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Strength of selection on the *Trpc2* gene predicts accessory olfactory bulb form in bat vomeronasal evolution

LAUREL R. YOHE[1]* and LILIANA M. DÁVALOS[1,2]

[1]*Department of Ecology and Evolution, Stony Brook University, Stony Brook, NY 11794, USA*
[2]*Consortium for Inter-Disciplinary Environmental Research, Stony Brook University, Stony Brook, NY 11794, USA*

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Vestigial characters are common across the tree of life, but the underlying evolutionary processes shaping phenotypic loss are poorly understood. The mammalian vomeronasal system, which detects social chemical cues important to fitness, is an impressive example of a sensory system lost multiple times. Three times more losses are inferred among bats than in other mammalian orders. We characterized the relationship between amino acid substitutions in a gene tightly linked to vomeronasal function (*Trpc2*) and the accessory olfactory bulb, a brain region that processes the detection of these vomeronasal chemical cues. By applying a phylogenetic logistic regression, we found a strong negative relationship between the branch lengths representing rates of codon changes in the *Trpc2* gene tree and the presence or absence of an accessory olfactory bulb. Longer branch lengths predict loss of the accessory olfactory bulb, suggesting selection has relaxed on the system as a whole. Based on this relationship, we predicted the absence of an accessory olfactory bulb in 19 bat species with unknown morphology. Several species with predicted losses have specialized skull morphology, suggesting a potential tradeoff between adaptation in skull shape and maintenance of the vomeronasal system. This study offers a new approach to relate genetic mechanisms and phenotypes at a macroevolutionary scale.

ADDITIONAL KEYWORDS: bat chemosensation – gene tree – macroevolution – phylogenetic logistic regression – *Trpc2* – vestigial structure – vomeronasal system.

INTRODUCTION

The vestigialization and loss of morphological structures offers insight into the link between genes and phenotype, as the strong purifying selection on the genetic machinery necessary to maintain function of these phenotypes is no longer present (Fong, Kane & Culver, 1995). Phenotypic characters and genes in corresponding pathways may be evolving neutrally and will eventually be lost by mutation and drift. Thus, the molecular footprint of relaxed selection provides a quantitative measure of ancient evolutionary shifts and may relate to variation in morphology across species. Mutations accumulate in protein-coding genes no longer essential to sustaining the cellular functions of the morphological structure, eventually leading to pseudogenization (Marshall, Raff & Raff, 1994; Fong *et al.*, 1995). Substitutions in protein-coding genes evolving under relaxed selection can be connected to variation in phenotypic form, as has been demonstrated in numerous examples of relaxed selection of opsin genes in cave-dwelling or fossorial vertebrates (Wilkens & Strecker, 2003; Niemiller *et al.*, 2013; Emerling & Springer, 2014). Relaxed selection applies to the entirety of the system, including relevant pathways and their constituent genes (as long as this is their sole function). Thus, if a gene is connected to the function of a known phenotype, even if it is not the gene underlying the morphogenesis of that structure, we can expect relaxed selection to be reflected in both the amino acid substitution rate and the degradation of the phenotype (Fong *et al.*, 1995). We can then calculate the codon substitution rates of the same gene across species with diverse phenotypes to predict the functionality or, in the case of an organ, presence of the morphological structure or system of interest. A central objective of this study is to quantify this relationship between genetic components of a system and morphological function, and to predict the morphological phenotype in species for which it is unknown.

*Corresponding author. E-mail: laurel.yohe@stonybrook.edu

Testing for a relationship between degradation of a structure and its related genes requires a system with variation in both morphological form and molecular function. The mammalian vomeronasal system detects chemical cues involved in many social behaviours and reproduction, such as mating and courtship, maternal care and territoriality (Chamero, Leinders-Zufall & Zufall, 2012; Liberles, 2014). Although this sense processes signals that mediate many fitness-related behaviours, it is highly variable in function across lineages, and serves as an ideal model for the framework we are testing. An organism with a working vomeronasal system detects socially relevant chemical ligands via activation of G-protein-coupled receptors (e.g. *V1Rs*) expressed in the vomeronasal organ in the nose (Døving & Trotier, 1998; Dulac, 2000). This signal is primarily relayed to a region of the brain known as the accessory olfactory bulb, where it is processed and sent for interpretation to elicit an appropriate behavioural response. Although the vomeronasal system is highly conserved in most mammals, variation in function exists in many aquatic mammals, primates (including loss in humans) and bats (Wible & Bhatnagar, 1996; Bhatnagar & Meisami, 1998; Meisami & Bhatnagar, 1998). In bats, only three of the 20 bat families (Phyllostomidae, one genus in Mormoopidae, and Miniopteridae) have intact vomeronasal systems, including both a well-developed vomeronasal organ and accessory olfactory bulb. Even within phyllostomids, the largest bat family that retains function, some species have rudimentary morphology (Bhatnagar & Meisami, 1998).

In addition to variation in morphology, selection has been relaxed on many of the molecular mechanisms for detecting and signalling in the system. Of the pheromone-binding receptors, all published bat *V1R* homologues are pseudogenized (Young *et al.*, 2010). For the signal transduction of pheromone-binding, the β-isoform of the *Transient receptor potential cation channel, subfamily C, member 2* (*Trpc2*) gene encodes a membrane-bound ion channel that receives a signal from the activated vomeronasal receptor in species with an intact organ, and depolarizes the cell, which then transduces the signal to the brain (Mast, Brann & Fadool, 2010). This is the only known function of this isoform. Given its essential role in vomeronasal signal transduction, *Trpc2* is highly conserved in most mammals (Yu *et al.*, 2010). However, *Trpc2* is a pseudogene in many mammals with a degraded vomeronasal system (Zhang & Webb, 2003; Yu *et al.*, 2010; Zhao *et al.*, 2011). Within bats, *Trpc2* function has been lost independently at least 13 times, and the few bat families that possess functional signalling have been under strong purifying selection to maintain a functional channel since bats first evolved (Yohe *et al.*, 2017). It is not known whether other genetic machinery is intact aside from *Trpc2*. Compared to other groups that demonstrate single ancestral vomeronasal loss, the many independent events leading to the accumulation of mutations in *Trpc2* across the gene tree and varying times over which selection has relaxed provide an unusual opportunity to quantify variation in molecular function. This information can be extracted from the gene tree and used as a predictor of morphological phenotype. This predictive framework clarifies how vestigialization of a sensory system occurs in different lineages, such that we can now understand if losses of all parts of the system occur in synchrony. Connecting the repeated losses of the vomeronasal system to the natural history of the different groups that have lost function may also reveal new evolutionary processes behind vomeronasal loss, such as genetic drift or morphological constraints.

In this study, we use branch lengths that represent codon substitution rates of the *Trpc2* gene tree to model the presence or absence of the accessory olfactory bulb. Although *Trpc2* is not directly involved with the accessory olfactory bulb phenotype, the selective pressures operating on the *Trpc2* gene and accessory olfactory bulb function are related. As the response variable is binary and the residual variation may contain phylogenetic signal, we used a phylogenetic logistic regression to model the relationship between mutation accumulation in the *Trpc2* gene and morphological form (Ives & Garland, 2014). If selection has indeed relaxed on all components of the vomeronasal system, we expect species with a pseudogenized *Trpc2* or *Trpc2* genes that have accumulated many codon substitutions (i.e. long branch lengths) will lack accessory olfactory bulbs. Species with highly conserved *Trpc2* (short branch lengths) should have an intact accessory olfactory bulb, suggesting a fully functional vomeronasal system. By linking genetics and morphology at the phylogenetic scale, our analyses offer new insights into how selection, or lack thereof, simultaneously shapes the function of all parts of a system.

MATERIAL AND METHODS

Gene tree inference

We inferred a *Trpc2* gene tree that reflects codon rates of substitution and used these branch lengths as a continuous independent variable. Longer branch lengths indicate more codon substitutions. As non-synonymous changes accumulate across all sites, lineages with non-functional *Trpc2* sequences should have longer branch lengths and a higher probability of an absent accessory olfactory bulb. We used the alignment inferred from a recent paper on the molecular evolution of the *Trpc2* exon 2 gene (507 bp) in 115 bat

species and the mouse (*Mus musculus*) (Yohe *et al.*, 2017). We also included one additional bat sequence amplified from *Platrryhinus brachycephalus*, to give 116 bat species in total. Details on this amplification are provided in the Supporting Information, but we note that *P. brachycephalus* had an intact *Trpc2* reading frame. The second exon represents *c.* 20% of the coding region, is the longest exon encoding this isoform, and encodes for an ankyrin-repeat domain involved in protein–protein interactions and coiling mechanisms involved in gate-opening (Yu *et al.*, 2010). This region contained within the second exon is highly conserved among animals, such that the mammalian N-terminal region of this protein family shares over 30% amino acid identity with *Drosophila* homologues (Venkatachalam & Montell, 2007). We estimated the best-fit model of evolution for the *Trpc2* alignment using ModelOMatic v.1.01 under default parameters and including all nucleotide, amino acid and codon models considered by the program (Whelan *et al.*, 2015). We applied the best-fit codon model to infer a gene tree from the alignment. To infer the tree, we used a maximum-likelihood method implemented in GARLI v.2.01.067 (Zwickl, 2006) with eight search replicates. We performed 2400 bootstrap replicates calculated using the SumTrees function in the DendroPy v.4.0.3 python library (Sukumaran & Holder, 2010). The tree was rooted using the *Trpc2* exon 2 orthologue of *M. musculus*. This is a single exon and pseudogenizing mutations may be found in additional exons of the gene. If this were the case, however, we would expect selection to relax on the gene as a whole, and substitutions would accumulate at a higher rate than if the gene were under purifying selection.

The diagonals of the variance–covariance matrix of the gene tree are equivalent to the summed branch lengths from root to tip for each lineage. These lengths represent the amount of accumulated variation in the number of codon substitutions per site for each lineage. We calculated the variance–covariance matrix using the vcv.phylo() function in the 'ape' v. 4.0 package in R v.3.3.2 (Paradis, Claude & Strimmer, 2004; R Core Team, 2016), and extracted the diagonals of this matrix to serve as the branch length covariate.

Accessory olfactory bulb morphology

When a chemical cue is detected by a receptor in the vomeronasal organ, the signal transduction triggered by *Trpc2* relays the signal directly to the accessory olfactory bulb, a brain region that sits on the posterior side of the olfactory bulb. Because *Trpc2* is tightly linked to the signals sent to the accessory olfactory bulb, we expect a conserved *Trpc2* to be associated with a functional accessory olfactory bulb. We collected morphological data on the presence or absence of the accessory bulb from previously published studies (Frahm & Bhatnagar, 1980; Frahm, 1981; Bhatnagar & Meisami, 1998). We compiled a comprehensive list of volumes of accessory olfactory bulbs for bats, including presence and absence data, which is more readily available (N = 93). However, many of the bats had a volume of 0 (absent), while the distribution of volumes for bats with a present accessory olfactory bulb tended to cluster (Supporting Information, Fig. S1). The volume data are fairly bimodal and inter-individual variation of accessory olfactory bulb volume can vary by up to 25% (Meisami & Bhatnagar, 1998). Thus, we coded the response variable as a binary trait. We collected presence/absence of the accessory olfactory bulb for 51 species of bats with *Trpc2* sequence data. Figure 1 shows the species used in this analysis (for a full list see Table S1).

Phylogenetic logistic regression

We implemented the Markov chain Monte Carlo (MCMC) sampler for phylogenetic multivariate generalized linear mixed models in the package 'MCMCglmm' v.2.24 (Hadfield, 2010). The branch lengths of the gene tree were used as a covariate to test for an association with the dependent binary trait of the presence (1) or absence (0) of the accessory olfactory bulb. The branch length for each species i is the independent variable that predicts the mean value p, which takes the form of either presence (1) or absence (0) of the accessory olfactory bulb (AOB), such that:

$$\Pr\left(AOB_i = 1\right) = p$$

$$logit\left(p_i\right) = b_0 + b_1 * branch.length_i + s + u$$

where b_0 and b_1 are regression coefficients. The s and u terms are normally distributed random variables that represent two sources of variation, in which s accounts for phylogenetic variance and incorporates a covariance matrix based on the phylogeny and u is residual variation (Hadfield, 2010, 2015; Ives & Garland, 2014). The model required an ultrametric species tree. We pruned a combined tree of phyllostomids and all bats (Shi & Rabosky, 2015; Rojas, Warsi & Dávalos, 2016) to match the data set. We also ran the same analysis with two additional models: a phylogenetic logistic regression approach that is more sensitive to detecting phylogenetic signal (Ives & Garland, 2010), and the maximized penalized likelihood regression of the 'phylolm' v.2.5 package (Ho & Ané, 2014). Details of model implementation for additional methods are available in the Supporting Information.

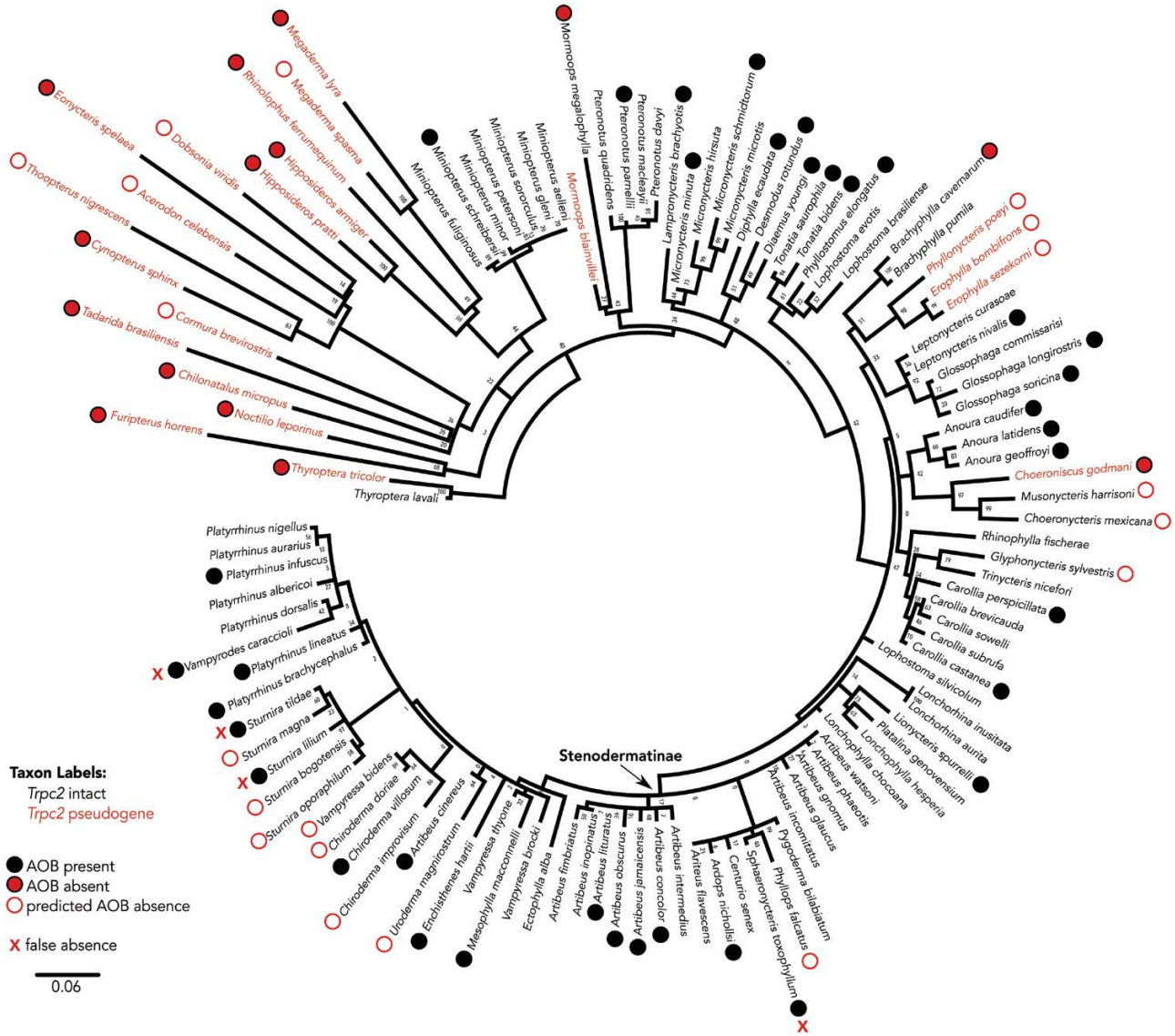

Figure 1. *Trpc2* gene tree estimated with a codon model of evolution. Red taxonomic labels indicate genes in which there was a premature stop codon or frameshift indel, while black labels depict taxa in which the *Trpc2* reading frame is intact. Details of the shared history of inactivating mutations is available in our previous study (Yohe *et al.*, 2017). Numbers at nodes indicate bootstrap support. Black circles indicate species that are known to possess an intact accessory olfactory bulb (AOB). Red closed circles indicate species known to lack an AOB. Open red circles indicate species predicted by our model that have a low probability of possessing an intact AOB. Red X's indicate the prediction of a false absence, i.e. our model predicted the AOB to be absent but it is observed to be present.

Prediction of accessory olfactory bulb presence

If there is a relationship between branch length and accessory olfactory bulb presence, the *Trpc2* gene tree branch lengths can predict the probability of accessory olfactory bulb presence/absence for species with sequence data lacking morphological observations. This requires a test of model performance to establish a well-supported threshold at which the sensitivity and specificity of the model are maximized. Using the posterior means of the b_0 and b_1 coefficients, we predicted the probability of accessory olfactory bulb presence for species with known branch lengths (Fig. 1). We estimated the true positive rate and false positive rate through a classifier performance approach using the 'ROCR' v.1.0.7 package in R (Sing *et al.*, 2005). This was done by comparing the predicted values to the observed values for species with known morphology. This serves as a

cross-validation, using species with known morphology to corroborate our predictions (solid circles in Fig. 1). Using the 'performance()' function, we evaluated model performance by quantifying the true and false positive rates, area under the curve (AUC) and model accuracy. We also wanted to evaluate how branch length-based predictions compared to simply using binary *Trpc2* pseudogenization as a predictor for morphology loss, and also calculated AUC values for *Trpc2* pseudogenization as a predictor of accessory olfactory bulb absence.

RESULTS

Gene tree inference

The best-fit model of evolution that reflected codon substitutions was a two-rate (different rates for transitions and transversions) model with equal codon frequencies and a four-category parameter of rate variation in the ratio of non-synonymous to synonymous substitutions across sites. The resulting gene tree is presented in Figure 1. Root-to-tip branch length estimates were extracted from the gene tree and resulted in a mean length of 0.31 substitutions and a standard deviation of 0.07 for 117 species. Branching relationships were similar to those of the species tree (Shi & Rabosky, 2015; Rojas *et al.*, 2016), although these was discordant in several places. Discordance is reflected in the low bootstrap values observed throughout the tree (Fig. 1), probably because of the lack of informative characters from a single exon sequence only containing amino acid changes.

Morphology

Of the 116 *Trpc2* bat sequences sampled, 51 species had morphological data for the presence or absence of an accessory olfactory bulb. About one-quarter of the species lacked an accessory olfactory bulb (N = 14), and losses were mainly distributed throughout all non-phyllostomid and non-miniopterid bat families in our sample. Two phyllostomid species (*Brachyphylla cavernarum* and *Choeroniscus godmani*) lacked an accessory olfactory bulb. The only two non-phyllostomid species with an intact accessory olfactory bulb were *Miniopterus schreibersii* and *Pteronotus parnellii*.

Phylogenetic logistic regression

All models achieved successful optimization and converged on similar solutions (see Supporting Information for details on all model results; Table S2 for model comparisons; Fig. S2 for convergence plots). The model we present here, described by Hadfield (2010), estimated a positive mean intercept b_0 = 14.52 (95% highest posterior density intervals: 3.80, 26.60), and a negative mean slope b_1 = −46.58 (−81.50, −11.07) (Fig. 2). The estimated G-structure reflecting phylogenetic signal was 4.66 (1.1×10^{-3}, 10.7). All parameter estimates had an effective sampling size of greater than 1990.

Prediction of morphology

To predict morphology, we first validated the robustness of the model. The model had high sensitivity (true positive rate = 0.89) and moderate specificity (true negative rate = 0.86), and a reliable AUC value (0.89), which suggests the model can classify the binary response depending solely on the *Trpc2* branch length. Plots from these analyses are available in Figure S3. *Trpc2* pseudogenization resulted in a slightly higher, although comparable AUC value (0.93). At a maximum accuracy of 0.88, we determined an optimal probability cutoff (0.36), and a species falling below this threshold is predicted to lack an accessory olfactory bulb. Based on this threshold, 19 bat species were predicted to lack an accessory olfactory bulb. Comparing the robustness of this threshold to observed values, four species (*Sturnira lilium*, *Sturnira tildae*, *Vampyrodes caraccioli* and *Sphaeronycteris toxophyllum*) were falsely predicted to lack an accessory olfactory bulb (Fig. 1). There were no false predictions of accessory olfactory bulb presence, and observed absences were correctly predicted by the model. This suggests our model reliably predicts the presence of an accessory olfactory bulb but overestimates its absence.

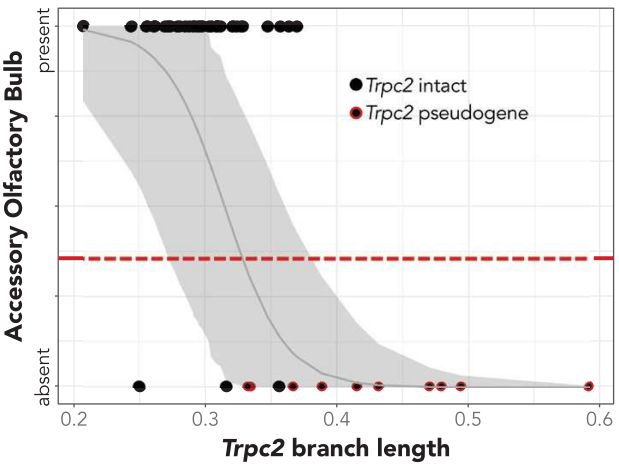

Figure 2. Fitted line of phylogenetic logistic regression to the observed data. The 95% highest posterior density intervals are represented by the grey shading. The red dotted line indicates the cutoff threshold (0.36) of predicted accessory olfactory bulb absence. Species below this threshold, for which the morphology was unknown, were predicted to lack an accessory olfactory bulb. The results of these predictions are presented in Figure 1. The black dots with red circles indicate species in which *Trpc2* is a pseudogene.

DISCUSSION

When a morphological structure becomes vestigial, genetic pathways tightly associated with the system, no longer under strong selection to maintain function, lose function as well (Fong *et al.*, 1995). However, observations of rudimentary structures and pseudogenized genetic machinery are often qualitative, and the extent to which they relate or cross-predict a relevant phenotype is poorly known. Here, we quantify the relationship between genetics and morphological function by using gene tree branch lengths to model the function of a variable mammalian sensory system.

Loss of the accessory olfactory bulb relates to non-synonymous substitutions, and eventual loss of function, of *Trpc2*. We found a strong negative relationship between *Trpc2* branch lengths inferred from a codon substitution rate gene tree and the presence of the accessory olfactory bulb. A reading frame indicated by a stop codon or frameshift mutation suggests the vestigialization of a system (Marshall *et al.*, 1994; Fong *et al.*, 1995), yet a phenotype or a gene may experience relaxed selection before a stop codon or indel mutation occurs. Our approach offers a new way to predict the presence or absence of a structure using information from codon replacements in the coding sequence without requiring mutations disrupting the reading frame. From the negative relationship and sensitivity analyses, the results suggest after a certain threshold of *Trpc2* codon substitutions, the brain region associated with receiving the vomeronasal signal is no longer present (Fig. 2). Although complete *Trpc2* pseudogenization had stronger predictive power than using codon substitution rates (AUC = 0.93 vs. 0.89), both models are reliable. Our approach of using branch lengths as a covariate allows us to detect relaxed selection in species that have not yet accumulated an inactivating mutation. There were a few species falsely predicted to lack an accessory olfactory bulb (Fig. 1), but these discrepancies occur at the steepest region of the logistic curve (Fig. 2), making the cutoff somewhat arbitrary. Thus, we propose that complete vestigialization of the vomeronasal system results in an 'all or nothing' pattern, in which the species either possesses a well-conserved *Trpc2* and an intact accessory olfactory bulb, or completely loses function of both. We emphasize that the neutrally evolving or disrupted *Trpc2* gene is not involved in the morphogenesis of the accessory olfactory bulb. Rather, because the structure and the ion channel are intimately linked in the same sensory function, we have established a relationship between the strength of selection on the two.

The resulting model predicted 19 species with unknown morphology to lack an accessory olfactory bulb. Many of these species had a disrupted *Trpc2* gene (Fig. 2) – although this information was not included in the regression, only the branch lengths – but a few other groups were more surprising. Although *Trpc2* is under strong purifying selection in many phyllostomids (Yohe *et al.*, 2017), there were at least two instances of pseudogenization within this family, one within Caribbean nectarivorous bats, and one within a highly specialized continental nectarivore, *Choeroniscus godmani*. The model in this paper also predicted the genera sister to *Choeroniscus* and, surprisingly, several lineages of frugivorous bats in the Stenodermatinae subfamily to lack the accessory olfactory bulb (Fig. 1). Although their *Trpc2* reading frame is not disrupted, the long branch lengths indicate rapid evolution and selection may have been relaxed on these clades.

Relaxed selection on the vomeronasal system in bats may be more pervasive than previously thought. These predictions provide the opportunity to explore the evolutionary processes that may underlie vomeronasal loss. Loss is often attributed to the irrelevance of the sensory system to the functional ecology of the species (Lahti *et al.*, 2009). Several other mammalian clades have lost function of their vomeronasal organ, including within cetaceans and catarrhine primates. Selection has probably been relaxed on the vomeronasal system with ancestral shifts to aquatic environments or diurnality and increased reliance on vision in these groups, and these shifts are coupled with loss of function of the system (Zhang & Webb, 2003; McGowen, Gatesy & Wildman, 2014). The explanation in bats remains elusive. Hypotheses of shifts to a volant lifestyle or tradeoffs with other extreme bat sensory adaptations have been rejected, as these are all decoupled from the many instances of vomeronasal losses that have occurred throughout bat diversification (Yohe *et al.*, 2017). This is particularly puzzling, as many bat species that have lost function still maintain pheromone-mediated behaviours, such as scent-marking of pups or the use of chemical cues for mate attraction (Gustin & McCracken, 1987; Murray & Fleming, 2008). Given the maintenance of pheromone-mediated behaviours, relaxed selection may not be the sole process responsible for bat vomeronasal degradation.

Besides *Glyphonycteris sylvestris*, whose sensor status is unknown, the model predicts loss of function in nectarivorous and frugivorous phyllostomids that also possess extreme skull morphologies and nose-leaf specializations (Arita, 1990; Dumont *et al.*, 2009, 2012; Monteiro & Nogueira, 2010). For example, the palate of *Musonycteris harrisoni* spans 50% of its skull length, resulting in the greatest palate length to width ratio of all bats and a tongue that nearly stretches the length of its body (Tellez & Ortega, 1999). Members of the Stenodermatinae family have long been regarded as considerable outliers as well, with significant

shifts at the base of their radiation in bite force and complementary skull morphology relative to all other bats (Dumont *et al.*, 2012, 2014; Rossoni *et al.*, 2017). While many of these stenodermatines have accessory olfactory bulbs, a large number of these species are outliers in accessory olfactory bulb volume, falling within the lower 30% of the size distribution (Frahm, 1981; Meisami & Bhatnagar, 1998). If other traits are more essential to survival and reproduction than an accessory sensory system, developmental constraints and adaptation may influence sensory loss. These processes need not be mutually exclusive (Lahti *et al.*, 2009; Rétaux & Casane, 2013). The redundancy between the main olfactory and vomeronasal system may be enough to compensate for this loss (Keller *et al.*, 2009; Omura & Mombaerts, 2014; Baum & Cherry, 2015). Given the tightly constrained energy budget of bats, especially those feeding almost exclusively on nectar, and considering it is energetically expensive to maintain sensory tissues (Niven & Laughlin, 2008), selection in favour of primarily relying on a single olfactory system may have led to vomeronasal loss in these species.

The constraints within the skull, combined with potential sensory redundancy, or reliance on alternative means to locate food and mates, hint at strong positive selection on skull morphologies relevant to feeding ecologies. Selection to maintain function of the costly vomeronasal system that sits in the most anterior region of the nasal cavity may have relaxed. In the Mexican tetra (*Astyanax mexicanus*), for example, the evolutionary processes leading to vision loss in cave-dwelling fish are more complex than previously appreciated, as adaptive selection for alternative cave phenotypes may be coupled with relaxed selection on the eyes in a dark environment (Bradic *et al.*, 2012). There is positive selection for larger jaws and more teeth to enhance foraging behaviour. Yet, because of the developmental chronosequence of jaw morphogenesis that is followed by lens formation, selection for a larger jaw may inhibit proper eye development, as long as selection for vision is lacking (Rétaux & Casane, 2013). In fact, it may even be adaptive to reduce eye development to make room for a larger jaw. Whether morphological constraints or sensory redundancy explain vomeronasal loss in bats remains to be tested with genome-wide data and comparative analyses. Furthermore, these processes might not be mutually exclusive and may simultaneously influence the evolution of the system.

Vestigial characters and the loss of traits are evidence of changes in selective constraints eventually leading to neutral evolution (Fong *et al.*, 1995). However, the genetic mechanisms associated with phenotypic loss and the evolutionary trajectory of selective constraints is poorly understood. We show a gene closely associated with system function can predict a key morphological component of a sensory system. Our approach may be useful in predicting degradation of particular phenotypes if a gene critical to function of a system has evidence of independent losses within a group, as with the *Oca2* gene and pigmentation loss in blind cavefishes (Protas *et al.*, 2006), or *C4orf26* and toothlessness in mammals (Springer *et al.*, 2016). In plants, one could predict losses of chloroplast genes in plants with suggested loss of photosynthesis, such as in the parasitic vine-like genus *Crustata* in which retention and loss of photosynthesis is cryptic (McNeal *et al.*, 2007a, b). Parallel studies in those systems may predict new species in which loss might have occurred or reveal times in which selection may be relaxing on a genetic component that relates to a complex phenotypic trait.

Our results suggest selection acts simultaneously on all parts of the vomeronasal system, linking genotype to phenotype, even when the former does not encode the latter. The phyllostomid species lacking an accessory olfactory bulb, or predicted to have lost it, appear to share extreme cranial phenotypes, suggesting potential tradeoffs between the functional demands in the skull and expensive but redundant sensory tissues. Finally, we emphasize further evidence on the functional redundancy of the two olfactory systems, and the cost of maintaining an intact vomeronasal system, is needed to test this new hypothesis.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:
Table S1. Table of bat species with known morphology of the accessory olfactory bulb.
Table S2. Mean parameter estimates from the different phylogenetic logistic regression methods described in the main text and Supplementary Methods.
Figure S1. Histogram of published volumes of the accessory olfactory bulb for 91 bat species.
Figure S2. Convergence and density plots of parameter estimates from Hadfield's (2010) model, from the results presented in the main text.
Figure S3. Plots from the area under the curve (AUC) analyses for statistical robustness.

SHARED DATA

Data deposited in the Dryad Digital Repository (Yohe & Davalos, 2018; doi:10.5061/dryad.6p6v0).