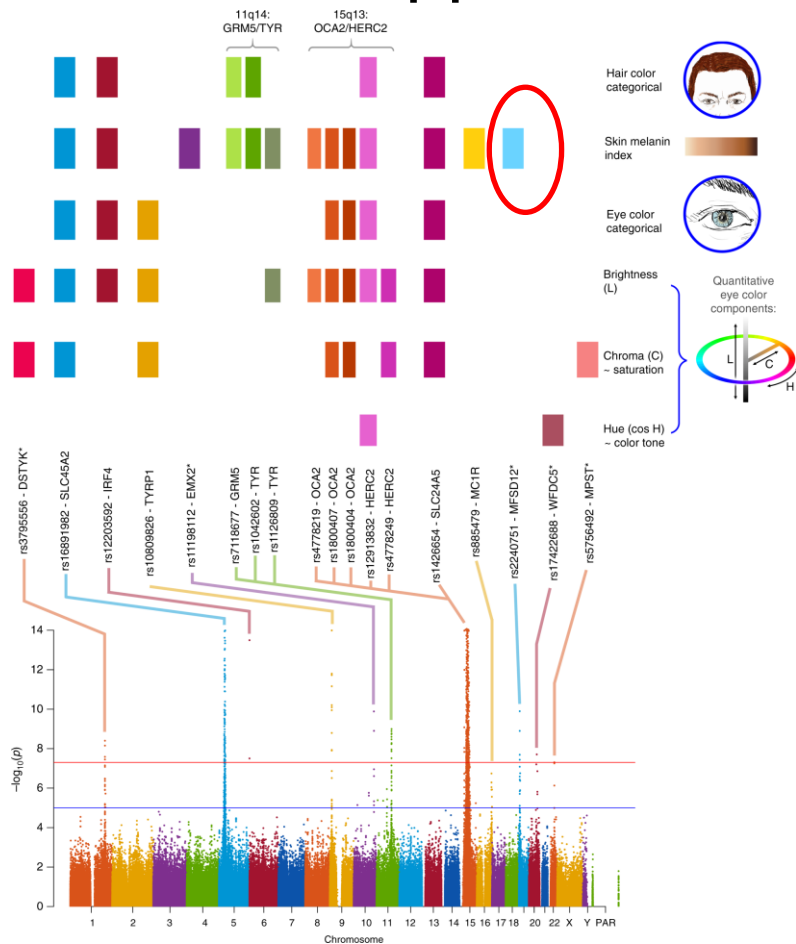
GWAS

Principle:

- many individuals (hundreds to thousands)
 - A trait is quantified for all individuals (case-control, quantitative)
 - Many markers (usually SNP-array or Whole-genome)
- ⇒ Locus-by-locus association test
- ⇒ Polygenic scores

Warning: need to account for population structure

Trait-based approaches

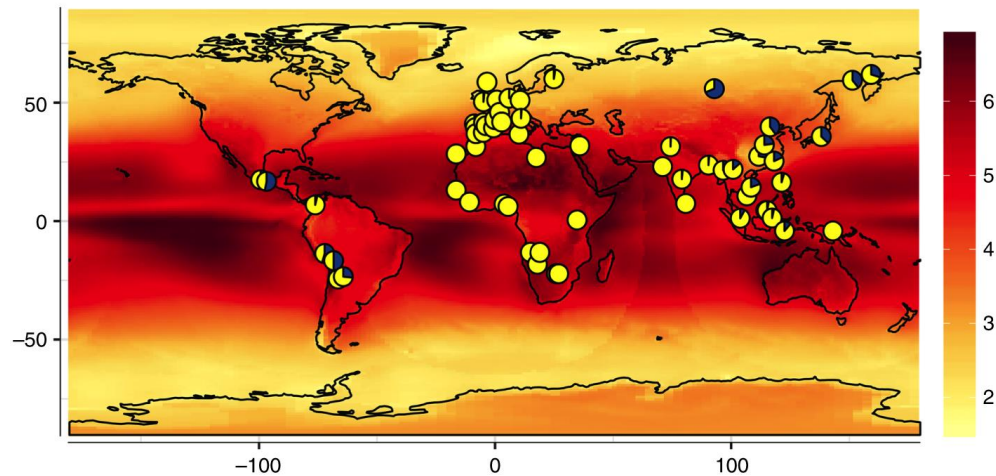


GWAS

6357 individuals

730,525 SNPs

pigmentation of skin and eyes



Adhikari, K., Mendoza-Revilla, J., Sohail, A. *et al.* A GWAS in Latin Americans highlights the convergent evolution of lighter skin pigmentation in Eurasia. *Nat Commun* **10**, 358 (2019). <https://doi.org/10.1038/s41467-018-08147-0>

Trait-based approaches

QTL (quantitative trait loci)

QTL analyses are usually based on family-design with crosses (or recombinant lines or pedigree) between individuals with the phenotype of interest.

Linkage maps can be build with genetic markers. A medium density is ok so RAD-seq is well-suited for that.

Then, the association between genotype and phenotype are tested in the progeny

Trait-based approaches

Huber, B., Whibley, A., Poul, Y. *et al.* Conservatism and novelty in the genetic architecture of adaptation in *Heliconius* butterflies. *Heredity* **114**, 515–524 (2015).
<https://doi.org/10.1038/hdy.2015.22>

QTL (quantitative trait loci)

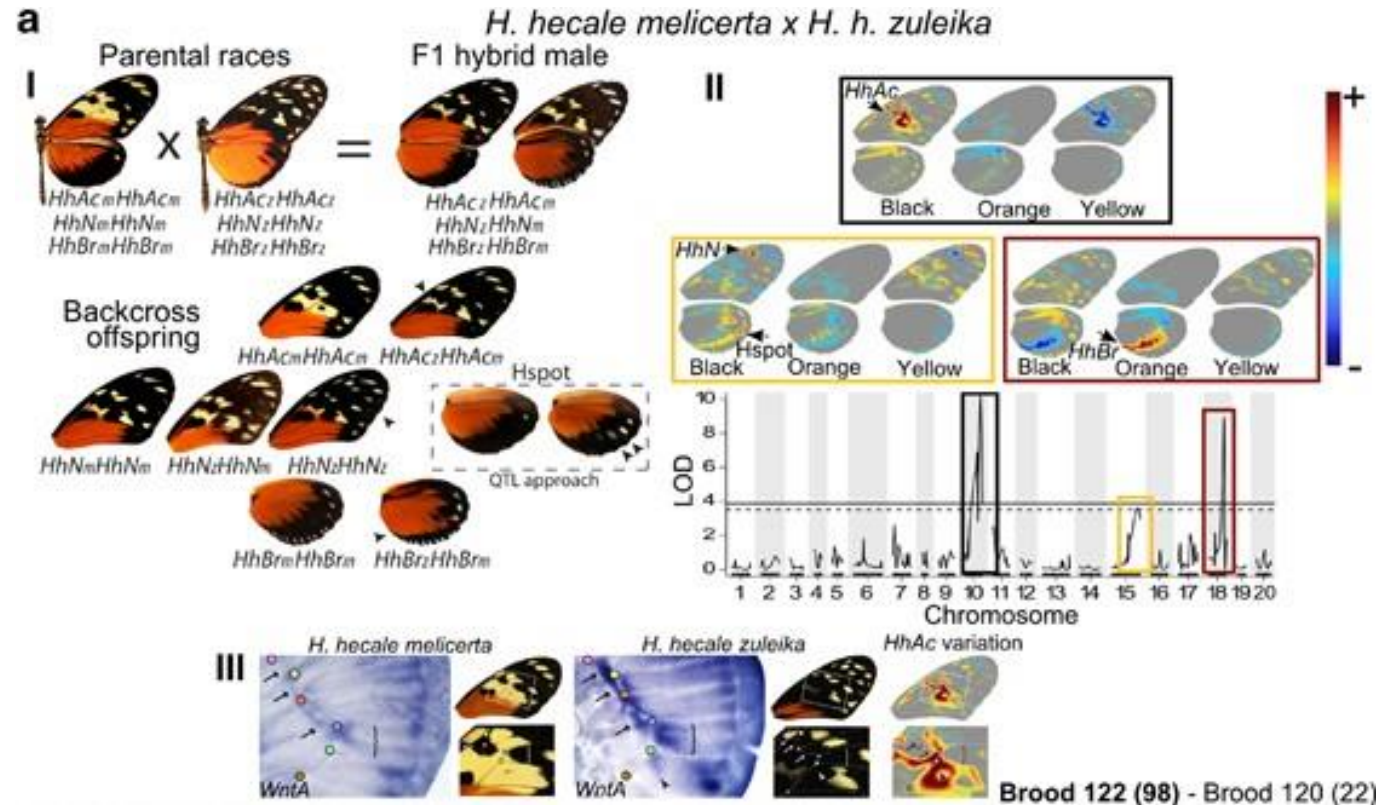
Targeted crosses
and back-crosses

RAD-seq

Image analysis for
colour pattern

Multivariate
associations

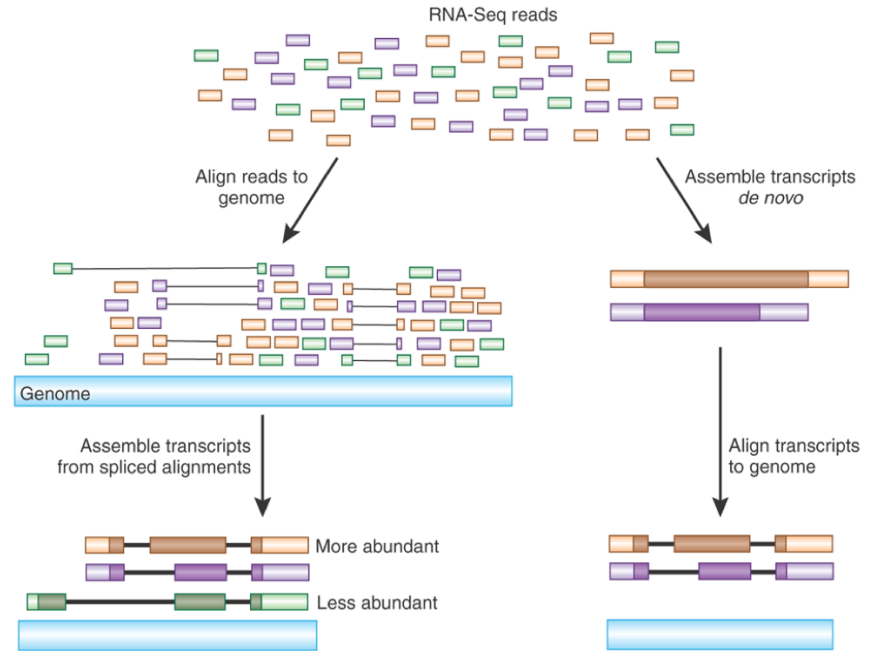
+ in situ hybridisation
of WntA transcripts



Multi-omics

Transcriptomics

- Quantify RNA expression
- Can be done on the whole-organism or specific tissue or single-cell
- qPCR on genes of interest or RNA-seq of all transcripts
- Need to build a transcriptome (collection of transcripts)



⇒ Compare expression levels between two groups (differential expression)

⇒ Find co-expressed genes

⇒ eQTL

⇒ Allele-specific expression, isoform expression

Multi-omics

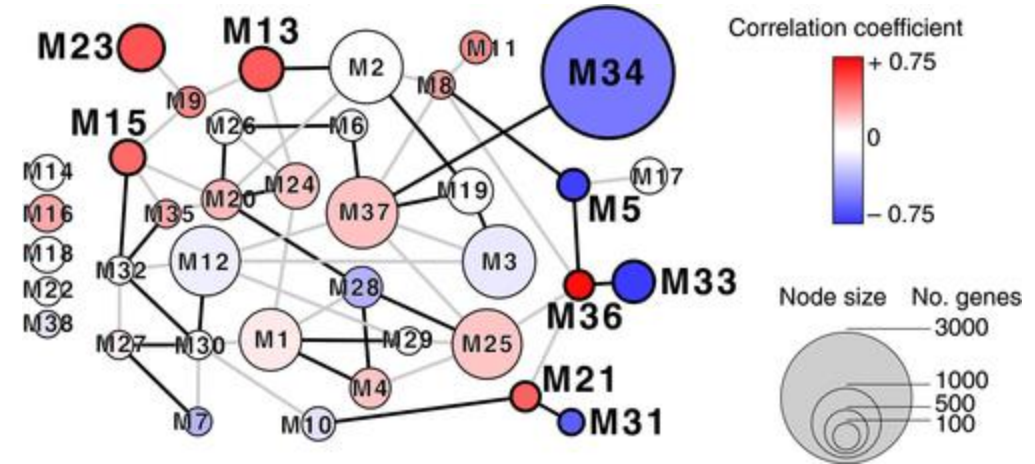
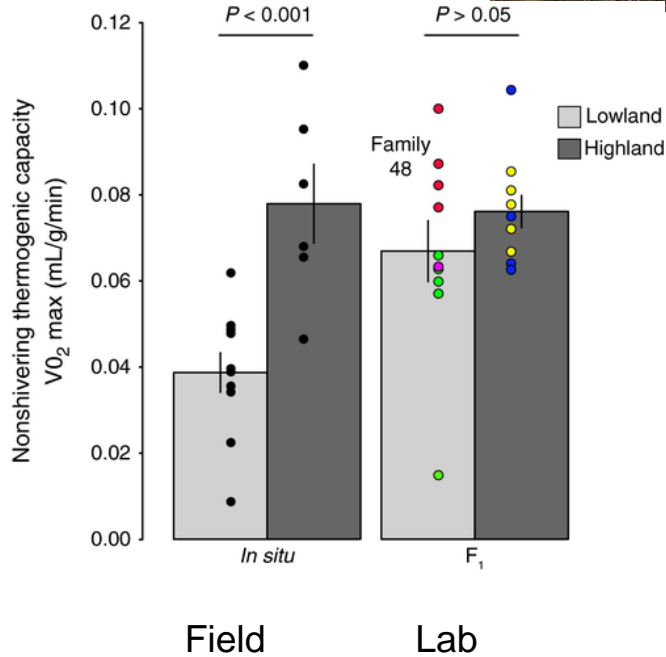
Transcriptomics



RNA seq of brown adipose tissue
19 samples

⇒ Genes co-expression network

⇒ Which modules are associated with thermogenic performance?



Velotta, J.P., Jones, J., Wolf, C.J. and Cheviron, Z.A. (2016), Transcriptomic plasticity in brown adipose tissue contributes to an enhanced capacity for nonshivering thermogenesis in deer mice. *Mol Ecol*, 25: 2870-2886. doi:[10.1111/mec.13661](https://doi.org/10.1111/mec.13661)

Multi-omics



Transcriptomics

⇒ Enrichment analysis for GO terms

GO term	Description	<i>P</i>	<i>q</i>	Enrichment
GO:0001525	Angiogenesis	2.42E-09	4.37E-06	3.67
GO:0048514	Blood vessel morphogenesis	8.13E-05	1.03E-02	4.01
GO:0007219	<i>Notch</i> signalling pathway	8.61E-05	1.07E-02	3.7
GO:0043547	Positive regulation of GTPase activity	4.66E-07	2.19E-04	2.76
GO:1904018	Positive regulation of vasculature development	1.05E-04	1.27E-02	3.41
GO:0045765	Regulation of angiogenesis	1.25E-07	8.31E-05	3.68
GO:0070372	Regulation of ERK1 and ERK2 cascade	6.79E-05	9.15E-03	2.93
GO:0048729	Tissue morphogenesis	2.94E-07	1.69E-04	2.82
GO:0001570	Vasculogenesis	4.13E-04	3.74E-02	3.94

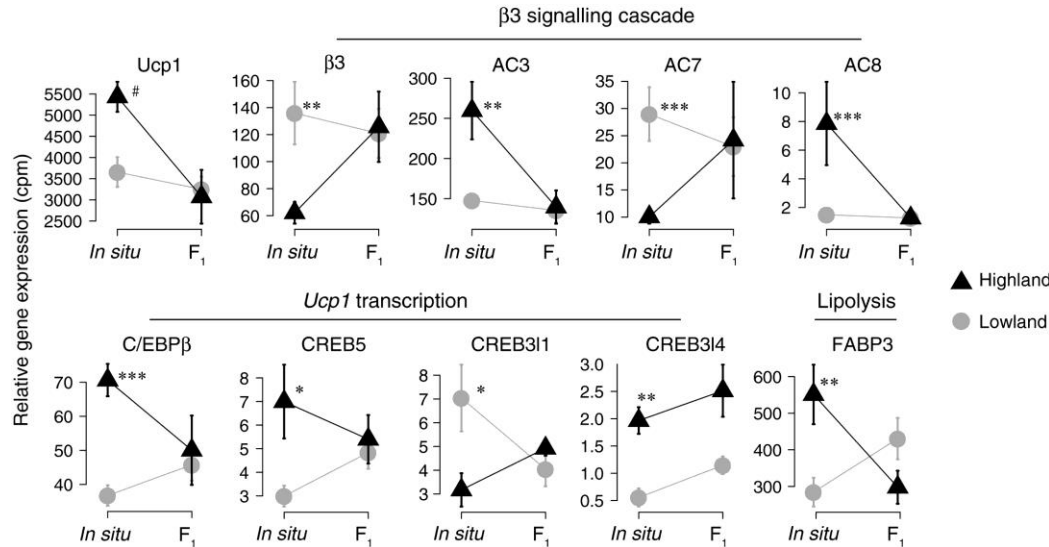
Velotta, J.P., Jones, J., Wolf, C.J. and Cheviron, Z.A. (2016), Transcriptomic plasticity in brown adipose tissue contributes to an enhanced capacity for nonshivering thermogenesis in deer mice. *Mol Ecol*, 25: 2870-2886. doi:[10.1111/mec.13661](https://doi.org/10.1111/mec.13661)

Multi-omics

Transcriptomics



⇒ Candidate genes



- β3 adrenergic receptor signalling cascade
- *Ucp1* transcription
- lipolysis

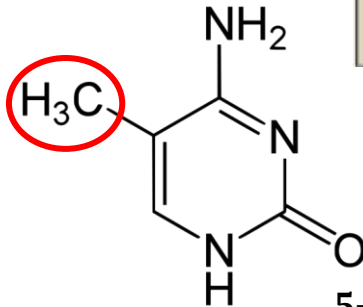
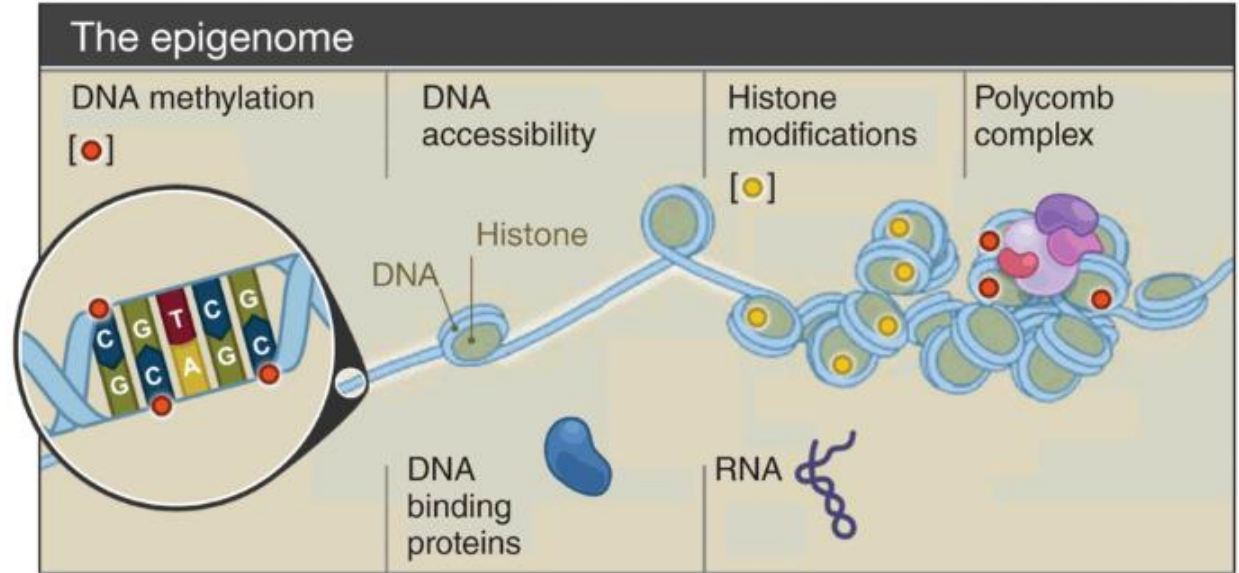
⇒ facilitate non-shivering thermogenesis within brown adipocytes

Multi-omics

Epi-genomics

Epigenome is change to the DNA or histones, can be plastic and/or heritable, vary between tissues

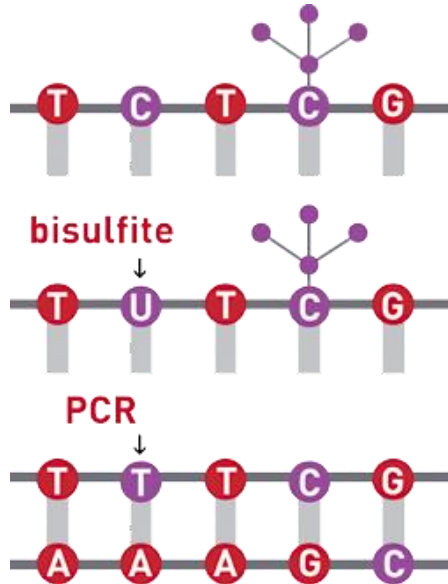
The most accessible aspect for studies in ecology and evolution will be DNA methylation



5-Méthylcytosine

Multi-omics

Epi-genomics : DNA methylation



use of bisulfite treatment of DNA before routine sequencing

⇒ Unmethylated C are converted into T

⇒ Remaining C are the ones that were methylated

100%	30%	✗
C	C	C
C	C	T
C	T	T
C	T	T
C	T	T
C	T	T
C/C	C/C	C/T

Studied variation is the % of methylated cytosine at a given position

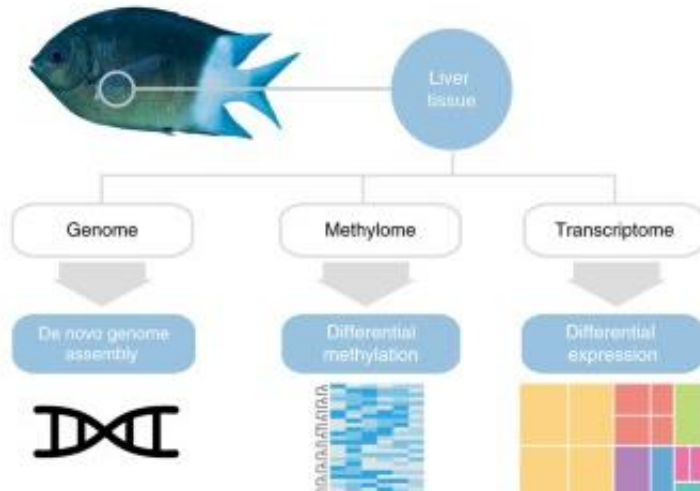
Cannot look at sites with C/T polymorphism

⇒ Variation in methylation level between tissues, between samples, between sexes, between populations, between ecotypes, etc...

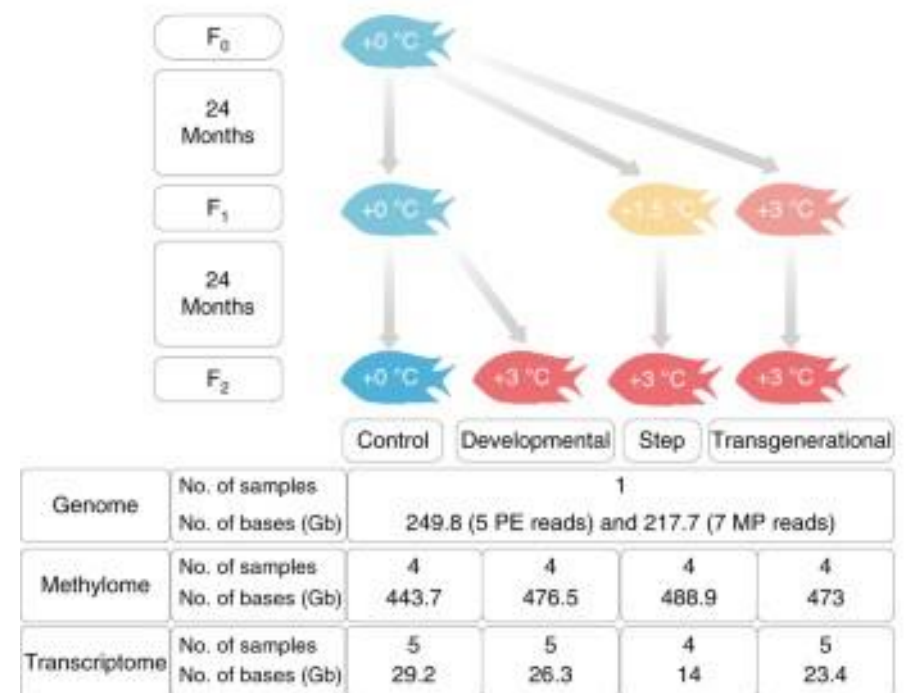
Multi-omics

Epi-genomics : DNA methylation

- coral reef fish, *Acanthochromis polyacanthus*
- liver genome, methylomes and transcriptomes



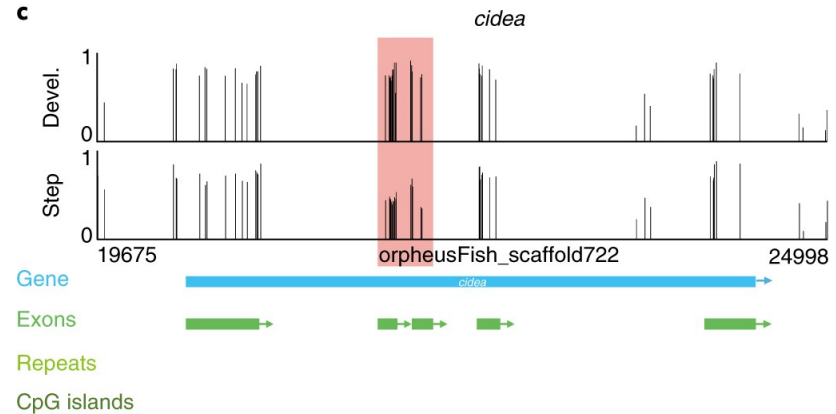
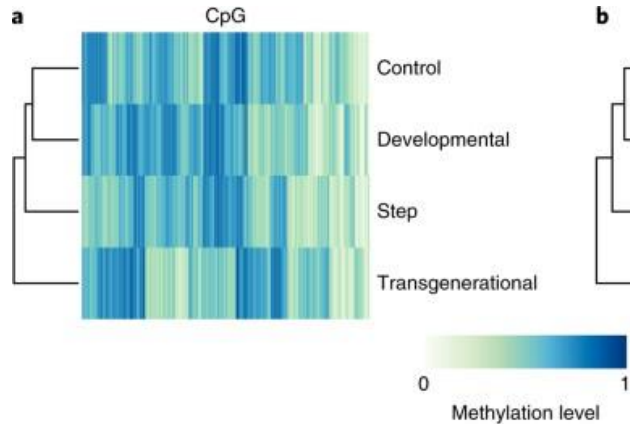
Exposed to current day (+0 °C) or future ocean temperatures (+3 °C) for one generation, two generations and incrementally across generations



Ryu, T., Veilleux, H.D., Donelson, J.M. *et al.* The epigenetic landscape of transgenerational acclimation to ocean warming. *Nature Clim Change* **8**, 504–509 (2018). <https://doi.org/10.1038/s41558-018-0159-0>

Multi-omics

Epi-genomics : DNA methylation



2,467 differentially methylated regions (DMRs) and 1,870 associated genes that respond to higher temperatures within and between generations

Some genes also show differential expression (but not many of them)

⇒ Association between DNA methylation and transgenerational acclimation to climate change

Ryu, T., Veilleux, H.D., Donelson, J.M. *et al.* The epigenetic landscape of transgenerational acclimation to ocean warming. *Nature Clim Change* **8**, 504–509 (2018).

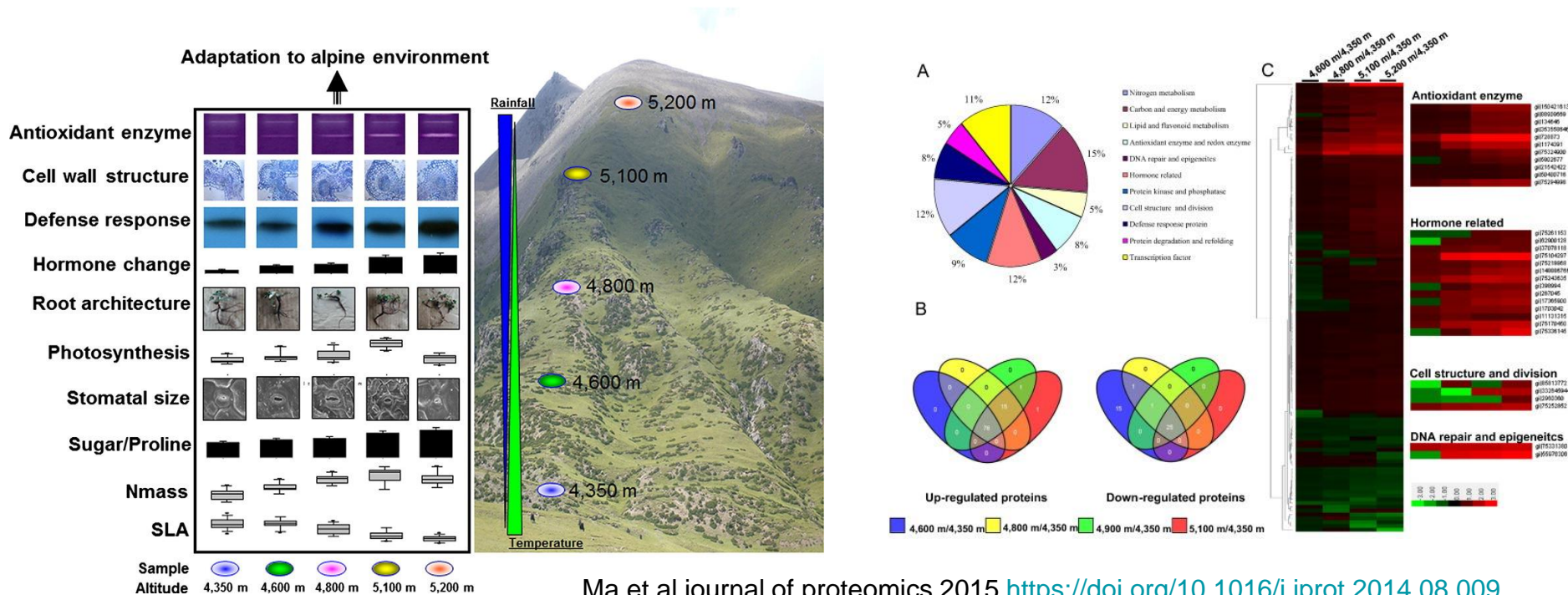
<https://doi.org/10.1038/s41558-018-0159-0>

Multi-omics

Proteomics : characterization of all proteins with large-scale mass spectrometry

⇒ an analysis of morphology and proteome along an altitudinal gradient

⇒ Identify pathways involved in adaptation (hormones, anti-oxydant, epi-genetic regulations, etc.



Experimental & genomics

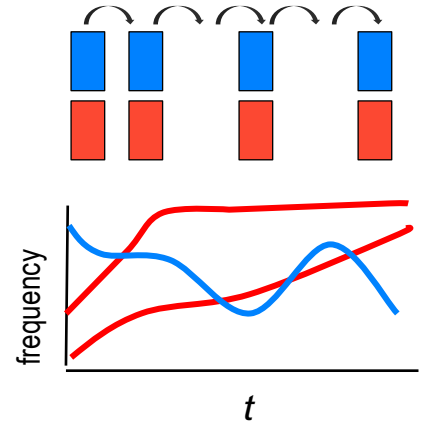
Experimental evolution / Experimental selection

From a starting population which is usually clonal (in yeast/bacteria) or highly-diverse (diploid species, drosophila, insects, etc), several replicates are kept under controlled conditions (one or several treatments) for a given number of generations (5 to 1 000...) Phenotypes can be regularly measured

With genomics:

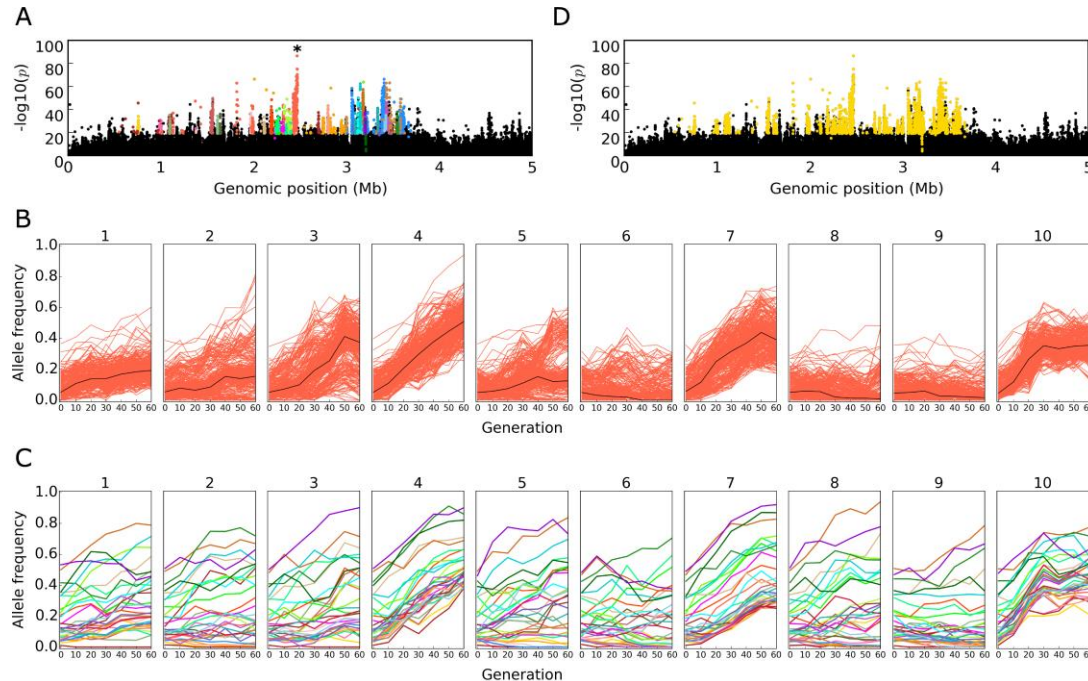
Usually pool-seq. The change of allelic frequencies is followed at regular intervals or contrasted between the beginning and the end of the experiment

⇒ Powerful way to follow evolution “under our eyes”!



Experimental & genomics

Experimental evolution / Experimental selection



Barghi N, Tobler R, Nolte V, Jakšić AM, Mallard F, Otte KA, et al. (2019) Genetic redundancy fuels polygenic adaptation in *Drosophila*. PLoS Biol 17(2): e3000128. <https://doi.org/10.1371/journal.pbio.3000128>

10 replicates of a *Drosophila simulans* population to a new temperature regime

Pool-seq

Convergent responses for several phenotypes

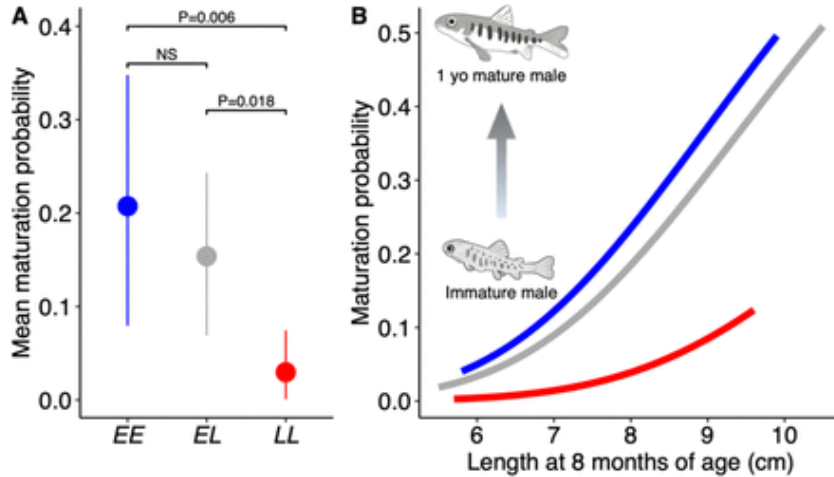
a strong polygenic response (99 selected alleles; mean $s = 0.059$)

⇒ redundancy : not the same loci contribute to the evolution of the same phenotypes between replicates

Experimental & genomics

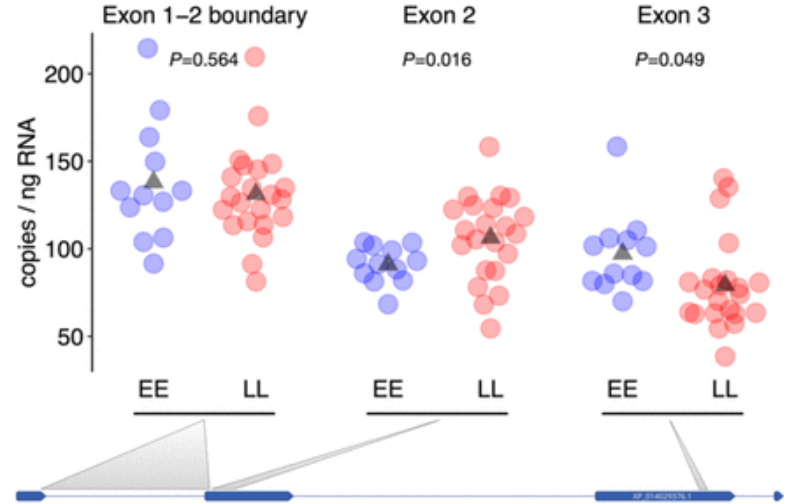
Cis-regulatory differences in isoform expression associate with life history strategy variation in Atlantic salmon
Jukka-Pekka Verta et al
bioRxiv 777300; doi: <https://doi.org/10.1101/777300>

Common-garden



A **candidate gene** from GWAS for age at maturity in Salmon : *vgl3*

⇒ Genotype and raise in controlled conditions 656 individuals



⇒ Transcriptomics : complex pattern of differential expression:
It depends on the exon...

⇒ A different isoform expressed in EE and LL : **isoform-specific expression**

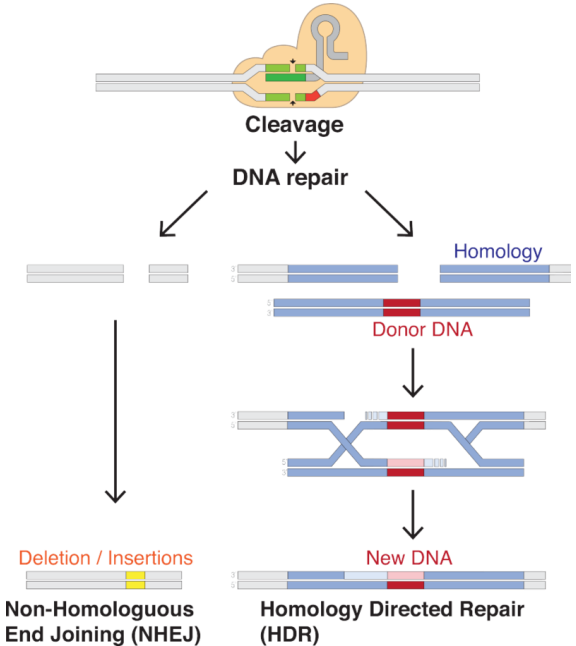
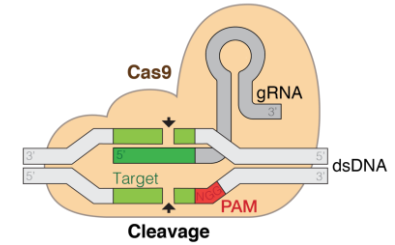
Experimental & genomics

Gene-editing

Target a candidate gene to silence it or change its sequence:

-> used to be possible only in model species

-> CRISPR-Cas9 is a game-changer



Experimental & genomics

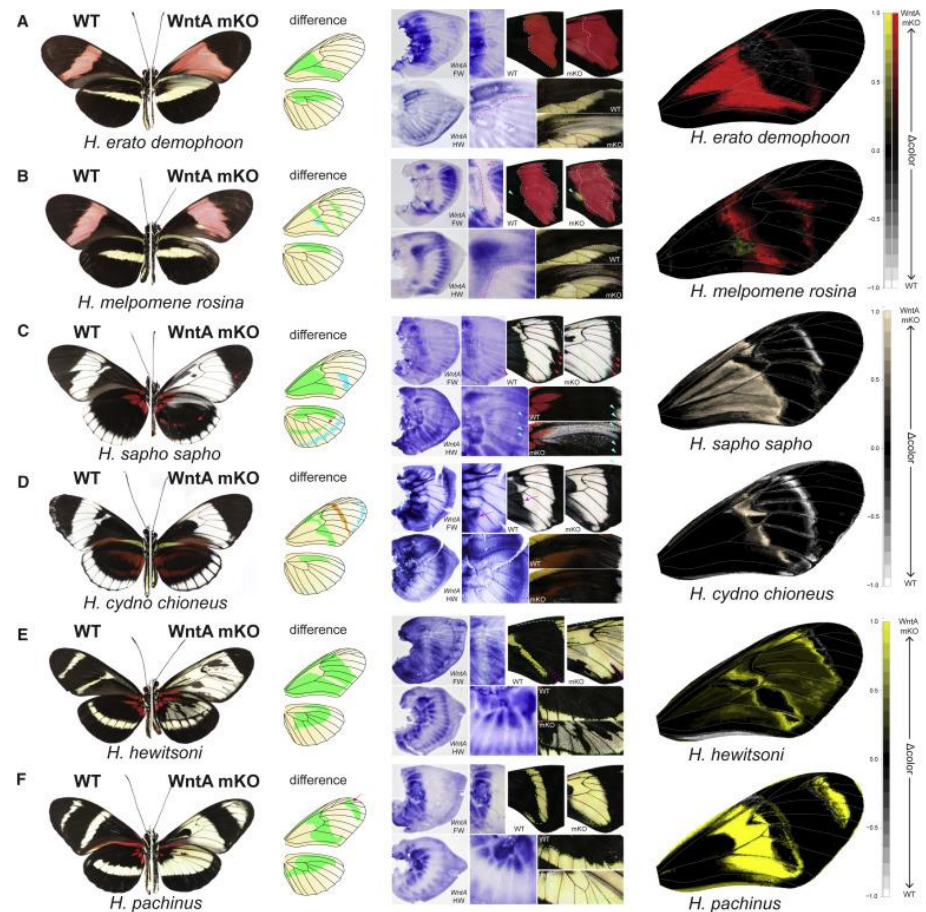
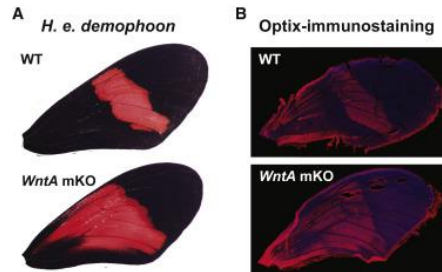
Gene-editing

Inject eggs and larva with CRISPR-CAs9 to make *WntA* KO mutant

-> generation 0: 30% with a mutant phenotype (indels in ~80% of PCR *WntA*)

- ⇒ Confirm the relation genotype/phenotype
- ⇒ Compare multiple species
- ⇒ Matches the area of influence during development

+ antibody immunostaining of transcripts



Concha, C., Wallbank, R. W., Hanly, J. J., Fenner, J., Livraghi, L., Rivera, E. S., ... & Morrison, C. (2019). Interplay between developmental flexibility and determinism in the evolution of mimetic *Heliconius* wing patterns. *Current Biology*, 29(23), 3996-4009. <https://doi.org/10.1016/j.cub.2019.10.010>

Studying adaptation... Integrative biology!

