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

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

As the ancestors of aquatic marine mammals adopted obligate aquatic lifestyles, they evolved many adaptive changes, such as those that improved locomotion and respiration in and perception of their new environment (1–3). 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 (4–6), bitter taste receptors in carnivorous tetrapods (7), and eyes in subterranean species (8–10). 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 eutharian mammals’ genomes in a 100-way vertebrate alignment (11). 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 through-out the mammalian phylogeny or by a model in which the loss rate was dependent upon the terrestrial or marine state of a given branch in a likelihood ratio test (LRT) (12). 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 (13). We used simulations to estimate empirical gene-specific P values and study-wide (multiple-test-corrected) false discovery rates (FDR) for all genes (13) (Table 1 and table 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 to S5). These results are consistent with previous behavioral, anatomical, and genetic studies indicating a reduction of smell and taste in marine mammals (5, 14, 15).

We also observed a notable pattern of convergent loss in the marine environment at Paraoxonase 1 (PON1) (Table 1) (13). PON1 encodes a bloodstream enzyme that reduces oxidative damage to lipids in low- and high-density lipoprotein (LDL and HDL) particles, potentially preventing atherosclerotic plaque formation (16, 17) (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 by-products.

Table 1. Top 10 manually validated genes with evidence for marine-specific loss. Loss rates represent the inferred instantaneous rates of transition from functional gene (1) to pseudogene (0) per unit branch length under the relevant model in BayesTraits (22, 23), restricted to a maximum value of 100 (the default).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Marine loss rate (independent)</th>
<th>Terrestrial loss rate (dependent)</th>
<th>LRT statistic</th>
<th>Empirical P value</th>
<th>FDR</th>
<th>Description of gene product</th>
</tr>
</thead>
<tbody>
<tr>
<td>PON1</td>
<td>0.672</td>
<td>49.7</td>
<td>0</td>
<td>22.24</td>
<td>3.08 × 10^-6</td>
<td>0.0154</td>
</tr>
<tr>
<td>OR1O21</td>
<td>1.15</td>
<td>100</td>
<td>0.467</td>
<td>19.99</td>
<td>7.25 × 10^-6</td>
<td>0.0201</td>
</tr>
<tr>
<td>OR8D4</td>
<td>1.25</td>
<td>100</td>
<td>0.510</td>
<td>19.21</td>
<td>1.60 × 10^-5</td>
<td>0.0201</td>
</tr>
<tr>
<td>TAS2R1</td>
<td>1.32</td>
<td>100</td>
<td>0.535</td>
<td>19.20</td>
<td>1.60 × 10^-5</td>
<td>0.0201</td>
</tr>
<tr>
<td>ORIF2P</td>
<td>2.03</td>
<td>100</td>
<td>1.18</td>
<td>15.86</td>
<td>5.40 × 10^-5</td>
<td>0.0831</td>
</tr>
<tr>
<td>GSTM1</td>
<td>1.48</td>
<td>100</td>
<td>0.762</td>
<td>15.82</td>
<td>3.90 × 10^-5</td>
<td>0.0831</td>
</tr>
<tr>
<td>OR6K2</td>
<td>2.02</td>
<td>100</td>
<td>1.22</td>
<td>15.79</td>
<td>4.50 × 10^-5</td>
<td>0.0831</td>
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<tr>
<td>OR5D1</td>
<td>1.13</td>
<td>49.3</td>
<td>0.466</td>
<td>15.59</td>
<td>8.60 × 10^-5</td>
<td>0.0831</td>
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<tr>
<td>TAAR5</td>
<td>1.17</td>
<td>48.2</td>
<td>0.484</td>
<td>15.16</td>
<td>9.90 × 10^-5</td>
<td>0.0936</td>
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<tr>
<td>OR4C13</td>
<td>1.77</td>
<td>100</td>
<td>0.915</td>
<td>14.88</td>
<td>7.00 × 10^-5</td>
<td>0.0972</td>
</tr>
</tbody>
</table>

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including chlorpyrifos oxon and diazoxon (Fig. 1B) (18). 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 and 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 (13) (Fig. 1C and Fig. S1). We observed shared genetic lesions among all sequenced cetaceans and a different shared lesion in sirenians (fig. S2), and the inferred ratio of nonsynonymous to synonymous substitutions (dN/dS) was not significantly different from one on the ancestral branches of both clades (cetacean ancestor dN/dS = 1.09, P = 0.79; sirenian ancestor dN/dS = 1.20, P = 0.057).

This suggests that PON1 lost functional constraint in the ancestral cetacean lineage soon after its split with the ancestral hipposidont lineage, approximately 53 million years (Ma) ago [95% confidence interval (CI) lower bound, 34.5 Ma ago (13, 19). In sirenians, functional loss occurred soon after the split with the ancestral elephantid lineage, approximately 64 Ma ago (lower bound, 41.7 Ma ago) (19).

In pinnipeds, we observed clear evidence of PON1 functional loss only among a subset of species within the family Phocidae, wherein Weddell seal and Hawaiian monk seal PON1 sequences contained nonshared 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-Otariidae split approximately 21 Ma ago (95% CI, 0 to 21 Ma ago). This incomplete loss may reflect either a difference between the selective environments experienced by pinnipeds and those experienced by other marine mammals or pinnipeds’ more recent colonization of the marine environment (pinnipeds, 24 Ma ago; cetaceans, 44.7 to 37.3 Ma ago; sirenians, 47.1 to 43.9 Ma ago) (20).

PON1’s functional loss in marine mammals may be related to its role in lipid metabolism via fatty acid beta-oxidation (21) (tables S6 and S7). The diets of both herbivorous and carnivorous aquatic mammals contain a higher proportion of ω-3 relative to ω-6 polyunsaturated fatty acids (PUFAs) than those of terrestrial mammals (22), and these PUFAs differ in their capacity to sustain oxidative damage (23). Marine and terrestrial mammals also have vastly different antioxidant profiles (24, 25), presumably because of the extreme oxidative-stress environments experienced during diving, with repeated cycles of hypoxia and reperfusion. Rewiring of either lipid metabolism or antioxidant networks in ancient marine mammals may 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 (26).

However, two semi-aquatic mammals, the sea otter and the beaver, which are more moderate divers (26), also have either lesions or substitutions at sites predicted to be necessary for PON1 function (fig. S2 and table S8).

Whatever the cause, loss of PON1 function may carry negative pleiotropic consequences for the health of marine mammals repeatedly exposed to man-made organophosphate compounds. PON1 alone is protective against the...

Bay has measured levels of chlorpyrifos as (Fig. 3). Limited sampling upstream of Manatee abut manatee protection zones and waterways reside (of Atlantic coast manatees migrate or seasonally In Brevard County, where an estimated 70% of agricultural land in Florida. (Right) Manatee protection zones, waterways, and agricultural land in Brevard County.

Given the sensitivity of Pon1−/− mice to organophosphate exposure (28), 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 (29, 30), 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/liter (31), and levels could be much higher directly after pesticide applications (32). Dugongs may be at risk of exposure to organophosphorus pesticides that are used in the sugarcane industry along the Queensland coast of Australia and have been detected at 5 to 270 pg/liter in coastal river systems (33). 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 (34). 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 trade-offs 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.

REFERENCES AND NOTES

13. Materials and methods are available as supplementary materials.
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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/361/6402/591/suppl/DC1
Materials and Methods
Figs. S1 to S6
Tables S1 to S13
References (36–105)
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Adaptive conflicts with the modern world

Mammals evolved in terrestrial environments. Those that now live in the marine environment have had to adapt to the particular selective pressures that this environment imposes. Meyer et al. surveyed the genomes of several marine mammal species to identify regions of convergent change. Multiple losses of the Paraoxonase 1 gene are evident in marine mammals, likely resulting from remodeling of lipid metabolism or antioxidant networks. The multiple occurrences of this loss of function across taxa indicate an evolutionary benefit. However, Paraoxonase 1 is the primary mammalian defense against organophosphorus toxicity. Marine mammals may be at a great disadvantage in the Anthropocene if run-off of this agricultural product into the marine environment continues.

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