Evolution of *V1R* Repertoires in Subterranean Rodents

Advanced article

Article Contents

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Pheromones are chemicals released and perceived by members of the same species, triggering sexual and social behaviours or physiological changes in diverse animals. Although rodents are the first mammals in which vomeronasal receptors and their major roles in pheromonal olfaction were identified, little is known about the evolution of vomeronasal receptor gene repertoires in subterranean rodents. Recent genome-wide surveys in rodents have shown that the number of functional vomeronasal type 1 receptor (*V1R*) genes was markedly reduced in phylogenetically distinct subterranean rodents compared with their superterranean relatives, possibly due to confined pheromonal signals in underground burrows. Interestingly, population genetic analysis proved that many *V1R* genes may have undergone positive selection rather than relaxed selection in one species of subterranean rodent. Exploration of functional roles of the reduced number of *V1R* genes would help to uncover the importance of pheromonal olfaction in subterranean rodents.

Introduction

Olfaction, or the sense of smell, is one of the five basic senses in animals. It detects chemicals that can identify territories, food, predators and mates and thus plays a crucial role in animals' survival and adaptation. **See also: Olfaction**. There are two olfactory systems in most terrestrial vertebrates: the main olfactory system (MOS) and the vomeronasal system (VNS) (Dulac and Torello, 2003). While the MOS mainly detects environmental odorants, the VNS is believed to play a major role in detecting intraspecific pheromones, despite the fact that the two olfactory systems have some overlapping functions (Grus and Zhang, 2008). Intraspecific pheromones are chemicals that convey information important for reproduction, mate choice, species and gender identification and social status. **See also: Mammalian Pheromones**. It has been well known that the majority of terrestrial vertebrates carry two large families of vomeronasal pheromone receptors: vomeronasal type 1 receptors (V1Rs) and vomeronasal type 2 receptors (V2Rs), both of which belong to seven-transmembrane G protein-coupled receptor (GPCR) families (Nei *et al*., 2008). The two types of receptors have distinct expression locations and gene structures: V1Rs are coexpressed with Gαi2 protein in the apical layer of the vomeronasal epithelium, whereas V2Rs are coexpressed with Gαo protein in the basal layer; Genes encoding V1Rs have a single exon, while those encodingV2Rs typically have six exons (Silva and Antunes, 2017). Functional separation of the two types of receptors was also observed: V1Rs are mainly involved in detecting air-borne molecules that are scattered in air, whereas V2Rs are commonly responsible for binding to water-soluble peptides that are abundant in aquatic environments (Shi and Zhang, 2007).

The two families of pheromone receptors, V1Rs and V2Rs, were originally discovered and characterised in mouse or rat (Dulac and Axel, 1995; Herrada and Dulac, 1997; Matsunami and Buck, 1997; Ryba and Tirindelli, 1997) but were not extensively described in other rodent species until recently many rodent genomes became available (Jiao *et al*., 2019). Rodents commonly use pheromonal signals in nature and in laboratories and thus have become one of the most thoroughly studied groups of mammals in pheromonal communication (Liberles, 2014). Because some rodents such as mouse and rat have been extensively used as laboratory animals, it is of fundamental importance to understand the role of pheromonal signalling in rodents. Of the two receptor families, V1Rs are of particular interest in rodents because nearly all rodent species live on land, where terrestrial mammals release air-borne pheromones that can be detected by their V1Rs (Boschat *et al*., 2002). Indeed, knockout mice lacking a cluster of 16 intact *V1R* genes showed substantial deficits in the expression of male sexual behaviour and maternal aggression (Del Punta *et al*., 2002); Comparative genomic analysis identified an expanded *V1R* repertoire in rodents, which

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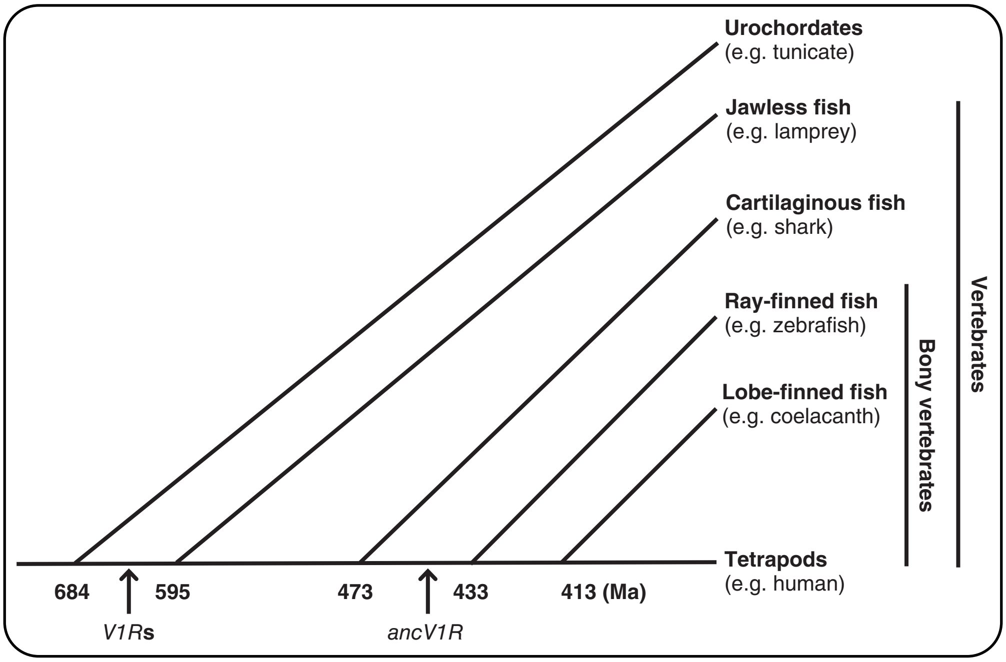


Figure 1 Origins of vertebrate *V1R* genes. Based on Hedges SB, Marin J, Suleski M et al. (2015). Tree of life reveals clock-like speciation and diversification., Molecular Biology and Evolution 32:835–845.

suggested that *V1R*s play a key role in pheromonal olfaction (Young *et al*., 2010). To date, *V1R* gene repertoires have been characterised in many surface-dwelling rodents (i.e. superterranean rodents) such as mouse, rat, kangaroo rat, squirrel and guinea pig (Wang *et al*., 2010; Young *et al*., 2010). In contrast, little is known about the evolution of vomeronasal receptor gene repertoires in subterranean rodents until recently (Jiao *et al*., 2019), although subterranean rodents are multiple rodent lineages that have independently evolved a subterranean lifestyle, making them excellent for studies of evolutionary convergence and divergence (Lacey *et al*., 2000).

Origin of V1R Repertoires in Vertebrates

Rodents are well known for their use of pheromonal olfaction, but they are not the only group of vertebrates relying on pheromonal signalling. Thus, the origin and evolution of pheromonal olfaction would help to understand its role in rodents. Pheromonal olfaction of vertebrates is mainly mediated by the VNS, which appears to be more difficult to ascertain by morphology than by genetic analysis, particularly in some species virtually lacking detectable morphological components of the VNS (Grus and Zhang, 2009). Using a comparative genomics approach, *V1R*s were identified from the sea lamprey (jawless fish) (Grus and Zhang, 2009), sharks (cartilaginous fish) (Grus and Zhang, 2009; Sharma *et al*., 2019), teleosts (ray-finned fish) (Shi and Zhang, 2007), coelacanth (lobe-finned fish) (Nikaido *et al*., 2013) and tetrapods (Young *et al*., 2010; Dong *et al*., 2012; Brykczynska *et al*., 2013) (**Figure 1**), with multiple lineage-specific expansions and contractions in mammals (Young *et al*., 2010). By contrast, no *V1R* was found in the genomes of the amphioxus (cephalochordates) and tunicate (urochordates) (Grus and Zhang, 2009), suggesting that the origin of *V1R*s occurred predating the divergence of vertebrates between 595 (jawless fish) and 684 (urochordates) million years ago (Ma) (Hedges *et al*., 2015) (**Figure 1**). However, the origin of *V2R*s arose between 473 (cartilaginous fish) and 595 (jawless fish) Ma (Grus and Zhang, 2009; Hedges *et al*., 2015), the timing of which is much later than the origin of *V1R*s. Thus, pheromonal olfaction mediated by *V1R*s is more ancient than that mediated by *V2R*s during the origin and evolution of vertebrates.

Contrary to olfactory receptors that have largely orthologous relationships among vertebrate species, vomeronasal pheromone receptors (*V1R*s and *V2R*s) are characterised by rapid gene turnover that resulted in virtually no one-to-one orthologs between species (Grus and Zhang, 2008). Interestingly, a recent study reported a previously uncharacterised *V1R* gene (termed *ancV1R*) that is shared among most bony vertebrates from ray- to lob-finned fish to tetrapods (Suzuki *et al*., 2018) (**Figure 1**). Phylogenetic and syntenic analyses showed that *ancV1R* appears to be an orthologous gene conserved across vertebrate lineages for more than 400 million years, with multiple independent losses coincided with the degeneration of the VNS in several tetrapod lineages such as higher primates, cetaceans and some reptiles (Suzuki *et al*., 2018). Such conservation of *ancV1R* further suggests the importance of pheromonal olfaction mediated by *V1R*s in vertebrate evolution.

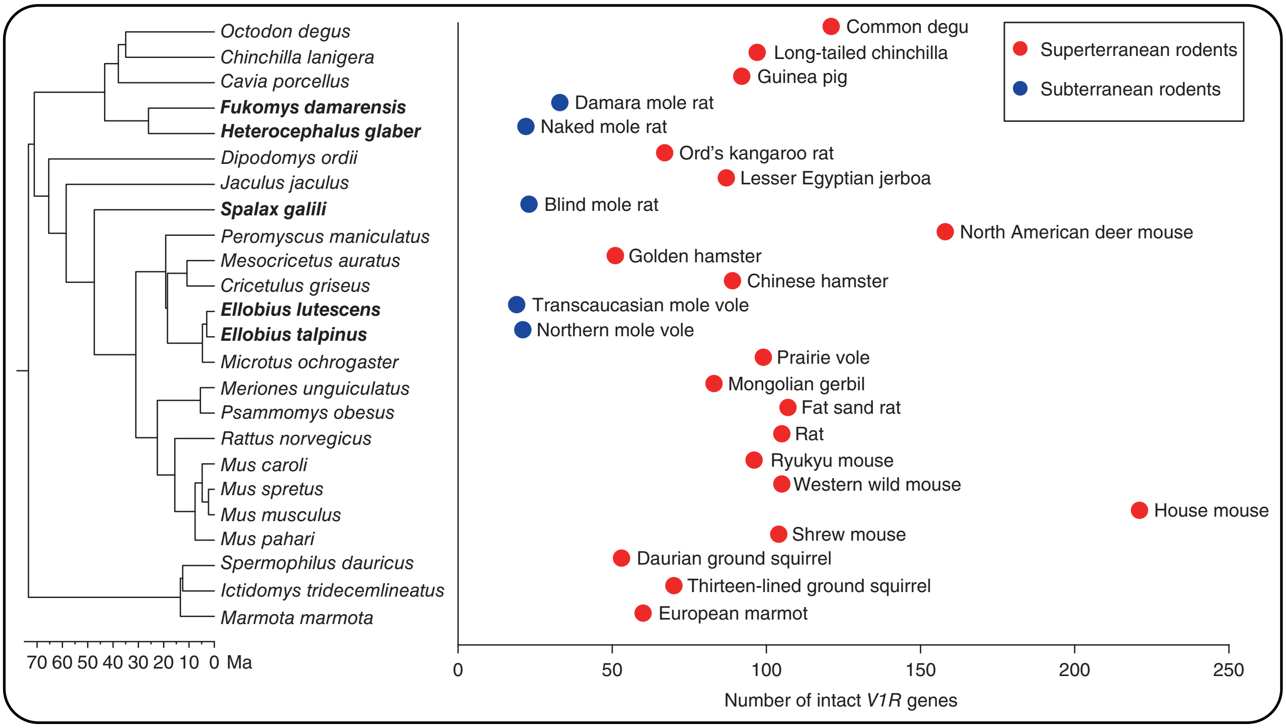
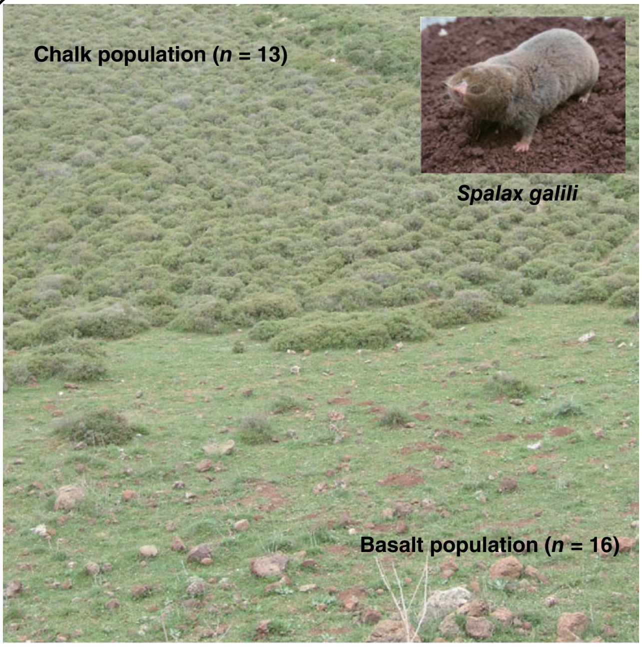


Figure 2 Repertoire sizes of intact *V1R*s in 24 rodents. These rodents are divided into two groups: superterranean rodents (red) and subterranean rodents (blue) based on their lifestyles. Jiao H, Hong W, Nevo E et al. (2019). Convergent reduction of V1R genes in subterranean rodents. BMC Evolutionary Biology. 19:176. Licensed under CC BY 4.0.

Evolutionary Reduction in V1R Repertoires in Subterranean Rodents

*V1R*s show extreme variability across diverse mammalian lineages with extensive gene gain and loss. For example, the platypus (283 intact genes) and mouse (239 intact genes) possess the largest *V1R* repertoires, followed by mouse lemur (214 intact genes) and rabbit (159 intact genes), whereas two bat species and the dolphin have the smallest repertoires with zero intact genes (Young *et al.*, 2010). The *V1R* repertoire size in each species roughly reflects the morphological complexity of their vomeronasal organs, but it seems that no single ecological factor can fully explain the gene expansion and contraction patterns (Grus *et al.*, 2005; Young *et al.*, 2010).

Rodents represent the most diversified mammalian order, accounting for approximately 40% of all mammal species and thus are expected to show a large variation in V1R repertoires. Surprisingly, five species of rodent examined previously only showed moderate variability, with the largest in mouse (239 intact genes) and the smallest in guinea pig (89 intact genes) (Young *et al.*, 2010). This observation probably cannot reflect the real diversity of *V1R* repertoires in rodents due to the sampling of very small number of species. Thanks to the rapid development of sequencing technology, a recent study examined currently available genomes of 24 rodent species spanning all major lineages of rodents (Jiao *et al.*, 2019). Specifically, mouse remains to contain the largest *V1R* repertoire (221 intact genes), while the Transcaucasian mole vole (*Ellobius lutescens*) has the smallest (19 intact genes) in rodents, with the intact *V1R* repertoire size varying by at least 11-fold among rodents (Jiao *et al.*, 2019) (**Figure 2**). Of these rodents, 19 are superterranean species, and the remaining five are subterranean rodents such as Damara mole rat (*Fukomys damarensis*), naked mole rat (*Heterocephalus glaber*), blind mole rat (*Spalax galili*), Transcaucasian mole vole (*E. lutescens*) and northern mole vole (*Ellobius talpinus*), representing at least three independent and divergent lineages (**Figure 2**). Intriguingly, subterranean rodents (mean 24, median 22) clearly have a much smaller size of intact V1R repertoire compared to their superterranean relatives (mean 98, median 96) (Jiao *et al.*, 2019) (**Figure 2**). The reduction in intact *V1R* repertoire size appears to have occurred repeatedly in all five examined species of subterranean rodents with divergent phylogenetic positions, which suggested that convergent reduction in pheromonal olfaction mediated by *V1R*s has independently occurred in diverse lineages of subterranean rodents (**Figure 2**). Indeed, phylogenetic analysis showed that different subterranean lineages tend to have different *V1R* gene groups, confirming that *V1R* reduction is independent in divergent lineages of subterranean rodents (Jiao *et al.*, 2019). However, it appears that the reduction in *V1R* repertoire size is not random in subterranean rodents, because there is a significant positive correlation between lifestyle and *V1R* repertoire size in rodents when two types of lifestyle (subterranean and superterranean)



(a)

Gene loci	F_{ST}	FDR
V1R1	0.153	0.003
V1R2	–0.019	0.621
V1R3	0.039	0.029
V1R4	0.026	0.330
V1R5	–0.011	0.621
V1R6	0.054	0.014
V1R7	0.063	0.161
V1R8	0.012	0.205
V1R9	0.127	0.000
V1R10	0.118	0.003
V1R11	0.07	0.060
V1R12	0.162	0.000
V1R13	0.136	0.000
V1R14	–0.001	0.388
V1R15	0.262	0.000
V1R16	0.301	0.000
V1R17	0.166	0.000
V1R18	0.185	0.026
V1R19	0.042	0.026
V1R20	0.21	0.019
V1R21	0.081	0.019
V1R22	*	*

(b)

Figure 3 Study subject and population differentiation of *V1R*s. (a) The blind mole rat *Spalax galili* inhabiting the chalk and basalt areas; 13 and 16 animals were sampled from the chalk and basalt soils, respectively. BMC Evolutionary Biology (Jiao *et al.* 2019). (b) Genetic differentiation of 22 *V1R*s between the two soil populations. Jiao H, Hong W, Nevo E *et al.* (2019). Convergent reduction of V1R genes in subterranean rodents. BMC, Evolutionary Biology. 19:176. Licensed under CC BY 4.0. F_{ST}, fixation index; FDR, *P*-value adjusted by false discovery rate (FDR). Significant *P*-values were underlined. *, F_{ST} was not able to be estimated because there is no polymorphism site in *V1R22*. Gene loci with *P*-values less than 0.05 are significantly differentiated between the two populations.

were considered, suggesting that the subterranean lifestyle plays a major role leading to the reduction in *V1R* repertoire in subterranean rodents (Jiao *et al*., 2019).

Why do subterranean rodents reduce their V1R repertoires in size? The reduction in *V1R* repertoires suggests that pheromonal olfaction mediated by *V1R*s must be reduced. Life underground allows the use of some sensory modalities but constrains the use of others. For instance, animals living in dark tunnels cannot rely on vision to perceive their surroundings. In fact, chemical signals must be confined to the tunnels within a burrow system (Lacey *et al*., 2000); thus, pheromonal olfaction mediated by *V1R*s may not be very useful for subterranean rodents. Indeed, these animals were proposed to rely heavily on seismic signals, which should be more effective because they can extend beyond the confines of the tunnels, although not all subterranean rodents can emit seismic signals (Lacey *et al*., 2000).

Positive Selection on V1R Repertoires in Populations of a Subterranean Rodent

The reduction in *V1R* repertoires has led to a hypothesis that V1Rs may have been functionally relaxed from functional constraints in subterranean rodents (Jiao *et al*., 2019). It would be straightforward to test this hypothesis using natural populations of subterranean rodents. Taking advantage of samples left in an earlier study (Li *et al*., 2015), Jiao *et al*. (2019) attempted to sequence all intact *V1R* genes in two natural populations of a subterranean rodent, the blind mole rat (*S. galili*) (**Figure 3**). *V1R* genes were compared with randomly selected noncoding regions that are assumed to be under neutral evolution. This work found that 14 of the 22 *V1R*s are significantly differentiated between populations (**Figure 3**), whereas only one of the 18 noncoding regions significantly differed (Jiao *et al*., 2019). The percentage of significantly differentiated *V1R*s (14/22 = 63.6%) is much greater than that of noncoding regions (1/18 = 5.6%), which strongly suggested that positive selection may have shaped the evolution of *V1R*s in subterranean rodents (Jiao *et al*., 2019). This finding supported the hypothesis that pheromonal olfaction mediated by the VNS may be involved in reproductive isolation of *S. galili*. Indeed, several behavioural examinations are consistent with the genetic analyses, although monitoring animal behaviours underground is particularly challenging (Jiao *et al*., 2019). For example, *S. galili* chose their mates with similar genetically determined odours (Tzur *et al*., 2009); reproductive isolation associated with olfaction was clearly involved in *Spalax* speciation across Israel (Heth and Nevo, 1981); Pheromones in the urine of male mole rats were demonstrated to attract females

(Menzies *et al*., 1992). However, the genetic analysis of *V1R*s in the blind mole rat is in stark contrast to that in the mouse, where the evolution of *V1R*s in natural populations is largely governed by purifying selection and random drift (Park *et al*., 2011). The disparity in *V1R* evolution between the mouse and the blind mole rat calls for more research that is needed to assess the general pattern of selection regime in subterranean rodents.

Conclusion

The number of functional *V1R* genes was markedly reduced in phylogenetically distinct subterranean rodents compared with their superterranean relatives, suggesting that pheromone detection mediated by *V1R* genes is commonly reduced in subterranean rodents, possibly due to confined pheromonal signals in underground burrows. Interestingly, population genetic analysis proved that many *V1R* genes may have undergone positive selection rather than relaxed selection in one species of subterranean rodent. Therefore, we call for in-depth studies of the functional roles of the reduced number of *V1R* genes, which would yield a better understanding of the significance of pheromonal olfaction among subterranean taxa.

Glossary

Evolutionary convergence The independent evolution of similar features in distantly related species, which creates analogous features that were not present in the last common ancestor of these species. A classic example is the recurrent evolution of flight, as flying insects, birds, pterosaurs and bats have independently evolved the flight ability.

Evolutionary divergence The evolution of different features in closely related species. A classic example is the case of Darwin's Finches, as Darwin discovered several different species of finch that shared a common ancestor in the Galápagos Islands.

Positive selection A mode of natural selection in which an extreme phenotype or genotype is favoured over other phenotypes or genotypes.

Purifying selection (also known as negative selection) A mode of natural selection in which deleterious phenotypes or genotypes are selectively removed.

Relaxed selection A mode of selection in which selection pressure is reduced or eliminated despite that it was formerly important for the maintenance of a particular trait.

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