Rat poison and bobcats: Application of gene expression in a natural population

Mouton Alice
FNRS Postdoctoral Researcher
University of Liege, Belgium

UCLA

Lecture Series in Ecology and Evolution
KU Leuven 12 February 2021

Outline

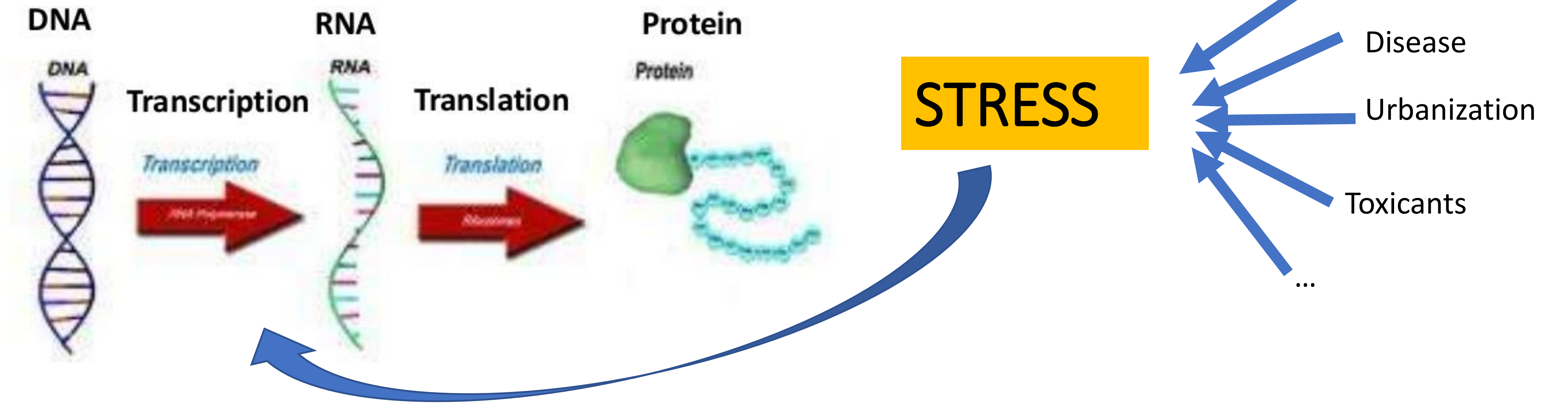
1. RNA-seq applied to wildlife
2. Background on rat poison and study system
3. Gene expression analyses in a bobcat population
4. Complementary study : methylation and rat poison
5. Perspectives

1. RNA-seq applied to wildlife

Design Experiment → **RNA Preparation** → **Prepare Libraries** → **Sequence** → **Analysis**

- **Design Experiment**: Set up the experiment to address your questions.
- **RNA Preparation**: Isolate and purify input RNA.
- **Prepare Libraries**: Convert the RNA to cDNA; add sequencing adapters.
- **Sequence**: Sequence cDNAs using a sequencing platform.
- **Analysis**: Analyze the resulting short-read sequences.

DNA → RNA → Protein



Gene Expression Modulates Organismal Response to Environmental Change

1. RNA-seq applied to wildlife

PLOS | PATHOGENS

RESEARCH ARTICLE

The White-Nose Syndrome Transcriptome: Activation of Anti-fungal Host Responses in Wing Tissue of Hibernating Little Brown Myotis

Kenneth A. Field[1]*, Joseph S. Johnson[1], Thomas M. Lilley[1], Sophia M. Reeder[1], Elizabeth J. Rogers[1], Melissa J. Behr[2], DeeAnn M. Reeder[1]

MOLECULAR ECOLOGY

Molecular Ecology (2012) **21**, 3110–3120 doi: 10.1111/j.1365-294X.2012.05481.x

FROM THE COVER

Only skin deep: shared genetic response to the deadly chytrid fungus in susceptible frog species

ERICA BREE ROSENBLUM,*†§ THOMAS J. POORTEN,*† MATTHEW SETTLES† and GORDON K. MURDOCH†‡

Diseases

Barakat *et al. BMC Plant Biology* 2012, **12**:38
http://www.biomedcentral.com/1471-2229/12/38

BMC Plant Biology

RESEARCH ARTICLE **Open Access**

Chestnut resistance to the blight disease: insights from transcriptome analysis

Behavior

PNAS

Social environment is associated with gene regulatory variation in the rhesus macaque immune system

Jenny Tung[a,1,2], Luis B. Barreiro[a,3], Zachary P. Johnson[b], Kasper D. Hansen[c], Vasiliki Michopoulos[b], Donna Toufexis[b,d], Katelyn Michelini[a], Mark E. Wilson[b], and Yoav Gilad[a,1]

[a]Department of Human Genetics, University of Chicago, Chicago, IL 60637; [b]Yerkes National Primate Research Center, Emory University, Atlanta, GA 30322; [c]Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21202; and [d]Department of Psychology, University of Vermont, Burlington, VT 05405

Edited by Gene E. Robinson, University of Illinois at Urbana-Champaign, Urbana, IL, and approved March 6, 2012 (received for review February 15, 2012)

Gene expression shifts in yellow-bellied marmots prior to natal dispersal FREE

Tiffany C Armenta, Steve W Cole, Daniel H Geschwind, Daniel T Blumstein, Robert K Wayne Author Notes

Behavioral Ecology, Volume 30, Issue 2, March/April 2019, Pages 267–277, https://doi.org/10.1093/beheco/ary175

MOLECULAR ECOLOGY

Molecular Ecology (2016) 25, 5680–5691 doi: 10.1111/mec.13879

Seasonal gene expression in a migratory songbird

RACHEL A. JOHNSTON,* KRISTINA L. PAXTON,†‡ FRANK R. MOORE,† ROBERT K. WAYNE* and THOMAS B. SMITH*§

Climate Change

ORIGINAL ARTICLE

Seedling response to water stress in valley oak (*Quercus lobata*) is shaped by different gene networks across populations

Alayna Mead, Juan Peñaloza Ramirez, Megan K. Bartlett, Jessica W. Wright, Lawren Sack, Victoria L. Sork

First published: 25 October 2019 | https://doi.org/10.1111/mec.15289

PLOS | ONE

RESEARCH ARTICLE

Transcriptomic Characterization of Tambaqui (*Colossoma macropomum*, Cuvier, 1818) Exposed to Three Climate Change Scenarios

Marcos Prado-Lima[1,2]*, Adalberto Luis Val[1]

SCIENTIFIC REPORTS

OPEN

Mediterranean versus Red sea corals facing climate change, a transcriptome analysis

Received: 04 August 2016
Accepted: 09 January 2017
Published: 09 February 2017

Keren Maor-Landaw[1], Hiba Waldman Ben-Asher[1], Sarit Karako-Lampert[1], Mali Salmon-Divon[2], Fiorella Prada[3], Erik Caroselli[3], Stefano Goffredo[3], Giuseppe Falini[4], Zvy Dubinsky[1] & Oren Levy[1]

ARTICLE

DOI: 10.1038/s41467-018-03384-9 OPEN

Strong phenotypic plasticity limits potential for evolutionary responses to climate change

Vicencio Oostra[1,2], Marjo Saastamoinen[3], Bas J. Zwaan[2] & Christopher W. Wheat[4]

1. RNA-seq applied to wildlife

SCIENTIFIC REPORTS

OPEN

Transcriptome analysis of a wild bird reveals physiological responses to the urban environment

Received: 12 October 2016
Accepted: 06 February 2017

Hannah Watson, Elin Videvall, Martin N. Andersson & Caroline Isaksson

Urbanization

OPEN ACCESS Freely available online
PLOS ONE

Signatures of Rapid Evolution in Urban and Rural Transcriptomes of White-Footed Mice (*Peromyscus leucopus*) in the New York Metropolitan Area

Stephen E. Harris[1], Jason Munshi-South[2*], Craig Obergfell[3], Rachel O'Neill[3]

Evo-devo

Transcriptomic and epigenomic characterization of the developing bat wing

Walter L Eckalbar, Stephen A Schlebusch, Mandy K Mason, Zoe Gill, Ash V Parker, Betty M Booker, Sierra Nishizaki, Christiane Muswamba-Nday, Elizabeth Terhune, Kimberly A Nevonen, Nadja Makki, Tara Friedrich, Julia E VanderMeer, Katherine S Pollard, Lucia Carbone, Jeff D Wall, Nicola Illing & Nadav Ahituv

Nature Genetics **48**, 528–536 (2016) | Download Citation

ORIGINAL ARTICLE | Full Access

Transcriptomic analysis of skin pigmentation variation in the Virginia opossum (*Didelphis virginiana*)

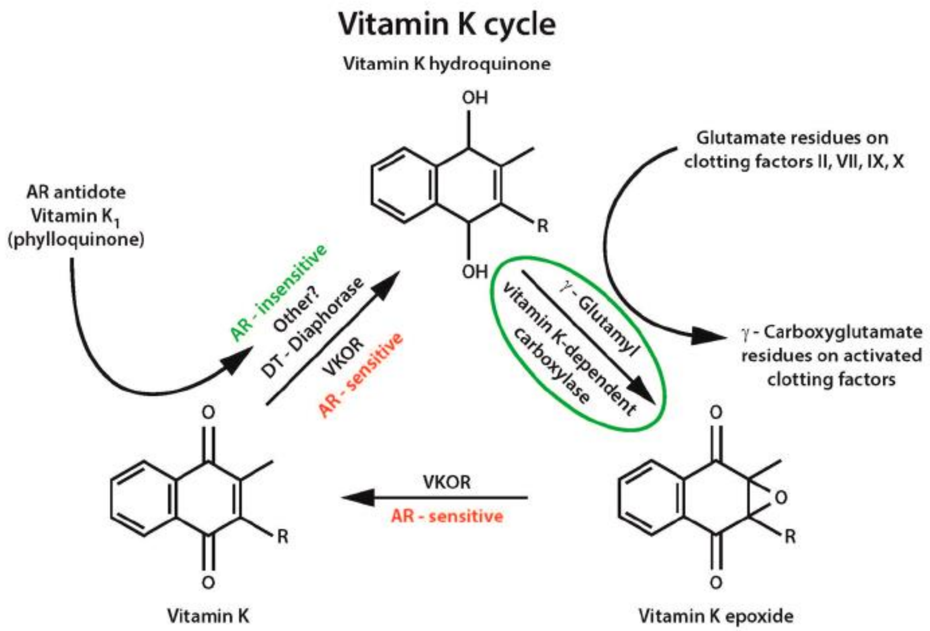
Sergio F. Nigenda-Morales, Yibo Hu, James C. Beasley, Hugo A. Ruiz-Piña, David Valenzuela-Galván, Robert K. Wayne

First published: 09 May 2018 | https://doi.org/10.1111/mec.14712 | Citations: 2

2. Background on rat poison

• Anticoagulant rodenticides (ARs) are used worldwide for vertebrate pest control in urban, suburban and agricultural settings.

• How do they work?

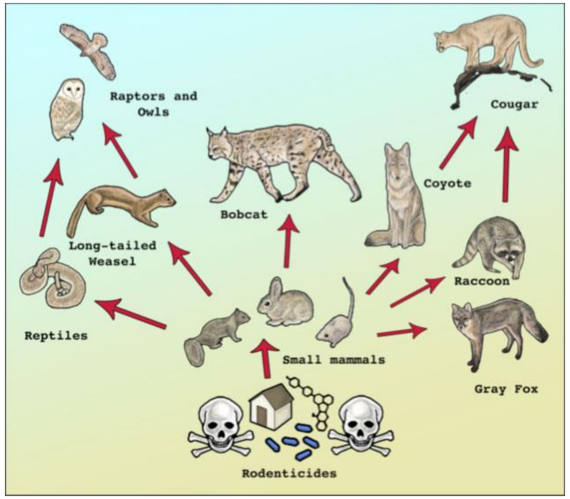


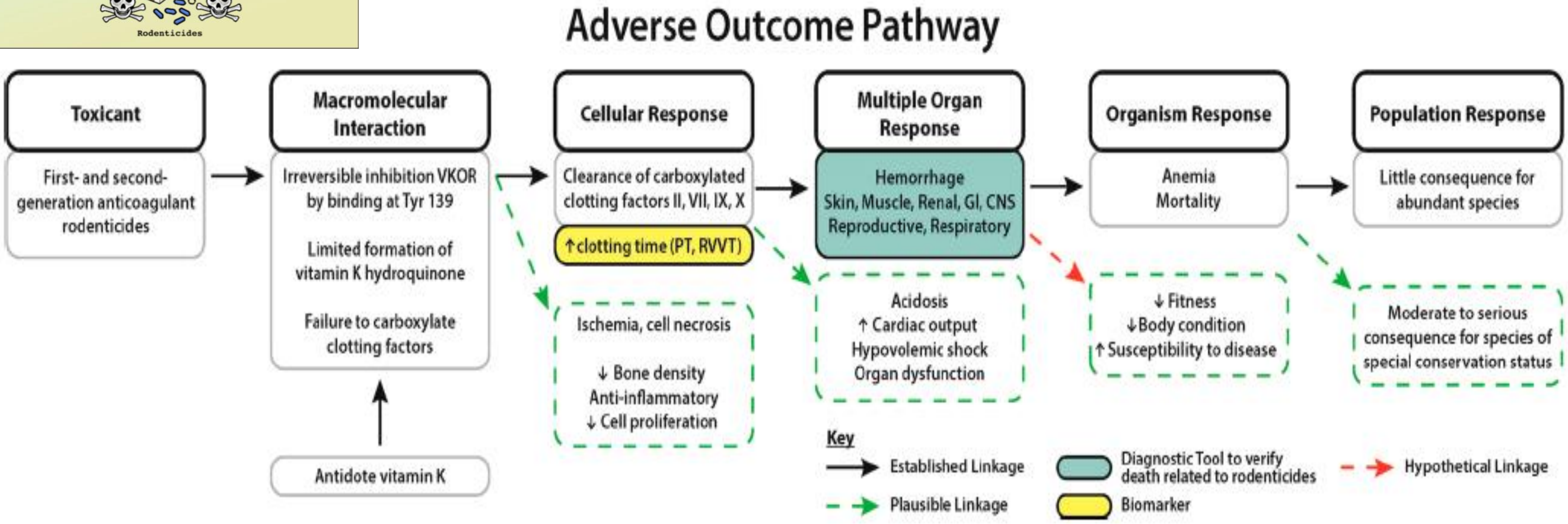
=> Internal bleeding/death

• Two generations of AR

- First Generation: Warfarin, chlorophacinone and diphacinone => several days of feeding (problem of rat resistant)
- Second Generation : bromadiolone, difenacoum, brodifacoum, flocoumafen, and difethialon => one single feed (highly toxic) and in some states/countries limited to professional pest companies

2. Background on rat poison : Secondary exposure





Env.Sci.Technol 2014,48,8433-8445

2. Background on rat poison: Secondary exposure

=> Anticoagulant rodenticides were detected in more than 90% of stoats and weasels in Denmark (Elmeros et 2011).

=> 62.8% of studied bird of prey with AR in Mediterranean region of Spain (Perea et al 2015)

⇒ Anticoagulants were detected in six of seven predatory species in Cape Town, South Africa (Serieys et al 2019).

⇒ AR residue in four mustelid species in France (Fournier-Chambrillon et al 2004)

⇒ Increasing parasite and pathogens in great bustards with AR (Lemus et al 2011)

⇒ Many more studies…

Problems

Many animals do not exhibit any outward signs of poisoning (sensitivity of AR varies among species),
Toxicants go undetected without specific testing

How to understand the effect on long-term exposure?
Long-term monitoring
How to measure exposure? What markers?
Need to sample exposed and unexposed alive animals!
Need to know other variables (sex, age, sample conditions) for confounding

2. Background on rat poison and study system

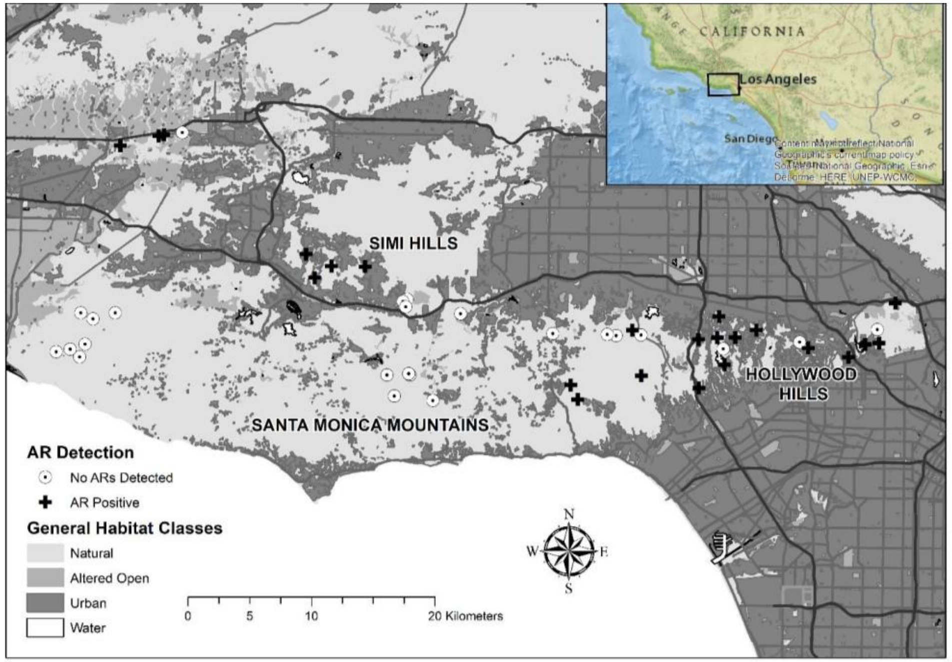
- ✓ Carnivores in the Santa Monica Mountains (and elsewhere) are chronically and pervasively exposed to ARs => in 2000, National Park Service started a carnivore monitoring (coyotes).

- ✓ In 2002-2005, rapid population decline due to notoedric mange in the bobcat population (50% of radiocollared animals died of mange in 2003) (Serieys et al. 2014).
- ✓ 90% bobcats detected positive to ARs
- ✓ Strong association between notoedric mange and exposure to AR (Riley et al. 2007)
- ✓ Primary cause of death from exposure (hemorrhaging) is not observed in bobcats as in other species=> but clotting time is the most frequent methods to assess AR exposure

Serieys et al 2013/ Serieys et al 2018 => Immunotoxic effects of AR

→ Measures of 65 biochemical markers of immune and organ function in 124 bobcats in the SMMNRP

data parameter	test (sample type)
anticoagulants	residue analysis for exposure (whole blood and serum)
	PIVKA clotting time (plasma)
	prothrombin clotting time (whole blood)
health parameters	general (whole blood and serum)
	immunophenotype (lymphocytes in whole blood profiled by flow cytometry)
	cytokine concentrations (serum)
	complete blood counts (whole blood)
	blood chemistries (serum)
B cell clonality	immunofixation (serum)
	PARR (whole blood)
pathogen infection or exposure	exposure/infection (whole blood and serum)
land use	percentage urban area (buffer zones)



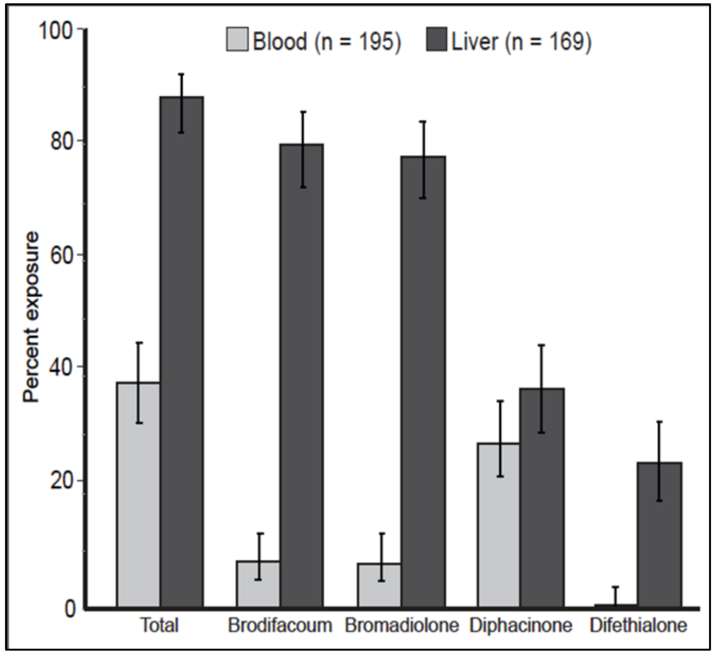
- Simultaneous immunostimulation and immunosuppression effects
- Evidence for immune dysregulation in animals exposed to ARs
- The broad spectrum of immune activation that we observe may compromise ability to mount a specific immune response.

3. Gene expression analyses in a bobcat population

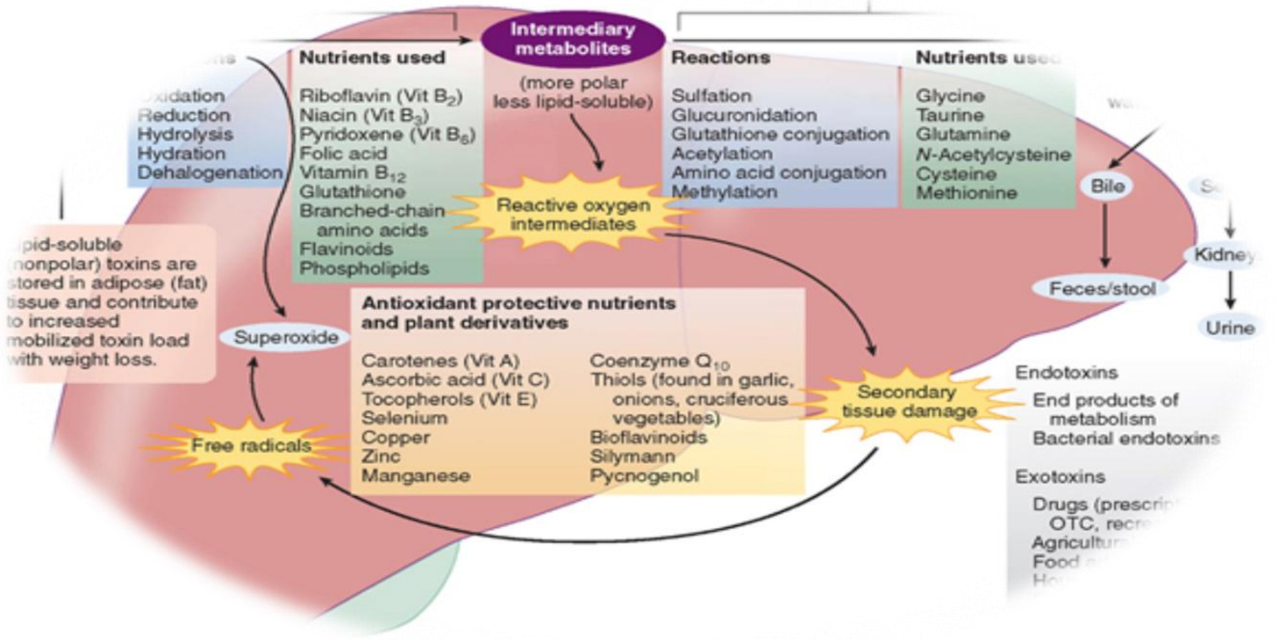
1. Do we have genes differentially expressed between bobcats exposed to ARs and not exposed?
2. What biological processes are they involved in?
3. Potential causal links between mange susceptibility and AR exposure in bobcats?

Challenges of RNA-Seq on natural populations

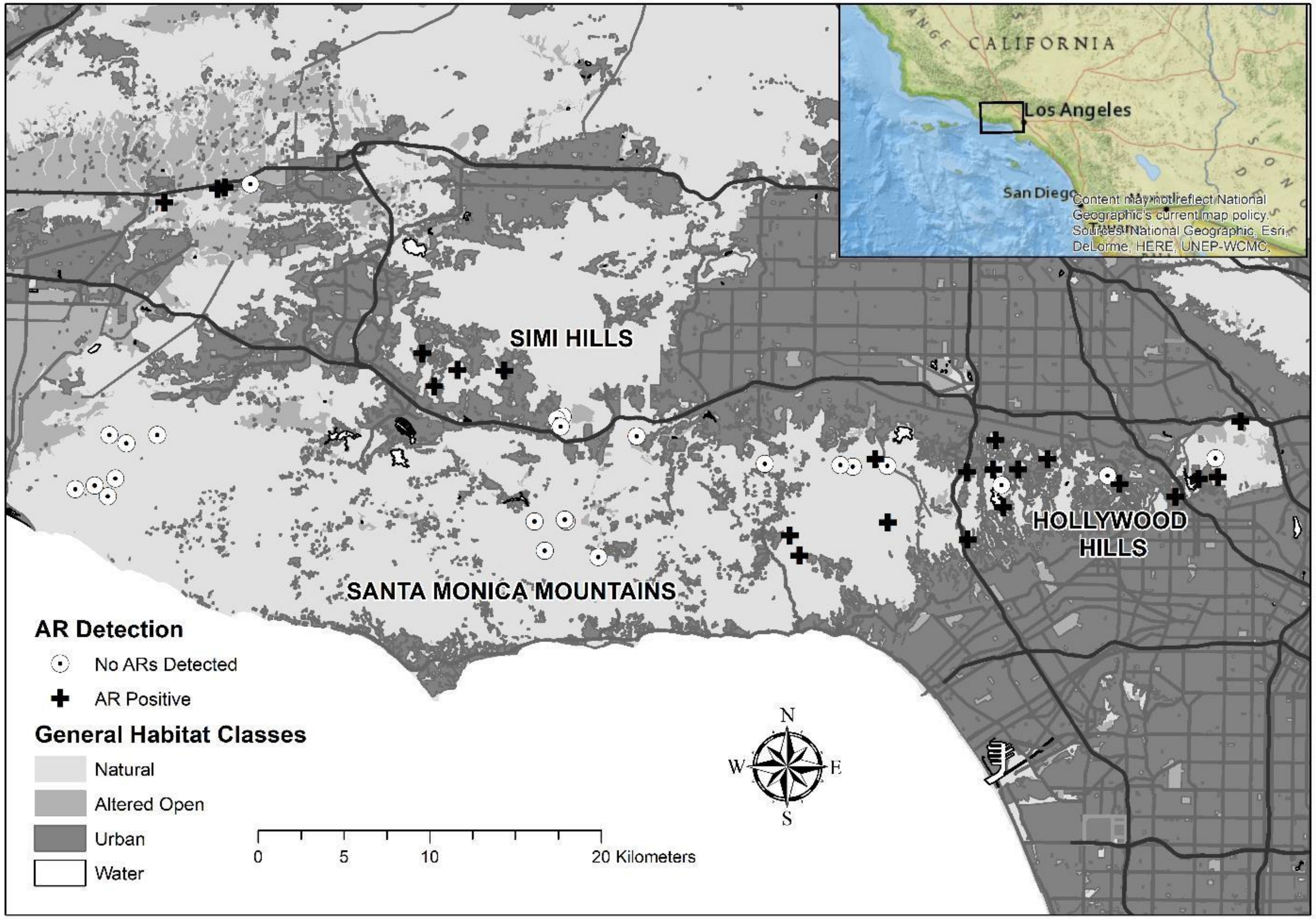
- Sampling is opportunistic- no knowledge of an animals experience of the environment prior to or after sampling (infection history, toxins not explicitly tested, etc.)
- AR detection in blood only indicates recent exposure, not lifetime exposure



- Can't target specific tissues (i.e. liver) to measure differential expression



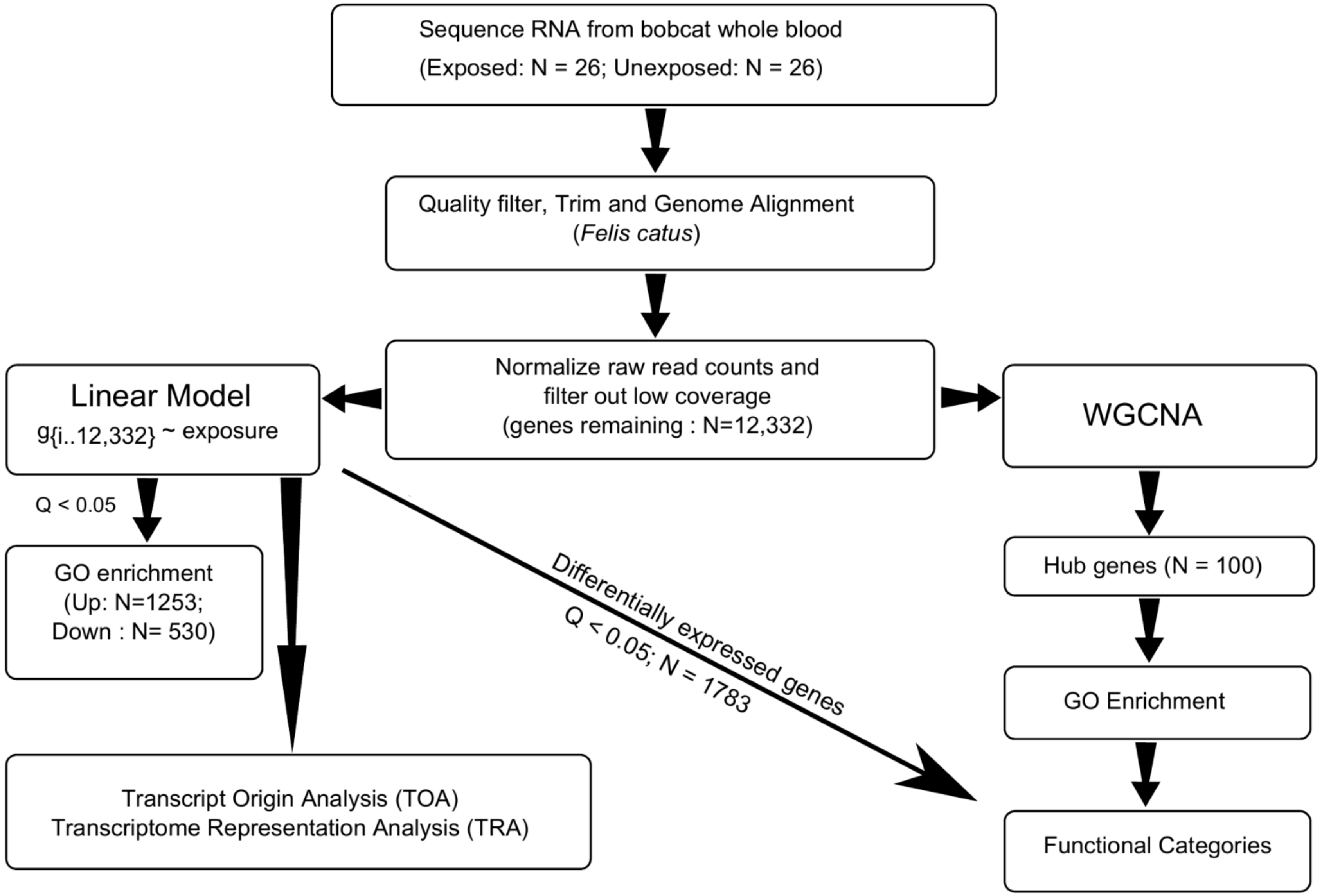
3. Gene expression analyses in a bobcat population



- ✓ 52 RNA samples from blood (26 exposed/ 26 non exposed)
- ✓ Balancing with age and sex
- ✓ Metadata (disease, RIN, sequencer, season,location…)

Fraser et al. (2018) *Molecular Ecology*

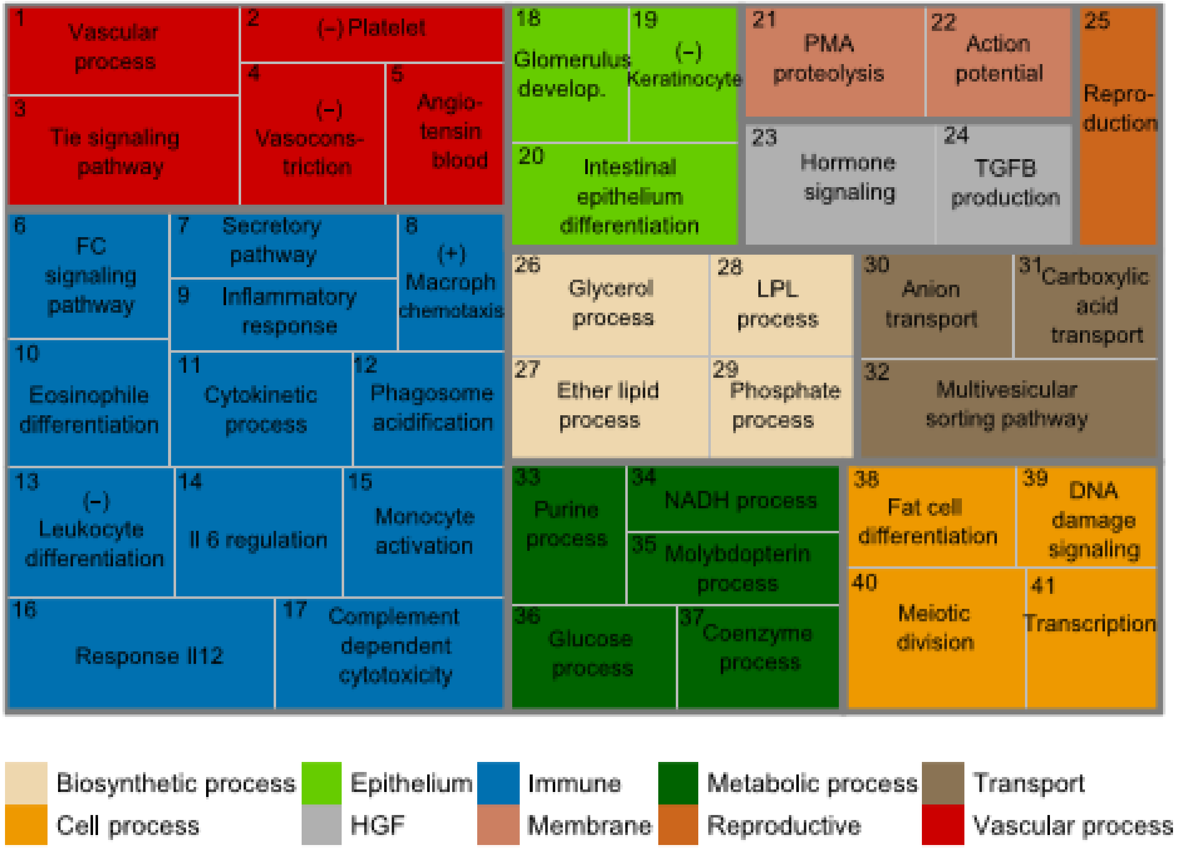
3. Gene expression analyses in a bobcat population



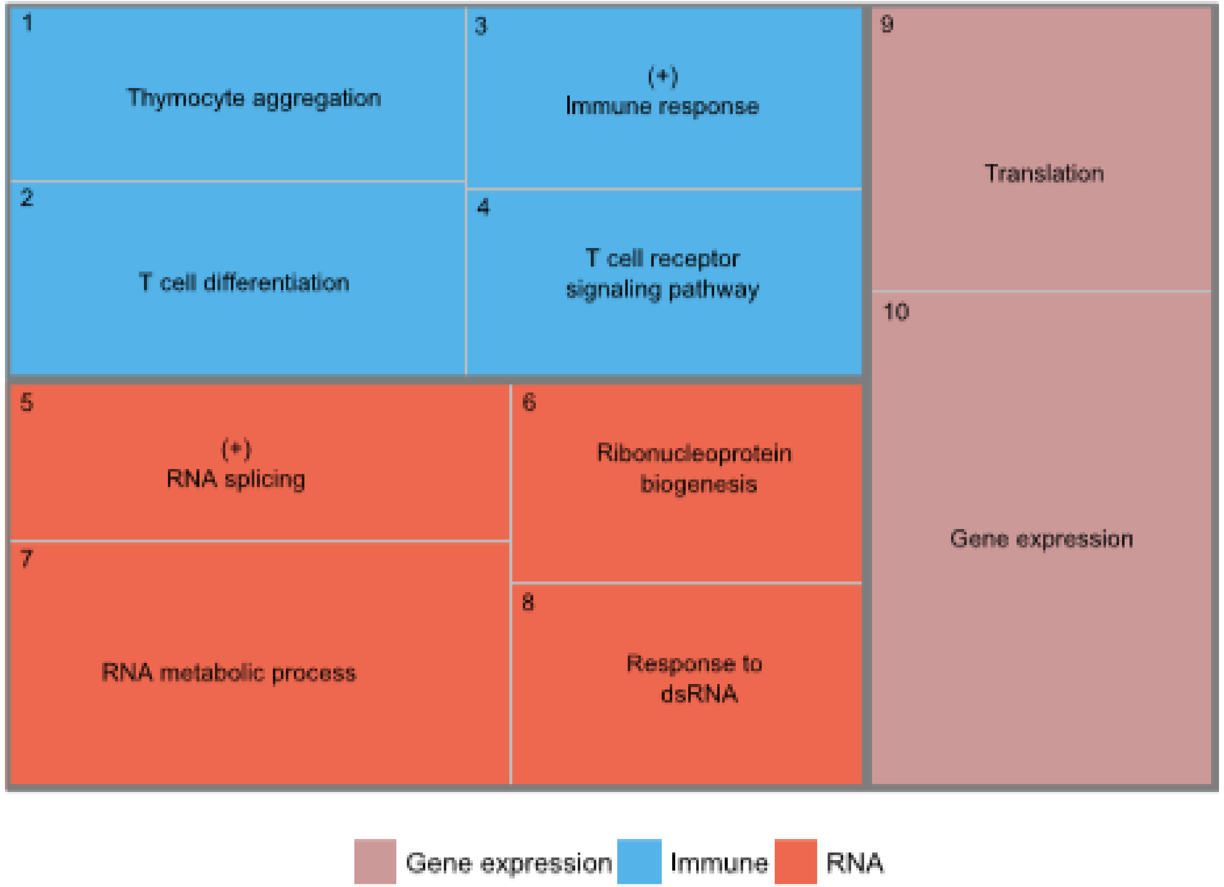
Fraser & Mouton et al. (2018) *Molecular Ecology*

3. Gene expression analyses in a bobcat population

Down regulated Genes

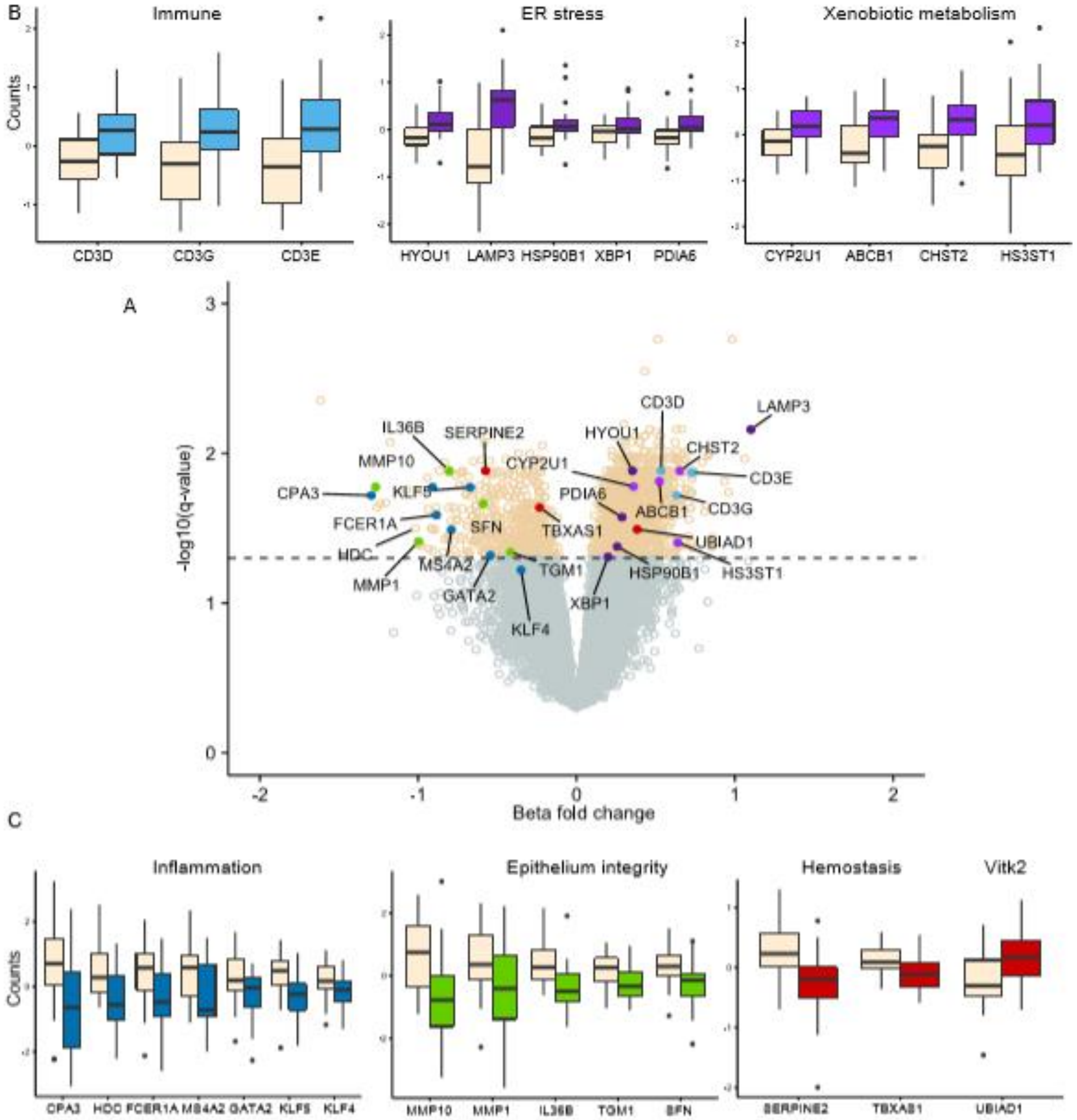


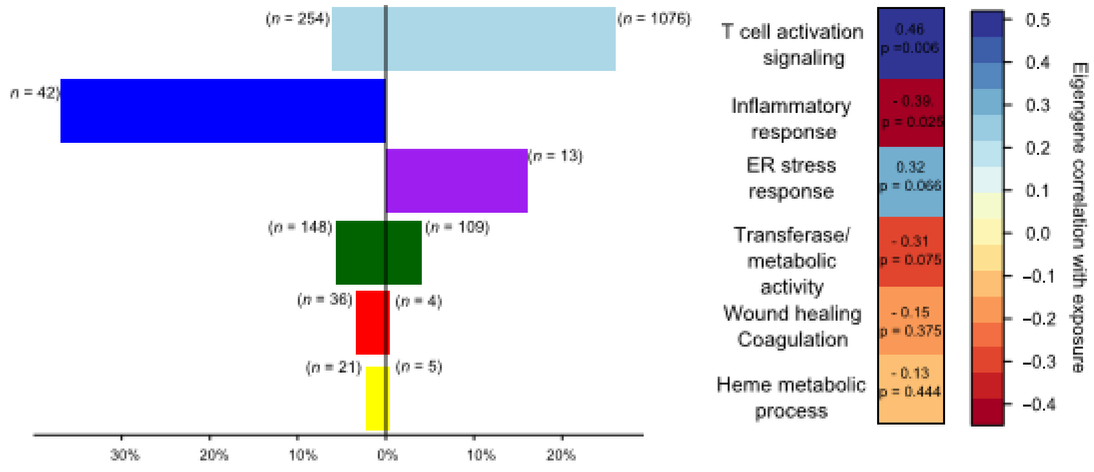
Up regulated Genes



Similarities with the immuno-assay study!

3. Gene expression analyses in a bobcat population





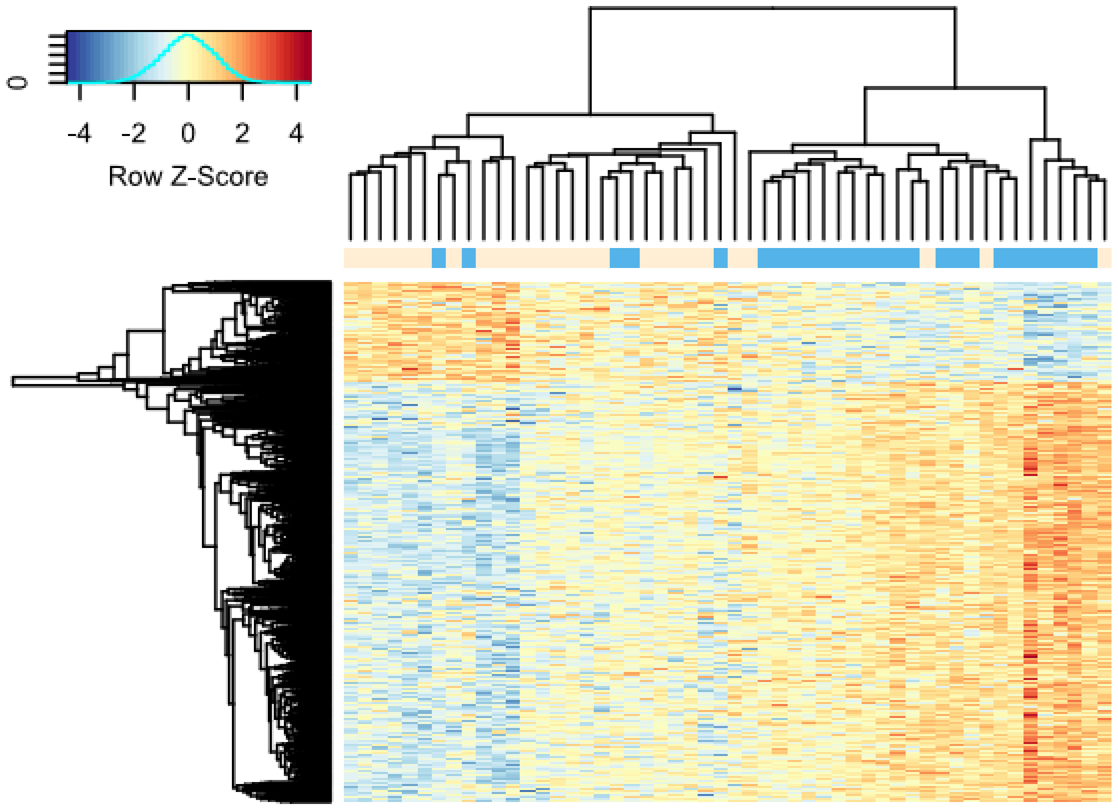
AR effect on immunity, ER stress, xenobiotic metabolism, epithelium integrity and hemostasis

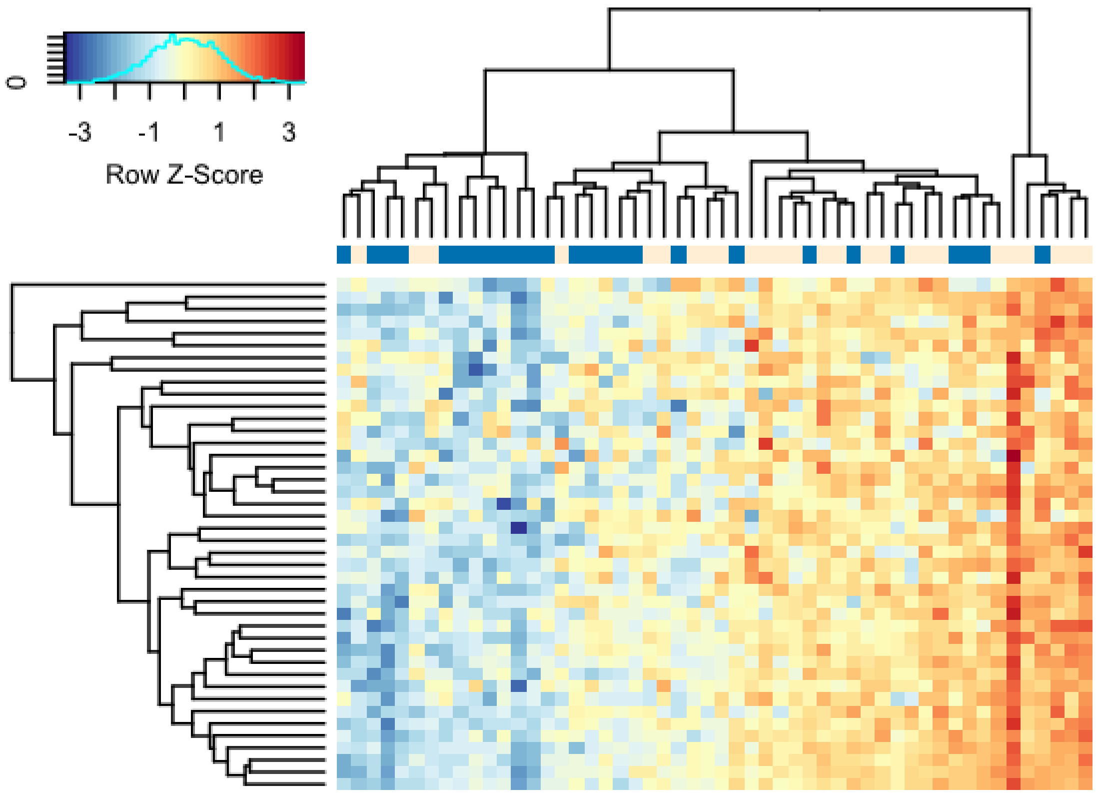
3. Gene expression analyses in a bobcat population

T cell activation

Inflammation process

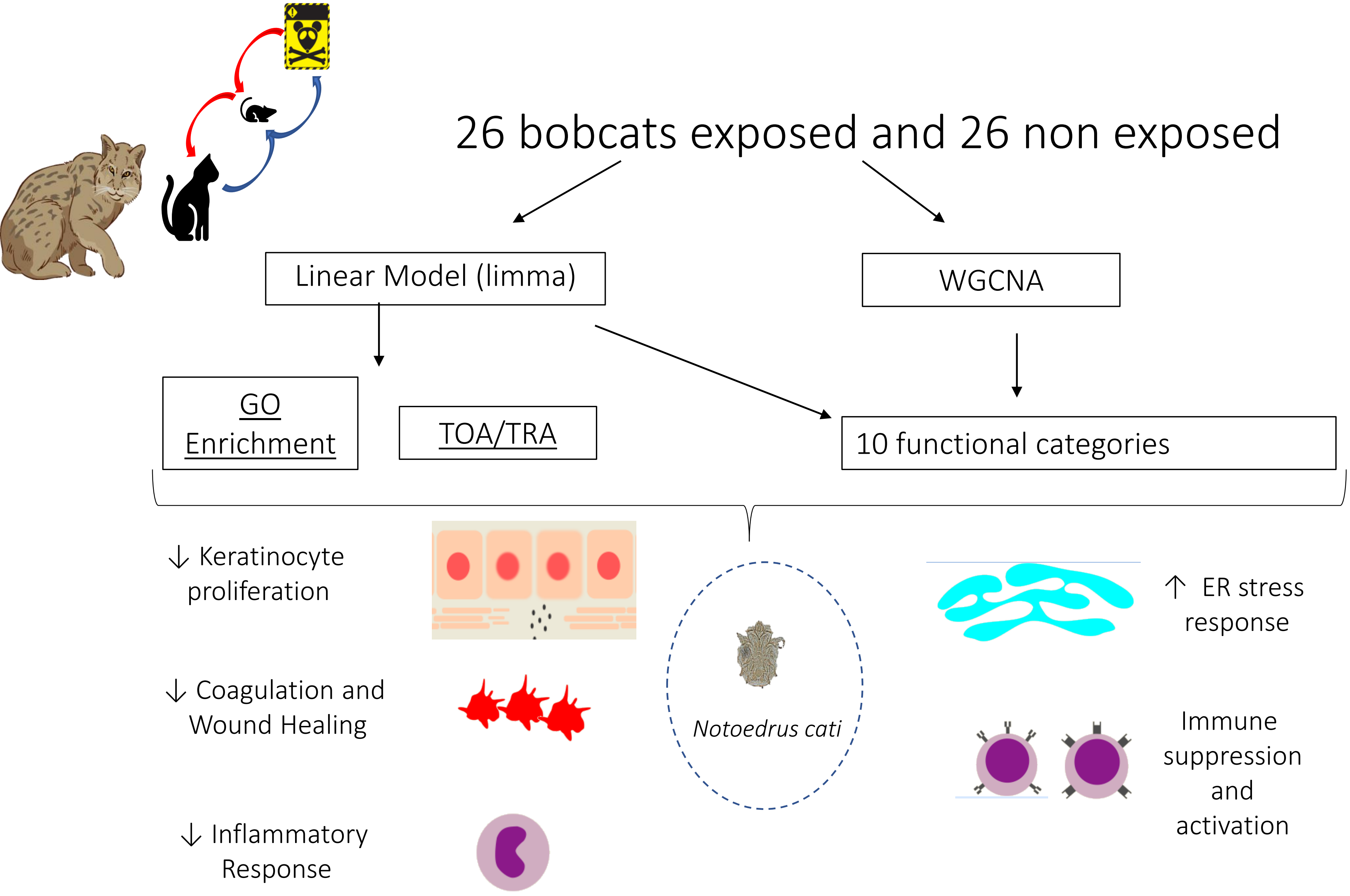
AR-negative (light color) and AR-positive (dark color)





Increasing expression T cell activation in AR positive bobcats
Decrease expression of inflammatory processes genes in AR positive bobcats

Fraser et al. (2018) *Molecular Ecology*

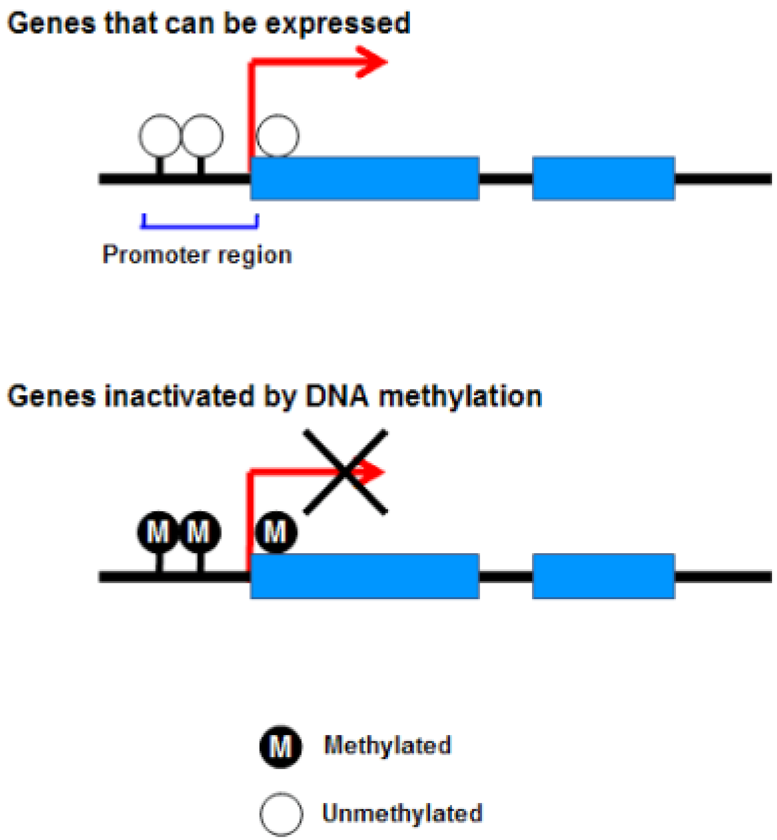


Impact on fitness

1) Decreased defense against extracellular pathogens and allergens
2) Immune activation leading to immune exhaustion
3) Increased cell death
4) Reduced epithelial integrity, more vulnerable to ectoparasites

Fraser *et al* 2018 Molecular Ecology

DNA methylation is a major gene regulatory mechanism in mammals



- Environmentally responsive (biotic stress, ..)
- Involved in aging
- Linked to many disease (e.g., cancer, diabetes, Alzheimer's)

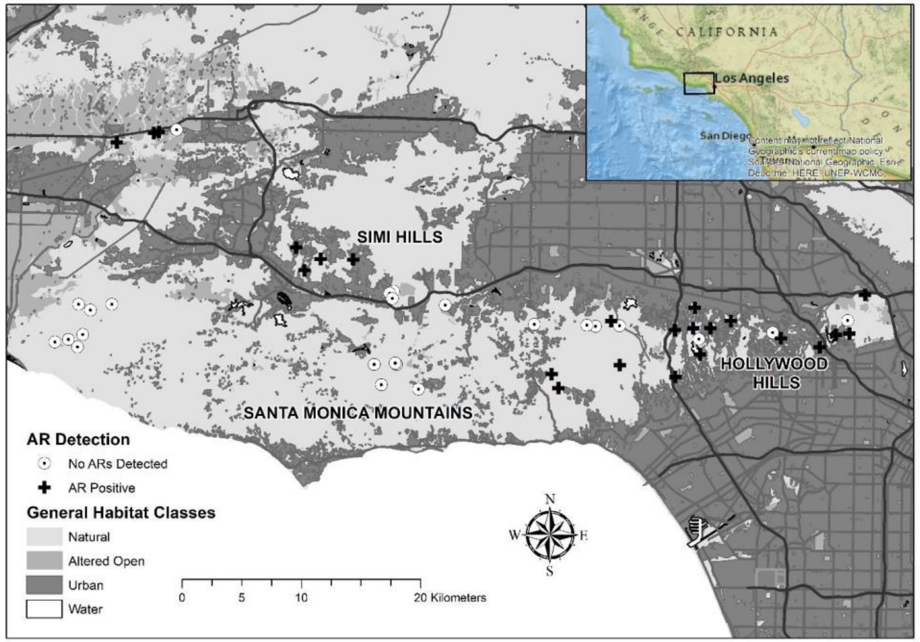
-- ...

Link exposure to ARs with gene expression patterns using a RNA-seq approach

↓

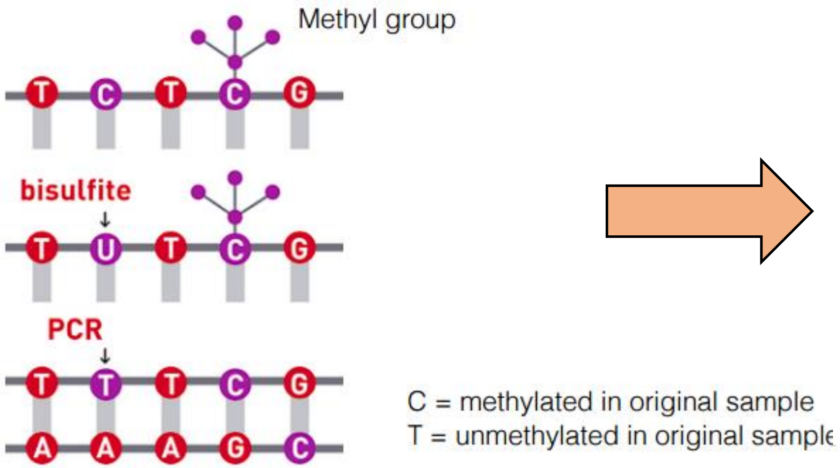
Exposure to ARs and methylation level?

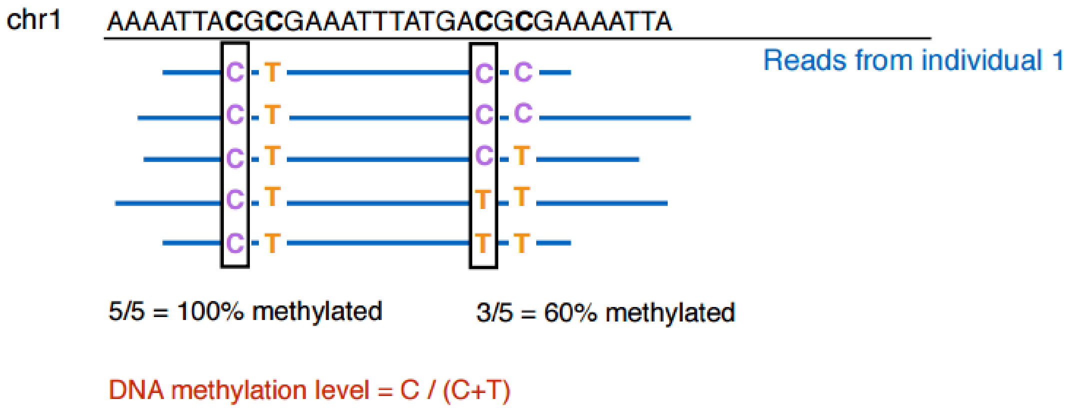
4. Complementary ongoing study : methylation and rat poison



Samples: 10 M « exposed » and 10 M « unexposed » bobcats
Whole blood DNA extraction

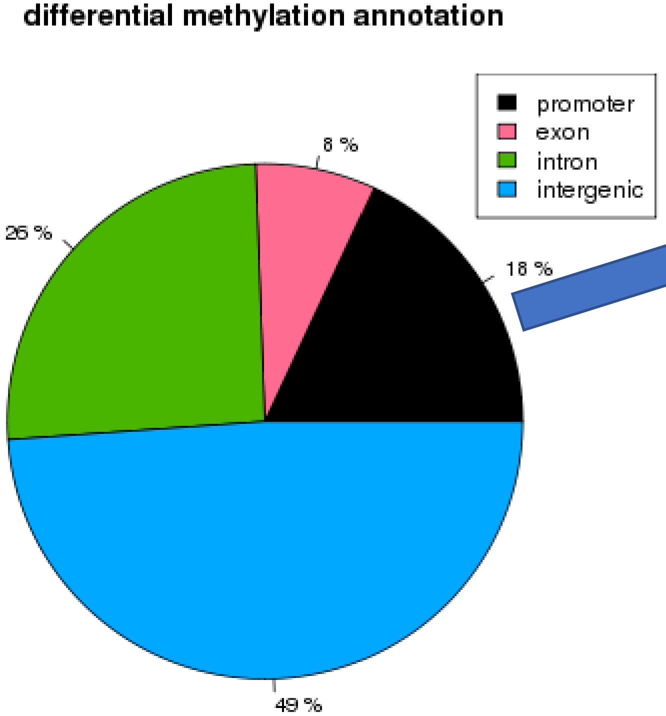
Methods: RRBS (Reduced Representation Bisulfite Sequencing) (Meissner et al. 2008; Gu et al. 2011; Boyle et al. 2012)
Macau (Differential methylation)





4. Complementary ongoing study : methylation and rat poison (preliminary results)

141 methylation sites differentially expressed (FDR< 0.1)



Genomation package

33 sites in promoter region=> closest gene => Gene ontology

- Regulation of cytokine (il6,il4,il2)
- Positive regulation of ER stress
- Macrophage differentiation
- T cell activation
- Regulation of platelet
- ….

Same GO than RNAseq analyses!!

15 genes in common with RNAseq => Biomarkers??

Conservation action

More studies on the sublethal effects of AR are needed!!

Los Angeles Times

CALIFORNIA

Two mountain lions found dead in Santa Monica Mountains had ingested rat poison

P-30 and P-53 were found dead in September and August, respectively. Both had rat poison in their systems. (National Park Service)

1 September 2020

California Legislature Passes Bill to Protect Wildlife From Super-toxic Rat Poisons

Moratorium on Dangerous Rodenticides Heads to Governor's Desk

SACRAMENTO, *Calif.*— The California legislature has passed a bill that would place a moratorium on super-toxic rodenticides until state agencies can develop better safeguards to protect wildlife from the dangerous, long-lasting poisons.

The California Ecosystems Protection Act (A.B. 1788) was passed late Monday just before the end of the session. The bill now goes to Gov. Gavin Newsom.

29 September 2020

Gov. Newsom Signs Bill Protecting Wild Animals from Super-toxic Rat Poisons

California Leads the Nation on Safeguards for Second-generation Anticoagulant Rodenticides

SACRAMENTO, *Calif.*—Gov. Gavin Newsom signed the California Ecosystems Protection Act (AB 1788) into law today, placing greater restrictions — with limited exceptions — on the use of second-generation anticoagulant rodenticides to protect the state's native wildlife.

The bill, introduced by Assemblymember Richard Bloom (D-Santa Monica), requires state regulators to reduce the threats to nontarget wildlife before the restrictions on second-generation anticoagulant rodenticides can be lifted.

Acknowledgements

- Dr. Devaughn Fraser (US Fish and Wildlife)
- Dr. Laurel Serieys (Cape town Uni.)
- Dr. Steve Cole (UCLA)
- Dr. Seth Riley (NPS)
- Dr. Robert Wayne (UCLA)
- Dr Scott Carver (Tasmania Uni.)
- Dr Sue Vandewoude (Colorado State Uni.)
- Wayne Lab (UCLA)
- Dr Bridgett Vonholdt (Princeton)
- Dr Matteo Pellegrini (UCLA)
- Dr Marco Morselli (UCLA)

UCLA QCBio Collaboratory

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The Belgian American Educational Foundation

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