Vomeronasal Receptors in Vertebrates and the Evolution of Pheromone Detection

Liliana Silva[1] and Agostinho Antunes[1,2,*]

[1]CIIMAR/CIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, 4050-208 Porto, Portugal; email: aantunes@ciimar.up.pt

[2]Department of Biology, Faculty of Sciences, University of Porto, 4169-007 Porto, Portugal

Annu. Rev. Anim. Biosci. 2017. 5:353–70

First published online as a Review in Advance on November 28, 2016

The *Annual Review of Animal Biosciences* is online at animal.annualreviews.org

This article's doi: 10.1146/annurev-animal-022516-022801

*Corresponding author

Keywords

vomeronasal organ, VNO, vomeronasal receptor, VR, transient receptor potential channel 2, TRPC2, olfactory receptors related to class A GPCRs, Ora, olfactory receptor related to class C GPCRs, OlfC

Abstract

Pheromones were identified as chemical signals used for intraspecific communication in insects (e.g., sexual attraction) in the 1950s. However, only almost 40 years later the vomeronasal receptors type-1 (V1R) and type-2 (V2R) were identified, usually associated with the presence of a vomeronasal organ (VNO). VRs are widespread in amphibians, reptiles, and mammals, but birds lost the VNO. Similarly, fishes lack VRs and a VNO but can still detect pheromones, instead using the olfactory receptors related to class A and class C G protein–coupled receptors. Here, we review recent evidence on VR repertoire contraction/expansion in vertebrates. We assess the association between VNO development and VR repertoire size. Phylogenetic relationships and selective pressures illuminate the dynamic evolutionary history of the VRs in vertebrates.

INTRODUCTION

VR: vomeronasal receptor

VNO: vomeronasal organ

V1R: vomeronasal receptor type-1

V2R: vomeronasal receptor type-2

GPCR: G protein–coupled receptor

Pheromones were identified in the 1950s as "substances secreted by an individual to the outside, being perceived by another individual of the same species and causing a specific reaction" (1, p. 55), changing behavior and physiology (2) in ways involving sexual recognition, mating (3), and neuroendocrine responses (4). Vomeronasal receptors (VRs) and their main role in pheromone detection were first characterized in rodents (5–8). Subsequent studies concluded that in the majority of vertebrates pheromones and some kairomones are detected by VRs (9, 10), not excluding the possibility that the vomeronasal system detects nonpheromonal stimuli (e.g., molecules responsible for smell) and/or that some pheromones elicit responses in other systems, like the olfactory system (11) (see sidebar titled Pheromones and Kairomones: Widespread Use from Invertebrates to Vertebrates; **Figure 1**).

VRs are mainly expressed in the anatomically well-defined bony capsule on the anterior nasal septum, the vomeronasal organ (VNO) (19) (**Figure 2**). The vomeronasal system is directly linked with limbic brain structures important in chemical communication (20). The two superfamilies of VRs [type-1 (V1R) and type-2 (V2R)] have different expression locations and gene structures (21) (**Figure 1**). Both receptors belong to the seven-transmembrane G protein–coupled receptor (GPCR) family, but whereas V1Rs with Gαi2-coupled protein are expressed in the apical layer of the vomeronasal epithelium and have axonal projections to the anterior accessory olfactory bulb, the V2Rs with Gαo-coupled protein are expressed in the basal layer of the vomeronasal epithelium with axonal projections to the posterior accessory olfactory bulb (2, 3, 19, 21, 22) (**Figure 2**).

Structurally, V1Rs have short N-terminal extracellular domains and V2Rs have long N-terminal extracellular domains, usually linked to H2-Mv molecules (nonclassical class I major histocompatibility Mhc genes) (**Figure 2**). The V1Rs are encoded by genes with a single exon, whereas V2R genes usually have six exons (**Figure 2**). At the functional level, V1Rs are associated with the detection of small volatile molecules involved in gender discrimination and

PHEROMONES AND KAIROMONES: WIDESPREAD USE FROM INVERTEBRATES TO VERTEBRATES

Recognition of chemical substances, like pheromones and kairomones, is extremely important for species survival (4, 12, 13), because these molecules are involved in communication between and within species (**Figure 1**).

In invertebrates, like worms and insects, the pheromones are usually ascarosides or long hydrocarbon chain molecules (14). The volatile pheromones of invertebrates are usually detected by odorant receptors (15), whereas the less-volatile pheromones might be detected by gustatory receptors and/or PPK ion channels (14). By contrast, vertebrates use small proteins or peptides for pheromonal communication (14), detected by specific receptors, which allow increasing the degree of complexity and specificity of pheromone recognition (16).

Along with pheromones, vertebrates and invertebrates can also interpret chemical cues using kairomones, which are similar to pheromones but have a heterospecific effect (9). Kairomonal communication is widespread among vertebrates and invertebrates with important roles in host and ectoparasite relationships, such as attraction of tick *Amblyomma americanum* by uric acid excreted from reptiles and birds (17). The predator-prey relationship is also affected by kairomones with disadvantages for the signaler and advantages for the receiver (9). For example, the chemical cues released by the Eurasian otter, which feeds mainly on salmon, are detected by young salmon, teaching them to recognize predators (17). Kairomones have been used previously for pest control of invertebrates, namely with the construction of traps using lizard (*Varanus niloticus*) kairomones to attract the vector of the sleeping sickness fly (*Glossina fuscipes fuscipes*) (18).

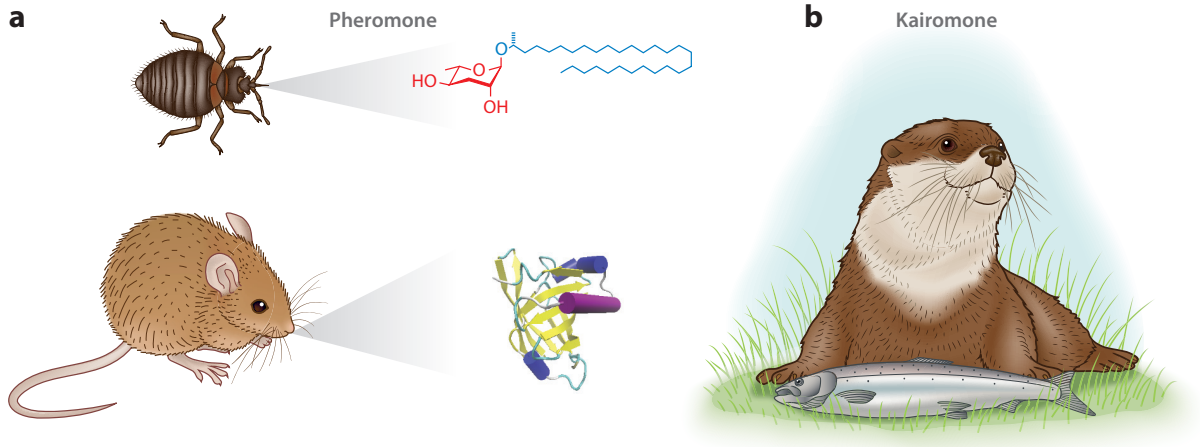

Figure 1

Widespread use of pheromones and kairomones. (*a*) In chemical intraspecies communication, vertebrates, like mice, use small peptides as pheromones, which are much larger and more complex than the small sugars of the pheromones used by invertebrates, such as insects. (*b*) For interspecies communication, vertebrates and invertebrates use kairomones, which are sensed by different species and have special relevancy inside prey-predator relationships. For example, young salmon can detect chemical cues of Eurasian otters. By recognizing their predator, the salmon can escape more efficiently (17).

sexual behaviors, whereas V2Rs are involved in detection of water-soluble peptides and control of pheromone-induced male-male aggression (2, 3, 19, 21, 22).

In *Xenopus tropicalis*, V1R genes are expressed in the main olfactory epithelium and not in the VNO. As VRs likely appeared first in amphibians, the main olfactory epithelium could have been the primary place for V1R expression, whereas the earlier-diverging genes (like some V2Rs of *X. tropicalis*) became expressed in the VNO (23, 24).

The TRPC2 gene encodes a transient receptor potential channel 2, crucial in neuronal signaling in the VNO (25, 26). The male TRPC2 knockout mouse has difficulties in gender discriminating, thus losing the natural attack behavior toward other males (27). TRPC2 is present across mammals, reptiles, and amphibians (**Figure 3**) and is located in a well-conserved syntenic region with NUMA1, IL18BP, and Rnf121 genes flanking its tail region and Art genes flanking its head region (**Figure 3**). Fishes also present the conserved TRPC2 gene, but the conserved synteny with other tetrapods is restricted to the Rnf121 gene in the flanking region (**Figure 3**, ***fishes***). The pseudogenization of TRPC2 in birds and Old World monkeys has been connected with absence of vomeronasal communication (2, 28). In fact, no TRPC2 genes were detected in bird genomes (29) (**Figure 3**, ***birds***), and within primates, humans possess only a TRPC2 pseudogene (**Figure 3**, ***primates***). Despite being Old World monkeys, the orangutan and rhesus macaque were found to have three TRPC2 copies (**Figure 3**, ***primates***), which could be related to an additional role of this gene in the induction of acrosomal reaction during the fertilization process (25, 26).

The vomeronasal system appeared after the emergence of tetrapods (28) (**Figure 4**) [birds have subsequently lost VRs (2, 4)], and no V1R or V2R genes or pseudogenes were found in fishes (30–32). However, fishes sense pheromones using a different class of GPCRs: olfactory receptors related to class A GPCRs (Ora) and class C GPCRs (OlfC) (32–36).

In this review, we discuss hypotheses explaining the evolution of VR genes, namely the influence of the water-to-land life transition. Moreover, we assess the selective pressures acting on VRs and

TRPC2: transient receptor potential channel 2

Ora: olfactory receptor related to class A GPCRs

OlfC: olfactory receptor related to class C GPCRs

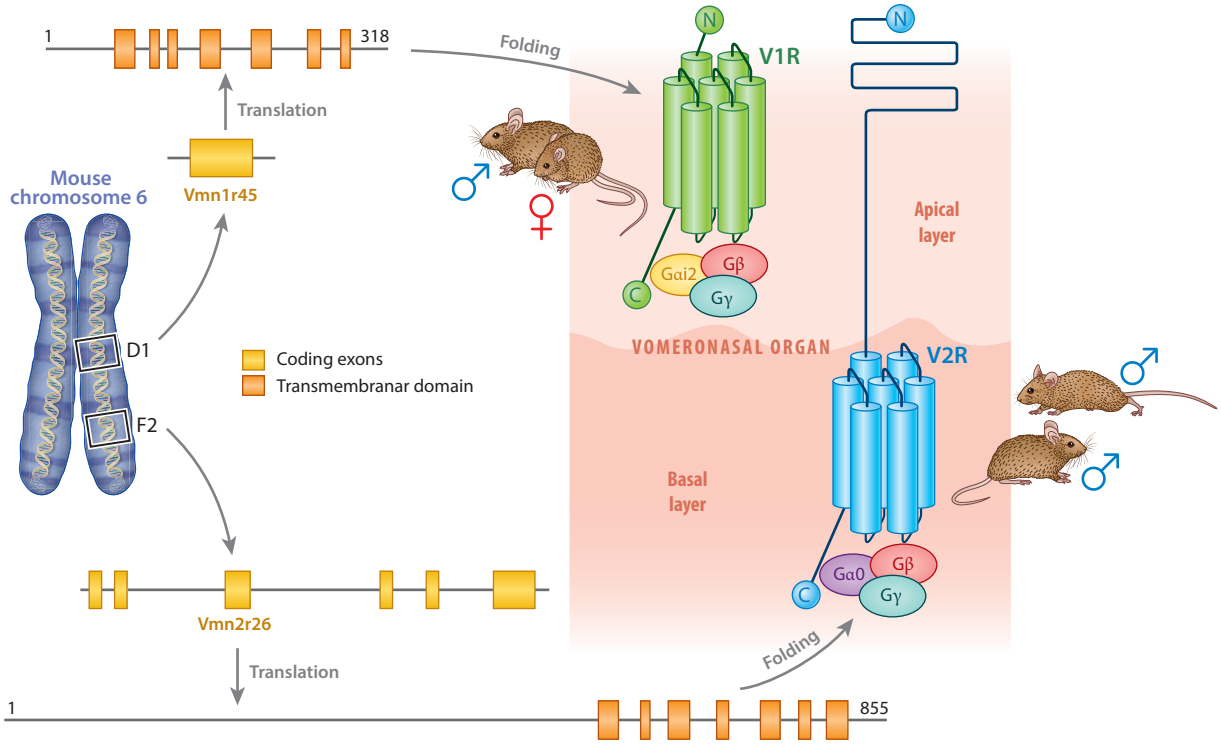

Figure 2

General characteristics of vomeronasal receptors (example of two rodent genes). Vomeronasal receptors type-1 (V1Rs) are encoded by single exons and are small when compared with vomeronasal receptors type-2 (V2Rs), which are encoded by six exons and possess a large N-terminal region. Both genes are expressed in the nasal cavity, but V1R is located in the apical layer, whereas V2R is in the basal layer. In rodents, V1Rs are involved in gender discrimination, whereas V2Rs are orientated for male aggression.

the relation between well-developed VNOs and extensive repertoires of VRs. Finally, we review pheromone detection in fishes using related receptors.

WATER-TO-LAND TRANSITION DRIVING VOMERONASAL RECEPTOR EVOLUTION: FACTS AND CONTRADICTIONS

V1Rs are commonly related to detection of small volatile molecules scattered in air, and V2Rs usually recognize molecules that are soluble in water, so they recognize molecules scattered in aquatic environments. Thus, it was suggested that during the transition of tetrapods from water to land, the V1R repertoires would have expanded to efficiently detect airborne ligands, whereas V2R repertoires would have contracted (2).

In fact, rodents have an extensive V1R repertoire (**Figure 4*b***) responsible for crucial functions (37, 38), including the recognition of urinary volatile steroids (37). Deletion of a cluster of V1R genes in mice can cause dramatic behavioral alterations, including reduced male libido and inappropriate maternal aggressive behavior (39). Rodents also show a great repertoire of V2R genes (40), though it is smaller compared to the number of V1R elements (4, 22).

The same discrepancy in V1R:V2R ratio is also visible in other mammals (**Figure 4*b***). In primates, the V1R repertoire is directly correlated with the anatomical development of the VNO. Indeed, strepsirrhines, like bushbabies and mouse lemurs, have well-developed VNOs and a high

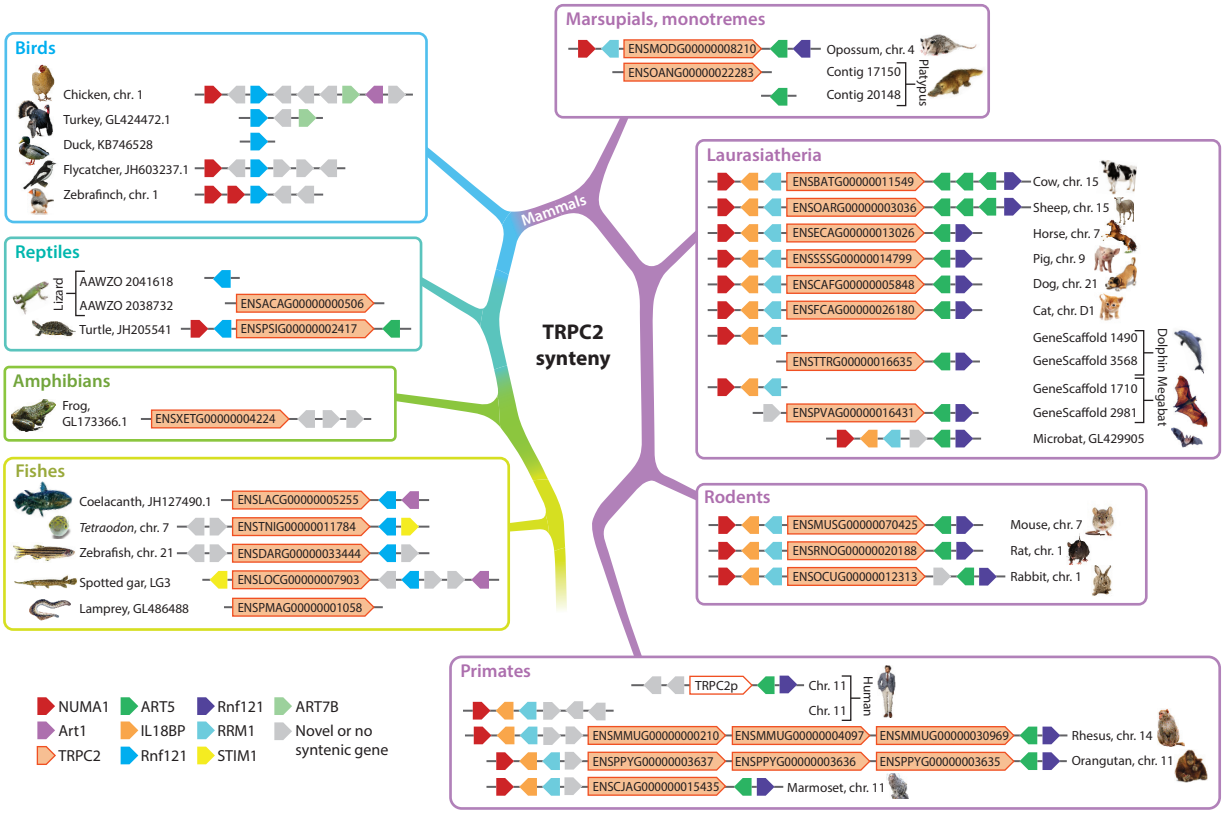

Figure 3

Transient receptor potential channel 2 (TRPC2) genes show conserved synteny across vertebrates. TRPC2 genes were detected in mammals, amphibians, reptiles, and fishes. No homologous genes or pseudogenes have been observed in birds.

V1R repertoire (41). In the platyrrhine marmoset, a medium-size VNO and a small V1R repertoire (25) have been related to important social behaviors, including recognition of group members and their reproductive status (42). By contrast, primates are known for lacking typically functional V2R genes (2, 4, 22, 43, 44). Surprisingly, two putatively functional V2R genes were detected in the platyrrhine marmoset (2, 45) (**Figure 3**, ***primates***).

Within ruminants, the cow has a well-developed VNO (46) and approximately 40 V1R genes (25, 32, 47) (**Figure 4*b***). In goats and sheep, the partially available genomes revealed 23 and 21 cow-similar V1R genes, respectively, but these figures may increase with better-quality genomes (43). Goat and sheep V1R genes have orthologs with the same family distribution in cross-species ruminant counterparts, suggesting an evolutionary conservation for the same/closely related chemical compounds (43). However, no functional V2R genes have been found in ruminants (4, 22, 43) (see sidebar titled Dog Domestication and Contraction of the Vomeronasal Repertoire; **Figure 5**).

Even in more basal mammalian species, like marsupials and monotremes, some divergence occurred in the ratio of V1R:V2R genes (**Figure 4*b***). High repertoires, with above 90 V1R genes, were reported in gray short-tailed opossum and tammar wallaby (2, 4, 25), which are related to the many and well-developed VNOs present in these species (48, 49). Similar to marsupials, the monotreme platypus has a high number of V1R genes (~280) (25). Those V1R genes form monophyletic groups that arose via gene duplication, suggesting species-specific adaptations to

DOG DOMESTICATION AND CONTRACTION OF THE VOMERONASAL REPERTOIRE

In carnivores, although dogs have an organized VNO with all characteristic elements (67) and the presence of the TRPC2 gene (**Figure 3**, **Laurasiatheria**), no V2R genes and only a small V1R repertoire with less than 10 genes have been reported (2, 4, 22, 25, 68, 69). This is unexpected given the high socialization and individual-specific interactions within dogs (70).

The deterioration of the V1R dog genes could have happened after dog domestication (**Figure 5**) (71–73). However, the wolf has the same inactivated genes as the dog, making it unlikely that domestication has caused V1R loss (25). However, other domesticated carnivores like cats, which are closely related to dogs, have a well-developed VNO (74) and medium-size V1R repertoire (28 elements) (25). Similarly, ruminants like the cow and sheep also went through a domestication process (75) but present a medium-size V1R gene repertoire, which gives strength to this hypothesis.

improve the pheromonal communication system in marsupials and monotremes (48, 50–52). This could have been due to offsprings' need to reach milk shortly after birth or hatching (48, 53). The opossum has 86 V2R genes (slightly less than the number of V1R genes) (2, 22), but the platypus has only 15 V2R genes (4, 47), which is low given the high duplication rate of V1R genes.

In amphibians, an opposite scenario occurs, with a high number of V2R genes detected relative to V1R genes (**Figure 4*b***). For example, the red-legged salamander (*Plethodon shermani*) has a highly dynamic VNO, which varies in size according to season (54), and expresses 34 V2R genes (55). The ligands to these receptors are still unknown, but salamanders use chemical cues in social and reproductive interactions (55–57), and V2Rs are also hypothesized to play a major role in their summer foraging (58). Difficulty in isolating and amplifying V1R genes (due to the many molecular similarities with other GPCRs) in amphibians precludes the precise characterization of their V1R repertoire, but it is believed to be small (55). Frogs are well adapted to both terrestrial and aquatic environments (59), being able to use both water-soluble and volatile chemicals as pheromones (60). *X. tropicalis* shows a gene expansion of more than 330 V2R genes (61), suggesting an increased importance of pheromonal communication (58). However, no volatile pheromones have been identified in frogs, and only 21 putatively V1R genes were identified in *X. tropicalis* (2, 47, 60).

Some of the previous examples give strength to the hypothesis of VR dynamic evolution driven by the water-to-land transition, but contradictory evidence in squamate reptiles is emerging. Although snakes can perceive an extensive collection of environmental chemical cues with their tongue (62, 63) and have a well-developed VNO (64–66), only four V1R genes were identified (**Figure 3**, ***birds***), which contrasts with large V2R repertoires (109 and 216 genes in *Pantherophis guttatus* and *Python molurus bivittatus*, respectively). This suggests either (*a*) an ancestral small V1R repertoire that did not expand in squamates or (*b*) a large V1R ancestral repertoire that contracted to the few remnants detected today (47). As the squamate V1R repertoire is not expanded, the hypothesized expansion of V1R/contraction of V2R related to the water-to-land vertebrate transition loses credibility (47). Further studies in reptiles are needed to fully understand how VR genes are evolving and which factors influenced the expansion or contraction of VR genes among species.

SELECTIVE PRESSURE IN THE EVOLUTION OF VOMERONASAL RECEPTORS

Gene evolution is often assessed based on the proportion of sites with nonsynonymous substitutions (Ka) relative to the sites with synonymous substitutions (Ks), the Ka/Ks ratio. A Ka/Ks

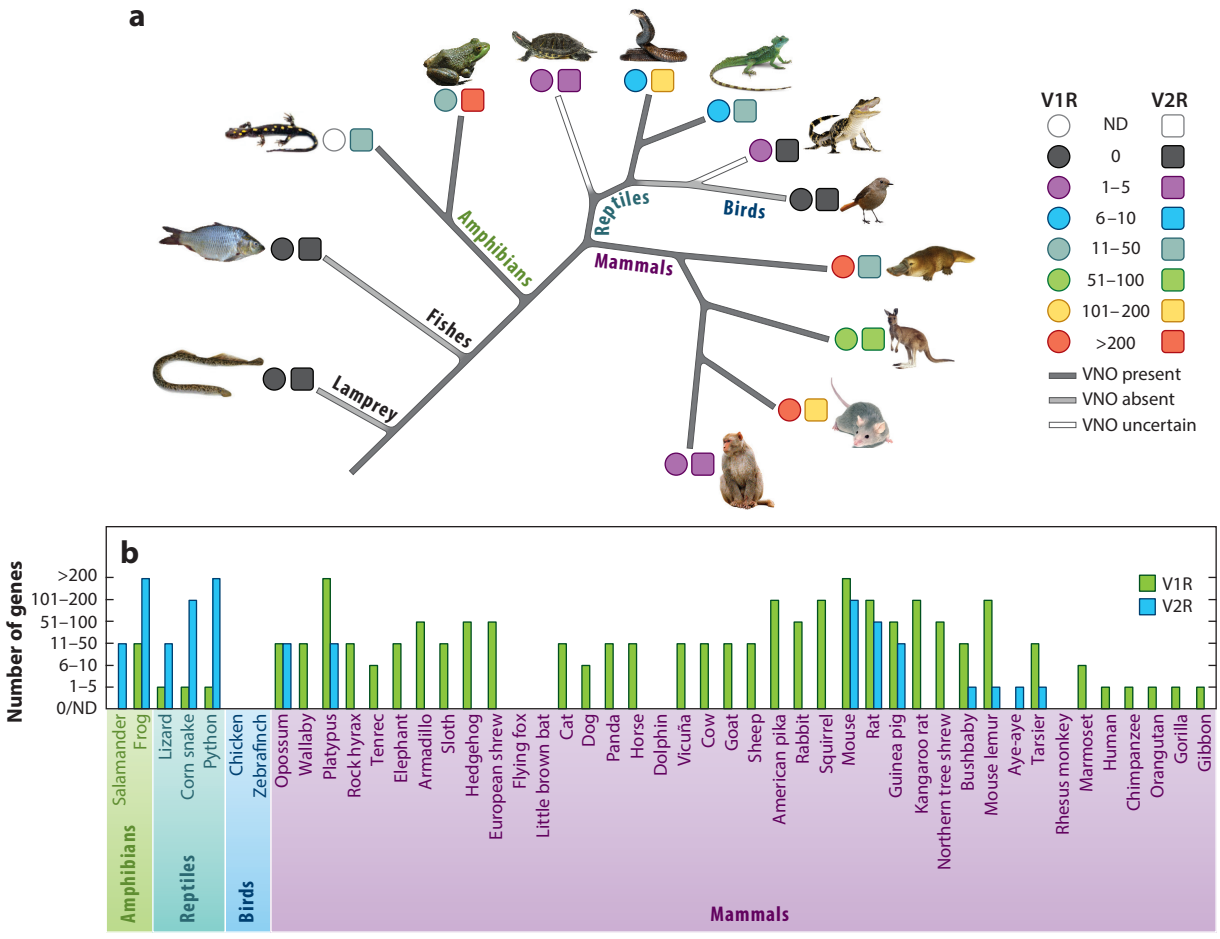

Figure 4

Distribution of the vomeronasal organ (VNO) and vomeronasal receptors (VRs) among vertebrates. (*a*) Distribution of VNOs and receptors among vertebrates. The presence of a VNO (*dark gray branches*) is connected with the existence of vomeronasal receptor type-1 (V1R) and/or vomeronasal receptor type-2 (V2R) genes, except in turtles and crocodiles, which have reported VRs but no available information on the presence of VNOs. The color spectrum of circles and squares reflects the gene repertoire size. In species that have lost a VNO (*light gray branches*), no VRs have been identified, suggesting a strong relationship between presence of organs and receptors. (*b*) Variation in V1R and V2R repertoire number among vertebrates (2, 4, 24, 25, 43, 47). Green bars represent the number of V1R genes, and blue bars represent the number of V2R genes. Nonmammalian species usually have more V2R than V1R genes, whereas in mammals the V1Rs are more common.

ratio lower than 1 indicates that the protein is under purifying selection. Ka/Ks > 1 suggests that positive selection favored the retention of beneficial mutations. Although the majority of genes are under strong to moderate purifying selection, genes involved in reproduction, host defense, and immune response are usually under positive selection (76).

VRs are very dynamic genes with rapid rates of gene duplication, gene conversion, lineage-specific expansions, deletions, and pseudogenizations (77). Early studies in rodent V1Rs detected a few accelerated sites located mostly in the extracellular loops and in fourth and seventh transmembranar domains (78), suggesting that V1R genes are under positive selection (39, 79). It was also hypothesized that positive selection pressure may maintain functional genes close

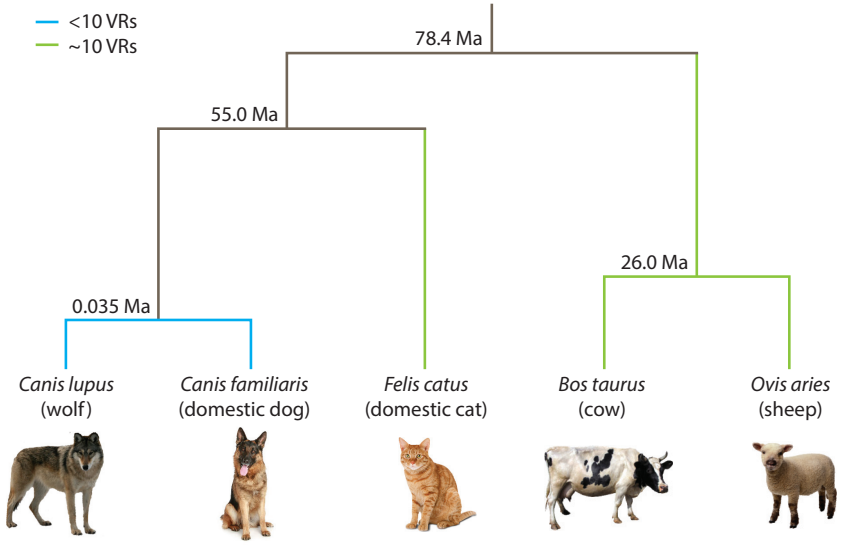

Figure 5

Wolf and dog split. The split between contemporary wolves and the wolf populations contributing to dog domestication occurred 35,000 years ago, before dog domestication (72). The domestication process does not seem to have interfered much with the inactivation of vomeronasal receptor (VR) genes and the small VR repertoire, as dog and wolf have similar gene repertoires, with less than 10 functional genes (*blue lines*) (73). By contrast, other domesticated species, such as the domestic cat and Bovidae representatives, which are phylogenetically close to carnivores, exhibit a medium-size repertoire with approximately 40 genes (*green lines*) (72, 75).

together in the genome because they are coregulated and share regulatory domains (80). However, if pheromones were evolving rapidly, this would create a strong selective pressure in recognition systems to quickly adapt to pheromone changes (81). All these studies used paralogous sequences precluding inference of positive selection between orthologs (21). Recently, it was proposed that despite occasional events of positive selection, the evolution of rodent V1Rs is largely ruled by purifying selection and random drift (82). In other species, only a few residues were found to be under positive selection (41). The analysis of a small number of rodent V2R genes identified some codons under positive selection mainly in extracellular domains (39). Subsequent studies suggested the presence of only weak purifying selection and/or positive selection acting in the N-terminal region of rodent V2Rs that is assumed to be the ligand-binding domain (19). In mouse lemur V2Rs, only one of the reported genes had some codons under positive selection (45). Further studies are required, namely using nonmammalian species, to understand the evolution of genes involved in pheromonal communication.

PRESENCE OF THE VOMERONASAL ORGAN AND VOMERONASAL COMMUNICATION: A STRONG CONNECTION

The vomeronasal system is found in many vertebrates, but punctual taxa or even large families have lost the VNO and/or vomeronasal communication (**Figure 4*a***). Here we highlight some of the best-known examples.

The vomeronasal system is absent in birds. The chicken lacks a VNO (83), and no V1R or V2R genes or their pseudogenes have yet been detected. Also, neither TRPC2 genes nor their

pseudogenes have been detected in the chicken or other birds (29) (**Figure 3**, ***birds***). This suggests the absence of VNO communication in birds. The pseudogenization of genes involved in these transduction pathways likely occurred so long ago that it cannot be identifiable in bird genomes (2). Birds are not anosmic, and chemical odorants are important for orientation, food detection, and nest location (84), but no evidence of pheromonal communication has been detected to date (85). Birds have an excellent visual and acoustic acuity (85), which is more relevant for flying than olfaction and may explain in part their vomeronasal system degeneration.

Hominids and Old World monkeys lost or have a very rudimentary VNO (86), which is associated with the absence of/small V1R repertoires (25, 87), suggesting that none of these species extensively use chemical cues to communicate (42). Moreover, the decline of pheromonal communication in catarrhines is coincident with the evolution of trichromatic color vision and the dominance of the primate visual system (77, 80).

A developed VNO has been reported only in *Miniopterus*, *Pteronotus*, and phyllostomid bats (88), the VNO being rudimentary or absent in all other bat species (88, 89). Flying fox and little brown bat do not have a VNO (88) and have lost V1R genes (90, 91). Bats possess only TRPC2 pseudogenes (90, 91), but the flying fox bat has one annotated TRPC2 gene in the Ensembl database (**Figure 3**, **Laurasiatheria**), which could be related to other roles of this gene in reproduction. Contrasting with the situation in primates, the loss of vomeronasal function in bats does not appear to be related to sensory trade-off because absence of VR is widespread in echolocation and nonecholocation taxa in dichromatic and monochromatic bats (90).

Chemical communication is widely believed to be unimportant in aquatic mammals, and the vomeronasal system was reported as being completely absent in sea cows, some seals, and all cetaceans (92–94). In dolphins, no VRs were detected (25, 95, 96), but the TRPC2 gene was retained, probably owing to dual function in the fecundation process (**Figure 3**, **Laurasiatheria**).

Currently, species that do not present VNOs also lack VRs, supporting the hypothesis that the expression of VRs is strictly connected with the presence of an organized structure, the VNO. However, analyses of newly sequenced genomes of key species are needed to further support these findings.

HOW CAN PHEROMONES BE DETECTED WHEN THE VNO IS ABSENT? THE CASE OF FISH

Vertebrates have used pheromone communication since early on, as sea lampreys have sexual (97) and migratory pheromones (98, 99). Because only tetrapods show an organized vomeronasal system, how do other vertebrates, like fishes or sea lampreys, detect pheromones? Fishes lack a VNO (30–32), and pheromone-detection genes are expressed in a pseudostratified olfactory rosette epithelium (32, 100–103) (**Figure 6**).

Teleost fishes' VRs were initially named V1R-like and V2R-like receptors, but they were later renamed ORAs (32) and OlfCs (36), respectively, because they represent independent monophyletic entities (30–32). Six ORA gene classes were identified in teleost fishes (**Figure 6**). Contrasting with VRs, Ora genes are not commonly ruled by duplication or pseudogenization (32, 102), being mostly influenced by strong negative selection (32, 104). Ora genes likely have similar function to V1Rs in the detection of chemical molecules, but the real function of Ora genes and their ligands remains unknown (32, 105).

OlfC genes show a reduced pseudogenization process but high size-repertoire variation among species (34). The OlfC genes are landmarked by neprilysin- and η-type phospholipase C–flanking genes (**Figure 7**) (34, 106). Besides pheromone sensing, the OlfC family can also act as amino acid–sensing receptors (101), which has so far been documented in zebrafish (36, 107–109)

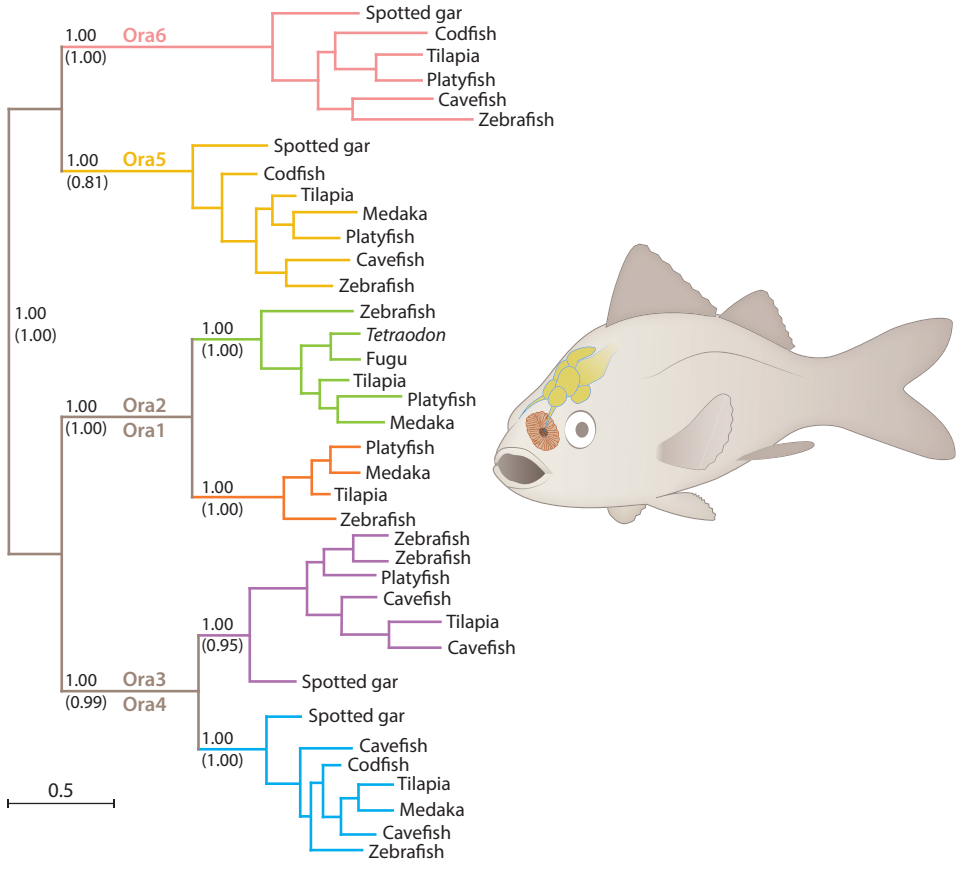

Figure 6

Phylogenetic relationships of the olfactory receptor related to class A G protein–coupled receptor (Ora) genes present in fishes. Ora receptors form three major clades (Ora1–Ora2, Ora3–Ora4, Ora5–Ora6) (32, 35), suggesting the presence of three ancestral genes. Upper bootstrap values correspond to MrBayes tree analysis using the GTR+I+G model and 100,000 replications (discarding the first 25% of results). Lower values correspond to the bootstrap PhyML tree analysis using the GTR+I+G model and 1,000 bootstraps. The Ora receptors are expressed in olfactory rosettes that connect with the brain via sensorial neurons.

(**Figure 8**). The large OlfC repertoire of the cichlid *Haplochromis chilotes* may have contributed to its extraordinary feeding behavior diversification by perceiving a wide range of amino acids (106). OlfC genes are ruled by negative selection (36).

Similar to teleost fishes, sea lampreys (*Petromyzon marinus*) lack an organized VNO (110). Some lamprey V1R-like genes have an olfactory epithelium expression (111). V2R-like genes were not detected in sea lampreys, suggesting an origin 600 Mya after the separation of jawed and jawless vertebrates, as V2R-like genes are absent in both urochordates (112) and cephalochordates (113).

CONCLUDING REMARKS

Over the past 20 years, significant advances in the understanding of the vomeronasal system have been obtained, in particular, the characterization of the VNO, the isolation of VRs, and the identification of putative ligands among several vertebrate species. Not all vertebrates possess the VNO

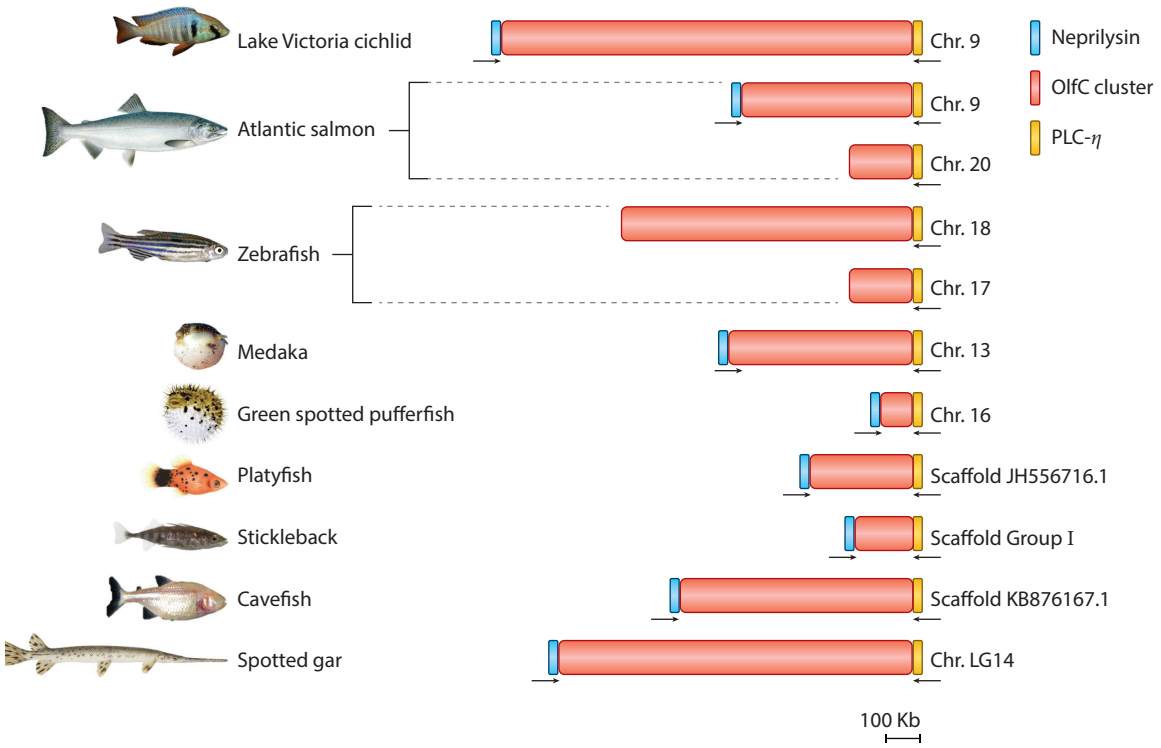

Figure 7

The chromosomal location of the olfactory receptor gene cluster related to class C G protein–coupled receptor (OlfC) gene clusters that show conserved synteny together with flanking genes across different fish genomes. The OlfC clusters are large and in well-defined regions inside fish genomes (31). The η-type phospholipase C (PLC-η) gene is present at the end of all clusters, whereas the neprilysin gene is usually in the beginning of clusters. Zebrafish and Atlantic salmon present OlfC genes in two different chromosomes. Information for medaka, green spotted pufferfish, platyfish, stickleback, cavefish, and spotted gar was retrieved directly from the Ensembl database, whereas Lake Victoria cichlid, Atlantic salmon, and zebrafish information was based on published studies (34, 106).

and receptors, and their presence can be very heterogeneous across species. In mammals, there is a strict relationship between the degree of VNO development and VR repertoire size. However, many doubts persist regarding the function of VRs and the physiological and morphological features of the vomeronasal system. Most of the available studies focus on rodents, but chemical communication in other mammals is yet to be described. The recent discovery of functional V2R genes in mouse lemur (45) increases the chance that new receptors may be detected in other mammalian species in which pheromonal communication was devalued previously.

The big gap in vomeronasal system understanding is in reptiles, in which, despite the importance of pheromones for foraging, avoidance of predators (114), and social relationships (115, 116), little is known about the evolution and distribution of the VNO, ligands, and the exact number of receptor genes. The distribution pattern of VRs in snakes is contradictory with what would be primarily expected for terrestrial species. Because VR genes were identified in some reptiles (**Figure 3**), but evidence of the VNO is lacking in others, like chameleons, further genomic studies in lizards would be fundamental to understand pheromonal detection within the suborder Iguania. Furthermore, no consensual opinion exists on vomeronasal development in turtles. Whereas some turtle species have a large VNO (117), others have only a vomeronasal

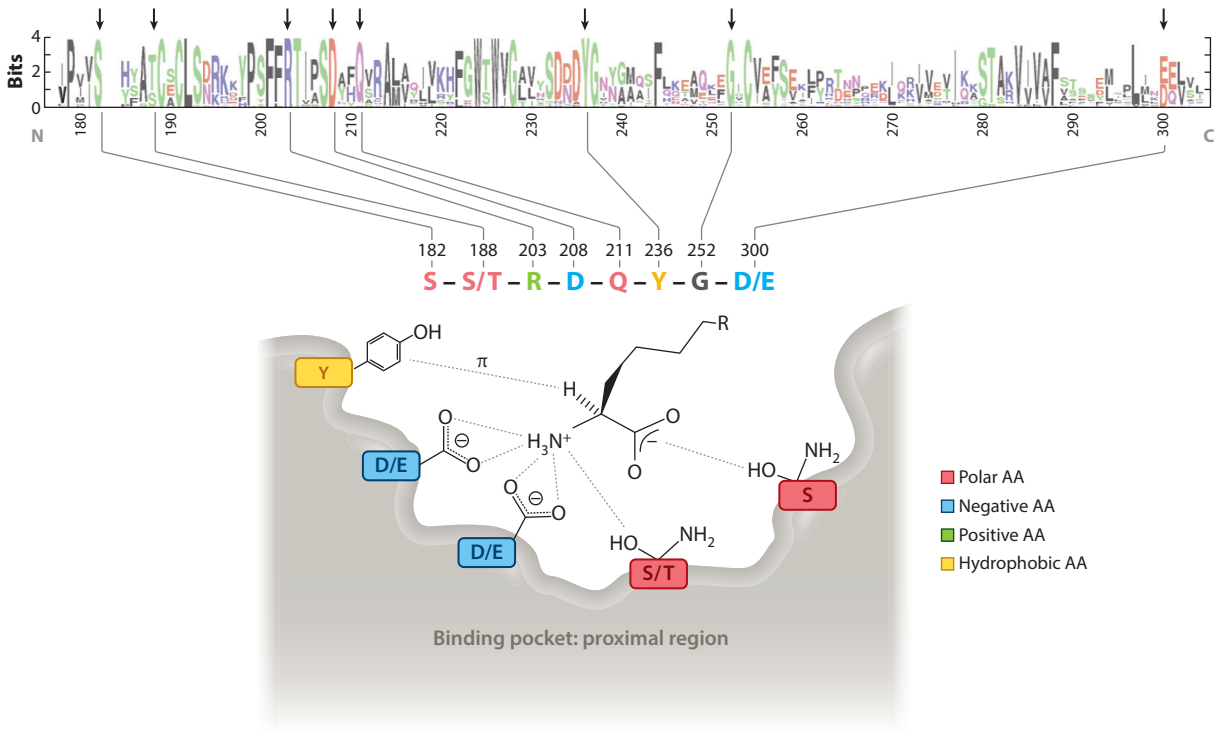

Figure 8

Amino acid (AA)-sensing ligand-binding receptor signature motif. The sequence logo, based on zebrafish olfactory receptor related to class C G protein–coupled receptor (OlfC) gene alignment (31), shows the conservation of eight AAs involved in the AA-sensing ligand-binding receptor signature motif (31). This signature is well conserved in the proximal pocket, a region known to interact with glycine moiety of AAs. The stronger and more probable AA interactions are represented in the binding pocket (109), suggesting that OlfC genes are able to detect and discriminate between a diverse spectrum of AAs (36).

epithelium (118, 119), and some aquatic turtles have even lost their VNO (120). Recently, three turtle genomes were published (121, 122), but only a few VRs were detected (one V1R in softshell turtle and green sea turtle and two V2R genes in softshell turtle) (123). Thus, the function, diversification, and evolution of VRs in turtles still remain mostly unknown. Similarly, crocodiles exhibit only one V1R across three different species (*Crocodylus porosus*, *Gavialis gangeticus*, and *Alligator mississippiensis*), and no V2R has been detected (123).

Another controversial aspect is the kind of selective pressure ruling the evolution of VR genes. Although many mammalian species have the VR repertoire described, the ligands for those receptors are still unknown. Identification of specific ligands for each VR could allow understanding of sexual attraction in mammals, which might be insightful in understanding chemical sense disorders and stimulating captive breeding.

In fishes, identification of the Ora and OlfC genes supports the idea that the original vomeronasal system is tetrapod specific and that fishes use an alternative method for pheromonal communication. However, chemical communication could be more straightforward than commonly assumed, and further studies are needed to clarify the role of positive or negative selection on these genes. For example, coelacanths belong to the lobe-finned fishes, being between teleost fishes and tetrapods and sharing several characteristics with land tetrapods (124). The recent

release of the coelacanth genome (125) may help elucidate the sensorial systems bridging fishes and highly developed tetrapods. Ongoing efforts for the genome sequencing of other relevant vertebrate lineages (126) will ultimately shed light on the evolutionary complexity of the vomeronasal system in vertebrates.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

A.A. was partially supported by the Strategic Funding UID/Multi/04423/2013 through national funds provided by the Fundação para a Ciência e a Tecnologia (FCT) and European Regional Development Fund in the framework of the program PT2020 and the FCT project PTDC/AAG-GLO/6887/2014 (POCI-01-0124-FEDER-016845).

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