Ancient convergent losses of the *Paraoxonase 1* gene could render marine mammals susceptible to organophosphate pesticides



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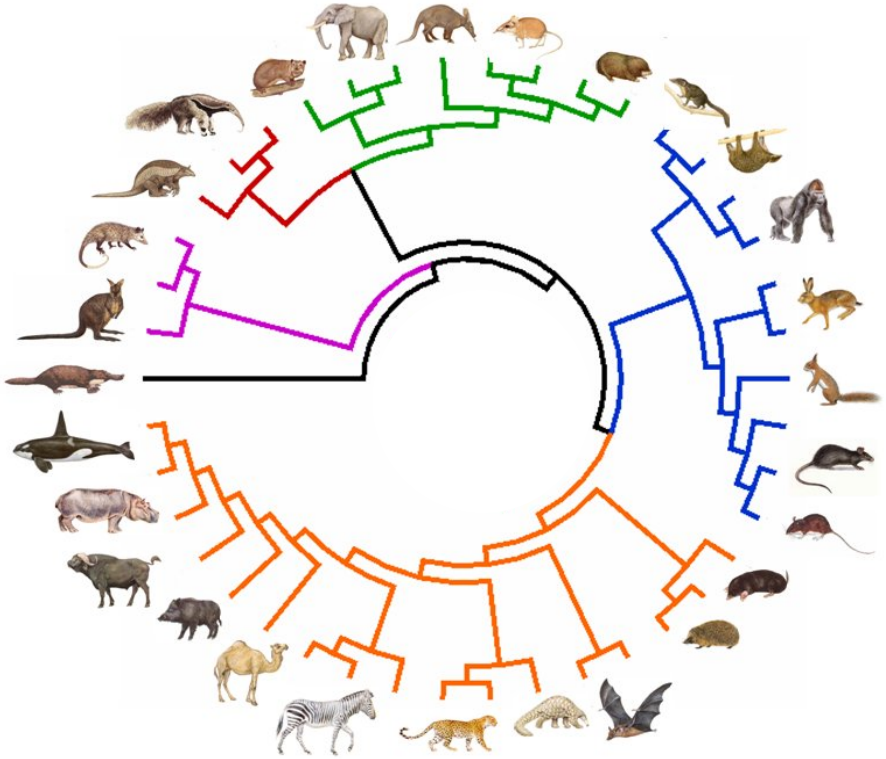
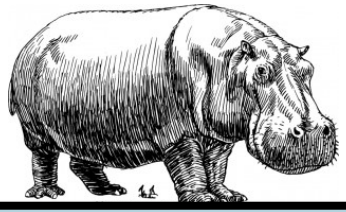
Mammalian Diversity and Genomics

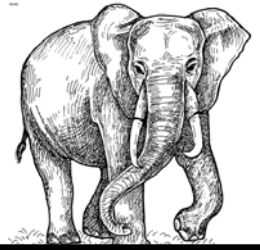
Mammals have adapted to a wide range of habitats over the past 100 Million years.

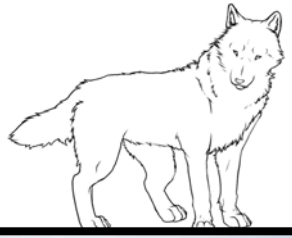
- Which functions are altered in a new environment?
- Can we reveal novel genes and regulatory regions mediating biomedical phenotypes?



Convergent Evolution in Marine Mammals





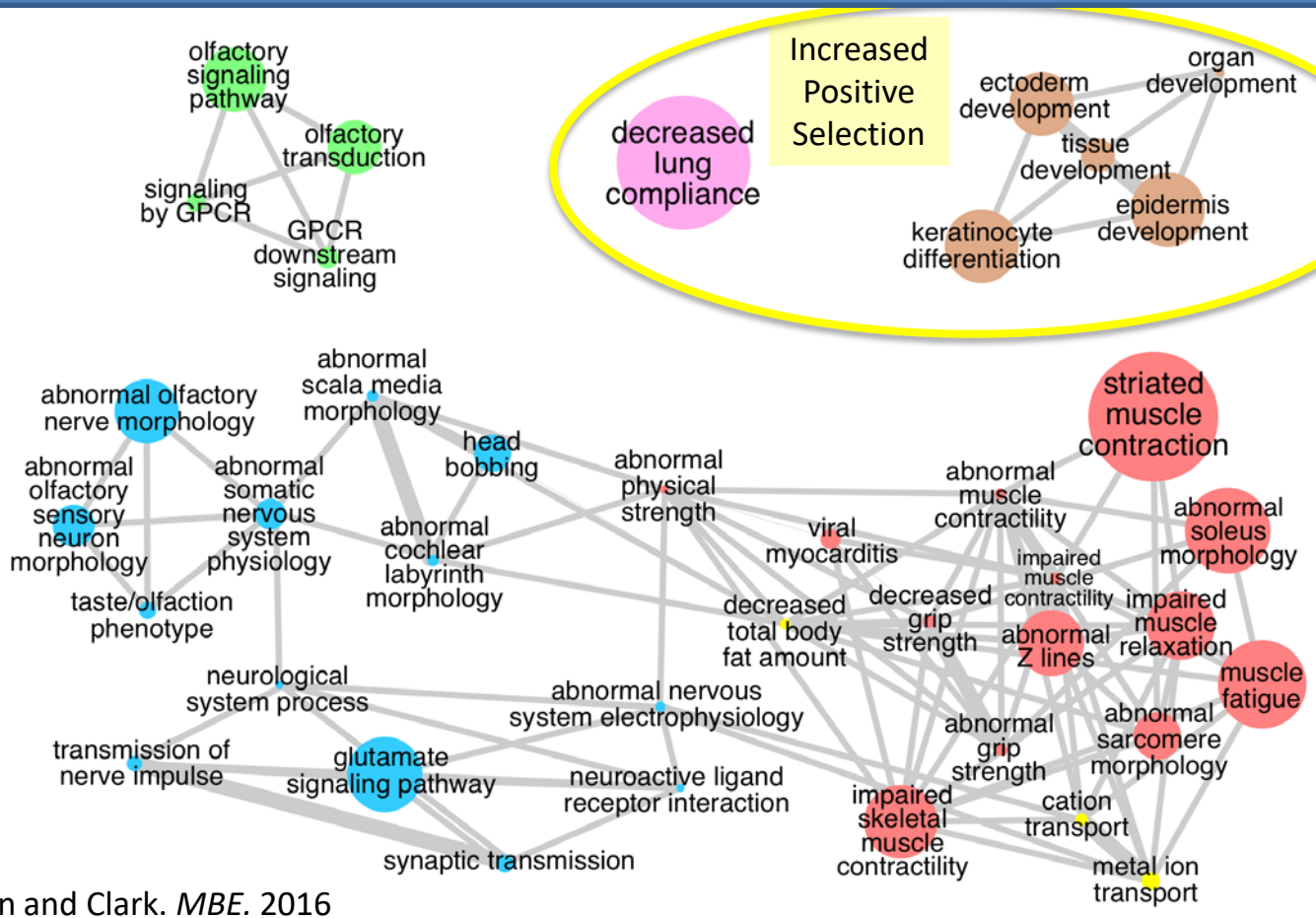


Goals:

Identify genetic changes underlying these adaptive traits.

Reveal as yet undiscovered adaptations.

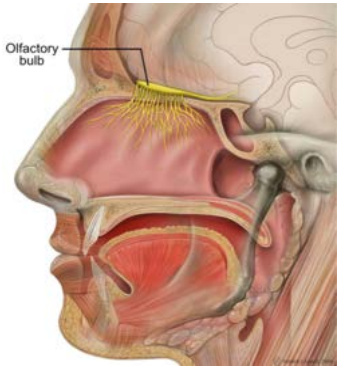
Functions enriched among marine-accelerated genes



Chikina, Robinson and Clark. *MBE.* 2016

Taste and Smell Are Reduced in Marine Mammals

- Olfactory receptors were lost *en masse*
  - Dolphins and whales have no olfactory bulb!



- Taste was known to be absent in cetaceans.
- We have shown that it is likely convergently reduced in manatees and pinnipeds.
  - Could this be because they swallow food whole?
  - Do they rely on visual cues instead?

Genes whose **loss** correlates with the marine environment

Gene presence (1) / absence (0)

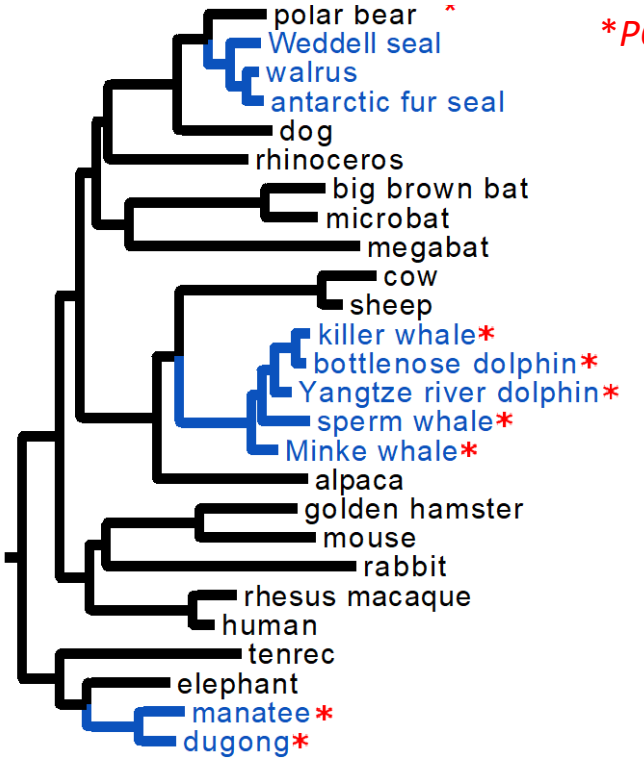
Gene	dolphin	killer whale	Weddell seal	Pacific walrus	manatee	chimp	human	gorilla	orangutan	gibbon	Rhesus macaque	crab-eating macaque	baboon	green monkey	marmoset	squirrel monkey	bushbaby	Chinese tree shrew	ground squirrel	Egyptian jerboa	prairie vole	Chinese hamster	golden hamster	mouse	rat	naked mole-rat	Guinea pig	chinchilla	brush-tailed rat
PON1	0	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
ANKRD23	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
XRCC6	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
OR9Q1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
PROS1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
FGD2	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
EMBP1	0	1	0	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
KRT6A	1	0	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CCDC170	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
GJD4	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Gene	rabbit	pika	pig	alpaca	bactrian camel	Tibetan antelope	cow	sheep	domestic goat	horse	white rhinoceros	cat	dog	ferret	panda	black flying-fox	megabat	David's Myotis bat	microbat	big brown bat	hedgehog	shrew	star-nosed mole	elephant	Cape elephant shrew	Cape golden mole	tenrec	aardvark	armadillo
PON1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
ANKRD23	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	0
XRCC6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
OR9Q1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
PROS1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
FGD2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
EMBP1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1
KRT6A	1	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CCDC170	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
GJD4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

(via BayesTraits by Mark Pagel)

	Independent model		Dependent model				
Gene	Log likelihood	gene loss rate	Log likelihood	marine loss rate	terrestrial loss rate	P-value	Description
PON1	-26.7294	2.31	-16.3059	100.00	0.00	4.97E-06	paraoxonase 1
ANKRD23	-33.0866	4.90	-25.7259	100.00	2.52	1.25E-04	ankyrin repeat domain 23 (skeletal muscle)
XRCC6	-25.3000	1.51	-18.0866	59.75	0.00	1.46E-04	X-ray repair cross-complementing 6 (Ku70)
OR9Q1	-25.4322	1.51	-18.2294	59.37	0.00	1.47E-04	olfactory receptor
PROS1	-25.4304	1.51	-18.2478	58.62	0.00	1.51E-04	protein S (alpha)
FGD2	-25.5627	1.51	-18.3903	58.26	0.00	1.52E-04	FYVE, RhoGEF and PH domain containing 2
EMBP1	-38.7641	3.81	-31.6236	100.00	1.58	1.57E-04	embigin pseudogene 1
KRT6A	-33.7456	3.89	-26.6269	100.00	1.61	1.61E-04	keratin 6A (epithelial, type II cytokeratin)

PON1 gene loss viewed across mammals



**PON1* contains lesions*

Has Pon1 been inactivated in manatees and dugongs?

Sample: 7 manatees from Florida

3 dugongs from Australia

Jerrica Jamison

Wynn Meyer

Pittsburgh Zoo & PPG Aquarium

Joe Gaspard

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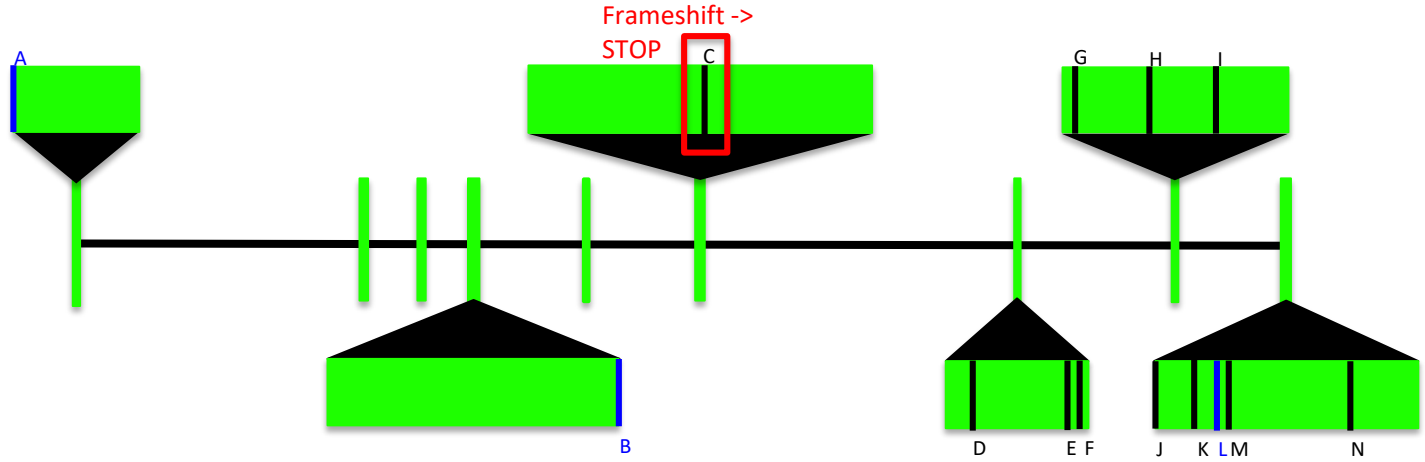
USGS Gainsville

Janet Lanyon
University of Queensland

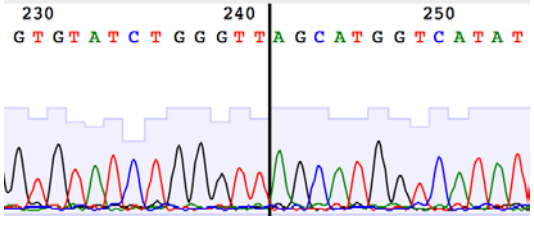
Predicted Lesions in Manatee PON1



Lesion Not Dependent on Frameshift

Lesion Dependent on Frameshift

Manatee

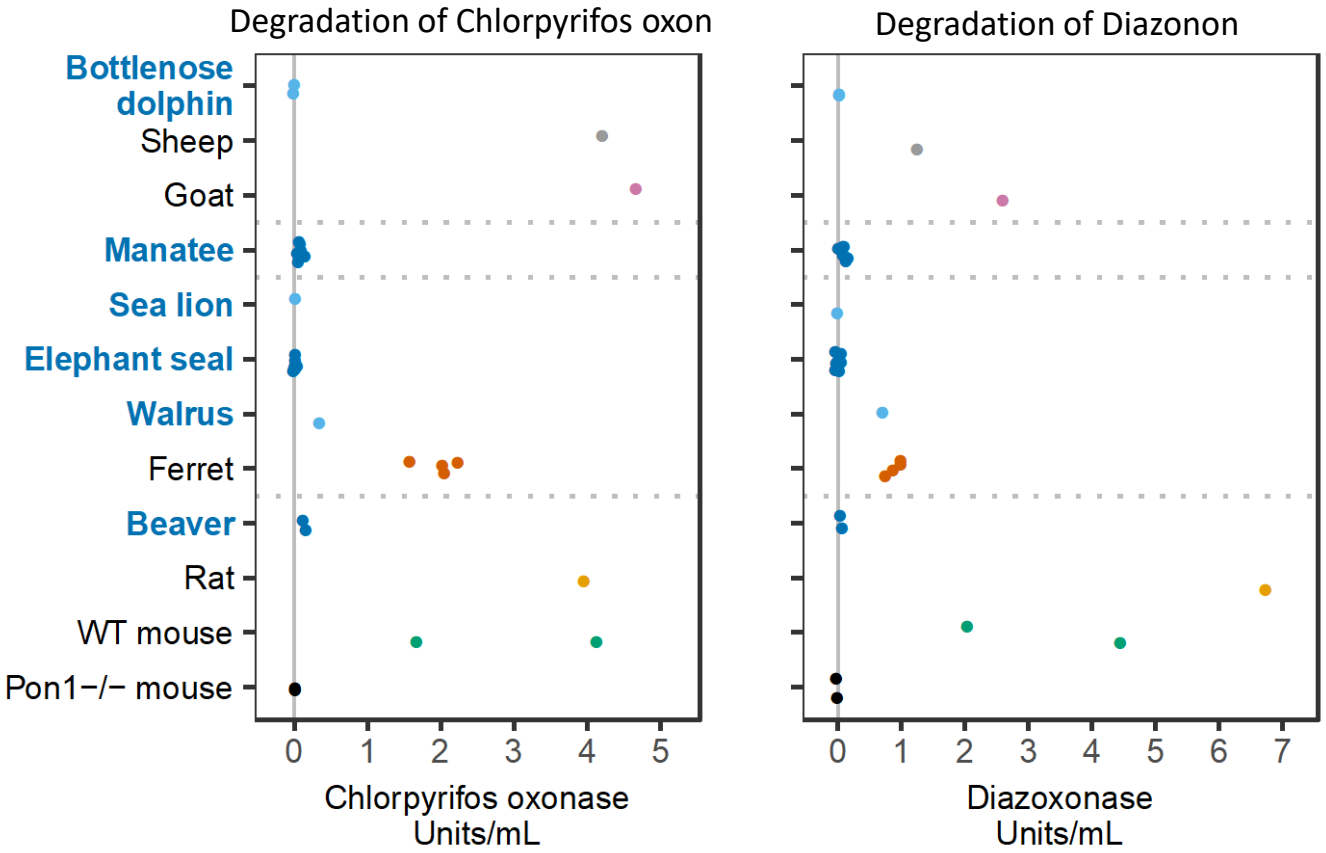


Jerrica Jamison

Summary of *PON1* Genetic Lesions in Mammals

	Loss-of-function substitution	Ablated Start	Ablated Start	Ablated Start	Frameshift	Early Stop	Frameshift	Frameshift	Frameshift	Frameshift	Frameshift	Early Stop	Ablated Splice Site + Frameshift	Ablated Splice Site	Early Stop	Frameshift	Earyly Stop	Frameshift	Frameshift	Frameshift	Ablated Splice Site	Frameshift	Early Stop	Early Stop	Frameshift	dN/dS ratio
	Exon	1	1	1	1	2	2	4	3	4	4	4	4/5	4/5	5	6	6	6	6	8	8/9	9	9	9	9	
	Human nt position	1	2	3	28	79	99	175	272	319	331	367	370	371	448	508	580	597	670	789	910	916	946	963	985	
Cetaceans	**Bottlenose Dolphin**				■	■	■	■	■										■		■			■		**1.57**
	River Dolphin				■	■	■	■	■										■	■	■			■		0.39
	Killer Whale				■	■	■	■	■							■	■		■		■			■		**1.17**
	Sperm Whale			■	■	■	■	■	■										■		■			■	■	**2.1**
	Minke Whale				■	■	■	■	■										■		■			■		**2.81**

Pon1 biochemical activity is absent in most aquatic mammals



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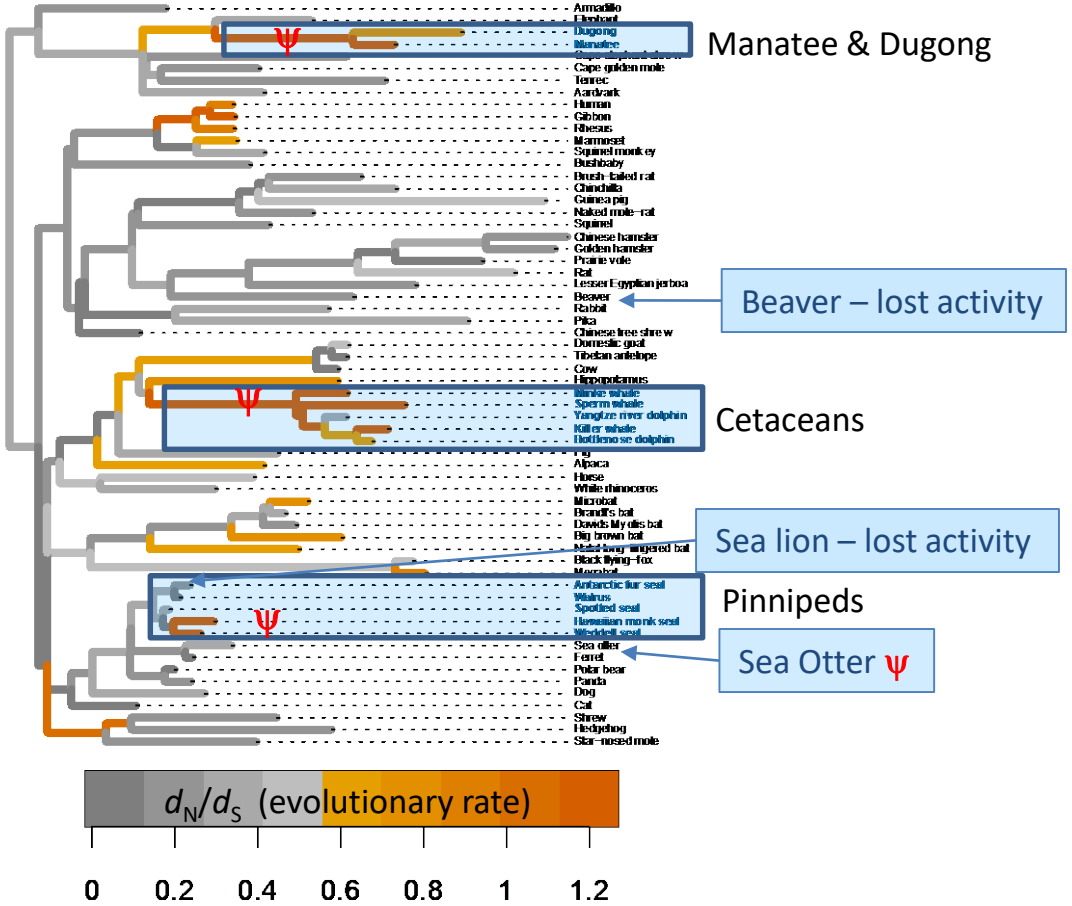
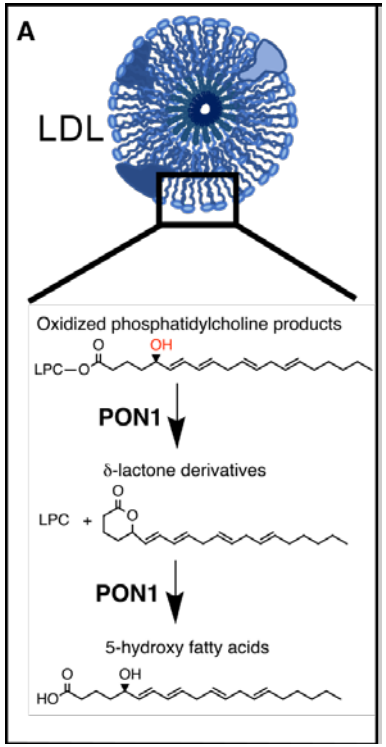
PON1 gene loss viewed across mammals

ψ = pseudogenization



What does Paraoxonase 1 (Pon1) do?

Mitigates damage to oxidized lipids in bloodstream



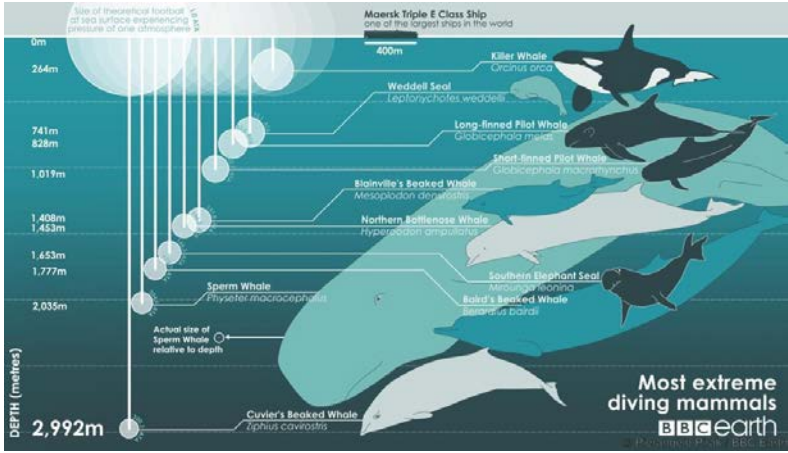
Human polymorphisms in *PON1* are associated with vascular disease.

Pon1 ⊣ Oxidized lipids → Inflammation → Atherosclerosis

Why was PON1 lost in aquatic mammals?

Hypothesis 1: Diving and oxidative stress

- PON1 repairs oxidative damage
- Diving is stressful!
- Repeated oxygen depletion & reperfusion
- Functional changes in antioxidants in diving species

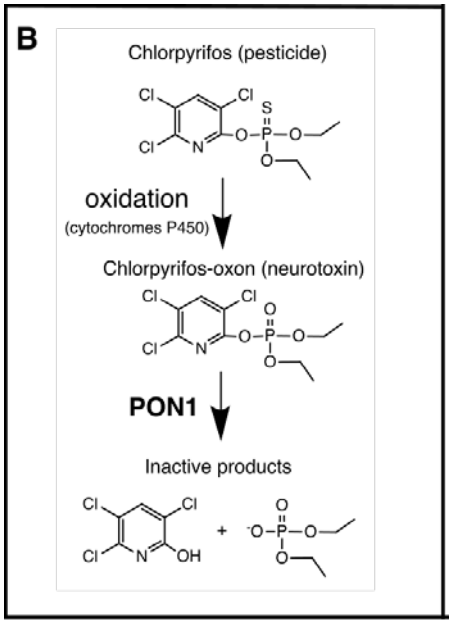


Hypothesis 2: Shift in dietary fatty acid intake

- Aquatic ecosystems have high ω-3/ω-6 fatty acid ratios
- Differences in rates of oxidation

What else does Pon1 do?

Breaks down organophosphate pesticides



Pon1 degrades the oxon forms of these pesticides:

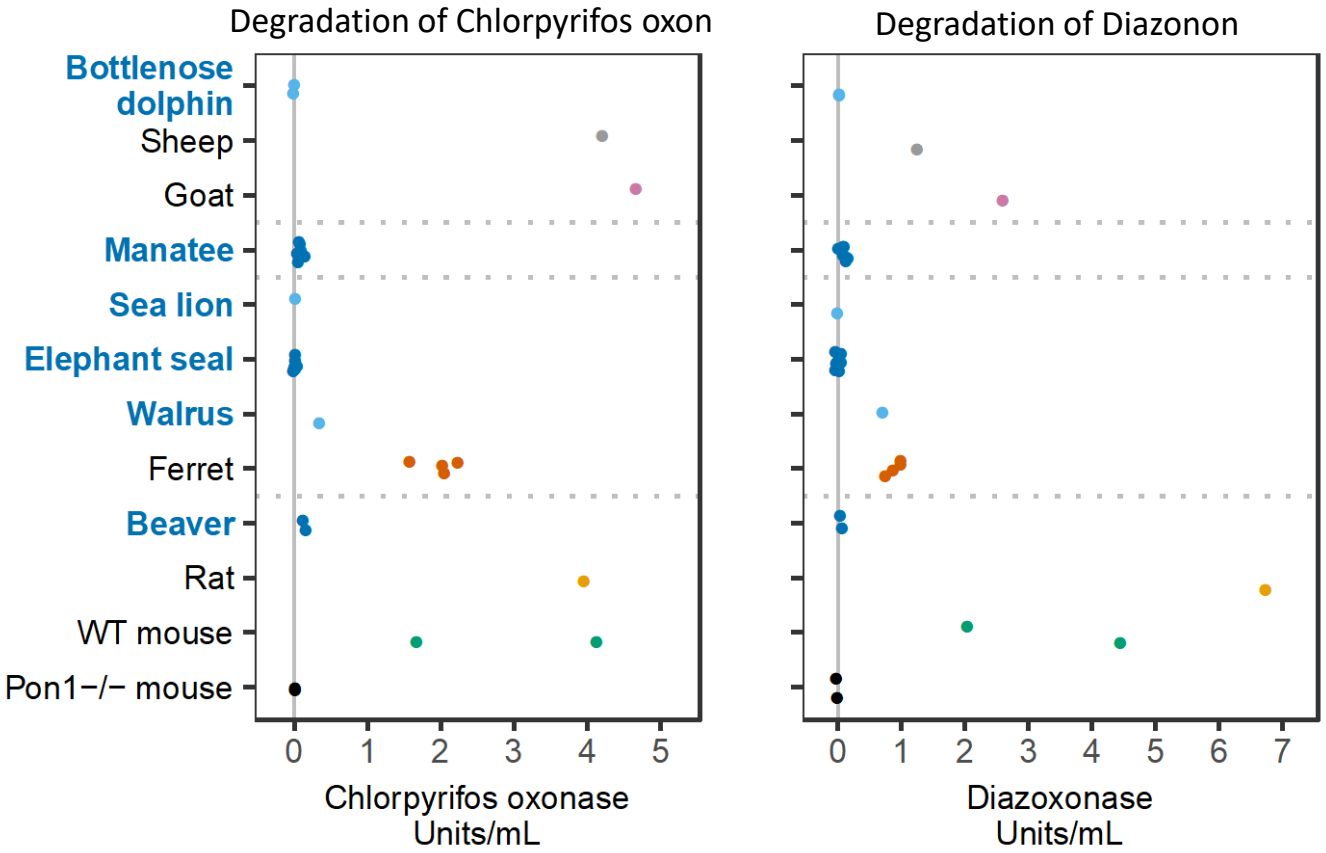
- Chlorpyrifos
- Diazonon
- Malathion

Pon1 is the main line of defense.

Mice lacking Pon1 are highly sensitive to these organophosphates.

Organophosphates are effective pesticides because insects lack Pon1.

Pon1 biochemical activity is absent in most aquatic mammals



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Potential implications for Pon1 loss in aquatic mammals

- Acute Exposure
  - Neurotoxin, Inhibits acetylcholine esterase
  - Kills *pon1*$^{-/-}$ knock-out mice
- Agricultural run-off
  - Chlorpyrifos
    - Widely used in agriculture
    - Cognitive delay in children of farmworkers
    - Ban recently reversed
  - Malathion
    - Agricultural & Residential (Most commonly used in USA)
    - Mosquito control
  - Naled
    - Recent increase in use for mosquito control
- Chronic Exposure
  - Potentially concentrated through bioloading through food chain
  - Potentially passed to infants through milk

Potential implications for Pon1 loss in aquatic mammals

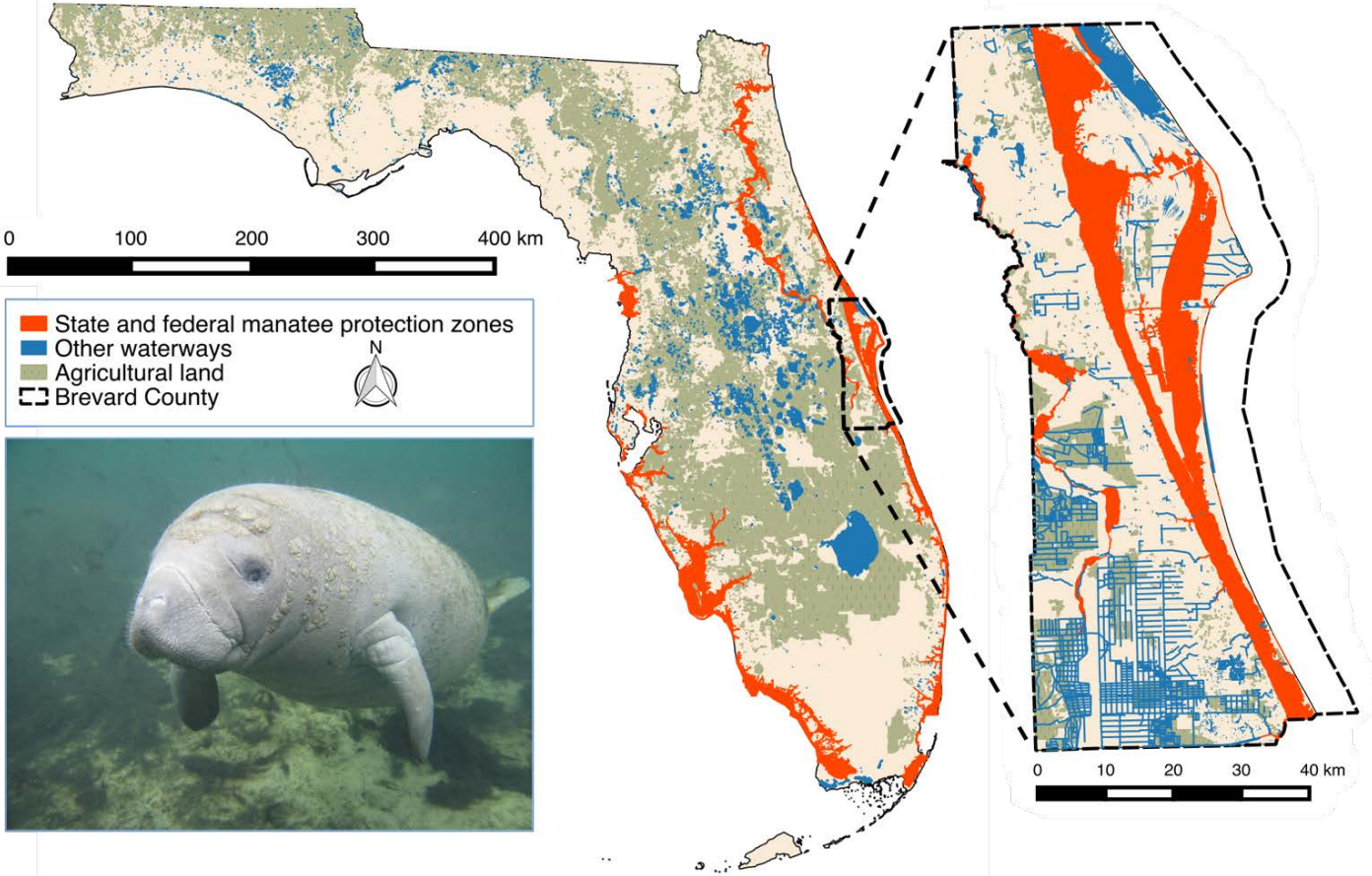


Photo:
Robert Bonde
USGS, Gainesville

Maps:
Stacy E Woods
Johns Hopkins University

Organophosphates in marine mammal waterways

- Carriger & Rand. *Ecotoxicology* 2008

Chlorpyrifos found in Canal C-111 upstream of Manatee bay (S. Biscayne Bay)

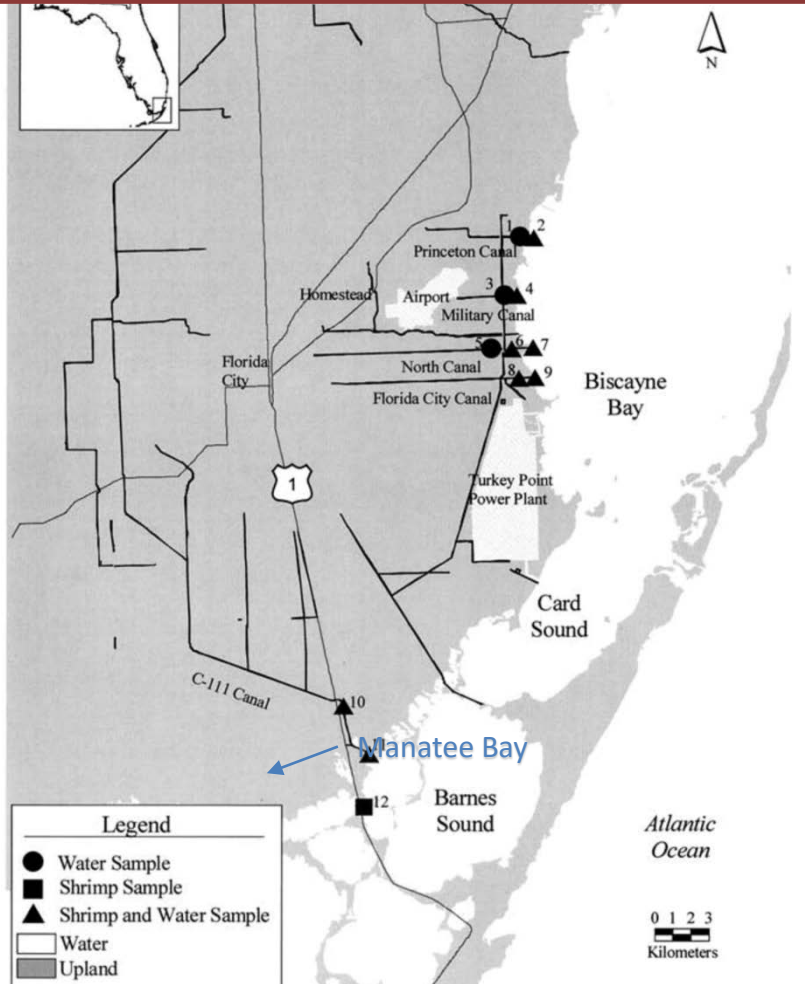
- Scott et al. *J of Agricultural and Food Chem.* 2002

Chlorpyrifos found in Southern Florida waterways (Canal C-111)

- Key et al. *Arch. Environ. Contam. Toxicol.* 2003

Chlorpyrifos, malathion, and diazinon found in 2 canals (Military and North) draining into Biscayne Bay

(Key et al. 2003)



Action Items

Associate Pon1 loss with relevant phenotypes

- Study more aquatic mammals (pinnipeds, nutria, river otters…)
  - *We need aquatic mammal blood samples*
- Contrast diving abilities & markers of oxidative stress
- Contrast diets / lipid intake

Test markers of organophosphate exposure

- Acetyl cholinesterase in red blood cells
- *Blood samples from 1 species across locations and times*

Monitor organophosphates in waterways

- *Anyone interested?*

Follow markers of organophosphate exposure

We need blood samples from a species across locations and times

Create model of potential exposure in waterways.

- Surrounding land use, Pesticide application, Degradation time, Water flow

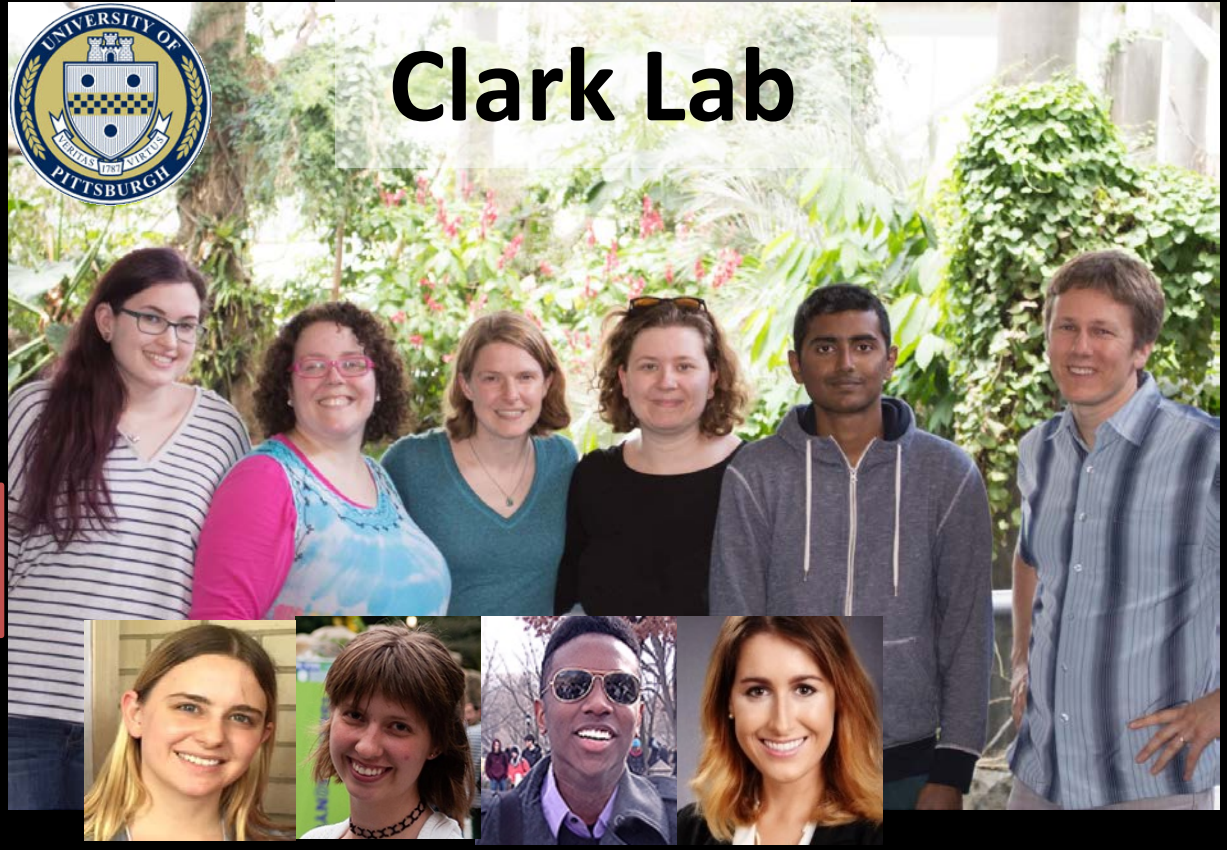
Blood-based marker of exposure in many animals

- Acetyl cholinesterase activity in red blood cells (Standard for agricultural workers)
- Compare to “non-exposed” baseline. (Use waterway model)
- Is marker stronger in animals predicted to be exposed?

Model Study - Key et al. *Arch. Environ. Contam. Toxicol.* 2003

- Acetyl cholinesterase activity is lower in shrimp from exposed waterways.

Clark Lab



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