Gene diversity and phylogenetic relationships in parrots

inferred from the nuclear seventh intron of β-fibrinogen gene sequences

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Introduction

Parrots consist of 350 species and three subfamilies (Cactuinae, Loriinae, and Psittacinae) according to systematics of Forshaw (1989); mainly distributed in the tropics of the world (Sibley and Alquist, 1995; del Hoyo *et al.*, 1997; Juniper and Parr, 1998). Recently, population of parrots decreased drastically and some species are becoming endangered to extinction. Therefore, conservation of parrot diversity is important. On the other hand, relationships among parrots are still insufficient. In particular, information on gene diversity and relationships of parrots on molecular level is required. As far as known, there is no information about relationships in parrots based on nuclear gene.

Although several recent authors have provided information on the phylogenetic utility of some nuclear exons, these sequences probably evolve too slowly for many comparisons at the species or generic level. Nuclear-gene introns sequence data have several properties that would seemingly make them ideal for phylogenetic studies, because they evolve more rapidly than exons (Prychitko and Moore, 2003). Introns are also attractive candidates for phylogenetic analysis because of their abundance in the nuclear genome, their convenient lengths, and potentially easy amplification by the PCR (Prychitko and Moore, 1997).

β-Fibrinogen is one of the nuclear genes consisting of exons and introns. Single seventh intron of β-fibrinogen gene is known as a non protein-coding gene (Prychitco and Moore, 1997) and has been described with regard to phylogenetic utility for birds at generic level (Johson and Clayton, 2000). Analysis of nuclear intron demonstrated the ability of the seventh intron of β-fibrinogen for the phylogenetic studies (Prychitco and Moore, 1997).

In my previous studies, cytochrome-*b* and nuclear exon RAG-1 genes sequence data were analyzed. In this study, I employed the seventh intron of the β-fibrinogen gene (β-FIB7) to obtain gene diversity and to construct phylogenetic relationships among parrots including some species from Africa, Australia, and South America.

Materials and Methods

Blood or tissue samples were obtained from 60 individual of 50 species of avian family Psittacidae (Psittaciformes) representing 27 genera and three subfamilies Loriinae, Psittacinae, and Cacatuinae (Forshaw, 1989). DNAs were extracted from each blood or tissue sample. A single fragment of intron7 fibrinogen gene was amplified using a primer pair (FIB-B17U and FIB-B17L) designed by Prychitko and Moore (1997) in PCR condition of 94 °C at 5 minute, 35 cycles of [94 °C- 30 sec., 46 °C-30 sec., 72 °C-60 sec.]. Amplified fragments were purified using PEG (polyethelene glycol) method and then DNA sequences of each sample were obtained. Alignment for the sequences data was done following the DNASIS version 2. Nucleotide substitutions and distances were calculated using DNAsa software. Then, PAUP* was used to reconstruct the

phylogenetic relationships with maximum parsimony (MP), neigbor-joining (NJ) methods.

Results

DNA sequence characters

Based on aligned sequences, several indels occurred in β-FIB7 of parrots, ranging from 1 to 189 bp. Indels were more common and concentrated toward the middle of the intron. Due to the presence of several indels, the fragment length of the FIB7 in parrots varied from 661 bp (*Agafornis*) to 850 bp (*Prioniturus platurnus*): from 808 to 817 bp in cockatoos (Cacatuinae), from 793 to 794 in lories and lorikeets (Loriinae), and from 804 to 850 in Psittacinae. In comparison with outgroups (*Columba* and *Accipiter*), total aligned fragments consisted of 1398 characters.

The mean of base frequencies of β-fibrinogen intron-7 was 28.67 % adenine, 21.63 % cytosine, 18.25 % guanine, and 31.45 % thymine. A test of homogeneity across taxa obtained by PAUP* was not significant. Unlike woodpeckers (Prychitco and Moore, 2000) and doves (Johson and Clayton, 2000) in which β-fibrinogen7 is AT rich. Nucleotide substitutions ranged from 2 to 115 sites. When all characters including indels were analyzed, there were 947 monomorphic sites, 451 polymorphic sites, and 300 parsimony- informative sites. Based on the sequence data obtained, there were variations in the sequences of the seventh intron of the β-fibrinogen gene in parrots. For instance, three individuals of *C. sanguinea* showed three haplotypes, and three individuals of *Eclectus roratus* exhibited three haplotypes.

Implication on the phylogenetic relationships among parrots

Maximum parsimony (MP) and neighbor joining (NJ) analyses produced phylogenetic trees shown in Figure 1. At subfamily level, both trees suggested that cockatoos (*Nymphicus, Probosciger,* and *Cacatua*) emerged as a monophyletic group. Monophyly of Loriinae (*Charmosyna, Chalcopsitta, Eos, Lorius, Pseudeos, and Trichoglossus*) was supported by only 52 % of bootstrap value in the NJ tree, but it was not supported by bootstrap value in the MP tree. Other parrots belonging to Psittacinae (*Agapornis, Alisterus, Aprosmictus, Anodorhynchus, Amazona, Ara, Aratinga, Cyclopsitta, Eclectus, Loriculus, Melopsittacus, Platycercus, Prioniturus, Psittaculirostris, Psittacus, Psittrichas, and Tanygnathus*) are paraphyletic.

Afro-Asian (Forshaw, 1989) parrots (*Loriculus* and *Agaforsnis*) emerged as a sister group. This result also suggested that parrots from South America (*Amazona, Ara, Aratinga,* and *Anodorynchus*) were grouped together as a monophyletic group and appeared to have emerged from *Psittacus erithacus* of Africa, while *Melopsittacus undulatus* of Australia emerged as a sister group of *Cyclopsitta* and *Psittaculirostris*.

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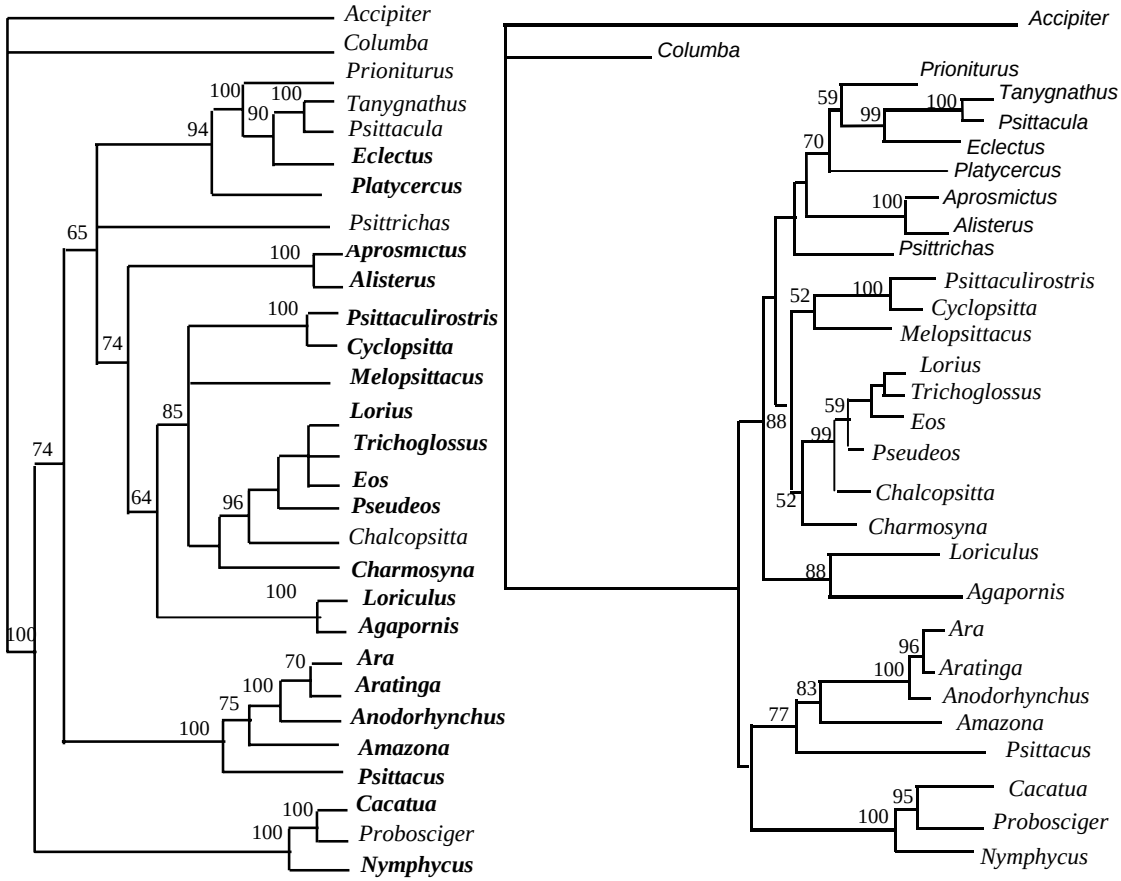

Fig. 1: Maximum-parsimony (left) and neighbor-joining (right) trees of parrots, based on sequence data of the seventh intron of the β-fibrinogen gene.