MIDDLE EAST FALCON RESEARCH GROUP

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Molecular phylogeny of the saker (*Falco cherrug*) and other desert falcons

Michael Wink. Universität Heidelberg, Germany

Abstract

Nucleotide sequences of the mitochondrial cytochrome b gene were used to reconstruct the phylogenetic relationships between falcons, Sagittariidae, Cathartidae, Accipitridae and some members of the Ciconiiformes (sensu Sibley & Monroe) and within the falcon family. Falcons represent a monophyletic group which do not share a close ancestry with the other families of raptors. Within the falcon family Caracaras, and Hierofalcons are separated from Merlins, Peregrines, Kestrels and the other falcons. In contrast to most other birds studied, Saker cytochrome b sequences occur in three haplotypes: Haplotype III shows a genetic distance of 0.5 to *F. jugger* and 1.3% to *F. biarmicus*; haplotype II has a distance of 0.3% to *F. rusticolus* and a third haplotype (I) is distantly (4.3%) related to *F. peregrinus*. It is suggested that haplotype III represents the "true" Saker, whereas haplotypes I and II were caused by hybridisation in the evolutionary past. Although these first data need to be corroborated with material from birds of certified origin, it can be speculated already that haplotype II represents the Altai falcon.

Introduction

In the last decade several molecular techniques have been developed which allow to study the ecology and evolution of birds in much greater detail and precision than ever before. Depending on the level of relatedness ("evolutionary window") to be analysed, different markers have to be applied (Table 1): For paternity studies (mating systems), DNA fingerprinting with multilocus-probes (minisatellites, oligonucleotides), singlelocus probes (microsatellites; singlecopy genes), and PCR methods such as RAPD (Randomly amplified polymorphic DNA) or microsatellite-PCR are the methods of choice. The same methods can be used to identify and to trace the origin of an individual raptor, that is kept in an aviary. For systematic and phylogenetic studies, sequences of marker genes allow the reconstruction of phylogenetic relationships between species, genera and families of birds. If population differences are to be analysed, then the mitochondrial D-loop region would be the appropriate marker. For relationships between species and genera, usually the mitochondrial cytochrome b gene (or other mtDNA) is an excellent marker. Higher order relationships are often difficult to assess and more conservative genes or DNA-DNA hybridisation would be more appropriate than cytochrome b (Meyer 1994). For a comprehensive description of the use of molecular markers in ecology and evolution the reader is referred to Hoelzel (1992) and especially Avise (1994); techniques are explained in Ertlich (1989), Innis et al.
The development of the polymerase chain reaction (short PCR) has far-reaching implications for most fields of biology and medicine (Table 2). Because of its speed and sensitivity the method has already revolutionised modern science, since PCR allows the rapid and specific amplification of any piece of DNA to such a degree that this DNA can be further processed by electrophoresis, sequencing or cloning (Eitrich, 1989; Innis et al., 1990; Zimmermann et al., 1992; Wink & Wehrle, 1994; Avise, 1994).

The principles of PCR are schematically explained in Fig. 1: Total DNA is heated to >94 °C so that both strands become separated ("melting" or "denaturation" of DNA). If PCR primers are present (these are small pieces [usually 15 to 30 bases long] of single-stranded DNA) and the temperature is lowered to <55 °C, an annealing of PCR primers occurs with homologous regions of the target DNA (for which they have been tailored). Then the temperature is increased to 72 °C at which Taq-polymerase from the thermophilic microorganism, *Thermus aquaticus*, has its reaction optimum. Starting at the respective PCR primers the corresponding DNA strands are copied. The reaction is terminated after 30 to 60 seconds by heating the vial to 94 °C. Then a new cycle of annealing and synthesis follows, which is repeated ca 30 times. Since the amount of amplified PCR product is doubled every cycle, about $10^9$ copies of a piece of DNA are theoretically obtained after 30 cycles (Fig. 1 B). Since usually primers or nucleotides become depleted during the last cycles and the Taq polymerase looses its activity, the reactions is no longer exponential in the end. Even $10^8$ copies of DNA are sufficient to perform further analysis, such as electrophoresis, restriction analysis or nucleotide sequencing.

The crucial element of PCR is the sequence of the PCR primers. A prerequisite usually is that the sequence of the target gene must be known. The better their homology to the nucleotide sequence of the target DNA the more efficient and specific the following PCR. This is especially important, when PCR is used for medicinal diagnostics or the amplification of marker genes. In a few applications, PCR employs short primers of 10 bases length which do not bind to a specific gene but to several targets; the technique of RAPD (pronounced "rapid": randomly amplified polymorphic DNA) has become important for several areas of population genetics.

PCR will also revolutionise genetic studies of raptors and other birds and can be used to elucidate close genetic relationships, such as paternity as well as to reconstruct the phylogeny of genera, families or even phyla of organisms (Table 1 and 2). Some recent examples for the use of DNA sequences in bird systematics (mostly outside the raptor group) are: Baker (1992), Cooper et al., (1992), Edwards et al. (1991), Edwards and Wilson (1990), Kocher et al., (1989), Richman and Price (1992), Avise et al.,
The advantages of DNA sequences are several fold: the major problem of morphological studies is convergence, which can indicate a close relationship where there is none. During evolution mutations occurred in a rather random fashion; if they took place in a critical position which would make the corresponding protein useless, these mutants were usually eliminated and lost. If these mutations occurred at the third position in a codon they are usually “silent”, which means they do not lead to amino acid substitutions. These mutations are extremely useful for phylogenetic reconstructions since they are not subjected to selection pressure and reflect the evolutionary past. However, multiple mutations in third codon positions can occur which then lead to homoplasy (Avise, 1994). That the third codon position is indeed most variant can be seen from Table 3, which illustrates an alignment of partial sequences of the cytochrome b gene in falcons.

The Saker falcon (Falco cherrug J. E. GRAY, 1834) has been classified as a member of the Hierofalco group (which includes also the Gyr (F. rusticolus), the Lanner (F. biarmicus) and the Laggar (F. jugger). The taxonomy of the Saker appears to be complicated and controversial, since this taxon shows a high degree of morphological (both colour morphs and size) variation: Two subspecies have been recognised by Del Hoyo et al. (1994): F. c. cherrug occurring from Central Europe throughout SW Russia, Ukraine and Iran to the river Yenisey and foothills of Altai and wintering from Europe and NE Africa to NW India. F. c. milvipes occurring in SE Siberia, N-Mongolia and China and wintering from Iran to Nepal, NW India, Tibet and Central China. A dark colour morph which appears closer related to the Gyr falcon and which lives in SW Siberia, NW Mongolia and W China has been treated as “F. altaicus” (Sibley & Monroe, 1990; Ellis, 1995): other authors consider this taxon as a morph besides several other forms or races (Del Hoyo et al., 1994). Since some Saker populations suffer from human activities (e.g. falconry, hunting, changing habitats), it is important to establish and know the genetic status of the different subspecies, forms and populations.

In this study we have employed the nucleotide sequences of the cytochrome b gene to reconstruct the phylogenetic relationship of the Saker falcon within the Falconidae and that of falcons as compared to other raptors.
Material and methods

Sequence analysis

DNA isolation. PCR methods, DNA sequencing and phylogenetic reconstructions followed methods outlined in several previous papers (Seibold et al., 1993, 1995; Wink 1995; Wink et al., 1996). Sequence data and origins of samples have been documented in Seibold (1994) or in Wink (1995).

Nucleotide sequences were aligned with the cytochrome b sequence of G. gallus (Desjardins & Morais 1990). Phylogenetic trees were reconstructed using the maximum parsimony method (MP) with the phylogeny program PAUP 3.1.1 (Swofford 1993) and the distance method neighbour-joining (NJ) as implemented in program package MEGA 1.0 (Kumar et al. 1993). In the neighbour-joining analyses genetic distances were calculated based on Jukes Cantor or the Tamura-Nei method, which takes into account the strong transition-transversion and base composition bias found in our data. With PAUP, heuristic algorithms were employed. Bootstrap analyses (Felsenstein, 1985) were performed to evaluate the robustness of the frcations found: the higher the values, the better the support of a furcation by the sequence data.

Microsatellite PCR

DNA was amplified by PCR using the PCR primer CACCACCACCACCCAC which produces polymorphic bands in the Hierofalco group. PCR products were separated by agarose gel electrophoresis (1.4% agarose) and visualised under UV light (after staining with ethidium bromide).

Results and discussion

Phylogeny of the Falconiformes

In a first set of experiments we tried to reconstruct the phylogenetic relationships between falcons and other raptors based on more than 1000 basepairs of the mitochondrial cytochrome b gene. Since falcons have been united with the families Accipitridae, Pandionidae, Sagittariidae and Cathartidae in the order Falconiformes (Del Hoyo et al., 1994) or in the Infraorder Falconides (Sibley and Monroe, 1990), we would expect that the phylogenetic reconstructions show a close connection between these groups. As can be seen from Fig. 2, which represents a bootstrap analysis of 85 taxa and other members of the Ciconiiformes (sensu Sibley & Monroe, 1990) carried out with the distance method "neighbour joining", falcons represent a monophyletic clade (supported by a bootstrap value of 99%). The falcon clade does not
cluster together with the Accipitridae, nor do the Sagittariidae and Cathartidae, indicating that falcons, secretary birds and New World vultures constitute unique groups of raptors which do not share (a close) ancestry with the Accipitridae. It is doubtful therefore, whether the order Falconiformes represents a natural and phylogenetic assemblage (Wink, 1995). The finer structures within the phylogeny of the Accipitridae and Cathartidae have been discussed already in Seibold et al. (1995), Wink & Seibold (1996), Wink (1995) and Wink et al. (1996a,b). But a few significant results will be reviewed in the following:

Vultures

_Aegypius_ and _Torgos_ represent a related sister clade, which together with _Trigonoceps_ form a monophyletic group. In view of their genetic similarities it needs to be discussed in the future whether they really represent monotypic genera as normally assumed (Sibley & Monroe, 1990; Del Hoyo et al., 1994). Members of the _Gyps_ (and _Pseudogyps_-complex are very closely related and also monophyletic. Although some morphological differences exist in the _Gyps_ complex (Del Hoyo et al., 1994), genetic distances imply that they are all members of one genus, e.g., _Gyps. Necrosyrtes_ clusters at the base of the _Aegypius Gyps_ complex and not with _Neophron_ as was sometimes suggested. _Circaetus gallicus_ is positioned at the base at the _Gyps-Aegypius_ complex and not with the eagles as one might expect.

_Gypaetus_ and _Neophron_ form another vulture clade and are not related to the other Old World vultures. A close analysis of skeletal and muscular characteristics and their postembryological development had already pointed out a common ancestry of both taxa (Jollie 1976, 1977; Thaler et al., 1986).

The New World Vultures and Condors _Vultur, Gymnogyps, Coragyps_ and _Cathartus_ cluster outside the Accipitridae and definitely unrelated to the Old World Vultures. Morphological data and DNA-DNA hybridisation studies have implied that New World vultures do not belong to the Accipitridae and that they and storks have shared common ancestry (Garrod, 1873; Ligon, 1967; König 1982; Rea, 1983; Sibley and Ahlquist, 1990). If we consider the genetic distances between New World vultures and Old World vultures or condors and storks or storks and Accipitridae distance values are between 14.89 to 15.89%. As can be seen from Figure 2, storks together with spoonbill, ibis, flamingo, pelican and shearwater appear in a common clade together with the New World vultures, indicating rather a heterogenous assemblage (see low basal bootstrap values). Summarising these evidences, we concluded that a close relationship of cathartid vultures with storks seems unlikely (more details in Wink, 1995).
Vultures thus appear in at least 3 positions in the phylogenetic tree, indicating that their similarities in lifestyle and biology is due to convergence and not to common descent (Avise et al., 1994, Wink 1995).

Eagles

Eagles comprise a heterogenous group of large raptors including among others “booted eagles” (genera *Aquila* and *Hieraaetus*), Martial eagle (*Polemaetus*), Short-toed snake eagles (*Circaetus*) and Sea eagles (genus *Haliaeetus*). As expected from morphological and anatomical evidence (Brown & Amadon, 1968; Cramp & Simmons, 1980; Del Hoyo et al., 1994), birds of the “booted-eagle”-complex and those of the *Haliaeetus*-complex are not closely related.

Within the *Aquila*-complex sensu stricto 3 clades are apparent: 1.) *A. nipalensis*, *A. rapax*, *A. heliaca* and *A. adalberti*; genetically, all 4 taxa are clearly differentiated and represent distinct species (Seibold et al., 1995). 2.) *A. pomarina* and *A. clanga* represent closely related sibbling species. 3.) *A. chrysaetos*, *A. verreauxii* and surprisingly *H. spilogaster* fall into a single clade. *H. pennatus* and *Polemaetus bellicosus* are closely related to the *Aquila*-complex; it needs to be established whether these genera can be maintained as genera of their own right or they should be included into *Aquila*.

The sea eagles represent a monophyletic group which is related to kites (*Milvus*) and buzzards (*Buteo*) but not to the booted eagles (*Aquila*-complex) (Wink et al., 1996): *H. albicilla* and *H. leucocephalus* represent sister species, to which *H. pelagicus* and *H. leucoryphus* are connected. A third clade (which occurs mainly in the southern hemisphere) includes *H. vocifer*, *H. leucogaster* and *H. sanfordi* (more details in Wink et al., 1996).

Kites, hawks, harriers and buzzards

Kites of the genus *Milvus* share ancestry with sea eagles and buzzards. Buzzards of genus *Buteo* and honey buzzards of the genus *Pernis* are obviously of polyphyletic origin and not related (more details in Wink & Seibold; 1995. Wink, 1995), which has also implied from morphological and behavioural differences between both groups (Cramp & Simmons, 1980; Del Hoyo et al., 1994). *Pernis* appears as a basal taxon of the eagle/ buzzard complex and is often clustered in or adjacent to the *Gypaetus Neophron* clade (Wink & Seibold, 1995; Wink, 1995). Buzzards of the genus *Buteo* and *Parabuteo* appear in a monophyletic clade. Genetic distances between the Old World (*B. buteo*, *B. rufouscucus*) and New World (*B. galapagoensis*, *B. lineatus*, *B. jamaicensis*) buzzards are relatively small suggesting a more recent speciation and dispersal in this group of raptors.
Goshawks (genus *Accipiter*) and harriers (genus *Circus*) appear to share ancestry with the buzzard/sea eagle/kite complex and usually cluster at the base of this clade. *Circus cyaneus hudsonius* from North America is already well separated from the Old World nominate form, *C.c. cyaneus* and might represent a distinct species.

**Phylogeny of the Falconidae**

Since the phylogenetic resolution within distinct clades can be reduced or even slightly distorted in trees which comprise as many and diverse taxa as in Fig. 2, we have independently inferred the phylogeny within the family Falconidae (Fig. 3 to 5) employing a smaller data set. The South American Caracaras cluster at the base of the falcon tree (Seibold et al., 1993; Heibig et al., 1993).

As can be seen from Figures 2 to 4, falcons can be divided in two larger clades, representing the Hierofalco group (including *F. rusticolus*, *F. jugger*, *F. biarmicus* and *F. cherrug* II & III) and the other falcons of the genus *Falco*. The Prairie falcon, *F. mexicanus* which had been included in the Hierofalco group by some authors, obviously does not cluster with the Desert falcon, but is closer to the Merlin/Peregrine assemblage (Fig. 2 and 3). This finding would agree with behavioural and karyological data (Del Hoyo et al., 1994).

The *Tinnunculus* group consists of *F. tinnunculus*, *F. naumanni*, and the African *F. punctatus* and *F. rupicoloides* but not of the North American *F. sparverius*. Morphological data had implied a close relationship between *F. sparverius* and *F. tinnunculus* (Cade, 1982) and this species has been considered to form a superspecies with *F. tinnunculus* (Sibley & Monroe, 1990). Although its true nearest relative has not been found yet without ambiguity (see low bootstrap values in Fig. 3), a *sparverius tinnunculus* relationship, however, is highly unlikely.

*F. eleonoraee, F. concolor* and *F. subbuteo* which share many biological characters form a closely related clade which had been recognised as a the separate subgenus Hypotriorchis. Here, the morphological and behavioural features and molecular data are in agreement.

The *Peregrinus*-complex consists of *F. peregrinus* (various subspecies cannot be distinguished with the cyt b gene: Fig. 5) and the closely related *F. pelegrinoides*. Since the differences between *F. pelegrinoides* and *F. peregrinus* are so small (genetic distance 0.6% for the full cyt b gene), it needs to be studied whether *F. pelegrinoides* represents a true species or rather a subspecies.
The North American Merlin, *F. c. columbarius* differs significantly from the European *F. c. aestival*. Genetic distances indicate that both taxa have been separated for more than 1 million years (if we assume a molecular clock of 2% sequence divergence per 1 million years; Shields & Wilson, 1987) and might have reached species status already.

The Red-footed falcon (*F. vespertinus*) has been considered to be closely related to the Amur falcon (*F. amurensis*) which substitutes *F. vespertinus* in East Asia (Sibley & Monroe, 1990). As reported earlier (Wink et al., 1996) both taxa are indeed closely related and form genetically distinct sister species.

The systematic position of *F. femoralis, F. sparverius, F. columbarius*, and *F. vespertinus* could not be resolved with certainty, as can be seen from the rather low bootstrap values in both the MP and NJ reconstructions (Fig. 2 to 4).

Phylogenetic relationships of the saker falcon

As can be seen from Fig. 2 to 5, the Saker Falcon appears in 3 different haplotypes. Type I occurs in the "Peregrine assemblage", Type II is closely related to the Gyr falcon and Type III is in the Laggar/Lanner clade and closely related to *F. jugger*.

Since the numbers of mutations correlate with time ("molecular clock hypothesis"), we can assume that high genetic distances (e.g. a high number of nucleotide substitutions between a species pair) indicate a long divergence time. It has been calculated that a 2% genetic distance in mitochondrial genes corresponds to roughly 1 million years of divergence (Shields and Wilson, 1987). Although the calibration of this clock is very crude, nevertheless this equation can be useful to interpret the Saker data. Since *F. peregrinus* and *F. cherrug* I are separated by 4.5%, both species should have separated from a common ancestor, more than 2 million years ago. Since birds of type I have a Saker phenotype, type I can only be derived from a hybridisation of ancestral Sakers with ancestral Peregrines. Later, the hybrids must have introgressed into the Saker population. A similar explanation applies to *F. cherrug* II (distance to *F. rusticolus* 0.3%), whose ancestor must have hybridised with the Gyr falcon about 150,000 years ago. *F. cherrug* III appears to represent the "true" Saker and was separated from the Laggar about 200,000 and from the Lanner about 650,000 years ago (respective distances are 0.5 and 1.3%) (Fig. 4).

In order to test whether these 3 haplotypes correlate with morphs or geographic origins, we have sequenced 9 birds of Type I, 7 birds of Type II and 5 birds of Type III (only partial sequences of 300 bp lengths were analysed). As can be seen from a Neighbour joining analyses (Fig. 5) the 3 haplotypes
remain separated and no intraspecific variation occurs within the three haplotypes. Geographical origins
do not reveal a clear pattern, i.e. all haplotypes appear to be distributed to some degree all over the
species' range. But this impression might be an artefact, since the origin of several birds (which came
from various sources) is certainly doubtful and incorrect.

In another set of experiments we have used a different approach in that we have employed
microsatellite PCR to analyse genetic variation of nuclear DNA (as opposed to the mt DNA assessed with
the cytochrome b gene). As can be seen from Fig. 6 and Table 4, several bands are generated which reveal
the relatedness between F. peregrinus, F. jugger, F. biarmicus, F. rusticolus and the 3 F. cherrug
haplotypes. These data are very preliminary but indicate that there are alleles combining F. rusticolus with
F. cherrug I, II, and III and others which show a close connection of F. cherrug I, II, and III with F.
jugger and F. biarmicus. On the other hand there are bands which are unique for F. peregrinus, F.
rusticolus, F. biarmicus, F. jugger and F. cherrug, respectively, implying that these 5 taxa represent
distinctive genetic units.

Conclusions

Our data clearly show that falcons represent a monophyletic clade separated from other raptors.
Within the falcon family, the Saker represents an enigma since it occurs in 3 distinct haplotypes. Since the
origins of the birds studied appear to be unreliable and also their morphology is partly unrecorded, we
cannot relate genetic data with colour morphs and geographical origin. We suggest that all 3 haplotypes
come from falcons with a Saker phenotype. Assuming that these 3 haplotypes constantly interbreed, we
should expect that various morphological traits (which were introduced into the Saker by former
hybridisations with the Peregrine and the Gyr Falcon) should turn up every now and then (especially if the
corresponding alleles are recessive): A morphological variation would have been postulated for the Saker
if only the genetic data were available; and indeed variability is most obvious trait in the Saker in Nature.
Since the hybridisation between Gyr and Saker occurred some 150000 years ago (if the molecular clock
can be applied for this case) then we should expect that Gyr falcon traits, should be visible in birds derived
from this line. It is tempting to speculate that the Altai falcon, which has many Gyr falcon traits (Ellis
1995), is represented by haplotype II. Work is underway with samples from birds of certified origin
(collected by D. Ellis and R. Kenward) to clarify this issue.

Acknowledgements

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References


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Table 2. PCR as a key technique in the study of the molecular ecology, genetics and evolution of birds

**BLOOD, TISSUE SAMPLE, FEATHER**

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+----------------+----------------+----------------+----------------+----------------+
|                 | DNA            | PCR            | RAPD           | MICRO-          |
| BLOOD, TISSUE   |                |                |                |                |
| SAMPLE, FEATHER|                |                |                |                |
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**RAPID**

- Parentage
- Sex determination
- Population genetics
- Phylogeny

**MICRO-**

- Parentage
- Sex determination
- Population genetics
- Phylogeny

**SSCP**

- Parentage
- Sex determination
- Population genetics
- Phylogeny

**SEQUENCING**

- Parentage
- Sex determination
- Population genetics
- Phylogeny
| Species                | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | Sequence | 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Alignment of nucleotide sequences of the cytochrome b gene of falcons

Note that most nucleotide substitutions occur at 3rd codon positions which are marked with an *.

Table 3. Alignment of nucleotide sequences of the cytochrome b gene of falcons

Note that most nucleotide substitutions occur at 3rd codon positions which are marked with an *;
Table 4. Microsatellite analysis of Desert falcons

*= Band present; (*) band present in some but not all birds; - = band not present

Individuals analyzed were as in Fig. 5.

<table>
<thead>
<tr>
<th>DNA band</th>
<th>Peregrine</th>
<th>Laggar</th>
<th>Gyr</th>
<th>Saker</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td>1</td>
<td>*</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>*</td>
<td>(*)</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>*</td>
<td>(*)</td>
<td>(*)</td>
</tr>
<tr>
<td>11</td>
<td>-</td>
<td>*</td>
<td>(*)</td>
<td>(*)</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
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<td>-</td>
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</tr>
<tr>
<td>13</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>*</td>
</tr>
</tbody>
</table>
Figure 1. Schematic illustration of PCR

A. Changes between denaturation, primer annealing, polymerase reaction

B. Exponential multiplication of PCR products during PCR
Figure 2. Phylogenetic reconstruction of the families Falconidae, Sagittariidae, Cathartidae and Accipitridae in relation to some members of the Ciconiiformes inferred from nucleotide sequences of the cytochrome b gene (c 1000 bp)

Analysis by the distance method Neighbour Joining (distance algorithm: Jukes Cantor). Numbers indicate bootstrap values obtained from 500 replications. Branch lengths are proportional to the number of nucleotide substitutions and divergence time.
Figure 3. Phylogenetic relationships within the Falconidae based on 1020 bp of the cytochrome b gene. Other procedures as in figure 2.
Figure 4. Phylogenetic relationships within the Falconidae

Analysis by the character state method Maximum parsimony (Swofford 1993); heuristic analysis and 100 bootstrap replications. Tree lengths 541 steps (min. 355, max. 1066 steps); CI = 0.656, RI = 0.738, HI = 0.344.

Illustration as a phylogram, in which branch lengths are proportional to divergence time (indicated in % nucleotide substitution for the Saker); Values below branches are bootstrap values, those above indicate the number of nucleotide substitutions.
Figure 5. Relationships between the three Saker haplotypes and a few selected other falcons (F. jugger, F. biarmicus, F. rusticulus, F. peregrinus and some subspecies; F. chicquera and F. eleonorae).

Analysis was based on 300 bp of the cytochrome b gene; origins of the Sakers is indicated after each individual (note that these origins can be wrong!); other conditions as in Fig. 2.
Saker I
Saker I
Saker II
Saker II
F. tinnunculus
F. pelegrinoides
F. peregrinus
F. rusticulus
F. biarmicus
F. biarmicus
F. juggar
Saker III
Saker III
Saker III
Saker III
Saker III
Saker III

Figure 6. Illustration of a separation of microsatellite PCR products by agarose gel electrophoresis.
Roundtable discussion on the systematics and taxonomy of the saker falcon

Conclusions and recommendations proposed by Professor Clayton White with suggestions by Professor Michael Wink

The results of investigations on the systematics and taxonomy of the saker falcon can be summarised as follows and suggest that the following approach would be most profitable.

1. The phenotypic morphological variation of sakers is highly variable but the extent of the variation on a geographical basis is unknown at present. It may be determinable but the variation may also prove to be broadly individual with slight differences geographically but along broad, gradual lines. This needs to be determined.

2. The molecular variation based on DNA studies suggest that there are at least three genetic subpopulations. So far such variation has not been able to be aligned with any specific phenotypically distinct group. The molecular variation may have a geographic basis but this is unknown.

3. It seems that large samples of blood specimens should be screened to see if, with sample size, a correlation can be made between haplotype and phenotype.

4. If this can be done then a geographic basis of both molecular and phenotypic variation might be assigned.

5. It follows then, that it would be possible to monitor the falcons taken into captivity to see if there is random taking of all molecular types and if birds from across the geographic range are being harvested or whether they come from certain geographic regions.

6. Probably, the molecular type will be reflected in the phenotype of the bird.

7. Such data would be necessary to properly manage the saker for long term use without adverse impact on any one local population. The goal being to maintain the genetic diversity (biodiversity if you will) within the species, which is the main goal of conservation biology.