Olfactory Receptor Genes: Evolution

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There are ~1000 olfactory receptor genes in mammals, forming the largest multigene family. Identification of the entire repertoires of olfactory receptor genes from various species and extensive phylogenetic analyses revealed dynamic change of this gene family in evolution.

Advanced article

Article Contents

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Introduction

Among the five senses, olfaction, the sense of smell, may seem to be the least important for us. However, for most animals, olfaction is essential to survival. Olfactory signals are used to find foods, identify mates and offspring, recognize territories and avoid danger. It is often said that humans can discriminate more than 10 000 different odours. Odour molecules in the environment are detected by olfactory receptors (ORs). Mammals have as many as ~1000 different OR genes, which comprise about 4% of the entire proteome and form the largest multigene family. The relationships between odour molecules and ORs are not one-to-one, but multiple-to-multiple. That is, one OR recognizes multiple odorants and one odorant is recognized by multiple ORs, but different odorants are recognized by different combination of ORs. This 'combinatorial coding' scheme could allow the distinction of an almost unlimited number of odorants as possible combinations of ~1000 ORs. OR genes were first discovered by Linda Buck and Richard Axel from rats (Buck and Axel, 1991). This discovery opened the door for molecular studies of the olfactory system, and they were awarded the 2004 Nobel Prize in Physiology or Medicine for this achievement.

OR genes are mainly expressed in sensory neurons of the olfactory epithelium in the nasal cavity. It is generally thought that each olfactory neuron expresses only one functional OR gene among ~1000 genes. The detailed molecular mechanism of this 'one neuron–one receptor rule' is still unclear, but it has been proposed that one functional OR gene is stochastically chosen in each OR neuron and its expression prevents the activation of other OR genes through negative feedback regulation (Serizawa *et al*., 2003). OR genes are also expressed in testis, and these ORs may mediate sperm chemotaxis (Spehr *et al*., 2003). Moreover, several studies suggested that OR genes are expressed in various nonolfactory tissues such as the tongue, brain, placenta, etc. However, there might be no functional significance for such 'ectopic expression' of OR genes (Feldmesser *et al*., 2006).

OR genes belong to a superfamily of G protein-coupled receptors (GPCRs) that have seven transmembrane α-helical regions. Binding of an odour molecule to an OR activates a G protein and subsequent signalling cascades. OR genes do not have any introns in their coding regions. However, the number of exons in the 5′ untranslated region can be variable among OR genes, and these noncoding exons are often alternatively spliced to generate various different messenger ribonucleic acid (mRNA) isoforms. However, the biological significance of the presence of multiple transcription isoforms is unclear. **See also**: G Protein-coupled Receptors; Olfaction; Olfactory Receptors

OR Genes in the Human Genome

Functional genes and pseudogenes

Availability of the whole genome sequences allowed us to examine the entire repertoires of OR genes in various species. By conducting homology searches, ~800 OR genes were identified from the nearly complete human genome (**Table 1**; Niimura and Nei, 2003). Interestingly, more than half of them are pseudogenes. All mammalian OR genes have sequence similarities to one another and share common motifs, and thus OR genes can be unambiguously distinguished from other GPCR genes. Here functional genes and pseudogenes were distinguished from each other on the basis of their sequences. A sequence that has an intact open reading frame from a putative initiation codon to a stop codon was regarded to be a functional gene. However, if a sequence contains nonsense or frameshift mutations, or long deletions, it was regarded as a pseudogene. Recently, it was reported that most (~80%) of human OR genes that are annotated to be functional are actually expressed in the olfactory epithelium, while a significantly smaller but a considerable fraction of OR pseudogenes (~67%) are also expressed in the olfactory epithelium (Zhang *et al*., 2007). Therefore, it is quite difficult to know whether a given gene is truly functional or not.

Genomic clusters and phylogenetic relationships

Figure 1a shows the genomic locations of OR genes in the human genome. This figure indicates that OR genes exist as many genomic clusters, and they are scattered all over the

Table 1 OR genes in various mammals

Order	Species	Functional genes	Truncated genes	Pseudogenes	Total	Fraction of pseudogenes (%)
Primates	Human	387	0	415	802	51.7
	Chimpanzee	380	19	414	813	50.9
	Macaque	309	17	280	606	46.2
Rodentia	Mouse	1035	28	328	1391	23.6
	Rat	1207	52	508	1767	28.7
Carnivora	Dog	811	11	278	1100	25.3
Cetartiodactyla	Cow	970	182	977	2129	45.9
Marsupialia	Opossum	1188	10	294	1492	19.7
Monotremata	Platypus	265	83	370	718	51.5

Truncated genes were assumed to be functional for the calculation of the fraction of pseudogenes. Reproduced from Niimura and Nei (2007).

chromosomes except chromosomes 20 and Y. More than 40% of all OR genes are located on chromosome 11. **Figure 1b** represents a chromosomal map of a genomic cluster located on chromosome 3, and **Figure 1c** is a phylogenetic tree for all OR genes contained in this cluster. This tree indicates that evolutionarily closely related genes are located also closely on a chromosome. Therefore, these genomic clusters appear to have been generated by repeated tandem gene duplications.

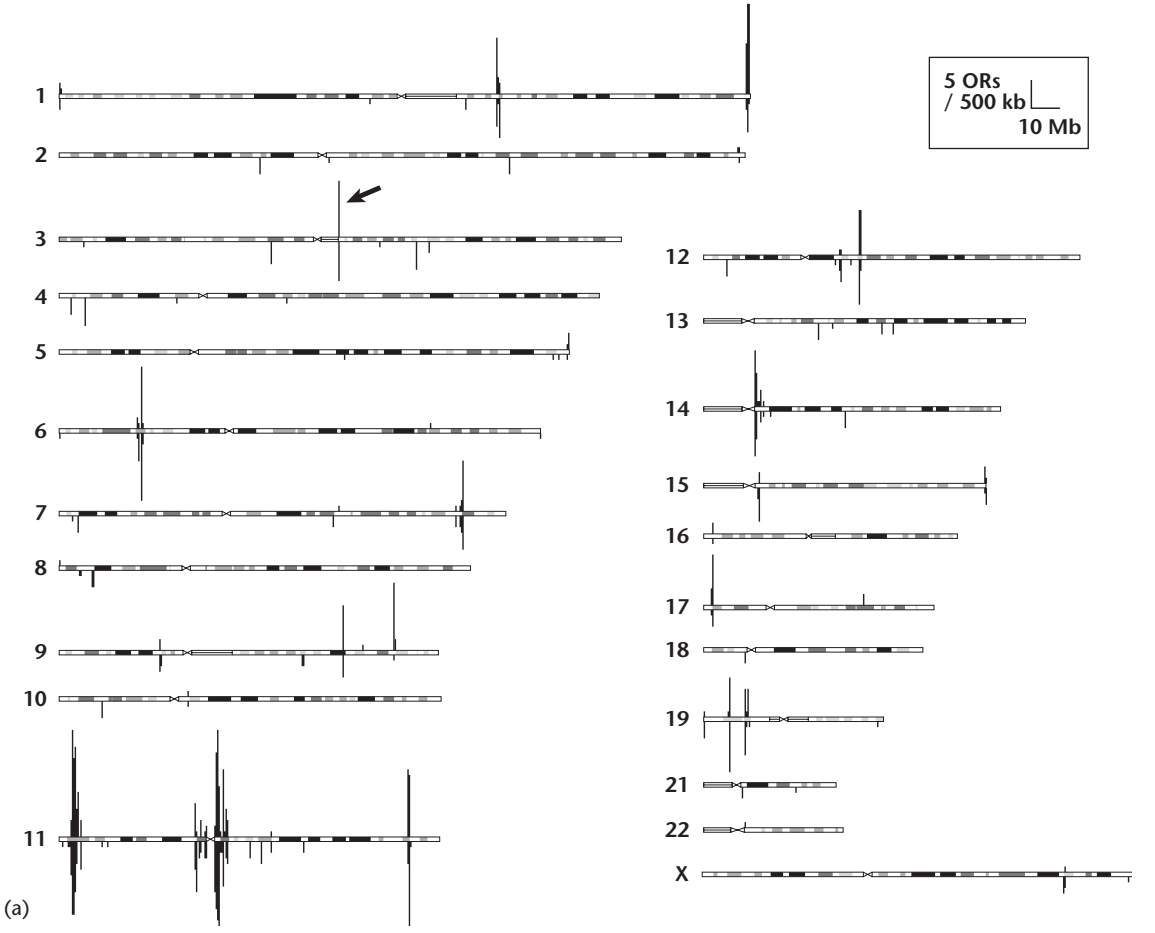
However, the relationships between genomic locations and phylogenetic kinships are usually more complicated. **Figure 2a** shows a phylogenetic tree constructed by using all functional OR genes in humans. Mammalian OR genes can be classified into Class I and Class II genes by sequence similarity. Further classification of OR genes into subgroups is not straightforward, because their phylogenetic relationships are not completely resolved. For this reason, here we consider only phylogenetic clades that are statistically well supported ($>90\%$ bootstrap values) and contain $\geqslant 5$ member genes. Each of the phylogenetic clades is denoted by using a different alphabet (**Figure 2a**). **Figure 2b** shows the organization of several OR genomic clusters. For example, the cluster named cluster 1.5 contains genes belonging to clade B, C and L. The genes belonging to each clade form a tandem array. However, these three clades are distantly related in the phylogenetic tree (**Figure 2a**). Moreover, genes belonging to clade A are located in several different clusters, which are present in different chromosomes (**Figure 2b**). This kind of relationship between genomic clusters and phylogenetic clades is often observed. These observations can be explained by assuming that several chromosomal rearrangements have occurred at the regions of OR gene clusters. As a result, the OR genes contained in different genomic clusters appear to have been shuffled.

OR Genes in Primates and Other Mammals

OR genes in primates

Table 1 shows the numbers of OR genes in several mammalian species for which the draft genome sequences are available. This table indicates that the numbers of OR genes are highly variable among different mammals. Primates have much smaller numbers (300–400) of functional OR genes than other mammals ($\sim$1000) except for platypuses (see later). For example, rats have more than three times larger repertoire of functional OR genes than humans. Moreover, the fractions of pseudogenes in primates ($\sim$50%) are much higher than those in rodents, dogs and opossums ($<30\%$). However, for the comparison of the fractions of pseudogenes among different species, some caution is necessary, because low-quality genome sequences tend to give an underestimate of the number of functional genes. Especially, if genome sequences contain many short contigs, a large number of functional genes are expected to be truncated at the ends of contigs. For this reason, the numbers of truncated genes, which could become functional when the genome sequence is completed, are shown separately in **Table 1**. The fractions of pseudogenes were estimated under the assumption that all truncated genes are functional. Large numbers of truncated genes were identified from the platypus and cow genomes,

Figure 1 Distribution of OR genes on human chromosomes. (a) Vertical bars above and below the chromosomes represent locations of functional OR genes and OR pseudogenes, respectively. The height of each bar indicates the number of OR genes present in a nonoverlapping 500-kb window. (b) Distribution of OR genes in a 0.6-Mb region indicated by an arrow in (a) on chromosome 3. 'ψ' represents a pseudogene. (c) Neighbour-joining phylogenetic tree for the genes contained in a genomic cluster shown in (b). Each gene is indicated by a number in (b). A scale bar shows the number of amino acid substitutions per site. Modified from Niimura and Nei (2003). Copyright (2003) National Academy of Sciences, U.S.A.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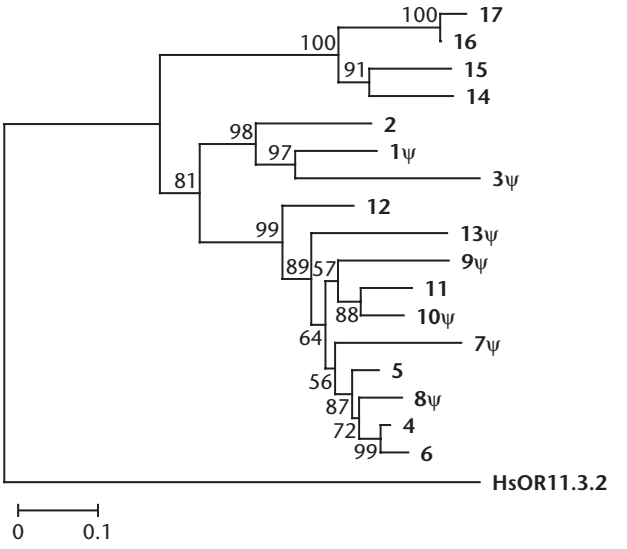
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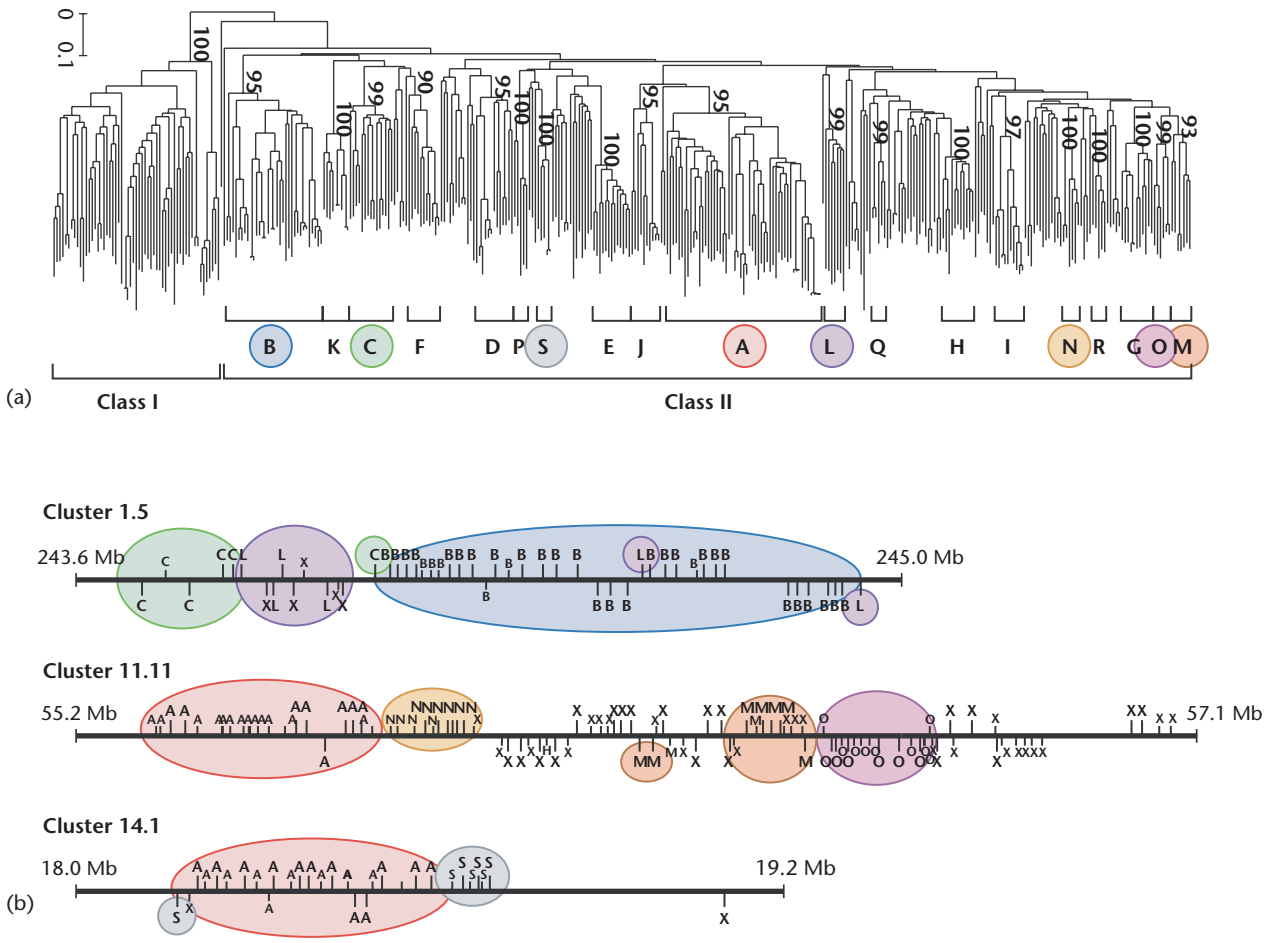

Figure 2 Relationships between genomic clusters and phylogenetic clades. (a) Neighbour-joining phylogenetic tree for all functional OR genes in humans. These genes are classified into Class I and Class II genes, and Class II genes are further classified into clades A–S. Several genes remain unclassified. (b) Arrangement of OR genes in three genomic clusters, which are located on chromosomes 1, 11 and 14. Vertical bars above and below the horizontal line indicate OR genes in opposite transcriptional orientations. Alphabets indicate the phylogenetic clades defined in (a). 'X' represents an unclassified gene. A pseudogene is shown by a shorter bar. Modified from Niimura and Nei (2003). Copyright (2003) National Academy of Sciences, U.S.A.

reflecting a relatively low quality of their genome sequences.

The number of OR genes and the fraction of OR pseudogenes in chimpanzees are very similar to those in humans, though macaques have a considerably smaller number of OR genes than humans and chimpanzees. It is unclear whether chimpanzees have a better olfactory ability than humans or not. Comparative anatomical studies suggested that the olfactory bulb, the first brain region for processing olfactory information, is much larger in chimpanzees than in humans. However, to our knowledge, there is no quantitative analysis for the comparison of olfactory ability between the two species.

Why primates have smaller numbers of functional OR genes and higher fractions of pseudogenes than other mammals such as rodents? This is intuitively likely, since it is generally thought that higher primates are vision-oriented animals and their ability of olfaction has been retrogressed. Actually, the olfactory bulb and the olfactory epithelium in primates are known to be proportionately smaller than those in most other mammals. Gilad *et al.*, (2004, 2007) estimated the fraction of OR pseudogenes for 19 primate species by sequencing 100 OR genes that were chosen randomly. They found that catarrhines, which includes humans, apes and Old World monkeys (OWMs), have a significantly higher fraction of pseudogenes ($>30\%$) than most of New World monkeys (NWMs) and prosimians ($\sim 20\%$; see **Figure 3**). Among NWMs, there is one exceptional species, the howler monkey, which showed a fraction of pseudogenes ($\sim 30\%$) as high as OWMs. Interestingly, catarrhines and the howler monkey share another phenotype: full trichromatic vision. In catarrhines, trichromatic vision is mediated by three opsin molecules that are sensitive to different wavelength. The gene for the blue-sensitive opsin is on an autosome, whereas red and green opsin genes are on chromosome X. In contrast, most NWMs have one autosomal opsin gene and one X-linked opsin gene, but the X-linked gene is

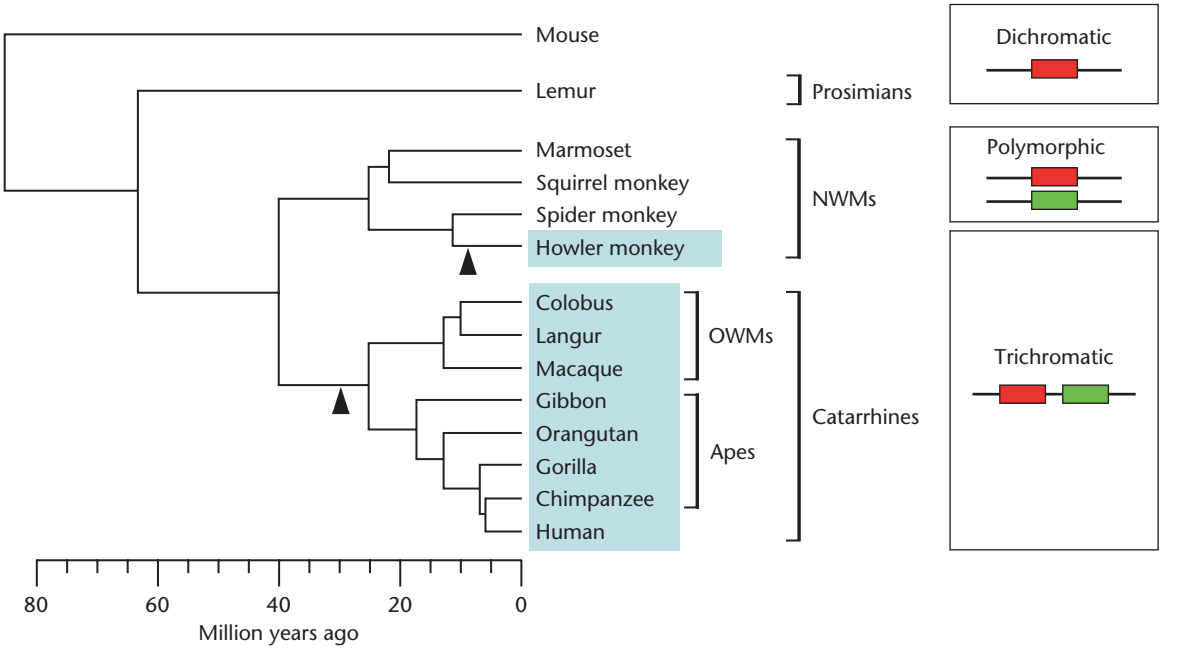

Figure 3 Phylogenetic tree of primates. Catarrhines and the howler monkey (shown in blue) have full trichromatic vision and show high fractions of OR pseudogenes. A triangle indicates a branch in which the duplication of red/green opsin genes and deterioration of OR genes occurred. A blue opsin gene is not shown.

polymorphic. Therefore, heterozygous females can possess trichromatic vision, but all males and homozygous females are dichromatic. The sole exception among NWMs is the howler monkey, which has duplicated opsin genes. Thus, full trichromatic vision arose twice independently in the evolution of primates, once in the common ancestor of catarrhines and once in the lineage of the howler monkey (**Figure 3**). From these observations, it was proposed that the loss of OR genes coincides with the acquisition of full trichromatic vision (Gilad *et al.*, 2004). This suggests a tight link between the olfactory system and the visual system. **See also**: Primates: Phylogenetics; Visual Pigment Genes: Evolution

OR genes in other mammals

Table 1 indicates that the platypus as well as primates have a smaller number of functional OR genes and a larger fraction of pseudogenes than other mammals. The platypus belongs to monotremes, which are egg-laying mammals and were diverged, the earliest in mammalian evolution. The observations of the small number of functional OR genes and the large fraction of pseudogenes in the platypus may be explained in the following way. First, platypuses are semi-aquatic. Mammalian OR genes have been adapted to detecting airborne odorants, and thus they might be useless in the water (see later). The second reason would be the presence of a bill sense. A platypus's bill is not just a bill as in a duck, but it is a sophisticated combination of electroreception and mechanoreception. It is known that platypuses can find prey with their eyes, ears and nostrils closed. This situation is reminiscent of toothed whales (dolphins), which have completely lost the olfactory system and instead have developed the echolocation system to adapt to the full aquatic life. In fact, there are data suggesting that the fraction of OR pseudogenes in toothed whales is extremely high. **See also**: Monotremata

However, the relationship between the number of OR genes and the environmental factor is not always clear. Dogs are supposed to have a very keen sense of smell, but they do not have necessarily a large repertoire of OR genes (see **Table 1**). The reason is unclear, but it is possible that the number of OR genes in each species is determined not only by the environmental needs but also by random elements that are evolutionarily neutral.

To investigate the evolutionary change of the number of OR genes in mammals, recently we estimated the numbers of genes in the ancestral species and those of gene gains and losses for each branch in the phylogenetic tree of mammals (Niimura and Nei, 2007). The results (**Figure 4a**) showed that (i) gene expansion occurred in the placental lineage each time after it diverged from monotremes and from marsupials and (ii) hundreds of gains and losses of OR genes have occurred in an order-specific manner. The latter finding is consistent with the observation that every species examined has a large number of pseudogenes (**Table 1**). Moreover, this finding means that, although the current numbers of functional OR genes in several mammalian species are similar (~1000), their OR gene repertoires have been highly diversified. Therefore, the spectrum of detectable odorants appears to be quite different among different mammalian species.

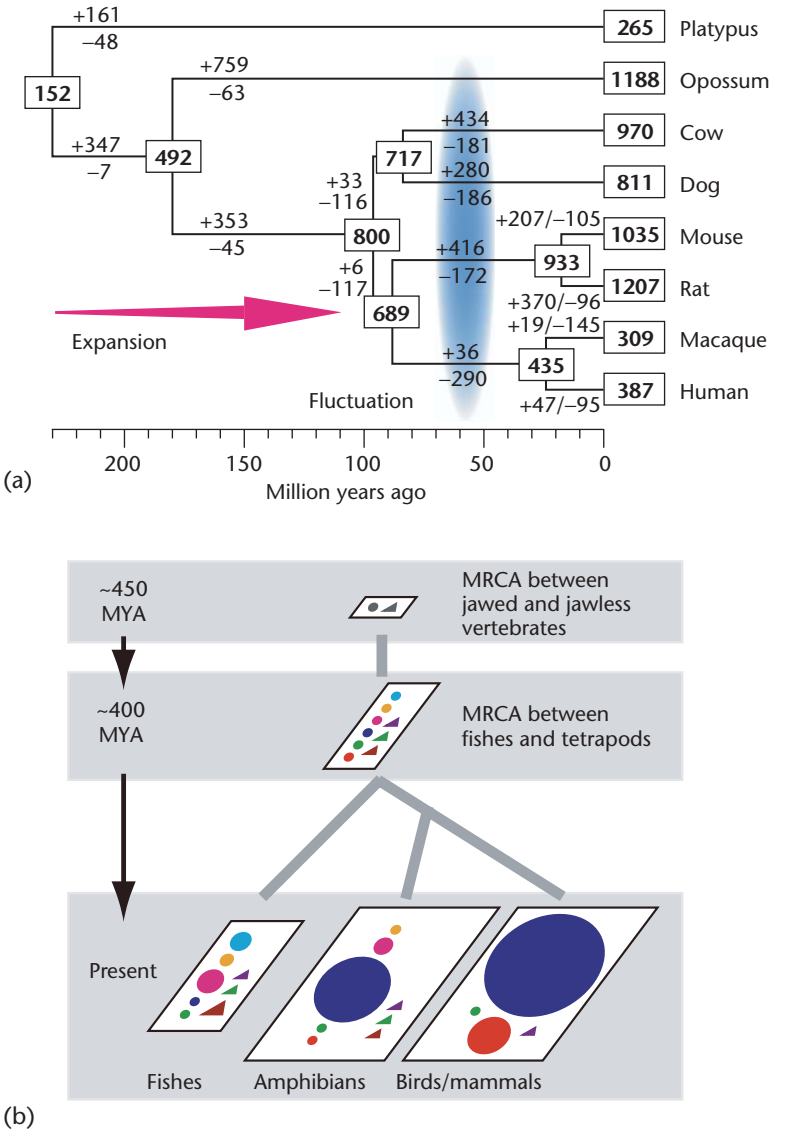

Figure 4 Evolutionary dynamics of OR genes in mammals (a) and in vertebrates (b). (a) The numbers in rectangles are those of functional OR genes in the extant species or in the ancestral species. The numbers with plus and minus signs indicate those of gene gains and losses, respectively, along each branch. Modified from Niimura and Nei (2007). (b) The MRCA between jawed and jawless vertebrates and that between fishes and tetrapods were estimated to have had at least two and nine functional OR genes. The size of a coloured circle or triangle at the bottom represents the number of genes originated from each ancestral gene in the MRCA between fishes and tetrapods. MYA, million years ago. Modified from Niimura and Nei (2005). Copyright (2005) National Academy of Sciences, U.S.A.

This kind of dynamic gains and losses of member genes in a multigene family is called birth-and-death evolution. In this model, new genes are created by gene duplication, and some duplicated genes are maintained in the genome for a long time, whereas others are deleted or become nonfunctional through deleterious mutations. This model was first proposed to explain the evolutionary pattern of major histocompatibility complex (MHC) genes that are involved in the immune system (Nei *et al.*, 1997). It is now known that most multigene families are subject to birth-and-death evolution to some extent, but OR genes provide one of the most extreme examples of this mode of evolution.

OR Genes in Vertebrates and Invertebrates

OR genes in vertebrates

OR genes are present in all vertebrate species. Several OR genes were identified from the lamprey, which is the most primitive vertebrate species. As shown in **Table 2**, fishes have much smaller numbers of OR genes (~100) than mammals. Fishes recognize water-soluble odorants such as amino acids, bile acids, sex steroids and prostaglandins.

Table 2 Sizes of chemosensory receptor genes in vertebrates

	OR	TAAR	V1R	V2R	T1R	T2R
Human	387 (415)[a]	6 (3)[b]	5 (115)[c]	0 (20)[d]	3[e]	25 (11)[e]
Chimpanzee	380 (433)[f]	3 (6)[b]	0 (116)[g]	0 (17)[d]	?	?
Mouse	1035 (356)[a]	15 (1)[b]	187 (121)[c]	121 (158)[d]	3[e]	35 (6)[e]
Rat	1207 (560)[a]	17 (2)[b]	106 (66)[c]	79 (142)[d]	3[e]	37 (5)[e]
Dog	811 (289)[a]	2 (2)[h]	8 (33)[c]	0 (9)[d]	3[e]	15 (5)[e]
Cow	970 (1159)[a]	17 (9)[h]	40 (45)[c]	0 (16)[d]	?	12 (15)[e]
Opossum	1118 (304)[a]	22 (0)[h]	98 (30)[c]	86 (79)[d]	3[e]	26 (5)[e]
Platypus	265 (453)[a]	4 (1)[h]	270 (579)[h]	15 (112)[h]	?	?
Chicken	82 (476)[i]	3 (2)[j]	0 (0)[c]	0 (0)[c]	2[e]	3 (0)[e]
Frog	410 (478)[i]	6 (1)[j]	21 (2)[c]	249 (448)[c]	0[e]	49 (12)[e]
Pufferfish	44 (54)[i]	13 (6)[j]	1 (0)[c]	18 (29)[c]	5[e]	4 (0)[e]
Zebrafish	102 (35)[i]	109 (10)[j]	2 (0)[c]	44 (8)[c]	1[e]	4 (0)[e]

The numbers of functional genes and pseudogenes (in parentheses) are shown for each species. The number of truncated genes in **Table 1** is included in that of pseudogenes, because truncated genes and pseudogenes were not distinguished in the studies other than Niimura and Nei (2007). The numbers of pseudogenes for T1R genes have not been determined.

[a]Niimura and Nei (2007)
[b]Lindemann *et al*. (2005)
[c]Shi and Zhang (2007)
[d]Young and Trask (2007)
[e]Shi and Zhang (2006)
[f]Go and Niimura (unpublished)
[g]Young *et al*. (2005)
[h]Grus *et al*. (2007)
[i]Niimura and Nei (2005)
[j]Hashiguchi and Nishida (2007)

Extensive phylogenetic analyses of vertebrate OR genes revealed that the OR genes in fishes are much more diverse than those in mammals regardless of the smaller repertoires of OR genes in fishes than in mammals (Niimura and Nei, 2005). Vertebrate OR genes were classified into nine groups, each of which was originated from at least one ancestral gene in the most recent common ancestor (MRCA) between fishes and tetrapods. Interestingly, almost all mammalian and avian OR genes belong to only two out of the nine groups. (These two groups correspond to Class I and Class II in **Figure 2a**.) The evolutionary scenario of vertebrate OR genes was inferred in the following way (**Figure 4b**). Fishes currently retain eight out of nine group genes that were present in the MRCA between fishes and tetrapods, presumably because their environment has not substantially changed from that of the MRCA. In the tetrapod lineage, two groups of genes appear to have acquired the ability to detect airborne odorants at the time of terrestrial adaptation. Because the importance of olfactory information is apparently larger in terrestrial organisms than in marine organisms, the OR genes have enormously expanded in the former by repeated gene duplications. In mammals and birds, the genes specific to water-soluble odorants have been eliminated from the genome, because they are useless for terrestrial life. However, amphibians retain the genes for both water-soluble and airborne odorants, reflecting that they have adapted to both aquatic and terrestrial environments. Therefore, OR multigene families in vertebrates are characterized by dynamic birth-and-death evolution.

OR genes in invertebrates

There are also genes named 'olfactory receptors' or 'ORs' in insects. Insect OR genes are functionally similar to vertebrate OR genes and are also GPCRs having seven transmembrane regions. However, OR genes in insects and those in vertebrates do not show any significant sequence similarity to each other and are thought to be of distinct evolutionary origin. Moreover, unlike vertebrate OR genes, coding regions of insect OR genes are interrupted by many introns. There are $\sim$60 OR genes in the *Drosophila* genome, and these genes are expressed in the antenna and the maxillary palps. Insect OR genes are also subject to birth-and-death evolution, but it was reported that *Drosophila* OR genes are evolutionarily more stable than mammalian OR genes (Nozawa and Nei, 2007).

Other Chemosensory Receptor Genes

Detection of chemosensory stimuli in vertebrates is mediated by five more multigene families in addition to ORs (**Table 2**). They are trace amino-associated receptors (TAARs), two families of vomeronasal receptors (V1Rs and V2Rs) and two families of taste receptors (T1Rs and T2Rs). All of these genes are GPCRs. TAARs have some sequence similarities to ORs, though TAARs are more closely related to receptors for neurotransmitters such as dopamine or serotonin. V1Rs and V2Rs are homologous to T2Rs and T1Rs, respectively. However, ORs/TAARs,

V1Rs/T2Rs and V2Rs/T1Rs do not show any sequence similarities to one another and are thought to be evolutionarily unrelated. **See also**: Chemosensory Systems

TAARs were originally identified as receptors for 'trace amines' in the brain. Trace amines designate a collection of amines that are present in the central nervous system at very low concentrations. Trace amines were suspected to be involved in psychiatric disorder, and TAARs have been postulated to play a role in depression and schizophrenia. Recently, it was revealed that TAARs are a second class of chemosensory receptors that are expressed in the olfactory epithelium in mice (Liberles and Buck, 2006). Like ORs, TAARs appear not to be coexpressed with other TAARs in a neuron. Some mouse TAARs recognize volatile amines that are present in urine. The number of TAAR genes in a mammalian genome is much smaller than that of OR genes, but zebrafish have a slightly larger repertoire of TAARs than ORs (**Table 2**).

Most mammals possess a second olfactory system called the vomeronasal system. Vomeronasal receptors are expressed in the vomeronasal organ (VNO), which is located at the base of the nasal cavity and is distinct from the main olfactory epithelium expressing OR genes. Previously the VNO was regarded to be specialized for pheromone detection. However, now it is thought that the olfactory system and the vomeronasal system have some overlapping functions. The VNO is vestigial in human adults. Although the VNO develops in the human fetus, it degenerates before birth. Bioinformatic analysis revealed that there are a few intact V1R genes and >100 V1R pseudogenes in the human genome (**Table 2**). However, these intact V1R genes are apparently nonfunctional, because *TPR2*, an ion channel gene that is essential for VNO function in mice, is pseudogenized in the catarrhine lineage. Therefore, the deterioration of the vomeronasal system in primates might have occurred in parallel to the inactivation of OR genes mentioned earlier. No intact V2R genes were found from primates, dogs and cows. Fishes do not have the vomeronasal organ, but both V1R and V2R genes are expressed in the olfactory epithelium in fishes. **See also**: Mammalian Pheromones; Pheromones in Vertebrates

Taste receptors are expressed in a taste bud of the tongue. There are five modalities of tastes, sweet, sour, bitter, salty and umami. Umami is a taste of L-glutamate. Umami means 'deliciousness' in Japanese, and it was discovered by a Japanese chemist Kikunae Ikeda in 1909. Of these five modalities, salty and sour tastes are detected by ion channels. However, sweet and umami tastes are perceived by T1Rs, and a bitter taste is recognized by T2Rs. In general, mammals have only three T1R genes (*T1R1, T1R2* and *T1R3*). Interestingly, T1R1 and T1R3 form a heterodimer to function as an umami receptor, while a T1R2/T1R3 heterodimer broadly responds to sweet tastants. The sizes of T2R gene repertoires are generally much larger than those of T1Rs (**Table 2**), reflecting the importance of bitter taste perception, which enables animals to avoid toxic and harmful substances. **See also**: Chemosensory Systems; Taste: Cellular Basis

As shown in **Table 2**, the numbers of V1R, V2R and T2R genes are quite variable among different species. Phylogenetic analyses revealed that these gene families also show lineage-specific expansions or contractions, and thus they are subject to birth-and-death evolution. It appears that there are some tendencies that are common to these gene families. For instance, the numbers of genes in primates are generally small, suggesting that the capability of all three chemosensory systems for olfactory, pheromone and taste perception have been declined in the primate lineage. However, gene repertoires in rodents and opossums are generally large. Moreover, chickens tend to have small repertoires. Therefore, the evolutionary changes of the numbers of chemosensory receptor genes might be correlated to one another. However, platypuses and fishes have a large repertoire of V1Rs and TAARs, respectively, although their gene repertories are generally small. Further analyses should be required to investigate the correlation among evolutionary changes of chemosensory receptor gene families in more details.

Conclusions

Higher primates generally have smaller number of OR genes and higher fractions of pseudogenes compared with other mammals. This observation reflects that primates are vision-oriented animals. Apparently they lost many OR genes in substitution with the acquisition of trichromatic vision. The sizes of OR gene repertories are highly variable among different mammalian species, and hundreds of gains and losses of OR genes were estimated to have occurred during mammalian evolution. It appears that OR genes largely expanded after the time of terrestrial adaptation in the tetrapod lineage by repeated gene duplications. OR and other chemosensory receptor multigene families are characterized by dynamic birth-and-death evolution. The numbers of chemosensory receptor genes have been determined primarily in response to environmental needs, although they may also be affected by random factors.

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