The Evolution of Pheromonal Communication

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Abstract

Small-brained rodents have been the principle focus for pheromonal research and have provided comprehensive insights into the chemosensory mechanisms that underpin pheromonal communication and the hugely important roles that pheromones play in behavioural regulation. However, pheromonal communication does not start or end with the mouse and the rat, and work in amphibians reveals much about the likely evolutionary origins of the chemosensory systems that mediate pheromonal effects. The dual olfactory organs (the main olfactory epithelium and the vomeronasal organ), their receptors and their separate projection pathways appear to have ancient evolutionary origins, appearing in the aquatic ancestors of all tetrapods during the Devonian period and so pre-dating the transition to land. While the vomeronasal organ has long been considered an exclusively pheromonal organ, accumulating evidence indicates that it is not the sole channel for the transduction of pheromonal information and that both olfactory systems have been co-opted for the detection of different pheromone signals over the course of evolution. This has also led to great diversity in the vomeronasal and olfactory receptor families, with enormous levels of gene diversity and inactivation of genes in different species. Finally, the evolution of trichromacy as well as huge increases in social complexity have minimised the role of pheromones in the lives of primates, leading to the total inactivation of the vomeronasal system in catarrhine primates while the brain increased in size and behaviour became emancipated from hormonal regulation.

Introduction

Fifty years ago, Karlson and Lüscher [43] coined the term 'pheromone' to describe chemicals released by animals into the environment which have consistent effects on the behaviour or physiology of conspecifics, akin to the effects of hormones on the internal physiology of the individual. In so doing they recognised that pheromones are distinct from other semiochemicals which communicate biological information whether intended or not and whether to a conspecific or heterospecific. Since then, research across different disciplines has enormously advanced our understanding of the chemistry of pheromones, the sensory systems which detect them and the neurochemical pathways which transduce these signals. The importance of pheromonal communication has evolved over time and varies considerably between species, and this has significant effects on pheromonal signals and the sensory systems that detect them. Major evolutionary changes such as the transition from water to land have had a huge effect on pheromonal communication in tetrapods, changing the key chemical property of pheromones from solubility to volatility, modifying the mechanisms of pheromone release into these mediums, and resulting in major changes in morphology and function of sensory organs, as well as in the receptors that bind pheromone ligands [24]. However, other less obvious evolutionary changes have also had considerable consequences for the importance of pheromones in behaviour, particularly in mammals. This review is intended to provide an overview of how pheromonal communication in tetrapods has changed and of the factors that are thought to have driven these adaptations. The majority of research into pheromonal communication in vertebrates has focused on mammals, and particularly on rodents. It is this work that has illuminated the fundamental processes involved in the detection of pheromonal signals by chemosensory organs, the molecular biology of the signal transduction of the signals in these sensory systems, and the forebrain pathways that are involved in triggering behavioural responses to pheromones. However, to understand the evolutionary roots of the systems at work in mammals, we must consider pheromonal communication in other animal classes, such as amphibians, which can tell us much about the evolution of the dual olfactory systems and their origins in the ancestors of tetrapods [23]. While pheromonal communication is a key regulatory component of behaviour in small-brained mammals, it has

diminished in importance in larger-brained primates, where the evolution of trichromacy and the expansion of the cortex have combined to reduce the role of pheromones in modulating endocrine responses and regulating behaviour [15]. The evolutionary pressures of new ecological niches and increased social complexity drove the expansion of the cortex in primates and a consequent decline in the importance of pheromonal regulation of behaviour.

Pheromonal communication in small-brained mammals

Pheromonal signals

Pheromones are major modulators of behaviour in most mammals, regulating behaviours including reproduction, maternal care, aggression and alarm responses (see table 1). Pheromones have traditionally been defined as either 'releasers' which trigger immediate short-term behavioural responses, or 'primers' which trigger medium to long-term changes in behaviour or physiology. A third class of pheromones, 'signallers', has been added to these classical categories to describe chemosignals which carry information about an individual and induce changes in behaviour or physiology which depend on the identities of the animals sending and receiving the signal. However, as researchers have identified specific pheromones and the behaviours they influence, so these clear categories have become somewhat muddied, and they are not always an aid to our understanding of the phenomena being studied. For instance, the testosterone-dependent constituents of male mouse urine, 2-sec-butyl-4,5-dihydrothiazole and 2,3-dehydro-exo-brevicomin act as releaser pheromones on other male mice, eliciting aggressive displays [70]. However, these compounds can also act as primer pheromones, modulating receptivity and inducing oestrus in female mice [42]. The effects of male urine on female reproductive physiology in mice are further modulated by other pheromones. Recently mated females lose their pregnancy and are brought back into oestrus if exposed to the urine of an unfamiliar male, a phenomenon known as the Bruce effect [12]. During mating, the female learns the olfactory signature of the mating male, after which his urine will not induce this pregnancy failure. The signals which mediate this effect are MHC

class-1 peptides found in male urine [56], and the addition of unfamiliar MHC peptides to the mating male's urine will restore pregnancy blocking properties to it once more, indicating that they are communicating identity. These examples show that pheromones do not simply induce reliable behavioural changes, but that the messages they carry are dependent on the identities of the signaller and the receiver at multiple levels, from gender to individuality, and that these interact to produce different effects.

The properties of pheromones themselves have evolved according to both the medium of signal transmission, and the message to be conveyed. In aquatic amphibians, pheromones must disperse through water and so the key chemical property for signalling molecules is water solubility. The pheromones identified in these species to date are polar proteins and peptides which are water soluble and thus effective signals in the aquatic environment [53]. Terrestrial mammals face different challenges and the signals they employ can be either volatile, or non-volatile, depending on the information to be transmitted. Complex messages such as individual identity require a complex signal to ensure that the message is conveyed accurately, hence these tend to be mixtures of large, involatile molecules, such as the mixtures of large lipocalin proteins in mouse urine known as mouse urinary proteins (MUPs) which convey individual identity in urine marks [8]. Simpler messages, such as the gender of an individual, do not require complex signals *per se*, and some such pheromones are small volatile molecules that disperse through the air for easy detection. The volatile steroid androstenone in boar saliva induces lordosis in receptive sows [18, 19], while the small thiol (methylthio)methanethiol in male mouse urine is a potent attractant for female mice [59]. Rabbit milk contains 2-methylbut-2-enal, a volatile aldehyde which induces nipple-searching behaviour in pups [76]. As rabbit mothers only letdown milk once per day for very short periods, the presence of a pheromone which attracts pups to the nipple ensures suckling behaviour will occur at the same time as milk release. As 2-methylbut-2-enal is volatile, it will disperse easily through the nest, increasing the likelihood that pups will detect the signal.

Pheromone detection

These different types of signals must be detected, whether volatile or involatile, and most mammals have two olfactory organs – the main olfactory epithelium (MOE), and the vomeronasal organ (VNO) which detect semiochemicals and then process this information in separate neural pathways. A functional role has been identified for a recently discovered third chemosensory organ, the Grueneberg ganglion, which appears to mediate behavioural responses to alarm pheromones in rodents [9], however the majority of behaviourally-significant chemosignals are detected by either the MOE or VNO. These two sensory organs have their own distinct primary projection targets, connecting to the main olfactory bulb (MOB) and the accessory olfactory bulb (AOB) respectively, which each then connect to different forebrain nuclei comprising the main olfactory system and the vomeronasal system [11]. The MOB sends projections to secondary processing areas including the piriform cortex, the anterior olfactory nucleus and the cortical amygdala, while the AOB has bidirectional connections with the medial amygdala and the bed nucleus of stria terminalis with further output to the ventral hypothalamus [28]. While the two systems converge at the level of the amygdala [82], the segregation of the two systems and the extensive functional differences between them have given rise to the traditional view that the MOE detects volatile odorants in the environment while the VNO detects non-volatile pheromones [14]. These functions were seen as being the exclusive domain of each sensory system, however as discussed above it is not only non-volatile compounds that act as pheromones in mammals [6], and recent evidence suggests that while there are functional differences between the two systems, there is also overlap between the two olfactory systems in their roles in pheromone detection. Both the VNO and MOE have recently been shown to respond to certain volatile odours [87] while MOE sensory neurons have also been shown to be activated by involatile peptides which were previously thought to be detected solely through the VNO [78]. These data indicate that the two olfactory systems do not operate in mutually exclusive sensory domains, however the majority of pheromonal effects in mammals are still thought to be mediated via the VNO [11] and it is only the MOE that has both the appropriate morphology and the complexity of sensory receptor to act as a chemosensor for general airborne odours.

The work of Jianzhi Zhang and colleagues on the comparative evolution of VNO and MOE receptors suggests that there have been different selection pressures on receptor families in the two olfactory organs and that MOE receptors have evolved to detect a broad range of odorants, while VNO receptors have evolved to detect limited groups of ligands [35]. This so called 'differential tuning' hypothesis suggests that MOE receptors broadly function to detect environmental cues while VNO receptors detect species-specific cues such as pheromones. As such the VNO receptors would be expected to vary considerably from species to species, as they bind species-specific ligands, while the genes encoding MOE receptors which detect environmental cues should remain relatively stable. Comparative sequence analysis indicates that this is the case, and that MOE receptor gene sequences are well conserved across vertebrates, while far more species-specific VNO receptor genes exist, indicating more rapid evolution of the VNO receptor repertoire, and suggesting that the VNO plays a more important role in species-specific communication.

The vomeronasal system in mammals

The VNO is a blind-ended, fluid-filled tube which opens into the basal part of the nasal cavity [20] and is lined with microvillar receptor neurons which send axonal projections to mitral cells in the accessory olfactory bulb [49]. Many mammalian species engage in extensive physical investigation of scent marks and of conspecifics, exposing them to semiochemicals which are pumped into the lumen of the VNO when the animal is aroused [67]. The flehmen response seen in ungulates in which the top lip is curled back towards the nose during such investigations is thought to be behavioural adaptation to increase vomeronasal exposure to stimulating semiochemicals. Vomeronasal-stimulating ligands then bind to receptors on the surface of the sensory neurons, triggering action potentials in these cells which project to the AOB. Two distinct families of receptors have been identified in vomeronasal sensory neurons in rodents, the vomeronasal type 1 receptors (V1Rs) and the vomeronasal type 2 receptors (V2Rs), both of which encode G protein-coupled seven-transmembrane proteins [21, 39, 64, 75]. V1Rs and V2Rs share little sequence homology, are coupled to different signalling molecules (the V1Rs to the

$G\alpha_{i2}$-protein and the V2Rs to the $G\alpha_o$-protein) and are thought to have a very ancient evolutionary origins [49]. There is also a topographical difference in expression of the two receptor gene families, V1Rs being expressed in the apical part of the VNO and V2Rs in the basal part [37]. The V1R-expressing apical receptor neurons project exclusively to the anterior AOB while the V2R-expressing basal receptors project to the posterior AOB. These two subdivisions of the VNO and AOB respond differentially to pheromonal stimuli in mice [10] and information processed in the two parts of vomeronasal pathway only appears to converge when their projections overlap in the amygdala [83]. As well as differing in their structure, signalling proteins and expression, the two families of vomeronasal receptors also respond to different stimuli. The V1R-expressing sensory neurons in the apical part of the VNO respond to the volatile pheromones 2,3-dehydro-exo-brevicomin and 2-sec-butyl-4,5-dihydrothiazole [55]which are found in mouse urine and thought to be conveyed into the lumen of the VNO bound to MUPs. The VNO is known to mediate the Bruce effect [60], and it is the V2R-expressing receptor neurons in the basal VNO which appear to bind the high-molecular weight MHC-class 1 proteins that signal identity in this behavioural response [56]. The different ligands that these receptors bind suggest that the two classes of vomeronasal receptor neurons regulate divergent pheromonally-regulated behaviours, and that the detection and primary processing of these signals is carried out separately by the two portions of the vomeronasal system. The volatile and non-volatile ligands that V1Rs and V2Rs respectively respond to and the lack of sequence homology in their genes point to separate evolutionary origins for these receptors, possibly as chemoreceptors for small and large molecules, and this appears to be reflected in their roles in the detection of different pheromonal cues [77]. It should be noted that the segregated vomeronasal system in mice appears to be a sophisticated chemosensory adaptation and that many mammals that are less reliant on pheromones as behavioural regulators do not have this functional division in the VNO and AOB. The V2Rs in many non-rodents have become pseudogenes and do not appear to be expressed at all in the VNO [90].

Activation of the vomeronasal system by semiochemicals has been demonstrated in electrophysiological studies in awake animals [61] which show that physical investigation of conspecifics

increases firing in AOB neurons and that these responses are also selective, individual neurons exhibiting consistent responses depending on the gender or strain of the stimulus mouse. Gene knockout studies have also shown a clear role for the VNO in behavioural responses to pheromones. Genetic ablation of even a subset of V1Rs themselves also has significant effects on behaviours that are thought to be pheromonally mediated, reducing maternal aggression in female mice and sexual behaviour in males [17]. Gene knockout of the VNO-specific Trpc2 ion channel blocks signalling in all V1R neurons, and studies of mice carrying such a knockout have shown that pheromone-mediated behaviours such as male-male aggression are abolished by the knockout [80]. The signal transduction mechanism in V2R-expressing neurons appears to be different and does not involve the Trpc2 channel, as *Trpc2*-knockout mice remain sensitive to MHC-class 1 peptides and exhibit the pregnancy block behaviour which these semiochemicals trigger and which are mediated through V2R-expressing vomeronasal sensory neurons in the basal VNO [46]. Lesions to the VNO have also been shown to disrupt pheromonally-induced behaviours, such as the absence of oestrous cyclicity that occurs in group-housed female mice (the Lee-Boot effect), and the induction of early puberty in young female mice that are exposed to male odours (the Vandenbergh effect) [47]. Surgical ablation of the VNO has dramatic effects on reproductive behaviours that are dependent on pheromones, compromising copulatory behaviour in both female [45] and male rodents [66, 71].

The two vomeronasal receptor gene families vary enormously across mammalian species, and the number of functional vomeronasal receptor genes appears to correlate with VNO complexity. In a comparison of different mammalian species, there was a positive correlation between VNO morphological complexity and the number of intact V1R genes present in the genome [33]. Somewhat surprisingly, the largest V1R receptor gene family (with 270 functional genes) has been identified in the platypus, one of only a handful of extant egg-laying monotreme species. Furthermore, there are far fewer functional V2R genes in the platypus than in eutherian mammals with large V1R gene repertoires, such as rats and mice, indicating that the vomeronasal system has a different functional organisation in the platypus. The picture is further complicated by the apparent degeneration of main olfactory receptor

genes in the platypus and the fact that the AOB is larger than the MOB in this species. In eutherian mammals, OR genes outnumber V1Rs several fold (for example, mice have 1037 functional OR genes and 187 functional V1R genes), however in the platypus V1R genes are more numerous [34] suggesting that vomeronasal function may be of greater significance than main olfactory function in this species. The vomeronasal system is completely absent in marine mammals [62], and so the presence of a complex VNO and large V1R gene family was somewhat surprising. In the platypus, the VNO does not connect to the nasal cavity but to the mouth, and when foraging underwater, the platypus seals its eyes, ears and nostrils, and uses its bill to detect prey. If the VNO of the platypus is involved in general chemosensation in this species and not specifically pheromonal communication, the increased repertoire of V1Rs may be an adaptation to underwater foraging. While the VNO appears to function primarily as a pheromonal organ in rodents, it may have evolved to fulfil a different role in this very unusual semi-aquatic monotreme.

The main olfactory system and pheromones

Although the vomeronasal system has been the main focus for pheromonal research, some volatile mammalian pheromones have long been known to be detected by the main olfactory system. The volatile boar pheromone androstenone continues to induce lordosis in female sows when the VNO is blocked, demonstrating that it is detected by the MOE [19]. The nipple-search behaviour of rabbit pups is elicited by a volatile pheromone, 2-methylbut-2-enal, and removal of the VNO has no effect on this behaviour while ablation of the MOE abolishes it completely [40]. Male sexual behaviour has also been shown to be generally dependent on a functioning MOE, as chemical ablation severely disrupts both investigatory and copulatory behaviours in male mice [44]. Recent electrophysiological work has shown that the volatile male mouse pheromone (methylthio)methanethiol clear responses in the MOB of female mice, indicating that the main olfactory system may mediate the attraction of females to this pheromone in mice [59]. Some pheromonal signals may also be processed in parallel by the two olfactory systems, as calcium imaging studies with nasal tissue slices have shown that there are sensory neurons that respond to

MHC peptides in both the VNO and the MOE [78]. Evidence at both the behavioural and cellular level shows that some pheromones are detected by the MOE and processed by the main olfactory system leading to increasing questioning of the view that pheromonal communication in mammals is the exclusive preserve of the vomeronasal system [6].

The MOE consists primarily of ciliated olfactory sensor neurons which line the nasal cavity [29]. The MOE and VNO have long been known to differ at the cellular level, with the receptors of each sensory system being expressed in ciliated or microvillar receptor neurons respectively, although a small sub-population of microvillar cells have been identified in the mouse MOE [27]. Like the vomeronasal receptors, the receptors of the MOE are G protein-coupled seven-transmembrane proteins, however they are evolutionarily distinct from both the V1Rs and V2Rs and can themselves be grouped into two separate gene families. The olfactory receptors (ORs) are encoded by approximately 1000 genes in rodents and are the largest gene family in mammals, accounting for up to 2% of the genome in mice [13], while the trace amine-associated receptors (TAARs) are a second, smaller family of olfactory receptors in the main epithelium which were more recently identified [57]. The diversity of the OR gene family and the receptors they encode provide sensitivity to a huge range of different odorants in rodents. The size of the OR gene family itself varies enormously across mammalian species [69], indicating that it has been under very different selection pressures in different species and that there is substantial variation in olfactory acuity in mammals. While this does not indicate a changing role for the MOE in pheromonal communication, it does show that the relative importance of chemosignals in the lives of mammals has shifted up and down in different species; changes that may also have affected the roles of pheromones in behavioural regulation.

The signal transduction mechanisms in olfactory sensory neurons differ significantly from those at work in vomeronasal sensory neurons. Both ORs and TAARs are bound to a MOE-specific G-protein, G_{olf}, which is released when ligands bind to the receptor, resulting in the opening of a cyclic nucleotide-gated ion channel (CNG) that is specific to the main olfactory system. Subsequent calcium influx depolarises the olfactory receptor neuron membrane triggering an action potential. This signalling

pathway has been the focus of genetic manipulations which have offered further evidence that the MOE plays a more significant role than previously thought in pheromonally-mediated behaviours such as reproduction. Knocking out the A2 subunit of the CNG channel abolishes main olfactory ability and has profound effects on male reproductive capability. *Cnga2*$^{-/-}$ mutant males do not initiate aggression with other males, but also show no social or sexual interest in females whatsoever and fail to show any mounting behaviour in tests [63]. This contrasts with the effects of the vomeronasal-specific *Trpc2*$^{-/-}$ knockout, which disrupts male-male aggression but does not abolish copulation, but instead results in indiscriminate mounting of both males and females [80]. These gene knockouts affecting the two olfactory systems suggest that both are required for proper induction of pheromonal behaviours such as intermale aggression and mating and that the MOE may detect volatile female odours, arousing interest and stimulating active investigation and subsequent pumping of semiochemicals into the VNO lumen. While the very existence of two separate olfactory systems suggests divergent functions for them, it is clear that certain behaviours are dependent on the detection of different semiochemicals by both systems. While the vomeronasal system still appears to be the main pathway for the processing of pheromonal signals, the main olfactory system has also evolved to detect certain pheromonal cues and may function synergistically with the vomeronasal system in initiating some pheromone-dependent behaviours.

Pheromonal communication in amphibians

Amphibian pheromones

While the majority of research into MOE and VNO function has been conducted in mammals, these dual olfactory systems appear to have evolved in ancestral tetrapods, meaning that a great deal can be learnt about these evolutionary origins by studying pheromonal communication in amphibians. There are three main extant groups of amphibians, the anurans (toads and frogs), the caudates/urodeles (newts and salamanders) and the caecilians. The salamanders and frogs have been the main subjects for research into amphibian chemosensation and the VNO and MOE have been found in most species examined, suggesting that these organs evolved in the common ancestors of amphibians and mammals before these

lineages branched apart over 350 million years ago in the Devonian period. The last common ancestor of amphibians and amniotes was aquatic and as such, any shared features of chemosensation in amphibians and mammals are likely to have evolved in this ancestor, before the transition from water to land [23]. The significance of pheromones for behavioural regulation in amphibians has become clear in recent years, however as amphibians spend part or all of their lives in water, pheromonal communication in this aquatic environment poses different challenges to those experienced by terrestrial mammals. Solubility rather than volatility is the key chemical property of pheromones, olfactory organs must be adapted for chemosensation in water rather than air and the physical properties of water make durable scent marking more difficult. The first amphibian pheromone, sodefrin, was identified over ten years ago and others have since been described in newts, salamanders and frogs. Sodefrin is a small decapeptide, secreted from the cloacal gland of the male Japanese fire-bellied newt *Cynops pyrrhogaster* [51] which attracts females when dissolved in water at concentrations as dilute as 0.1pM. Its effects are species-specific and it does not attract females of the related sword-tail newt, *Cynops ensicauda* which instead are attracted by silefrin, a similar decapeptide that differs at two residues and which is secreted by males of that species [88]. The male magnificent tree-frog, *Litoria splendida*, has been shown to secrete a small peptide, splendipherin, which diffuses through water and attracts females of the same species but not others [84]. Male salamanders of the genus *Plethodon* (the lungless salamanders) have also been shown to use reproductive pheromones, secreting skin-borne semiochemicals which induce receptivity in females during courtship [74]. As compounds that either diffuse through water, or are secreted onto permanently moist skin (in the case of *Plethodon* salamanders), all of these pheromones are polar proteins or peptides which are water-soluble and thus suitable for signalling in the aquatic environment in which amphibians spend at least part of their lives (see table 1).

Amphibian olfactory systems

Most amphibians have the same dual olfactory system seen in many other tetrapods, with a MOE lining the nasal cavity and projecting to the main olfactory bulb, and a VNO that projects to the accessory olfactory bulb [23]. As in mammals, these two olfactory systems also have similarly segregated

connections to distinct forebrain processing pathways, with separate projections that only appear to converge at the level of the amygdale [6], indicating that the evolution of both the separate chemosensory organs and their distinct projection pathways must have occurred in a common ancestor [68]. The vomeronasal pathway in both amphibians and mammals involves the same nuclei, with the VNO connecting to the AOB, which sends its major projection to the medial amygdala and then on to the hypothalamus, suggesting that this circuit has been conserved in amphibians and mammals [37]. The VNO is thought to mediate many of the pheromonal effects described so far in amphibians and has been shown to respond to several amphibian pheromones. Sodefrin causes a marked increase in neural activity in the VNO when applied to the vomeronasal epithelium of female *Cynops pyrrhogaster* [52]. Furthermore, it evokes no response when applied to the vomeronasal epithelium of either sexually immature females or males, suggesting that the behavioural responses seen in mature females are mediated by detection at the vomeronasal epithelium. The protein pheromones secreted by male plethodontid salamanders have been shown to activate vomeronasal receptor neurons, but not olfactory receptor neurons in females of the species [86]. Moreover, application of these peptide pheromones to the snout of females increases expression of the immediate early gene c-Fos in areas of the brain thought to be involved in reproduction and sexual receptivity [54], suggesting a functional link between the VNO and the reproductive brain in these salamanders. The neural pathways in the vomeronasal system appear to be similar in amphibians and mammals, indicating that the pheromones that modulate courtship and reproduction in these two classes of tetrapod may act through the same forebrain circuits.

Amphibian chemosensory receptors

In mammals, the VNO is lined exclusively with microvillar receptor neurons and the MOE with ciliated receptor neurons, morphological differences which suggest that the receptor cells have divergent evolutionary origins and that there are functional chemosensory differences between receptor neurons in the two systems. However, while the amphibian VNO is lined exclusively with microvillar receptor neurons, the MOE is only lined with ciliated olfactory sensory neurons in salamanders, and with a mixture of ciliated and microvillar receptor neurons in frogs [25]. This latter pattern of receptor cell

distribution resembles that of fish, which lack a vomeronasal system and in which the olfactory epithelium is lined with a mixture of ciliated and microvillar receptor neurons. Despite the lack of VNO in fish, olfactory receptor expression is segregated according to receptor neuron morphology, as in mammals. The olfactory-like receptors are expressed in ciliated olfactory neurons, while vomeronasal-like receptors are expressed in microvillar receptor neurons [38]. As with receptor cell type, it appears that segregation of vomeronasal and olfactory receptors that is seen in mammals is not as strictly defined in amphibians, and specifically in frogs. While ORs are expressed in the MOE and the expression of V2Rs is restricted to the vomeronasal organ of the African clawed frog *Xenopus laevis*, [36], V1Rs are not expressed in the VNO, but rather are found in the MOE, specifically in the middle cavity (MC) portion of the MOE which is thought to be specialised for the detection of water-borne chemosignals [16]. There is very little expression of the V1Rs in the principle cavity (PC) portion of the MOE which is adapted for the detection of air-borne odorants in these amphibians, suggesting that although the V1Rs are not expressed in the VNO, they may still be binding water-borne ligands, rather than volatile odorants. *Xenopus* is a fully aquatic amphibian, and the presence of V1Rs in the water-exposed MOE suggests that it may be used for general chemosensation. If this is the case, there would appear to be some similarity with the role that V1Rs might play in chemosensation in the platypus, as discussed previously. While this is simple conjecture and there is as yet no experimental data to support this possibility, the different receptor families are likely to have evolved to bind specific kinds of ligands, and the V1Rs may perform a similar chemosensory function in aquatic frogs and platypuses, despite being expressed in different organs. Such functional similarity would be likely to be the result of convergent evolution, rather than conservation of function, as monotremes diverged from the main mammalian lineage approximately 200 MYA, some 250 MYA after amphibians and mammals diverged. The specialisations exhibited by the platypus are not common to mammals, and so are likely to have evolved as adaptations to its freshwater habitat, one which is not dissimilar from that of *Xenopus* in the wild.The differential expression patterns of these receptor families in tetrapods suggest that the strict segregation of V1Rs and V2Rs to the VNO might have occurred in an ancestral mammal, and not in the shared ancestor of mammals and amphibians.

The absence of the VNO in fish, its presence in terrestrial mammals and its loss in marine mammals have been cited as evidence that this organ evolved as an adaptation to terrestrial life [7], however the presence of the VNO in amphibians suggests that this is not the case . As the branching of the amphibian and amniote classes of tetrapods occurred before the transition to life on land, the VNO must have evolved initially in an aquatic environment. While the amphibian species listed above have all been shown to secrete and respond to pheromones, apparently detected by the VNO, these species are all semi-aquatic, raising the possibility that the vomeronasal organ might have evolved separately in amniotes and in amphibians that spend at least part of their adult lives on land. The absence of the VNO in the aquatic proteid amphibians would seem to lend this theory credence, however a functional VNO is found both in fully aquatic axolotls and amphiumid salamanders [26] indicating that life underwater is not a barrier to VNO function. It should also be noted that the oceans and seas in which VNO-lacking marine mammals [65] live are very different environments to the freshwater streams and ponds that many amphibians live in. The diluting power of the oceans would render chemical signalling ineffective, as would the lack of solid substrates in the open seas on which marine mammals might deposit scent marks. Moreover, the VNO is also present in the pre-metamorphosis larval stage of frogs and salamanders [25], offering strong support to the theory that the VNO did not evolve as an adaptation to life on land, but in the aquatic ancestor of both amphibians and amniotes.

Pheromonal communication in large-brained mammals

Dual olfactory systems in mammals

The evolving importance of pheromonal communication in mammals has driven major changes in chemosensation, which are reflected in the functions of the dual olfactory systems and in the diversity of the gene families that encode MOE and VNO receptors in different species. While most mammals have both olfactory systems, the vomeronasal system is not present in marine mammals [65], bats [85], old world monkeys and great apes (catarrhine primates)[49]. While the aquatic and arboreal environments inhabited by marine mammals and bats are thought to have led to the decreased importance of

pheromonal communication and general olfaction in these mammals, the absence of a VNO in primates appears to not simply be due to the practicalities of signalling in a particular environment. Instead, changes in visual capacity, ecological niche, social organisation and brain size appear to have all had a role in the reduced importance of pheromonal communication in these animals, resulting in the loss of the accessory olfactory system as well as reduced complexity in the main olfactory system [15]. Changes in brain size and organisation in primates have led to changes in behavioural regulation, with increasing emancipation of behaviour from hormonal and pheromonal control.

Pheromones are thought to play a role in behavioural regulation in prosimians and new world monkeys [1, 2], although the effects of pheromones on behaviour do not appear to be as significant in primates as in small-brained mammals [3]. While prosimians and new world monkeys possess a VNO and functioning vomeronasal system, the VNO and AOB appear to have become vestigial in catarrhine primates approximately 23 million years ago. Furthermore, there has also been a decline in the complexity of the main olfactory system, indicating reduced importance for chemosignals in these primates. While functional V2R genes are absent in both primates and some mammals which have retained the vomeronasal system [90], other genetic markers of the vomeronasal system have also become pseudogenes in catarrhine primates, indicating neutral evolutionary pressure on this system. Both the VNO-specific ion channel *Trpc2* and the V1R gene family have undergone extensive inactivation in catarrhine primates [58, 91]. In humans, approximately 200 V1R genes have been identified of which only 5 have an intact open reading frame and a potential coding sequence [73], while none of the V1R sequences in the chimpanzee appear to be functional [89]. The pseudogenisation of V1Rs and V2Rs, as well as the *Trpc2* ion channel that is a fundamental component of V1R signal transduction is strong evidence that a functional VNO was lost in the ancestor of all old world primates and great apes. The main olfactory system has also seen extensive pseudogenisation of the OR gene family – only 70% of OR genes in old world monkeys and 40 % of OR genes in humans are functional [30]. These changes OR gene family size and the absence of a functional vomeronasal system in catarrhine primates all indicate a diminished role for olfaction and pheromones in these large-brained mammals.

Trichromacy, social complexity and pheromones

The inactivation of the vomeronasal system and increasing pseudogenisation of OR genes occurred concurrently with the evolution of trichromacy in catarrhine primates [32, 91] and the evolution of a more sophisticated visual system was a significant driver in the decline of chemosensory systems in these primates. Trichromacy allowed primates to occupy new niches, moving from a nocturnal to a diurnal lifestyle and also to a frugivorous diet and the selection of food based on cues such as colour [81]. Such changes appear to have driven a considerable expansion of the visual cortex in these primates, as well as a simultaneous decline in size of olfactory brain structures [4] indicating the changes in relative importance of these two senses. However it is not just shifts in circadian rhythm and diet which led to these changes, but shifts in social organisation too. In catarrhine primates, visual cues such as brightly coloured sexual skin are indicators of female receptivity and male dominance, and have supplanted semiochemicals as behavioural signals. Comparative of analysis of MOB and AOB size in different primates has shown that while ecological factors correlate strongly with the reduction in MOB size in primates, social factors such as group size and mating system correlate with AOB size in non-catarrhine primates (the AOB being absent in catarrhine primates) [5]. This suggests that while trichromacy may have reduced the importance of olfaction in general in primates, increased social complexity played a specific role in the shift away from the pheromonal regulation of social and sexual interactions. Primates have undergone considerable encephalization over the course of evolution, particularly in the cortex, and social group size and complexity shows a strong correlation with neocortical size across primate species. There has also been a simultaneous decline in the size of the hypothalamus and limbic brain that mediates hormonal control of behaviour and receives significant olfactory and vomeronasal input in non-primates [48], and this shift in relative size of the limbic brain and cortex is indicative of major changes in the regulation of behaviour from hormonal to cortical control [50]. The increasing social complexity of primate species are thought to have driven primate brain evolution [22] which has resulted in the emancipation of behaviour from hormonal control. Sexual behaviour is used not just for reproduction during periods of female receptivity in many catarrhine primates, but also for the reinforcement of male-

female social relationships at any time, while maternal care extends well beyond weaning and is independent of the hormones of the perinatal period. The evolution of a reduced role for hormones in regulating social and sexual behaviours in primates has also involved considerable reduction in the importance of pheromones as triggers for these hormonal changes and behavioural responses and increasing modulation of behaviour by social factors. The greatly reduced role for pheromones in primates, as well as changes in ecological niche and diet, has removed the functional evolutionary constraints on the dual olfactory systems and led to the disappearance of the vomeronasal system and the decrease in complexity of the main olfactory system in catarrhine primates.

Human pheromones

The possible regulation of human behaviour by pheromones is a topic that arouses interest and controversy in equal measure in the scientific community as well as the wider public. Several studies have demonstrated physiological and psychological responses to semiochemicals in humans, however it is clear that these are very different to the effects that pheromones exert on behaviour in small-brained mammals. The odours of females in the ovulatory phase of the menstrual cycle have been shown to shift the menstrual cycle of other women [79], while levels of luteinising hormone and ratings of mood in women appear to be affected by male secretions [72]. It has also been reported that women exhibit preferences for MHC-derived odours that differ from their own [41], however there is as yet no evidence that this plays a role in sexual and social behaviour in humans. While these studies show behavioural effects of human semiochemicals, they also arouse considerable controversy given the limited chemosensory array that humans have compared to small-brained mammals. While the VNO appears during development in foetal humans, its main function is the guidance of migrating GnRH neurons in the early brain [11], and it does not appear to persist in the adult human. Furthermore, the critical genes that code for vomeronasal function have undergone widespread inactivation in the human genome. All V2R [90] and almost all V1R genes [73] in the human genome appear to be non-functional, and while 4 potentially functional V1Rs have been identified, the *Trpc2* ion channel on which V1R-mediated signal

transduction is dependent is also a pseudogene [58]. While human semiochemicals are thus likely to be sensed via the MOE, this structure also shows a reduction in receptor repertoire complexity and more than 60% of human ORs are pseudogenes [31]. The comparatively impoverished olfactory senses of humans are not suggestive of a species in which pheromones play a significant role, and given the role that complex social groupings appear to have had in reducing the importance of olfaction in primates, the incredibly sophisticated and nuanced social relationships of humans are unlikely to be reliant to any significant degree on pheromones as triggers for simple behavioural responses.

Conclusion

Pheromonal communication in tetrapods has evolved to regulate diverse behaviours in very different species, employing diverse signals to communicate both very complex and very simple messages. While some behavioural responses in some species are robust and consistent, others are shaped by the identities of the signaller and receiver and the relationships between them. These signals are detected primarily by the VNO in mammals, however the MOE also plays a significant role in the detection of certain volatile pheromones. These two chemosensory systems appear to have ancient evolutionary origins as evidenced by their presence in aquatic amphibians. While there appears to be strong separation of MOE and VNO function in small-brained rodents, the variety of function and receptor expression that is seen in other mammalian orders and in other tetrapods suggest that the VNO did not evolve as a purely pheromonal organ in ancestral tetrapods and that it should not be viewed as such. The role of pheromones in behaviour in large-brained primates has declined enormously, to the extent that discussions of pheromonal effects on human behaviour arouse considerable controversy. What is clear is that a functioning VNO is absent in old world monkeys and primates and that the key genes of the vomeronasal system have undergone extensive inactivation. This process appears to have been driven by the evolution of trichromacy, allowing for large shifts in ecological niche and diet, and huge subsequent changes in social organisation, brain size and the resulting emancipation of behaviour from hormonal and pheromonal control.

References

[1] Aujard F, Schilling A, Perret M. Gonadotropin-releasing hormone (GnRH) immunoreactive neurons in male mouse lemurs following removal of the vomeronasal organ. Brain Res, 2005;1043: 247-250.

[2] Barrett J, Abbott DH, George LM. Extension of reproductive suppression by pheromonal cues in subordinate female marmoset monkeys, *Callithrix jacchus*. J Reprod Fertil, 1990;90: 411-8.

[3] Barrett J, Abbott DH, George LM. Sensory cues and the suppression of reproduction in subordinate female marmoset monkeys, *Callithrix jacchus*. J Reprod Fertil, 1993;97: 301-10.

[4] Barton RA, Purvis A, Harvey PH. Evolutionary radiation of visual and olfactory brain systems in primates, bats and insectivores. Philos Trans R Soc Lond B Biol Sci, 1995;348: 381-392.

[5] Barton RA. Olfactory evolution and behavioral ecology in primates. Am J Primatol, 2006;68: 545-58.

[6] Baxi KN, Dorries KM, Eisthen HL. Is the vomeronasal system really specialized for detecting pheromones? Trends Neurosci, 2006;29: 1-7.

[7] Bertmar G. Evolution of vomeronasal organs in vertebrates. Evolution, 1981;35: 359-366.

[8] Beynon RJ, Hurst JL. Multiple roles of major urinary proteins in the house mouse, *Mus domesticus*. Biochem Soc Trans, 2003;31: 142-146.

[9] Brechbuhl J, Klaey M, Broillet MC. Grueneberg ganglion cells mediate alarm pheromone detection in mice. Science, 2008;321: 1092-5.

[10] Brennan PA, Schellinck HM, Keverne EB. Patterns of expression of the immediate-early gene egr-1 in the accessory olfactory bulb of female mice exposed to pheromonal constituents of male urine. Neuroscience, 1999;90: 1463-70.

[11] Brennan PA, Kendrick KM. Mammalian social odours: attraction and individual recognition. Philos Trans R Soc Lond B Biol Sci, 2006;361: 2061-78.

[12] Bruce HM. An exteroceptive block to pregnancy in the mouse. Nature, 1959;184: 105.

[13] Buck L, Axel R. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. Cell, 1991;65: 175-87.

[14] Buck LB. The molecular architecture of odor and pheromone sensing in mammals. Cell, 2000;100: 611-618.

[15] Curley JP, Keverne EB. Genes, brains and mammalian social bonds. Trends Ecol Evol, 2005;20: 561-7.

[16] Date-Ito A, Ohara H, Ichikawa M, Mori Y, Hagino-Yamagishi K. *Xenopus* V1R vomeronasal receptor family is expressed in the main olfactory system. Chem Senses, 2008;33: 339-346.

[17] Del Punta K, Leinders-Zufall T, Rodriguez I, Jukam D, Wysocki CJ, Ogawa S, Zufall F, Mombaerts P. Deficient pheromone responses in mice lacking a cluster of vomeronasal receptor genes. Nature, 2002;419: 70-4.

[18] Dorries KM, Adkins-Regan E, Halpern BP. Olfactory sensitivity to the pheromone, androstenone, is sexually dimorphic in the pig. Physiol Behav, 1995;57: 255-9.

[19] Dorries KM, Adkins-Regan E, Halpern BP. Sensitivity and behavioral responses to the pheromone androstenone are not mediated by the vomeronasal organ in domestic pigs. Brain Behav Evol, 1997;49: 53-62.

[20] Doving KB, Trotier D. Structure and function of the vomeronasal organ. J Exp Biol, 1998;201: 2913-25.

[21] Dulac C, Axel R. A novel family of genes encoding putative pheromone receptors in mammals. Cell, 1995;83: 195-206.

[22] Dunbar RIM, Shultz S. Evolution in the social brain. Science, 2007;317: 1344-1347.

[23] Eisthen H, L. Evolution of vertebrate olfactory systems. Brain Behav Evol, 1997;50: 222-233.

[24] Eisthen H, L. The goldfish knows: Olfactory receptor cell morphology predicts receptor gene expression. J Comp Neurol, 2004;477: 341-346.

[25] Eisthen HL. Phylogeny of the vomeronasal system and of receptor cell types in the olfactory and vomeronasal epithelia of vertebrates. Microsc Res Tech, 1992;23: 1-21.

[26] Eisthen HL. Presence of the vomeronasal system in aquatic salamanders. Philos Trans R Soc Lond B Biol Sci, 2000;355: 1209-13.

[27] Elsaesser R, Montani G, Tirindelli R, Paysan J. Phosphatidyl-inositide signalling proteins in a novel class of sensory cells in the mammalian olfactory epithelium. Eur J Neurosci, 2005;21: 2692-700.

[28] Fernandez-Fewell GD, Meredith M. c-Fos expression in vomeronasal pathways of mated or pheromone- stimulated male golden hamsters: contributions from vomeronasal sensory input and expression related to mating performance. J Neurosci, 1994;14: 3643-3654.

[29] Firestein S. How the olfactory system makes sense of scents. Nature, 2001;413: 211-8.

[30] Gilad Y, Bustamante CD, Lancet D, Paabo S. Natural selection on the olfactory receptor gene family in humans and chimpanzees. Am J Hum Genet, 2003;73: 489-501.

[31] Gilad Y, Man O, Paabo S, Lancet D. Human specific loss of olfactory receptor genes. Proc Natl Acad Sci USA, 2003;100: 3324-7.

[32] Gilad Y, Przeworski M, Lancet D. Loss of olfactory receptor genes coincides with the acquisition of full trichromatic vision in primates. PLoS Biol, 2004;2: E5.

[33] Grus WE, Shi P, Zhang YP, Zhang J. Dramatic variation of the vomeronasal pheromone receptor gene repertoire among five orders of placental and marsupial mammals. Proc Natl Acad Sci USA, 2005;102: 5767-72.

[34] Grus WE, Shi P, Zhang J. Largest vertebrate vomeronasal type 1 receptor gene repertoire in the semiaquatic platypus. Mol Biol Evol, 2007;24: 2153-2157.

[35] Grus WE, Zhang J. Distinct evolutionary patterns between chemoreceptors of 2 vertebrate olfactory systems and the differential tuning hypothesis. Mol Biol Evol, 2008;25: 1593-601.

[36] Hagino-Yamagishi K, Moriya K, Kubo H, Wakabayashi Y, Isobe N, Saito S, Ichikawa M, Yazaki K. Expression of vomeronasal receptor genes in *Xenopus laevis*. J Comp Neurol, 2004;472: 246-256.

[37] Halpern M, Martínez-Marcos A. Structure and function of the vomeronasal system: an update. Prog Neurobiol, 2003;70: 245-318.

[38] Hansen A, Anderson KT, Finger TE. Differential distribution of olfactory receptor neurons in goldfish: structural and molecular correlates. J Comp Neurol, 2004;477: 347-59.

[39] Herrada G, Dulac C. A novel family of putative pheromone receptors in mammals with a topographically organized and sexually dimorphic distribution. Cell, 1997;90: 763-73.

[40] Hudson R, Distel H. Pheromonal release of suckling in rabbits does not depend on the vomeronasal organ. Physiol Behav, 1986;37: 123-8.

[41] Jacob S, McClintock MK, Zelano B, Ober C. Paternally inherited HLA alleles are associated with women's choice of male odor. Nat Genet, 2002;30: 175-9.

[42] Jemiolo B, Harvey S, Novotny M. Promotion of the Whitten effect in female mice by synthetic analogs of male urinary constituents. Proc Natl Acad Sci USA, 1986;83: 4576-9.

[43] Karlson P, Luscher M. 'Pheromones': a new term for a class of biologically active substances. Nature, 1959;183: 55-6.

[44] Keller M, Douhard Q, Baum MJ, Bakker J. Sexual experience does not compensate for the disruptive effects of zinc sulfate--lesioning of the main olfactory epithelium on sexual behavior in male mice. Chem Senses, 2006;31: 753-62.

[45] Keller M, Pierman S, Douhard Q, Baum MJ, Bakker J. The vomeronasal organ is required for the expression of lordosis behaviour, but not sex discrimination in female mice. Eur J Neurosci, 2006;23: 521-30.

[46] Kelliher KR, Spehr M, Li XH, Zufall F, Leinders-Zufall T. Pheromonal recognition memory induced by TRPC2-independent vomeronasal sensing. Eur J Neurosci, 2006;23: 3385-90.

[47] Keverne EB. Pheromonal influences on the endocrine regulation of reproduction. Trends Neurosci, 1983;6: 381.

[48] Keverne EB, Martel FL, Nevison CM. Primate brain evolution: genetic and functional considerations. Proc R Soc Lond B Biol Sci, 1996;263: 689-96.

[49] Keverne EB. The vomeronasal organ. Science, 1999;286: 716-20.

[50] Keverne EB. Brain evolution, chemosensory processing, and behavior. Nutr Rev, 2004;62: S218-23; discussion S224-41.

[51] Kikuyama S, Toyoda F, Ohmiya Y, Matsuda K, Tanaka S, Hayashi H. Sodefrin: a female-attracting peptide pheromone in newt cloacal glands. Science, 1995;267: 1643-5.

[52] Kikuyama S, Yamamoto K, Iwata T, Toyoda F. Peptide and protein pheromones in amphibians. Comp Biochem Physiol Part B Biochem Mol Biol, 2002;132: 69-74.

[53] Kikuyama S, Nakada T, Toyoda F, Iwata T, Yamamoto K, Conlon JM. Amphibian Pheromones and Endocrine Control of Their Secretion. Ann NY Acad Sci, 2005;1040: 123-130.

[54] Laberge F, Feldhoff RC, Feldhoff PW, Houck LD. Courtship pheromone-induced c-Fos-like immunolabeling in the female salamander brain. Neuroscience, 2008;151: 329-339.

[55] Leinders-Zufall T, Lane AP, Puche AC, Ma W, Novotny MV, Shipley MT, Zufall F. Ultrasensitive pheromone detection by mammalian vomeronasal neurons. Nature, 2000;405: 792-6.

[56] Leinders-Zufall T, Brennan P, Widmayer P, S PC, Maul-Pavicic A, Jager M, Li XH, Breer H, Zufall F, Boehm T. MHC class I peptides as chemosensory signals in the vomeronasal organ. Science, 2004;306: 1033-7.

[57] Liberles SD, Buck LB. A second class of chemosensory receptors in the olfactory epithelium. Nature, 2006;442: 645-50.

[58] Liman ER, Innan H. Relaxed selective pressure on an essential component of pheromone transduction in primate evolution. Proc Natl Acad Sci USA, 2003;100: 3328-32.

[59] Lin DY, Zhang S-Z, Block E, Katz LC. Encoding social signals in the mouse main olfactory bulb. Nature, 2005;434: 470-477.

[60] Lloyd-Thomas A, Keverne EB. Role of the brain and accessory olfactory system in the block to pregnancy in mice. Neuroscience, 1982;7: 907-13.

[61] Luo M, Fee MS, Katz LC. Encoding pheromonal signals in the accessory olfactory bulb of behaving mice. Science, 2003;299: 1196-1201.

[62] Mackay-Sim A, Duvall D, Graves BM. The West Indian manatee (*Trichechus manatus*) lacks a vomeronasal organ. Brain Behav Evol, 1985;27: 186-94.

[63] Mandiyan VS, Coats JK, Shah NM. Deficits in sexual and aggressive behaviors in *Cnga2* mutant mice. Nat Neurosci, 2005;8: 1660-2.

[64] Matsunami H, Buck LB. A multigene family encoding a diverse array of putative pheromone receptors in mammals. Cell, 1997;90: 775-84.

[65] Meisami E, Bhatnagar KP. Structure and diversity in mammalian accessory olfactory bulb. Microsc Res Tech, 1998;43: 476-499.

[66] Meredith M. Vomeronasal organ removal before sexual experience impairs male hamster mating behavior. Physiol Behav, 1986;36: 737-43.

[67] Meredith M. Chronic recording of vomeronasal pump activation in awake behaving hamsters. Physiol Behav, 1994;56: 345-54.

[68] Moreno N, Gonzalez A. Evolution of the amygdaloid complex in vertebrates, with special reference to the anamnio-amniotic transition. J Anat, 2007;211: 151-63.

[69] Niimura Y, Nei M. Evolutionary dynamics of olfactory and other chemosensory receptor genes in vertebrates. J Hum Genet, 2006;51: 505-517.

[70] Novotny M, Harvey S, Jemiolo B, Alberts J. Synthetic pheromones that promote inter-male aggression in mice. Proc Natl Acad Sci USA, 1985;82: 2059-61.

[71] Powers JB, Winans SS. Vomeronasal organ: critical role in mediating sexual behavior of the male hamster. Science, 1975;187: 961-3.

[72] Preti G, Wysocki CJ, Barnhart KT, Sondheimer SJ, Leyden JJ. Male axillary extracts contain pheromones that affect pulsatile secretion of luteinizing hormone and mood in women recipients. Biol Reprod, 2003;68: 2107-13.

[73] Rodriguez I, Mombaerts P. Novel human vomeronasal receptor-like genes reveal species-specific families. Curr Biol, 2002;12: R409-R411.

[74] Rollmann SM, Houck LD, Feldhoff RC. Proteinaceous pheromone affecting female receptivity in a terrestrial salamander. Science, 1999;285: 1907-1909.

[75] Ryba NJ, Tirindelli R. A new multigene family of putative pheromone receptors. Neuron, 1997;19: 371-9.

[76] Schaal B, Coureaud G, Langlois D, Ginies C, Semon E, Perrier G. Chemical and behavioural characterization of the rabbit mammary pheromone. Nature, 2003;424: 68-72.

[77] Shi P, Zhang J. Comparative genomic analysis identifies an evolutionary shift of vomeronasal receptor gene repertoires in the vertebrate transition from water to land. Genome Res, 2007;17: 166-174.

[78] Spehr M, Kelliher KR, Li XH, Boehm T, Leinders-Zufall T, Zufall F. Essential role of the main olfactory system in social recognition of major histocompatibility complex peptide ligands. J Neurosci, 2006;26: 1961-70.

[79] Stern K, McClintock MK. Regulation of ovulation by human pheromones. Nature, 1998;392: 177-9.

[80] Stowers L, Holy TE, Meister M, Dulac C, Koentges G. Loss of sex discrimination and male-male aggression in mice deficient for TRP2. Science, 2002;295: 1493-500.

[81] Surridge AK, Osorio D, Mundy NI. Evolution and selection of trichromatic vision in primates. Trends Ecol Evol, 2003;18: 198-205.

[82] Swanson LW, Petrovich GD. What is the amygdala? Trends Neurosci, 1998;21: 323-331.

[83] von Campenhausen H, Mori K. Convergence of segregated pheromonal pathways from the accessory olfactory bulb to the cortex in the mouse. Eur J Neurosci, 2000;12: 33-46.

[84] Wabnitz PA, Bowie JH, Tyler MJ, Wallace JC, Smith BP. Differences in the skin peptides of the male and female Australian tree frog *Litoria splendida*. Eur J Biochem, 2000;267: 269-275.

[85] Wible JR, Bhatnagar KP. Chiropteran vomeronasal complex and the interfamilial relationships of bats. J Mamm Evol, 1996;3: 285-314.

[86] Wirsig-Wiechmann CR, Houck LD, Feldhoff PW, Feldhoff RC. Pheromonal activation of vomeronasal neurons in plethodontid salamanders. Brain Res, 2002;952: 335-344.

[87] Xu F, Schaefer M, Kida I, Schafer J, Liu N, Rothman DL, Hyder F, Restrepo D, Shepherd GM. Simultaneous activation of mouse main and accessory olfactory bulbs by odors or pheromones. J Comp Neurol, 2005;489: 491-500.

[88] Yamamoto K, Kawai Y, Hayashi T, Ohe Y, Hayashi H, Toyoda F, Kawahara G, Iwata T, Kikuyama S. Silefrin, a sodefrin-like pheromone in the abdominal gland of the sword-tailed newt, *Cynops ensicauda*. FEBS Lett, 2000;472: 267-70.

[89] Young JM, Kambere M, Trask BJ, Lane RP. Divergent V1R repertoires in five species: Amplification in rodents, decimation in primates, and a surprisingly small repertoire in dogs. Genome Res, 2005;15: 231-40.

[90] Young JM, Trask BJ. V2R gene families degenerated in primates, dog and cow, but expanded in opossum. Trends Genet, 2007;23: 212-215.

[91] Zhang J, Webb DM. Evolutionary deterioration of the vomeronasal pheromone transduction pathway in catarrhine primates. Proc Natl Acad Sci USA, 2003;100: 8337-41.

table

Pheromone	Species	Signal Type	Detector	Effect of Signal
sodefrin	Japanese fire-bellied newt	water-soluble	VNO	Attraction of females
silefrin	sword-tail newt	water-soluble	unknown	Attraction of females
splendipherin	magnificent tree frog	water-soluble	unknown	Attraction of females
plethodon receptivity factor	plethodontid salamanders	water-soluble	VNO	Induction of female receptivity
(methylthio) methanethiol	Mouse	volatile	MOE	Attraction of females
2-sec-butyl-4,5-dihydrothiazole	Mouse	volatile (but usually bound to protein)	VNO	Puberty acceleration, induction of female receptivity, intermale aggression
2,3-dehydro-exo-brevicomin	Mouse	volatile (but usually bound to protein)	VNO	Puberty acceleration, induction of female receptivity, intermale aggression
mouse urinary proteins	Mouse	non-volatile (also binds volatiles ligands)	VNO	Identity signaling, intermale aggression, male urine marking
MHC class-1 proteins	Mouse	non-volatile (also binds volatiles ligands)	MOE & VNO	Identity signaling, pregnancy block
androstenone	Pig	volatile	MOE	Induction of female lordosis
2-methylbut-2-enal	Rabbit	volatile	MOE	Nipple-seeking behaviour in pups

Table 1. A summary of the properties of several well characterized pheromone signaling compounds in amphibians and mammals, showing the different types of signaling molecule employed by different species to communicate different messages in different environments. The behavioural responses induced by each pheromone, as well as the sensory organ which they stimulate are also shown.

Table(s)

Feature	Amphibians	Small-brained mammals	Large-brained primates	Ancestral tetrapod?
Pheromones	proteins & peptides	steroids, aldehydes, thiols, thiazoles, proteins	Unknown	proteins & peptides
Pheromone properties	Water-soluble	Predominantly involatile	Unknown	Water-soluble
VNO	Yes	Yes	Only during embryogenesis	Yes
MOE	Yes	Yes	Yes	Yes
VNO cells	microvillar	microvillar	None	microvillar
MOE cells	microvillar & ciliated	ciliated	ciliated	microvillar & ciliated
OR expression	MOE ciliated cells	MOE ciliated cells	MOE ciliated cells	MOE ciliated cells
V1R expression	MOE microvillar cells	VNO microvillar cells	No – pseudogenes	microvillar cells
V2R expression	VNO microvillar cells	VNO microvillar cells	No – pseudogenes	microvillar cells
Vomeronasal pathway	VNO-AOB-medial amygdala-hypothalamus	VNO-AOB-medial amygdala-hypothalamus	No	VNO-AOB-medial amygdala-hypothalamus

Table 2. A summary of the major features of pheromonal communication in amphibians, mammals and a putative common ancestor. The properties of pheromones themselves, as well as the organization of the olfactory systems that sense them are listed.