BIOLOGY LETTERS

royalsocietypublishing.org/journal/rsbl

Research

Cite this article: Martinez Q, Courcelle M, Douzery E, Fabre P-H. 2023 When morphology does not fit the genomes: the case of rodent olfaction. *Biol. Lett.* **19**: 20230080. https://doi.org/10.1098/rsbl.2023.0080

Received: 20 September 2022
Accepted: 24 March 2023

Subject Areas:
evolution, molecular biology, ecology, behaviour, neuroscience

Keywords:
olfactory turbinal bones, genomics, functional olfactory receptor genes, olfactory capabilities, anatomy, mammals

Author for correspondence:
Quentin Martinez
e-mail: quentinmartinezphoto@gmail.com

Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.6534012.

THE ROYAL SOCIETY PUBLISHING

Downloaded from https://royalsocietypublishing.org/ on 07 February 2024

Evolutionary biology

When morphology does not fit the genomes: the case of rodent olfaction

Quentin Martinez[1,2], Maxime Courcelle[1], Emmanuel Douzery[1] and Pierre-Henri Fabre[1,3,4,5]

[1]Institut des Sciences de l'Évolution (ISEM, UMR 5554 CNRS-IRD-UM-EPHE), Université de Montpellier, Place E. Bataillon - CC 064 - 34095, Montpellier Cedex 5, France
[2]Staatliches Museum für Naturkunde Stuttgart DE-70191, Stuttgart, Germany
[3]Mammal Section, Department of Life Sciences, The Natural History Museum, London SW7 5DB, UK
[4]Institut Universitaire de France (IUF), Paris, France
[5]Division of Vertebrate Zoology (Mammalogy), American Museum of Natural History, Central Park West, 79th St., New York, NY 10024-5192, USA

QM, 0000-0002-7127-4012; ED, 0000-0001-5286-647X

Linking genes to phenotypes has been a major question in evolutionary biology for the last decades. In the genomic era, few studies attempted to link olfactory-related genes to different anatomical proxies. However, they found very inconsistent results. This study is the first to investigate a potential relation between olfactory turbinals and olfactory receptor (OR) genes. We demonstrated that despite the use of similar methodology in the acquisition of data, OR genes do not correlate with the relative and the absolute surface area of olfactory turbinals. These results challenged the interpretations of several studies based on different proxies related to olfaction and their potential relation to olfactory capabilities.

1. Introduction

The sense of olfaction is key to the survival of most mammals [1–3]. However, there is still a large gap in our understanding of how olfactory performance relates to its genomic and anatomical bases, and whether and how olfactory genetics and morphology differentially covary among species that have contrasting olfactory capabilities. Olfactory turbinals (turbinals covered with olfactory epithelium) are considered the main olfactory bony system. Olfactory sensory neurons from these areas project posteriorly via olfactory nerves through the cribriform plate and join the glomeruli from the main olfactory bulb [1]. It is assumed that olfactory turbinals are uniformly covered with olfactory receptors (ORs), therefore the absolute surface area of olfactory turbinals may reflect the absolute number of ORs [4–8]. Comparative phylogenetic analyses demonstrated a relation between the relative size of olfactory turbinals and species ecology such as diet [4,7] or lifestyle [4,8]. At the genomic level, OR genes encode the most represented chemosensory receptors in mammals, and constitute the largest multigenic superfamily categorized into about 13 gene families [3,9,10]. Moreover, the number of functional and pseudogenized OR genes and their composition vary highly along mammalian phylogeny [3,9] and with species ecology [9–15]. This is for example the case for diet [10,13,15] or ecological lifestyle [11,14,16,17]. Therefore, OR genes are expected to covary with morphological olfactory-related proxies such as olfactory turbinals.

Linking genes to phenotypes has been a major question in evolutionary biology for the last decades. Few studies focused on olfaction, evaluated the correlation between genetic and morphological proxies and yielded contrasting results [18–20]. However, to date, none of them investigated the potential relation between olfactory turbinals and OR genes. The major obstacles to address these issues have been to access and accurately quantify the internal olfactory organs as

© 2023 The Author(s) Published by the Royal Society. All rights reserved.

Downloaded from https://royalsocietypublishing.org/ on 07 February 2024

well as the limited number of high-quality, available mammalian genomes. However, in 2022, more than 500 mammalian genomes became mineable, and genome quality has increased exponentially [21]. Similarly, X-ray micro-computed tomography (micro-CT) has become more accessible, resulting in an unprecedented expansion of three-dimensional data for internal anatomical structures [22]. This provides ample opportunities to fill the gap in our understanding of the basis and evolution of olfaction in mammals.

In the most diversified mammalian order, the rodents [23], we investigated the potential relation between the two main proxies for olfaction: (1) OR genes and (2) olfactory turbinals. For this, we used standardized genomics and morphological data, carefully acquired by similar investigators and using identical methods.

2. Results

No significant correlations were found between (i) the number of functional OR genes and the relative olfactory turbinal surface area (figure 2*a*, $s = 0.33$, $r^2 = -0.01$, $p = 0.35$), (ii) the number of functional OR genes and the absolute olfactory turbinal surface area (figure 2*b*, $s = 0.04$, $r^2 = -0.01$, $p = 0.39$), (iii) the number of functional OR genes and the skull length (figure 2*c*, $s = 0.05$, $r^2 = -0.04$, $p = 0.63$), (iv) the total number of OR genes and the skull length (figure 2*d*, $s = 0.21$, $r^2 = -0.04$, $p = 0.18$), (v) the number of functional OR genes and the number of olfactory turbinals (figure 2*e*, $s = -0.03$, $r^2 = -0.05$, $p = 0.94$), (vi) the total number of OR genes and the number of olfactory turbinals (figure 2*f*, $s = 0.07$, $r^2 = -0.05$, $p = 0.90$), (vii) the relative olfactory turbinal surface area and the number of olfactory turbinals (figure 2*g*, $s = -0.08$, $r^2 = -0.04$, $p = 0.72$), and (viii) the absolute olfactory turbinal surface area and the number of olfactory turbinals (figure 2*h*, $s = 3.06$, $r^2 = 0.11$, $p = 0.07$). Conversely, there is a significant correlation between: (i) the total turbinal surface area and the skull length (figure 2*i*, $s = 2.82$, $r^2 = 0.96$, $p = 7.01 \times 10^{-16}$), (ii) the absolute olfactory turbinal surface area and the skull length (figure 2*j*, $s = 2.41$, $r^2 = 0.94$, $p = 3.25 \times 10^{-14}$), and (iii) the absolute olfactory turbinals surface area and the total turbinal surface area (figure 2*k*, $s = 0.86$, $r^2 = 0.98$, $p = 2.20 \times 10^{-16}$). The inclusion or exclusion of the lamina semicircularis (ls) did not change the results (electronic supplementary material, tables S1–S15). This reinforces the relevance of our results given the current available genomes and scanned specimens.

Species with a similar number of functional OR genes may have different values of the relative olfactory turbinal surface area (figure 2*a*). Conversely, species with comparable values of the relative olfactory turbinal surface area may have a different number of functional OR genes (figure 2*a*).

When the 13 different families of functional OR genes are independently analysed, none of them significantly correlates with the relative olfactory turbinal surface area that used total turbinal surface area for sizing (electronic supplementary material, tables S1–S15). When skull length is used for sizing, the relative olfactory turbinal surface area significantly correlates with the number of functional OR genes of the following families: 1S55, 2S11, 2S1.3.7 and 2S4 (electronic supplementary material, figure S1). However, r squared values are very low (less than 0.29) and these significant correlations are only based on the relative olfactory turbinals surface area that used skull length for sizing (see electronic supplementary material, Methods S1.).

3. Discussion

(a) Lack of link between genomic and morphology

Despite their key function in olfaction, functional OR genes and the relative surface area of olfactory turbinals did not significantly correlate with each other in Rodentia (figure 2*a*). Interestingly, species with a similar number of functional OR genes may have different values of the relative (or absolute) olfactory turbinal surface area, and conversely (figures 1 and 2*a*,*b*). The absolute surface area of olfactory turbinals mostly reflects allometry (figure 2*b*,*h*,*j*, [5–8]). The smallest taxa (*Heterocephalus*, *Microtus*, *Mus*, *Muscardinus*) have the lowest values while the largest species (*Castor*, *Hydrochoerus*, *Hystrix*, *Myocastor*) have the highest ones. Indeed, absolute olfactory turbinal surface area is significatively correlated with skull length (figure 2*j*, [7,8]). The number of functional OR genes and the total number of OR genes are independent of species size since they did not correlate with skull length (figure 2*c*,*d*). This pattern may be the result of different selective pressures, such as drift, phylogenetic inertia as well as environmental constraints [3,13,14]. As an example, the amphibious mammals were largely studied in the light of their olfactory turbinal reductions [4,8] and their large number of OR pseudogenes and/or different OR gene compositions [11–13,16,24–27]. When amphibious and terrestrial species are compared, their patterns differ depending on whether we look at the functional OR genes or the relative surface area of olfactory turbinals. For example, the amphibious *Castor*, *Hydrochoerus* and *Ondatra* have the lowest values for the relative olfactory turbinal surface area while they have an intermediate number of functional OR genes (figures 1 and 2*a*). According to the ecological lifestyle, when the mean value of the sampled species is considered, there is a tendency for the reduction of the number of functional OR genes in amphibious species for ORs class I, ORs class II and all ORs (electronic supplementary material, figure S3). However, our dataset lacks closely related terrestrial species to go further in our interpretation. Indeed, the more phylogenetically distant the species are, the more likely it is that the observed differences are due to this distance and not to ecology. Such an accurate comparison can be approached with the amphibious *Ondatra* and the terrestrial *Microtus*, both species from the subfamily of the Arvicolinae. Again, when amphibious and terrestrial species are compared, their patterns differ depending on whether we look at the genomics or morphological proxies. The relative surface area of olfactory turbinals is markedly lower in *Ondatra* than in *Microtus* (figures 1 and 2*a*). This is a complete opposite pattern for the number of functional OR genes, which are higher in *Ondatra* than in *Microtus* (figures 1 and 2*a*). Our results contrast with the significant correlation between the relative surface area of the cribriform plate and the number of functional OR genes across Mammalia [19]. This is surprising knowing that the absolute and the relative surface area of olfactory turbinals significantly correlated with different proxies of the cribriform plate of carnivora and some myrmecophagous mammals [28,29]. The cribriform plate is often described as a potential proxy for olfactory function [28]

Downloaded from https://royalsocietypublishing.org/ on 07 February 2024

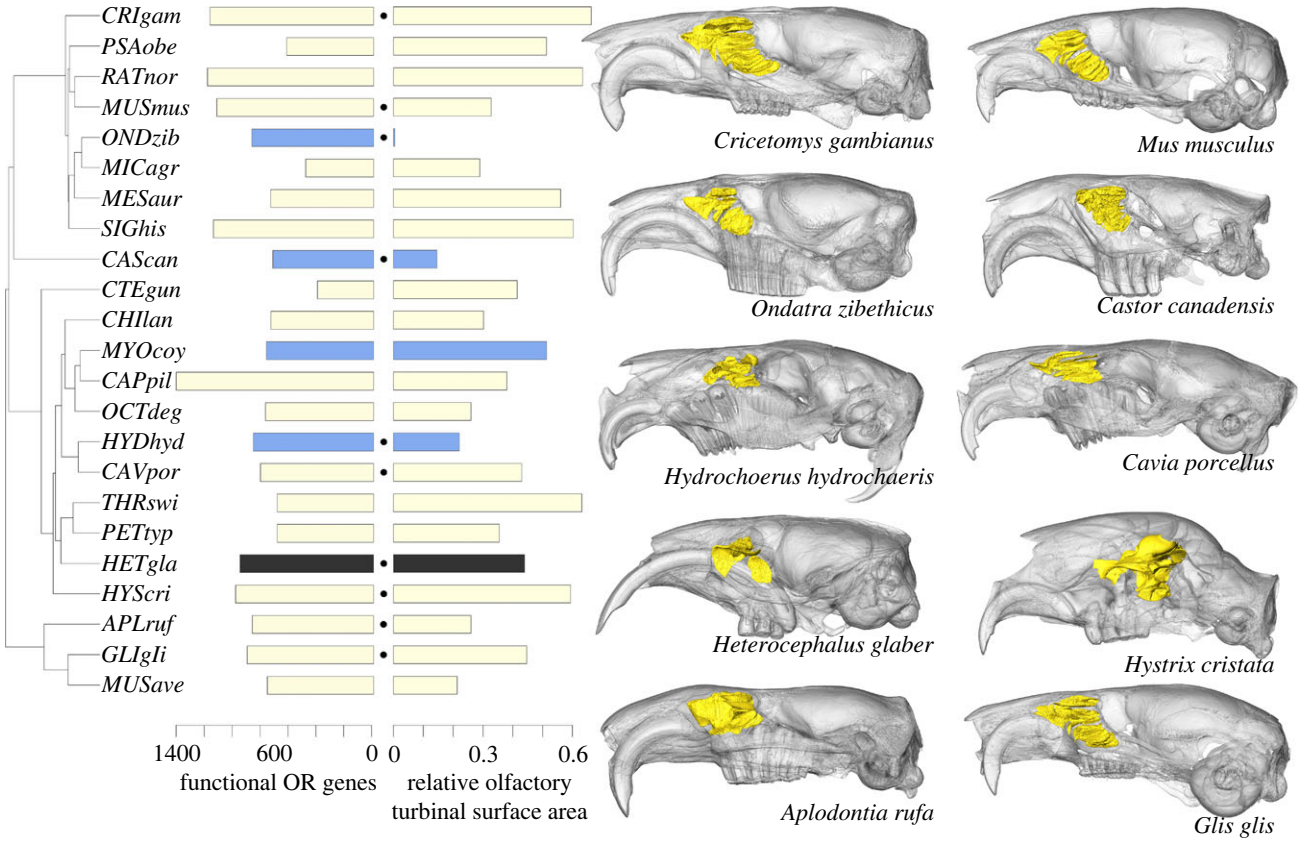

Figure 1. Inconsistent pattern between the functional OR genes and the relative olfactory turbinal surface area. Phylogeny of the sampled species with bar plots of their functional OR genes and relative olfactory turbinal surface area as well as three-dimensional representations of their skull and olfactory turbinals. The relative olfactory turbinal surface area corresponds to the residuals of the PGLS between the absolute olfactory turbinal surface area and the total turbinal surface area. Black dots represent the illustrated species. Bar plot colours: beige = terrestrial, blue = amphibious, black = subterranean species.

because it is a proxy for the cross section of olfactory nerves that originate from the olfactory turbinals and join the olfactory bulb through this plate. Similarly, olfactory turbinals are described as a potential proxy for olfactory function [4,8] because it is assumed that they reflect olfactory epithelium surface area and therefore ORs.

The absence of significant correlations between the two main genomics and morphological proxies for olfaction may be explained by several factors. First, it is possible that olfactory turbinals are not uniformly covered by ORs [30] and/or that their degree of receptive range varies [31]. Second, the degree of neural connectivity may also vary between organs and species [32]. Third, the olfactory epithelium cover may vary between species and mammalian orders [30,33,34]. Also, some evidence in Carnivora suggested that some parts of the olfactory turbinals may be selected for respiration [35]. However, in rodents, such variation is mostly discussed for the lamina semicircularis (ls) and we demonstrated that with our current dataset, its inclusion or exclusion did not change the results (electronic supplementary material, tables S1–S15, see also electronic supplementary material, Methods S1.). Finally, it is possible that OR genes do not represent the different expression levels leading to different olfactory capabilities [36]. A recent study in phyllostomid bats demonstrated that there is no relation between the evolutionary rates of OR genes extracted from transcriptomes and diet whereas there may exist a relation between olfactory epithelium and diet [37]. In addition, whereas classes I and II of OR genes have been postulated to bind mostly water-borne and air-borne odorant molecules [9,38], the different OR gene families have mostly been distinguished on the basis of their DNA sequence alignment and phylogenetic analysis, and await for more functional characterization.

(b) Estimating and linking olfactory capabilities with morphology and genomes

Our study demonstrated that at the taxonomic scale of rodents, the number of olfactory turbinals does not correlate with potential proxies for olfactory functions such as the number of functional OR genes, and the relative and the absolute olfactory turbinal surface area (figure 2*e*,*g*,*h*). This finding matches with the general hypothesis that the number of turbinals is mostly related to phylogenetic inertia while their relative size and complexity is related to species ecology and olfactory performance [4,6–8,33,34,39,40].

Olfactory capabilities are commonly divided in two major components: (1) sensitivity, the ability to detect odours at low concentration and (2) discrimination, the ability to distinguish between two similar odours [4,41]. Van Valkenburgh *et al*. ([4] citing [42,43] hypothesized that the relative surface area of olfactory turbinals may neither correlate with olfactory discrimination nor with sensitivity. Therefore, they suggested that it may characterize the diversity of odorants that can be perceived. However, Martinez *et al*. [7] demonstrated that highly specialized worm-eating rodents have significantly higher relative surface area and complexity of olfactory turbinals as compared to their close omnivorous and carnivorous relatives. This may be also the

Downloaded from https://royalsocietypublishing.org/ on 07 February 2024

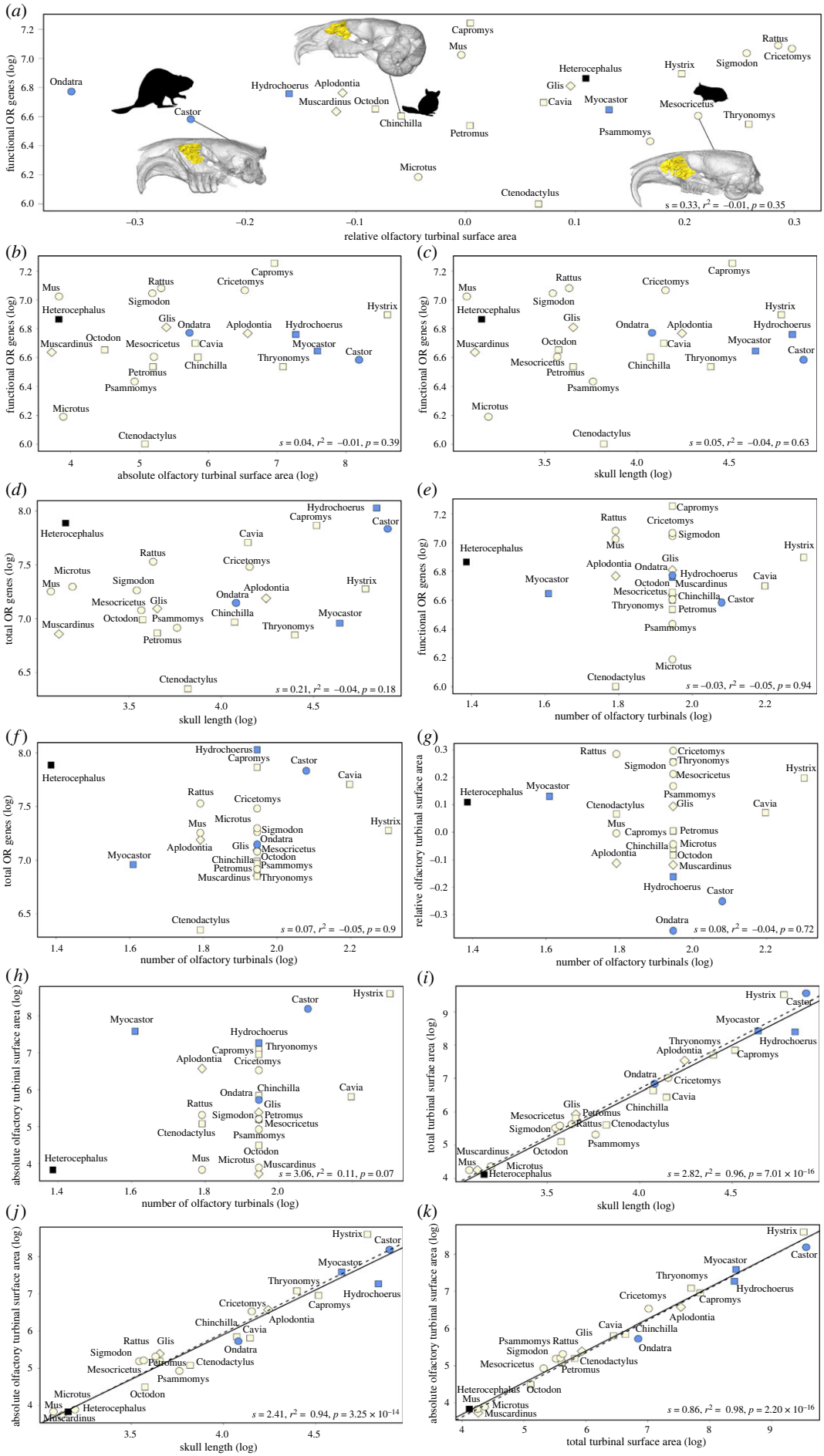

Figure 2. Absence of significant correlations between the morphology and the genomes. Linear regressions (continuous lines) and PGLS (dashed lines) between genomic and morphological proxies for olfaction. Species silhouettes and three-dimensional representations of the skull and the olfactory turbinals in *Castor*, *Chinchilla* and *Mesocricetus*. Symbols: circle = mouse-related clade, square = Ctenohystrica clade, diamond = squirrel-related clade.

Downloaded from https://royalsocietypublishing.org/ on 07 February 2024

case in Carnivora where large-bodied hypercarnivores such as wolf (*Canis lupus*) and wolverine (*Gulo gulo*) have very large olfactory turbinals [6]. These results suggest that the relative size of olfactory turbinals may be linked to olfactory sensitivity. However, the very small number of studies linking olfactory performance with the relative size of olfactory organs leaves many open questions [44]. In addition, the mechanisms of olfaction are still not fully understood [45]. For example, a single odorant molecule can be detected by a specialized receptor, or by multiple receptors operating independently or in combination. Conversely, a single olfactory receptor can also bind to several odorant molecules [10,46–48]. To complicate things further, odorant molecules with different structures may be perceived as a single odour and different odorant molecules with a similar structure may be perceived as the same odour, while odorant molecules with similar structure may be perceived as different odours [10,46–48]. However, as an example, the millions or even trillions of olfactory stimuli that humans can detect cannot be explained solely by our approximately 400 functional OR genes, even with combinatorial models [49,50]. In this context, other theories have re-emerged like the highly debated vibration theory of olfaction based on quantum physics [45,51–56]. This implies that olfactory molecules could be detected thanks to their vibration frequency instead of their shape [57]. In this context, it is challenging to interpret how the number of OR genes impact the olfactory capabilities. For this, it will be critical to map the level of gene expression and their composition on the olfactory turbinals [15,58].

4. Material and methods

In this study, we used the same methodology of data acquisition both for morphological data and genomic pipeline to extract OR genes (see discussion in the electronic supplementary material, Methods S1). Undamaged specimens of 32 individuals belonging to 23 species were selected from museums (electronic supplementary material, table S16) and scanned using high-resolution X-ray micro-computed tomography. Left respiratory and olfactory turbinals were segmented following Martinez *et al*. [7,8] with AvizoLite 2020.1 (VSG Inc.). Mean values were used when multiple individuals of a species were sampled. OR genes were extracted from genomic assemblies *in silico*, using the same bioinformatic procedures for each species (electronic supplementary material, Methods S1). The total number of OR genes is the sum of functional and pseudogenes OR genes. We performed linear regression and phylogenetic generalized least squares (PGLS) between the different variables (figure 2, electronic supplementary material, figures S1 and S2, electronic supplementary material, tables S1–S15). This was performed with the *gls* function from the package nlme [59]. We plotted linear regressions (continuous lines) and PGLS lines (dashed lines) when a significant correlation exists. The relative olfactory turbinal surface area corresponds to the residuals of the PGLS between the absolute olfactory turbinal surface area and the total turbinal surface area. The total turbinal surface area corresponds to the sum of respiratory and olfactory turbinal surface area. We used the phylogeny from Upham *et al*. [60] and pruned to match the species in our dataset.

Data accessibility. All data are available in the main text or the supplementary materials. We provided an online link for the Data S1.

The data are provided in the electronic supplementary material [61].

Authors' contributions. M.Q.: conceptualization, data curation, formal analysis, investigation, methodology, writing—original draft; M.C.: conceptualization, data curation, formal analysis, investigation, writing—original draft; E.D.: conceptualization, data curation, formal analysis, investigation, writing—original draft; P.-H.F.: conceptualization, data curation, formal analysis, investigation, writing—original draft.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

Funding. We acknowledge the MRI platform member of the national infrastructure France-BioImaging supported by the French National Research Agency (grant no. ANR-10-INBS-04, Investments for the future), the labex CEMEB (grant no. ANR-10-LABX-0004) and NUMEV (grant no. ANR-10-LABX-0020). This research was funded by l'Agence Nationale de la Recherche (Défi des autres savoirs, grant nos DS10, ANR-17-CE02-0005 RHINOGRAD 2017), Synthesis of Systematic Resources (SYNTHESYS, financed by European Community Research Infrastructure Action, grant nos GB-TAF-5737, GB-TAF-6945 and GB-TAF-1316 to the National History Museum London, NHM UK), and the Alexander von Humboldt foundation (Q.M., under the grant no. FRA-1222365-HFST-P). This is a contribution of ISEM 2023-047.

Acknowledgements. We are grateful to the following people for different types of support, including discussions and access to specimens: R. Allio, P. G. Cox, F. Delscuc, S. Ferreira-Cardoso, M. Laska, M. Lovy, C. Molinier, J. Okrouhlík, R. Šumbera.

References

1. Negus V. 1958 *The comparative anatomy and physiology of the nose and paranasal sinuses*. London, UK: E. & S. Livingstone.
2. Stoddart DM. 1980 *The ecology of vertebrate olfaction*. New York, NY: Chapman & Hall.
3. Niimura Y, Nei M. 2007 Extensive gains and losses of olfactory receptor genes in mammalian evolution. *PLoS ONE* **2**, e708. (doi:10.1371/journal.pone.0000708)
4. Van Valkenburgh B, Curtis A, Samuels JX, Bird D, Fulkerson B, Meachen-Samuels J, Slater GJ. 2011 Aquatic adaptations in the nose of carnivorans: evidence from the turbinates. *J. Anat.* **218**, 298–310. (doi:10.1111/j.1469-7580.2010.01329.x)
5. Van Valkenburgh B, Pang B, Bird D, Curtis A, Yee K, Wysocki C, Craven BA. 2014 Respiratory and olfactory turbinals in feliform and caniform carnivorans: the influence of snout length. *Anat. Rec.* **297**, 2065–2079. (doi:10.1002/ar.23026)
6. Green PA, Valkenburgh B, Pang B, Bird D, Rowe T, Curtis A. 2012 Respiratory and olfactory turbinal size in canid and arctoid carnivorans. *J. Anat.* **221**, 609–621. (doi:10.1111/j.1469-7580.2012.01570.x)
7. Martinez Q, Lebrun R, Achmadi AS, Esselstyn JA, Evans AR, Heaney LR, Miguez RP, Rowe KC, Fabre P-H. 2018 Convergent evolution of an extreme dietary specialisation, the olfactory system of worm-eating rodents. *Sci. Rep.* **8**, 17806. (doi:10.1038/s41598-018-35827-0)
8. Martinez Q, Clavel J, Esselstyn JA, Achmadi AS, Grohé C, Pirot N, Fabre P-H. 2020 Convergent evolution of olfactory and thermoregulatory capacities in small amphibious mammals. *Proc. Natl Acad. Sci. USA* **117**, 8958–8965. (doi:10.1073/pnas.1917836117)
9. Hayden S, Bekaert M, Crider TA, Mariani S, Murphy WJ, Teeling EC. 2010 Ecological adaptation determines functional mammalian olfactory subgenomes. *Genome Res.* **20**, 1–9. (doi:10.1101/gr.099416.109)
10. Hayden S, Teeling EC. 2014 The molecular biology of vertebrate olfaction. *Anat. Rec.* **297**, 2216–2226. (doi:10.1002/ar.23031)

Downloaded from https://royalsocietypublishing.org/ on 07 February 2024

11. Kishida T, Kubota S, Shirayama Y, Fukami H. 2007 The olfactory receptor gene repertoires in secondary-adapted marine vertebrates: evidence for reduction of the functional proportions in cetaceans. *Biol. Lett.* **3**, 428–430. (doi:10.1098/rsbl.2007.0191)
12. Kishida T, Thewissen J, Hayakawa T, Imai H, Agata K. 2015 Aquatic adaptation and the evolution of smell and taste in whales. *Zool. Lett.* **1**, 9. (doi:10.1186/s40851-014-0002-z)
13. Hughes GM, Boston ESM, Finarelli JA, Murphy WJ, Higgins DG, Teeling EC. 2018 The birth and death of olfactory receptor gene families in mammalian niche adaptation. *Mol. Biol. Evol.* **35**, 1390–1406. (doi:10.1093/molbev/msy028)
14. Courcelle M, Fabre P-H, Douzery E. Submitted. Phylogeny, ecology and gene families covariation shaped the olfactory subgenome of rodents.
15. Yohe LR, Leiser-Miller LB, Kaliszewska ZA, Donat P, Santana SE, Dávalos LM. 2021 Diversity in olfactory receptor repertoires is associated with dietary specialization in a genus of frugivorous bat. *G3* 11.10. (https://doi.org/10.1093/g3journal/jkab260).
16. Niimura Y. 2009 On the origin and evolution of vertebrate olfactory receptor genes: comparative genome analysis among 23 chordate species. *Genome Biol. Evol.* **1**, 34–44. (doi:10.1093/gbe/evp003)
17. Stathopoulos S, Bishop JM, O'Ryan C. 2014 Genetic signatures for enhanced olfaction in the African mole-rats. *PLoS ONE* **9**, e93336. (doi:10.1371/journal.pone.0093336)
18. Garrett EC, Steiper ME. 2014 Strong links between genomic and anatomical diversity in both mammalian olfactory chemosensory systems. *Proc. R. Soc. B* **281**, 20132828. (doi:10.1098/rspb.2013.2828)
19. Bird DJ, Murphy WJ, Fox-Rosales L, Hamid I, Eagle RA, Van Valkenburgh B. 2018 Olfaction written in bone: cribriform plate size parallels olfactory receptor gene repertoires in Mammalia. *Proc. R. Soc. B* **285**, 20180100. (doi:10.1098/rspb.2018.0100)
20. Hecker N, Lächele U, Stuckas H, Giere P, Hiller M. 2019 Convergent vomeronasal system reduction in mammals coincides with convergent losses of calcium signalling and odorant-degrading genes. *Mol. Ecol.* **28**, 3656–3668. (doi:10.1111/mec.15180)
21. Allio R, Tilak M-K, Scornavacca C, Avenant NL, Kitchener AC, Corre E, Nabholz B, Delsuc F. 2021 High-quality carnivoran genomes from roadkill samples enable comparative species delineation in aardwolf and bat-eared fox. *Elife* **10**, e63167. (doi:10.7554/eLife.63167)
22. Boyer D, Gunnell G, Kaufman S, McGeary T. 2016 Morphosource: archiving and sharing 3-D digital specimen data. *Paleontol. Soc. Pap.* **22**, 157–181. (doi:10.1017/scs.2017.13)
23. D'Elía G, Fabre P-H, Lessa E. 2019 Rodent systematics in an age of discovery: recent advances and prospects. *J. Mammal.* **100**, 852–871. (doi:10.1093/jmammal/gyy179)
24. Freitag J, Ludwig G, Andreini I, Rössler P, Breer H. 1998 Olfactory receptors in aquatic and terrestrial vertebrates. *J. Comp. Physiol. [A]* **183**, 635–650. (doi:10.1007/s003590050287)
25. Zhou X *et al.* 2013 Baiji genomes reveal low genetic variability and new insights into secondary aquatic adaptations. *Nat. Commun.* **4**, 2708. (doi:10.1038/ncomms3708)
26. Springer MS, Gatesy J. 2017 Inactivation of the olfactory marker protein (OMP) gene in river dolphins and other odontocete cetaceans. *Mol. Phylogenet. Evol.* **109**, 375–387. (doi:10.1016/j.ympev.2017.01.020)
27. Beichman AC *et al.* 2019 Aquatic adaptation and depleted diversity: a deep dive into the genomes of the sea otter and giant otter. *Mol. Biol. Evol.* **36**, 2631–2655. (doi:10.1093/molbev/msz101)
28. Bird DJ, Amirkhanian A, Pang B, Van Valkenburgh B. 2014 Quantifying the cribriform plate: influences of allometry, function, and phylogeny in carnivora. *Anat. Rec.* **297**, 2080–2092. (doi:10.1002/ar.23032)
29. Hautier L, Garland K, Ferreira-Cardoso S, Wright M, Martinez Q, Fabre P-H, Lebrun R, Frederic D. 2019 Sniffing out covariation patterns in the olfactory system of myrmecophagous mammals *J. Morphol.* **280**, S133.
30. Smith TD, Bhatnagar KP, Tuladhar P, Burrows AM. 2004 Distribution of olfactory epithelium in the primate nasal cavity: are microsmia and macrosmia valid morphological concepts? *Anat. Rec. A. Discov. Mol. Cell. Evol. Biol.* **281A**, 1173–1181. (doi:10.1002/ar.a.20122)
31. Kaupp UB. 2010 Olfactory signalling in vertebrates and insects: differences and commonalities. *Nat. Rev. Neurosci.* **11**, 188–200. (doi:10.1038/nrn2789)
32. Hildebrand JG, Shepherd GM. 1997 Mechanisms of olfactory discrimination: converging evidence from common principles across phyla. *Annu. Rev. Neurosci.* **20**, 595–631. (doi:10.1146/annurev.neuro.20.1.595)
33. Ruf I. 2020 Ontogenetic transformations of the ethmoidal region in Muroidea (Rodentia, Mammalia): new insights from perinatal stages. *Vertebr. Zool.* **70**, 383–415.
34. Yee KK, Craven BA, Wysocki CJ, Van Valkenburgh B. 2016 Comparative morphology and histology of the nasal fossa in four mammals: gray squirrel, bobcat, coyote, and white-tailed deer. *Anat. Rec.* **299**, 840–852. (doi:10.1002/ar.23352)
35. Pang B, Yee KK, Lischka FW, Rawson NE, Haskins ME, Wysocki CJ, Craven BA, Van Valkenburgh B. 2016 The influence of nasal airflow on respiratory and olfactory epithelial distribution in felids. *J. Exp. Biol.* **219**, 1866–1874.
36. Young JM, Shykind BM, Lane RP, Tonnes-Priddy L, Ross JA, Walker M, Williams EM, Trask BJ. 2003 Odorant receptor expressed sequence tags demonstrate olfactory expression of over 400 genes, extensive alternate splicing and unequal expression levels. *Genome Biol.* **4**, R71. (doi:10.1186/gb-2003-4-11-r71)
37. Yohe LR *et al.* 2022 Ecological constraints on highly evolvable olfactory receptor genes and morphology in neotropical bats. *Evolution* **76**, 2347–2360. (doi:10.1111/evo.14591)
38. Freitag J, Krieger J, Strotmann J, Breer H. 1995 Two classes of olfactory receptors in *Xenopus laevis*. *Neuron* **15**, 1383–1392. (doi:10.1016/0896-6273(95)90016-0)
39. Macrini T. 2012 Comparative morphology of the internal nasal skeleton of adult marsupials based on X-ray computed tomography. *Bull. Am. Mus. Nat. Hist.* **365**, 1–91. (doi:10.1206/365.1)
40. Ruf I. 2014 Comparative anatomy and systematic implications of the turbinal skeleton in Lagomorpha (mammalia). *Anat. Rec.* **297**, 2031–2046. (doi:10.1002/ar.23027)
41. Van Valkenburgh B, Smith TD, Craven BA. 2014 Tour of a Labyrinth: exploring the vertebrate nose. *Anat. Rec.* **297**, 1975–1984. (doi:10.1002/ar.23021)
42. Kowalewsky S, Dambach M, Mauck B, Dehnhardt G. 2006 High olfactory sensitivity for dimethyl sulphide in harbour seals. *Biol. Lett.* **2**, 106–109. (doi:10.1098/rsbl.2005.0380)
43. Laska M. 2005 The number of functional olfactory receptor genes and the relative size of olfactory brain structures are poor predictors of olfactory discrimination performance with enantiomers. *Chem. Senses.* **30**, 171–175. (doi:10.1093/chemse/bji013)
44. Pihlström H, Fortelius M, Hemilä S, Forsman R, Reuter T. 2005 Scaling of mammalian ethmoid bones can predict olfactory organ size and performance. *Proc. R. Soc. B* **272**, 957–962. (doi:10.1098/rspb.2004.2993)
45. Vosshall LB. 2015 Laying a controversial smell theory to rest. *Proc. Natl Acad. Sci. USA* **112**, 6525–6526. (doi:10.1073/pnas.1507103112)
46. Malnic B, Hirono J, Sato T, Buck LB. 1999 Combinatorial receptor codes for odors. *Cell* **96**, 713–723. (doi:10.1016/S0092-8674(00)80581-4)
47. Niimura Y. 2012 Olfactory receptor multigene family in vertebrates: from the viewpoint of evolutionary genomics. *Curr. Genomics* **13**, 103–114. (doi:10.2174/138920212799860706)
48. Yohe LR, Brand P. 2018 Evolutionary ecology of chemosensation and its role in sensory drive. *Curr. Zool.* **64**, 525–533. (doi:10.1093/cz/zoy048)
49. Bushdid C, Magnasco MO, Vosshall LB, Keller A. 2014 Humans can discriminate more than 1 trillion olfactory stimuli. *Science* **343**, 1370–1372. (doi:10.1126/science.1249168)
50. Gerkin RC, Castro JB. 2015 The number of olfactory stimuli that humans can discriminate is still unknown. *Elife* **4**, e08127. (doi:10.7554/eLife.08127)
51. Dyson GM. 1928 Some aspects of the vibration theory of odor. In *Perfumery and essential oil record*, vol. 19, pp. 456–459. London, UK: G. Street & Company, Limited.
52. Turin L. 1996 A spectroscopic mechanism for primary olfactory reception. *Chem. Senses* **21**, 773–791. (doi:10.1093/chemse/21.6.773)
53. Franco MI, Turin L, Mershin A, Skoulakis EMC. 2011 Molecular vibration-sensing component in *Drosophila melanogaster* olfaction. *Proc. Natl Acad. Sci. USA* **108**, 3797–3802. (doi:10.1073/pnas.1012293108)

54. Block E *et al.* 2015 Vibrational theory of olfaction is implausible. *Proc. Natl Acad. Sci. USA* **112**, E3155–E3155.
55. Block E *et al.* 2015 Implausibility of the vibrational theory of olfaction. *Proc. Natl Acad. Sci. USA* **112**, E2766–E2774.
56. Turin L, Gane S, Georganakis D, Maniati K, Skoulakis EMC. 2015 Plausibility of the vibrational theory of olfaction. *Proc. Natl Acad. Sci. USA* **112**, E3154–E3154. (doi:10.1073/pnas.1508035112)
57. Brookes JC. 2017 Quantum effects in biology: golden rule in enzymes, olfaction, photosynthesis and magnetodetection. *Proc. R. Soc. Math. Phys. Eng. Sci.* **473**, 20160822.
58. Hayden S, Bekaert M, Goodbla A, Murphy WJ, Dávalos LM, Teeling EC. 2014 A cluster of olfactory receptor genes linked to frugivory in bats. *Mol. Biol. Evol.* **31**, 917–927. (doi:10.1093/molbev/msu043)
59. Pinheiro J, Bates D, DebRoy S, Sarkar D. 2006 Linear and nonlinear mixed effects models. R Package. See https://svn.r-project.org/R-packages/trunk/nlme/.
60. Upham NS, Esselstyn JA, Jetz W. 2019 Inferring the mammal tree: species-level sets of phylogenies for questions in ecology, evolution, and conservation. *PLoS Biol.* **17**, e3000494. (doi:10.1371/journal.pbio.3000494)
61. Martinez Q, Courcelle M, Douzery E, Fabre P-H. 2023 When morphology does not fit the genomes: the case of rodent olfaction. Figshare. (doi:10.6084/m9.figshare.c.6534012)

Downloaded from https://royalsocietypublishing.org/ on 07 February 2024