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Coupling and decoupling of evolutionary mode between X- and Y-chromosomal red-green opsin genes in owl monkeys

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Abstract

We previously discovered Y-chromosomal red-green opsin genes in two types of owl monkeys with different chromosomal characteristics. In one type, the Y-linked opsin gene is a single-copy intact gene and in the other, the genes exist as multiple pseudogenes on a Y/autosome fusion chromosome. In the present study, we first distinguished the two types of monkeys as distinct allopatric species on the basis of karyotypic characteristics: *Aotus lemurinus griseimembra* (Karyotype III, diploid chromosome number [$2n$]=53) and *Aotus azarae boliviensis* (Karyotype VI; male $2n$=49; female $2n$=50), belonging to the northern and southern species groups, respectively, separated by the Amazon River system. Our sequence analysis revealed a common L1-Alu-Alu insertion between the two species in the 3′-flanking region of the X-linked opsin genes. The insertion was absent in the Y-linked opsin genes and in the human red and green opsin genes, indicating that it occurred in the X copy before the split into northern and southern species and after the X to Y duplication, i.e. duplication preceded speciation. We also show that in the northern species, the Y-linked opsin gene has evolved concomitantly with the X-linked copy whereas in the southern species, the Y-autosome fusion possibly led to decoupling evolutionary processes between X- and Y-linked copies and subsequent degeneration and duplications of the Y-linked opsin gene.

Keywords: *Aotus*; New World monkeys; Visual pigments; Gene conversion; Chromosomal fusion

1. Introduction

Photoreceptor cells in the vertebrate retina are distinguished into rods and cones. Rods work for dim light (scotopic) vision while cones work for daylight (photopic) vision. Color vision, an entity of photopic vision, can be achieved only with the presence of at least two spectrally distinct types of cones. Owl monkeys (*Aotus*), a genus of the New World monkeys (platyrrhine primates), are the only nocturnal higher primates (simians). They have features specialized for nocturnal vision. Their orbit size is the largest among simians. This, together with a relative increase in rod density (peak density 325,000/mm^2) compared to that of diurnal simians (~180,000/mm^2), supports their high visual sensitivity and enhanced scotopic acuity (Wikler and Rakic, 1990). In addition, they lack a foveola (a major central peak in cone density in the retina characteristic of diurnal primates). In diurnal simians, peak cone density is ~200,000/mm^2 (Wikler and Rakic, 1990) whereas peak cone density is only ~7000/mm^2 in owl monkeys, comparable to that in bushbaby (~8500/mm^2), a nocturnal prosimian. Owl monkeys have only one type of red-green cone visual pigment maximally sensitive to 539 nm (Hiramatsu et al., 2004); the blue visual pigment has been lost due to disruptive mutations (Jacobs et al., 1996) and the allelic polymorphism of the red-green opsins, a common feature of most New World monkeys, is

Abbreviations: bp, base pair(s); cDNA, complementary DNA; CDR, coding region; kb, kilobase(s); M/LWS, middle-to-long wavelength-sensitive; PAR, pseudoautosomal region.

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absent (Jacobs et al., 1993). These features preclude the possibility of color vision in owl monkeys and appear to support only a level of photopic acuity significantly inferior to that of diurnal simians.

Despite these nocturnal features, owl monkeys lack a tapetum lucidum, a reflective retinal structure in back of the photoreceptor layer and characteristic of nocturnal mammals. The rod density, though higher than that in diurnal primates, is relatively low compared to that in the nocturnal bushbaby (~450,000/mm^2) (Wikler and Rakic, 1990). Based on these characteristics, owl monkeys are considered to have a diurnal ancestry (Fleagle, 1999). Although utilizing a variety of auditory and olfactory signals, owl monkeys appear to be highly dependent on vision because their social behaviors (including intra-group calling and playing and inter-group fighting) almost exclusively occur under bright moon light; insect foraging also occurs at dawn, dusk and on moonlit nights (Wright, 1994). Owl monkeys are one of the most successful genera among New World monkeys, broadly distributed across South America from Panama to northern Argentina (Fleagle, 1999). They are adept leapers found in a variety of forest habitats and are primarily frugivorous, supplemented by both foliage and insects.

Phylogenetically, owl monkeys have traditionally been linked with the titi monkeys (*Callicebus*). Molecular studies, however, have classified them in the family Cebidae, in the subfamily Aotinae, together with Callitrichinae (marmosets and tamarins) and Cebinae (capuchins and squirrel monkeys) (Schneider, 2000). Owl monkeys can be divided into northern (gray-necked) and southern (red-necked) groups, widely inhabiting regions north and south of the Amazon River system, respectively. The northern and southern groups are comprised of four and five allopatric species, respectively, and are distinguished by karyotypes, geographic origins and pelage patterns (Hershkovitz, 1983) though details of the intra-group taxonomy are still controversial (Pieczarka et al., 1993). Pre-mating reproductive isolation between the two species groups appears to be established in the natural habitat since hybrids have not been found in river-bend cutoffs where species from the two groups are sympatric, though the two groups can be crossed in captivity with reduced fertility (Pieczarka et al., 1992). The karyotypical diversity is one of the most conspicuous characteristics among owl monkeys, exhibiting many intra- and inter-specific chromosomal variations, both numerical and structural; chromosome diploid numbers ranging from 46 to 58 in 18 karyotypes can be assigned to general karyotypically-defined taxa (Torres et al., 1998). Characteristic of the southern species group, three widely-distributed species (*Aotus nigriceps*, *Aotus azarae*, and *Aotus infulatus*) have been documented to have a Y/autosome fusion chromosome (Pieczarka and Nagamachi, 1988; Ma et al., 1989; Pieczarka et al., 1993).

In addition to the authentic X-chromosomal gene, we have recently identified extra red-green opsin genes on the Y chromosome in captive owl monkeys maintained in an institutional breeding colony (Kawamura et al., 2002). In Kawamura et al. (2002), two genes of red-green opsin were identified in one male monkey (no. 14), only one of which was transmitted to a single daughter. In situ hybridization indicated an XY location of these genes. The Y-linked opsin gene was found to have no structural defect (denoted 14Y). Multiple red-green opsin genes were identified in another male monkey (no. 29); one appeared X-linked and four others appeared Y-linked on the basis of inheritance patterns. These genes were also mapped to the sex chromosomes by in situ hybridization. The four Y-linked genes were pseudogenes (29Ys) and the Y chromosome was found to be fused with an autosome (Kawamura et al., 2002). The two types of monkeys were both classified as a single, widespread polytypic species, *Aotus trivirgatus*, which is now recognized as being comprised of multiple species as described above (Hershkovitz, 1983). Therefore, our previous species identification needs to be reconsidered by more detailed chromosomal characterization.

In the present study, we aimed to determine the species origins of two types of the owl monkeys (types 14 and 29) by re-examining their karyotypes. On the basis of the species relationships, we then sought to determine the translocation origin of the two types of Y-linked opsin genes by extensive DNA sequence analysis in order to gain better insight into the evolution of Y-chromosomal red-green opsin genes.

2. Materials and methods

2.1. Owl monkeys

The two types of owl monkeys (types 14 and 29) were found in a breeding colony in the Primate Research Institute of Kyoto University, Japan, on the basis of genomic organization of the red-green opsin genes in our previous study (Kawamura et al., 2002). Briefly, type 29 has multiple red-green opsin genes (29Y-1, 29Y-2, 29Y-3 and 29Y-4) with premature stop codons on the Y chromosome that is fused to an autosome. Type 14 has a canonical Y chromosome and has an intact red-green opsin gene (14Y). The X-linked opsin genes of type 14 (14X) and type 29 (29X) can be distinguished by their restriction site distribution. Founders of type 29 monkeys have a Bolivian origin; the origin of type 14 has not been recorded. All founder owl monkeys were introduced to the Institute in 1973–1977. Until the work of Hershkovitz (1983), owl monkeys were generally thought to contain a single widespread polytypic species, *A. trivirgatus*, and the founder monkeys were all recorded with this species name.

2.2. Chromosomal typing

Peripheral blood lymphocytes from four male owl monkeys (no. 14 from type 14 and nos. 24, 28 and 44 from type 29; see Kawamura et al., 2002 for their kin