Genetic Evidence for the Coexistence of Pheromone Perception and Full Trichromatic Vision in Howler Monkeys

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Vertebrate pheromones are water-soluble chemicals perceived mainly by the vomeronasal organ (VNO) for intraspecific communications. Humans, apes, and Old World (OW) monkeys lack functional genes responsible for the pheromone signal transduction and are generally insensitive to vomeronasal pheromones. It has been hypothesized that the evolutionary deterioration of pheromone sensitivity occurred because pheromone communication became redundant after the emergence of full trichromatic color vision via the duplication of the X-chromosome–linked red/green opsin gene in the common ancestor of hominoids and OW monkeys. Interestingly, full trichromacy also evolved in the New World (NW) howler monkeys via an independent duplication of the same gene. Here we sequenced from three species of howler monkeys an essential component of the VNO pheromone transduction pathway, the gene encoding the ion channel TRP2. In contrast to those of hominoids and OW monkeys, the howler TRP2 sequences have none of the characteristics of pseudogenes. This and other observations indicate that howler monkeys have maintained both their systems of pheromone communication and full trichromatic vision, suggesting that the presence of full trichromacy alone does not lead to the loss of pheromone communication. We suggest that the ecological differences between OW and NW primates, particularly in habitat selection, may have also affected the evolution of pheromone perception.

Introduction

Pheromones are used by individuals of the same species to elicit behavioral or physiological changes such as male-male aggression, puberty, estrus, and induction of mating (Keverne 1999). Pheromones are perceived primarily by the vomeronasal organ (VNO), an organ situated at the base of the nasal cavity and separated from the main olfactory epithelium (MOE) that senses volatile odorants (Keverne 1999). Anatomical, physiological, behavioral, and genetic data generally show the lack of VNO-mediated pheromone sensitivity in humans, apes, and Old World (OW) monkeys, whereas other placental mammals are known to be sensitive to pheromones (Loo 1973; Stoddart 1980; Keverne 1999; Meredith 2001; Zhang and Webb 2003; Liman and Innan 2003; Dulac and Torello 2003). Some behavioral studies, nevertheless suggest that humans may still be responsive to certain pheromones such that women living in close proximity tend to have synchronized menstrual cycles (Stern and McClintock 1998), although there is no evidence that this is a VNO-mediated process. Evolutionary genetic studies of components of the VNO pheromone transduction pathway suggested that the loss of pheromone sensitivity in primates occurred shortly before the separation of hominoids and OW monkeys ~23 MYA, but after the divergence of these organisms from New World (NW) monkeys ~35 MYA (Zhang and Webb 2003; Liman and Innan 2003). It is of substantial interest to understand why this important communication channel was inactivated and whether its function in eliciting behavioral and reproductive changes has been replaced by other sensory mechanisms. An evolutionary hypothesis, gradually developed over the past 20 years, asserts that the role of pheromone communication was replaced by color vision in hominoids and OW monkeys (Dixson 1983; Liman and Innan 2003; Zhang and Webb 2003). In these organisms, color vision is conferred by three genes that respectively encode the blue-sensitive, green-sensitive, and red-sensitive opsins (Nathans, Thomas, and Hogness 1986). The green and red genes are located on the X chromosome, whereas the blue gene is autosomal. The green and red genes were generated by gene duplication in the common ancestor of hominoids and OW monkeys (Yokoyama and Yokoyama 1989). In most NW monkeys all males are dichromatic because they have one blue gene and one red/green gene. But females can be trichromatic, as a green allele and a red allele may coexist at the red/green opsin locus in a heterozygote (Boissinot et al. 1998). The duplication of the red/green gene made both sexes trichromatic; hereafter referred to as full trichromacy. Trichromacy allows yellow, orange, pink, and red hues to be distinctly perceived and may have been fixed because it helped primates detect young leaves and ripened fruits against a background of dappled foliage (reviewed in Surridge, Osorio, and Mundy 2003). It is noteworthy that females of many OW monkeys and hominoids develop a prominent reddening and swelling of the sexual skin surrounding their perineum around the time of ovulation (Dixson 1983). With full trichromatic vision, subtle color changes of female sexual skins can be perceived by males, which may have facilitated the evolution of sexual swelling and a new visual-based signaling-sensory mechanism (Zhang and Webb 2003). This new system may be preferred to the pheromone system, as colors can be detected from a distance whereas pheromones are likely sensed only by physical contact, at least for mammals (Luo, Fee, and Katz 2003). In other words, the emergence of full trichromacy may have made pheromone communication unnecessary. Comparative data can be used to test this hypothesis. For instance, nonprimate placental

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mammals are known to be dichromatic and they are pheromone sensitive. In contrast, birds have tetrachromatic vision and develop colorful plumages at sexual maturity, but they lack VNOs and are pheromone insensitive (Stoddart 1980; Keverne 1999; Zhang and Webb 2003). In the last few years, it has been discovered that one genus of NW monkeys, *Alouatta* (howler monkeys), are also full trichromatic (Jacobs et al. 1996). An independent duplication of the red/green opsin gene apparently occurred in the common ancestor of howler monkeys after they diverged from other NW monkeys, as all howler monkey species, but no other NW monkey species so far examined, are full trichromatic (Jacobs et al. 1996; Kainz, Neitz, and Neitz 1998; Jacobs and Deegan 2001). This provides an opportunity for the examination of the hypothesis on the relationship between color vision and pheromone sensitivity.

Many genes involved in the VNO pheromone transduction pathway have been identified (Zufall, Kelliher, and Leinders-Zufall 2002; Rodriguez 2003). Among them, the TRP2 gene and pheromone receptor genes appear to be unique to this pathway and thus may be used as genetic markers for studying the evolution of pheromone sensitivity. We chose to study only the TRP2 gene here because pheromone receptor genes form large gene families (Dulac and Axel 1995; Ryba and Tirindelli 1997; Herrada and Dulac 1997; Matsunami and Buck 1997) that are difficult to fully characterize without the availability of a genome sequence. TRP2 is an ion channel of the transient receptor potential family (Liman, Corey, and Dulac 1999). Disruption of the TRP2 gene in mice hampers pheromone perception and causes dramatic changes in sexual and social behaviors (Stowers et al. 2002; Leypold et al. 2002). In all hominoids and OW monkeys surveyed, TRP2 is a pseudogene without an open reading frame (ORF; Zhang and Webb 2003; Liman and Innan 2003). Three of the 13 exons in TRP2 have been sequenced in an individual of *Alouatta seniculus*, and no ORF-breaking mutations were found (Liman and Innan 2003). But this does not preclude the presence of ORF-breaking mutations in any of the other 10 exons. We here sequenced all 13 exons from three species of howler monkeys. The sequence data, as well as our subsequent evolutionary analysis, suggest that TRP2 is functional in howler monkeys.

Materials and Methods

Polymerase Chain Reaction (PCR) and Sequencing

We amplified all 13 exons of the TRP2 gene by PCR from the genomic DNAs of mantled howler (*Alouatta palliata*), red-handed howler (*A. belzebul*), and Venezuelan red howler (*A. seniculus*). The PCR was conducted with MasterTaq under conditions recommended by the manufacturer (Eppendorf, Hamburg, Germany). The products were purified and sequenced from both directions using the dideoxy chain termination method with automated sequencer. The PCR primers and conditions follow Zhang and Webb (2003).

DNA Sequence Analysis

The complete TRP2 gene sequences of tamarin (*Saguinus oedipus*), squirrel monkey (*Saimiri sciureus*), owl monkey (*Aotus trivirgatus*), saki (*Pithecia irrorata*), and spider monkey (*Ateles geoffroyi*) were obtained from Zhang and Webb (2003). The sequences were analyzed based on an established phylogeny of the NW monkeys involved. Ancestral gene sequences at all interior nodes of the tree were inferred by the distance-based Bayesian method (Zhang and Nei 1997). Synonymous and nonsynonymous substitutions were then counted for each tree branch. We also used a likelihood-based method (Yang 1998) to analyze the synonymous and nonsynonymous substitution rates.

Computer Simulation

To examine how long it takes for a nonfunctional TRP2 to lose its ORF, we adopted the computer simulation approach of Zhang and Webb (2003). The speed with which an ORF becomes disrupted depends in large part on the sequence of the ORF, rate of point mutations, and rate of indel (insertion/deletion) mutations. We used a point mutation rate of 2.2×10^{-9} per site per year, as estimated from large genomic data sets of mammals (Kumar and Subramanian 2002). The relative mutational frequencies among the four nucleotides are assumed to be equal, as they have only a negligible effect on the simulation result. We assumed that all indels with sizes that are multiples of three nucleotides ($3n$ indels) do not disrupt an ORF. This simplifies our simulation but does not affect our results, because the majority of indels generated by mutations have small sizes ($\leq$6 nucleotides; Zhang and Webb 2003). It has been estimated from genomic comparisons between the human and the chimpanzee (Britten 2002) and between human and baboon (Silva and Kondrashov 2002) that the mutation rate of indels is about 1.0×10^{-10} per site per year, of which 17% are $3n$ indels (Zhang and Webb 2003; Podlaha and Zhang 2003). A simulation was then performed for 20,000 replications with an NW monkey TRP2 coding sequence and the above parameters. Under no functional constraints, the substitution rate is identical to the mutation rate and mutations are assumed to be random. An ORF is interrupted when a non-$3n$ indel or a nonsense point mutation occurs. We thus determined $t_{1/2}$ of an ORF, or the time required for an intact ORF to be interrupted in half of the simulation replications. The computer program Pseudogene (Zhang and Webb 2003) was used.

Results

Howler Monkey TRP2 Genes Have Complete ORFs

We sequenced all 13 exons of the TRP2 gene from one individual of each of the three species: *A. palliata*, *A. belzebul*, and *A. seniculus*. Howler monkeys are separated into Mesoamerican and South American groups by both geographic distribution and phylogeny (Cortés-Ortiz et al. 2003). Both groups are represented in our samples, as *A. palliata* is Mesoamerican and *A. belzebul* and *A. seniculus* are South American. The entire TRP2 gene retains an

intact ORF in each of the three species surveyed (fig. 1). One interpretation of this result is that TRP2 is functional in howler monkeys. An alternative interpretation is that the loss of TRP2 function is so recent that the ORF has not yet been hit by deleterious substitutions. To evaluate this alternative hypothesis, we simulated the neutral evolution of TRP2 under no selection. Using realistic rates of point mutations (Kumar and Subramanian 2002) and indel mutations (Podlaha and Zhang 2003) that were previously estimated from primate or mammalian genomic comparisons (see *Materials and Methods*), we found that the half-life of TRP2 is about 1.5 Myr. That is, after 1.5 Myr, there is 50% chance that TRP2 still retains its ORF. Molecular dating suggests that howler monkeys diverged from their closest evolutionary relatives (spider moneys and woolly monkeys) about 16 MYA, and that the Mesoamerican and South American howler monkeys were separated about 7 MYA (Cortés-Ortiz et al. 2003). Because full trichromacy emerged in the common ancestor of howler monkeys (Jacobs et al. 1996), one could assume that TRP2 lost its function between 7 and 16 MYA. Under such an assumption, the probability that TRP2 retains its ORF today is 3.9×10^{-2} to 6.2×10^{-4}. The probability that it retains the ORF in all three howler monkey species should be even lower, and this probability is 2.4×10^{-6} if TRP2 lost its function 16 MYA and 3.9×10^{-4} if it lost the function 7 MYA. These computations are based on an additional assumption that the two South American howler monkeys studied here diverged ~5 MYA, which has been estimated with molecular dating (Cortés-Ortiz et al. 2003). In any case, the results suggest that the alternative hypothesis that TRP2 retains its ORF by chance is improbable. Rather, the howler monkey TRP2 gene is likely maintained because of its function.

Howler Monkey TRP2 Genes Are Under Functional Constraints and Purifying Selection

Further evidence for the functionality of howler monkey TRP2 may be gained by an examination of the number of nonsynonymous substitutions per nonsynonymous site (d_N) and that of synonymous substitutions per synonymous site (d_S). A functionally important gene is under functional constraint and purifying selection, which prevents deleterious nonsynonymous substitutions from fixation but generally does not affect synonymous substitutions, leading to a d_N/d_S ratio smaller than 1 (Nei and Kumar 2000). By contrast, a pseudogene is not under functional constraint and has a d_N/d_S ratio of approximately 1. We analyzed the TRP2 genes of three howler monkeys and five other NW monkeys from a phylogeny-based approach (Zhang, Kumar, and Nei 1997). The phylogeny of the eight monkeys is relatively well established, except for the interrelationships of the owl monkey, squirrel monkey, and tamarin (Goodman et al. 1998; Singer et al. 2003; Steiper and Ruvolo 2003). We here assumed that the owl monkey and squirrel monkey form a clade in exclusion of the tamarin, a conclusion supported by the TRP2 data. Use of alternative trees does not change our conclusion. Using this tree, we inferred the ancestral TRP2 gene sequences for all interior nodes and counted the numbers of synonymous (s) and nonsynonymous (n) substitutions for each tree branch. We separate the tree branches into three groups, as indicated in figure 2. The first group includes those branches that link the three howler monkeys; the second group contains only one branch, which links the common ancestor of howlers with the common ancestor of the spider monkey and howlers; and the third group consists of all other branches of the tree. The n/s ratio for the three groups is 1.10, 0.58, and 0.71, respectively. Although the n/s ratio for group 1 appears higher than those of the other two groups, their differences are statistically insignificant ($P > 0.1$). No statistically significant differences are found even when any two of the three groups are combined and then compared with the third group ($P > 0.1$). These results suggest no change in natural selection on TRP2 among the NW monkeys examined. The potential numbers of synonymous and nonsynonymous sites for the NW monkey TRP2 sequences are 1,809.85 and 842.15, respectively, estimated by the modified Nei-Gojobori method (Zhang, Rosenberg, and Nei 1998). Thus the above three n/s ratios correspond to d_N/d_S ratios of 0.51, 0.27, and 0.33, respectively. All of them are significantly lower than 1 ($P < 0.05$, Fisher's test), indicating the presence of purifying selection.

We also applied a likelihood-based analysis, which does not depend on the inference of ancestral sequences (Yang 1998). In this analysis, we first assumed that all branches have the same w ($=d_N/d_S$) value, which was estimated to be 0.24 (table 1). This model (I) is not significantly worse than model II, which allows different w values across branches. We also examined models III, IV, and V. Model III allows three w values for each of the three groups of branches mentioned earlier. Model IV allows one w for group 1 and 2 branches and a second w for group 3 braches. Model V allows one w for group 1 branches and a second w for group 2 and 3 branches. No statistically significant difference in likelihood is found among any of the five models (table 1). Thus, consistent with the non-likelihood–based analyses, likelihood analysis also suggests that natural selection on TRP2 has been relatively constant among species of NW monkeys. Furthermore, the presence of purifying selection on TRP2 of howlers is indicated by the likelihood analysis as well, because both the w values of group 1 branches and that of group 1 and 2 branches are significantly smaller than 1 (see the comparison between models IV and VI and that between V and VII in table 1).

Discussion

In this study, we sequenced and analyzed the TRP2 gene from three species of howler monkeys in an attempt to examine the relationship between the evolution of full trichromatic vision and the deterioration of pheromone communication. It has been hypothesized that the loss of pheromone sensitivity in OW monkeys and hominoids was related to the emergence of full trichromatic vision, which, in conjunction with the evolution of sexual swelling, led to a visual-based signaling-sensory system that made pheromone sensitivity redundant (Zhang and Webb 2003).

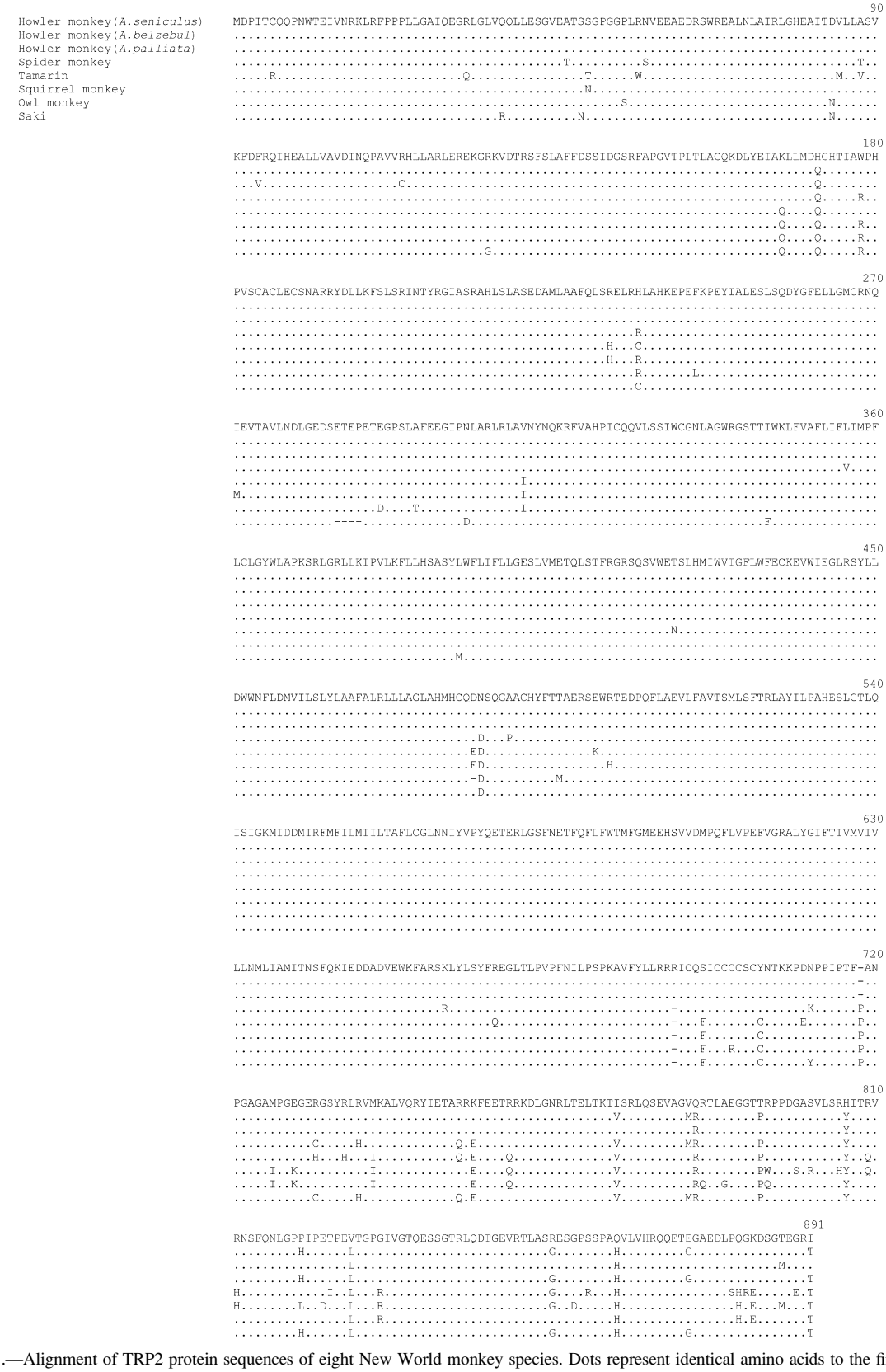

FIG. 1.—Alignment of TRP2 protein sequences of eight New World monkey species. Dots represent identical amino acids to the first sequence, and dashes represent alignment gaps.

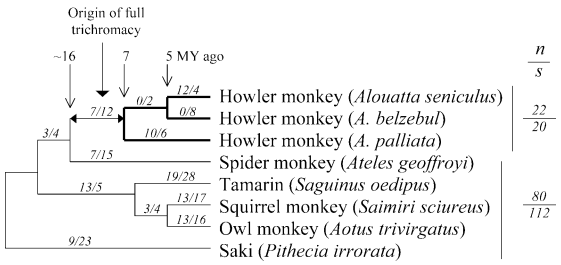

FIG. 2.—Nucleotide substitutions in the evolution of New World monkey TRP2 genes. Shown on each branch is the estimated number of nonsynonymous nucleotide substitutions (n), followed by the number of synonymous substitutions (s). The tree branches are separated into three groups. Group 1 branches are shown in boldface. There is only one branch belonging to group 2, and it is shown with arrowheads at both ends. All other branches form group 3. The total numbers of nonsynonymous and synonymous substitutions for group 1 and 3 branches are shown on the right-hand side of the figure. The species divergence times follow Cortés-Ortiz et al. (2003).

Because howler monkeys, a genus of NW monkeys, independently evolved full trichromacy (Jacobs et al. 1996), the above hypothesis predicts that these monkeys should also lose pheromone sensitivity as in OW monkeys and hominoids. Our present study, however, shows that the TRP2 gene, which encodes a critical component of the VNO pheromone transduction pathway, is intact in howlers and is under purifying selection, which strongly suggests that pheromone sensitivity is retained in howler monkeys. Previous behavioral studies also support this view. For example, it has been observed that male howlers smell and lick the female genital area (Glander 1980); males rub their own urine on their tails, legs, feet, and hands (Milton 1975); females in estrus rub their vaginal area on a branch and then males approach and smell the branch and stay around the females (Cortés-Ortiz 1998). Carrera-Sanchez (1993) reported that males and females smell the spots of urine of other individuals and that males smell and lick the flow of urine coming from females. Cortés-Ortiz (1998) observed that when a female is receptive, she may present her back to the male and drop some urine, and the male immediately sniffs the urine spots or the genital area of the female and then copulates with her. Similar rubbing, marking, sniffing, and/or licking behaviors have been observed in other NW moneys (Candland, Blumer, and Mumford 1980; Epple 1985; Epple and Smith 1985; Hennesey et al. 1978; Klein 1971; Mac and Kleiman 1978) but are rarely seen in OW monkeys and hominoids. In addition, anatomical examinations showed that NW monkeys, including howlers, have the VNO (Maier 1980; Hunter, Fleming, and Dixson 1984; Taniguchi et al. 1992). Thus, the genetic, behavioral, and anatomical data all indicate the presence of pheromone sensitivity in howlers. It may therefore be concluded that the emergence of full trichromacy does not make the pheromone communication dispensable in howlers.

The above conclusion may have two implications: (1) it may indicate that there is no correlation between the presence of trichromacy and lack of pheromone sensitivity, and (2) it may suggest that the phylogenetic concordance of the two traits in non-primate placental mammals, birds, and OW monkeys and hominoids is simply a coincidence. Alternatively, our results may indicate that although trichromacy is related to the loss of pheromone sensitivity, additional factors are also required for the replacement of the pheromone system by color vision. It is worth noting that the origin of trichromacy merely provides a new sensory mechanism, which will have to be coupled with a signaling mechanism to establish a new signaling-sensory channel to replace pheromone communication. In OW monkeys and hominoids, sexual swelling emits the visual signal, and in birds, colorful plumages serve the same function. In NW monkeys, including howlers, no

Table 1
Likelihood Ratio Tests of Various Hypotheses on w ($=d_N/d_S$) of Tree Branches in Figure 1

Hypotheses	w	ln$L$	np[a]
I. All branches have the same w	$w = 0.24$	−4949.69	15
II. Each branch has a unique w		−4946.07	27
III. Branch groups 1, 2, and 3 all have a different w	$w_1 = 0.27$, $w_2 = 0.21$, $w_3 = 0.24$	−4949.63	17
IV. Branch groups 1 and 2 have w_{12}, and group 3 has w_3	$w_{12} = 0.25$, $w_3 = 0.24$	−4949.68	16
V. Branch group 1 has w_1, and groups 2 and 3 have w_{23}	$w_1 = 0.27$, $w_{23} = 0.24$	−4949.65	16
VI. Branch groups 1 and 2 have $w_{12} = 1$, and group 3 has w_3	$w_{12} = 1$, $w_3 = 0.24$	−4963.14	15
VII. Branch group 1 has $w_1 = 1$, and groups 2 and 3 have w_{23}	$w_1 = 1$, $w_{23} = 0.24$	−4958.35	15

Hypothesis Testing[b]	Δ[c]	Degree of Freedom	Probability
II vs. I	7.24	12	0.84
III vs. I	0.12	2	0.94
IV vs. I	0.02	1	0.89
V vs. I	0.08	1	0.78
II vs. III	7.12	10	0.71
III vs. IV	0.10	1	0.75
III vs. V	0.04	1	0.84
IV vs. VI	26.9	1	2×10^{-7}
V vs. VII	17.4	1	3×10^{-5}

[a] Number of free parameters.
[b] Tests of whether the hypothesis listed first is significantly better than the one that follows.
[c] Twice the difference between ln$L$ of the hypothesis listed first and that of the hypothesis listed second.

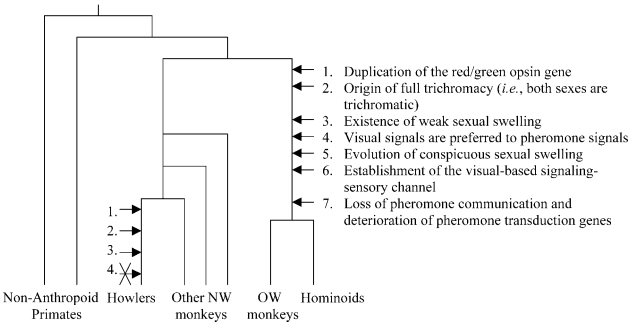

FIG. 3.—A model of the evolution of color vision and pheromone sensitivity in primates. An arrowhead with crosses indicates an unrealized event.

true sexual skins have been found (Dixson 1983). In certain NW monkey species, mild swelling may appear during estrus (Sillen-Tullberg and Moller 1993), but neither the color nor the size of this swelling is comparable to that in OW monkeys and hominoids (Dixson 1983). In open environments, visual signals may be preferred to pheromones because the former can be perceived at a distance whereas the latter requires physical contact. In closed areas such as dense forests, the advantage of visual signals over pheromone signals may diminish, as visual signals are more difficult to transmit. With this difference in mind, it is interesting to note that OW monkeys and hominoids are generally terrestrial and live in more open forests and savannas, while NW monkeys generally live in more dense tropical rainforests and are arboreal (Fleagle 1999). Such ecological differences may have provided a selective advantage for visual signals over pheromone signals in OW but not NW primates, thereby producing the difference in evolutionary force for sexual skin between the two groups of primates.

Taking into account this ecological factor, we propose a revised model of the impact of full trichromacy on the evolution of pheromone sensitivity in primates (fig. 3). According to this model, a critical step, advantage of visual signals over pheromone signals, did not occur in howlers, but occurred in OW monkeys and hominids and led to the difference in pheromone use between the two groups of primates. Further work is required to critically evaluate and possibly test this new model. It is also interesting to note that exaggerated sexual swellings were lost in some OW monkey and hominid species, most of which do not have multi-male social groups (Nunn 1999). Thus, while in our model visual signaling via sexual swelling is prerequisite for the loss of the pheromone sensory system, the visual signal may be subsequently lost in the absence of multi-male mating. It has been suggested that concealed ovulation can be advantageous to females in a single-male mating system, because it will promote paternal care (Sillen-Tullberg and Moller 1993; Nunn 1999).

In the analysis of incomplete TRP2 gene sequences of primates, Liman and Innan (2003) noticed a lower d_N/d_S ratio in rodents than in NW monkeys. With the availability of the full-length TRP2 sequences, we reevaluated their result. We found that $d_N/d_S = 0.10$ between the mouse and rat and equals 0.35 among the eight NW monkeys shown in figure 2; this difference is statistically significant ($P < 0.01$). Figure 1 shows that in NW monkey TRP2 sequences the C-terminus is more variable than other parts of the sequences. The same is also true in the mouse and rat TRP2 sequences. No apparent alteration in relative substitution rate among regions of TRP2 is found when the NW monkey sequences and rodent sequences are compared. A possible explanation of the above result of a d_N/d_S difference between NW monkeys and rodents is that TRP2 is under more relaxed functional constraints in NW monkeys than in rodents, as suggested by Liman and Innan (2003). However, previous studies showed that d_N/d_S is on average 1.5–2 times higher in primates than in rodents for average nuclear genes, probably because of fixation of slightly deleterious nonsynonymous mutations in primates, as the population size is believed to be smaller in primates than in rodents (Ohta 1995; Zhang 2000; Eyre-Walker et al. 2002). If this genome-wide factor is considered, the TRP2-gene–specific difference in d_N/d_S between NW monkeys and rodents becomes much smaller. From a biological point of view, if pheromone transduction is still used in NW monkeys, it is difficult to imagine how the functional constraint on TRP2 can be relaxed, as it would still need to be fully functional. More studies of proteins that interact with TRP2 may shed light on this puzzle.

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Literature Cited

Boissinot, S., Y. Tan, S. K. Shyue, H. Schneider, I. Sampaio, K. Neiswanger, D. Hewett-Emmett, and W. H. Li. 1998. Origins and antiquity of X-linked triallelic color vision systems in New World monkeys. Proc. Natl. Acad. Sci. USA **95**:13749–13754.

Britten, R. J. 2002. Divergence between samples of chimpanzee and human DNA sequences is 5%, counting indels. Proc. Natl. Acad. Sci. USA **99**:13633–13635.

Candland, D. K., Blumer, E. S., and M. D. Mumford. 1980. Urine as communicator in a New World primate Saimiri sciureus. Anim. Learn. Behav. **8**:468–480.

Carrera-Sánchez, E. 1993. Etograma del Mono Aullador (Alouatta palliata mexicana Merriam, 1902) en la Isla agaltepec, Lago de Catemaco, Veracruz. BSc. Thesis. Universidad Veracruzana. Xalapa, Veracruz, Mexico.

Cortés-Ortiz, L. 1998. Sistema de apareamiento y comportamiento sexual del mono aullador (Alouatta palliata mexicana) en semilibertad. MSc. Thesis. Instituto de Neuroetología. Universidad Veracruzana. Xalapa, Veracruz, Mexico.

Cortés-Ortiz, L., E. Bermingham, C. Rico, E. Rodriguez-Luna, I. Sampaio, and M. Ruiz-Garcia. 2003. Molecular systematics and biogeography of the Neotropical monkey genus, Alouatta. Mol. Phylogenet. Evol. **26**:64–81.

Dixson, A. F. 1983. Observations on the evolution and behavioral significance of "sexual skin" in female primates. Adv. Study Behav. **13**:63–106.

Dulac, C., and R. Axel. 1995. A novel family of genes encoding putative pheromone receptors in mammals. Cell **83**:195–206.

Dulac, C., and A. T. Torello. 2003. Molecular detection of pheromone signals in mammals: from genes to behavior. Nat. Rev. Genet. **4**:551–562.

Epple, G. 1985. The Primates I: Order Anthropoidea. Pp. 739–769 *in* R. E., Brown and W.D. MacDonald, eds., Social odours in mammals. Clarendon Press, Oxford, U.K.

Epple, G., and A. B. Smith. 1985. The Primates II: a case of study of the saddle back tamarin. Pp. 739–769 *in* R. E. Brown and W. D. MacDonald, eds., Social odours in mammals. Clarendon Press, Oxford, U.K.

Eyre-Walker, A., P. D. Keightley, N. G. Smith, and D. Gaffney. 2002. Quantifying the slightly deleterious mutation model of molecular evolution. Mol. Biol. Evol. **19**:2142–2149.

Fleagle, J. G. 1999. Primate adaptation and evolution. Academic Press, San Diego, Calif.

Glander, K. E. 1980. Reproduction and population growth in free-ranging mantled howling monkeys. Am. J. Phys. Anthropol. **53**:25–36.

Goodman, M., C. A. Porter, J. Czelusniak, S. L. Page, H. Schneider, J. Shoshani, G. Gunnell, and C. P. Groves. 1998. Toward a phylogenetic classification of Primates based on DNA evidence complemented by fossil evidence. Mol. Phylogenet. Evol. **9**:585–598.

Hennesey, M. B., C. L. Coe, S. P. Mendoza, E. P. Lowe, and S. Levine. 1978. Scent marking and olfactory investigatory behavior in the squirrel monkey (Saimiri sciureus). Behav. Biol. **24**:57–67.

Herrada, G., and C. Dulac. 1997. A novel family of putative pheromone receptors in mammals with a topographically organized and sexually dimorphic distribution. Cell **90**:763–773.

Hunter, A. J., D. Fleming, and A. F. Dixson. 1984. The structure of the vomeronasal organ and nasopalatine ducts in Aotus trivirgatus and some other primate species. J. Anat. **138**: 217–226.

Jacobs, G. H., and J. F. Deegan, 2001. Photopigments and colour vision in New World monkeys from the family Atelidae. Proc. R. Soc. Lond. Ser. B. Biol. Sci. **268**:695–702.

Jacobs, G. H., M. Neitz, J. F. Deegan, and J. Neitz. 1996. Trichromatic colour vision in New World monkeys. Nature **382**:156–158.

Kainz, P. M., J. Neitz, and M. Neitz. 1998. Recent evolution of uniform trichromacy in a New World monkey. Vision Res. **38**:3315–3320.

Keverne, E. B. 1999. The vomeronasal organ. Science **286**: 716–720.

Klein, L. L. 1971. Observations on copulation and seasonal reproduction of two species of spider monkeys Ateles belzebuth and A. geoffroyi. Folia Primatol. **15**:233–248.

Kumar, S., and S. Subramanian. 2002. Mutation rates in mammalian genomes. Proc. Natl. Acad. Sci. USA **99**:803–808.

Leypold, B. G., C. R. Yu, T. Leinders-Zufall, M. M. Kim, F. Zufall, and R. Axel. 2002. Altered sexual and social behaviors in trp2 mutant mice. Proc. Natl. Acad. Sci. USA **99**:6376–6381.

Liman, E. R., D. P. Corey, and C. Dulac. 1999. TRP2: a candidate transduction channel for mammalian pheromone sensory signaling. Proc. Natl. Acad. Sci. USA **96**:5791–5796.

Liman, E. R., and H. Innan. 2003. Relaxed selective pressure on an essential component of pheromone transduction in primate evolution. Proc. Natl. Acad. Sci. USA **100**:3328–3332.

Loo, S. K. 1973. A comparative study of the nasal fossa of four nonhuman primates. Folia. Primatol. **20**:410–422.

Luo, M., M. S. Fee, and L. C. Katz. 2003. Encoding pheromonal signals in the accessory olfactory bulb of behaving mice. Science **299**:1196–201.

Mac, D. S., and D. G. Kleiman. 1978. Distribution of scent marks in different contexts in captive lion tamarins Leonthopitecus rosalia (Primates). Pp. 181–188 *in* H. Rothe, H. J. Wolters, and J. P. Hearn, eds., Biology and behavior of marmosets. Eigenverlag Rothe, Göttingen, Germany.

Maier, W. 1980. Nasal structures in Old and New World primates. Pp. 219–241 *in* R. L. Ciochon and A. B. Chiarelli, eds., Evolutionary biology of the New World monkeys and continental drift. Plenum Press, New York.

Matsunami, H., and L. B. Buck. 1997. A multigene family encoding a diverse array of putative pheromone receptors in mammals. Cell **90**:775–784.

Meredith, M. 2001. Human vomeronasal function: a critical review of best and worst cases. Chem. Senses **26**: 433–445.

Milton, K. 1975. Urine-rubbing behavior of a solitary male howler monkey Alouatta palliata. Folia Primatol. **23**: 105–112.

Nathans, J., D. Thomas, and D. S. Hogness. 1986. Molecular genetics of human color vision: the genes encoding blue, green, and red pigments. Science **232**:193–202.

Nei, M., and S. Kumar. 2000. Molecular evolution and phylogenetics. Oxford University Press, New York.

Nunn, C. L. 1999. The evolution of exaggerated sexual swellings in primates and the graded-signal hypothesis. Anim. Behav. **58**:229–246.

Ohta, T. 1995. Synonymous and nonsynonymous substitutions in mammalian genes and the nearly neutral theory. J. Mol. Evol. **40**:56–63.

Podlaha, O., and J. Zhang. 2003. Positive selection on protein-length in the evolution of a primate sperm ion channel. Proc. Natl. Acad. Sci. USA **100**:12241–12246.

Rodriguez, I. 2003. Nosing into pheromone detectors. Nat. Neurosci. **6**:438–440.

Ryba, N. J., and R. Tirindelli. 1997. A new multigene family of putative pheromone receptors. Neuron **19**:371–379.

Sillen-Tullberg, B., and A. P. Moller. 1993. The relationship between concealed ovulation and mating systems in anthropoid primates. Am. Nat. **141**:1–25.

Silva, J. C., and A. S. Kondrashov. 2002. Patterns in spontaneous mutation revealed by human-baboon sequence comparison. Trends Genet. **18**:544–547.

Singer, S. S., J. Schmitz, C. Schwiegk, and H. Zischler. 2003. Molecular cladistic markers in New World monkey phylogeny (Platyrrhini, Primates). Mol. Phylogenet. Evol. **26**:490–501.

Steiper, M. E., and M. Ruvolo. 2003. New World monkey phylogeny based on X-linked G6PD DNA sequences. Mol. Phylogenet. Evol. **27**:121–130.

Stern, K., and M. K. McClintock. 1998. Regulation of ovulation by human pheromones. Nature **392**:177–179.

Stoddart, D. M. 1980. The ecology of vertebrate olfaction. Chapman and Hall, London.

Stowers, L., T. E. Holy, M. Meister, C. Dulac, and G. Koentges. 2002. Loss of sex discrimination and male-male aggression in mice deficient for TRP2. Science **295**:1493–1500.

Surridge, A. K., D. Osorio, and N. I. Mundy. 2003. Evolution and selection of trichromatic vision in primates. Trends Ecol. Evol. **51**:198–205.

Taniguchi, K., Y. Matsusaki, K. Ogawa, and T. R. Saito. 1992. Fine structure of the vomeronasal organ in the common marmoset (Callithrix jacchus). Folia. Primatol. **59**:169–176.

Yang, Z. 1998. Likelihood ratio tests for detecting positive selection and application to primate lysozyme evolution. Mol. Biol. Evol. **15**:568–573.

Yokoyama, S., and R. Yokoyama. 1989. Molecular evolution of human visual pigment genes. Mol. Biol. Evol. **6**:186–197.

Zhang, J. 2000. Rates of conservative and radical nonsynonymous nucleotide substitutions in mammalian nuclear genes. J. Mol. Evol. **50**:56–68.

Zhang, J., and M. Nei. 1997. Accuracies of ancestral amino acid sequences inferred by the parsimony, likelihood, and distance methods. J. Mol. Evol. **44**(Suppl 1):S139–S146.

Zhang, J., S. Kumar, and M. Nei. 1997. Small-sample tests of episodic adaptive evolution: a case study of primate lysozymes. Mol. Biol. Evol. **14**:1335–1338.

Zhang, J., H. F. Rosenberg, and M. Nei. 1998. Positive Darwinian selection after gene duplication in primate ribonuclease genes. Proc. Natl. Acad. Sci. USA **95**:3708–3713.

Zhang, J., and D. M. Webb. 2003. Evolutionary deterioration of the vomeronasal pheromone transduction pathway in catarrhine primates. Proc. Natl. Acad. Sci. USA **100**:8337–8341.

Zufall, F., K. R. Kelliher, and T. Leinders-Zufall. 2002. Pheromone detection by mammalian vomeronasal neurons. Microsc. Res. Tech. **58**:251–260.

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