

Reconstruction of evolutionary changes in fat and toxin consumption reveals associations with gene losses in mammals: A case study for the lipase inhibitor *PNLIPRP1* and the xenobiotic receptor *NR1I3*

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Abstract

The inactivation of ancestral protein-coding genes (gene loss) can be associated with phenotypic modifications. Within placental mammals, repeated losses of *PNLIPRP1* (gene inhibiting fat digestion) occurred preferentially in strictly herbivorous species, whereas repeated *NR1I3* losses (gene involved in detoxification) occurred preferentially in strictly carnivorous species. It was hypothesized that lower fat contents of herbivorous diets and lower toxin contents of carnivorous diets cause relaxed selection pressure on these genes, resulting in the accumulation of mutations and ultimately to convergent gene losses. However, because herbivorous and carnivorous diets differ vastly in their composition, a fine-grained analysis is required for hypothesis testing. We generated a trait matrix recording diet and semi-quantitative estimates of fat and toxin consumption for 52 placental species. By including data from 31 fossil taxa, we reconstructed the ancestral diets in major lineages (*grundplan* reconstruction). We found support that *PNLIPRP1* loss is primarily associated with low levels of fat intake

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and not simply with herbivory/carnivory. In particular, *PNLIPRP1* loss also occurred in carnivorous lineages feeding on a fat-poor diet, suggesting that the loss of this gene may be beneficial for occupying ecological niches characterized by fat-poor food resources. Similarly, we demonstrated that carnivorous species are indeed less exposed to diet-related toxins, suggesting that the loss of *NR1I3* and related genes (*NR1I2* and *UGT1A6*) resulted from relaxed selection pressure. This study illustrates the need of detailed phenotype studies to obtain a deeper understanding of factors underlying gene losses and to progress in understanding genomic causes of phenotypic variation in mammals.

KEYWORDS

dietary composition, fatty acids, forward genomics, *grundplan* reconstruction, plant toxins

1 | INTRODUCTION

Recent advances in comparative genomics revealed that the loss of genes can be associated with phenotypic variability in all phyla of prokaryotic and eukaryotic organisms (review in Albalat & Cañestro, 2016). The term *gene loss* describes the inactivation of a coding gene, such that it is unlikely that these gene remnants can still produce a functional protein. Gene loss can occur by the deletion of many or all exons from the genome. Alternatively, as frequently observed in mammals, gene loss can result from the accumulation of mutations that inactivate the reading frame by creating premature stop codons, reading frame shifts or dysfunctional splice sites (examples in Sharma et al., 2020). In contrast to processed pseudogenes, where an often non-functional copy of an existing functional gene is produced, gene loss leads to a reduced functional gene repertoire encoded in the genome of the organism. Many cases of gene loss are believed to be the consequence of relaxed selection pressure on preserving the function of the gene, resulting from phenotypic changes (e.g., regressive evolution; review in Albalat & Cañestro, 2016). The same authors also review examples where the inactivation of genes contributes to novel phenotypes that can be adaptive and might, for instance, be a precondition for occupying new ecological niches (e.g., less-is-more hypothesis). Thus, exploring evolutionary conditions that allow the accumulation of mutations, which eventually result in gene loss is important for the understanding of molecular and organismic evolution.

Gene loss was observed to be associated with many phenotypic modifications in placental mammals, for example, with the reduction of structures such as the vomeronasal system (Hecker et al., 2019a), the eyes (Emerling, 2018; Prudent et al., 2016), loss of teeth (Meredith et al., 2009, 2014) or immune receptors (Sharma et al., 2020). Recent studies revealed that gene losses can be associated with adaptation to environments and hence to ecological niche occupation as well as dietary specialization. For example, Emerling et al. (2018) performed a targeted screen for the presence of different chitinase gene paralogs (*CHIA1–5*) among 107 mammalian species and showed that the number of lost *CHIA* copies primarily correlates with the proportion

of invertebrates in the diet. Furthermore, gene losses observed in bats (Chiroptera) and whales (Cetacea) likely contributed to adaptations to sugar-rich diets or the aquatic environment (Huelsmann et al., 2019; Sharma et al., 2018).

As dietary specialization is a key process in mammalian evolution, Hecker et al. (2019b) tested on a coarse scale how gene loss is associated with diet in placental mammals. They performed an unbiased screening for genomic changes associated with two feeding styles: herbivory and carnivory. Among other genes, *PNLIPRP1* (pancreatic lipase-related protein 1) was identified as being preferentially lost in herbivorous species, and *NR1I3* (nuclear receptor subfamily 1 group I member 3) was found to be preferentially lost in carnivorous mammals (Hecker et al., 2019b). *PNLIPRP1* is expressed in the pancreas and functions as a lipase inhibitor. Experimental inactivation of this gene in knockout mice led to an increased lipase activity and caused increased body fat mass gain (Ren et al., 2011). Consequently, Hecker et al. (2019b) hypothesized that the preferential loss of this inhibitor in herbivores may reflect the ability to efficiently digest fatty acids from a generally fat-poor diet. The second gene, *NR1I3*, is expressed in the liver and intestine and encodes a key receptor involved in detoxification (Tolson & Wang, 2010), leading to the hypothesis that preferentially carnivorous species lost this gene as their diets should go along a lower exposure to diet-related toxins (Hecker et al., 2019b). This implies that low toxin exposure is associated with lower selection pressure on the *NR1I3* gene locus, resulting in the accumulation of mutations and finally in gene loss.

These hypotheses have not been tested yet. It is still not known whether a fat-poor or an almost toxin-free diet are per se the factors primarily associated with the convergent loss of *PNLIPRP1* and *NR1I3*, irrespective of the rather coarse categories herbivory and carnivory. Furthermore, if such associations exist, it can be post hoc tested whether the loss of these genes might be the key driver for the exploitation of novel food resources during evolution. This provides the opportunity to discover examples of adaptive gene loss events (Albalat & Cañestro, 2016). The broad definition of herbivorous and carnivorous diet in the original analyses by Hecker et al. (2019b) does not allow testing these hypotheses. In particular, the study grouped

different mammalian species under the term *herbivore*, regardless of whether they feed on, for example, roots, leaves, seeds or fruits. Similarly, the 'carnivore' category composed a heterogeneous group of animals feeding on different food sources such as meat, fish or insects. Species-specific herbivorous or carnivorous diets are expected to differ substantially in their composition. However, detailed information about the specific dietary constituents (or proportion of) is not easily accessible from either printed or digital data sources.

To fill these gaps, the present study reconstructs the diet composition of the same 52 placental species analysed by Hecker et al. (2019b) and adds 31 fossil taxa in addition. Our main goal is to assess whether the true amount of fat and diet-related toxins consumed is associated with the presence or absence of *PNLIPRP1* and *NR1I3*. This includes testing the following hypotheses derived from Hecker et al. (2019b): (a) If an association between fat consumption and *PNLIPRP1* loss exists, we expect that the few carnivorous mammals, which lost the gene, feed on a low-fat diet. Conversely, (b) herbivorous mammals, which retained the *PNLIPRP1* gene in exceptional cases, are expected to feed on a high-fat diet. And (c) if diet-related toxin content and *NR1I3* presence/absence are interrelated, we expect those carnivorous species that retained this gene to tolerate some level of toxin in their diet. In order to reach these goals, we performed an extensive literature review to reconstruct species-specific diets, which then allowed to develop five diet composition models. Subsequently, semi-quantitative estimates of the overall fat and toxin content in the diet of extant and fossil taxa were derived. These estimates were used as the basis for our *grundplan* reconstruction to infer how the fat and diet-related toxin consumption changed within mammalian lineages. Overall, hypothesis testing was performed by associating gene losses with the overall diet-related fat (*PNLIPRP1*) and toxin (*NR1I3*) content. This was done by comparing extant species with each other and by comparing stem members of mammalian lineages with their extant relatives.

2 | MATERIAL AND METHODS

2.1 | Species under consideration, phylogeny, taxonomy and gene loss information

Diet composition was primarily reconstructed for the same extant 52 placental mammals used by Hecker et al. (2019b); (Figure 1). Additionally, in order to perform the *grundplan* reconstruction (Figure 2; see below), a total of 31 fossil taxa were considered. They fulfilled the following criteria: state-of-the-art reconstructed and well-supported inference of diet as well as known geological age and a phylogenetic position close to the base of the extant species and lineages considered in this study (Table S1). Phylogenetic relationships between extant and extinct taxa as required for *grundplan* reconstruction were inferred as follows: the phylogenetic relationships between extant placental mammals as given by Hecker et al. (2019b) were used as a basis. Subsequently, extinct species and taxa were positioned into this tree according to literature information

(Table S1). Gene loss observations in extant species follow the results of the genomic analyses by Hecker et al. (2019b), which also includes their classification as independent, polymorphic or ancestral loss (see supplemental material by Hecker et al., 2019b).

2.2 | Recording feeding habits and dietary components in extant species based on diet composition models

Recording feeding habits and dietary components as distinct categories in a trait matrix (recording as traits and states) is one approach to associate gene loss with species-specific dietary components such as fat or toxins (Stefen et al., 2021). However, definitions and the level of details given on feeding habit categories or description of main dietary components varies from study to study (e.g., Hecker et al., 2019b; Román-Palacios et al., 2019). Our study uses four independent but complementary diet composition models that describe diet from different perspectives. This ensures the best possible description of species-specific diet, which can be retrieved from the literature. These models categorize feeding habits and dietary components according to definitions commonly used in mammalogy (e.g., Eisenberg, 1981; Pineda-Munoz & Alroy, 2014; Ungar, 2010). These models are outlined in Figure 1, and a full description is given in Table S2. The *fine-scaled model* (Model M1) allows a qualitative description of feeding habits (e.g., granivory and piscivory) and is based on the actual dietary components (e.g., seeds and fish). It distinguishes 19 different feeding habits (recorded as traits), which can have two states: absent (dietary component is not consumed) and present (dietary component is consumed). With this model, the species-specific diet composition can be described as a qualitative combination of multiple feeding habits (traits). The three other diet composition models code the diet in a quantitative way. The *quantitative model* (Model M2) describes 10 different dietary components as traits (e.g., seeds and tetrapods) and quantifies their proportion based on five states, from none (intake = 0%) to exclusively (intake = 100%). The *diet model* (Model M3) uses one trait (diet) and describes the feeding habits as one of six possible states (e.g., carnivore and herbivore) focusing on the major type of food according to field observations. Finally, the *stomach content model* (Model M4) uses also one trait (stomach content) and describes dietary components based on one of nine possible states (e.g., invertebrates and green plants). Similar to the diet model, it focuses on the major proportion of food actually found in the stomach. Primary data were collected from the literature, and the results of trait recording are deposited as part of a larger trait matrix (Stefen et al., 2021; <https://www.morphobase.de/?MaTrics-Mx-v1>).

2.3 | Overall fat score for extant species

For semi-quantitative estimates of species-specific fat consumption, an overall fat score was developed. This score integrates qualitative

Herbivorous species
Carnivorous species






















| Fine scaled model (M3) | | Quantitative model (M2) | | Diet model (M3) | | Stomach content model (M4) | |
|---|---------|---|---------------|---|----------------|---|---------------|
|  | Absent |  | None |  | Carnivore |  | Invertebrates |
|  | Present |  | Occasionally |  | Herbivore |  | Vertebrates |
|  | Missing |  | Regularly |  | Omnivore |  | Green plants |
| | |  | Predominantly |  | Insectivore |  | Missing |
| | |  | Exclusively |  | Myrmecophagous | | |
| | |  | Missing |  | Piscivore | | |
| | |  | |  | Missina | | |

FIGURE 1 Trait matrix recording of feeding habits and main dietary components for 52 extant species according to four diet composition models: the fine-scaled model (M1) lists 19 feeding habits as traits (head row) describing its presence or absence (two states) in columns; the quantitative model (M2) lists 10 feeding habits as traits (head row) with five states describing quantity in columns; the diet model (M3) uses one trait (diet; head row) and describes the feeding habits as six states in the columns focusing only on the major type of food according to field observations; the stomach content model (M4) uses one trait (stomach content; head row) and describes the feeding habits based on nine states focusing on the major proportion of food found in the stomach. Additional information is provided as reference to the study by Hecker et al. (2019b) as original gross scale assignment of species to herbivorous (green) and carnivorous (red) lineages (not used for analytical approaches in this study)

and quantitative fat content information associated with feeding habits and/or main dietary components from our diet composition models (M1–M4) and characterizes the species-specific diet fat content. It was introduced to associate fat consumption with the presence or absence of the *PNLIPRP1* gene.

First, feeding habits and main dietary components in Models M1–M4 were assigned to semi-quantitative estimates of fat content (Table S3). This was done by categorizing information from the literature as 'high', 'medium' and 'low' fat content. Many feeding habits and dietary components were unambiguously assignable to either 'high' (e.g., granivory in M1 and insectivore/myrmecophagous in M3) or 'low' (e.g., rhizovory in M1 and Trochozoa in M2). However, feeding habits and dietary components were also observed to have a highly variable fat content (e.g., fish in M2: <1% fat [haddock, *Melanogrammus* sp.] to 25% fat [herring, *Clupea* sp.]). Thus, the primary assignment was 'medium'.

Second, species-specific records about diet according to Models M1–M4 were used and associated with the respective semi-quantitative estimates of fat content (Table S3). In order to obtain a single fat score, information obtained from all four models M1–M4 had to be integrated. If the assignments in all four models were uniformly either 'high' or 'low', the overall diet-related fat score was defined accordingly. However, if feeding habits or dietary components were assigned to 'medium', a more comprehensive analysis was performed. For instance, the dietary component 'fish' (M2; primary assignment 'medium') includes low-fat fish (e.g., *Melanogrammus* sp. with <1% fat) as well as high-fat fish (e.g., Anguilliformes and Clupeiformes with up to 25% fat; Arentz, 2020). If the species assigned to this dietary component actually selects predominantly low-fat fish or high-fat fish, they were secondarily reassigned to 'low' or 'high'. However, if species indeed ingest a mixed diet (e.g., low- and high-fat fish in similar proportions), the assignment was kept at 'medium'. The comprehensive analysis was performed based on the detailed literature review.

2.4 | Overall toxin score for extant species

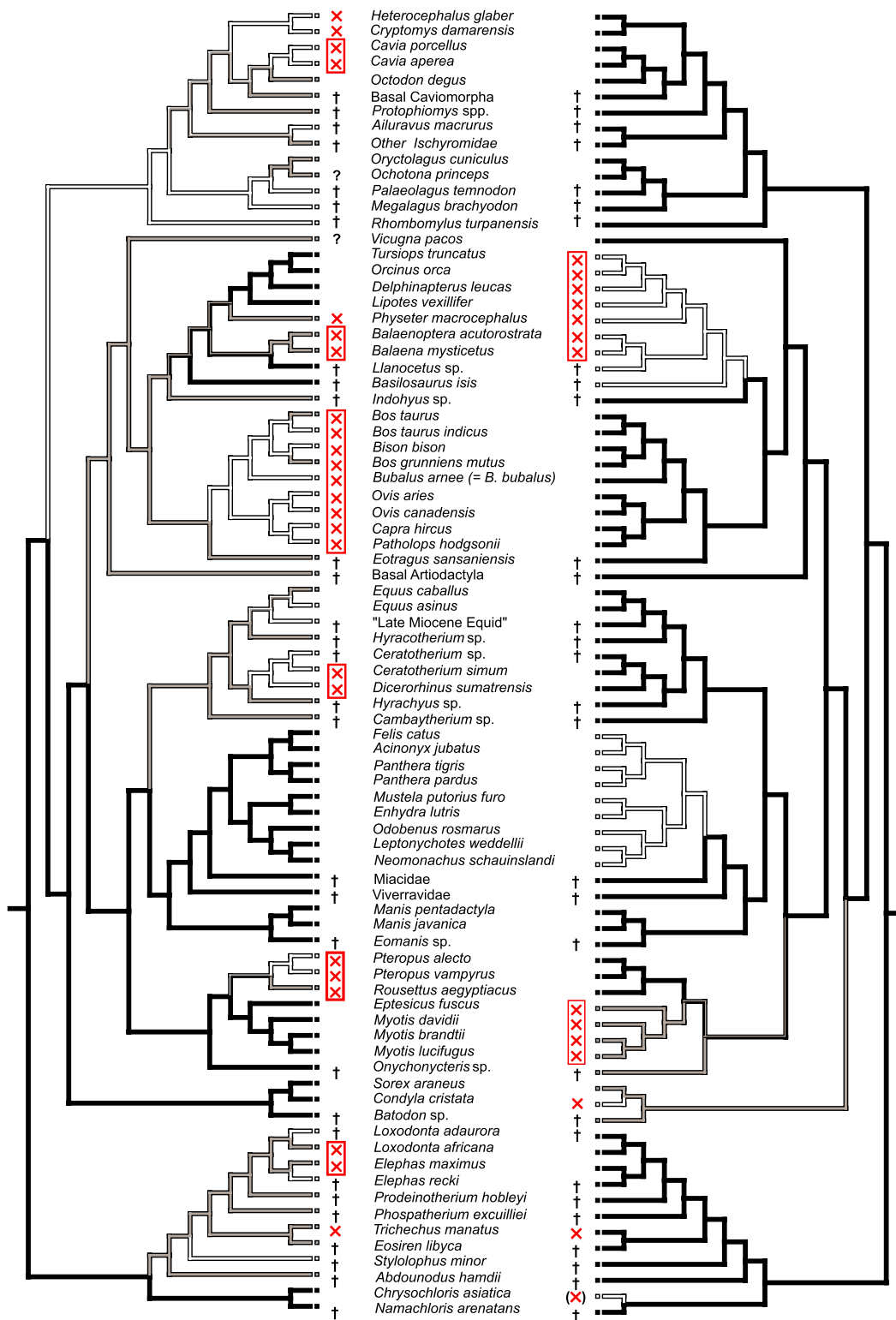
Overall toxin scores provide semi-quantitative estimates for the species-specific toxin consumption. This follows the same objectives and methodological outlines as described for the overall fat score (see above) and aims at associating species-specific toxin consumption with the presence/absence of the *NR1I3* gene.

In a first step, literature information about toxins allowed associating feeding habits and dietary components described by Models M1–M4 to semi-quantitative estimates using the three levels 'low', 'medium' and 'high' (Table S4). We considered only toxins, which are naturally present in the diet but/and neglected environmental toxic substances (xenobiotics s.l.), which are omnipresent in nature or originate from anthropogenic environmental pollution (Soucek, 2017). Because phytotoxins are present in all plant tissues, herbivorous diets were generally assigned to 'high' in all four diet composition models. Diets consisting of animal tissues were primarily considered to be free from toxic substances (Beasley, 1999; Kakehi et al., 2015; Kim et al., 2016; Shrestha et al., 2011). However, our literature review identified the following potential diet-related toxin sources in an otherwise carnivorous diet: plant material ingested occasionally or on purpose (e.g., maintenance of gut function), cadaveric poison in carcasses, plant material as gut and stomach content of herbivorous prey and animal prey with defence toxins (e.g., social insects; Table S4). It is hardly identifiable in which proportions these sources contribute to the total toxin uptake of a species from the categories of Models M1–M4 (Figure 1; e.g., uptake of gut and stomach content associated with carnivory [M1] or uptake of defence toxins associated with insectivory [M1]). Thus, further literature reviews became necessary. Carnivorous species were assigned to category 'high' in cases where dietary components include ants and their toxin-producing organs (e.g., myrmecophagy, M1). Animal-based diet, which contains insects (e.g., insectivorous, M1) or terrestrial arthropods (e.g., arthropods, M2), was assigned to 'medium', as the production of chemical substances is heterogeneously distributed within the entire group. The same assignment was performed in cases where carcasses are consumed (e.g., scavenger, M1; Discher et al., 2009; Harborne, 1999; Ma et al., 2017; Rainio et al., 2012; Richardson & Levitan, 1994). As the occasional uptake of toxins in all other carnivorous diets cannot unambiguously be excluded, they were assigned to 'low'.

Subsequently, semi-quantitative toxin content estimates (Models M1–M4) were integrated to obtain an overall toxin score. As described for diet fat content, if assignments for all models were uniformly 'high' or 'low', the overall score was accordingly. A deepened analysis based on literature research was performed for mammals with a 'medium' toxin level. If quantitative information from models M2–M4 suggested the predominant consumption of a diet with 'high' or 'low' toxin levels, a reassignment was performed. Otherwise, the toxin score 'medium' was accepted.

Fat (PNLIPRP1)

Toxin (NR1I3)



Low

Medium

High

X Gene loss

XX Ancestral gene loss

X(Polymorphic gene loss

? Missing or uncertain data

† Extinct species

FIGURE 2 Phylogenetic relationship and *grundplan* reconstruction referring to diet-related fat (left panel) and toxin (right panel) consumption of 52 extant and 31 fossil (†) placental species/taxa. Topology of extant species was adopted from Hecker et al. (2019b), and the position of fossil species/taxa is the result of literature research (see Table S1 for references). Left: Presence or absence of the *PNLIPRP1* gene for extant species according to Hecker et al. (2019b). Overall diet fat scores (squares) indicate a semi-quantitative estimate of species-specific fat consumption using three categories: low (white), medium (grey) and high (black). The same three categories were used for *grundplan* reconstruction, which provides information about ancestral states along the branches and nodes of the tree. Right: Presence or absence of the *NR1I3* gene is indicated for extant species according to Hecker et al. (2019b). Overall toxin scores (squares) indicate a semi-quantitative estimate of species-specific diet-related toxin consumption using three categories: low (white), medium (grey) and high (black), and these three categories were also used for *grundplan* reconstruction (ancestral states along the branches and nodes of the tree)

2.5 | *Grundplan* reconstruction of diet-related fat and toxin consumption

Grundplan reconstruction aimed at estimating the fat and toxin consumption associated with the diet of stem members of mammalian lineages (ancestral state analysis). This allowed analysing how the fat and toxin consumption changed within mammalian lineages and enabled associating the evolutionary change of diet with the phylogenetic origin of *PNLIPRP1* and *NR1I3* gene losses (Hecker et al., 2019b; ancestral gene losses also shown in Figure 2).

First, paleo diets of fossil species (Figure 2) were derived from state-of-the-art diet reconstructions (see also selection criteria for fossils above). Because paleo diets cannot be determined as specifically as modern ones (e.g., 'soft vegetation' and 'abrasive plant items'), they could not be described using Models M1–M4. Consequently, an additional diet composition model was introduced exclusively for fossil species, that is, *grundplan model* (Model M5; Table S5). It breaks down dietary components (e.g., grasses and browse) in five traits, which have two states like in the fine-scaled model (M1): absent (not part of the diet) and present (in any proportion in the diet). Then, semi-quantitative estimates for the fat content ('high', 'medium' and 'low'; Table S3) and diet-related toxins ('low', 'medium' and 'high'; Table S4) in the paleo diets were performed in analogy to the approach described for extant species. These estimates have the same meaning for fossils as overall fat and toxin scores for extant species. Finally, the overall fat and toxin scores of both, extant species and fossils, were used for the *grundplan* reconstruction. This was executed in Mesquite (version 3.61; Maddison & Maddison, 2019) using the parsimony model. Ancestral state reconstruction is based on the tree topology used by Hecker et al. (2019b). The characters were treated as unordered, and the 'Trace Character History' option was used to graphically map the evolution history of a character on the tree.

3 | RESULTS

3.1 | Associations between fat consumption and *PNLIPRP1* loss in extant species

Records of feeding habits and dietary components for 52 placental species (Figure 1; Tables S6–S9) allowed estimating the species-specific fat scores (Figure 2; Table S10). All extant species with a 'high' overall diet-related fat score possess the *PNLIPRP1* gene (22

of 22 species: 100%), whereas gene loss was preferentially observed among species whose diet contains a 'low' (15 of 16 extant species: 93.8%) or 'medium' amount of fat (nine of 14 extant species: 64.3%). This summary excludes two extant species (*Ochotona princeps* and *Vicugna pacos*) for which the previous genomic analyses did not unambiguously identify *PNLIPRP1* loss (Figure 2). Two hypotheses related to *PNLIPRP1* were tested. First, whether carnivorous mammals, which lost *PNLIPRP1*, feed on a diet that contains less fat than those carnivorous species that possess the gene. This pattern was observed among whales (Cetacea) and applies to the species *Physeter macrocephalus*, *Balaenoptera acutorostrata* and *Balaena mysticetus*. They mainly prey on taxa characterized by a medium fat content, for example, plankton and Trochozoa (Figure 1; Tables S6–S9). In contrast, all other analysed whale species of our analysis feed on a high-fat diet (i.e., actinopterygian and elasmobranchian fishes; Figure 1; Tables S6–S9) and retained the gene.

A second hypothesis tested whether herbivorous mammals, which retained *PNLIPRP1*, show a higher fat consumption compared with herbivorous mammals that lost this gene. This applies to the rodent *Octodon degus* and the lagomorph *Oryctolagus cuniculus*, whose diets were assigned a 'medium' fat score according to the recurring ingestion of seeds and nuts (Figure 1; granivory, M1; seeds, M2; Tables S6–S9), dietary components with high fat content (Figure 2; Table S10). In contrast, the remaining four rodents lost the *PNLIPRP1* gene and feed on a low-fat diet predominantly consisting of roots (rhizovory; *Heterocephalus glaber* and *Cryptomys damarensis*) and grasses (graminivory; *Cavia aperea* and *Cavia porcellus*; Figures 1 and 2; Tables S6–S9). Although this example proves that the retention of *PNLIPRP1* can indeed be associated with higher fat consumption in Glires (Rodentia and Lagomorpha), such relationship was not observed within Perissodactyla. Indeed, the overall fat consumption of the two equine species that retained *PNLIPRP1* was recorded as 'medium' (*Equus caballus*) and even 'low' (*Equus asinus*; Figures 1 and 2; Tables S6–S9).

3.2 | Lineage-specific evolution of fat consumption and association with *PNLIPRP1* gene

Our *grundplan* reconstruction allows putting overall fat consumption and the loss of *PNLIPRP1* in an evolutionary context (Figure 2). It became possible after analysing the diet of 31 extinct placental taxa and assigning diet-related fat scores to them (Table S11).

Parsimony analysis revealed that the last common ancestors of the Placentalia in our sample fed most likely on a 'high' fat content diet (Figure 2). The same analysis suggests that early Glires (Rodentia and Lagomorpha) fed on a diet characterized by a 'low' fat content, which is supported by the stem Glires †*Rhombomylus turpanensis* (late early Eocene, eating soft vegetation; Table S11). Yet in early Rodentia, the signal is mixed with a 'medium' fat content in the food of some of its stem members (e.g., late Palaeocene/early Oligocene, †*Ischyromyidae*, eating seeds and nuts; Table S11). Accordingly, the lineage represented by *H. glaber* and *C. damarensis* and the lineage represented by both *Cavia* species (*C. porcellus* and *C. aperea*) secondarily reduced their fat intake to 'low' (Figure 2). This happened independently in the two lineages since *O. degus*, the basal Caviomorpha (late middle Eocene, †*Cachiyacu* spp. and †*Canaanimys maquiensis*, general herbivory; Table S11), and the lineage represented by †*Protophiomys* spp. (late middle Eocene/early late Eocene, general herbivory; Table S11) continued feeding on a 'medium' fat content food. Therefore, this study demonstrates that the independent loss of *PNLIPRP1* in *H. glaber*, *C. damarensis* and the two *Cavia* species is associated with the independent change of dietary fat content from 'medium' to 'low' within Rodentia.

Within Lagomorpha, the presence of *PNLIPRP1* in extant *O. cuniculus* and *O. princeps* is associated with a 'medium' fat content in their diet. This is in contrast to their fossil relatives (i.e., †*Palaeolagus temnodon* and †*Megalagus brachyodon*, from the late Eocene, mainly frugivorous and folivorous; Table S11), which were assigned to a 'low' diet-related fat score (Figure 2). Because the 'low' fat consumption is considered the ancestral state within Glires (see above), the evolution towards a 'medium' fat content diet is the derived condition (apomorphy).

Among whales (Cetacea), the usually 'high' dietary fat content got reduced to a 'medium' level only in *P. macrocephalus*, and within the mysticete lineage represented by *B. acutorostrata* and *B. mysticetus*. These three species are also the only whales to have lost their *PNLIPRP1* gene (Figure 2). The basal mysticete †*Llanocetus* sp. (late Eocene) was a macrophagous predator, and the archaeocete †*Basilosaurus isis* (late Eocene), one of the closest relatives to the crown Cetacea, was a carnivorous predator, which means that they were both high-fat feeders (Table S11). Hence, the reduction of dietary fat content in these three extant species, as well as the associated loss of *PNLIPRP1*, happened convergently within Cetacea.

Grundplan reconstruction within Bovidae suggests that the last common ancestor of the crown Bovidae in our analysis fed on a low-fat diet (Figure 2). This is associated with the loss of *PNLIPRP1* in this ancestral species as suggested by genomic patterns (Figure 2; Hecker et al., 2019b). It is interesting to note that the absence of the lipase inhibitor gene does not generally prevent a secondary return to a 'medium' fat diet as seen in the two species *Bos taurus* and *Bos grunniens mutus* (Figures 1 and 2; rapeseed and mosses; Tables S7–S11).

The last common ancestor of the two investigated Perissodactyla lineages Equidae (represented by *E. caballus* and *E. asinus*) and Rhinocerotidae (represented by *Ceratotherium simum* and

Dicerorhinus sumatrensis) was reconstructed with a 'medium' overall fat score. This is similar to that of its early Eocene stem taxon †*Cambaytherium thewissi* (Figure 2). The *grundplan* reconstruction shows on the one hand an independent change towards low-fat diet within Rhinocerotidae that is associated with *PNLIPRP1* loss. In contrast, *PNLIPRP1* is retained in Equidae but is associated to 'medium' (*E. caballus*) as well as a change towards 'low' (*E. asinus*) fat consumption. *Grundplan* reconstruction further shows that the change from 'medium' to 'low' fat consumption in both taxa occurred convergently. This is because the stem taxa of both clades, that is, †*Hyracotherium* sp. (late Eocene/Oligocene) and †*Hyrachyus* sp. (early/middle Eocene), show a 'medium' diet-related fat score similar to their ancestor (†*C. thewissi*; see above).

Early Palaeocene Chiroptera were specialized in hunting invertebrates (†*Onychonycteris* sp., late early Eocene; Table S11), and the fat content of their diet was scored as 'high'. Since all Microchiroptera in our study also feed on 'high'-fat diet, the ancestral character state for Chiroptera was reconstructed as 'high' (Figure 2). Accordingly, extant Megachiroptera evolved towards a fat-reduced ('medium' and 'low') herbivorous diet. This reduction of fat intake is accompanied by the expected loss of *PNLIPRP1*.

Within Afrotheria, the *grundplan* reconstruction using the parsimony approach suggests that the last common ancestor fed on a 'high'-fat diet. There is strong scientific support that the insectivorous side of the clade depends on a 'high'-fat diet. This clade is represented in our study by *Chrysochloris asiatica* (Table S10) and the oldest known golden-mole (†*Namachloris arenatans*; late Eocene, fed on termites and soft invertebrates; Table S11). Change to a 'medium' fat score occurred in the sister clade (Figure 2), which is represented by herbivorous (s.l.) afrotherian species (Paenungulatomorpha). This clade includes extant species, which lost *PNLIPRP1* independently, that is, *Loxodonta africana* and *Elephas maximus* on one hand and *Trichechus manatus* on the other hand (Figure 2; Table S11). Although many extinct species were classified in the same fat score category (e.g., †*Abdounodus hamdi*, middle Palaeocene; browse; Table S11), there are also examples of taxa that evolved towards 'low' fat consumption such as †*Stylophorus minor* (early Eocene; hyper-specialized folivores; Table S11; Gheerbrant et al., 2018). Other examples are the close relatives of the extant African and Asian elephants †*Loxodonta adaurora* (latest Miocene/Pliocene) and †*Elephas recki* (early Pliocene/middle Pleistocene), respectively, which fed on grass ('low' diet-related fat score). There is, however, an alternative scenario to the convergent shift in fat consumption within Paenungulatomorpha. A detailed literature review revealed a shift in the plant-based diet of Elephantidae, which were mainly browsers until the late Miocene, but became predominantly grazers 7 million years ago (Cerling et al., 2005; Figure 3). The modern elephants, *L. africana* and *E. maximus*, have reversed this trend <1 million years ago (Cerling et al., 2005) and independently returned to a browse-dominated diet (i.e., 'medium' diet-related fat score). Consequently, the ancestral character reconstruction for the Elephantidae in our analysis (late Miocene to recent) should rather be a 'low' diet-related fat score (Figure 3), a score that is strongly correlated to the loss of

the *PNLIPRP1* gene. Under this hypothesis, a secondary return to 'medium' fat diet occurred, similar to what was observed in Bovidae (see above).

In summary, *grundplan* reconstruction revealed an independent reduction of the overall fat consumption in 10 lineages. This was associated with *PNLIPRP1* gene loss in nine cases: Rodentia (two lineages), Cetacea (two lineages), Bovidae, Perissodactyla (one lineage), Chiroptera (one lineage) and Afrotheria (two lineages). No association between reduction in fat consumption and gene loss was observed in *E. asinus* (Equidae). The same *grundplan* reconstruction also revealed cases where fat consumption increased during evolution. This happened despite of *PNLIPRP1* loss (Bovidae: *B. taurus* and *B. grunniens mutus*; Chiroptera: *Rousettus aegyptiacus*; Afrotheria: *L. africana* and *E. maximus*) or was entirely independent from any gene loss event in the respective species, lineages and sister taxa (Lagomorpha).

3.3 | Associations between diet-related toxin consumption and *NR1I3* loss in extant species

Diet records for 52 extant placental species (Figure 1; Tables S6–S9) were used to assign overall toxin scores (Figure 2; Table S12) and to associate species-specific toxin consumption with presence or absence of the *NR1I3* gene. All extant placental mammal species, which are consistently exposed to diet-related toxins (assigned to 'high'; 29 species, 100%), possess the xenobiotic receptor gene *NR1I3*. Conversely, the gene is preferentially lost in species showing a 'medium' or 'low' exposure to toxins (13 of 23 species, 56.5%). This means that not all species with the 'medium' or 'low' score have lost

NR1I3, and these species predominantly belong to the taxa Carnivora (all species under consideration) and Lipotyphla (*Sorex araneus*).

Further data exploration was performed in the light of the hypothesis that carnivorous species retaining *NR1I3* (representing Carnivora, Pholidota and Lipotyphla) ingest diet-related toxins. Small amounts of grasses consumed by Carnivora species are considered to be sufficient to be recorded in our models (graminivory, M1; green plants, M2; e.g., *Felis catus*, *Panthera tigris* and *Panthera pardus*; Figure 1; Tables S6–S9). But it is largely believed that grasses are ingested by some Carnivora to mitigate intestinal infections or manage intestinal parasites (see Franck & Farid, 2020 and references herein) rather than for their nutritional value. Furthermore, carrion is also ingested by *F. catus*, *P. tigris*, *P. pardus* and *Odobenus rosmarus* (scavenger, M1; Figure 1; Tables S6–S9). Yet tigers, as well as other large size felids, do not consume the gastrointestinal contents of their prey (e.g., Delibes et al., 2011; Labisky & Boulay, 1998; Schaller, 1967; Stahler et al., 2006; Sunquist, 1981; Vucetich et al., 2012). This part of the carcasses certainly contains toxic substances, and it is not well known whether predators would 'behaviorally avoid the consumption of maggots or different parts of a carrion to the effects of toxins' (Blandford et al., 2019, p. 6). However, Carnivora, which consume small-sized prey (e.g., mice) as a whole therefore come into contact with toxins (e.g., plant material from gut and stomach), which justified their assignment to a 'low' score, instead of a total absence of toxin in their diet.

With respect to Pholidota, plants were reported to occur in the diet of *Manis javanica* (but it is considered as a by-product during feeding on ant and termite mounds; Hua et al., 2015). However, both studied *Manis* species are consuming a high amount of animal defence toxins in their myrmecophagous diet, which justifies the assignment 'high'. Finally, plant material is part of the diet of Lipotyphla species. For instance, the diet of *S. araneus* includes plant material (e.g., folivory and granivory M1; Figure 1; Tables S6–S9). Furthermore, *Condylura cristata* was reported to ingest small amounts of 'unspecified vegetable matter', but it is questionable if the ingestion was intentional (Hamilton, 1931; Figure 1; Tables S6–S9). Consequently, the proportion of toxins in the diet was scored as 'medium' in *S. araneus* and as 'low' in *C. cristata* (Figure 2; Table S12). In our sample, *S. araneus* is the only example for a species that consumes only 'medium' amounts of toxins but still retains *NR1I3*. Conversely, *C. cristata* shows the expected association of a toxin-'low' diet with the loss of the xenobiotic receptor.

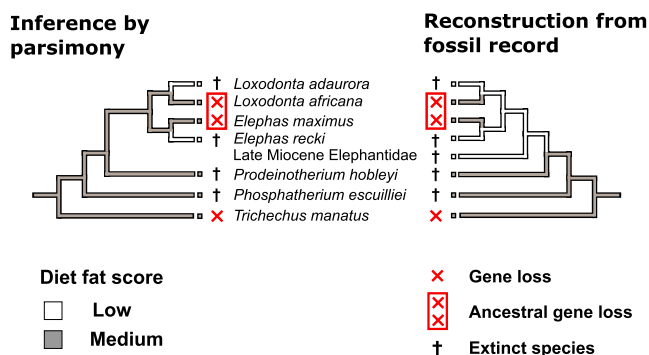


FIGURE 3 Comparison between two different approaches of *grundplan* reconstruction referring to the diet-related fat content for selected extant and fossil (†) Afrotheria. Left: Ancestral states according to parsimony analyses as semi-quantitative estimates indicating low (white) or medium (grey) diet-related fat consumption along branches and nodes of the tree. Right: Ancestral states of diet-related fat consumption according to literature reports about fossil records given as semi-quantitative estimates indicating low (white) or medium (grey) consumption along branches and nodes of the tree. The information referring to parsimony analyses (left) is extracted from Figure 2

3.4 | Lineage-specific evolution of diet-related toxin consumption and association with *NR1I3* gene

Grundplan reconstruction of toxin consumption became possible after assigning the diet of 52 extant species (Table S12) and 31 extinct species (Table S11) to overall toxin scores. We used parsimony analysis to infer ancestral states and to test whether *NR1I3* loss observed in some of these lineages could be associated with a reduced exposure to diet-related toxins during evolution (Figure 2).

The analysis revealed that the last common ancestor of the Placentalia in our sample habitually consumed diet-related toxins ('high' state). Consequently, major changes in toxin uptake were identified in lineages including carnivorous mammals, that is, Cetacea, Carnivora, Pholidota, Chiroptera, Lipotyphla and Afrotheria (Figure 2).

While extant Cetacea were assigned to a 'low' toxin diet, the middle Eocene extinct sister group to Cetacea (*†Indohyus* sp.; Table S11) still ingested a terrestrial food like browse and invertebrates. Hence, its diet-related toxin level was 'high', like in every remaining member of Artiodactyla in our sample (Bovidae, *V. pacos*). Our literature review shows that the diet of *†Indohyus* differed significantly from that of late Eocene whales like *†B. isis* (carnivorous; Table S11) and *†Llanocetus* sp. (microphagous predator; Table S11) because they had already transitioned to fully aquatic species, with the associated habitat change. Their now marine prey (aquatic mammals and fish; Table S11) no longer exposed them to major amounts of toxins. Thus, these species were assigned to a 'low' score, like each extant whale in our analysis (Table S12). Moreover, we observed the loss of the *NR1I3* gene in all extant Cetacea.

Our *grundplan* reconstruction estimated that the common ancestor of Carnivora and Pholidota (Ferae) had the ancestral state 'high' in respect to toxin uptake, but that crown Ferae (Carnivora and Pholidota) followed two different paths. Early Pholidota (e.g., *†Eomanis* spp., early-middle Eocene; Table S11), like their extant representatives, are specialized on social insects (i.e., myrmecophagy), which often use toxic substances for defence (toxin score 'high'; Table S12). This path is associated with the retention of the *NR1I3* gene. This is similar to the Palaeocene stem Carnivoramorpha (*†Viverravidae* and *†Miacidae*), which preyed on vertebrates but were still exposed to toxins through their omnivorous food supplements (e.g., fruits and invertebrates; toxin score 'high' in both cases; Figure 2; Table S11). Conversely, the extant Carnivora species in our sample feed on a 'low' toxin diet without having lost the *NR1I3* gene. This provides an example that shifts towards reduced toxin uptake are not necessarily associated with *NR1I3* gene loss (Figure 2).

In contrast, *grundplan* reconstruction reveals an association between *NR1I3* loss and reduced toxin consumption in Chiroptera. Indeed, all herbivorous Chiroptera (Megachiroptera) are exposed to diet-related toxins and have retained their *NR1I3* gene. This is different in the insectivorous Chiroptera (Microchiroptera), which have reduced their toxin exposure ('medium' toxin score), but have lost the gene (Figure 2). Because the basal Chiroptera *†Onychonycteris* sp. (late early Eocene, insectivorous; Table S11) was also exposed to a 'medium' amount of diet-related toxins, the parsimony analysis assumes that the last common ancestor of extant Chiroptera had a 'medium' toxin uptake (Figure 2). Following this prediction, crown Chiroptera evolved following either an increased consumption of diet-related toxins (Megachiroptera) and retained the *NR1I3* gene or specialized further in a strictly insectivorous diet with a reduced toxin intake associated with gene loss (Microchiroptera; Figure 2).

Presuming that toxin uptake was 'high' in the diet of ancestral Placentalia (see above), our analysis shows that the lineage

Lipotyphla shifted towards a 'medium' diet-related toxin exposure. Indeed, the extinct sister taxon of the Lipotyphla (late Cretaceous/early Palaeocene *†Batodon* sp., insectivorous; Table S11) already shows a diet scored as 'medium'. Likewise, the extant *S. araneus* is best described as consuming a 'medium' amount of toxins, while *NR1I3* is retained. Conversely, the extant species *C. cristata* evolved towards a 'low' toxin diet and has lost the gene. Thus, the overall pattern within Lipotyphla is a decrease of toxin intake, but gene loss is only observed in the extreme case of *C. cristata*.

Finally, an apparent association between 'low' level toxin consumption and *NR1I3* gene loss was also observed in Afrotheria. Whereas all Afrotheria consuming 'high' toxin levels possess *NR1I3*, the insectivorous tenrecoid *C. asiatica* feeds on a 'low' toxin diet and has lost the gene. Given that the earliest tenrecoid in our sample (late Eocene, *†N. arenatans*) fed on a toxin-rich diet ('high'; Table S11), *NR1I3* gene loss is associated with the reduction of toxin uptake within Tenrecoidea.

In summary, *grundplan* reconstruction revealed an independent reduction of the overall toxin consumption in five lineages. This was associated with *NR1I3* gene loss in four cases: Cetacea, Chiroptera (Microchiroptera), Lipotyphla (*C. cristata*) and Afrotheria (*C. asiatica*), but no association was observed in Carnivora. The same *grundplan* reconstruction also revealed a secondary increase in toxin uptake in case of Megachiroptera, a taxon that retained *NR1I3*.

4 | DISCUSSION

4.1 | Convergent loss of *PNLIPRP1* and *NR1I3* is associated with low fat and toxin consumption

Reconstruction of diet and exploring the evolution of fat and toxin uptake for 52 extant and 31 extinct placental species were prerequisites to addressing the overall question of this study. This enabled us demonstrating that the loss of *PNLIPRP1* and *NR1I3* is preferentially associated with the reduced consumption of specific diet compounds and is independent from a rather coarse classification into carnivorous or herbivorous mammals (Hecker et al., 2019b). In fact, *PNLIPRP1* was preferentially lost in extant species with 'low' or 'medium' fat consumption, and *grundplan* reconstruction showed an association between *PNLIPRP1* loss and the reduction of fat uptake. Likewise, we show that *PNLIPRP1* is preferentially retained in species with a high-fat herbivorous diet, which is especially well shown within Glires (Rodentia and Lagomorpha), whereas Equidae appear to be an exception at first glance (Figure 2). Similarly, our study showed that *NR1I3* gene loss occurred convergently only in extant species that feed on diet with a toxin content classified as 'low' or 'medium', whereas *grundplan* reconstruction showed an association with reduced dietary toxin intake. The independence of *NR1I3* gene loss from the carnivorous–herbivorous classification was again demonstrated by testing our specific hypothesis. While showing a straightforward association between retention of *NR1I3* in some carnivorous species with an increased toxin consumption (Pholidota

and Lipotyphla), a more complex explanation will be forwarded for the presence of *NR1I3* in Carnivora (see below).

Overall, the results of our study illustrate how a change in feeding habit and dietary composition can co-evolve with genomic changes, that is, the reduction of the genomes' functional repertoire by gene loss. This motivates discussing the biological significance and evolution of *PNLIPRP1* and *NR1I3* gene loss in more detail, not least to explain exceptions listed above (see paragraphs below). In fact, we explore whether the presence of, for instance, *PNLIPRP1* in Equidae and *NR1I3* in Carnivora can be explained by the inherent variability present in every biological system. Alternatively, these exceptions may indicate the existence of processes mimicking the effect of gene loss. We further consider whether a reduced functional repertoire could be a consequence or a precondition of diet change during evolution. In fact, if genes lose their biological function under novel evolutionary conditions (e.g., reduced availability of fat- or toxin-rich food items), neutral evolution will also lead to the accumulation of destructive mutations. This may ultimately result in a non-functional gene (gene loss according to the 'use-it-or-lose-it' principle; neutral evolution hypothesis; Gerland & Hwa, 2009). Alternatively, the loss of gene function may result in novel phenotypes that are adaptive ('less-is-more hypothesis'; adaptive evolution; Albalat & Cañestro, 2016). This may, for instance, allow the occupation of ecological niches offering a specific food quality with respect to fat and toxin content. However, irrespective of whether loss-of-function mutations were fixed by genetic drift or selection, a reduced functional repertoire of the genome is expected to shape the evolutionary history of species and lineages, for example, by potentially constraining secondary diet changes associated with the increase of fat and toxin consumption.

4.2 | Biological significance and evolution of *PNLIPRP1* gene loss

Hecker et al. (2019b) suggested a protective function of the lipase inhibitor *PNLIPRP1* against excessive fat digestion and putative fitness reduction due to obesity. Under a neutral evolution hypothesis, one would assume that *PNLIPRP1* loses its protective function in species that adapted to fat-poor diet. Consequently, genetic drift may have caused the fixation of destructive mutations under relaxed selection presumably enhanced by varying population sizes during phylogenesis. Alternatively, it is tempting to forward the idea that the loss of a lipase inhibitor may be a key factor that enables the sufficient supply with fatty acids, given that lipids can be a limited resource in both herbivorous and carnivorous diets. Consequently, the loss of this gene might be a necessary prerequisite to occupy ecological niches characterized by fat-poor food sources. In addition, the loss of *PNLIPRP1* may serve as an adaptive strategy that allows the fast gain of body fat mass when facing seasonal variation of food availability or quality and/or after seasonal fasting. Hence, positive selection may have led to the fixation of destructive mutations resulting in gene loss and consequently in the maintenance of a fitness optimum (adaptive evolution hypothesis as outlined above).

There are, however, a number of aspects that need to be further explored prior to accepting the adaptive evolution hypothesis. Although the reduction of fat uptake was associated with *PNLIPRP1* loss in nine lineages (see above), the equid lineage has retained a functional gene copy despite of feeding on fat-poor diet. However, Hecker et al. (2019b) reanalysed transcriptomic data and found a case study for *E. caballus* showing that the pancreas-specific gene is not expressed anymore (Jayne et al., 2002). Given that regulatory inactivation of a gene is functionally equivalent to gene loss, it may turn out to be common in Equidae and enable the recruitment of fat-poor diet.

Another aspect is that alternative evolutionary strategies exist that allow a more efficient recruitment of fatty acids from a generally fat-poor diet. These alternatives may have similar and complementary biological effects like *PNLIPRP1* loss. For instance, it is difficult to quantify the amount of fat that is being recruited apart from the uptake of the main dietary components as recorded in this study. In fact, strictly herbivorous species are known to feed on animal tissue on occasion for various reasons (Tables S6–S9). For instance, insects are ingested by the primarily frugivorous *R. aegyptiacus* (Barclay et al., 2006; Del Vaglio et al., 2011), and arthropods are of minor importance for *O. degus* (Meserve, 1981). Furthermore, *H. glaber* increases its mineral balance through feeding on buried ungulate bones (Jarvis & Sherman, 2002), and *T. manatus* picks up sessile molluscs and crustaceans like barnacles from hulls and is reported to select the fleshy parts of caught actinopterygian fishes from gillnets (Courbis & Worthy, 2003; Powell, 1978). Another example for fat recruitment that is not traceable by recording main dietary components comes from the physiology of herbivorous species. Body plan evolution resulted in various adaptations of the digestive tract that increase the efficiency of fatty acid recruitment. For instance, fermentation of plant material (e.g., cellulose) in the digestive tract of Ruminantia (rumen and foregut fermenters) or Perissodactyla (caecum and hindgut fermenters) by microorganisms results in low molecular fatty acids (volatile fatty acids, e.g., propionic acid and butyric acid), which become part of the lipid metabolism of the species. Similarly, there is experimental evidence for a positive regulation of the lipid metabolism by caecotrophy in Rodentia and Lagomorpha (Wang et al., 2019). A final example for alternative evolutionary strategies refers to the diet-specific fat type composition. For instance, food sources differ not only in their total amount of polyunsaturated fatty acid (PUFA) and monounsaturated fatty acid (MUFA) but also in their ratio (Clausen et al., 2009). This is an interesting aspect to explore given that MUFA and PUFA have different abilities to control the activity and expression of the pancreatic lipase, an enzyme that is competitively inhibited by *PNLIPRP1* (e.g., Birk et al., 2004; Ricketts & Brannon, 1994; Sabb et al., 1986). Furthermore, mammalian lipases are known to be selective towards MUFA/PUFA (review in Kurtovic et al., 2009), and it is known that different PUFA types can be hydrolyzed at different speed (Akanbi et al., 2014). Thus, it needs to be explored to what extent the total amount of fat recruitment solely depends on the fat type itself, irrespective of the (additional) effects resulting from the absence of a lipase inhibitor.

While promoting further functional studies on extant species, we provide support that in-depth phylogenetic analyses (e.g., molecular clock investigations in combination with paleo diet reconstruction) will contribute to an evolutionary understanding of *PNLIPRP1* loss. For instance, although *PNLIPRP1* loss was found to be an ancestral state of all extant Bovidae and convergently occurred within Cetacea, *PNLIPRP1* must have been present in basal Artiodactyla. Thus, it becomes an interesting evolutionary question whether gene loss is associated with the shift from browsing (\dagger *Eotragus sansaniensis*; Figure 2; Table S11) to grazing (extant Bovidae; Figures 1 and 2; Tables S6–S9). This point in evolution does not only mark a diet change but also the occupation of novel environments; a criterion that is in congruence with the adaptive evolution hypothesis (see above). In fact, middle Miocene basal Bovidae like \dagger *E. sansaniensis* lived in an environment free of grassland (Solounias and Moelleken, 1994). Grassland became a dominant ecological niche only later in earth history (late Miocene; Strömberg, 2011), which must have been occupied by bovid species that carried the ancestral *PNLIPRP1* loss described by Hecker et al. (2019b; compare extant Bovidae in Figure 2). Similar arguments count for exploring the evolutionary history of *PNLIPRP1* loss in both extant elephant species. This is because fossil analyses predict a shift from ‘medium’ to ‘low’-fat diet in late Miocene Elephantidae (Figure 3). Under the hypothesis that evolutionary shifts in nutrition strategies are associated with *PNLIPRP1* gene loss in Bovidae and Elephantidae, the absence of *PNLIPRP1* does not constrain the secondary increase of fat consumption to a ‘medium’ level.

4.3 | Biological significance of *NR1I3* gene loss

Hecker et al. (2019b) drew up the hypothesis that the detoxification mechanisms are more important for herbivorous than for carnivorous mammals. This motivated to explore more thoroughly why some carnivorous species still retain *NR1I3* (Pholidota, Carnivora and some Lipotyphla). Our study showed that carnivorous taxa are exposed to diet-related toxins but to a conspicuously lower extent compared with herbivores. Although this statement is primarily derived from diet analyses of the limited number of species used in our study (Figure 1), there is a need to discuss this result in a broader context. Particularly, the order Carnivora includes species, which cover a wider nutrition spectrum. For instance, species like *Arctictis binturong* (binturong) or *Potos flavus* (kinkajou) are specialized frugivores (Lambert et al., 2014). Other families not represented here cover a variety of different ecological niches, from myrmecophagy to scavenger (Hyaenidae) or from carnivory to graminivory (e.g., bamboo; Ursidae). Some of these diets definitively expose these species to toxins (see, for instance, Lambert et al., 2014; Zhou et al., 2020), which in turn supports our assumption that the diet of Carnivora is not totally toxin free. Consequently, it stands to reason that basic detoxification abilities must be essential for carnivorous species. With this conclusion in mind, we presume that a reduced toxin exposure is primarily associated with relaxed selection pressure

on entire detoxification pathways (e.g., pathways associated with *NR1I3*). Focusing on entire pathways instead of single molecules becomes explainable if one considers that *NR1I3* is an upstream receptor along with its paralog *NR1I2*, and both molecules interact with downstream effectors such as the *UGT1A6* enzyme. We assume that a relaxed selection pressure allows the random accumulation of mutations on different members of the same pathway. If destructive mutations cause gene loss of one member, the detoxification capacity is downregulated. At the same time, the strength of negative selection on the remaining pathway molecules will increase and results in the maintenance of a basic detoxification level. This hypothesis is consistent with the situation observed in different genomic screenings. For example, different representatives of Carnivora (Felidae such as *F. catus*, *Acinonyx jubatus*, *P. tigris* and *P. pardus* but also *Mustela putorius furo*, *Enhydra lutris*, *O. rosmarus*, *Leptonychotes weddellii* and *Neomonachus schauinslandi*) still have the two nuclear receptors *NR1I3* and *NR1I2* (Hecker et al., 2019b) but lost *UGT1A6*. A similar situation was observed in *S. araneus*, which possesses *NR1I3* (Figure 1) but lost *NR1I2* (Hecker et al., 2019b).

While forwarding the idea that the lineage-specific loss or retention of different genes (*NR1I3*, *NR1I2* and *UGT1A6*) is the result from an interplay of relaxed and negative selection, we argue that gene losses associated with detoxification pathways cause evolutionary constraints. Our study revealed that extant Megachiroptera evolved from insectivorous ancestors (‘medium’) towards a diet strictly consisting of browse and fruits and thus with a higher toxin content (‘high’), whereas extant Microchiroptera retained a ‘medium’ insectivorous diet. Given that early bats kept the *NR1I3* gene (Figure 2; Hecker et al., 2019b), it was put to full use by Megachiroptera. Conversely, the extant Microchiroptera continued their evolution and became insectivorous specialists that must avoid high level toxin intake, because their loss of the *NR1I3* gene makes them more susceptible to such substance. To do so, they would have to select their insect prey carefully because several of them can be toxic (e.g., arctiid moths; see Blest, 1964; Dunning, 1968). Aposematic insects advertise their toxicity (or fake it) with colourful but also acoustic signals (see, for instance, Dunning & Kruger, 1995; Joron, 2009). A particular example of co-evolution actually involves the ultrasonic acoustic system in echolocating bats (i.e., Microchiroptera) and their prey (see review by Conner & Corcoran, 2012). Because \dagger *Onychonycteris* sp. (late early Eocene) was not yet capable of echolocating its prey (Simmons et al., 2008), the ultrasonic specialization seen in Microchiroptera probably evolved along with their non-toxic prey selection using acoustic signals (see Conner & Corcoran, 2012). We propose that the reduction of toxin consumption was associated with relaxed selection on the *NR1I3* locus in Microchiroptera and hence with the accumulation of destructive mutations, which eventually led to gene loss. This adds one more aspect to an otherwise already classical example of co-evolution. Another constraint that is highlighted here has a more applied aspect. The fact that members of the detoxification pathway are lost in Carnivora (e.g., *UGT1A6* in Felidae and Phocidae; see above) makes them more susceptible to

environmental pollutants than herbivorous mammals (Kakehi et al., 2015). This can be interpreted as the burden of gene loss associated with an adaptation to low diet-related toxin consumption during evolution. Accordingly, this susceptibility to toxin should be considered when assessing the level of threat experienced by Carnivora species (e.g., International Union for Conservation of Nature Red List of Threatened Species) and especially when modelling the impact of human settlements on their population dynamics.

5 | CONCLUSIONS AND FUTURE PERSPECTIVES

This study is a post hoc analysis of a previous comparative genomic study (Hecker et al., 2019b) employing a multitrait matrix. This conceptual approach successfully revisited previous assumptions that the loss of *PNLIPRP1* and *NR1I3* is primarily associated with particular feeding habits, that is, carnivory and herbivory. One of the methodological key features of this study is that our in-depth analysis of phenotypes (feeding habits and uptake of main dietary components) is not only focused on extant mammalian species. The inclusion of informative fossil taxa (state-of-the-art diet reconstruction) allowed considering ancestral conditions (*grundplan* reconstruction). The advantage of this methodological aspect is best exemplified in our study when we showed that *grundplan* reconstruction can be misleading when solely based on inference approaches such as parsimony analyses. Specifically, the loss of *PNLIPRP1* in Elephantidae (Figure 3) became explainable when the fossil record was considered. This demonstrated an independent adaptation to fat-poor grasses in Proboscidea and Sirenia, whose common ancestor ingested fat-containing browse. Furthermore, we argue that the combination of molecular phylogenetics and paleo diet reconstruction is a particularly well-suited conceptual approach to test the coincidence of gene loss and ecological niche occupation. Although this is showcased in our study of *PNLIPRP1*, such approach is also relevant for research on detoxification molecules. By recording dietary components and feeding habits in a fully documented and publicly available data repository (Morph-D-Base; *MaTrics*: <https://www.morphdbase.de/?MaTrics-Mx-v1>, Stefen et al., 2021), the data collection used for this study can be applied to further post hoc studies of comparative genomic data (e.g., initiatives such as Zoonomia; Genereux et al., 2020; <https://zoonomiaproject.org/>). Our study also revealed the substantial lack of knowledge about diet, diet composition and feeding habits. These research gaps ranged between 1% and up to 60%, depending on the requirements of the diet composition models M1–M4. Apart from nutrition, the knowledge gap on general/basic phenotypic information is substantial for mammals (Stefen et al., 2021). Nonetheless, the present study serves as an example that the combination of comparative genomics (e.g., Forward Genomics) and comparative trait analyses is a powerful tool. It generates new insights into genomic and phenotypic

evolution and provides knowledge with implication for applied science. To fully exploit this scientific concept, phenotype analyses of fossil and extant species should experience the same impetus and dynamic as currently experienced by genomic studies.

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CONFLICT OF INTEREST

The authors have declared that no competing interests exist.

AUTHOR CONTRIBUTIONS

I. R., T. L., S. O., C. S. and H. S. conceived the project and elaborated the study design; data were collected by F. W., I. R., T. L., R. H., S. O., C. S. and C. St., and the formal data analysis was performed by H. S. and F. W. The manuscript was drafted by H. S. and F. W. (including visualization of results as tables and figures), and all authors contributed to review and editing of the final manuscript. All authors approved the final manuscript.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

There are no original experimental data (e.g., DNA sequences, measurements etc.) associated with this study.

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REFERENCES

- Akanbi, T. O., Sincliar, A. J., & Barrow, C. J. (2014). Pancreatic lipase selectively hydrolyses DPA over EPA and DHA due to location of double bonds in the fatty acid rather than regioselectivity. *Food Chemistry*, 160, 61–66. <https://doi.org/10.1016/j.foodchem.2014.03.092>
- Albalat, R., & Cañestro, C. (2016). Evolution by gene loss. *Nature Reviews Genetics*, 17, 379–391. <https://doi.org/10.1038/nrg.2016.39>
- Arentz, F. (2020). *Fettfisch oder Magerfisch?* Available from <https://arentz-fisch.de/content/fischkennen/FettMager.php> Accessed 29th of September 2020

- Barclay, R. M. R., Barclay, L. E., & Jacobs, D. S. (2006). Deliberate insectivory by the fruit bat *Rousettus aegyptiacus*. *Acta Chiropterologica*, 8, 549–553.
- Beasley, V. (1999). *Absorption, distribution, metabolism, and elimination: Differences among species*. International Veterinary Information Service.
- Birk, R. Z., Regan, K. S., Boyle-Roden, E., & Brannon, P. M. (2004). Pancreatic lipase and its related protein 2 are regulated by dietary polyunsaturated fat during the postnatal development of rats. *Pediatric Research*, 56, 256–262.
- Blandford, M. I., Katouli, M., Gilby, B. L., O'Dea, C., Olds, A. D., & Schlacher, T. A. (2019). Not all rotten fish stink: Microbial changes in decaying carcasses increase cytotoxicity and potential risks to animal scavengers. *Estuarine, Coastal and Shelf Science*, 227, 106350.
- Blest, A. D. (1964). Protective display and sound production in some New World arctiid and ctenuchid moths. *Zoologica*, 49, 161–181.
- Cerling, T. E., Harris, J. M., & Leakey, M. G. (2005). Environmentally driven dietary adaptations in African mammals. In J. R. Ehleringer, M. D. Dearing, & T. E. Cerling (Eds.), *History of atmospheric CO₂ and the impacts on plants, animals, and ecosystems* (pp. 258–272). Springer.
- Clauss, M., Grum, C., & Hatt, J.-M. (2009). Polyunsaturated fatty acid content in adipose tissue in foregut and hindgut fermenting mammalian herbivores: A literature survey. *Mammalian Biology*, 74, 153–158. <https://doi.org/10.1016/j.mambio.2008.04.004>
- Conner, W. E., & Corcoran, A. J. (2012). Sound strategies: the 65-million-year-old battle between bats and insects. *Annual Review of Entomology*, 57, 21–39.
- Courbis, S. S., & Worthy, G. A. J. (2003). Opportunistic carnivory by Florida manatees (*Trichechus manatus latirostris*). *Aquatic Mammals*, 29, 104–107.
- Del Vaglio, M. A., Nicolaou, H., Bosso, L., & Russo, D. (2011). Feeding habits of the Egyptian fruit bat *Rousettus aegyptiacus* on Cyprus Island: A first assessment. *Hystrix, The Italian Journal of Mammalogy*, 22, 281–289.
- Delibes, M., Calzada, J., Chavez, C., Revilla, E., Ribeiro, B. A., Prado, D., Keller, C., & Palomares, F. (2011). Unusual observation of an ocelot (*Leopardus pardalis*) eating an adult Linnaeus's two-toed sloth (*Choloepus didactylus*). *Mammalian Biology*, 76, 240–241. <https://doi.org/10.1016/j.mambio.2010.08.006>
- Discher, S., Burse, A., Tolzin-Banasch, K., Heinemann, S. H., Pasteels, J. M., & Boland, W. (2009). A versatile transport network for sequestering and excreting plant glycosides in leaf beetles provides an evolutionary flexible defense strategy. *ChemBioChem*, 10, 2223–2229. <https://doi.org/10.1002/cbic.200900226>
- Dunning, D. C. (1968). Warning sounds of moths. *Zeitschrift Für Tierpsychologie*, 25, 129–138.
- Dunning, D. C., & Kruger, M. (1995). Aposematic sounds in African moths. *Biotropica*, 27, 227–231. <https://doi.org/10.2307/2388998>
- Eisenberg, J. F. (1981). *The mammalian radiations. An analysis of trends in evolution, adaptation, and behaviour*. The Athlone Press.
- Emerling, C. A. (2018). Regressed but not gone: Patterns of vision gene loss and retention in subterranean mammals. *Integrative and Comparative Biology*, 58, 441–451.
- Emerling, C. A., Delsuc, F., & Nachman, M. W. (2018). Chitinase genes (CHIAs) provide genomic footprints of a post-Cretaceous dietary radiation in placental mammals. *Science Advances*, 4, eaar6478.
- Franck, A. R., & Farid, A. (2020). Many species of the Carnivora consume grass and other fibrous plant tissues. *Belgian Journal of Zoology*, 150, 1–70.
- Genereux, D. P., Serres, A., Armstrong, J., Johnson, J., Marinescu, V. D., Muren, E., Juan, D. V., Bejerano, G., Casewell, N. R., Chemnick, L. G., Damas, J., Di Palma, F., Diekhans, M., Fiddes, I. T., Garber, M., Gladyshev, V. N., Goodman, L., Haerty, W., Houck, M. L., ... Consortium Z. (2020). A comparative genomics multitool for scientific discovery and conservation. *Nature*, 587, 240–+.
- Gerland, U., & Hwa, T. (2009). Evolutionary selection between alternative moes of gene regulation. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 8841–8846.
- Gheerbrant, E., Schmitt, A., & Kocsis, L. (2018). Early African fossils elucidate the origin of embriothopod mammals. *Current Biology*, 28, 2167–2173.
- Hamilton, W. J. Jr. (1931). Habits of the star-nosed mole, *Condylura cristata*. *Journal of Mammalogy*, 12, 345–355.
- Harborne, J. B. (1999). Plant chemical ecology. In D. Barton, K. Nakanshi, & O. Meth-Cohn (Eds.), *Comprehensive natural products chemistry* (Vol. 8, pp. 137–196. Elsevier.
- Hecker, N., Lächele, U., Stuckas, H., Giere, P., & Hiller, M. (2019a). Convergent vomeronasal system reduction in mammals coincides with convergent losses of calcium signalling and odorant-degrading genes. *Molecular Ecology*, 28, 3656–3668. <https://doi.org/10.1111/mec.15180>
- Hecker, N., Sharma, V., & Hiller, M. (2019b). Convergent gene losses illuminate metabolic and physiological changes in herbivores and carnivores. *Proceedings of the National Academy of Sciences of the United States of America*, 116, 3036–3041. <https://doi.org/10.1073/pnas.1818504116>
- Hua, L., Gong, S., Wang, F., Li, W., Ge, Y., Li, X., & Hou, F. (2015). Captive breeding of pangolins: Current status, problems and future prospects. *ZooKeys*, 507, 99–114. <https://doi.org/10.3897/zookeys.507.697099-114>
- Huelsmann, M., Hecker, N., Springer, M. S., Gatesy, J., Sharma, V., & Hiller, M. (2019). Genes lost during the transition from land to water in cetaceans highlight genomic changes associated with aquatic adaptations. *Science Advances*, 5, eaaw6671. <https://doi.org/10.1126/sciadv.aaw6671>
- Jarvis, J. U. M., & Sherman, P. W. (2002). Heterocephalus glaber. *Mammalian Species*, 706, 1–9. [https://doi.org/10.1644/1545-1410\(2002\)706<0001:HG>2.0.CO;2](https://doi.org/10.1644/1545-1410(2002)706<0001:HG>2.0.CO;2)
- Jayne, S., Kerfelec, B., Foglizzo, E., Chapus, C., & Crenon, I. (2002). High expression in adult horse of PLRP2 displaying a low phospholipase activity. *Biochimica Et Biophysica Acta*, 1594, 255–265. [https://doi.org/10.1016/S0167-4838\(01\)00309-0](https://doi.org/10.1016/S0167-4838(01)00309-0)
- Joron, M. (2009). Aposematic coloration. In V. H. Resh & R. Carde (Eds.), *Encyclopedia of insects* (2nd ed., pp. 33–38). Academic Press.
- Takehi, M., Ikenaka, Y., Nakayama, S. M., Kawai, Y. K., Watanabe, K. P., Mizukawa, H., Nomiya, K., Tanabe, S., & Ishizuka, M. (2015). Uridine diphosphate-glucuronosyltransferase (UGT) xenobiotic metabolizing activity and genetic evolution in pinniped species. *Toxicological Sciences*, 147, 360–369. <https://doi.org/10.1093/toxsci/kfv144>
- Kim, S., Cho, Y. S., Kim, H. M., Chung, O., Kim, H., Jho, S., Seomun, H., Kim, J., Bang, W. Y., Kim, C., An, J., Bae, C. H., Bhak, Y., Jeon, S., Yoon, H., Kim, Y., Jun, J., Lee, H., Cho, S., ... Yeo, J. H. (2016). Comparison of carnivore, omnivore, and herbivore mammalian genomes with a new leopard assembly. *Genome Biology*, 17, 211.
- Kurtovic, I., Marshall, S. N., Rhao, X., & Simpson, B. K. (2009). Lipases from mammals and fishes. *Reviews in Fisheries Science*, 17, 18–40. <https://doi.org/10.1080/10641260802031322>
- Labisky, R. F., & Boulay, M. C. (1998). Behaviors of bobcats preying on white-tailed deer in the everglades. *The American Midland Naturalist*, 139, 275–281.
- Lambert, J. E., Fellner, V., McKenney, E., & Hartstone-Rose, A. (2014). Binturong (*Arctictis binturong*) and kinkajou (*Potos flavus*) digestive strategy: Implications for interpreting frugivory in Carnivora and Primates. *PLoS One*, 9, e105415. <https://doi.org/10.1371/journal.pone.0105415>
- Ma, J. E., Li, L. M., Jiang, H. Y., Zhang, X. J., Li, J., Li, G. Y., Yuan, L. H., Wu, J., & Chen, J. P. (2017). Transcriptomic analysis identifies genes and pathways related to myrmecophagy in the Malayan pangolin (*Manis javanica*). *PeerJ*, 5, e4140.

- Maddison, W. P., & Maddison, D. R. (2019). *Mesquite: A modular system for evolutionary analysis. Version 3.61*. <http://www.mesquiteproject.org>
- Meredith, R. W., Gatesy, J., Murphy, W. J., Ryder, O. A., & Springer, M. S. (2009). Molecular decay of the tooth gene Enamelin (ENAM) mirrors the loss of enamel in the fossil record of placental mammals. *PLoS Genetics*, 5, e1000634. <https://doi.org/10.1371/journal.pgen.1000634>
- Meredith, R. W., Zhang, G., Gilbert, M. T. P., Jarvis, E. D., & Springer, M. S. (2014). Evidence for a single loss of mineralized teeth in the common avian ancestor. *Science*, 346, 1254390–1254396. <https://doi.org/10.1126/science.1254390>
- Meserve, P. L. (1981). Trophic relationships among small mammals in a Chilean semiarid thorn scrub community. *Journal of Mammalogy*, 62, 304–314. <https://doi.org/10.2307/1380707>
- Pineda-Munoz, S., & Alroy, J. (2014). Dietary characterization of terrestrial mammals. *Proceedings of the Royal Society B: Biological Sciences*, 281, 20141173. <https://doi.org/10.1098/rspb.2014.1173>
- Powell, J. A. Jr. (1978). Evidence of carnivory in manatees (*Trichechus manatus*). *Journal of Mammalogy*, 59, 442. <https://doi.org/10.2307/1379938>
- Prudent, X., Parra, G., Schwede, P., Roscito, J. G., & Hiller, M. (2016). Controlling for phylogenetic relatedness and evolutionary rates improves the discovery of associations between species' phenotypic and genomic differences. *Molecular Biology and Evolution*, 33, 2135–2150. <https://doi.org/10.1093/molbev/msw098>
- Rainio, M. J., Kanerva, M., Wahlberg, N., Nikinmaa, M., & Eeva, T. (2012). Variation of basal EROD activities in ten passerine bird species—Relationships with diet and migration status. *PLoS One*, 7, e33926. <https://doi.org/10.1371/journal.pone.0033926>
- Ren, J., Chen, Z., Zhang, W., Li, L., Sun, R., Deng, C., Fei, Z., Sheng, Z., Wang, L., Sun, X., Wang, Z., & Fei, J. (2011). Increased fat mass and insulin resistance in mice lacking pancreatic lipase-related protein 1. *Journal of Nutritional Biochemistry*, 22, 691–698. <https://doi.org/10.1016/j.jnutbio.2010.06.002>
- Richardson, P. R. K., & Levitan, C. D. (1994). Tolerance of aardwolves to defense secretions of *Trinervitermes trinervoides*. *Journal of Mammalogy*, 75, 84–91. <https://doi.org/10.2307/1382238>
- Ricketts, J., & Brannon, P. M. (1994). Amount and type of dietary fat regulate pancreatic lipase gene expression in rats. *The Journal of Nutrition*, 124, 1166–1171. <https://doi.org/10.1093/jn/124.8.1166>
- Román-Palacios, C., Scholl, J. P., & Wiens, J. J. (2019). Evolution of diet across the animal tree of life. *Evolution Letters*, 3, 339–347. <https://doi.org/10.1002/evl3.127>
- Sabb, J. E., Godfrey, P. M., & Brannon, P. M. (1986). Adaptive response of rat pancreatic lipase to dietary fat: Effects of amount and type of fat. *The Journal of Nutrition*, 116, 892–899. <https://doi.org/10.1093/jn/116.5.892>
- Schaller, G. B. (1967). *The deer and the tiger. A study of wildlife in India*. University of Chicago Press.
- Sharma, V., Hecker, N., Roscito, J. G., Foerster, L., Langer, B. E., & Hiller, M. (2018). A genomics approach reveals insights into the importance of gene losses for mammalian adaptations. *Nature Communications*, 9, 1215. <https://doi.org/10.1038/s41467-018-03667-1>
- Sharma, V., Hecker, V., Walther, F., Stuckas, H., & Hiller, M. (2020). Convergent losses of TLR5 suggest altered extracellular flagellin detection in four mammalian lineages. *Molecular Biology and Evolution*, 37, 1847–1854. <https://doi.org/10.1093/molbev/msaa058>
- Shrestha, B., Reed, J. M., Starks, P. T., Kaufman, G. E., Goldstone, J. V., Roelke, M. E., O'Brien, S. J., Koepfli, K.-P., Frank, L. G., & Court, M. H. (2011). Evolution of a major drug metabolizing enzyme defect in the domestic cat and other Felidae: Phylogenetic timing and the role of hypercarnivory. *PLoS One*, 6, e18046. <https://doi.org/10.1371/journal.pone.0018046>
- Simmons, N. B., Seymour, K. L., Habersetzer, J., & Gunnell, G. F. (2008). Primitive Early Eocene bat from Wyoming and the evolution of flight and echolocation. *Nature*, 451, 818–821.
- Solounias, N., & Moelleken, S. M. (1994). Differences in diet between two archaic ruminant species from Sansan, France. *Historical Biology*, 7, 203–220. <https://doi.org/10.1080/10292389409380454>
- Soucek, P. (2017). Xenobiotics. In M. Schwab (Ed.), *Encyclopedia of cancer*. Springer.
- Stahler, D. R., Smith, D. W., & Guernsey, D. S. (2006). Foraging and feeding ecology of the gray wolf (*Canis lupus*): lessons from Yellowstone National Park, Wyoming, USA. *The Journal of Nutrition*, 136, 1923–1926. <https://doi.org/10.1093/jn/136.7.1923>
- Stefen, C., Wagner, F., Asztalos, M., Giere, P., Grobe, P., Hiller, M., Hofmann, R. R., Jähde, M., Lächele, U., Lehmann, T., Ortman, S., Peters, B., Ruf, I., Schiffmann, C., Thier, N., Unterhitzberger, G., Vogt, L., Rudolf, M., Wehner, P., & Stuckas, H. (2021). Phenotyping in the era of genomics: MaTrics—A digital character matrix to document mammalian phenotypic traits coded numerically. *Mammalian Biology*, in press. <https://doi.org/10.1007/s42991-021-00192-5>
- Strömberg, C. A. (2011). Evolution of grasses and grassland ecosystems. *Annual Review of Earth and Planetary Sciences*, 39, 517–544. <https://doi.org/10.1146/annurev-earth-040809-152402>
- Sunquist, M. E. (1981). *The social organization of tigers (Panthera tigris) in Royal Chitawan National Park, Nepal*. Smithsonian Institution Press.
- Tolson, A. H., & Wang, H. (2010). Regulation of drug-metabolizing enzymes by xenobiotic receptors: PXR and CAR. *Advanced Drug Delivery Reviews*, 62, 1238–1249. <https://doi.org/10.1016/j.addr.2010.08.006>
- Ungar, P. S. (2010). ch. 3: Food and feeding. In P. S. Ungar (Ed.), *Mammal teeth: Origin, evolution, and diversity* (pp. 26–38). The Johns Hopkins University Press.
- Vucetich, J. A., Vucetich, L. M., & Peterson, R. O. (2012). The causes and consequences of partial prey consumption by wolves preying on moose. *Behavioral Ecology and Sociobiology*, 66, 295–303. <https://doi.org/10.1007/s00265-011-1277-0>
- Wang, Y., Xu, H., Sun, G., Xue, M., Sun, S., Huang, T. Z. J., Loo, J. J., & Li, M. (2019). Transcriptome analysis of the effects of fasting caecotrophy on hepatic lipid metabolism in New Zealand rabbits. *Animals*, 9, 648.
- Zhou, C., Liu, Y., Qiao, L., Lan, Y., Price, M., Meng, Y., Yang, N., & Yue, B. (2020). Genome-wide analyses provide insights into the scavenging lifestyle of the striped Hyena (*Hyaena hyaena*). *DNA and Cell Biology*, 39, 1872–1885.

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