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Ancient convergent losses of *Paraoxonase 1* yield potential risks for modern marine mammals

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Abstract

Mammals diversified by colonizing drastically different environments, with each transition yielding numerous molecular changes including losses of protein function. While not initially deleterious, these losses could subsequently carry deleterious pleiotropic consequences. Here we

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Author contributions: NLC, MC, CEF, and WKM designed the study. RB, DEC, JG, JML, and CEF provided samples and reagents. JJ, JM, and RR performed laboratory experiments. WKM, JJ, RP, AK, CK, and NLC performed analyses. WKM, SEW, RP, AK, and NLC generated figures. WKM, CEF, and NLC wrote the paper.

Competing interests: The authors declare no competing financial interests.

Data and materials availability: The data reported in this paper are tabulated in the Supplementary Materials. Resequencing data for *PON1* coding sequence in dugong is available in GenBank (accession MF197755). Scripts used in analyses are available at https://github.com/nclark-lab/MarineFxLoss. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Supplementary Materials:
Materials and Methods
Figures S1–S6
Tables S2, S3, S6, S8, S11, and S12*
*Tables S1, S4, S5, S7, S9, S10, and S13 are available as Excel files.
References (33–103)

use phylogenetic methods to identify convergent functional losses across independent marine mammal lineages. In one extreme case, *Paraoxonase 1* (*PON1*) accrued lesions in all marine lineages, while remaining intact in all terrestrial mammals. These lesions coincide with PON1 enzymatic activity loss in marine species' blood plasma. This convergent loss is likely explained by parallel shifts in marine ancestors' lipid metabolism and/or bloodstream oxidative environment affecting PON1's role in fatty acid oxidation. PON1 loss also eliminates marine mammals' main defense against neurotoxicity from specific man-made organophosphorus compounds, implying potential risks in modern environments.

One Sentence Summary:

Organophosphate toxicity may threaten modern marine mammals due to their ancestors' repeated loss of PON1 for oxidative or metabolic reasons.

As the ancestors of aquatic marine mammals adopted obligate aquatic lifestyles, they evolved many adaptive changes, such as those that improved locomotion in and perception of their new environment (1, 2). Many of these morphological and physiological changes occurred in parallel in distinct lineages of marine mammals, including cetaceans, pinnipeds, and sirenians. Although convergent trait changes are frequently adaptive, environmental transitions can also result in non-adaptive convergent trait loss due to release from functional constraint. Examples of convergently reduced or lost traits include olfaction in marine mammals (3–5), bitter taste receptors in carnivorous tetrapods (6), and eyes in subterranean species (7–9). Any convergent evolutionary change in the context of a given environment can carry negative consequences in a different environment as a result of pleiotropy (one genetic locus influencing multiple phenotypes).

To characterize how mammals responded to selective pressures imposed by the marine environment, we identified genes that convergently lost function in marine mammals. We identified candidate pseudogenes with observed early stop codons and/or frameshifts (genetic lesions) in 58 eutherian mammals' genomes in a 100-way vertebrate alignment (http://genome.ucsc.edu/). Using our predicted pseudogene calls, we then tested, for each gene, whether its pattern of functional loss was better explained by a model with one loss rate throughout the mammalian phylogeny or by a model in which the loss rate was dependent upon the terrestrial/marine state of a given branch, using a likelihood ratio test (LRT) (10). To ensure that our results were not strongly influenced by errors in pseudogene calling, we performed manual checks of lesion calls against reference genomes for our top genes, along with comparisons of pseudogene calls at highly conserved genes for marine and terrestrial species (11). We used simulations to estimate empirical gene-specific *P*-values and study-wide (multiple-test-corrected) false discovery rates (FDR) for all genes (11) (Tables 1 and S1). The set of genes with the strongest evidence for a higher loss rate on marine lineages was strongly enriched for functions related to chemosensation, driven by many olfactory and taste receptors (Tables S2–S5). These results are consistent with previous behavioral, anatomical, and genetic studies indicating a reduction of smell and taste in marine mammals (4, 12, 13).

We also observed a striking pattern of convergent loss in the marine environment at *Paraoxonase 1* (*PON1*) (Table 1) (11). *PON1* encodes a bloodstream enzyme that reduces oxidative damage to lipids in low- and high-density lipoprotein particles (LDL and HDL, respectively), potentially preventing atherosclerotic plaque formation (14, 15) (Fig. 1A). PON1 also hydrolyzes the oxon forms of specific organophosphate compounds, such that it is the main line of defense against some man-made pesticide byproducts, including chlorpyrifos oxon and diazoxon (Fig. 1B) (16). The *PON1* coding sequence contains genetic lesions in the cetacean, pinniped, and sirenian lineages but is intact in all 53 terrestrial mammal genomes surveyed (Fig. 1C; Table S1).

To estimate when PON1 function was lost in the three marine mammal clades, we obtained *PON1* sequences for 14 additional species including three cetaceans, the dugong, and two pinnipeds, and we estimated evolutionary rates across the mammalian phylogeny (11) (Figs. 1C and S1). We observed shared genetic lesions among all sequenced cetaceans and a different shared lesion in sirenians (Fig. S2), and the inferred ratio of non-synonymous-to-synonymous substitutions (d_N/d_S) was not significantly different from one on the ancestral branches of both clades (cetacean ancestor $d_N/d_S = 1.09$, $P = 0.79$; sirenian ancestor $d_N/d_S = 1.20$, $P = 0.57$). This suggests that PON1 lost functional constraint in the ancestral cetacean lineage soon after its split with the ancestral hippopotamid lineage, approximately 53 MYA (95% confidence interval lower bound: 34.5 MYA) (11, 17). In sirenians, functional loss occurred soon after their split with the ancestral elephantid lineage, approximately 64 MYA (lower bound 41.7 MYA) (17).

In pinnipeds, we observed clear evidence of PON1 functional loss only among a subset of species within family Phocidae, wherein Weddell seal and Hawaiian monk seal *PON1* sequences contained non-shared genetic lesions (Fig. S2). Because these branches are short, it is difficult to estimate precisely when functional loss occurred in pinnipeds; however, there was likely at least one loss since the Phocidae:Otarioidea split approximately 21 MYA (95% CI: 0 – 21 MYA). This incomplete loss could reflect either a difference between the selective environments experienced by pinnipeds and those experienced by other marine mammals, or it could reflect pinnipeds' more recent colonization of the marine environment (pinnipeds: 24 MYA, cetaceans: 44.7 – 37.3 MYA, sirenians: 47.1 – 43.9 MYA) (18).

PON1's functional loss in marine mammals may be related to its role in lipid metabolism via fatty acid beta-oxidation (19) (Tables S6 and S7). Compared to their terrestrial relatives, the diets of both herbivorous and carnivorous aquatic mammals contain a higher proportion of w-3 relative to ω−6 polyunsaturated fatty acids (PUFAs) (20), and these PUFAs differ in their capacity to sustain oxidative damage (21). Marine and terrestrial mammals also have vastly different antioxidant profiles (22, 23), presumably due to the extreme oxidative stress experienced during diving, with repeated cycles of hypoxia and reperfusion. Rewiring of either lipid metabolism or antioxidant networks in ancient marine mammals could have obviated the function of PON1. Supporting the antioxidant hypothesis, the Weddell seal, which carries *PON1* lesions, is one of the longest diving pinnipeds known, in contrast to the shorter diving walrus and Antarctic fur seal, which lack lesions but share an aquatic diet (24). However, two semi-aquatic mammals, the sea otter and the beaver, which are more

moderate divers (24), also have either lesions or substitutions at sites predicted to be necessary for PON1 function (Fig. S2; Table S8).

Whatever the cause, loss of PON1 function could carry negative pleiotropic consequences for the health of marine mammals repeatedly exposed to man-made organophosphate compounds. PON1 alone is protective against the highly toxic oxon forms of the heavily used pesticides chlorpyrifos and diazinon; these oxons are formed from the parent compounds in the environment and *in vivo* by cytochromes P450 (25) (Fig. 1B). We tested blood plasma from six marine and semi-aquatic species for capacity to hydrolyze these and other PON1 substrates (Figs. 2 and S3). The plasma from all but one of the assayed marine and semi-aquatic species showed activity levels against the PON1 substrates that more closely resembled those of the *Pon1* knockout *(Pon1−/−)* mouse than those of terrestrial outgroups. Thus, the genetic deterioration of *PON1* has left these species without a mechanism to break down specific neurotoxic compounds.

Given the sensitivity of $Pon1^{-/-}$ mice to organophosphate exposure (26), the inability of most marine mammal plasma to detoxify organophosphates suggests the potential for neurotoxicity if sufficient levels of these compounds accumulate in these animals' habitats or food sources. In Florida, agricultural use of organophosphate pesticides is common, and runoff can drain into manatee habitats. In Brevard County, where an estimated 70% of Atlantic Coast manatees migrate or seasonally reside (27, 28), agricultural lands frequently abut manatee protection zones and waterways (Fig. 3). Limited sampling upstream of Manatee Bay has measured levels of chlorpyrifos as high as 0.023 μg/L (29), and levels could be much higher directly after pesticide applications (30). Dugongs may be at risk of exposure to organophosphorus pesticides that are used in the sugar cane industry along the Queensland coast and have been detected at 5 – 270 pg/L in coastal river systems (31). Carnivorous marine mammals may also ingest these compounds through their diets of invertebrates and fish, which have shown evidence of bioaccumulation of organophosphates in Arctic populations (32). In order to improve our understanding of the extent of exposure and attendant risk marine mammals face, we recommend increased monitoring of marine mammal habitats, as well as the testing of tissues from deceased animals for biomarkers of organophosphate exposure.

The presence of these potential risks to many marine mammals due to their loss of PON1 function provides a clear example of the tradeoffs possible in evolution: although PON1 functional loss was not deleterious and may even have been beneficial in ancestral marine environments, it may carry detrimental fitness consequences in modern environments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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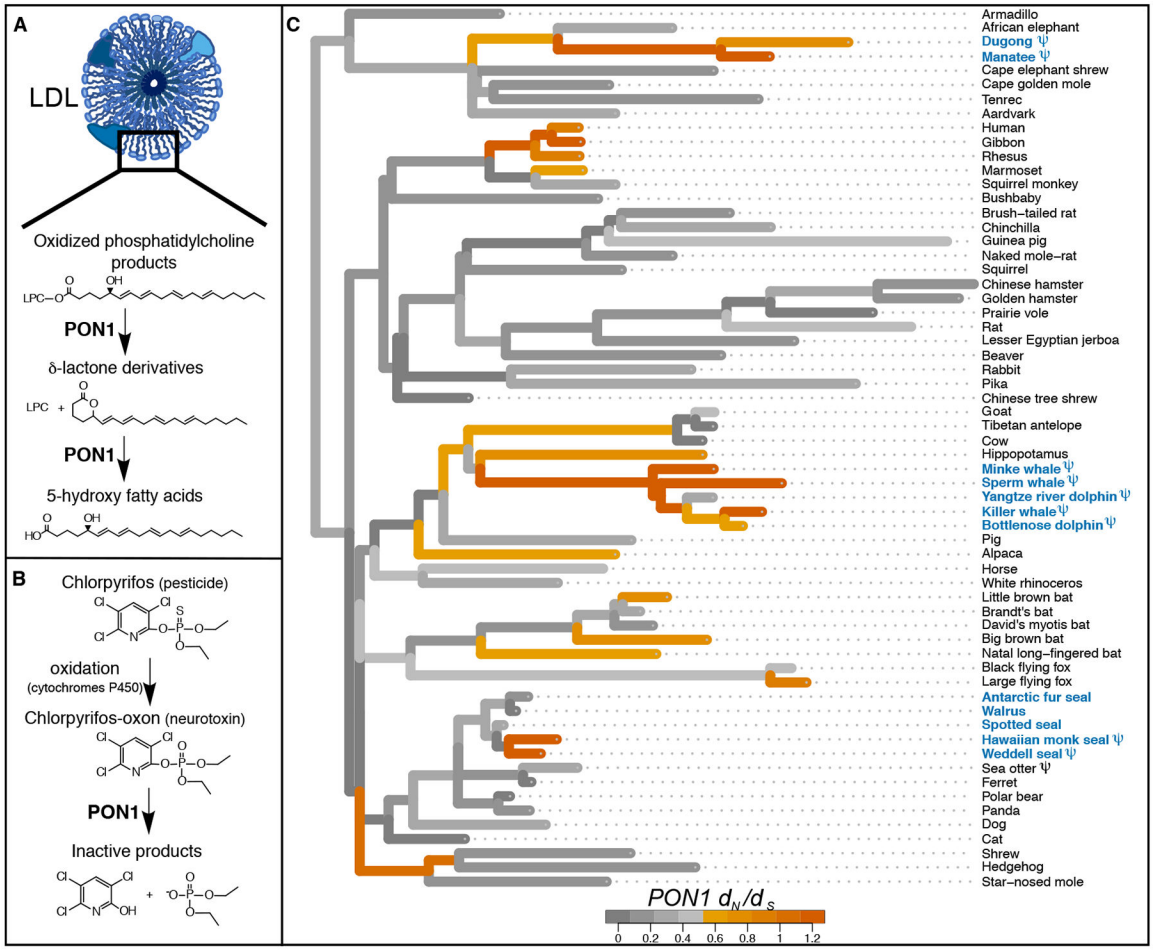

Fig. 1. PON1 functions and evolutionary history.

Illustration of PON1's proposed roles in **(A)** preventing oxidative damage to low- and high-density lipoproteins (14, 15) and **(B)** detoxifying the oxon byproduct/metabolite of a common organophosphorus pesticide, chlorpyrifos (25). **(C)** Evolutionary rate of *PON1* coding sequence across the phylogeny of 62 eutherian mammals. Branch lengths represent d_N, and colors represent d_N/d_S (see color legend). d_N/d_S values greater than 1.2 were set to 1.2. Blue: marine species. ψ: genetic lesion(s) present.

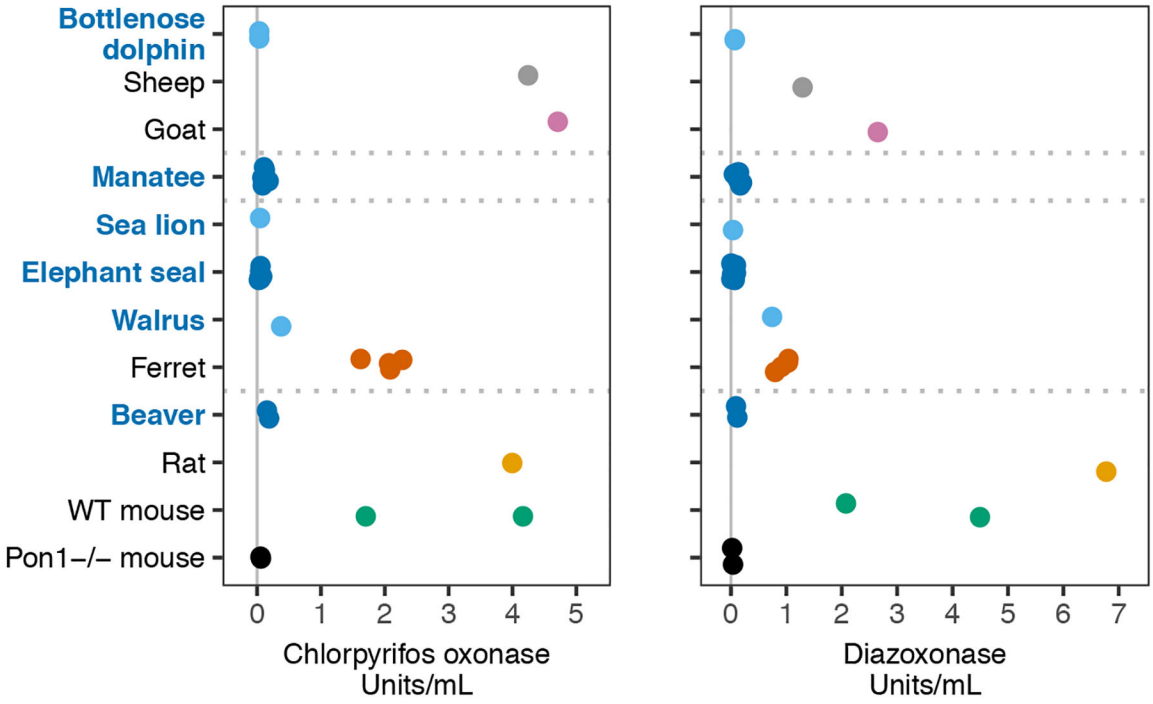

Fig. 2. Blood plasma enzymatic activity against two organophosphate-derived substrates.

Points represent rates of hydrolysis of chlorpyrifos oxon (left) or diazoxon (right) in μmol/min/mL for plasma from marine and semi-aquatic species (in blue) and terrestrial outgroups. Values for sheep, goat, and rat are from Furlong et al. (33), who performed assays under the same experimental conditions as in this study. Control assays of alkaline phosphatase activity show samples were not degraded (Fig. S3).

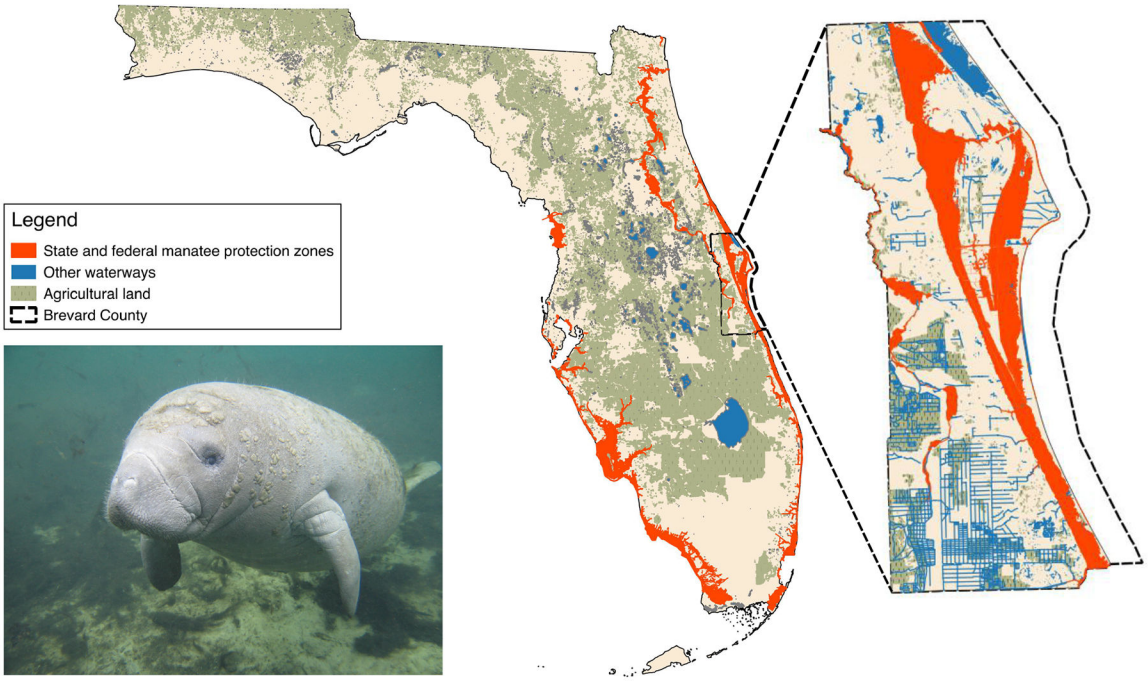

Fig. 3. Manatee and adjacency of its habitat to agricultural land use.

Left: Florida manatee (photo by Robert K. Bonde, 2006). Center: Manatee protection zones and agricultural land in Florida. Right: Manatee protection zones, waterways, and agricultural land in Brevard County.

Table 1.

Top 10 manually validated genes with evidence for marine-specific loss

Gene	Loss rate (independent)	Marine loss rate (dependent)	Terrestrial loss rate (dependent)	LRT statistic	Empirical P-value	FDR	Description
PON1	0.672	49.7	0	22.24	3.08×10^{-6}	0.0154	paraoxonase 1
ORIOZI	1.15	100	0.467	19.99	7.25×10^{-6}	0.0201	olfactory receptor
OR8D4	1.25	100	0.510	19.21	1.60×10^{-5}	0.0201	olfactory receptor
TAS2R1	1.32	100	0.535	19.20	1.60×10^{-5}	0.0201	taste receptor
OR1F2P	2.03	100	1.18	15.86	5.40×10^{-5}	0.0831	olfactory receptor
GSTM1	1.48	100	0.762	15.82	3.90×10^{-5}	0.0831	glutathione S-transferase mu 1
OR6K2	2.02	100	1.22	15.79	4.50×10^{-5}	0.0831	olfactory receptor
OR51D1	1.13	49.3	0.466	15.59	8.60×10^{-5}	0.0831	olfactory receptor
TAAR5	1.17	48.2	0.484	15.16	9.90×10^{-5}	0.0936	trace amine associated receptor 5
OR4C13	1.77	100	0.915	14.88	7.00×10^{-5}	0.0972	olfactory receptor