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Does the number of functional olfactory receptor genes predict olfactory sensitivity and discrimination performance in mammals?

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Abstract

The number of functional genes coding for olfactory receptors differs markedly between species and has repeatedly been suggested to be predictive of a species' olfactory capabilities. To test this assumption, we compiled a database of all published olfactory detection threshold values in mammals and used three sets of data on olfactory discrimination performance that employed the same structurally related monomolecular odour pairs with different mammal species. We extracted the number of functional olfactory receptor genes of the 20 mammal species for which we found data on olfactory sensitivity and/or olfactory discrimination performance from the Chordata Olfactory Receptor Database. We found that the overall olfactory detection thresholds significantly correlate with the number of functional olfactory receptor genes. Similarly, the overall proportion of successfully discriminated monomolecular odour pairs significantly correlates with the number of functional olfactory receptor genes. These results provide the first statistically robust evidence for the relationship between olfactory capabilities and their genomics correlates. However, when analysed individually, of the 44 monomolecular odourants for which data on olfactory sensitivity from at least five mammal species are available, only five yielded a significant correlation between olfactory detection thresholds and the number of functional olfactory receptors genes. Also, for the olfactory discrimination performance, no significant correlation was found for any of the 74 relationships between the proportion of successfully discriminated monomolecular odour pairs and the number of functional olfactory receptor genes. While only a rather limited amount of data on olfactory detection thresholds and olfactory discrimination scores in a rather limited number of mammal species is available so far, we conclude that the number of functional olfactory receptor genes may be a predictor of olfactory sensitivity and discrimination performance in mammals.

Keywords: functional olfactory receptor genes, mammals, olfactory sensitivity, olfactory discrimination performance, correlational analysis

Introduction

It is well established that species differ in their olfactory capabilities (e.g., Laska, 2017; Wackermannová et al., 2016). However, the mechanisms underlying such between-species differences in olfactory sensitivity, olfactory discrimination performance, olfactory learning and memory, as well as reliance upon olfactory cues in a variety of behavioral contexts remain controversial.

Several neuroanatomical features, such as the size of the olfactory epithelium (e.g., Nummela et al., 2013), the number of olfactory receptor cells (e.g., Bressel et al., 2016), the absolute and/or the relative size of the olfactory turbinals (e.g., Green et al., 2012; Martinez et al., 2018, 2020, Martinez, Courcelle, et al., 2023; Van Valkenburgh et al., 2011, 2014), the morphology of the nasal cavity with regard to olfactory airflow (e.g., Eiting et al., 2015), the absolute and/or relative size of the cribriform plate (e.g., Bird et al., 2014, 2018; Pihlström et al., 2005), the absolute and/or relative size of the olfactory bulbs (e.g., Barton, 2006; Smith et al., 2007), or the number of glomeruli within the olfactory bulbs (e.g., Ngwenya et al., 2011), have been suggested to correlate with a species' olfactory performance. Similarly, some genetic features such as the total number of functional olfactory receptor genes (e.g., Niimura et al., 2018) have been used to predict a species' olfactory capabilities. All of these attempts to explain between-species differences in any aspect of olfactory performance have so far met with only limited success, with some studies supporting the notion of an existing correlation between the neuroanatomical or genetic feature in question and a given olfactory capability (e.g., Rizvanovic et al., 2013) and other studies failing to do so (e.g., Laska et al., 2005). Possible reasons for this include, but are not restricted to, the limited number of species that have been tested on a given olfactory capability, the limited number of odour stimuli that have been tested with different species, and the limited number of species for which data on the neuroanatomical or genetic feature in question are available.

Recent advances in genome sequencing and annotation of olfactory receptor genes have markedly increased the number of species for which data on the number of functional and non-functional olfactory receptor genes are now at hand (Han et al., 2022). As the former rather than the latter of these two genetic features determines the breadth of a species' olfactory receptor repertoire, we assessed whether the number of functional olfactory receptor genes may correlate with (a) olfactory sensitivity and/or (b) olfactory discrimination

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performance with structurally related monomolecular odourants in mammals.

For olfactory sensitivity, the reasoning underlying this potential relationship is that species expressing a higher number of olfactory receptor types should be able to detect odour molecules at lower concentrations and thus have a higher olfactory sensitivity compared with species expressing a lower number of olfactory receptor types due to an increased likelihood that a given type of odour molecule should find suitable olfactory receptors with which it can interact (see Mombaerts, 2001; Rouquier & Giorgi, 2007). Recent research has demonstrated that, when presented with very low, peri-threshold concentrations of an odourant, only one or at most few glomeruli are triggered in the olfactory bulb (Burton et al., 2022). Therefore, from a genomics perspective, it is likely that olfactory sensitivity to a specific odourant is closely associated with the expression level of a specific functional olfactory receptor gene.

For olfactory discrimination, species expressing a higher number of olfactory receptor types should be able to perform finer discriminations between perceptually and/or structurally similar odour stimuli compared to species expressing a lower number of olfactory receptor types due to an increased ability to generate detailed patterns of neural activation in the olfactory bulb and in higher centers of the olfactory-processing pathway.

To this end, we compiled a database of all published olfactory detection threshold values in mammals and of all published olfactory discrimination data that employed the same monomolecular odour pairs with different mammal species. Based on the findings of previous studies, we hypothesized that the olfactory sensitivity of mammals would not correlate with the number of functional olfactory receptor genes, whereas the olfactory discrimination performance would (e.g., Laska & Shepherd, 2007; Laska et al., 2005; Rizvanovic et al., 2013; Sarrafchi et al., 2013).

Materials and methods

Data collection

For olfactory sensitivity, we compiled a database of all published olfactory detection threshold values in non-human mammals obtained using operant conditioning procedures. They were extracted from Laska (2017) and updated with recent findings (Supplementary Table S1). As most studies on olfactory sensitivity in non-human mammals employed only a low number of individuals, we decided to use the lowest individual threshold values reported per odourant and species. Human olfactory detection threshold values were extracted from van Gemert (2011) and updated with recent findings (Supplementary Table S1). As most studies on olfactory sensitivity in human subjects only report mean threshold values, we used the lowest mean threshold values reported per odourant. All threshold values were converted to log parts per million (log ppm) of the gas phase (Supplementary Table S2). For our analyses, we only used the 44 odourants for which threshold values from at least five species were available.

For olfactory discrimination performance, we used three published data sets that reported on olfactory discrimination performance with structurally related monomolecular odourants in human and non-human mammals. They were extracted from Laska (2017) and updated with recent findings (Supplementary Table S1). The three data sets comprise data on olfactory discrimination performance with (A) aliphatic odourants sharing the same functional group but differing in carbon chain length, (B) aliphatic odourants sharing the same carbon chain length but differing in the functional group, and (C) pairs of enantiomers, that is, chiral stereoisomers. For our correlational analyses, we considered the overall success rate of a given species with a given data set, that is, the proportion of successfully discriminated odour pairs with data set A, or B, or C, respectively (level 1; Supplementary Table S3), the proportion of successfully discriminated odour pairs within data sets A and B sharing either the same functional group (data set A, level 2; Supplementary Table S4) or sharing the same carbon chain length (data set B, level 2; Supplementary Table S4), and the success rate with each individual odour pair pairs with data set A, or B, or C, respectively (level 3; Supplementary Tables S5, S6, and S7, respectively). In cases when all species considered here succeeded with discriminating a given odour pair (or a given set of odour pairs), correlational analysis was not applicable. However, since all species considered here differ from each other in their number of functional olfactory receptor genes, the correlation may also be considered statistically non-significant. Similarly, in cases when data for a given odour pair (or a given set of odour pairs) were available for less than three species, correlational analysis was not applicable.

All data on both olfactory sensitivity and olfactory discrimination performance considered in the present study were based on operant conditioning procedures. It is widely accepted that this paradigm is the gold standard in animal olfactory psychophysics (Pearce, 2008) and thus allows for accurate and comparable data. In particular, this experimental approach keeps the best possible control over an animal's motivation, thus minimizing the risk of differences in motivation affecting olfactory performance (Hastings, 2003).

The number of functional olfactory receptor genes of the 20 mammal species for which we found data on olfactory sensitivity and/or olfactory discrimination performance was extracted from the Chordata Olfactory Receptor Database (CORD, Han et al., 2022). With two of the 20 species (*Saimiri sciureus* and *Arctocephalus pusillus*), we had to use the data of their closest relatives (*Saimiri boliviensis* and *Arctocephalus gazella*) as the number of olfactory receptor genes for the exact species was not available. The 20 available species include five species of bats, four species of primates, four species of carnivorans, two species of eulipotyphlans, two species of rodents, one species of rabbit, one species of pig, and one species of elephant. To perform additional tests (see below), we also extracted from CORD the number of functional olfactory receptor genes of the 396 species that match the mammal phylogeny of Upham et al. (2019).

Statistical assessment of the overall olfactory sensitivity and discrimination performance

For olfactory sensitivity and olfactory discrimination performance levels 3A, B, and C, we conducted logistic regressions predicting olfactory detection threshold values and discrimination success with trial-ranked count of the number of functional olfactory receptors genes (Figure 1, Supplementary Table S8). To do this, for each series of performance tests, we removed species that were not tested and ranked the remaining species according to their number of functional olfactory receptor genes from the lowest to the highest value. This ranking is based on the hypothesis that species with a high

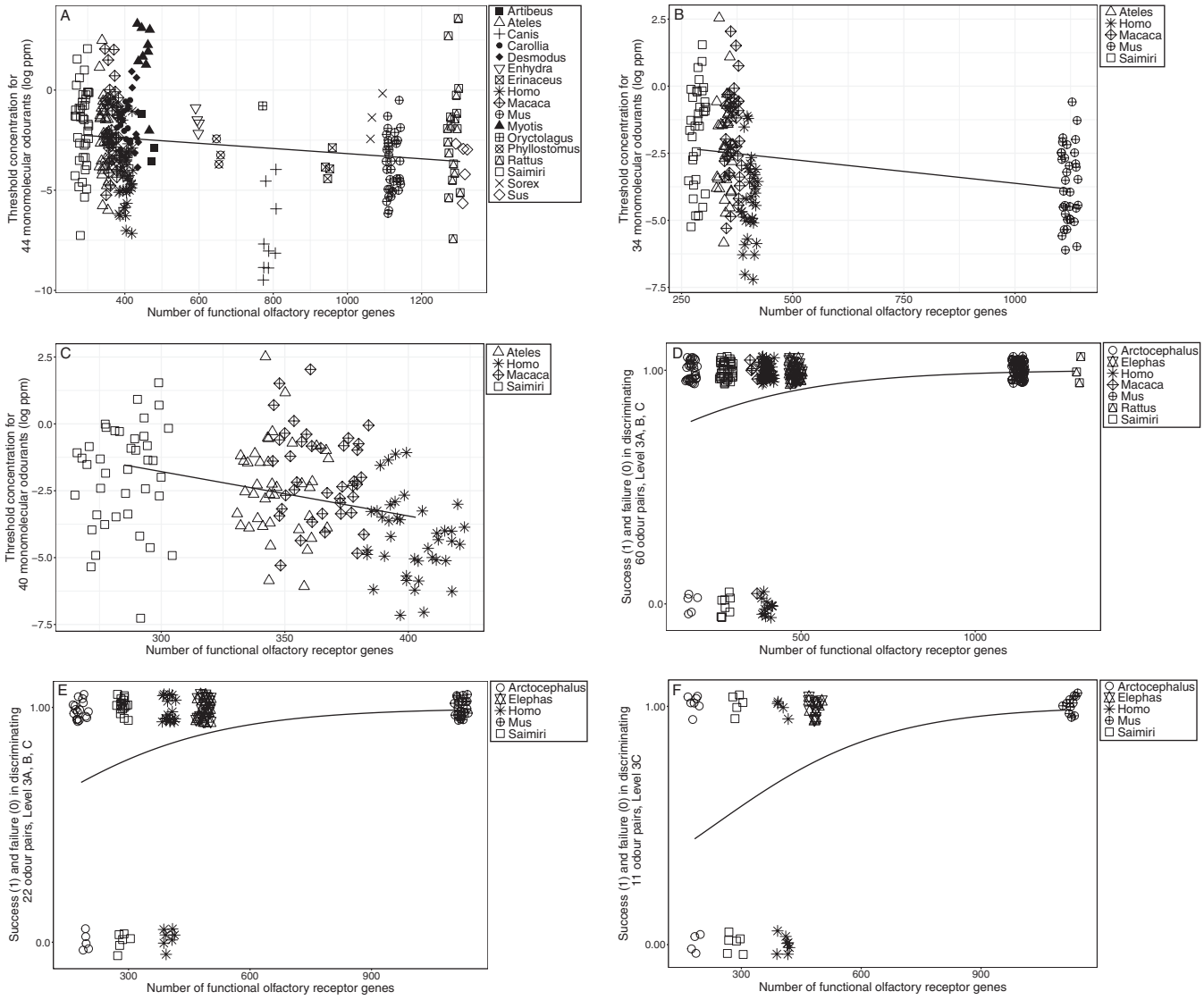

Figure 1. Monotonic correlations and logistic regressions between olfactory detection threshold values (A–C) or success and failure in discriminating odour pairs (D–F) and the number of functional olfactory receptor genes. (A) Unbalanced overall data set of 44 monomolecular odourants and 18 species (P = 3.651e-05 and 7.450e-04). (B) Balanced subset of 34 monomolecular odourants and five species (P = 1.309e-09 and 1.454e-04). (C) Balanced subset of 40 monomolecular odourants and four species (P = 6.029e-07 and 2.233e-06). (D) Unbalanced overall data set, level 3A, B, C (see Method) of 60 odour pairs and seven species (P = 0.002 and 0.005). (E) Balanced subset, levels 3A, B, C (see Method) of 22 odour pairs and five species (P = 0.003 and 0.021). (F) Balanced subset, level 3C (see Method) of 11 odour pairs and five species (P = 0. 003 and 0.026). The first P-values are based on Spearman's rank-correlation test, while the second is based on model comparison employing the genus as a random factor (see Method and Supplementary Table S8). For visualization purposes, random noise was added to the values (see Method). The logistic regression lines and the statistics did not comprise this random noise.

number of functional olfactory receptor genes may have high olfactory capabilities in sensitivity and/or discrimination. Then, we merged all the trial-ranked counts, respectively, for olfactory sensitivity and olfactory discrimination performance and performed model comparisons. We considered olfactory performance as the dependent variable (y) and the number of functional olfactory receptor genes as the predictor variable (x). For olfactory sensitivity, we compared a linear mixed-effects model (lmer) considering the genus as the random effect and a linear model (lm) that did not consider the genus. Both models were compared using an ANOVA, then another ANOVA was applied to the best-fitting model. The normality of the residuals of the models was checked with the Shapiro test.

For olfactory discrimination performance, we compared a generalized linear mixed-effects model (glmer) considering the genus as the random effect and a generalized linear model (glm) that did not consider the genus. In both cases, we considered the family binomial as a function of the model. Again, both models were compared using an ANOVA, then another ANOVA was applied to the best-fitting model. The linearity of the model was graphically checked in R (R Core Team, 2017). We tested the heteroscedasticity of the model with the Breusch–Pagan test. All these tests were conducted with the R packages *tests* and *lme4* (Bates et al., 2015). The overall data sets are unbalanced, with some species that were tested for more monomolecular odourants and/or odour pairs than others, which potentially biases the results. Therefore,

we conducted the previously described analyses on balanced subsets where all the analysed species were tested for the same monomolecular odourants and/or odour pairs (Figure 1, Supplementary Table S8). To provide comparable results with previous studies (e.g., Laska & Shepherd, 2007; Rizvanovic et al., 2013) as well as to perform a non-parametric test when the validity condition of the previous tests has not been met, we also performed Spearman's rank-correlation tests with the functions *cor.test* from the *stats* R package (Supplementary Table S8). Figure 1 was performed with the R package *ggplot2* (Wickham, 2016). For the plots, random noise was added to the values in order to be able to distinguish the overlapping points. This was performed with the option *position jitter* from the R package *ggplot2* (Wickham, 2016). The logistic regression line and the statistics did not comprise this random noise.

A different approach to handling the data involves transforming the raw values for the number of functional olfactory receptor genes into numerical rankings. These rankings range from "one," corresponding to the species with the lowest number of functional olfactory receptor genes, to the maximum number. Finally, for some statistical approaches, it is also possible to exclude trials wherein all species successfully discriminated odour pairs. Subsequently, we conduct the previous statistical analyses while implementing these two alternative transformations and subsets.

One-by-one statistical assessments of olfactory sensitivity and discrimination performance

To provide a more precise statistical assessment of the potential link between the olfactory performance and its potential genomics correlate, we also tested the relationship between the number of functional olfactory receptor genes and each independent test with the different monomolecular odourants and odour pairs. In this case, we conducted Spearman's rank-correlation (Supplementary Tables S2, S3, S4, S5, and S7). For olfactory sensitivity, correlation analyses were performed using the number of functional olfactory receptor genes and the olfactory detection threshold values of 44 odourants (Figure 2, Supplementary Figures S1 and S2, Supplementary Table S2). For olfactory discrimination, a total of 74 correlation analyses were performed with the number of functional olfactory receptor genes and the proportion of successfully discriminated odour pairs according to the three levels mentioned above (Figure 2, Supplementary Figure S2, Supplementary Tables S3, S4, S5, and S7). For the olfactory sensitivity and the olfactory discrimination level 3C, the *P*-values were also adjusted for multiple comparisons with the functions *p.adjust* from the *stats* R package (Supplementary Tables S2 and S7). Figure 2 was performed with the *ggplot2* (Wickham, 2016) and *ggsunburst* (available at https://github.com/didacs/ggsunburst) R packages.

Phylogenetic signal and statistical power

Due to the low number of species (5–14 for olfactory sensitivity with a given odourant and 3–7 for olfactory discrimination with a given odour pair or a given set of odour pairs), it is not recommended to perform phylogenetic generalized least squares (PGLS, see Münkemüller et al., 2012). However, for demonstrative purposes, we conducted PGLS on the previously tested relationships (Supplementary Figure S2, Supplementary Tables S2, S3, S4, S5, and S7). This was performed with the function *pgls* from the R package *caper* (Orme et al., 2023) and using the transformation parameter lambda with a value bounded between 0 and 1 (Supplementary Data S1). For this, we used a maximum clade credibility (MCC) phylogeny obtained from 10,000 trees sampled in the posterior distribution of Upham et al. (2019) and pruned to match the 20 species in our data set as well as the 396 species that match the CORD database. The MCC consensus tree was inferred using TreeAnnotator v.2.6.6 (Bouckaert et al., 2014) with a 25% burn-in. For each category and level of olfactory performance, we tested if we have the power to distinguish between different models of evolution. For this, we followed Boettiger et al. (2012) and compared the distributions of the likelihood ratio statistic for two evolutionary models (relying on Brownian motion and white noise, respectively) fitted to the residuals of the correlation between the number of functional olfactory receptor genes and the threshold detection of the *n*-butanoic acid as well as for the success of odour pairs discrimination (Supplementary Figure S3; R packages *caper* (Orme et al., 2023), *dplyr*, *ggplot2* (Wickham, 2016), *pmc* (Boettiger et al., 2012), and *tidyr*; Supplementary Data S1). We computed this check in the subsets that comprise the higher number of species in each category and level of olfactory performance tests.

The phylogenetic signal of the relationship between the olfactory performance and the number of functional olfactory receptor genes was tested with the Pagel's lambda (Pagel, 1999) and the *phylosig* function from the R package *phytools* (Revell, 2012). We computed it for the subsets that comprise the higher number of species in each category and level of olfactory performance test. For this, we used the residuals of the linear model between these performance tests and the number of functional olfactory receptor genes (Supplementary Table S9). For demonstrative purposes, we also tested the phylogenetic inertia of the number of functional olfactory receptor genes for the data sets that comprise 20 and 396 species, respectively (Supplementary Table S9).

For all the previously listed analyses, we estimated the number of species or points (for the overall performance tests analyses) required to reach a sufficient statistical power in our correlations (Figure 2, Supplementary Figure S2, Supplementary Tables S2, S3, S4, S5, S7, and S8). This was performed with *pwr* function from the R package *pwr* (Champely et al., 2017), with a power level of 80% and 50%, respectively (Supplementary Data S1).

Results

Overall olfactory sensitivity performance

For the relationship between the number of functional olfactory receptor genes and the overall olfactory sensitivity, the model considering the genus as the random effect is always the best fit with a significant difference. This is true for the complete unbalanced data set as well as with the balanced subsets considering at least two, four, five, and six genera (Supplementary Table S8). The ANOVA of the model and the Spearman's rank-correlation test are non-significant for the subset considering at least six genera (but considering only six monomolecular odourants). However, in this case, the statistical power does not meet the criteria neither at the 50% nor at the 80% power levels. For the complete data set and all the other subsets, the ANOVAs of the model and the Spearman's rank-correlation tests are significant, and the statistical power

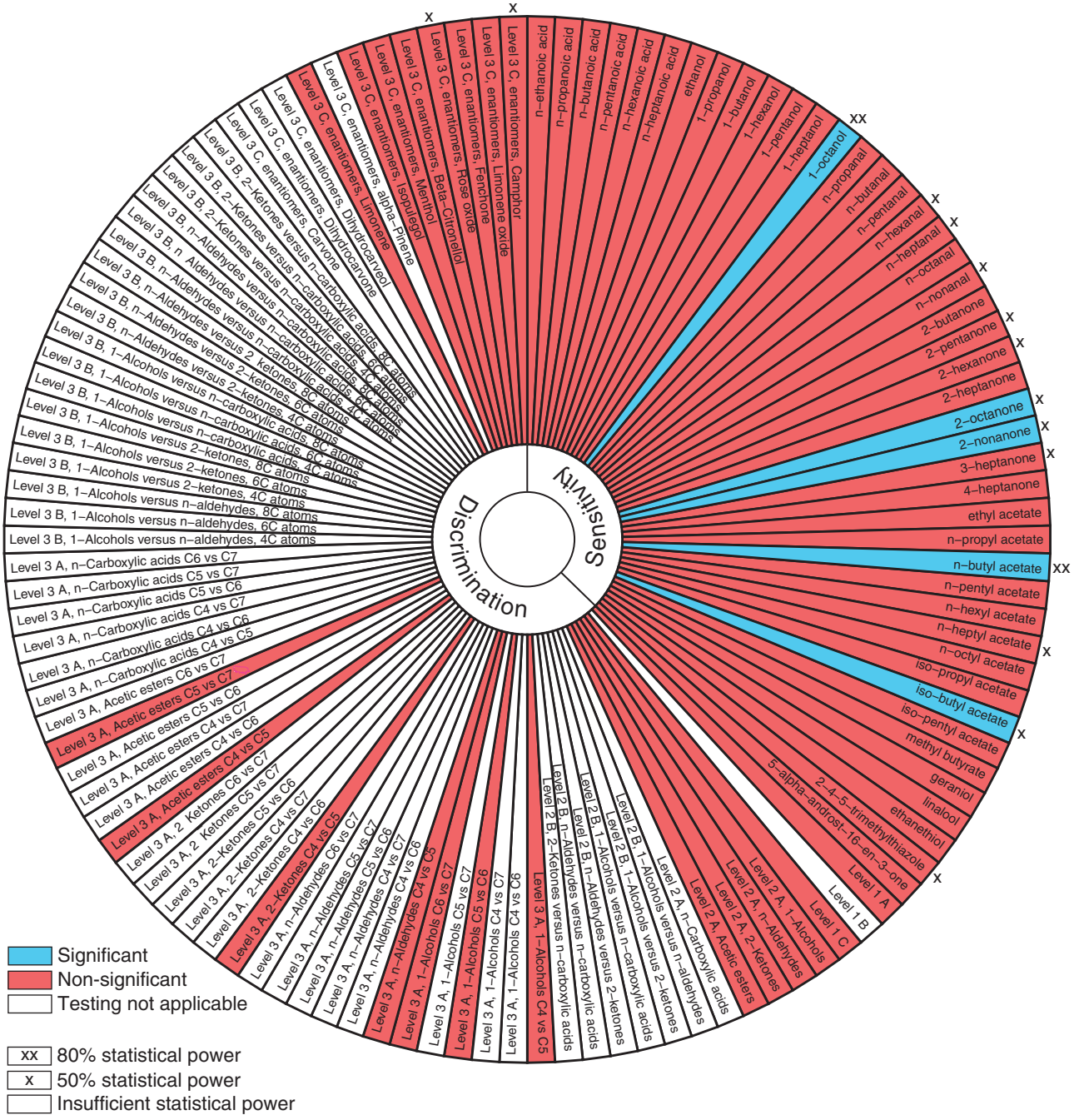

Figure 2. Plot of the 128 monotonic correlations performed against the number of functional olfactory receptor genes. In red, the 70 non-significant correlations; in white, the 53 not applicable correlations (i.e., when all included species succeeded with discriminating a given odour pair or a given set of odour pairs); and in blue, the 5 significant correlations. X indicates that a statistical power at the 50% level is met, while XX indicates that it is met at the 80% level. The five significant correlations turned out to be non-significant when the *P*-values were adjusted with Holm and Bonferroni corrections (see Supplementary Table S2).

meets the criteria at both the 50% and 80% power levels (Figure 1A–C, Supplementary Table S8).

Overall olfactory discrimination performance

For the relationship between the number of functional olfactory receptor genes and the overall olfactory discrimination performance levels 3A, B, and C, there are no significant differences between the models that considered or did not consider the genus as the random effect. This is true for all complete (unbalanced) data sets as well as the balanced subsets (Supplementary Table S8). The ANOVAs of the model and the Spearman's rank-correlation tests are significant for the complete data set level 3C as well as for the complete data set merging the levels 3A, B, C (Figure 1D, Supplementary Table S8). The ANOVAs of the model and the Spearman's rank-correlation tests are significant for the subsets considering at least four and five genera for the data set merging the levels 3A, B, and C (Figure 1E, Supplementary Table S8). The ANOVAs of the model and the Spearman's rank-correlation tests are significant for the subset considering at least five

genera for the level 3C (Figure 1F, Supplementary Table S8). All of these significant tests had a sufficient statistical power (for both the 50% and 80% power levels; Supplementary Table S8). All the remaining tests are non-significant, and none of them achieved the required statistical power (neither at 50% nor at 80% power levels; see Supplementary Table S8). Finally, when considering the alternative transformation where raw values for the number of functional olfactory receptor genes are converted into numerical rankings ranging from one to the maximum number of species, the overarching trends persist (Supplementary Table S10). Similar trends also persist when excluding trials wherein all species successfully discriminated odour pairs (Supplementary Table S11).

Fine-scale olfactory sensitivity

Among the 44 monomolecular odourants considered, only five were found to yield a significant correlation between olfactory detection thresholds and the number of functional olfactory receptor genes (Figure 2, Supplementary Table S2). The number of functional olfactory receptor genes significantly correlated positively with the threshold values for *n*-butyl acetate ($r = 0.883$, $P = 0.009$), whereas it significantly correlated negatively for 1-octanol ($r = -0.975$, $P = 0.005$), 2-octanone ($r = -1$, $P = 0.017$), 2-nonanone ($r = -1$, $P = 0.017$), and iso-butyl acetate ($r = -0.886$, $P = 0.033$). Although mostly not significant (39/44), 13 odourants tended to be positively associated with the number of functional olfactory receptor genes, and 31 tended to be negatively associated. The five significant tests exhibited a statistical power of 50% (Figure 2, Supplementary Table S2). For the 80% power threshold, the correlations with 1-octanol and *n*-butyl acetate meet the statistical power criteria, while the others fail to meet the criteria but are close (only one additional genus required, Figure 2, Supplementary Table S2). The five significant correlations turned out to be non-significant when the *P*-values were adjusted with Holm and Bonferroni corrections (Supplementary Table S2). Among the five identified significant correlations, only the association with *n*-butyl acetate retains its statistical significance when running a PGLS analysis. In addition to the five previously identified significant correlations, the 2,4,5-trimethylthiazole presents a significant correlation with the PGLS analysis (Supplementary Figure S2, Supplementary Table S2). However, a phylogenetic Monte Carlo approach suggests that the data at hand are not suitable for meaningfully fitting phylogenetically informed models (Supplementary Figure S3).

Fine-scale olfactory discrimination

Among the 74 correlations that we performed between the proportion of successfully discriminated odour pairs and the number of functional olfactory receptor genes, none was found to be statistically significant ($0.000 < r < 0.866$, $P > 0.050$ in all cases; Figure 2, Supplementary Tables S3, S4. S5, S6, and S7). This was true when considering all three sets of structurally related monomolecular odour pairs combined, separately, and subdivided by functional moiety, as well as when considering each odour pair individually (Supplementary Tables S3, S4, S5, S6, and S7). Although not significant, all the associations tended to be positive. When the level 3C is considered (pairs of enantiomers), the correlations with the rose oxide and the camphor had a sufficient statistical power at the 50% level (Figure 2, Supplementary Table S7). All the other correlations did not achieve the required statistical power, neither at the 50% nor at the 80% levels (Figure 2, Supplementary Tables S3, S4, S5, S6, and S7). Finally, all the correlations remain non-significant running PGLS analyses (Supplementary Figure S2, Supplementary Tables S3, S4. S5, and S7). Again, a phylogenetic Monte Carlo approach suggests that the data at hand are not suitable for meaningfully fitting phylogenetically informed models (Supplementary Figure S3).

Phylogenetic inertia

The number of functional olfactory receptor genes exhibited a strong and significant phylogenetic inertia for both 20 and 396 species (lambda 0.99 and 0.91, respectively, Supplementary Table S9). The interpretation of phylogenetic inertia's value is not conclusive for the residuals of the models that incorporate the number of functional olfactory receptor genes and the olfactory performance because none of the *P*-values reach statistical significance (Supplementary Table S9).

Discussion

The results of the present study demonstrate that when the currently available data are merged to reach a sufficient statistical power, there is a significant relationship between the number of functional olfactory receptor genes and olfactory sensitivity as well as with olfactory discrimination performance. However, this relationship becomes mostly non-significant when olfactory performance tests are independently analysed for each individual monomolecular odourant and odour pair.

Olfactory sensitivity

Several studies have put forth the proposition that the number of functional olfactory receptor genes should be predictive of a species' olfactory sensitivity (e.g., Mombaerts, 2001; Rouquier & Giorgi, 2007). However, none of these studies provided experimental evidence supporting this notion.

Here we provide the first experimental and statistically supported evidence for a potential relationship between olfactory sensitivity and the number of functional olfactory receptor genes. With the currently available data, we showed that most species that have lower olfactory detection thresholds for the tested monomolecular odourants have a higher number of functional olfactory receptor genes (Figure 1A–C, Supplementary Table S8). However, at a finer scale, the results are quite different. Indeed, among the 44 monomolecular odourants considered in the present study, only five yielded a significant correlation between olfactory detection thresholds and the number of functional olfactory receptor genes (Figure 2, Supplementary Table S2). Interestingly, these five significant correlations are either one of the rare correlations with sufficient statistically power or they come remarkably close to achieving such statistical power. Such a contrast in the results between the overall sensitivity and the fine-scale analyses may hence be explained by the difference in the statistical power. Furthermore, this disparate outcome could potentially be attributed to the scale of the analyses, which can significantly influence the results. As an example, the number of olfactory turbinals significantly correlates with the number of functional olfactory receptor genes at the scale of placental mammals (64 species representing most of the living orders), while it turns out to be non-significant at the scale of rodents (23 species from 15 families, Martinez, Amson, et al.,

2023; Martinez, Courcelle, et al., 2023). Among the five significant correlations with monomolecular odourants, *n*-butyl acetate and iso-butyl acetate are both major components in a wide variety of fruit odours (e.g., Hui, 2010; Maarse, 1991). Similarly, 1-octanol has been reported as a frequently occurring constituent of essential oils (Burdock, 2009). The two aliphatic ketones, 2-octanone and 2-nonanone, are widely found in body-borne odours of mammals, including urine and faeces, and are thought to serve as chemosignals in social communication (Laska, 2014). At this point, we can only speculate that the behavioural relevance of a given odour stimulus, whether in the context of dietary specialization or in the context of chemical communication, rather than the number of functional olfactory receptor genes may determine a species' olfactory sensitivity for these odour stimuli. If subsequent research validates that only these five monomolecular odourants demonstrate a noteworthy correlation, this might provide evidence that many types of functional olfactory receptor genes are sensitive to these odourants.

Several caveats should be considered with regard to the potential relationship between olfactory sensitivity and the number of functional olfactory receptor genes. First, the total number of olfactory receptor cells is known to vary markedly between species (e.g., Bressel et al., 2016; Günterschulze, 1979). Accordingly, a species expressing a relatively low number of olfactory receptor types (due to a relatively low number of functional olfactory receptor genes) might be able to, at least partly, compensate for this by having a relatively high number of olfactory receptor cells. Second, the degree of neural connectivity within structures involved in olfactory processing is known to vary markedly between species (e.g., Hildebrand & Shepherd, 1997; Migliore et al., 2014). Accordingly, a species expressing a relatively low number of olfactory receptor types might be able to, at least partly, compensate for this by having a relatively high degree of neural connectivity. Third, it is well established that olfactory receptors differ markedly in their molecular receptive range, that is, in the breadth of the spectrum of ligands that they respond to (e.g., Kaupp, 2010; Saito et al., 2009). Accordingly, a species expressing a relatively low number of olfactory receptor types might be able to, at least partly, compensate for this by having a relatively high proportion of broadly tuned olfactory receptor types. Lastly, the number of the functional olfactory receptor genes might not account for the different levels of expression and therefore for the potentially different olfactory performance (e.g., Young et al., 2003). Future studies should therefore consider neuroanatomical features such as the total number of olfactory receptor cells or the degree of neural connectivity in olfactory brain structures; the neurophysiological properties of olfactory receptors such as their molecular receptive range as covariates; and the gene composition, families, and expression levels when assessing potential correlations between the number of functional olfactory receptor genes and a species' olfactory sensitivity.

Olfactory discrimination

Similar to olfactory sensitivity, several studies proposed that the number of functional olfactory receptor genes should be predictive of a species' olfactory discrimination performance (e.g., Breer, 2003; Concas et al., 2021; Hildebrand & Shepherd, 1997; Niimura et al., 2018). To the best of our knowledge, only two studies so far experimentally assessed this notion: Laska and Shepherd (2007) reported a significant positive correlation between the number of functional olfactory receptor genes and the ability to discriminate enantiomeric odour pairs (Spearman, $r_s = +0.81$, $P < 0.05$), and using a similar set of enantiomeric odour pairs, Rizvanovic et al. (2013) reported a positive correlation that fell just short of statistical significance (Spearman, $r_s = +0.78$, $P = 0.057$).

Here, our results support a potential relationship between olfactory discrimination success and the number of functional olfactory receptor genes. With the currently available data, we demonstrate that, in general, species that have a higher rate of success in discriminating odour pairs have a higher number of functional olfactory receptor genes. However, at a finer scale, the results are again quite different, as we found no significant correlations between the number of functional olfactory receptor genes and any of the three data sets on olfactory discrimination performance with structurally related monomolecular odourants.

In these last 74 non-significant correlations, almost all of them fail to meet a sufficient statistical power. In addition, the same caveats mentioned above also apply to a species' olfactory discrimination performance.

Is "bigger" necessarily "better?"

The notion that "*bigger is better*" has a long tradition in comparative neuroanatomy. Whereas studies assessing possible correlations between the size of certain brain components such as the neocortex and certain cognitive functions often found significant positive correlations (e.g., van Benson-Amram et al., 2016; van Schaik et al., 2012), corresponding studies looking into possible links between brain (component) size and sensory functions often yielded mixed results, with some studies reporting positive correlations and other studies failing to do so (e.g., Hofman, 2014; Safi et al., 2005).

More recently, the same notion has become popular in comparative genetic studies. The idea that "bigger"—in terms of the number of genes coding for sensory receptors—is "better" has, for example, been put forward not only in olfaction but also in color vision. Some studies claimed that tetrachromatic subjects would be able to see a 100-fold higher number of colors compared to trichromatic subjects (McCrone, 2002; Neitz et al., 2001). However, this claim received little, if any, experimental support so far, both in human subjects (Jordan & Mollon, 2019) and in non-human models (Jacobs, 2018).

The contrasting results we obtained from both large- and fine-scale analyses raise the question as to why mammals differ so markedly in their number of functional olfactory receptor genes. It is commonly agreed that evolutionary selective pressures acted, and continue to act, upon the olfactory sub-genome of a given species, which ultimately resulted, and continues to result, in an expansion or reduction of either the total number of functional genes coding for olfactory receptors and/or the number and diversity of olfactory receptor gene families that are currently present in a species (Hayden et al., 2010; Hughes et al., 2018; Niimura & Nei, 2007; Niimura et al., 2014). These selective pressures include, but are probably not restricted to, a species' habitat (e.g., aquatic, semi-aquatic, or terrestrial), activity pattern (e.g., diurnal or nocturnal), dietary specialization (e.g., frugivorous, carnivorous, folivorous), and reliance upon olfactory cues in behavioral contexts such as spatial orientation, foraging and food selection, social communication, mate choice, and predator avoidance (e.g., Laska and Hernandez Salazar, 2015). Recent studies demonstrated a reduction in the number of functional

olfactory receptor genes in aquatic mammals relative to terrestrial mammals (e.g., Liu et al., 2019) and distinct patterns among olfactory receptor gene families as a function of dietary specialization in bats (e.g., Hayden et al., 2014; Yohe et al., 2022) lend support to this idea. Future studies should therefore not only consider the total number of functional olfactory receptor genes when assessing potential correlations with olfactory sensitivity or discrimination performance but also the size of certain gene families that are thought to be linked to selective pressures such as a species' habitat, diet, and olfactory social communication.

Mammals exhibit varying olfactory capabilities and the way they perceive their surroundings through odours may not exhibit consistent patterns across the phylogeny. This may be explained by a complex interplay of multifactorial processes and a myriad of underlying components that drive olfactory capabilities. For instance, mammals exhibit significant variations in both the size and morphology of their olfactory organs. Several previous studies have acknowledged the potential trade-off between different senses, encompassing both morphological and genomic dimensions (Gilad et al., 2004; Hall et al., 2021; Martinez & Naas, 2021; Martinez et al., 2020; Martinez, Amson, et al., 2023; Martinez, Okrouhlík, et al., 2023; Mutumi et al., 2023; Van Valkenburgh et al., 2014; Yohe et al., 2020, 2022). Further studies may investigate the potential link between olfactory performance, genomics correlates, and the olfactory organs.

Limitations of our study

We are aware that our study has several limitations that need to be taken into consideration: the total number of mammal species for which olfactory detection threshold values using operant conditioning procedures have been published so far is 20, which is less than 0.3% of the approximately 6,000 species of mammals that live today (Burgin et al., 2018). These 20 species represent only 7 of the 27 orders of extant mammals. Accordingly, some orders of mammals and, concomitantly, certain ecological niches are clearly over- or underrepresented in our data. Similarly, the number of monomolecular odourants for which olfactory detection threshold values have been determined in at least five mammal species so far ($n = 44$) and the number of sets of structurally related monomolecular odourants that have been employed in tests of olfactory discrimination performance with more than two mammal species so far is rather limited. Accordingly, it is apparent that any generalization with regard to the correlations between the number of functional olfactory receptor genes and olfactory performance of mammals reported here should be seen as a first tentative conclusion to be corroborated with the examination of additional species.

Furthermore, when evaluating the fine-scale analyses with monotonic correlations conducted across 2–14 species, our statistical power remains limited. Therefore, we cannot exclude that the addition of new species could change our results. However, it is unlikely that the number of species for which olfactory capability data are available will significantly increase in the next few years. The data compiled for this study (20 species in total) cover more than 70 years of research.

Also, the number of functional olfactory receptor genes is an informative proxy but presents a significant limitation in our ability to formulate comprehensive hypotheses about the intricate mechanisms responsible for olfactory capabilities. Subsequent research endeavours should delve into this area with a comprehensive analysis of the functional olfactory receptor gene composition as well as the overall diversity of chemoreceptor genes.

Our findings regarding olfactory sensitivity and discrimination performance challenge the simplistic view of olfaction. However, a third aspect that is missing in this study is the range of odourants that may be detectable for a given number of functional olfactory receptor genes. Indeed, it is possible that a higher number of functional olfactory receptor genes would allow an animal to detect a broader range of odourants. However, in the light of the 5.8 million volatile monomolecular odourants known today (CAS Registry, https://www.cas.org/cas-data/cas-registry), such a hypothesis is almost impossible to test experimentally and an answer can only be approximated with an electrophysiology approach.

Nevertheless, we can conclude that our study, based on this limited set of published data, provides at least some correlation with sufficient statistical power, suggesting that the number of functional olfactory receptor genes may be predictive of a species' olfactory sensitivity and/or discrimination performance.

Supplementary material

Supplementary material is available at *Journal of Evolutionary Biology* online.

Data availability

All data are incorporated into the article and its online Supplementary Material, openly available in Dryad at https://doi.org/10.5061/dryad.73n5tb33v.

Author contributions

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Conflicts of interest

The authors have no conflicts of interest to declare.

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