Remarkable diversity of mammalian pheromone receptor repertoires

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At the center of animal species survival is the ability of individuals to identify members of their own species and to discriminate between the genders of these members to procreate. This basic biological task is, in mammals, mostly mediated by the exchange of pheromonal information and performed by the olfactory system. More precisely, an elongated tubular structure, the vomeronasal organ, located in the nasal cavity and filled with sensory neurons, is mainly responsible for the detection of intraspecies chemosensory signals (1). Receptors responsible for the recognition of pheromones (2, 3) and expressed by vomeronasal sensory neurons are G protein-coupled receptors, termed V1r receptors (4). The genes encoding these latter are particularly numerous in rodents (>100) and form a very diverse superfamily (5–8). In a recent issue of PNAS, Grus *et al*. (9) reported the identification of the V1r pheromone receptor repertoires pertaining to multiple orders of marsupial and placental mammals, and they observed striking variations in terms of repertoire size and content between these orders. Such extent of variability in the mammalian class for a given gene family is unusual, to say the least, and forces us to reflect on the nature of the evolutionary forces that led to such unequally distributed chemosensory tools among mammals.

Mammals constitute a large group, which includes monotremes, marsupials, and eutherians, these latter comprising species apparently as unrelated as bats and whales. Our current view of the molecular chemosensory tools (i.e., the chemosensory receptor gene repertoires) available to the different mammalian species is extremely limited, due to the very few genomes sequenced. The approach taken by Grus *et al*. (9) consisted of digging into the recently sequenced genomes of multiple mammalian species pertaining to both marsupials and eutherians, including dog, cow, and opossum. They extracted novel V1r pheromone receptor genes, some of which formed species-specific families, extending previous observations in other species (8, 10, 11). Their results, and another almost coincident publication that reports very similar findings (11), indicate a remarkable V1r repertoire size variation of >20-fold in placental mammals, corresponding to a functional V1r repertoire size of only 8 genes in dogs and 160–190 genes in mice. Importantly, the authors did not limit their search to only potentially functional V1r pheromone receptor genes, but also they identified V1r pseudogenes in the different species, information that helped them to evaluate the V1r repertoires present in ancestor species. Grus *et al*. also included in their survey a noneutherian species, the opossum, a marsupial. They found that this species not only amplified and diversified its own V1r repertoire, but also it possesses eight species-specific families. The extraordinary diversification of V1r repertoires in rodents, which apparently postdates the separation of carnivores and rodents (9, 11), is therefore not restricted only to this latter order (the dog data may have led to this conclusion) but also occurred in other distant orders, although possibly not on the same scale.

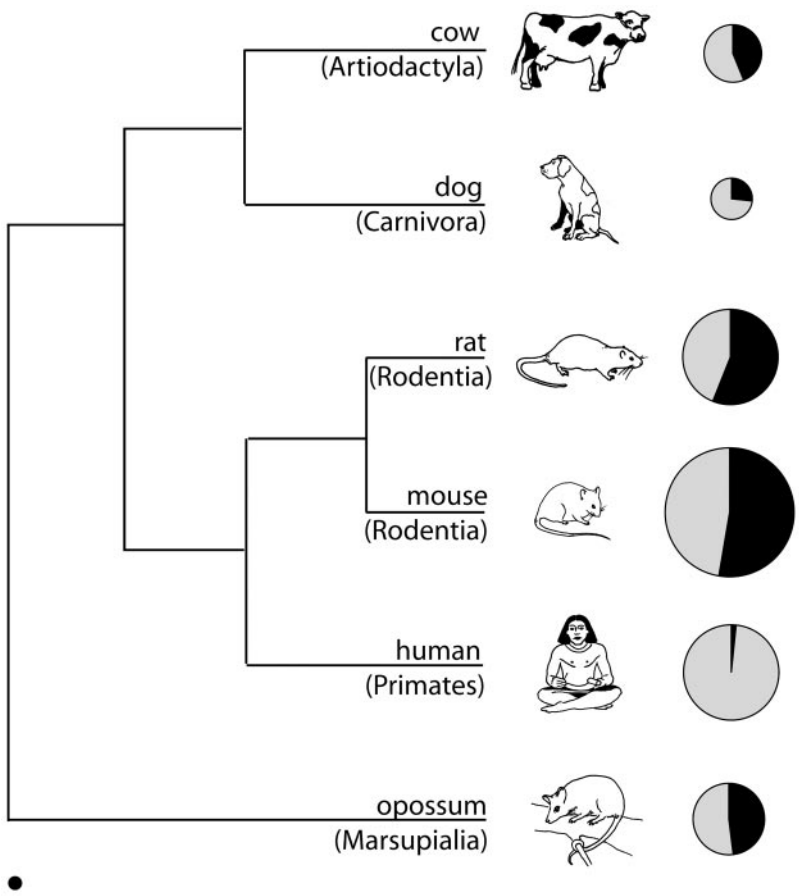

Fig. 1. Variability of the V1r repertoire size among six mammalian species. Pies represent the complete V1r repertoires corresponding to each species, and their surface the number of V1r genes. The surface of the disk on the lower left corner of the figure corresponds to one gene. Black and gray indicate potentially functional V1r genes and pseudogenes, respectively.

How does a gene repertoire become divergent between two species? To put it simply, two types of events can result in gene family size variations: gene loss (involving, for example, deletion of gene

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clusters or pseudogenization events) and gene duplication (a very common event, although the fixation of duplicated sequences is very uncertain), which, respectively, lead to the shrinkage or the expansion of a given repertoire. Grus *et al*. (9) and others (7, 8, 10–12) showed evidence that both types of events have been involved in the shaping of mammalian V1r repertoires, with a dynamic interplay between gene birth and death, leading to a massive presence of V1r pseudogenes in most species analyzed (Fig. 1). Complex genomic rearrangements leading to major gene family expansions or collapses are often observed between distant species, but the common ancestor of some species analyzed by Grus *et al*. lived relatively recently (≈10–30 and 75–95 million years ago for mouse-rat and mouse-dog, respectively). So not only are mammalian V1r repertoires remarkably variable in terms of size and content, but also these differences appeared particularly rapidly.

Pheromone receptor genes are not the only genes to exhibit extreme diversity between species, although, at the level reported by Grus *et al*. (9), it may be unique. The recent sequencing of rodent genomes pointed indeed to a few gene families subject to rapid expansions, most of them being involved in immunity, detoxification, or reproductive or, yes, chemosensory function (13, 14). Positive Darwinian selective pressure (i.e., a selective pressure enhancing diversity) often accompanied the expansion of these gene families, including V1rs (7, 8, 15–17). One may easily understand a selective pressure on gene number and variation in the immune system: those adaptable to multiple and ever-changing pathogens are naturally favored. But what about pheromone receptor genes? Pheromone perception is, for mammals, a main part of reproduction, because it allows them to identify a mate and respond to it in an adequate way. With this in mind, it may seem that a tool that consists in variable pheromone receptor repertoires may allow different species to perceive and respond differently to the same chemosensory stimulus and therefore help to maintain, or maybe even establish, an effective species barrier that may prevent useless energy expenditure.

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Now, concerning the variability of the repertoire size, Grus *et al*. (9) in their recent study suggest that different species, depending on their use of the vomeronasal system (supposedly reflected by the complexity of their vomeronasal neurosensory epithelium), maintained a corresponding bouquet of receptors. The authors support their view by a good correlation between the development of the vomeronasal system and the expansion of the V1r repertoire of the species analyzed. Future identification of mammalian V1r repertoires should put this hypothesis to the test, keeping in mind that the functional implications of chemosensory receptor repertoire size on the ability to detect and discriminate chemicals are unknown. On the other hand (but this possibility is not exclusive), some species could have developed specific receptors related to specialized tasks dependent, for example, on their ecological niche, even nonrelated to pheromone perception. The observation that some vomeronasal receptor transcripts are found in spermatozoa (18) could thus not simply be the result of loosely controlled and nonfunctional transcription. Alternatively, one could also consider the extensive V1r amplification in rodents as the reflection of an uncontrolled phenomenon that led to a large and redundant repertoire. Finally, another exciting and, again, nonexclusive view could be the unequal involvement, depending on the species, of non-V1r receptors to mediate pheromonal interactions, leading to a corresponding amplification, degradation, or nonexpansion of the V1r repertoire.

Should we need more evidence that complete genome sequencing of numerous, even extremely related species represents invaluable information, the reports by Grus *et al*. (9) in a recent issue of PNAS and Young *et al*. (11) remind us that relatively simple comparisons of gene repertoires may lead to quite unexpected and stimulating findings and, in our particular case, to a significant insight into the unequal use made by mammalian species of an initially common set of molecular devices to communicate.

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