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Mitochondrial Cytochrome *b* Gene of the Korean Subspecies of the Common Kestrel (*Falco tinnunculus*)

Jin Hee Lee, Shi Hyun Ryu and Hee Cheon Park*

Department of Biology, College of Natural Sciences, Kyungpook National University,
Daegu 702-701, Korea

ABSTRACT

The mitochondrial cytochrome *b* gene of the Korean subspecies (*Falco tinnunculus interstinctus*) of the common kestrel has been analyzed. According to the molecular phylogeny of six subspecies of common kestrel, six subspecies of the common kestrel were divided into two clades: the first clade is composed of the South African subspecies (*F. t. rupicolus*) and the second clade includes 5 subspecies (*F. t. tinnunculus, F. t. rufescens, F. t. interstinctus, F. t. canariensis* and *F. t. dacotiae*) of the common kestrel. Korean subspecies, *F. t. interstinctus* was closely related to *F. t. rufescens* and original subspecies *F. t. tinnunculus*.

Key words: *Falco tinnunculus interstinctus*, mitochondrial cytochrome *b* gene, phylogeny, Korea

INTRODUCTION

The genus *Falco* in the family Falconidae includes 39 species worldwide and is morphologically divided into three groups: falcon, hobby, and kestrel groups (Ferguson-Lees and Christie, 2001). The falcon group has a rather small body size, long tail feathers, and short curved beak. Of 13 species recorded to the present, only one species is found in the New World, while the other twelve species are found in the Old World (Village, 1990). The common kestrel (*Falco tinnunculus*) in the falcon group is a typical kestrel well adapted to the variable habitats like a desert, mountain, farmland and urban area, and has eleven subspecies based on morphological and geographical characteristics (Ferguson-Lees and Christie, 2001) (Fig. 1). However, there is only one subspecies of the common kestrel, *F. t. interstinctus* found in Northeast Asia (The Korean Society of Systematic Zoology, 1997).

Until now, the mitochondrial cytochrome b gene sequences of the following five subspecies of the common kestrel have been identified in the GenBank: *F. t. canariensis, F. t. dacotiae, F. t. rufescens, F. t. rupicolus* and *F. t. tinnunculus* (Groombridge et al., 2002).

We have analyzed the mitochondrial cytochrome *b* gene of the Northeastern Asian subspecies, *F. t. interstinctus*, collected from Korea and reconstructed its molecular phylogeny among the subspecies of the common kestrel.

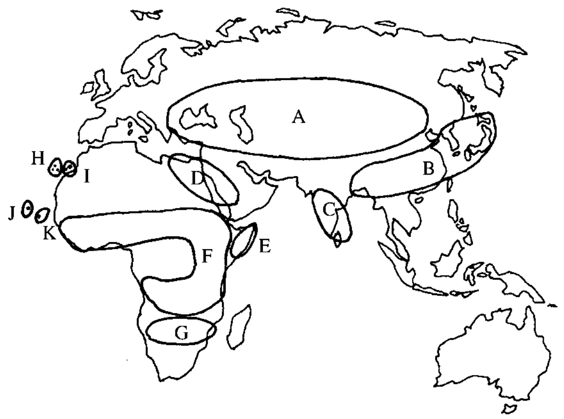

Fig. 1. Geographical distribution of the eleven subspecies of the common kestrel (*Falco tinnunculus*). An asterisk (*) indicates subspecies of common kestrel used in this study. A, *Falco tinnunculus tinnunculus**; B, *F. t. interstinctus**; C, *F. t. objurgatus*; D, *F. t. rupicolaeformis*; E, *F. t. archeri*; F, *F. t. rufescens**; G, *F. t. rupicolus**; H, *F. t. canariensis**; I, *F. t. dacotiae**; J, *F. t. neglectus*; K, *F. t. alexandri*.

MATERIAL AND METHODS

Samples

Two specimens of the Northeastern Asian subspecies of the common kestrel, *F. t. interstinctus* collected from Gyeongju, Korea and stocked at the Wildlife Rescue Center of Kyungpook National University, were used for this study.

Molecular techniques

Total genomic DNA was extracted from frozen tissues of

***To whom correspondence should be addressed**
Tel: 82-53-950-5351, Fax: 82-53-953-3066
E-mail: heecheon@knu.ac.kr

Table 1. GenBank accession numbers of the 6 subspecies and 8 species in the genus *Falco*

Species		Accession No.
Falco cenchroides	Australian Kestrel	AF279475
F. naumanni	Lesser Kestrel	AF279465
F. newtoni	Malagasy Spotted Kestrel	AF279476
F. rupicoloides	White-eyed Kestrel	AF279466
F. sparverius	American Kestrel	U83306
F. tinnunculus canariensis	Common Kestrel	AF279472
F. t. dacotiae	Common Kestrel	AF279473
F. t. rufescens	Common Kestrel	AF279468
F. t. rupicolus	Common Kestrel	AF279470
F. t. tinnunculus	Common Kestrel	AF279469
**F. t. interstinctus*	Common Kestrel	AY390349
outgroup		
F. peregrinus	Peregrine Falcon	AF090338
F. vespertinus	Western Red-footed Falcon	U83311

*Species from which mitochondrial cytochrome *b* genes were newly sequenced in this study

the specimens using of DNeasy Tissue System kit (QIAGEN). Forward and reverse primers to amplify the mitochondrial cytochrome *b* gene were as follows: mt-A (L-14995);

5-CTCCCGCCCCATCCAACATCTCAGCATGATGAAACTTCG-3, mt-F (H-16065); 5-CTAAGAAGGGTGGAGTCTTCAGTTTTTGGTTTACA AGAC-3 (Desjardins and Morais, 1990). PCR was performed under the following conditions: denaturation at 94°C for 1 min, annealing at 52°C for 1 min, and extension at 72°C for 1 min for 35cycles. PCR products were purified using QIAquick PCR Purification kits (QIAGEN) and then sequenced with automatic DNA sequencer (ABI 310 model, Perkin Elmer Co.).

Phylogenetic analyses

The mitochondrial cytochrome *b* gene sequences of five subspecies (*F. t. canariensis, F. t. dacotiae, F. t. rufescens, F. t. rupicolus* and *F. t. tinnunculus*) of common kestrel and seven species in the genus *Falco* were obtained from GenBank (Table 1). Eight species including the Korean subspecies *F. t. interstinctus* were used for phylogenetic analyses.

The mitochondrial cytochrome *b* sequences were aligned using the CLUSTAL X (Thompson et al., 1997). Phylogenetic analyses were performed with PAUP* 4.0b10 (Swofford, 2002) using maximum likelihood (ML) analysis. The ML analysis was conducted with transition (Ts)/transversion (Tv) ratio of 2 and HKY 85 variant (Hasegawa et al., 1985). Supports for the resulting nodes were tested using quartet puzzling with 1000 steps. *Falco peregrinus* and *F. vespertinus* were used as outgroup (Groombridge et al., 2002).

RESULTS AND DISCUSSION

There was no individual variation on the sequences of mitochondrial cytochrome *b* of Northeastern Asian subspecies, *F. t. interstinctus* (GenBank Accession Number AY390349).

The total length of the aligned Cyt *b* sequences data of six subspecies and eight species of the genus *Falco* was 998bp. Of 998 characters, 760 characters were constant. Of 238 variable sites, 104 positions were phylogenetically informative. Average frequencies of four nucleotide in the genus *Falco* were A-29.25%, C-34.47%, G-12.68%, and T-23.6%. The low level of G composition is similar to those found in other birds species: Cranes, A-28.3%, C-34.6%, G-12.2%, T-24.9% (Krajewski and King, 1996); Owls, A-26.4%, C-35.8%, G-13.9%, T-23.8% (Ryu and Park, 2003); Blue bird, A-27.1%, C-35.8%, G-12.3%, T-24.8% (Kirchman et al., 2001); and Swallow, A-27.4%, C-35.1%, G-13.2%, T-24.3% (Whittingham et al., 2002).

There were two clades in the ML tree among the subspecies of the common kestrel. One clade contains only one subspecies, namely, the South African common kestrel subspecies, *F. t. rupicolus* grouped with the Australian falcon species, *F. cenchroides*. The other clade includes five common kestrel subspecies, *F. t. tinnunculus, F. t. rufescens, F. t. interstinctus, F. t. canariensis*, and *F. t. dacotiae* (Fig. 2).

The South African subspecies, *F. t. rupicolus* was appa-

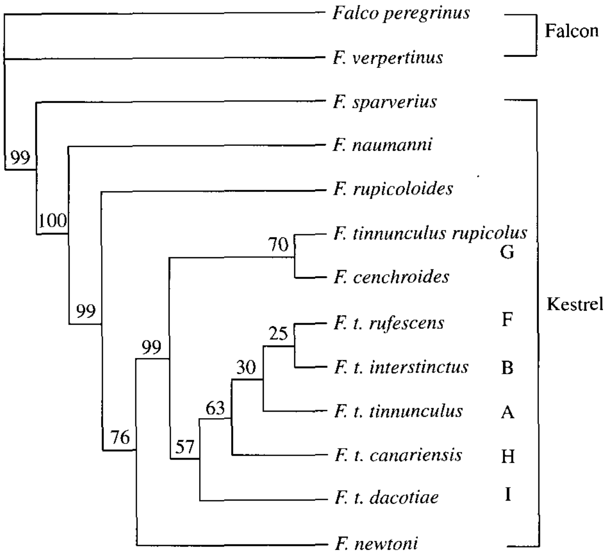

Fig. 2. Maximum likelihood tree based on mitochondrial cytochrome *b* gene sequences of the genus *Falco* including Korean common kestrel subspecies. See Fig. 1 for the geographical distribution of the subspecies.

rently separated from the other five subspecies with large nucleotide differences of 13bp (1.3%). However, there were just average 2.8 nucleotide differences (0.28%) between the five subspecies except the South African subspecies. South African subspecies is different in the pattern of their feathers from other five subspecies, and having especially short tail feather and tarsus (Cade, 1982; Clark, 1995; Perlo, 1999; Ferguson-Lees and Christie, 2001). Additionally, ecologically this subspecies is often found in arid areas (Van Zyl, 1997) compared with the nominate subspecies, *F. t. tinnunculus*. This South African subspecies showed obviously differences in morphological, ecological, and genetic characteristics compared to five other common kestrel subspecies.

The Northeastern Asian subspecies, *F. t. interstinctus* is closely related to the Central African subspecies, *F. t. rufescens*, which belongs to the same clade with just two nucleotide differences at site 31 and 946, although these two subspecies are distributed in the distant and different continents, Eurasia and Africa. Additionally, both subspecies are closely related to nominate subspecies, *F. t. tinnunculus* with only one nucleotide difference.

There was few nucleotide differences between subspecies of the common kestrel, although we confirmed a rather reasonable phylogenetic tree showing the subspecies relationship of the common kestrel in the genus *Falco*.

Therefore, to clarify the taxonomic position and phylogeny of the subspecies in the common kestrel, *F. tinnunculus*, by morphological characteristics, such as body size and color pattern of the feathers and by the geographical division (Ferguson-Lees and Christie, 2001; Perlo, 2002; Stevenson and Fanshawe, 2002), it needs more molecular data of other subspecies of the common kestrel, as well as, from other gene sequences.

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