Degeneration of Olfactory Receptor Gene Repertories in Primates: No Direct Link to Full Trichromatic Vision

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Abstract

Odor molecules in the environment are detected by olfactory receptors (ORs), being encoded by a large multigene family in mammalian genomes. It is generally thought that primates are vision oriented and dependent weakly on olfaction. Previous studies suggested that Old World monkeys (OWMs) and hominoids lost many functional OR genes after the divergence from New World monkeys (NWMs) due to the acquisition of well-developed trichromatic vision. To examine this hypothesis, here we analyzed OR gene repertoires of five primate species including NWMs, OWMs, and hominoids for which high-coverage genome sequences are available, together with two prosimians and tree shrews with low-coverage genomes. The results showed no significant differences in the number of functional OR genes between NWMs (marmosets) and OWMs/hominoids. Two independent analyses, identification of orthologous genes among the five primates and estimation of the numbers of ancestral genes by the reconciled tree method, did not support a sudden loss of OR genes at the branch of the OWMs/hominoids ancestor but suggested a gradual loss in every lineage. Moreover, we found that humans retain larger numbers of ancestral OR genes that were in the common ancestor of NWMs/OWMs/hominoids than orangutans and macaques and that the OR gene repertoire in humans is more similar to that of marmosets than those of orangutans and macaques. These results suggest that the degeneration of OR genes in primates cannot simply be explained by the acquisition of trichromatic vision, and our sense of smell may not be inferior to other primate species.

Key words: olfactory receptor, primate evolution, color vision, smell sense, multigene family, gene loss.

Introduction

Olfaction, the sense of smell, plays an important role for finding foods, mating, avoiding danger, and social behaviors in animals. Odor molecules in the environment are detected by olfactory receptors (ORs), which are mainly expressed in sensory neurons of olfactory epithelia in nasal cavities and initiate a neuronal response that triggers the perception of smell (Buck and Axel 1991; Mombaerts 2004; Niimura and Nei 2006; Nei et al. 2008). Vertebrate ORs are members of G protein–coupled receptors containing seven hydrophobic transmembrane domains and are responsible for G protein–mediated transduction of olfactory signals. OR genes are typically single coding-exon genes and 310 codons long on average. It is well known that OR genes comprise the largest multigene family in mammalian genomes. However, bioinformatic analyses using the draft genome sequences of various organisms revealed that the numbers of OR genes are quite variable among different species (Niimura and Nei 2005b, 2007; Niimura 2009). For example, humans, chimpanzees, and macaques have $<$400 functional OR genes, whereas mice, rats, and opossums have $>$1,000 (Glusman et al. 2001; Zozulya et al. 2001; Zhang and Firestein 2002; Niimura and Nei 2003, 2007; Go and Niimura 2008). Moreover, the fractions of OR pseudogenes vary extensively among species, and the former group of animals show a much larger fraction of pseudogenes (46–52%) than the latter (20–29%) (Niimura and Nei 2007; Go and Niimura 2008).

Primates are generally regarded as animals depending on powerful visual sense. Therefore, it is often said that olfactory abilities in primates are relatively unimportant and have retrogressed. Traditionally, primates were classified into two suborders, the Prosimii, which includes lemurs, lorises, and tarsiers, and the Anthropoidea, which includes New World monkeys (NWMs), Old World monkeys (OWMs), and hominoids (human and apes). However, there is another classification of primates into two suborders, strepsirrhines (lemurs and lorises), meaning "curved nose," and the haplorhines (tarsiers, NWMs, OWMs, and hominoids), meaning "simple nose," based on the features of a nose shape. Recent molecular studies revealed the monophyly of haplorhines (Schmitz et al. 2001; Matsui et al. 2009), supporting the latter classification. Strepsirrhines are characterized by the presence of the rhinarium, the moist and naked surface around the tip of the nose, which is also present in many mammals such as cats or dogs (Martin 1990). The rhinarium is very sensitive and useful to olfaction, being able to detect the direction of odors. It is also known that developments of vision on the basis of the structure of the brain and eyes are more marked in haplorhines than in strepsirrhines (Barton 2006). Moreover,

most strepsirrhine species are nocturnal, whereas most haplorhines are diurnal, and color vision systems are well developed only in haplorhines (see below). These observations suggest a decreased reliance on olfaction in haplorhines compared with strepsirrhines.

The evolution of color vision systems in primates has been thoroughly investigated. Among haplorhines, tarsiers are dichromats as most of other mammals and strepsirrhines (some strepsirrhine species are monochromats). On the other hand, all species of catarrhines (OWMs and hominoids) are trichromats. Trichromatic vision is mediated by three opsins that are activated by different wavelengths: the short-wavelength opsin (S-opsin) on an autosome and the medium-wavelength opsin (M-opsin) and long-wavelength opsin (L-opsin) on X chromosome. NWMs are unique in that their color vision is highly polymorphic. In NWMs, typically red–green color vision is controlled by multiple alleles at a single M/L-opsin gene locus on X chromosome (Jacobs 1996). Therefore, heterozygous females are trichromatic, whereas homozygous females and all males are dichromatic. The allelic compositions of M/L-opsin genes widely vary among NWMs, ranging from diallelic in spider monkeys and wooly monkeys to pentallelic in dusky titi (Jacobs 2007). Among NWMs, howler monkeys are exceptional because they have M- and L-opsin genes residing at two separate loci (Jacobs et al. 1996). Therefore, in primate evolution, a full trichromatic vision system has evolved twice independently in the common ancestor of catarrhines and in howler monkeys.

To examine a possible link between color vision and olfaction in primate evolution, Gilad et al. (2004) examined OR gene sequences in 19 primate species including strepsirrhines, NWMs, OWMs, and hominoids. In the study, they sequenced 100 OR genes that were randomly chosen from each species and found that the fractions of OR pseudogenes in catarrhines and the howler monkey are significantly higher than the other NWMs and strepsirrhines (for correction, see Gilad et al. 2007). From this observation, they hypothesized that primates have lost the function of OR genes due to the acquisition of full trichromatic vision. Here we call this hypothesis "color vision priority hypothesis" (Nei et al. 2008). Gilad et al. (2004) also argued that the fraction of OR pseudogenes is significantly higher in humans than in other hominoids and OWMs, which has been reported in other studies by the same group as well (Gilad et al. 2003, 2005). However, our recent study using the high-quality whole-genome sequences of chimpanzees and the comparison with human OR genes suggested that there are no significant differences in the number of OR genes and the fraction of pseudogenes between humans and chimpanzees (Go and Niimura 2008). This might imply that the random sequencing strategy may not be very accurate due to some unexpected biases. Therefore, the timing of olfactory degeneration in the primate evolution is still unclear, and the color vision priority hypothesis should be investigated using the whole-genome sequences.

Now high-coverage (6×) whole-genome sequences of Sumatran orangutans and common marmosets are available. The common marmoset belongs to NWMs and has three alleles at an L/M-opsin gene locus (Kawamura et al. 2001). Because the OR gene repertoires of humans, chimpanzees, and macaques (belonging to OWMs) have been identified in previous studies (Niimura and Nei 2007; Go and Niimura 2008), we can now examine the color vision priority hypothesis at the whole-genome level. Moreover, the whole-genome sequences of two strepsirrhine species, mouse lemurs (belonging to lemurs) and bush babies (belonging to lorises), and those of tree shrews are available, though these genomes are at low coverage (<2×). The tree shrew belongs to the order Scandentia, which is regarded to be a close relative of primates (Murphy et al. 2007). The mouse lemur and the tree shrew are dichromats, whereas the bush baby is a monochromat (Perry et al. 2007). In this study, we analyze and compare the OR gene repertoires of the seven primate species and the tree shrew and show that the color vision priority hypothesis is not supported.

Materials and Methods

Data

The draft genome sequences of the Sumatran orangutan (*Pongo pygmaeus abelii*, ponAbe2, released in July 2007; 6× coverage) and the common marmoset (*Callithrix jacchus*, calJac1, released in June 2007; 6× coverage) were retrieved from the Web site of the Genome Sequencing Center at Washington University School of Medicine (http://genome.wustl.edu/), and those of the mouse lemur (*Microcebus murinus*, micMur1, released in June 2007; 1.93× coverage), the bush baby (*Otolemur garnettii*, otoGar1, released in May 2006; 1.5× coverage), and the northern tree shrew (*Tupaia belanger*, tupBel1, released in April 2006; 1.54× coverage) were downloaded from the Web site of the Broad Institute (http://www.broadinstitute.org/). The updated version of the human genome sequences (hg18, released in March 2006; International Human Genome Sequencing Consortium 2001) was obtained from the University of California Santa Cruz Web site (http://genome.ucsc.edu/). We also used OR genes from the rhesus macaque (*Macaca mulatta*, rheMac2, released in January 2006; Rhesus Macaque Genome Sequencing and Analysis Consortium 2007) and the chimpanzee (*Pan troglodytes*, panTro2, released in March 2006; Chimpanzee Sequencing and Analysis Consortium 2005) identified in Niimura and Nei (2007) and Go and Niimura (2008), respectively.

Identification of Orthologous Genes

The method for the identification of OR genes from genome sequences was described in supplementary text S1, Supplementary Material online. We identified orthologous OR gene sets among five species (marmosets, macaques, orangutans, chimpanzees, and humans), each of which was originated from one ancestral OR gene in the most recent common ancestor (MRCA) of these five species (supplementary table S1, Supplementary Material online).

Because the total number of OR genes in the five species is so large, we first classified intact OR genes into phylogenetic clades and treated each of them separately. The previous studies identified 20 clades (Class I clade and 19 Class II clades named A–S) that were supported with high (>90%) bootstrap values for human (Niimura and Nei 2003) and macaque (Niimura and Nei 2007) OR genes. The classification of intact OR genes from chimpanzees, orangutans, and marmosets were conducted without any ambiguity using phylogenetic trees of these genes together with genes from humans or macaques. Some Class II genes remained unclassified (Niimura and Nei 2003).

We constructed a phylogenetic tree (see supplementary text S1, Supplementary Material online) for each of the 20 clades separately using all intact OR genes from two species (e.g., marmoset and human) out of the five species. Unclassified Class II genes were also treated separately. Eight genes each of which was chosen from Clades A–H were used as the outgroup for each tree. From these phylogenetic trees, we extracted candidate orthologous genes of the two species by taking monophyletic clades that contained genes from both of the species and were supported with >90% bootstrap values. When such a clade was nested within another clade, smaller one was used. The above processes were conducted for all possible combinations of two out of the five species (i.e., ten combinations).

We then examined each of the candidate orthologous gene pairs from the two species by using the values of synonymous substitutions per synonymous site, dS. For one-to-multiple or multiple-to-multiple relationships, all possible pairs of genes from the two species were examined. dS values were calculated by the modified Nei–Gojobori method (Nei and Gojobori 1986) from pairwise alignments constructed using ClustalW (Thompson et al. 1994). The distribution of dS values showed the presence of some outliers because paralogous gene pairs were also contained. Excluding such outliers and visually inspecting phylogenetic trees containing genes from the five species, we determined the threshold dS values to be 0.4, 0.3, 0.2, and 0.1 for the comparisons between marmosets and the other species, macaque–orangutan/chimpanzee/human comparisons, orangutan–chimpanzee/human comparisons, and human–chimpanzee comparisons, respectively (supplementary fig. S1, Supplementary Material online). The gene pairs showing dS values larger than the threshold values were eliminated, and the remaining gene pairs were regarded as putative orthologs between two species.

Truncated genes were treated in the following way. Using each of a truncated gene as a query, BlastP searches (Altschul et al. 1997) were conducted for all intact genes from four species except for the species having the truncated gene. The dS value was calculated between the truncated gene and the best-hit sequence showing the lowest *E* value for each species using the above-mentioned method. When the dS value is smaller than the above-mentioned threshold value, the truncated gene and the best-hit intact gene were regarded as putative orthologs to each other.

In this way, orthologous relationships of intact and truncated genes were examined for all possible combinations of two species out of the five species. We then merged the two-species orthologous relationships into five-species orthologous relationships. Finally, we again inspected phylogenetic trees containing all intact genes from the five species to see whether the genes in each of the five-species orthologous relationships were considered to have originated from a single ancestral gene in the MRCA among the five species. We investigated a phylogenetic clade (named clade X) formed by the genes from each of the five-species orthologous relationships. When a marmoset gene was nested in a subclade supported with a >90% bootstrap value within the clade X, the genes in the subclade (rather than the clade X) were regarded to be orthologous to one another. By these processes, we obtained 438 orthologous gene sets containing genes from at least two out of the five species.

As for the remaining 118 species-specific (intact and truncated) genes, some of them were generated by gene duplications that occurred after the divergence between marmosets and the other four species. To identify such paralogous genes, we conducted self-against-self BlastP searches (Altschul et al. 1997) using all the 118 genes. When the dS value between a given gene and its best hit was smaller than 0.4, these genes were regarded to have been originated from one gene in the MRCA of the five species and were assigned into the same orthologous gene set.

Results

Number of OR Genes

Table 1 indicates the numbers of OR genes in seven primate species and the tree shrew. OR gene repertories in chimpanzees and macaques were identified in the previous studies (Niimura and Nei 2007; Go and Niimura 2008), and those in the other species were newly identified in this study. The amino acid sequences of OR genes in these species are given in supplementary data set S1 (Supplementary Material online). We also renewed the human OR gene repertoire by using the updated version of the human genome and obtained a slightly larger number of OR genes than the previous study (Niimura and Nei 2003). In table 1, truncated genes represent partial intact sequences that are located at the contig ends (Niimura and Nei 2007), and they may become intact if the genome sequences are completed. Because the genome sequences of mouse lemurs, bush babies, and tree shrews are at low coverage, the numbers of truncated genes in these three species are very large. On the other hand, the numbers of truncated genes in four species (chimpanzees, orangutans, macaques, and marmosets) with high-coverage (~6×) genomes are small, suggesting that the total numbers of intact and truncated genes in these species would give accurate estimates of the numbers of functional genes. In this study, we examine OR genes identified from humans and the four species with high-coverage genomes in detail. The results in table 1 showed that the estimated numbers of functional OR

Table 1. Number of OR Genes in Seven Primate Species and the Tree Shrew.

Species	Human	Chimpanzee[a]	Orangutan	Macaque[b]	Marmoset	Bush Baby	Mouse Lemur	Tree Shrew
Intact genes	396	380	296	309	366	356	361	563
Class I	61	64	58	36	82	76	81	81
Class II	335	316	238	273	284	280	280	482
Fraction of class I genes (%)	15.4	16.8	19.6	11.7	22.4	21.3	22.4	14.4
Truncated genes	0	19	37	17	27	215	280	402
Intact + truncated genes	396[c]	399[c]	333[c]	326[c]	393[c]	571	641	965
Pseudogenes	425	414	488	280	231	370	339	1154
Fraction of pseudogenes (%)	51.8	50.9	59.4	46.2	37.0	39.3	34.6	54.5
H* pseudogenes	86	85	71	8	1	—	—	—
Fraction of pseudogenes excluding H* (%)	46.1	45.2	55.6	44.7	36.9	—	—	—
Total	821	813	821	606	624	941	980	2119
Genome coverage	Complete	6×	6×	5.1×	6×	1.5×	1.93×	1.54×

[a] From Go and Niimura (2008).
[b] From Niimura and Nei (2007).
[c] Estimated number of functional OR genes.

genes in these five primate species are similar (320–400 genes), though the numbers for orangutans and macaques are relatively small.

We, however, found that the numbers of OR pseudogenes are quite different among the five species. Hominoids (humans, chimpanzees, and orangutans) have significantly larger numbers of pseudogenes than macaques and marmosets. Especially, orangutans have more than twice as many pseudogenes as marmosets. Due to the variation in number of pseudogenes, the total number of OR genes and the fractions of pseudogenes are also quite variable. Because marmosets have a relatively large number of functional genes but the smallest number of pseudogenes among the five species, the fraction of pseudogenes in marmosets is significantly lower than those in the other four species. This observation is consistent with the previous study (Gilad et al. 2004) in which a significant difference in the fraction of OR pseudogenes between NWMs and OWMs/hominoids was suggested. However, we should note that there are no significant differences between NWMs and OWMs/hominoids in the estimated number of functional OR genes.

It is known that humans have a group of pseudogenes named 7E (Newman and Trask 2003) or H* pseudogenes (Niimura and Nei 2005a), which seem to have been generated by gene duplications after they were pseudogenized (Niimura and Nei 2005a). Table 1 shows that hominoids have much larger numbers of H* pseudogenes than OWMs or NWMs, suggesting that the duplication events of H* pseudogenes were activated in the hominoid lineage. This was also supported by a phylogenetic analysis (supplementary fig. S2, Supplementary Material online). After excluding H* pseudogenes, the fraction of pseudogenes in marmosets is still significantly smaller than the other four species, whereas that in orangutans is significantly higher than the other species.

Evolutionary Changes of OR Genes

To delineate evolutionary changes of OR gene repertoires in primates, we identified orthologous genes that are originated from the same gene in the MRCA among five species of NWMs, OWMs, and hominoids. In this analysis, we used both intact and truncated genes and identified 551 orthologous gene sets among the five species (supplementary table S1, Supplementary Material online). Therefore, it was estimated that the MRCA between NWMs and OWMs/hominoids had ~550 functional OR genes. Supplementary table S2 (Supplementary Material online) shows the presence or absence of the orthologous genes in each species. We found that 11.3% (62/551) of the orthologous gene sets are shared in all the five species. Among the 62 orthologous gene sets, 34 sets are one-to-one, that is, each species have one gene and there are no lineage-specific gene duplications.

Figure 1 illustrates the numbers of genes that are absent in each species among the 551 orthologous gene sets. Each species has lost >200 functional OR genes that were present in the MRCA. Among the five species, orangutans have lost the largest number of OR genes (283 genes) compared with their MRCA. On the other hand, humans, chimpanzees, and marmosets have lost similar numbers of OR genes. This means that humans and chimpanzees retain the ancestral OR genes in the MRCA to the same extent as marmosets. The number of species-specific OR gene losses, that is, the number of OR genes that is absent in one species but is present in the other four species is 31, 35, 48, 13, and 14 for marmosets, macaques, orangutans, chimpanzees, and humans, respectively (supplementary table S2, Supplementary Material online). Therefore, the number of species-specific gene losses is the largest in the orangutan lineage, regardless of relatively recent divergence of orangutans among the five species. This is consistent with the above observation that the extent of OR gene degeneration is the most prominent in the orangutan lineage.

To see evolutionary changes of OR gene repertoires in the five species, we estimated the number of OR gene losses in each branch of their phylogeny under the parsimony principle (fig. 1) using the results in supplementary table S2, Supplementary Material online. The analysis suggested that the number of OR gene losses (51 genes) occurred in the ancestral branch of catarrhines (indicated by an arrowhead in fig. 1) is not particularly large compared with other

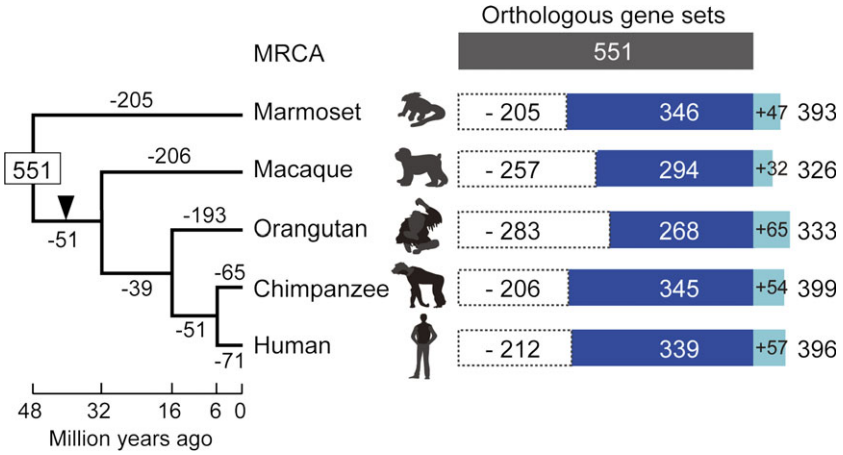

Fig. 1. Losses of orthologous OR gene sets among five primate species. The number of ancestral OR genes in the MRCA among the five species that have been lost in each species are shown in a box surrounded by a broken line. The number of OR gene losses in each branch in the primate evolution is shown at the branch. These numbers were calculated from supplementary table S2 (Supplementary Material online) using Dollo parsimonious principle (Le Quesne 1974; Farris 1977), in which gene losses are considered to be irreversible. For example, the number of OR gene losses at the branch indicated by an arrowhead (51 genes) is in the category "10000" in supplementary table S2 (Supplementary Material online). As another example, the number in the marmoset lineage (205 genes) was calculated as the summation of the numbers in categories $0n_2n_3n_4n_5$, where n_2–n_5 are 0 or 1, in supplementary table S2 (Supplementary Material online). The number of functional OR genes in each species is shown at the right. The number with a plus sign in a box at the right is the estimated number of gene duplications in each lineage from the MRCA. These numbers were calculated from the number of orthologous gene sets (551 genes), the number of OR gene losses, and the number of functional OR genes in each species. For example, in the case of marmosets, the number (47 genes) was obtained as 393 − (551 − 205). The evolutionary timescale is shown at the bottom. The divergence times were obtained from Hedges et al. (2006) and Matsui et al. (2009).

branches leading to humans, which does not support the scenario that an extensive loss of OR genes had occurred in the common ancestor of catarrhines that acquired full trichromatic vision. Rather, it appears that OR genes were gradually lost in every branch from the MRCA to humans. This observation is inconsistent with the color vision priority hypothesis.

We also estimated the numbers of gene gains and losses in the evolution of the five species using the reconciled tree method (Niimura and Nei 2007) (fig. 2). Note that in this analysis both gene gains and gene losses in each branch were considered. Moreover, here we used intact OR genes from mouse lemurs, bush babies, and tree shrews as outgroups for accurate estimation (see Discussion). The results showed that the MRCA of the five species had ~530 functional OR genes, which is consistent with the result in figure 1. Furthermore, this analysis again did not support an abrupt loss of OR genes in the ancestral lineage of catarrhines after the divergence from NWMs.

Pairwise Comparison of OR Gene Repertoires

Diagrams in figure 3 illustrate the extent of commonality in OR gene repertoires between any combinations of two out of the five primate species. Figure 3*A* and *B* indicates the numbers of orthologous gene sets among the five species mentioned above and those of OR genes in the extant species, respectively. For example, among the 551 orthologous gene sets, 274 contained both human and chimpanzee genes, whereas 65 and 71 contained either human or chimpanzee genes (see fig. 3*A*). Therefore, of the ancestral OR genes in the NWMs/OWMs/hominoids MRCA that survive in the human or chimpanzee lineages, 66.8% [=274/(274 + 65 + 71)] are shared between the two species. Because of gene duplication events, the 274 ancestral OR genes in the MRCA generated 306 and 304 OR genes in the human and chimpanzee lineages, respectively, and the 65 and 71 genes in the MRCA yielded 90 and 95 genes in humans and chimpanzees, respectively (see fig. 3*B*). Therefore, on average, 76.7% [=(306/396 + 304/299)/2] of the human or chimpanzee OR gene repertoires are common to each other (Go and Niimura 2008).

Out of ten pairwise comparisons among the five species, human and chimpanzee OR gene repertoires are the most similar to each other (fig. 3*A* and *B*), which is expected because they are evolutionarily the most closely related among the five species. However, interestingly, the second most similar comparison is that between humans and marmosets. In other words, the human OR gene repertoire is more similar to that of marmosets than that in orangutans or macaques, although humans and marmosets are distantly related among the five species.

Discussion

In this study, we found that the number of functional OR genes (table 1) and the extent of OR gene losses (fig. 1) in marmosets are similar to those in humans or chimpanzees. Moreover, by examining orthologous OR gene sets among five primate species, we showed that loss of OR genes was not marked in the ancestral branch of catarrhines. The reconciled tree method using OR genes from eight species indicated that the number of functional OR genes in the NWMs/OWMs/hominoids MRCA was similar to that in

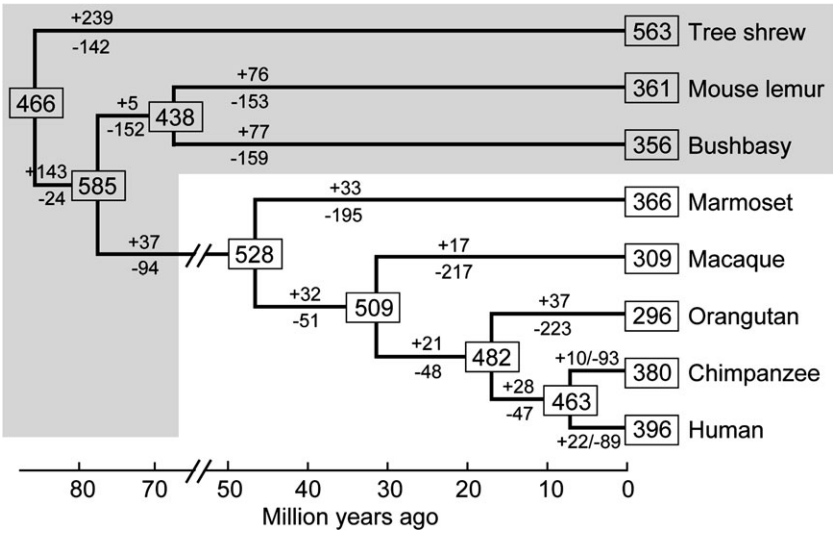

Fig. 2. Evolutionary changes of the number of OR genes in haplorhines. The numbers of OR genes in the ancestral species and those of gene gains and losses in each branch were estimated by the reconciled tree method, which was described in detail in Niimura and Nei (2007). The number of functional OR genes in the extant or ancestral species is shown in a rectangular box, and those of gene gains and losses in each branch are indicated with a plus sign and a minus sign, respectively, at the branch. All intact OR genes from seven primate species and the tree shrew were used in this analysis. Note that, however, OR genes from mouse lemurs, bush babies, and tree shrews were used as the outgroup of those from marmosets, macaques, orangutans, chimpanzees, and humans for accurate estimation of the numbers in the evolution of the latter five species. Because the genome sequences of the former three species are at low coverage, the estimated numbers involved with these species are inaccurate and are shaded in gray. We used 70% bootstrap condensed trees of OR genes for the estimation (Niimura and Nei 2007), but the results were essentially the same when 90% or 50% bootstrap condensed trees were used (supplementary fig. S4, Supplementary Material online). The divergence times were obtained from Hedges et al. (2006) and Matsui et al. (2009).

the OWMs/hominoids MRCA. These two independent analyses gave a similar estimation of the number of functional OR genes in the MRCA of the five species (~550), suggesting that the estimation is reliable. Both analyses suggested gradual OR gene losses in primate evolution rather than a sudden loss at the branch of the catarrhine ancestor after the divergence from NWMs. All these observations do not support the color vision priority hypothesis, which predicts a gap between NWMs and catarrhines.

The results shown in table 1 may indicate a significant difference in the fraction of OR pseudogenes between NWMs and catarrhines, which is consistent with Gilad et al. (2004). However, the fraction of OR pseudogenes is not necessarily negatively correlated with the number of functional OR genes (table 1) because pseudogenes can be easily lost during evolution. Therefore, the fraction of OR pseudogenes is not a good indicator of the olfactory ability in a species. In fact, humans, chimpanzees, and orangutans retain numerous pseudogenes, probably because these pseudogenes were generated relatively recently. For example, there are many gene losses in the human lineage after the divergence from chimpanzees as shown in figure 2. On the other hand, relatively small numbers of OR pseudogenes in macaques and marmosets would imply that pseudogenization events had occurred in more ancient time, being unable to be detected any more.

We also found that the OR gene repertoire in humans is more similar to that in marmosets than that in orangutans or macaques. This observation is unexpected because marmosets are much more distantly related to humans than orangutans and macaques. In contrast to the previous assertion of human-specific OR gene losses (Gilad et al. 2003, 2005), orangutans and macaques have lost more OR genes than humans. Orangutans have the smallest number of functional OR genes among the species examined. It is known that a pericentric inversion occurred in the orangutan lineage after the divergence from human/African great apes in chromosome 11 (Müller and Wienberg 2001), on which the largest number of OR genes (~40% in the case of humans; Niimura and Nei 2003) are present among all chromosomes. We therefore examined the synteny of chromosome 11 between humans and orangutans and found a deletion of a large OR gene cluster near the centromere (supplementary fig. S3, Supplementary Material online). However, our knowledge of the relationships between ORs and odorous ligands is still quite limited (Saito et al. 2009), and thus the ecological significance of this radical loss of OR genes in orangutans is unclear at this stage.

One caveat of our analyses is the usage of low-coverage genome sequences. However, the results present in this study are essentially based on five primate species with high-coverage genomes. In the analysis of OR gene gains/losses by the reconciled tree method (fig. 2), we used OR genes from eight species. However, we should note that OR genes from mouse lemurs, bush babies, and tree shrews were used as the outgroup of those from the five species for estimating the numbers of OR gene gains/losses in the evolution of NWMs and catarrhines. For this analysis, usage of the outgroup species is essential. Because the reconciled

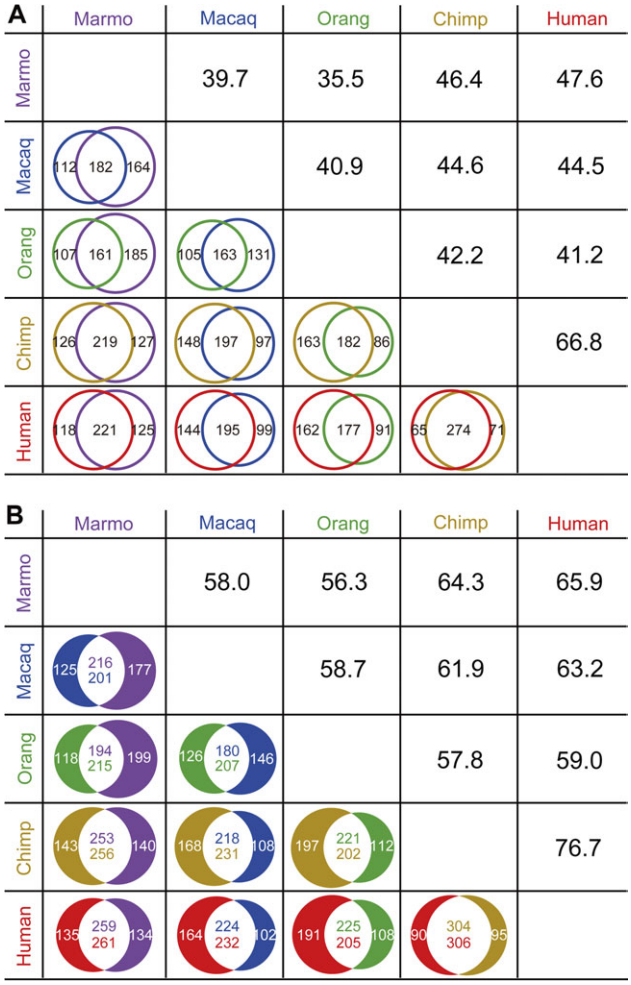

Fig. 3. Pairwise comparisons of OR gene repertoires among the five species. (A) A Venn diagram at the bottom left indicates the number of orthologous gene sets among the five species that are present in both or either of the two species. A colored circle represents a species shown in the same color. The number at the upper right indicates the fraction (in percentage) of the number of orthologous gene sets that are present in both the two species to the number of orthologous gene sets that are present in at least one species. Marmo, marmoset; Macaq, macaque; Orang, orangutan; Chimp, chimpanzee. (B) A diagram indicates the number of (intact or truncated) OR genes that are shared between two species and those of species-specific OR genes. A colored crescent represents a species shown in the same color. The number at the upper right indicates the mean percentage of the genes in one species that are shared with the other species.

tree method gives a minimum estimate of the number of ancestral genes that is consistent with a phylogenetic tree, when there are many gene losses in the lineage of the most basal species (marmosets in this case), the number of genes in the MRCA of all species tends to be underestimated. In fact, if the same analysis was conducted using OR genes from only the five species, the number of OR genes in the NWMs/OWMs/hominoids MRCA was considerably underestimated (416 genes for the usage of 70% bootstrap condensed trees).

Although the numbers of OR genes for the three species with low-coverage genomes are inaccurate, the results in table 1 indicate a gap in the number of functional OR genes between haplorhines and strepsirrhines. This observation is consistent with the fact that only strepsirrhines have the rhinarium (see Introduction). The sizes of olfactory bulbs vary widely among primates and are thought to be correlated with the olfactory ability. Barton (2006) found that the relative size of the olfactory bulb is substantially greater in strepsirrhines than in three haplorhine groups (NWMs, OWMs, and hominoids), whereas there are no clear differences among the three groups. Therefore, neuroanatomical studies also support the presence of a gap in olfactory ability between haplorhines and strepsirrhines rather than that between strepsirrhines/NWMs and OWMs/hominoids.

It is widely believed that trichromatic color vision is powerful for perceiving environmental signals such as foraging, predation, and mating. Dominy and Lucas (2001) reported that hominoids and OWMs discriminate young leaves only by using a red–green signal, which is correlated with protein levels and toughness of leaves. It was also reported that, for some species of NWMs, trichromats showed a better ability to find red objects against a green background than dichromats in experimental conditions (Caine and Mundy 2000; Smith et al. 2003). However, the advantage of trichromacy in NWMs is unclear. For example, Hiramatsu et al. (2008) carried out field observations of a group of wild spider monkeys in natural habitats; they did not find any significant differences in the foraging efficiency between dichromats and trichromats and rather found that the luminance contrast was the main determinant of the foraging efficiency. Moreover, Melin et al. (2007) showed that dichromatic capuchin monkeys are more efficient at detecting camouflaged, surface-dwelling insects than trichromatic monkeys in the wild. Other studies also suggested a superiority of dichromats to trichromats (Saito et al. 2005; Caine et al. 2009). Furthermore, several studies showed that primates use both color vision and olfaction for foraging, suggesting an interplay between the two senses (Laska et al. 2000, 2007; Hiramatsu et al. 2009; Melin et al. 2009).

In this study, it was suggested that the degeneration of OR gene repertories in primates cannot simply be explained by the acquisition of full trichromatic vision. To investigate the timing of olfactory degeneration in the primate evolution, it is particularly important to analyze the tarsier genome. We should note that, however, results obtained from representative species cannot be generalized because color vision systems in NWMs and strepsirrhines are highly diverse. Therefore, comparison of OR gene repertoires among various primate species should be necessary (see Genome 10K Community of Scientists 2009). It is also worth investigating intraspecific variation of OR gene repertoires in an NWM species in which trichromats and dichromats coexist. OR genes are known to be highly polymorphic among human individuals (Hasin-Brumshtein et al. 2009). In this respect, it is intriguing to note that humans are exceptionally polymorphic in color vision among catarrhines, that is, approximately 3–8% of human males are dichromatic (Deeb 2006), whereas color vision defects

are rare in nonhuman catarrhines (Onishi et al. 1999; Terao et al. 2005). Therefore, for further understanding of the genetic interaction between color vision and olfaction, comparison of the extent of OR gene diversity among different primate species would also be informative.

Supplementary Material

Supplementary figures S1–S4, tables S1–S2, data set S1, and text S1 are available at *Molecular Biology and Evolution* online (http://www.mbe.oxfordjournals.org/).

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References

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped Blast and PSI-Blast: a new generation of protein database search programs. *Nucleic Acids Res.* 25:3389–3402.

Barton RA. 2006. Olfactory evolution and behavioral ecology in primates. *Am J Primatol.* 68:545–558.

Buck L, Axel R. 1991. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell* 65:175–187.

Caine NG, Mundy NI. 2000. Demonstration of a foraging advantage for trichromatic marmosets (*Callithrix geoffroyi*) dependent on food colour. *Proc R Soc Lond B.* 267:439–444.

Caine NG, Osorio D, Mundy NI. Forthcoming. 2009. A foraging advantage for dichromatic marmosets (*Callithrix geoffroyi*) at low light intensity. *Biol Lett.* 6:36–38.

Chimpanzee Sequencing and Analysis Consortium. 2005. Initial sequence of the chimpanzee genome and comparison with the human genome. *Nature* 437:69–87.

Deeb SS. 2006. Genetics of variation in human color vision and the retinal cone mosaic. *Curr Opin Genet Dev.* 16:301–307.

Dominy NJ, Lucas PW. 2001. Ecological importance of trichromatic vision to primates. *Nature* 410:363–366.

Farris JS. 1977. Phylogenetic analysis under Dollo's law. *Syst Zool.* 26:77–88.

Genome 10K Community of Scientists. 2009. Genome 10K: a proposal to obtain whole-genome sequence for 10,000 vertebrate species. *J Hered.* 100:659–674.

Gilad Y, Man O, Glusman G. 2005. A comparison of the human and chimpanzee olfactory receptor gene repertoires. *Genome Res.* 15:224–230.

Gilad Y, Man O, Pääbo S, Lancet D. 2003. Human specific loss of olfactory receptor genes. *Proc Natl Acad Sci USA.* 100: 3324–3327.

Gilad Y, Przeworski M, Lancet D. 2004. Loss of olfactory receptor genes coincides with the acquisition of full trichromatic vision in primates. *PLoS Biol.* 2:e5.

Gilad Y, Wiebe V, Przeworski M, Lancet D, Pääbo S. 2007. Correction: loss of olfactory receptor genes coincides with the acquisition of full trichromatic vision in primates. *PLoS Biol.* 5:e148.

Glusman G, Yanai I, Rubin I, Lancet D. 2001. The complete human olfactory subgenome. *Genome Res.* 11:685–702.

Go Y, Niimura Y. 2008. Similar numbers but different repertoires of olfactory receptor genes in humans and chimpanzees. *Mol Biol Evol.* 25:1897–1907.

Hasin-Brumshtein Y, Lancet D, Olender T. 2009. Human olfaction: from genomic variation to phenotypic diversity. *Trends Genet.* 25:178–184.

Hedges SB, Dudley J, Kumar S. 2006. TimeTree: a public knowledge-base of divergence times among organisms. *Bioinformatics* 22:2971–2972.

Hiramatsu C, Melin AD, Aureli F, Schaffner CM, Vorobyev M, Kawamura S. 2009. Interplay of olfaction and vision in fruit foraging of spider monkeys. *Anim Behav.* 77:1421–1426.

Hiramatsu C, Melin AD, Aureli F, Schaffner CM, Vorobyev M, Matsumoto Y, Kawamura S. 2008. Importance of achromatic contrast in short-range fruit foraging of primates. *PLoS ONE.* 3:e3356.

International Human Genome Sequencing Consortium. 2001. Initial sequencing and analysis of the human genome. *Nature* 409:860–921.

Jacobs GH. 1996. Primate photopigments and primate color vision. *Proc Natl Acad Sci USA.* 93:577–581.

Jacobs GH. 2007. New World monkeys and color. *Int J Primatol.* 28:729–759.

Jacobs GH, Neitz M, Deegan JF, Neitz J. 1996. Trichromatic colour vision in New World monkeys. *Nature* 382:156–158.

Kawamura S, Hirai M, Takenaka O, Radlwimmer FB, Yokoyama S. 2001. Genomic and spectral analyses of long to middle wavelength-sensitive visual pigments of common marmoset (Callithrix jacchus). *Gene* 269:45–51.

Laska M, Freist P, Krause S. 2007. Which senses play a role in nonhuman primate food selection? A comparison between squirrel monkeys and spider monkeys. *Am J Primatol.* 69:282–294.

Laska M, Seibt A, Weber A. 2000. 'Microsmatic' primates revisited: olfactory sensitivity in the squirrel monkey. *Chem Senses.* 25:47–53.

Le Quesne WJ. 1974. The uniquely evolved character concept and its cladistic application. *Syst Zool.* 23:513–517.

Martin RD. 1990. Primate origins and evolution: a phylogenetic reconstruction. London: Chapman Hall. p. 327–329

Matsui A, Rakotondraparany F, Munechika I, Hasegawa M, Horai S. 2009. Molecular phylogeny and evolution of prosimians based on complete sequences of mitochondrial DNAs. *Gene* 441:53–66.

Melin AD, Fedigan LM, Hiramatsu C, Hiwatashi T, Parr N, Kawamura S. 2009. Fig foraging by dichromatic and trichromatic *Cebus capucinus* in a tropical dry forest. *Int J Primatol.* 30:753–775.

Melin AD, Fedigan LM, Hiramatsu C, Sendall C, Kawamura S. 2007. Effects of colour vision phenotype on insect capture by a free-ranging population of white-faced capuchins, *Cebus capucinus*. *Anim Behav.* 73:205–214.

Mombaerts P. 2004. Genes and ligands for odorant, vomeronasal and taste receptors. *Nat Rev Neurosci.* 5:263–278.

Müller S, Wienberg J. 2001. "Bar-coding" primate chromosomes: molecular cytogenetic screening for the ancestral hominoid karyotype. *Hum Genet.* 109:85–94.

Murphy WJ, Pringle TH, Crider TA, Springer MS, Miller W. 2007. Using genomic data to unravel the root of the placental mammal phylogeny. *Genome Res*. 17:413–421.

Nei M, Gojobori T. 1986. Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. *Mol Biol Evol*. 3:418–426.

Nei M, Niimura Y, Nozawa M. 2008. The evolution of animal chemosensory receptor gene repertoires: roles of chance and necessity. *Nat Rev Genet*. 9:951–963.

Newman T, Trask BJ. 2003. Complex evolution of 7E olfactory receptor genes in segmental duplications. *Genome Res*. 13: 781–793.

Niimura Y. 2009. On the origin and evolution of vertebrate olfactory receptor genes: comparative genome analysis among 23 chordate species. *Genome Biol Evol*. 1:34–44.

Niimura Y, Nei M. 2003. Evolution of olfactory receptor genes in the human genome. *Proc Natl Acad Sci USA*. 100:12235–12240.

Niimura Y, Nei M. 2005a. Comparative evolutionary analysis of olfactory receptor gene clusters between humans and mice. *Gene* 346:13–21.

Niimura Y, Nei M. 2005b. Evolutionary dynamics of olfactory receptor genes in fishes and tetrapods. *Proc Natl Acad Sci USA*. 102:6039–6044.

Niimura Y, Nei M. 2006. Evolutionary dynamics of olfactory and other chemosensory receptor genes in vertebrates. *J Hum Genet*. 51:505–517.

Niimura Y, Nei M. 2007. Extensive gains and losses of olfactory receptor genes in mammalian evolution. *PLoS ONE*. 2:e708.

Onishi A, Koike S, Ida M, et al. (13 co-authors). 1999. Dichromatism in macaque monkeys. *Nature* 402:139–140.

Perry GH, Martin RD, Verrelli BC. 2007. Signatures of functional constraint at aye-aye opsin genes: the potential of adaptive color vision in a nocturnal primate. *Mol Biol Evol*. 24:1963–1970.

Rhesus Macaque Genome Sequencing and Analysis Consortium. 2007. Evolutionary and biomedical insights from the rhesus macaque genome. *Science* 316:222–234.

Saito A, Mikami A, Kawamura S, et al. (13 co-authors). 2005. Advantage of dichromats over trichromats in discrimination of color-camouflaged stimuli in nonhuman primates. *Am J Primatol*. 67:425–436.

Saito H, Chi Q, Zhuang H, Matsunami H, Mainland JD. 2009. Odor coding by a mammalian receptor repertoire. *Sci Signal*. 2:ra9.

Schmitz J, Ohme M, Zischler H. 2001. SINE insertions in cladistic analyses and the phylogenetic affiliations of *Tarsius bancanus* to other primates. *Genetics* 157:777–784.

Smith AC, Buchanan-Smith HM, Surridge AK, Osorio D, Mundy NI. 2003. The effect of colour vision status on the detection and selection of fruits by tamarins (Saguinus spp.). *J Exp Biol*. 206:3159–3165.

Terao K, Mikami A, Saito A, et al. (15 co-authors). 2005. Identification of a protanomalous chimpanzee by molecular genetic and electroretinogram analyses. *Vision Res*. 45:1225–1235.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignments through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Res*. 22:4673–4680.

Zhang X, Firestein S. 2002. The olfactory receptor gene superfamily of the mouse. *Nat Neurosci*. 5:124–133.

Zozulya S, Echeverri F, Nguyen T. 2001. The human olfactory receptor repertoire. *Genome Biol*. 2:research0018.1–researc0018.12.