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Phylogeography and population structure of the saker falcon (*Falco cherrug*) and the influence of hybridization: mitochondrial and microsatellite data

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Abstract

Microsatellite as well as sequence analysis of the mitochondrial control region were applied to infer phylogeography and population genetic structure of the saker falcon (*Falco cherrug*). Furthermore, we compared the patterns of mitochondrial haplotypes with the variation of microsatellite alleles among the species of the hierofalcon complex (*F. cherrug*, *Falco rusticolus*, *Falco biarmicus*, *Falco jugger*) to test hypotheses on population history. Historical samples from museum specimens of *F. cherrug* were analysed together with samples from contemporary populations to investigate possible influences of hybrid falcons escaped from falconry on the genetic composition. In the mitochondrial DNA analysis, none of the four species represents a monophyletic group. Moreover, there are no clearly defined groups of haplotypes corresponding to taxonomic entities. In the microsatellite analysis most of the variation is shared between species and no clear differentiation by private alleles is found. Yet, with a Bayesian clustering method based on allele frequencies, a differentiation of *F. cherrug*, *F. rusticolus* and two geographic groups of *F. biarmicus* was detected. Results from both nuclear and mitochondrial markers are compatible with the previously postulated 'Out of Africa' hypothesis assuming an African origin of the hierofalcons. From an ancestral African population, *F. cherrug*, *F. rusticolus* and *F. jugger* split off in separate waves of immigration into Eurasia and South Asia. A combination of evolutionary processes, including incomplete lineage sorting as well as hybridization, may be responsible for the currently observed genetic patterns in hierofalcons.

Keywords: *Falco cherrug*, hierofalcons, hybridization, microsatellites, mitochondrial control region, phylogeography

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Introduction

Phylogeographic analyses have proven powerful in elucidating patterns of gene flow, hybridization, historical range fragmentation, range expansion, and speciation among many bird species (Newton 2003). Most speciation scenarios taking into account the role of Pleistocene glaciations suggest that ancestral populations were split by climate–induced ecological barriers and subsequently evolved in allopatry. Whether these changes lead to speciation, depends on the evolution of reproductive isolation before or after secondary contact (Liebers *et al*. 2004). In most cases, however, it is not known if such barriers have already been established or are just developing. Here genetic data can help to uncover the degree of differentiation and the influence of historical events like climatic shifts and ecological changes. Furthermore, they may reveal patterns of geographic variation within the gene pool of a species or a species complex and indications of incipient speciation. However, to obtain a comprehensive picture, the respective taxa should be investigated over their entire distribution range.

In the present study, we focus on a group of large falcons (genus *Falco*) which form a superspecies complex, the hierofalcons (Kleinschmidt 1901; C. M. White *et al*. in del Hoyo *et al*. 1994), which comprise the following species

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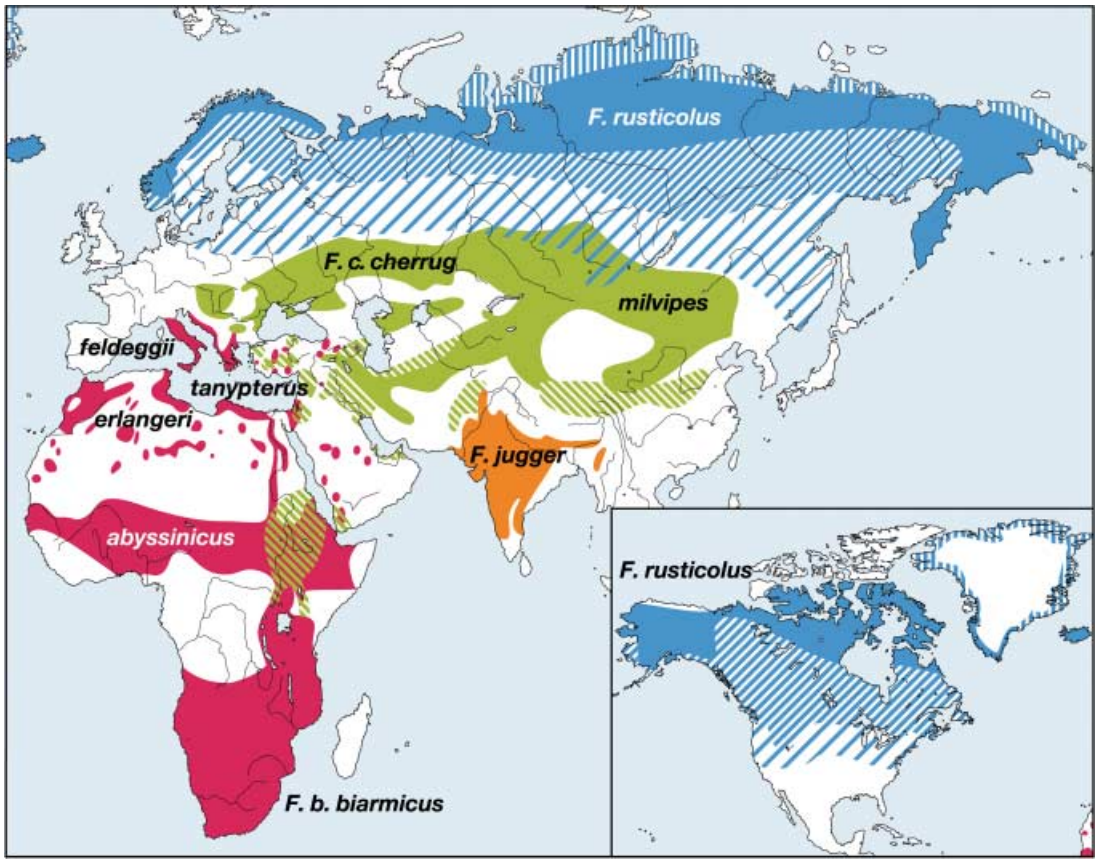

Fig. 1 Geographic distribution of the four hierofalcons at subspecies level (modified after Dementiev & Gladkov 1969; Ferguson-Lees & Christie 2001; Potapov & Sale 2005). Coloured areas are inhabited year-round, vertically hatched areas for breeding only, narrow diagonally hatched areas are normal wintering grounds, and diagonally wide hatched areas are extreme wintering ranges. Colour codes of the *Falco* species in the study areas: green, *F. cherrug*; purple, *F. biarmicus*; blue, *F. rusticolus*; orange, *F. jugger*.

(Fig. 1): saker falcon (*Falco cherrug*), lanner falcon (*Falco biarmicus*), laggar falcon (*Falco jugger*), and gyr falcon (*Falco rusticolus*). According to previous genetic studies, these hierofalcons are an assemblage of species not clearly differentiated in mitochondrial (mt) markers (control region, cytochrome *b*) analysed so far (Wink *et al*. 2004; Nittinger *et al*. 2005). The latter study was primarily focused on *F. biarmicus*, while the other hierofalcons were represented only by a limited number of individuals. Nevertheless, one striking outcome of that study was the occurrence of *F. cherrug* individuals in two distantly related haploclades. Therefore, we intended to confirm these findings by analysing a more extensive sample of *F. cherrug* comprising populations from a wide geographic range.

Falco cherrug is a species of the Palearctic avifauna favouring forest steppe, steppe and grassland, as well as open and dry habitats where scattered trees or electric pylons serve as breeding places. The migrating part of the population winters south of the breeding range (Fig. 1). Currently, two subspecies are distinguished (Ferguson-Lees & Christie 2001): *Falco c. cherrug* (east Europe to south-central Siberia) and *Falco c. milvipes* (from south-central Siberia to northeastern China). Even though only listed in Appendix II of the CITES (Convention on International Trade in Endangered Species of Wild Flora and Fauna), *F. cherrug* is classified as 'globally threatened' with an estimated population size of 3600–4400 pairs only (BirdLife International 2006). In eastern Asia (Mongolia, southern Siberia and northern China), the observed decline is partly due to the use of rodenticides which has decimated especially Brandt's vole (*Microtus brandti*) populations (Dawaa *et al*. 2005), one major food resource of *F. cherrug*. Additionally, as a consequence of indirect poisoning the *F. cherrug* population has crashed dramatically in this region (Potapov & Bailey 2003). Therefore, many breeding areas have become deserted, even in locations in Kazakhstan and Mongolia (Levin 2000), from where samples analysed in the present study have been obtained in the mid-1990s. Another problem for the wild populations is the use of trapped falcons for hunting, especially in Central Asian countries and the Middle East. Migrating birds were trapped, exported mostly to countries of the Arabian Peninsula, and released there after one

hunting season. However, due to drastic political, social and economic changes after the breakup of the Russian Federation, the access to the central Asian countries became facilitated and the increasing demand for saker falcons was accommodated by new trade routes. Thus, trappers intensified nest robbing and harvesting of adult and young falcons from the breeding populations throughout Asia (Levin 2000; Xiaodi & Fox 2003; Karyakin *et al*. 2004). In Central Europe, the population has suffered a drastic decline in the 1960s to 1970s caused by insecticides, habitat fragmentation, and human persecution (Baumgart 1991). Currently, populations have recovered in many areas. For example, in Hungary, the core area of the European distribution range, the population size was estimated at a maximum of 30 pairs in 1980 and at 145 pairs in 2002 (Bagyura *et al*. 2004). Present estimates for the Central European population range from 173 to 225 pairs (Mebs & Schmidt 2006). In recent years, captive breeding and training of large falcons for the international market has gained importance. Methods of artificial insemination were developed in the 1970s, allowing intercrosses even among large falcon species. In hierofalcons, hybridization is easily accomplished in various parental combinations giving rise to fertile offspring (Heidenreich *et al*. 1993). One severe problem with this practice is that a certain number of the hybrid falcons bred in captivity, mainly crosses between *F. rusticolus* and *F. cherrug*, escape within the breeding area of wild *F. cherrug* populations, a process that is still continuing in Central Europe today (Anonymus-1 2000). These hybrids may interbreed with wild individuals as it occasionally happened between *F. peregrinus* and *F. rusticolus*/*F. peregrinus* hybrids in Scandinavia and Germany (Lindberg & Nesje 2000).

One specific aim of the present study was to analyse the population structure of *F. cherrug* and to assess possible gene flow between the populations using microsatellite loci and variation in the mt control region (CR). Moreover, concerning the Central European *F. cherrug* population, we compared the genetic composition of historical samples collected before the 1970s with later samples to assess possible genetic changes since the time when cross-breeding through artificial insemination started. More general objectives were to compare the patterns of mt haplotype differentiation with the variation of nuclear microsatellites within the hierofalcon species complex to test previous hypotheses on population history (Nittinger *et al*. 2005) and the possible effect of Pleistocene glaciations on the phylogeographic structure of hierofalcons.

Materials and methods

Sampling

In total, 244 hierofalcons (see Appendix) were used, comprising *Falco cherrug* (n = 186, unrelated specimens covering a major portion of the distribution range), *Falco biarmicus* (n = 33), *Falco rusticolus* (n = 19), and *Falco jugger* (n = 5). Blood (n = 135), muscle tissue (n = 12) or feather (n = 60) samples were taken from contemporary living or dead individuals, whereas skin pieces of footpads (n = 37) were collected from museum specimens. Not all individuals could be successfully analysed with both marker systems (nuclear and mitochondrial); thus, the respective data sets differ in size (CR: n = 200, microsatellites n = 240). In general, the shorter microsatellite loci were easier to amplify than the mt CR fragment (460 bp). Because of the limited amount of DNA, four individuals could not be completely genotyped for the microsatellite loci. The mt data set also includes CR sequences from individuals analysed previously (Nittinger *et al*. 2005). According to their geographic origin, all samples were assigned to 12 groups which were treated as populations in the genetic analyses. *F. cherrug* populations originated from South Kazakhstan (SKA, n = 25), North Kazakhstan (NKA, n = 13), South Siberia (SSI, n = 5), Central Mongolia (CMO, n = 10), Eastern Mongolia (EMO, n = 46), and Central Europe (CEU, n = 82). The samples from Central Europe were further subdivided into contemporary (CEU-c, n = 60) and historical samples dated before 1970 (CEU-h, n = 22). For all other populations (*F. cherrug* as well as the other species), we did not distinguish between historical and contemporary samples. One individual from Pakistan, one from Afghanistan, and two individuals from Turkmenistan were pooled in a Central Asian group (CAS, n = 4). *F. c. 'altaicus'* was represented by two individuals (ALT, n = 2). The *F. biarmicus* samples were assigned to two groups, one comprising *F. b. biarmicus* and *F. b. abyssinicus* from South and East Africa (SEA, n = 25), and the other containing the remaining three subspecies (*F. b. erlangeri*, *F. b. tanypterus*, *F. b. feldeggii*) from the Mediterranean region (MED, n = 8). In *F. rusticolus*, although the samples represent a huge geographic area, the CR sequences proved to be homogeneous. Therefore, all samples of *F. rusticolus* (NEA, n = 19) from northern Europe and Asia as well as North America were pooled. The individuals of *F. jugger* (IND, n = 5) were pooled too. All samples were collected during the breeding season of the species, except two *F. cherrug* individuals (one each from Pakistan and Afghanistan).

DNA extraction, amplification and sequencing

DNA from blood or muscle tissue was extracted with DNeasy Tissue Kit (QIAGEN) following the manufacturer's instruction. DNA from dry study skins (museum specimens) and feathers was isolated using a 10% Chelex solution containing proteinase K (0.5 mg/mL). After incubation (4 h, 55 °C, with agitation) solutions were heated to 95 °C (5 min) and centrifuged (1 min). Extractions were then purified using the QIA Quick PCR Purification Kit (QIAGEN).

From 200 individuals a 456–458-bp fragment (including primers) of the mt CR could be amplified. The selected region contains a high number (97) of variable sites and extends from the central part of the CR to the repetitive section (positions 15663–16099 in *F. peregrinus*; Accession no. AF090338). Polymerase chain reaction (PCR) conditions for primer sequences (CR1+5′-AGGGCCATAACTTGGTTAATCC-3′/CR10–5′-ATGAAAGATAAGATAACGG-3′) and precautions to avoid (co)amplification of possible nuclear copies of mitochondrial DNA (mtDNA) are described in Nittinger *et al*. (2005). To prevent/detect contamination, which is especially critical for tissue from museum specimens, DNA of museum samples was extracted separately from fresh tissue and PCRs of museum samples were carried out separately. All plastic materials were UV-irradiated prior to use. Filter tips were used to avoid (*trans*)contamination. To detect contaminations, we performed control extractions with pure extraction buffer (without tissue) and negative controls in the PCR experiments. PCR products were cloned (TOPO TA cloning kit, Invitrogen) and sequenced in both directions (performed by MWG Biotech AG, Ebersberg, Germany). Sequences were visually inspected and alignments were produced manually. All mtDNA sequences are deposited in GenBank (for the Accession numbers, see Appendix).

Genotyping

We tested 26 microsatellite primer pairs, 18 developed for the peregrine falcon *Falco peregrinus* (Nesje *et al*. 2000) and eight developed for *F. rusticolus* (Nesje & Roed 2000), for their usability in hierofalcons (Nittinger 2004). Six loci proved polymorphic and showed clearly scorable bands in all species. Two hundred forty individuals (see Appendix) were successfully genotyped at these six microsatellite loci identified previously in *F. peregrinus*: Accession nos NVHfp54, AF118425; NVHfp74-4, AF118427; NVHfp82-2, AF118428; NVHfp92-1, AF118431 (Nesje *et al*. 2000); MSFp01, AF218771 (J. Fickel and S. Auls, unpublished); Fp347, AF448412 (E. Calonge, D. Parra and S. Dunner, unpublished). Primer sequences were taken from Nesje *et al*. (2000). For locus MSFp01 and locus Fp347, new primers were designed in the present study: fp01F 5′-GACTAAACTCTATTCAG-3′/fp01R 5′-TCGCAAAGCATTCTTGTC-3′; fp347F 5′-CAAGACAAGCAAAGGTGATG-3′/fp347R 5′-ATTTCCGTTCTCAACATGCC-3′. Microsatellite MSFp01 consists of a tetranucleotide repeat; the others comprise of dinucleotide repeats. The following amplification protocol was applied: denaturation 94 °C (3 min); a touchdown of 10 cycles of 95 °C (30 s), 65 °C (30 s) (with a stepwise decrease of 1 °C at each cycle), 72 °C (45 s); then 30 amplification cycles of 95 °C (30 s), 55 °C (30 s), and 72 °C (45 s), followed by an extension at 72 °C (10 min). PCR products were analysed in a Li-Cor 4200 sequencer. Amplifications of feather and museum samples were repeated up to four times to detect PCR errors and allelic dropout. Genotyping results were considered as reliable when two (heterozygotes) or three (homozygotes) in subsequent PCRs yielded the same result.

Mitochondrial DNA analyses

The sequences were aligned by eye. The alignment required only a single gap (1–3 bp) within a series of repeated T nucleotides, which was omitted from further analyses, resulting in a total length of 412 bp. A matrix including only the 96 polymorphic sites was used as input for constructing a median-joining network (Bandelt *et al*. 1999) with NETWORK version 4.111 (www.fluxus-engineering.com/sharenet.htm). Equal weights were assumed for each variable position (epsilon set to 0). An almost-identical network was obtained with the program TCS version 1.21 (Clement *et al*. 2000). An NJ tree was calculated with PAUP* 4.0b10 (Swofford 2002) taking *Falco tinnunculus* (Accession no. DQ144199) as an outgroup. With respect to the major clades the topology proved identical to those obtained in NJ and MP analyses (see Nittinger *et al*. 2005). Basic statistics of mtDNA diversity, including nucleotide and haplotype diversity, molecular variance (AMOVA) and pairwise F_{ST} estimates as well as Tajima's D were calculated with ARLEQUIN 2.0 (Schneider *et al*. 2000) and compared across all geographic groups.

Microsatellite analyses

Departures from Hardy–Weinberg equilibrium (HWE) and linkage equilibrium were tested using GENEPOP 3.4 (http://wbiomed.curtin.edu.au/genepop/). Genetic diversity parameters were computed with MICROSATELLITE ANALYSER 4.0 (MSA; Dieringer & Schlötterer 2003): number of alleles (*na*) and unbiased expected heterozygosity (H_E). Effective number of alleles was calculated as $ne = 1/(1 - H_E)$. For comparative purposes, a hierarchical AMOVA was performed for *F. cherrug* populations with ARLEQUIN. To examine the overall differentiation among hierofalcon species, we calculated a distance matrix with the program MSA using the proportion of shared alleles. NJ trees were calculated with PHYLIP (Felsenstein 1991). The genetic structure was further examined with a Bayesian clustering method and the admixture analysis implemented in BAPS 3.2 (Corander *et al*. 2003) and STRUCTURE (Pritchard *et al*. 2000). For these analyses, the small sample of *F. jugger* was excluded. In BAPS, evidence for admixture was considered 'significant' for individuals with a Bayesian P value < 0.05. To assess the differentiation between populations, we calculated F_{ST} values with the program MSA. Significance levels were determined by permuting genotypes 10 000 times among population pairs and the Bonferroni method was used to

Table 1 Mitochondrial control region diversity in all geographic groups (N > 2). Numbers in brackets are number of samples analysed

	Geographic groups					
	CEU-c (46)	CEU-h (16)	CAS (4)	NKA (13)	SKA (19)	CMO (10)
No. of haplotypes	22	10	4	5	5	9
Nucleotide diversity	0.00538 ± 0.00128	0.00533 ± 0.00095	0.01699 ± 0.00684	0.00591 ± 0.00258	0.00474 ± 0.00189	0.01149 ± 0.00199
Haplotype diversity	0.754 ± 0.071	0.882 ± 0.051	1.000 ± 0.177	0.538 ± 0.161	0.556 ± 0.130	0.978 ± 0.054
Tajima's *D*	–2.49360 *P* < 0.01	NS	NS	NS	NS	NS
	Geographic groups					
	EMO (36)	SSI (4)	MED (7)	SEA (23)	NEA (16)	IND (5)
No. of haplotypes	15	2	6	14	8	5
Nucleotide diversity	0.00995 ± 0.00103	0.00971 ± 0.00515	0.00670 ± 0.00141	0.01045 ± 0.00139	0.00291 ± 0.0077	0.00680 ± 0.00153
Haplotype diversity	0.788 ± 0.063	0.500 ± 0.265	0.952 ± 0.096	0.918 ± 0.045	0.758 ± 0.110	1.000 ± 0.126
Tajima's *D*	NS	NS	NS	NS	–1.81075 *P* < 0.05	NS

NS, not significant.

account for multiple testing. A principal component analysis (PCA) was performed using GENALEX 6 (Peakall & Smouse 2006). Moreover, we tested for correlation between geographic and genetic distances with a Mantel test implemented in GENALEX. Statistical significance was tested via 9999 random permutations.

Results

Analysis of the mitochondrial CR

In the 412-bp section of the CR, 97 polymorphic sites were detected in 200 hierofalcons analysed. Altogether, 87 different haplotypes were observed, several of them shared by different species (see Table S1, Supplementary material). In the NJ analysis (tree not shown), haplotypes are grouped into two major clades (A, B). This division is consistent with results obtained in Nittinger *et al*. (2005), although statistical support was low in all analyses. The border between clades A and B obtained in the trees is indicated in the unrooted network (Fig. 2). In each of the two groups (A and B), there is one prevalent haplotype from which many similar haplotypes branch off, the majority of them differing by only one or two substitutions. The two prevalent haplotypes are connected through a series of haplotypes with several ramifications. The minimal distance between the prevalent haplotypes is six mutational steps. None of the four species appears monophyletic, a finding that is in accordance with Nittinger *et al*. (2005). The central haplotype in group A (no. 69) is the most common and is found in all four species, representing a large geographic range extending over the entire Holarctic and south to India. It is the only haplotype that *F. rusticolus* shares with other species, except the rare haplotype no. 83 which also occurs in *F. jugger*. All nine haplotypes present in the 19 sampled *F. rusticolus* individuals belong to group A. Although these individuals cover the whole distribution range of the species, genetic variation is rather low (nucleotide diversity: 0.3%; Table 1). The five *F. jugger* individuals belong also to group A. Most haplotypes of *F. biarmicus* form a chain in the centre of the network connecting the central haplotypes of groups A and B. In addition, some haplotypes are dispersed at branches extending from the two central haplotypes. Group B contains only haplotypes of *F. cherrug* and *F. biarmicus*, but no haplotypes of group B are shared between the two species. The central haplotype (no. 1) occurs only in *F. cherrug*. Observed number of haplotypes, estimates of haplotype diversity, and nucleotide diversity for the geographic groups are shown in Table 1. Interestingly, the contemporary European *F. cherrug* and *F. rusticolus* showed significant departures from neutral expectations as indicated by Tajima's *D*.

Figure 3 shows the geographic distribution of group A and B haplotypes in *F. cherrug*. In Central Europe and northern Kazakhstan, group B haplotypes prevail, whereas the populations from Central Asia (Afghanistan, southern Kazakhstan, and Altai Mountains) and southern Siberia possess mainly group A haplotypes. Both haplotypes coexist at intermediate frequencies in eastern Asia (Mongolia). Thus, there is no obvious frequency cline in the west–east direction as it is observed with other bird species (e.g. *Anas platyrhynchos*: Kulikova *et al*. 2005). Consistent with this finding is the result of a hierarchical AMOVA. For this analysis, the populations were divided

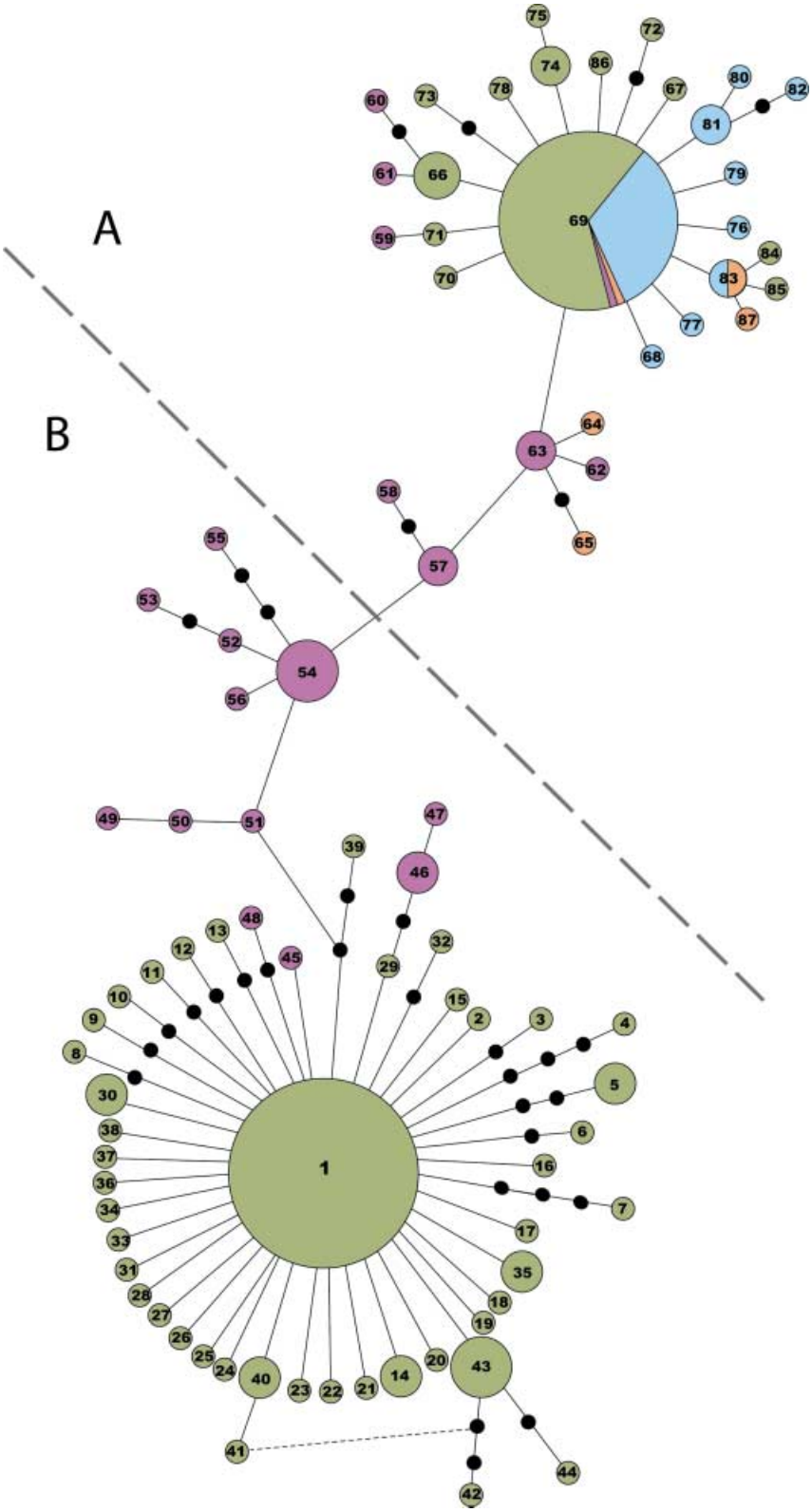

Fig. 2 Network of the 87 mitochondrial haplotypes of the four hierofalcon species. Each circle represents one haplotype, its size is proportional to the frequency of that haplotype. Small black dots stand for missing haplotypes and connecting lines represent single-mutation steps. Haplotype numbers correspond to those in the Appendix. The hatched line indicates the split between the two major haplotype groups (A and B). For colour codes, see Fig. 1.

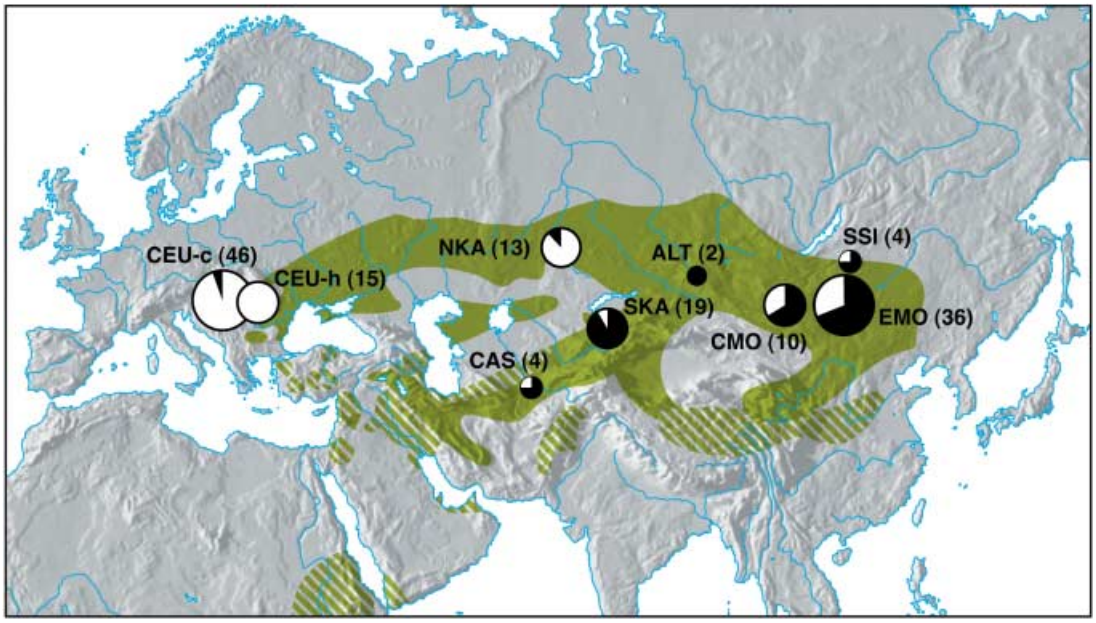

Fig. 3 Frequencies of group A (black) and B (white) haplotypes in populations of *F. cherrug*. Circles are scaled to reflect number of individuals. Population codes and numbers of individuals are indicated. Proportion of group A haplotype in populations: CEU-h, 0%; CEU-c, 9%; CAS, 75%; NKA, 15%; SKA, 89%; CMO, 30%; EMO, 31%; ALT, 100%; SSI, 75%. Green-coloured area represents the geographic distribution of *F. cherrug*. Diagonally hatched areas depict wintering grounds.

into an eastern and a western group corresponding to the ranges of the subspecies *F. c. cherrug* vs. *F. c. milvipes*, respectively. The component of genetic variance between these two groups is only 1%, in contrast to 11% among populations and 88% within populations. The historical population from Central Europe (CEU-h) differs from the contemporary one (CEU-c) by the absence of group A haplotypes; however, the diversity values were similar between the two temporal data sets. The ranges of significant F_{ST} values obtained in pairwise comparisons with the other populations proved also very similar (CEU-h: F_{ST} = 0.051–0.271; CEU-c: F_{ST} = 0.077–0.260), although they differed with respect to specific population comparisons. The highly significant F_{ST} values between most of the *F. cherrug* populations, especially between southern and northern Kazakhstan (F_{ST} = 0.423), can be explained by high variation in the presence of haplogroups A and B.

Microsatellite analysis

None of the geographic groups exhibited significant linkage disequilibrium or departures from HWE. Numbers, effective numbers, and frequencies of alleles in the 12 geographic groups are given in Table 2. The effective number of alleles was highest (6.0) in the Mediterranean *F. biarmicus*. In the *F. cherrug* populations this value ranged from 2.9 to 4.5 and the value observed in *F. rusticolus* falls into this range (3.9). The lowest value (1.8) was observed in *F. jugger*. Similar proportions are found with respect to average expected heterozygosity. However, it has to be noted that these results may be biased by the low sample size in some populations, especially *F. jugger*. No diagnostic alleles (i.e. species-specific alleles in high frequencies) characteristic for the respective species were detected at the six microsatellite loci. Private alleles with frequencies above 10% occurred only in *F. biarmicus* (locus MsFp01: alleles 152 and 156).

Based on the proportions of shared alleles, an NJ tree was calculated (Fig. 4). According to the tree, neither species nor populations are clearly differentiated, probably due to the low number of loci. Each of the five groups indicated in Fig. 4 contains individuals of at least two species. Group 1 is the most heterogeneous. One of its clusters comprises all four species, whereas in the remaining clusters only *F. cherrug* and *F. biarmicus* are found. All African representatives of *F. biarmicus* (SEA) in this group form a separate cluster except one individual from East Africa. The Mediterranean individuals of *F. biarmicus* (MED) are dispersed. Group 2 is divided into two clusters, one containing African individuals of *F. biarmicus*, the other individuals of *F. cherrug*. In group 3, there is one mixed cluster with *F. cherrug* and *F. rusticolus*, the two other clusters contain *F. cherrug* except one individual of *F. jugger*. Group 4 comprises mainly *F. cherrug*, but also four interspersed individuals of *F. biarmicus* (MED/Israel) and *F. rusticolus* and a small cluster of four *F. rusticolus* individuals. Finally, group 5

Table 2 Allele frequencies for the six microsatellite loci in 12 geographic groups of hierofalcons

Falco species		*cherrug*	*cherrug*	*cherrug*	*cherrug*	*cherrug*	*cherrug*	*cherrug*	*cherrug*	*biarmicus*	*biarmicus*	*rusticolus*	*jugger*
Population		CEU-c	CEU-h	CAS	NKA	SKA	CMO	EMO	SSI	MED	SEA	NEA	IND
Locus	Allele												
Fp347	133									0.063			
	135	0.008					0.100	0.033					
	137	0.350	0.281	0.375	0.577	0.400	0.300	0.326	0.400	0.125	0.460	0.056	0.125
	139	0.375	0.563	0.125	0.192	0.120	0.100	0.228	0.400	0.435	0.380	0.194	0.625
	141	0.083	0.063	0.125	0.038	0.040	0.150	0.043		0.125	0.120	0.028	0.125
	143	0.017	0.031					0.011		0.188	0.040		0.125
	149	0.042		0.125	0.077	0.080		0.043	0.100				
	151	0.033		0.125	0.115	0.260	0.200	0.174	0.100			0.333	
	153	0.075	0.063	0.125		0.040	0.150	0.130		0.063		0.389	
	155	0.017				0.060		0.011					
MSFp01	152									0.063	0.200		
	156									0.188	0.720		
	176	0.195	0.250	0.500	0.231	0.140	0.300	0.256			0.020	0.053	
	180											0.026	
	184	0.212	0.114	0.125	0.192	0.380	0.200	0.144	0.700			0.342	
	188	0.093	0.045		0.077	0.100		0.056		0.063	0.020	0.026	
	192	0.025	0.068		0.346	0.160	0.100	0.211		0.188			
	194											0.026	
	196	0.161	0.250	0.375	0.115	0.060	0.100	0.233	0.200		0.040	0.316	0.625
	198	0.017											
	200	0.195	0.227		0.038	0.120	0.150	0.100	0.100	0.438		0.211	
	204	0.093	0.045			0.040	0.150						0.375
	208	0.008								0.063			
Fp54	92											0.143	
	94		0.036										
	98		0.071							0.063	0.087		
	100	0.009				0.021		0.011	0.167	0.125	0.043		
	102	0.083	0.071		0.385	0.146	0.100	0.109			0.196	0.071	
	104	0.167	0.536	0.125	0.115	0.208	0.200	0.098		0.500	0.304	0.036	
	106	0.352	0.143	0.375	0.115	0.250	0.450	0.293	0.167		0.217	0.250	1.000
	108	0.213	0.036	0.250	0.231	0.229		0.239	0.333	0.187	0.043	0.036	
	110	0.093	0.036			0.042	0.250	0.130	0.167		0.021		
	112							0.043		0.063	0.087		
	114				0.077	0.042		0.022				0.036	
	116			0.250		0.021		0.022	0.167			0.143	
	118							0.011		0.063		0.071	
	120	0.019				0.042							
	122	0.009	0.036									0.143	
	124	0.019										0.036	
	126				0.038								
	128							0.011					
	130	0.037	0.036					0.011				0.036	
	134				0.038								
Fp82-2	122							0.014					
	130	0.012						0.027					
	132	0.110	0.042					0.014	0.250				
	134	0.036	0.083			0.028		0.068			0.435		
	136	0.131	0.250	0.167	0.500	0.389	0.278	0.135		0.200	0.304	0.100	
	138	0.083	0.042	0.167	0.056	0.083		0.068	0.500	0.300	0.022	0.100	
	140	0.095	0.083			0.056	0.278	0.027			0.065	0.433	
	142	0.048	0.042		0.111		0.111	0.135		0.100		0.067	
	144	0.024		0.167				0.041			0.022	0.067	
	146	0.155	0.083	0.333	0.056	0.056	0.167	0.041	0.250	0.100	0.022	0.033	0.500
	148	0.202	0.125	0.167	0.056	0.250	0.111	0.108			0.109	0.133	
	150	0.036	0.083		0.056	0.028	0.056	0.135		0.300	0.022		

Table 2 *Continued*

Falco species Population		*cherrug* CEU-c	*cherrug* CEU-h	*cherrug* CAS	*cherrug* NKA	*cherrug* SKA	*cherrug* CMO	*cherrug* EMO	*cherrug* SSI	*biarmicus* MED	*biarmicus* SEA	*rusticolus* NEA	*jugger* IND
	152	0.060	0.042			0.056		0.054					0.500
	154		0.125		0.056	0.028		0.014					
	156	0.012			0.111			0.041					
	158							0.027					
	160							0.014					
	162							0.014					
	164							0.027					
	166					0.028							
	172											0.067	
Fp92-1	102				0.038								0.250
	104											0.026	
	106					0.063						0.026	
	108	0.298	0.386	0.250	0.500	0.292	0.650	0.364	0.500	0.5625	0.540	0.263	0.750
	110	0.175	0.273	0.250	0.231	0.125	0.050	0.182	0.200	0.0625	0.320	0.263	
	112	0.377	0.114		0.192	0.167	0.200	0.182	0.100	0.250	0.080	0.026	
	114	0.105	0.045	0.250		0.083		0.023		0.0625	0.020	0.053	
	116		0.045			0.167	0.100	0.080	0.100			0.211	
	118	0.044	0.091	0.250	0.038	0.104		0.159	0.100		0.040	0.105	
	120		0.045					0.011				0.026	
	126									0.0625			
Fp79-4	142				0.038	0.040		0.011			0.020		
	144	0.009		0.167		0.020	0.050	0.011					0.125
	146	0.018					0.050			0.143	0.020		
	148	0.018	0.056	0.333		0.060		0.067	0.200		0.040	0.033	0.500
	150	0.327	0.444	0.167	0.385	0.440	0.300	0.267	0.400	0.357	0.060	0.500	
	152	0.218	0.250	0.167	0.231	0.120	0.450	0.367	0.400	0.214	0.320	0.333	0.125
	154	0.318	0.167		0.038	0.220	0.050	0.111		0.071	0.360	0.100	0.125
	156	0.082	0.028		0.077	0.039		0.089		0.214	0.100	0.333	0.125
	158	0.009	0.056	0.167	0.192			0.078			0.060		
	160					0.060	0.050				0.020		
	162				0.038		0.050						
N		60	22	4	13	25	10	46	5	8	25	19	4
na		54	45	27	38	48	33	61	23	33	39	45	16
ne		4.5	4.2	3.8	3.5	4.4	3.5	3.2	2.9	6.0	2.9	3.9	1.8
H_E		0.778	0.760	0.733	0.710	0.772	0.716	0.809	0.649	0.833	0.653	0.741	0.433

Populations of *F. cherrug*: CEU-c, Central Europe contemporary; CEU-h, Central Europe historical; CAS, Central Asia; NKA, North Kazakhstan; SKA, South Kazakhstan; CMO, Central Mongolia; EMO, Eastern Mongolia; SSI, South Siberia. *F. biarmicus*: MED, Mediterranean region; SEA, South and East Africa; *F. rusticolus*: NEA, Northern Europe, Asia and North America. *F. jugger*: IND, India. *N*, number of successfully analysed individuals; *na*, number of alleles detected; *ne*, effective number of alleles; H_E, expected average heterozygosity.

mainly consists of *F. cherrug* except one *F. biarmicus* (MED/Sicily) and five *F. rusticolus* individuals. The division into mt haplogroups (A, B) is not reflected by clades (or groups of clades) in the NJ tree based on microsatellites. To illustrate the assignment of *F. cherrug* individuals to the eight geographic populations, they are annotated with different symbols in Fig. 4. Obviously, there is no clear correspondence between geographic origin and genetically defined groups based on the proportion of shared alleles. This finding is confirmed by the result of a hierarchical AMOVA: 96% of the genetic variance can be attributed to variation within populations, 3% to differences between populations, while the component due to differences between the eastern and western groups of *F. cherrug* populations is close to 0%. Also in the BAPS analysis, which is based on differences in allele frequencies, no population structure within *F. cherrug* was detected (data not shown). Yet, four differentiated groups were obtained with this method: *F. cherrug*, *F. rusticolus* and two groups of *F. biarmicus* (SEA and MED) (*F. jugger* was excluded due to small sample size). In the BAPS admixture analysis, the probability of ancestry in one of these four groups was assessed for each individual. This analysis was made to detect genetic traces of past hybridization, as indicated by their admixture coefficients and the Bayesian

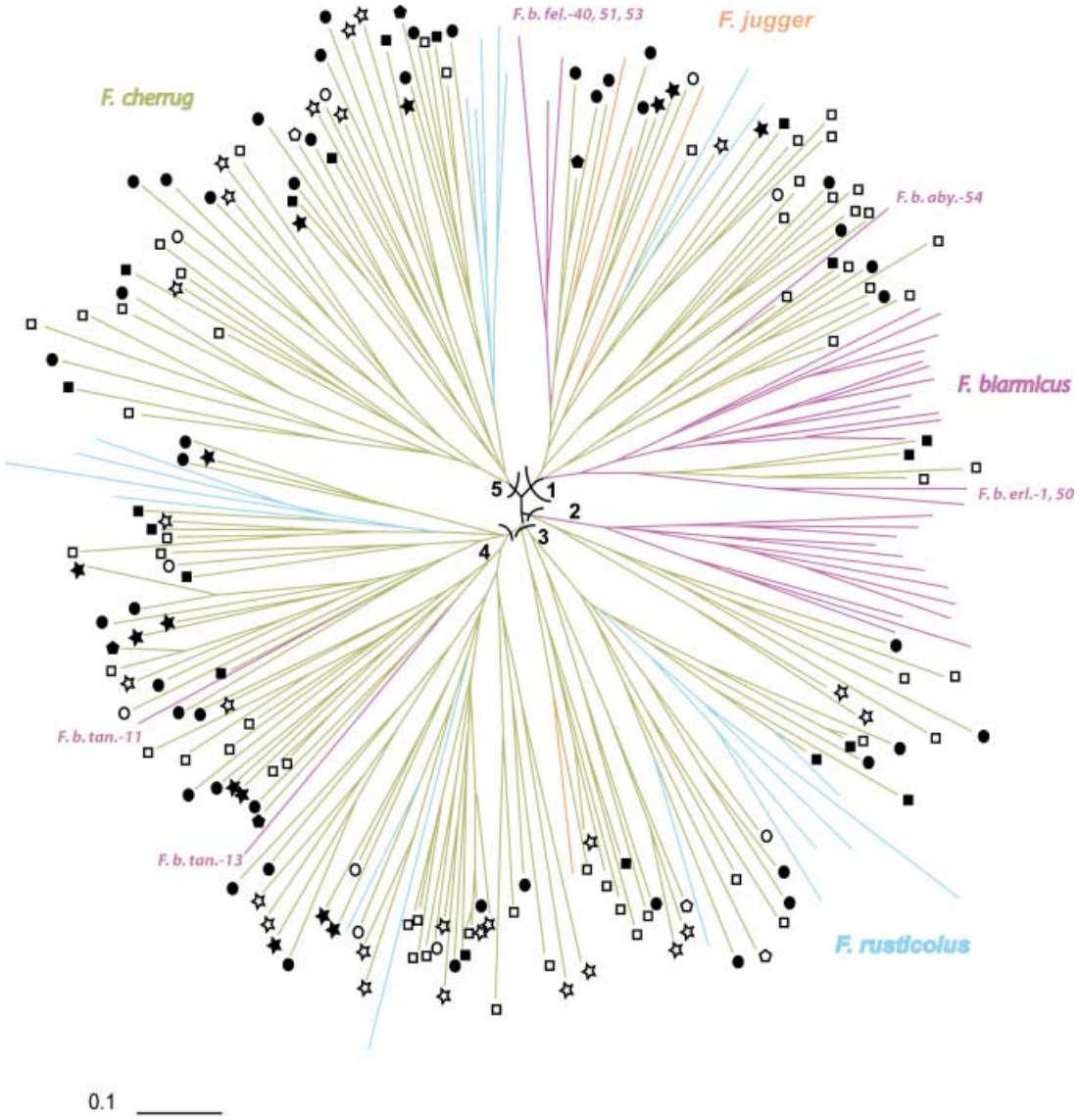

Fig. 4 NJ tree based on the proportion of shared microsatellite alleles among hierofalcon individuals. Colour codes for species correspond to those in Fig. 1. Numbers 1–5 indicate groups described in the text. Symbols indicate populations of *Falco cherrug*: black squares, CEU-h; white squares, CEU-c; black circles, EMO; white circles, CMO; black asterisks, NKA; white asterisks, SKA; black pentagons, SSI; white pentagons, CAS.

P values, i.e. to determine whether the genetic assignment contradicts the taxonomic assignment. The majority of individuals were correctly assigned with high probabilities (> 70%) to their respective taxonomic group. A total of 27 individuals (24 *F. cherrug*, two *F. rusticolus* and one *F. biarmicus*), for which admixture coefficients and Bayesian *P* values (< 0.05) indicated mixed ancestry in this test, were classified as potential hybrids or descendants of hybrids (Table S2, Supplementary material). Among them were eight (out of 56) Mongolian *F. c. milvipes* individuals (CMO, EMO) that were assigned to *F. rusticolus* with probabilities between 26% and 70%. In the Central European population of *F. cherrug* (Pannonian region, CEU-h, CEU-c) the admixture analysis identified 16 individuals (out of 82) as putative hybrids or hybrid descendants. They were assigned to either *F. rusticolus* or *F. biarmicus* (SEA, MED) with probabilities up to 91%. Moreover, two *F. rusticolus* individuals (NEA) obtained high *F. cherrug* admixture coefficients.

In contrast to the results of the BAPS analysis, no distinct groups were differentiated with the clustering analysis using STRUCTURE. The number of groups could not be determined without ambiguity. Therefore, it was not possible to perform an admixture test with this method.

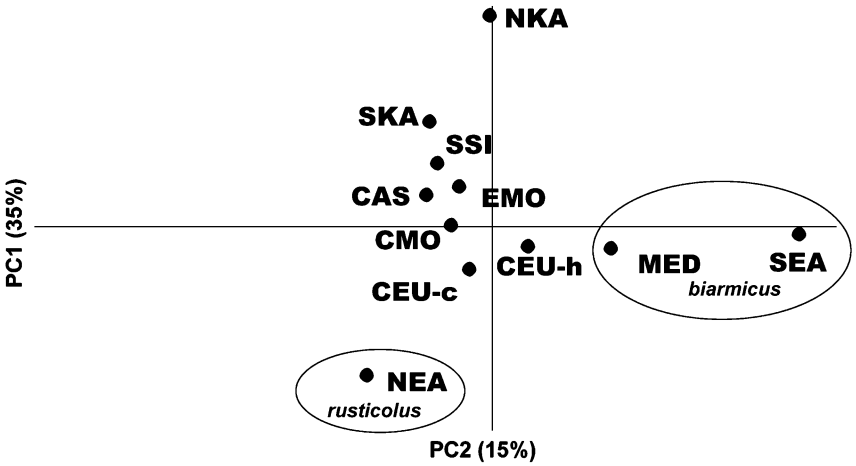

Fig. 5 Principal component analysis (PCA) based on microsatellite allele frequencies of hierofalcon populations plotted on the first two axes.

Table 3 F_{ST} values among populations: mtDNA (above diagonal) and microsatellites (below diagonal)

Populations	CEU-c	CEU-h	CAS	NKA	SKA	CMO	EMO	SSI	MED	SEA	NEA	IND
CEU-c	—	0.047*	−0.008	0.037	0.260*	0.077*	0.017	−0.122	0.124*	0.147*	0.198*	0.109
CEU-	0.025	—	0.009	0.195*	0.271*	0.051*	0.088*	−0.030	0.072*	0.100*	0.167*	0.054
CAS	−0.001	0.017	—	0.122	0.124	−0.054	−0.035	−0.333	−0.009	0.067	0.024	−0.052
NKA	0.051*	0.052	0.036	—	0.423*	0.237*	0.053	−0.034	0.322*	0.300*	0.359*	0.325*
SKA	0.025*	0.046*	0.010	0.018	—	0.150*	0.166*	0.344	0.220*	0.283*	0.021	0.177*
CMO	0.029	0.045	0.018	0.044	0.033	—	0.060	−0.019	0.018	0.079*	0.053	−0.016
EMO	0.020*	0.033*	−0.023	0.020	0.023	0.011	—	−0.066	0.124*	0.169*	0.133*	0.097
SSI	0.023	0.035	−0.081	0.044	0.053	0.034	−0.007	—	0.034	0.081	0.186	0.001
MED	0.050	0.015	0.076	0.078	0.072*	0.069	0.052*	0.047	—	0.082*	0.091*	−0.003
SEA	0.112*	0.101*	0.126	0.124*	0.130*	0.129*	0.111*	0.151*	0.109*	—	0.172*	0.064
NEA	0.069*	0.075*	0.033	0.111*	0.058*	0.049	0.051*	0.087	0.110*	0.181*	—	0.031
IND	0.069	0.105	0.016	0.191	0.146*	0.093	0.076	0.056	0.139	0.179*	0.142*	—

Designation of populations as in Table 2. *$P < 0.05$ (after Bonferroni correction).

A PCA based on microsatellite allele frequencies within populations was then employed to test which groups can be differentiated (Fig. 5). Population scores were plotted on two axes, which cumulatively explained 50% of the total genetic diversity (PC1: 35%, $P < 0.01$; PC2: 15%, $P > 0.05$). The diagram shows that *F. biarmicus* and *F. rusticolus* are separated from the populations of *F. cherrug*. The *F. cherrug* populations form a compact cluster except the population from North Kazakhstan (NKA).

Pairwise F_{ST} values are given in Table 3. Although 25 out of 66 comparisons deviate significantly from zero, the F_{ST} values are rather low. *F. biarmicus* (SEA) and *F. rusticolus* (NEA) show the highest values compared to the remaining populations (maximum value of 0.181 between SEA and NEA). With an overall F_{ST} of only 0.024, genetic differentiation between *F. cherrug* populations is very low. In a Mantel test, no significant correlation between F_{ST} values and geographic distances was detected among *F. cherrug* populations ($r^2 = 0.11$; $P = 0.109$).

Discussion

Phylogeographic structure and speciation in hierofalcons

Concerning the distribution of mtDNA haplotypes (412 bp of the CR), our results confirm earlier findings (Nittinger *et al*. 2005) based on much smaller samples of *Falco cherrug* and *Falco rusticolus*. None of the taxa (species/subspecies) corresponds to a distinct mtDNA haplogroup and none of the four *Falco* species, *F. cherrug*, *F. biarmicus*, *F. rusticolus* and *F. jugger*, appears as a monophyletic group in the CR network (Fig. 2). This lack of monophyly could be due to incomplete sorting of mtDNA lineages from a polymorphic ancestral gene pool and/or due to hybridization. From the observed pattern, it is not possible to distinguish unequivocally between these two possibilities, since in both cases we would expect similar patterns of shared haplotypes. Hybridization could have taken place either in the remote past (e.g. at the end of the last glaciation), where distribu-

tion ranges are not known, or even in recent times. Although our data provide hints about possible cases of recent hybridization (see below), the surprising picture of two distinct haplogroups within *F. cherrug* and the fact that *F. rusticolus* exclusively possesses A group haplotypes seem to be best explained by ancient hybridization events. The relationships found in hierofalcons as revealed by the distribution of taxa in the haplotype network do not match any general phylogeographic pattern described so far (e.g. Hewitt 1999). In our earlier study, we proposed that the hierofalcons originated in Africa (Nittinger *et al*. 2005). According to this 'Out of Africa' scenario, *F. cherrug*, *F. rusticolus* and *F. jugger* could have split from an ancestral African population in separate waves of immigration into Eurasia and South Asia. This might have occurred during interglacial periods. Thus, *F. cherrug* initially may have carried only haplotypes of group B, whereas *F. rusticolus* possessed group A haplotypes. The presence of haplogroup A in recent *F. cherrug* could be the result of successive introgression. Hybridization could have taken place at the end of the last glaciation, when both species probably expanded their ranges north and eastwards, and thus might have met in central Asia. Interestingly, mt gene flow between *F. cherrug* and *F. rusticolus* apparently was asymmetric since no *F. rusticolus* individuals belonging to group B were detected. Nevertheless, due to insufficient sampling, especially over large parts of Siberia, the *F. rusticolus* sample may not be representative. However, although our sample is comparatively small, it covers the whole distribution range of the species. A comprehensive study of *F. rusticolus* including more populations will be necessary to corroborate/falsify our hypotheses. The assumption of extensive postglacial range expansion is compatible with the finding of significant deviation from neutral expectations (negative Tajima's *D*) for *F. rusticolus* and the contemporary European *F. cherrug* population due to the fixation of rare mt haplotypes after a bottleneck. In the other groups, no significant deviation was observed, but *D* values were also negative.

Do the results obtained in hierofalcons confirm general assumptions concerning genetic variation such as an increasing diversity towards the south (Hewitt 2004)? Such a pattern was found, e.g. in gulls (genus *Larus*; Liebers & Helbig 2002) where northern lesser black-backed gulls show little genetic variation in mitochondrial CR sequences and a poor phylogeographic structure compared with southern yellow-legged gulls. According to this model, one would expect higher variation in *F. biarmicus* and *F. jugger* compared to the other two more northerly distributed species. From the mt diversity values (Table 1), this becomes apparent only with respect to *F. rusticolus* vs. *F. biarmicus*. (The comparatively high values of *F. jugger* are not considered because of the small sample size.) In contrast, *F. cherrug* is seemingly more variable: in most populations, nucleotide and haplotype diversities are higher than in *F. biarmicus*. However, this is due to the presence of the two distinct haplotypes (A, B) in all populations. If our hypothesis of substantial ancient introgression of A haplotypes (representing *F. rusticolus*) into *F. cherrug* is correct, the high level of sequence variation in *F. cherrug* is a secondary phenomenon. In this respect, the hierofalcons fit into the expected spatial pattern of diversity (Hewitt 2004).

Summing up, the existing mtDNA data do not provide a simple explanation for the present haplotype pattern in hierofalcons. Instead, a combination of evolutionary processes, including hybridization and incomplete lineage sorting, may be responsible for the current distribution of mtDNA haplotypes in hierofalcons. Since the mtDNA data tell us only the maternal part of the evolutionary history, we investigated also microsatellite markers to test whether differentiation exists in the nuclear genome. Yet, consistent with the mt study, we observed that most of the variation is shared between species and no clear differentiation by private alleles is found. As with the mt data, the effects of gene flow are difficult to distinguish from shared ancestral variation. Nevertheless, in the Bayesian clustering method BAPS a differentiation of *F. cherrug*, *F. rusticolus* and two groups of *F. biarmicus* (representing sub-Saharan Africa vs. Mediterranean populations) was detected. Assuming extensive gene flow in recent times, such differentiation would not be expected. Yet, since the differentiation, which is based mainly on allele frequencies, is not very pronounced, a certain degree of gene flow and/or ancient polymorphisms cannot be ruled out. The following facts suggest that ancient polymorphism might not be the only explanation: *F. biarmicus* possesses private microsatellite alleles and the South African population forms two compact branches in the NJ tree, while the Mediterranean *F. biarmicus* individuals are distributed in other branches of the tree (Fig. 4). Thus, low levels of gene flow between Mediterranean *F. biarmicus* and *F. cherrug* populations seem to be more likely. However, we cannot rule out the possibility of homoplastic mutations of microsatellite alleles which would lead to a similar picture. Nevertheless, homoplasy seems to be of minor importance at least for *F. biarmicus* as indicated by the BAPS analysis: the presence of two genetic groups corresponding with geographic regions, rather than one coherent group would not be expected assuming a high level of homoplasy. In summary, like the mt haplotypes, the microsatellite data are compatible with the scenario involving ancient and occasional recent hybridization between *F. cherrug*, *F. rusticolus* and *F. biarmicus* as postulated by Nittinger *et al*. (2005).

Recent hybridization?

Natural hybridization and introgression are well-documented phenomena in many bird species especially in contact zones

and viable hybrid offspring has been recorded in 10% of bird species (Helbig 2000; Randler 2004). In birds of prey, natural hybridization in contact zones has been rarely, but repeatedly, documented (Fefelov 2001; Löhmus & Väli 2001; Helbig *et al*. 2005).

Among hierofalcons, the possibility of recent natural hybridization can be ruled out for the allopatric species pairs *F. rusticolus*/*F. jugger* and *F. rusticolus*/*F. biarmicus*. However, it cannot be excluded among *F. biarmicus*, *F. cherrug*, and *F. jugger* (Eastham 2000; Eastham *et al*. 2001): individuals of *F. cherrug* are known to overwinter within the breeding area of northern populations of *F. biarmicus* (Balkans to Asia Minor and Near East) as well as in the southwestern parts of the breeding range of *F. jugger* (Pakistan, India). A few cases of hybridization between *F. cherrug* and *F. biarmicus* have been documented from their contact zone in Bulgaria (Boev & Dimitrov 1995). Another possible hybridization zone exists in Israel where *F. cherrug* individuals overwintering in the breeding area of the resident *F. biarmicus* may stay and hybridize. Moreover, for centuries *F. cherrug* individuals have been transferred to the Near East for falconry and released after the hunting season. In recent years, captive falcons from Arabic countries were brought to Central Asia to the breeding areas of *F. cherrug* to supplement the wild populations (Anonymus-2 2000). Even *F. biarmicus* and *F. jugger* could occasionally meet in southern Iran and Pakistan. Moreover, the wintering grounds of *F. rusticolus* and the northern breeding range of *F. cherrug* overlap in the Sayan, Altai and Baikal regions (southern Siberia). It is not unlikely that some *F. rusticolus* individuals may remain in their wintering area during the breeding season of *F. cherrug*, thus allowing interspecific hybridization (Moseikin & Ellis 2004; Potapov & Sale 2005).

Does the genetic data support the assumption of natural hybridization? A potential hybrid between *F. cherrug* and *F. biarmicus* reported by Boev & Dimitrov (1995) was included in our study (F.c.che-79), but neither microsatellite nor mt analyses indicate hybridization. Furthermore, according to the genetic data, two *F. b. tanypterus* individuals from Israel (F.b.tan-11, F.b.tan-13) could be considered as putative *F. biarmicus*/*F. cherrug* hybrids or hybrid descendants. They possess group B haplotypes, and in the NJ tree based on microsatellites (Fig. 4), these individuals are embedded in group 4 among *F. cherrug* individuals. Nevertheless, the admixture analysis did not identify them as potential hybrids. The admixture analysis indicated mixed ancestry for 27 individuals, suggesting that gene flow occurs in several regions. Several of these cases can hardly be explained by natural hybridization, e.g. four *F. cherrug* individuals that were assigned to South African *F. biarmicus* (SEA). But, given the fact that these results were not supported by the STRUCTURE analysis, they have to be considered with caution as the assignment test cannot be regarded as a proof of recent hybridization. Another problem is the limited sample size, especially of the historical Central European population. Besides the fact that traces of recent hybridization events may be blurred after a few generations, the reticulate genetic pattern of hierofalcons considerably hampers any attempt to assess the level of recent gene flow (natural or human-induced). Thus the question, whether there is a genetic influence of escaped hybrid falcons on natural population, remains open.

Population-genetic structure of F. cherrug *and discrimination of subspecies*

In several bird species, a genetic west–east differentiation in the Palearctic has been observed (e.g. Godoy *et al*. 2004). In contrast, although the range of *F. cherrug* extends from Central Europe to eastern Mongolia, no such pattern was found. It is remarkable that representatives of group B prevail in the western part of the distribution range (Fig. 3), whereas group A haplotypes are mainly found in the eastern and southern part. The topography of the area could provide an explanation: a putative postglacial expansion route of *F. cherrug* (carrying B haplotypes) from west to northeast followed the lower steppe areas to northern Kazakhstan, farther on along the foothills of the mountain ranges Tienshan and Altai, and from there through the Irtysh valley (Dzungaria) into eastern Mongolia and northern China. In the higher regions between Kazakhstan and Mongolia, *F. cherrug* met with the northerly distributed *F. rusticolus* (carrying A haplotypes) where hybridization took place. In the climatic conditions encountered in the cold steppes at higher altitudes, these hybrids may have been favoured by natural selection. *F. cherrug* populations expanding south towards Tibet and west towards Iran may have carried A haplotypes to these regions. An alternative explanation assuming recent introgression from released/escaped hybrid falcons in this region can be ruled out for our data set, because our samples were collected at a time before the introductions in Asia had started (end of 1990s; Anonymus-2 2000).

In the present study, we also addressed the genetic relationships among subspecies and populations of *F. cherrug* by comparing mitochondrial and nuclear DNA markers. The division of *F. cherrug* into subspecies is largely based on geographic variation of plumage colour and pattern at head, dorsal and ventral side, but these characters were interpreted differently by various authors (Brown & Amadon 1968; Glutz *et al*. 1971). In the past, up to six subspecies of *F. cherrug* have been described: *cyanopus* (Central Europe to Volga river), *cherrug* (Volga river to steppes of Minussinsk), *hendersoni* (Pamir and Himalaya to Gansu), *altaicus* (Sayan-Altai region), *milvipes* (Kirgisia to Mongolia), and *coatsi* (Iran to Tienshan). Today taxonomists usually distinguish only two subspecies, the western *F. c. cherrug* and the eastern *F. c. milvipes* (del Hoyo *et al*. 1994;

Eastham 1999; Ferguson-Lees & Christie 2001). However, neither the overall pattern of mt haplotype distribution nor the microsatellite analyses support any subspecific division, not even the separation of *F. c. cherrug* and *F. c. milvipes*. Besides genetic drift, a feasible explanation is that phenotypic and morphological traits characteristic for the subspecies evolved rather fast as adaptations to changing environmental conditions and hunting behaviour (Bulgin *et al*. 2003; Zink 2004). An example of fast morphological change in birds of prey (*F. cherrug*, *Accipiter gentilis*, *Aquila nipalensis*, *Aquila heliaca*) within the last century was described by Pererva & Grazhdankin (1994).

Since its description (Menzbier 1891), the enigmatic Altai falcon has been a matter of debate (Moseikin & Ellis 2004). Some authors treated it as a separate species (Menzbier 1891; Sushkin 1938; Ferguson-Lees & Christie 2001), while others (Dementiev 1951; Brown & Amadon 1968) dismissed that problem by relegating it to either a subspecies (Stresemann & Amadon 1979; Baumgart 1991) or a colour morph (Cade 1982) of *F. cherrug*. Eastham (2000) classified the Altai falcons as natural hybrids between *F. cherrug* and *F. rusticolus*, since they show intermediate phenotypes. Although the preferred tundra-like mountainous steppe habitat is still available, Altai falcons are almost extinct now because of intensive trapping for falconry. On the basis of our data (Fig. 2; haplotypes H-69, H-86), species or subspecies status can be rejected for the Altai falcon. To investigate a possible hybridogenic origin, mt data are not the appropriate markers, and unfortunately, the old material of the two individuals from the Altai region available to us was not of sufficient quality for microsatellite analysis. Moreover, a larger sample would be necessary.

Conclusions

Our analysis revealed that *Falco cherrug* and the other hierofalcons are genetically not clearly differentiated, implying that they are an evolutionary young group. Nevertheless, the aim of conservation should be to protect phenotypic diversity at population and subspecies level even if genetic differentiation in neutral markers is low (Gamauf *et al*. 2005). Are there practical consequences for *F. cherrug* populations? From the genetic point of view, the close phylogenetic relationships among hierofalcons does not allow to deduce from the data that recent hybridization or introgression through artificial hybridization takes place. On the other hand, the results point at possible recent hybridization events. Given these hints, genetic monitoring of *F. cherrug* populations should continue and further molecular markers should be established. In any case, since the effects of gene flow from uncontrolled sources into this globally threatened species are unpredictable, it seems advisable to prevent introgression from captive birds into natural populations. This could be achieved either by behavioural imprinting of the hybrid nestlings or by sterilization. Moreover, the deliberate release of captive bred hybrids into the breeding grounds of *F. cherrug* should be avoided, in Europe as well as in Central Asia.

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This work is part of Franziska Nittinger's dissertation research on phylogeography and population genetics of large falcons. She will continue studying speciation of birds at the Konrad Lorenz Institute for Ethology, Academy of Sciences, Austria. The research group of Elisabeth Haring, Anita Gamauf, and Wilhelm Pinsker is investigating phylogenetic relationships in various groups of birds.

Supplementary material

The following supplementary material is available for this article:

Table S1 Variable sites of a 412-bp section of the mt control region of hierofalcons and occurrence of haplotypes in the four species.

Table S2 Individuals with assumed mixed ancestry according to the admixture analysis (in percentage).

This material is available as part of the online article from:
http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-294X.2007.03245.x
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Appendix

Specimens investigated

Taxon	Code	Tissue	Origin, year	Source/voucher	Haplotype	HG/Mic	Pop	Accession no.
Falco cherrug								
F. c. cherrug	F.c.che-1	bl	Austria, Haringsee, 2002	H. Frey	H-1	B	CEU-c	DQ144166*
	F.c.che-2	fe	Austria, 1989	NMW 82961	H-12	B	CEU-c	
	F.c.che-3	fe	Austria, Hollabrunn, 1960	O. Knotzinger	H-43	B	CEU-h	
	F.c.che-4	fe	Austria, Zurndorf, 1987	A. Gamauf	H-38	B	CEU-c	
	F.c.che-5	fe	Slovakia, 1990	A. Gamauf	H-1	B	CEU-c	
	F.c.che-6	fe	Austria, Fischamend, 1999	A. Gamauf	H-78	B	CEU-c	
	F.c.che-10	fe	Austria, Nickelsdorf, 1999	H.-M. Berg	H-9	B	CEU-c	
	F.c.che-11	fe	Austria, 1989	W. Dolak	H-20	B	CEU-c	
	F.c.che-13	fe	Afghanistan, 1976	O. Knotzinger	H-74	A	CAS	DQ144170*
	F.c.che-15	fe	Austria, St Pölten, 1955	O. Knotzinger	—		CEU-h	
	F.c.che-16	ba	Austria, Vienna, 1860	NMW 36817	—		CEU-h	
	F.c.che-17	ba	Austria, Wiener Neustadt, 1929	NMW 57244	H-13	B	CEU-h	
	F.c.che-21	fe	Austria, Marchfeld, 1984	O. Knotzinger	—		CEU-c	
	F.c.che-22	ba	Austria, Groß Enzersdorf, 1895	NMW 36823	H-43	B	CEU-h	
	F.c.che-23	ba	Austria, Vienna, 1941	NMW 46461	H-15	B	CEU-h	
	F.c.che-24	ba	Croatia, Bjelovar, 1917	NWM 36821	H-5	B	CEU-h	
	F.c.che-25	ba	Austria, Gross Enzersdorf, 1932	NMW 73566	H-43	B	CEU-h	
	F.c.che-31	ba	Austria, Horn, 1895	NMW 12909	H-1	B	CEU-h	
	F.c.che-36	ba	Austria, Laxenburg, 1860	NMW 36818	H-30	B	CEU-h	
	F.c.che-38	ba	Austria, Laa/Thaya, 1953	NMW 60160	H-5	B	CEU-h	
	F.c.che-39	ba	Yugoslavia, 1949	NMW 52738	H-43	B	CEU-h	
	F.c.che-40	ba	Austria, Vienna, 1931	NMW 73565	H-1	B	CEU-h	
	F.c.che-41	ba	Austria, 1989	NMW 82962	H-5	B	CEU-c	
	F.c.che-42	fe	Austria, Zurndorf, 1977	NMW 75152	H-1	B	CEU-c	
	F.c.che-43	ba	Austria, Guntramsdorf, 1895	NMW 12902	H-43	B	CEU-h	
	F.c.che-45	ba	Austria, Vienna, 1929	NMW 36820	H-1	B	CEU-h	
	F.c.che-57	ba	Slovakia, Znaim, 1880	NMW 36822	—		CEU-h	
	F.c.che-59	ba	Croatia, Dalmatia, 1892	NMW 12727	H-44	B	CEU-h	
	F.c.che-60	ba	Romania, Banat, 1853	NMW 37737	—		CEU-h	
	F.c.che-61	ba	Austria, Mannswörth, 1850	NMW 37716	—		CEU-h	
	F.c.che-68	ba	Austria, Zurndorf, 1859	NMW 70609	—		CEU-h	
	F.c.che-70	fe	Czech Republic, 2002	R. Dosedel	H-78	A	CEU-c	
	F.c.che-71	fe	Czech Republic, 2001	R. Dosedel	H-14	B	CEU-c	
	F.c.che-72	mu	Slovakia, Bratislava, 2000	J. Chavko	H-41	B	CEU-c	
	F.c.che-79	fe	Bulgaria, Sandanski, 1884	NMNHS 5137	H-34	B	CEU-h	
	F.c.che-83	fe	Bulgaria, Sofia, 1910	NMNHS 4358	H-32	B	CEU-h	
	F.c.che-84	fe	Czech Republic, 1993	H. Senn	H-1	B	CEU-c	
	F.c.che-85	bl	Austria, Haringsee, 2002	H. Frey	H-1	B	CEU-c	
	F.c.che-87	bl	Austria, Münchendorf, 2002	A. Gamauf	H-1	B	CEU-c	
	F.c.che-89	ba	Kazakhstan, Zaissan, 1913	ZMMU 95290	H-14	B	NKA	
	F.c.che-94	mu	Slovakia, Bratislava, 1999	J. Chavko	H-29	B	CEU-c	
	F.c.che-95	mu	Slovakia, Zohor, 1989	J. Chavko	H-1	B	CEU-c	
	F.c.che-96	mu	Slovakia, 2001	J. Chavko	H-1	B	CEU-c	
	F.c.che-101	fe	Slovakia, Bratislava-Raca, 1989	J. Chavko	H-4	B	CEU-c	
	F.c.che-103	fe	Slovakia, Dol'any, 1980	J. Chavko	H-1	B	CEU-c	
	F.c.che-104	fe	Slovakia, Stupava, 1991	J. Chavko	H-35	B	CEU-c	
	F.c.che-105	fe	Slovakia, Solsonica, 1982	J. Chavko	H-1	B	CEU-c	
	F.c.che-106	fe	Slovakia, Sturovo, 1993	J. Chavko	H-10	B	CEU-c	
	F.c.che-108	fe	Slovakia, Bratislava-Rusovce, 1994	J. Chavko	H-30	B	CEU-c	
	F.c.che-109	fe	Slovakia, Stupava, 1990	J. Chavko	H-1	B	CEU-c	
	F.c.che-112	fe	Slovakia, Stupava, 1997	J. Chavko	—		CEU-c	
	F.c.che-125	fe	Slovakia, Stupava, 1991	J. Chavko	H-18	B	CEU-c	
	F.c.che-133	fe	Slovakia, Vrobe 1999	J. Chavko	H-11	B	CEU-c	
	F.c.che-136	fe	Slovakia, Vrbove, 2002	J. Chavko	H-69	A	CEU-c	
	F.c.che-138	fe	Slovakia, Zohor, 1996	J. Chavko	H-73	A	CEU-c	

Appendix *Continued*

Taxon	Code	Tissue	Origin, year	Source/voucher	Haplotype	HG/Mic	Pop	Accession no.
	F.c.che-142	fe	Slovakia, Solsonica, 1985	J. Chavko	H-1	B	CEU-c	
	F.c.che-151	fe	Slovakia, Vysoka, 1991	J. Chavko	H-1	B	CEU-c	
	F.c.che-154	fe	Slovakia, Trstin, 1980	J. Chavko	—		CEU-c	
	F.c.che-195	bl	Kazakhstan, Kugussem, 1993	R. Pfeffer	H-74	A	NKA	
	F.c.che-197	bl	Kazakhstan, Kurtschum, 1993	R. Pefffer	H-69	A	NKA	
	F.c.che-198	bl	Pakistan, 1992	W. Bednarek	H-69	A	CAS	
	F.c.che-206	fe	Austria, Ebreichsdorf, 2003	C. Kaipel	H-1	B	CEU-c	
	F.c.che-212	bl	Kazakhstan, Almaty, 1993	R. Kenward	H-75	A	SKA	
	F.c.che-213	bl	Kazakhstan, Almaty, 1993	R. Kenward	—		SKA	
	F.c.che-214	bl	Kazakhstan, Almaty, 1993	R. Kenward	—		SKA	
	F.c.che-215	bl	Kazakhstan, Almaty, 1993	R. Kenward	H-69	A	SKA	
	F.c.che-216	bl	Kazakhstan, Almaty, 1993	R. Kenward	H-69	A	SKA	
	F.c.che-217	bl	Kazakhstan, Almaty, 1993	R. Kenward	—		SKA	
	F.c.che-218	bl	Kazakhstan, Almaty, 1993	R. Kenward	—		SKA	
	F.c.che-219	bl	Kazakhstan, Almaty, 1993	R. Kenward	H-69	A	SKA	
	F.c.che-220	bl	Kazakhstan, Almaty, 1993	R. Kenward	H-69	A	SKA	
	F.c.che-221	bl	Kazakhstan, Almaty, 1993	R. Kenward	H-71	A	SKA	
	F.c.che-222	bl	Kazakhstan, Almaty, 1993	R. Kenward	H-69	A	SKA	
	F.c.che-223	bl	Kazakhstan, Almaty, 1993	R. Kenward	H-69	A	SKA	
	F.c.che-224	bl	Kazakhstan, Almaty, 1993	R. Kenward	H-69	A	SKA	
	F.c.che-225	bl	Kazakhstan, Almaty, 1993	R. Kenward	H-69	A	SKA	
	F.c.che-226	bl	Kazakhstan, Almaty, 1993	R. Kenward	H-69	A	SKA	
	F.c.che-227	bl	Kazakhstan, Almaty, 1993	R. Kenward	—		SKA	
	F.c.che-228	bl	Kazakhstan, Almaty, 1993	R. Kenward	H-69	A	SKA	
	F.c.che-229	bl	Kazakhstan, Almaty, 1993	R. Kenward	H-66	A	SKA	
	F.c.che-230	bl	Kazakhstan, Almaty, 1993	R. Kenward	—		SKA	
	F.c.che-231	bl	Kazakhstan, Almaty, 1993	R. Kenward	H-66	A	SKA	
	F.c.che-232	bl	Kazakhstan, Almaty, 1993	R. Kenward	H-69	A	SKA	
	F.c.che-234	bl	Kazakhstan, Almaty, 1993	R. Kenward	H-69	A	SKA	
	F.c.che-235	bl	Kazakhstan, Almaty, 1993	R. Kenward	H-69	A	SKA	
	F.c.che-236	bl	Kazakhstan, Almaty, 1993	R. Kenward	H-1	B	SKA	
	F.c.che-237	bl	Kazakhstan, Almaty, 1993	R. Kenward	H-1	B	SKA	
	F.c.che-238	bl	Kazakhstan, Naurzum, 1997	R. Kenward	H-27	B	NKA	
	F.c.che-239	bl	Kazakhstan, Naurzum, 1997	R. Kenward	H-1	B	NKA	
	F.c.che-242	bl	Kazakhstan, Naurzum, 1997	R. Kenward	H-1	B	NKA	
	F.c.che-243	bl	Kazakhstan, Naurzum, 1997	R. Kenward	H-1	B	NKA	
	F.c.che-244	bl	Kazakhstan, Naurzum, 1997	R. Kenward	H-1	B	NKA	
	F.c.che-245	bl	Kazakhstan, Naurzum, 1997	R. Kenward	H-1	B	NKA	
	F.c.che-247	bl	Kazakhstan, Naurzum, 1997	R. Kenward	H-1	B	NKA	
	F.c.che-248	bl	Kazakhstan, Naurzum, 1997	R. Kenward	H-1	B	NKA	
	F.c.che-261	bl	Kazakhstan, Tersek, 1997	R. Kenward	H-1	B	NKA	
	F.c.che-266	bl	Kazakhstan, Tersek, 1997	R. Kenward	H-1	B	NKA	
	F.c.che-287	mu	Austria, Ebreichsdorf, 2003	H.-M. Berg	H-1	B	CEU-c	
	F.c.che-289	bl	Hungary, Borsod-Abauj, 2002	J. Bagyura	H-1	B	CEU-c	
	F.c.che-290	bl	Hungary, Borsod-Abauj, 2002	J. Bagyura	—	CEU-c		
	F.c.che-291	bl	Hungary, Borsod-Abauj, 2002	J. Bagyura	—	CEU-c		
	F.c.che-292	bl	Hungary, Borsod-Abauj, 2002	J. Bagyura	—	CEU-c		
	F.c.che-293	bl	Hungary, Bacs-Kiskun, 2002	J. Bagyura	H-2	B	CEU-c	
	F.c.che-294	bl	Hungary, Heves, 2002	J. Bagyura	H-1	B	CEU-c	
	F.c.che-295	bl	Hungary, Heves, 2002	J. Bagyura	—	CEU-c		
	F.c.che-296	bl	Hungary, Hajdu-Bihar, 2002	J. Bagyura	H-1	B	CEU-c	
	F.c.che-297	bl	Hungary, Hajdu-Bihar, 2002	J. Bagyura	—	CEU-c		
	F.c.che-298	bl	Hungary, Hajdu-Bihar, 2002	J. Bagyura	—	CEU-c		
	F.c.che-299	bl	Hungary, Hajdu-Bihar, 2002	J. Bagyura	—	CEU-c		
	F.c.che-300	bl	Hungary, Hajdu-Bihar, 2002	J. Bagyura	—	CEU-c		
	F.c.che-301	bl	Hungary, Hajdu-Bihar, 2002	J. Bagyura	—	CEU-c		
	F.c.che-302	bl	Hungary, Hajdu-Bihar, 2002	J. Bagyura	—	CEU-c		

Appendix *Continued*

Taxon	Code	Tissue	Origin, year	Source/voucher	Haplotype	HG/Mic	Pop	Accession no.
	F.c.che-303	bl	Hungary, Jasz-Nagykun, 2002	J. Bagyura	H-23	B	CEU-c	
	F.c.che-304	bl	Hungary, Fejer, 2002	J. Bagyura	H-1	B	CEU-c	
	F.c.che-306	bl	Hungary, Györ-Moson, 2002	J. Bagyura	H-1	B	CEU-c	
	F.c.che-308	bl	Hungary, Hadju-Bihar, 2002	J. Bagyura	—	CEU-c		
	F.c.che-309	bl	Hungary, Pest, 2002	J. Bagyura	H-1	B	CEU-c	
	F.c.che-311	bl	Hungary, Bekes, 2002	J. Bagyura	H-21	B	CEU-c	
	F.c.che-313	bl	Hungary, Pest, 2002	J. Bagyura	H-31	B	CEU-c	
	F.c.che-315	fe	Czech Republic, Novi Prerov, 2003	P. Horak, D. Horal	H-1	B	CEU-c	
	F.c.che-316	fe	Czech Republic, Jeseniky, 2003	P. Horak, D. Horal	H-28	B	CEU-c	
	F.c.che-341	fe	Turkmenistan, 1955	ZMMU 96917	H-72	A	CAS	
	F.c.che-343	fe	Turkmenistan, 1951	ZMMU 96918	H-7	B	CAS	
	F.c.che-353	fe	Austria, Ebreichsdorf, 2003	A. Gamauf	H-3	B	CEU-c	
F. c. milvipes	F.c.mil-7	fe	Russia, Dauuria, 2001	A. Gamauf	H-69	A	SSI	
	F.c.mil-8	fe	Russia, Dauuria, 2001	A. Gamauf	H-69	A	SSI	DQ144179*
	F.c.mil-88	ba	Mongolia, Altai, 1946	ZMMU 95281	H-69	A/–	ALT	
	F.c.mil-91	ba	Mongolia, Altai, 1945	ZMMU 58635	H-86	A/–	ALT	
	F.c.mil-155	bl	Mongolia, 1994	D. Ellis	H-1	B	CMO	
	F.c.mil-156	bl	Mongolia, 1994	D. Ellis	H-33	B	CMO	
	F.c.mil-157	bl	Mongolia, 1994	D. Ellis	H-16	B	CMO	
	F.c.mil-158	bl	Mongolia, 1994	D. Ellis	H-17	B	CMO	DQ144171*
	F.c.mil-159	bl	Mongolia, 1994	D. Ellis	H-43	B	CMO	
	F.c.mil-160	bl	Mongolia, 1994	D. Ellis	H-69	A	CMO	
	F.c.mil-162	bl	Mongolia, 1994	D. Ellis	H-69	A	CMO	DQ144174*
	F.c.mil-163	bl	Mongolia, 1994	D. Ellis	H-40	B	CMO	
	F.c.mil-164	bl	Mongolia, 1994	D. Ellis	H-70	A	CMO	
	F.c.mil-165	bl	Mongolia, 1994	D. Ellis	H-6	B	CMO	
	F.c.mil-168	bl	Mongolia, 1995	D. Ellis	—		EMO	
	F.c.mil-169	bl	Mongolia, 1995	D. Ellis	H-1	B	EMO	
	F.c.mil-170	bl	Mongolia, 1995	D. Ellis	—		EMO	
	F.c.mil-171	bl	Mongolia, 1995	D. Ellis	H-85	A	EMO	
	F.c.mil-172	bl	Mongolia, 1995	D. Ellis	H-42	B	EMO	
	F.c.mil-173	bl	Mongolia, 1995	D. Ellis	H-1	B	EMO	
	F.c.mil-174	bl	Mongolia, 1995	D. Ellis	H-84	A	EMO	
	F.c.mil-175	bl	Mongolia, 1995	D. Ellis	—		EMO	
	F.c.mil-176	bl	Mongolia, 1995	D. Ellis	H-1	B	EMO	
	F.c.mil-177	bl	Mongolia, 1995	D. Ellis	H-1	B	EMO	
	F.c.mil-178	bl	Mongolia, 1995	D. Ellis	H-1	B	EMO	
	F.c.mil-179	bl	Mongolia, 1995	D. Ellis	H-8	B	EMO	
	F.c.mil-180	bl	Mongolia, 1995	D. Ellis	H-1	B	EMO	
	F.c.mil-181	bl	Mongolia, 1995	D. Ellis	H-1	B	EMO	
	F.c.mil-182	bl	Mongolia, 1995	D. Ellis	H-36	B	EMO	
	F.c.mil-183	bl	Mongolia, 1995	D. Ellis	H-1	B	EMO	
	F.c.mil-184	bl	Mongolia, 1995	D. Ellis	H-66	A	EMO	
	F.c.mil-185	bl	Mongolia, 1995	D. Ellis	H-69	A	EMO	
	F.c.mil-186	bl	Mongolia, 1995	D. Ellis	H-37	B	EMO	
	F.c.mil-188	bl	Mongolia, 1995	D. Ellis	—		EMO	
	F.c.mil-189	bl	Mongolia, 1995	D. Ellis	H-69	A	EMO	
	F.c.mil-190	mu	Mongolia, 1995	D. Ellis	H-69	A	EMO	DQ144175*
	F.c.mil-192	mu	Mongolia, 1995	D. Ellis	H-69	A	EMO	DQ144172*
	F.c.mil-193	mu	Mongolia, 1995	D. Ellis	—		EMO	
	F.c.mil-194	mu	Mongolia, 1995	D. Ellis	—		EMO	
	F.c.mil-199	bl	Russia, South Siberia, 1992	R. Pfeffer	H-69	A	SSI	DQ144173*
	F.c.mil-267	bl	Mongolia, 1997	D. Ellis	H-25	B	EMO	
	F.c.mil-268	bl	Mongolia, 1997	D. Ellis	H-69	A	EMO	
	F.c.mil-269	bl	Mongolia, 1997	D. Ellis	H-39	B	EMO	
	F.c.mil-270	bl	Mongolia, 1997	D. Ellis	H-69	A	EMO	
	F.c.mil-271	bl	Mongolia, 1997	D. Ellis	—		EMO	

Appendix *Continued*

Taxon	Code	Tissue	Origin, year	Source/voucher	Haplotype	HG/Mic	Pop	Accession no.
	F.c.mil-272	bl	Mongolia, 1997	D. Ellis	H-42	B	EMO	
	F.c.mil-273	bl	Mongolia, 1997	D. Ellis	H-24	B	EMO	
	F.c.mil-274	bl	Mongolia, 1997	D. Ellis	H-1	B	EMO	
	F.c.mil-275	bl	Mongolia, 1997	D. Ellis	H-19	B	EMO	
	F.c.mil-276	bl	Mongolia, 1997	D. Ellis	H-1	B	EMO	
	F.c.mil-277	bl	Mongolia, 1997	D. Ellis	H-69	A	EMO	DQ144176*
	F.c.mil-278	bl	Mongolia, 1997	D. Ellis	—		EMO	
	F.c.mil-279	bl	Mongolia, 1997	D. Ellis	H-1	B	EMO	
	F.c.mil-280	bl	Mongolia, 1997	D. Ellis	—		EMO	
	F.c.mil-281	bl	Mongolia, 1997	D. Ellis	H-1	B	EMO	
	F.c.mil-282	bl	Mongolia, 1997	D. Ellis	—		EMO	
	F.c.mil-283	bl	Mongolia, 1997	D. Ellis	H-67	A	EMO	
	F.c.mil-284	bl	Mongolia, 1997	D. Ellis	H-1	B	EMO	
	F.c.mil-285	bl	Mongolia, 1997	D. Ellis	H-1	B	EMO	
	F.c.mil-288	ba	Russia, Burjatia, Muchino, 1940	—	—		SSI	
	F.c.mil-319	ba	Mongolia, Ulan Bataar, 1962	ZMMU 95280	H-1	B	EMO	DQ144169*
	F.c.mil-321	ba	Mongolia, Hangai, 1945	ZMMU 95282	H-26	B	EMO	
	F.c.mil-330	ba	Russia, Krasnojarsk, 1966	ZMMU 97697	H-22	B	SSI	
Falco rusticolus								
	F.rus-2	fe	Russia, Anadyr, 2001	R. Probst	H-79	A	NEA	DQ144192*
	F.rus-4	fe	Canada, 1999	VUW	H-76	A	NEA	DQ144197*
	F.rus-8	ba	Greenland, Gothaard, 1906	NMW 30007	H-69	A	NEA	DQ144195*
	F.rus-10	fe	Iceland, Reykjavik, 1993	D. Hartmann	H-68	A	NEA	DQ144190*
	F.rus-12	fe	Canada, 2001	H. Lauermann	H-77	A	NEA	
	F.rus-13	bl	Russia, 1993	W. Bednarek	H-69	A	NEA	
	F.rus-14	bl	Greenland, 1993	W. Bedanrek	H-69	A	NEA	
	F.rus-16	mu	Norway, Tranøy, 1980	Tromsø Museum	H-69	A	NEA	DQ144191*
	F.rus-17	mu	Norway, Tromsø, 1996	Tromsø Museum	H-69	A	NEA	DQ144196*
	F.rus-18	mu	Norway, Tromsø, 1995	Tromsø Museum	H-83	A	NEA	
	F.rus-21	bl	North Sweden, 2001	P. Lindberg	H-69	A	NEA	DQ144193*
	F.rus-22	bl	North Sweden, 2001	P. Lindberg	H-69	A	NEA	DQ144194*
	F.rus-23	bl	North Sweden, 2001	P. Lindberg	—		NEA	—
	F.rus-31	fe	Norway, Børgefjell, 1994	J.-O. Gjershaug	—		NEA	—
	F.rus-32	fe	Norway, Børgefjell, 1997	J.-O. Gjershaug	H-82	A	NEA	
	F.rus-33	fe	Norway, Børgefjell, 1994	J.-O. Gjershaug	—		NEA	—
	F.rus-34	fe	Norway, Finnmark, 1991	J.-O. Gjershaug	H-81	A	NEA	
	F.rus-38	fe	Norway, Dovrefjell, 2003	J.-O. Gjershaug	H-81	A	NEA	
	F.rus-51	fe	Iceland, 1981	O. K. Nielsen	H-69	A	NEA	
Falco biarmicus								
F. b. abyssinicus	F.b.aby-2	fe	Ethiopia, Addis Abba, 1969	NMW 94038	H-46	B	SEA	DQ144142*
	F.b.aby-54	ba	Camerun, Yagoua, 1970	MRAC RG 73.15.A.386	H-47	B	SEA	DQ144162*
	F.b.aby-55	ba	Ethiopia, Ambo Schoa, 1956	PMJ/FSU 6116	H-46	B	SEA	DQ144163*
	F.b.aby-56	ba	Ethiopia, Ambo Fl. 1956	PMJ/FSU 7030	H-46	B	SEA	DQ144164*
F. b. biarmicus	F.b.bia-4	fe	South Africa, 2001	O. Knotzinger	H-45	B	SEA	DQ144155*
	F.b.bia-5	fe	South Africa, 2001	O. Knotzinger	H-48	B	SEA	DQ144158*
	F.b.bia-6	fe	South Africa, 2001	O. Knotzinger	H-54	B	SEA	DQ144165*
	F.b.bia-16	bl	South Africa, 1997	W. Bednarek	H-54	B	SEA	
	F.b.bia-17	bl	South Africa, 2001	A. Stephenson	H-51	B	SEA	DQ144139*
	F.b.bia-18	bl	South Africa, 2001	A. Stephenson	H-57	A	SEA	DQ144140*
	F.b.bia-19	bl	South Africa, 2001	A. Stephenson	H-54	B	SEA	DQ144141*
	F.b.bia-20	bl	South Africa, 2001	A. Stephenson	H-52	B	SEA	DQ144143*
	F.b.bia-21	bl	South Africa, 2001	A. Stephenson	H-55	B	SEA	DQ144144*
	F.b.bia-22	bl	South Africa, 2001	A. Stephenson	H-54	B	SEA	DQ144145*
	F.b.bia-23	bl	South Africa, 2001	A. Stephenson	H-57	A	SEA	DQ144146*
	F.b.bia-24	bl	South Africa, 2001	A. Stephenson	H-56	B	SEA	DQ144147*
	F.b.bia-25	bl	South Africa, 2001	A. Stephenson	H-54	B	SEA	DQ144148*
	F.b.bia-26	bl	South Africa, 2001	A. Stephenson	H-49	B	SEA	DQ144149*
	F.b.bia-27	bl	South Africa, 2001	A. Stephenson	H-54	B	SEA	DQ144150*

Appendix *Continued*

Taxon	Code	Tissue	Origin, year	Source/voucher	Haplotype	HG/Mic	Pop	Accession no.
	F.b.bia-28	bl	South Africa, 2001	A. Stephenson	H-50	B	SEA	DQ144151*
	F.b.bia-29	bl	South Africa, 2001	A. Stephenson	H-54	B	SEA	DQ144152*
	F.b.bia-30	bl	South Africa, 2001	A. Stephenson	H-58	A	SEA	DQ144153*
	F.b.bia-33	ba	Uganda, Kissenye, 1910	NMW 4135	H-53	B	SEA	DQ144154*
	F.b.bia-34	bl	Captivity, 1990	M. Heindl	—		SEA	*
	F.b.bia-35	bl	Captivity, 2001	M. Heindl	—		SEA	*
F. b. erlangeri	F.b.erl-1	fe	Morocco, 2000	A. Gamauf	—		MED	—
	F.b.erl-50	ba	Morocco, 2004	EBD	H-62	A	MED	DQ144159*
F. b. feldeggii	F.b.fel-40	fe	Italy, Tuskany, 2003	A. Frankoni	H-63	A	MED	DQ144156*
	F.b.fel-44	ba	Italy, Foggia, 1928	RMNH No. 26	H-63	A/–	MED	DQ144157*
	F.b.fel-51	ba	Italy, Sicily, 1987	W. Wurzinger	H-69	A	MED	DQ144160*
	F.b.fel-53	ba	Italy, Sicily, 1994	W. Wurzinger	H-59	A	MED	DQ144161*
F. b. tanypterus	F.b.tan-11	fe	Israel, Divshon, 2002	O. Hatzofe	H-60	A	MED	DQ144137*
	F.b.tan-13	fe	Israel, Divshon, 2002	O. Hatzofe	H-61	A	MED	DQ144138*
Falco jugger								
	F.jug-2	fe	India, 1980	O. Knotzinger	H-69	A/–	IND	DQ144181*
	F.jug-6	fe	India, 1989	H. Lauermann	H-65	A	IND	DQ144182*
	F.jug-7	ba	India, 1875	NMW 71294	H-83	A	IND	DQ144183*
	F.jug-8	ba	India, 1875	NMW 71292	H-87	A	IND	DQ144184*
	F.jug-13	fe	India, 1985	O. Knotzinger	H-64	A	IND	DQ144180*

Samples are listed according to species and subspecies names (taxonomy following del Hoyo *et al*. 1994). Tissue: bl, blood; fe, basal feather quill; sk, skin; ba, skin from foot pad; mu, muscle; locality and year of collection according to information from specimen's labels or tissue data bases. *, analysed already in Nittinger *et al*. (2005); Pop, population; HG, haplogroup; '—' in the Mic column indicates unsuccessful microsatellite amplification.
The Source column enumerates the names of collectors or vouchers from institutions: AMNH, American Museum of Natural History, New York, USA (P. Sweet); EBD, Estac'on Biologica de Donana, Sevilla, E (J. Cabot); NMW, Naturhistorisches Museum Wien, A (E. Bauernfeind); NMNHS, National Museum of Natural History, Sofia, BG (Z. Boev); PMJ/FSU, Phyletisches Museum Jena, D (M. Fischer); MRAC Musée Royal de l'Afrique Centrale, Tervuren, B (M. Louette); RMNH, Rijks Museum voor Natuurlike Historie/Naturalis, Leiden, NL (R. Dekker); Tromsø Museum, N (S.C. Hügel); VUW, Veterinämedizinische Universität Wien, A (H. Frey); ZMMU, Zoological Museum, Moscow Lomonosov State University, RUS (P. Tomkovich, I. Kalyakin).