

Phylogenetic relationships among Falcon species (genus *Falco*) according to DNA sequence variation of the cytochrome b gene

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ABSTRACT

A phylogeny of 17 taxa of the genus *Falco* plus two Accipitrids, an owl and the domestic chicken as outgroups was reconstructed based on 300 nucleotide positions of the mitochondrial cytochrome b gene. DNA was isolated from blood, the target gene was amplified using the polymerase chain reaction and sequenced directly. The results indicate that falcons are monophyletic and more closely related to Accipitridae than to owls or galliformes. Typical kestrels (*F. tinnunculus*, *F. naumanni*, *F. punctatus*), three members of the subgenus *Hypotriorchis* (*F. eleonora*, *F. concolor*, *F. subbuteo*) and three members of the subgenus *Hierofalco* (*F. rusticolus*, *F. biarmicus*, *F. jugger*) each form monophyletic groups. The latter subgenus, however, appears to be polyphyletic with *F. mexicanus* more closely related to the Peregrine Falcon than to the other members. In the Saker (*F. cherrug*) two very different mtDNA haplotypes were found, one almost identical to Gyrfalcon, the other one related to the Peregrine. This dichotomy can be explained by past hybridization, probably with *F. pelegrinoides*. Four taxa of the *F. peregrinus/pelegrinoides* complex were almost identical and may be conspecific. The position of *F. femoralis* and *F. columbarius* within the phylogeny is presently uncertain. The *F. rusticolus* group appears to be the oldest evolutionary lineage among the Falcon species studied. The advantages of DNA sequence data in reconstructing phylogenies and the taxonomic implications of the results are briefly discussed.

INTRODUCTION

Morphologically, the members of the genus *Falco* are a very homogeneous group of species, which makes it difficult to assess phylogenetic relationships within the genus (Cade 1982). Interest in resolving such relationships is more than 100 years old, as early treatments by Suschkin (1905) and Kleinschmidt (1901) demonstrate. However, most previous attempts had to rely on morphological and plumage characters (Jollie 1977; Boyce & White 1987), which are obvious adaptations

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to a certain way of life and are therefore prone to evolve convergently. Cade (1982) presented a hypothetical phylogenetic tree of the genus *Falco* in which the Australian Brown Falcon *F. berigora* is assumed to be closest to the common ancestor and kestrels are hypothesized to be most primitive among all other living falcons. Seven subgenera assumed to represent monophyletic groups are recognized.

Only recently have techniques become available to study directly the DNA sequences of birds and other organisms and to use these sequences, among other goals, to reconstruct phylogenies. Such data enable us to test some of the hypotheses implicit in Cade's (1982) phylogenetic tree of falcons:

- 1) is the grouping into subgenera correct in the sense that they do represent monophyletic groups?
- 2) what is the relationship among these subgenera and
- 3) which of them is closest to the common ancestor of all falcons?

We have determined and compared partial sequences of the mitochondrial cytochrome b gene to study the relationships of a total of 17 falcon taxa, mostly from the Palearctic. These include four sub- or semispecies of the Peregrine Falcon complex and five species of the subgenus *Hierofalco*, often regarded as a superspecies (Kleinschmidt 1923-1937; Baumgart 1975).

RATIONALE OF DNA STUDIES

There are a number of features that make DNA sequences superior to any other kind of data in assessing phylogenetic relationships (cf. Hillis & Moritz 1990):

- The variability at the DNA level is much higher than at the protein level, because the genetic code is redundant, i. e. several amino acids are coded for by up to six different codons.

- Therefore, most base substitutions that occur in protein-coding regions are so-called "silent" substitutions, i. e. they do not lead to an amino acid change in the protein. Per definition, silent substitutions cannot lead to convergent evolution, i. e. similarities observed at silent positions are due either to common descent or to chance (homoplasy).

Most recent DNA studies on bird phylogeny have utilized the mitochondrial genome (rather than chromosomal genes), mainly for two reasons: mtDNA is about ten times more variable than nuclear genes in higher vertebrates; it is inherited clonally and not subject to recombination, i. e. all individuals will be homozygous with respect to the locus studied. Among mitochondrial genes the cytochrome b sequence is well characterized and is now becoming a standard reference for phylogenetic and taxonomic studies in birds (Kocher *et al.* 1989; Edwards *et al.* 1991; Richman & Price 1992). Its advantages include a moderately high rate of base substitution, availability of near-universal primer sequences for PCR (polymerase chain reaction) amplification and a relatively large published data set for comparison (ref. see above). A disadvantage of mitochondrial DNA is that it does not directly identify hybrids, because it is inherited purely maternally.

LABORATORY METHODS AND DATA ANALYSIS

Small quantities of blood (100-200 μ l) were collected in EDTA-NaF preservation buffer (Arctander 1988) from captive and wild falcons in various countries (Table 1). The sampling procedure is very easy and does not harm the birds. Total DNA was isolated according to standard procedures and the cytochrome b gene was amplified using the polymerase chain reaction with primers modified from Kocher *et al.* (1989). The primary product was run and extracted from a 1 % agarose gel and reamplified asymmetrically with a primer ratio of 1:100. A portion of 300 nucleotides, i. e. about one third of the entire cytochrome b gene, was then sequenced directly with a third primer according to Sanger *et al.* (1977).

Sequences were aligned and rates of synonymous base substitutions estimated using the weighted pathway method (Li *et al.* 1985). Phylogenetic trees were constructed using the neighbour-joining procedure (Saitou & Nei 1987) of the program package PHYLIP (Felsenstein 1991) and the maximum parsimony method of PAUP 3.0 (Swofford 1991). To assess the Species

Table 1: List of species sequenced with origin and number of individuals analysed per species. F. = Falco.

Species	Origin	number
<i>Otus choliba</i>	Argentina	1
<i>Buteo buteo</i> , Common Buzzard	Germany	3
<i>Accipiter nisus</i> , Sparrowhawk	Germany, Israel	3
<i>F. jugger</i> , Lagger Falcon	India	1
<i>F. rusticolus</i> , Gyrfalcon	Greenland, Baltic Sea	3
<i>F. biarmicus</i> , Lanner	Italy, N-Africa	2
<i>F. femoralis</i> , Aplomado Falcon	Bolivia	1
<i>F. columbarius</i> , Merlin	Scandinavia	3
<i>F. naumanni</i> , Lesser Kestrel	Spain	3
<i>F. tinnunculus</i> , Eurasian Kestrel	Netherlands, Israel	4
<i>F. punctatus</i> , Mauritius Kestrel	Mauritius	4
<i>F. eleonora</i> , Eleonora's Falcon	Greece	2
<i>F. concolor</i> , Sooty Falcon	Saudi Arabia	2
<i>F. subbuteo</i> , Eurasian Hobby	Germany	3
<i>F. mexicanus</i> , Prairie Falcon	USA	1
<i>F. cherrug</i> , Saker	Pakistan, SE-Europe, Kazakhstan	8
<i>F. p. peregrinus</i> , Peregrine	Scotland	1
<i>F. p. calidus</i> , Peregrine	Saudi Arabia	1
<i>F. p. pelegrinoides</i> , Barbary Falcon	Saudi Arabia	3
<i>F. p. babylonicus</i> , Red-naped Shahin	Pakistan, Iran	3

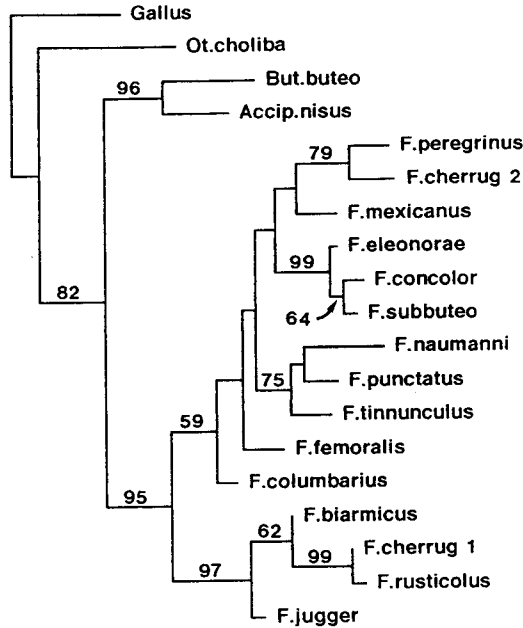
robustness of the various groupings within the phylogenetic tree we performed 260 parsimony bootstrap replicates with PAUP 3.0 (heuristic search, random sequence addition).

RESULTS

Excluding the primer regions, the analysis is based on a total of 300 nucleotide positions (Seibold *et al.* 1993). Of these, 119 were variable within the total set of species, 76 positions varied within the falcons. Between one and eight individuals were sequenced per species. Intraspecific sequence variation was negligible with a maximum of two nucleotide differences, but usually none (exception: Saker Falcon, see below). We used the domestic chicken (*Gallus gallus*; Desjardins & Morais 1990) and the Tropical Screech Owl (*Otus choliba*; Heidrich, Wink, König in prep.) as outgroups to root the partial phylogenetic tree of Falconiformes. Within the raptor part of the tree a Buzzard (*Buteo buteo*) and a Sparrowhawk (*Accipiter nisus*) were included as outgroups with respect to the falcons (Fig. 1). Branch lengths in the tree are proportional to genetic distances between taxa, but not necessarily to evolutionary divergence times (due to variation of substitution rates among lineages). Numbers given for some branches show the percent frequency with which the respective groupings were found by the parsimony bootstrap test. These values indicate the level of confidence with which each clade is supported by the data.

Fig. 1:

Phylogenetic tree of falcons (genus *Falco*) plus two other raptors (*Buteo buteo*, *Accipiter nisus*), chicken (*Gallus domesticus*) and an owl (*Otus choliba*) as outgroups. "*F. peregrinus*" includes the subspecies *peregrinus*, *calidus*, *pelegrinoides* and *babylonicus*. The tree is based on 300 nucleotide positions of the cytochrome b gene and was constructed using the heuristic search option (informative characters only) with "closest" sequence addition (PAUP 3.0). The neighbor-joining procedure (Saitou & Nei 1987) with non-synonymous substitution rates (Li et al. 1985) produced a tree with identical topology except that the positions of *A. femoralis* and *F. columbarius* were interchanged. Numbers are parsimony bootstrap values derived from 260 replicates (only values > 50 % are shown).



The results can be interpreted as follows: The 17 falcon taxa investigated represent a monophyletic group and are more closely related to other birds of prey (*Buteo*, *Accipiter*) than to owls or galliforms. Within the Genus *Falco* the typical kestrels (*F. tinnunculus*, *F. naumanni*, *F. punctatus*) on the one hand and all three members of the subgenus *Hypotriorchis* investigated (*F. subbuteo*, *F. eleonorae*, *F. concolor*) on the other hand are each other's closest relatives. Both these findings are in agreement with previous hypotheses, but the genetic distance between Common and Lesser Kestrels was relatively large for a species pair which is morphologically so similar. Interestingly, the Mauritius Kestrel was more similar to Lesser than to Common Kestrel, even though the latter breeds in Africa, much closer to Mauritius, than does the highly migratory, Palearctic Lesser Kestrel. The various taxa of the Peregrine complex not surprisingly fall into one clade, and among each other they are so similar that with the data available so far we are unable to resolve any species limits between them. If they are distinct species, they must be much more closely related among each other than any other species pair in this phylogeny.

The most surprising results of our study concern the subgenus *Hierofalco*, which according to Cade (1982) includes *F. cherrug*, *F. mexicanus*, *F. biarmicus*, *F. rusticolus* and *F. jugger*. Gyrfalcon, Lanner and Lager Falcon are closely related, but Prairie Falcon clusters with the Peregrine Falcon. Two very different mtDNA haplotypes were identified in the Saker: Type I was almost identical to the Gyrfalcon, Type II differed by 12 % of the nucleotide positions from Type I and clearly resembled the Peregrine. Birds carrying one or the other type originated from the same regions (SE-Europe, Kazakhstan, Pakistan) and there was no relationship with plumage pattern (e. g. barred versus unbarred upperparts). The bootstrap values indicate a probability of less than 3 % for the possibility that the branch leading to Gyrfalcon, Lanner, Lager and Type I Saker is an error

(Fig. 1).

Furthermore, the Gyrfalcon group seem to be the first lineage to have diverged from the ancestral Falcon stock, all other species studied were derived later. The positions of Merlin and Aplomado Falcon within the tree is rather uncertain, as indicated by the short branch-lengths; we cannot state with any degree of confidence which other species they are most closely related to, but they do seem to be similar to each other.

DISCUSSION

Our phylogenetic tree based on partial cytochrome b sequences agrees with previously suggested phylogenies in that typical kestrels, three members of the subgenus *Hypotriorchis* and the *rusticolus*-*jugger*-*biarmicus* group are each monophyletic. In the case of Hobby, Eleonora's and Sooty Falcon the obvious similarities in body proportions, colouration, hunting and migratory behaviour may actually be due to common descent and reflect the species' close evolutionary relationships (Walter 1979). It was unexpected that Hobby and Sooty Falcon clustered more closely than did Sooty and Eleonora's Falcon (although this grouping is not strongly supported, see Fig. 1). Based on distribution, morphometrics and biology, the latter two appear to be sister species (Walter 1979).

Sequence data of the large falcons of the subgenus *Hierofalco* provided some unexpected insights: the subgenus appears to be polyphyletic according to our data, because the Prairie Falcon is more closely related to the Peregrine than to Gyrfalcon or Lanner. The subgenus *Hierofalco* was defined by Kleinschmidt (1901) as a group of ecologically and morphologically similar species replacing each other in various parts of the world. They share similarities in plumage structure and pattern and body proportions (broad-based wings, long tail), which may obviously have evolved convergently as adaptations to similar hunting strategies in open terrain, where all these species live. In agreement with our findings, a chromosome study showed the Prairie Falcon to be more similar to Peregrine than to Gyrfalcon (Schmutz & Oliphant 1987). Courtship displays and vocalizations of Prairie Falcon include similarities to both Peregrine and Gyrfalcon (Wrege & Cade 1977).

Electrophoretic patterns of feather proteins (Olsen et al. 1989) and one type of mt-DNA sequence found in this study support the idea that the Saker Falcon also belongs to the subgenus *Hierofalco*. The fact that two very different mtDNA haplotypes occurred in Sakers, one similar to Gyrfalcon, the other to Peregrine Falcon, can be explained by past hybridization between male Sakers and female Peregrines and subsequent back-crosses with Sakers. Although very few such cases have been observed in the wild and Saker x Peregrine hybrids are sterile today (Heidenreich & Pfeffer, pers. comm.), this may not have been the case in the more distant past. The amount of sequence divergence between Peregrine and Saker Type II indicates that the hypothesized hybridization took place several million years ago. Since mitochondrial DNA is inherited clonally, the Peregrine-like haplotype may have persisted in the Saker population until today. The same scenario could potentially also account for the fairly close relationship of mtDNA sequences of Peregrine and Prairie Falcon (only 1 individual of the latter was studied), two species between which hybridization has been recorded in the wild (Oliphant 1991). If this was true, the subgenus *Hierofalco* may be monophyletic after all, but the hybridization must be phylogenetically very old, because the Prairie Falcon sequence differs from the Peregrine even more than the Saker Type II.

The DNA data confirm the very close relationship of the four *F. peregrinus/pelegrinoides* taxa studied. Barbary Falcon (*F. pelegrinoides*, incl. Red-naped Shahin, *F. p. babylonicus*) and Peregrine are very similar genetically and may be conspecific (see Weick 1989). More extensive sequence data will be needed to clarify whether any consistent differences between the two forms do exist. Perhaps they have become separated very recently or are on the way to speciation. Detailed field observations are required in the small areas where their breeding ranges overlap (in Morocco and perhaps northern India) to find out whether they behave as distinct biological species.

Contrary to earlier hypotheses (Suschkin 1905; Brown & Amadon 1968; Cade 1982), Kestrels do not seem to be close living relatives of the ancestral falcon. In the mitochondrial DNA tree this position is occupied by the Gyrfalcon group. Obviously, however, our tree is incomplete and the inclusion of South American, Australian and New Zealand falcons or the African Kestrels may

may show some other species to be more primitive.

We conclude that cytochrome b sequences are sufficiently variable to untangle intrageneric relationships of falcons. Although we so far studied less than half of all living species, the DNA data allowed a more detailed resolution of relationships than a previous protein electrophoretic study (Olsen et al. 1989). The case of two highly divergent genotypes in the Saker, however, illustrates how hybridization may lead to discrepancies between gene genealogy and species phylogeny, a problem one should always be aware of when interpreting molecular phylogenies (Avice 1989). Occasional hybridization between full biological species is fairly widespread among birds (Grant & Grant 1992) and even if hybrids are sterile today, they may have been fertile in the past.

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