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Genetic Analyses of the Red-footed Falcon (*Falco vespertinus*) – Population Structure and Alternative Reproductive Strategies

PhD értekezés

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Genetic Analyses of the Red-footed Falcon (*Falco vespertinus*) – Population Structure and Alternative Reproductive Strategies

PhD Thesis

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Contents

Abbreviations

1y and 2y = First- and second-year birds

Φ_{PT} = F_{ST} analogue which uses genealogical information of alleles

A = Allelic diversity

AD = Anno Domini

AFLP = Amplified fragment length polymorphism

ARS = Alternative reproductive strategies

Bp = Base pair

CHD1 = Chromodomain Helicase DNA-binding gene

DTT = 1,4-dithiothreitol

EPC = Extra-pair copulation

EPP = Extra-pair paternity

F_{null} = Frequency of null alleles

F_{ST} = Fixation index

F_{IS} = Inbreeding coefficient

H_e = Expected heterozygosity

H_o = Observed heterozygosity

HWE = Hardy-Weinberg equilibrium

IBP = Intraspecific or conspecific brood parasitism

IUCN = International Union for Conservation of Nature

K = Number of possible populations in Structure software

LINE = Long interspersed nuclear elements

LnP(D) = Log likelihood for each K

LOD score = Natural log of the overall likelihood ratio

mtDNA = Mitochondrial DNA

N_a = Number of alleles

PCR = Polymerase chain reaction

PG = Population genetic analyses

QP = Quasi-parasitism

Regions:

- BOR − Borsodi-mezőség
- CSAN – Csanádi-puszták
- CSER – Cserebökény
- HEV – Heves
- HOR – Hortobágy
- JAS – Jászság
- KISK – Kiskunság
- RO1 – Southeast region colonies of Romania
- RO2 – West region colonies of Romania
- VAS – Vásárhelyi-puszták

RFF = Red-footed Falcon

RFLP = Restriction fragment length polymorphism

SINE = Short interspersed nuclear elements

SNP = Single nucleotide polymorphism

SSRs = Short sequence repeats, microsatellites

STRs = Short tandem repeats, microsatellites

VNTRs = Variable number tandem repeats, microsatellites

General Introduction

One of the most divisive question in biology is the number of species on Earth. According to the latest estimate, at least 1-6 billion species might live on Earth today, while others say there is only 8.7 million (±1.3 million SE) eukaryotic species (Larsen et al. 2017, Mora et al. 2011). Still, we must be careful while estimating biodiversity, since the data might be deceptive. Besides, due to the increased rate of extinction, it is possible that we are losing 500-50 000 species (~0.01-1%) per decade (Costello et al. 2013). As for birds, the Red List of the International Union for Conservation of Nature (IUCN) currently considers 226 species as critically endangered and in total 14% of the bird species are believed to be threatened by extinction (https://www.iucnredlist.org/search?query=Birds&searchType=species). The Red List dates back to AD 1500 and since then at least 159 extinct bird species have been recorded. Moreover, due to the lack of data there is likely to be even more than that (Şekercioğlu et al. 2004). Parallel with human expansion, the class of birds has suffered great damage; just think of some of the well-known extinct species like the Dodo (*Raphus cucullatus*), the Elephant Bird (*Aepyornis maximus*) or the Upland Moa (*Megalapteryx didinus*). It is also well known that many of the extinct bird species were flightless and island species, but other characteristics also indicate the risk of extinction. Species characterized with low intrinsic reproduction rate, large territory requirement and migration can be more susceptible to extinction (Pimm 1988), as it was the case with the famous Passenger Pigeon (*Ectopistes migratorius)* of the American continent.

Nonetheless, biodiversity can be interpreted as variability within species, between species and between ecosystems (Feest et al. 2010, Convention on Biological Diversity 1992), thus biodiversity is not only threatened in terms of the number of species, but also in terms of genetics and ecosystems. Within species, variability (also known as genetic diversity) is hard to study, but its role in nature conservation is vital, especially in the case of rare species and small populations which are usually threatened, too. Preserving the genetic information of several billions of years of evolution is the aim of conservation genetics, which has become increasingly important in the ‘70s accompanied by heated debates. There were several doubts about the role of low genetic variability and many questioned if it can lead to the collapse of populations and species (Lande 1988). In the early 2000s, these doubts were dispelled, and it was proved that the loss of genetic variability causes the decline of evolutionary potential and consequently increases the risk of extinction (Frankham 2003, Spielman et al. 2004, Frankham 2005).

Genetic variability has strong correlations with many concepts related to conservation biology, like long term survival, genetic drift, inbreeding depression, and even taxonomic classification among others (Frankham 1996, Lacy 1997, Reed & Frankham 2003, Frankham 2005). More importantly, the consequences of low genetic variability are displayed not just in captive, but also in wild populations (O'Grady et al. 2006, Keller & Waller 2002). Accordingly, it became a key component of conservation, leading to the success of several species-protection programs, like in the case of the Californian Condor (*Gymnogyps californianus*) or the Mauritius Kestrel (*Falco punctatus*) (Ewing et al. 2007, Mace 2014).

Subsequently, besides conservation biological and population genetic questions, data of genetic diversity seems to be useful for other fields of science, too (Frankham et al. 2004). Application of genetic markers has become highly important in wildlife forensics, behavioural ecology or in mating system studies, as this kind of "molecular vision" gives insight to a world invisible via conventional field observation methods.

Previously used molecular methods included restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), DNA fingerprinting by minisatellites or the examination of certain parts of the mtDNA etc. (Frankham et al. 2004, Wink 2006). Nowadays, beside SNPs (single nucleotide polymorphism) the most commonly used molecular markers in such studies are the microsatellite loci (STRs, SSRs). However, the neutrality of microsatellites was refuted in some recent studies (Haasl & Payseur 2013, Bagshaw 2017), they have several characteristics – like showing Mendelian inheritance and hyper-variability – which make them highly convenient (Schlötterer et al. 2000, Selkoe & Toonen 2006, Ellegren 2004). Microsatellites can help to understand the demographical events and the genetic structure of populations; they provide information about taxonomy as well. Multilocus microsatellite genotyping is also frequently used by behavioural ecologists since microsatellites are used to create genetic "fingerprints". Due to their properties, it is possible to identify individuals uniquely together with the relationships between them (Queller et al. 1993). Developing species-specific markers is usually costly, but another advantage of microsatellites is that the primer binding regions (the so-called flanking regions) are usually conserved among closely related species, so the chance of cross-species amplification is usually high (Dawson et al. 2010, Selkoe & Toonen 2006, Primmer et al. 2005). By the application of microsatellites, conservation genetics has become a more widely used interdisciplinary field of science.

Present dissertation focuses on the Red-footed Falcon (*Falco vespertinus*, RFF), a small raptor species of the Great Hungarian Plain. The species has a high conservation value not just in Hungary, but also internationally due to global population decline. By 2006, the Hungarian RFF population dropped by 30-40% and the nesting habits changed from breeding in natural colonies to using artificial nestboxes (Keve & Szijj 1957, Fehérvári et al. 2008, Palatitz et al. 2015). Several programs were launched in order to relieve the damage caused by the breeding and feeding site shortage of the last century (http://falcoproject.eu/hu). Despite the fact that RFFs have been at the focus of conservation for a long time, the exploration of population structure, genetic mating system and the background mechanisms are still poorly known due to the limitations of field observations in the crowded rookeries. Later, the successfully introduced nestbox scheme − as a part of conservation actions – allowed sampling individuals with high certainty. The sparsely placed boxes with roofs and walls on three sides provided new possibilities to continue studying the species in term of conservation genetics. Describing the structure of the Hungarian colonies and analysing the mating system based on individual genotypes might be critical to preserve this characteristic species of the plains.

All things considered; this study contains three main topics of conservation genetics in the Red-footed Falcon. First, to widen the information provided by the cross-species markers, species-specific marker development was aimed for RFF. Second, their application in combination with cross-species markers in a further analysis about population structure. We analysed 100 individuals in order to sample the colonies of the Carpathian Basin and compare them with a colony beyond the Carpathians. Third, the usage of cross species- and species-specific markers to investigate the mating system of RFFs. We aimed to study the alternative reproductive strategies, especially focusing on extra-pair paternity (EPP) and intraspecific (or conspecific) brood parasitism (IBP) which has already known among other Falco species.

Results of this study present not just scientifically new data but may also form an integral part of conservation efforts in the protection of the Red-footed Falcon.

The Red-footed Falcon

Species Description and Distribution

The Red-footed Falcon (*Falco vespertinus*, RFF) is a small iconic raptor species of the Great Hungarian Plain. Its pigeon-sized body is 28-31 cm long and the wingspan is 65-78 cm. The average body mass of males is 120-180 g, and females are slightly bigger, weighing 130-210 g (Palatitz et al. 2018).

The species is characterized by a well noticeable sexual dimorphism. Males are easy to identify by colour: slate grey or 'blue' with red legs and rusty undertail coverts. The females' face is white with a black eye stripe and moustache. Their main colour is orange except the back and wings which are brown with dark transverse lines. Juveniles have a nestling plumage dominated by shades of brown. The underparts are light brown with dark strikes, wings are also dark brown, and the face is like that of females. The species got its English name for the red legs, while the Hungarian name comes from the typical 'blue' colour of males. This feature facilitates the sex determination in the field. Additionally, juveniles in the first (1y) and second year (2y) of their lives are also distinguishable based on the plumage. To determine the gender of younger individuals, molecular examination is needed.

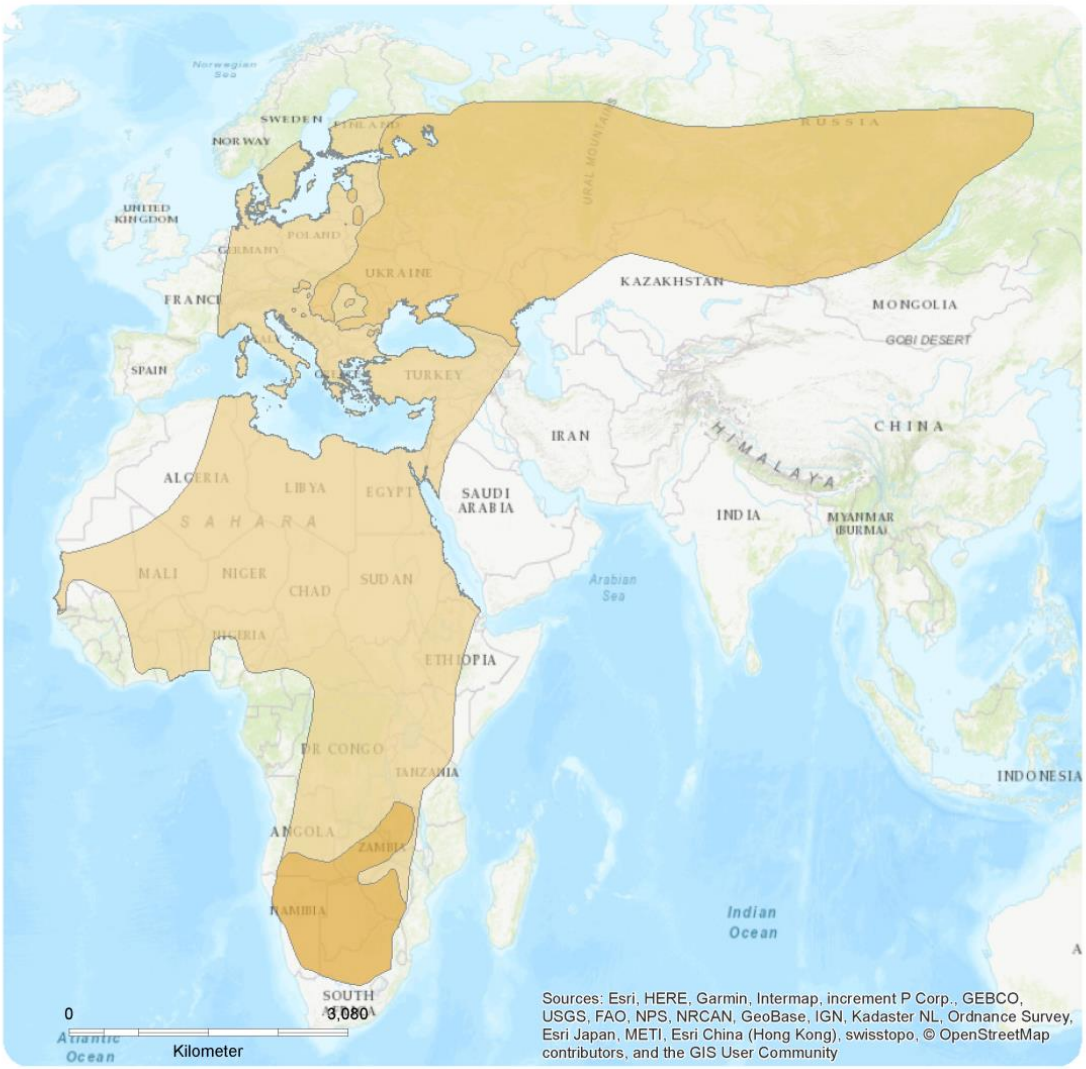
Figure 1. Red-footed Falcon female and male (Photo by Csaba Lóki)

The species belongs to the Falco genus and it has common ancestors with the Peregrine Falcon (*Falco peregrinus*), the Common Kestrel (*Falco tinnunculus*) and the Amur Falcon (*Falco amurensis*) (Wink & Siebold 1996). Due to their similarities and the wide distribution area of the RFF, the Amur Falcon was mistakenly recognised as a subspecies for a long time (Hoyo et al. 1995). However, based on phylogenetic analysis we currently regard the RFF as monotypic and the Amur Falcon as the closest relative (Fuchs et al. 2015).

The RFFs' range runs from Central Europe to Central and North Central Asia all the way to lake Baikal and Northern China. Its distribution area in the South spreads to Kazakhstan, while − except of some small colonies in Austria and Italy − Hungary is the westernmost border of its range (Figure 2). Most of the population breeds in the Russian steppe area, and approximately 30 000-64 000 pairs breed in Europe. According to the latest data, the global population is around 300 000-800 000 individuals, and it is decreasing rapidly (Birdlife International 2015, http://datazone.birdlife.org/species/factsheet/red-footed-falcon-falco-vespertinus/text, https://www.iucnredlist.org/species/22696432/131939286).

As a long-term migrant, the species leaves the breeding area through the Mediterranean Sea to Central and South Africa between the end of September and early October. Right before migration, RFFs tend to form groups of hundreds and thousands of individuals in pre-migration sites to start their annual journey together. According to a study (Katzner et al. 2016), the species follows a clockwise migration route and after spending the cold season of the northern hemisphere under favourable conditions, RFFs fly back in a loop − exposing themselves to high poaching risk through the Sahara and the Mediterranean area – around April and May (del Hoyo et al. 1994, Ferguson-Lees & Christie 2001, Katzner et al. 2016) (Figure 10).

Since the RFFs' diet includes mainly orthopterans and small rodents (e. g. Common vole - *Microtus arvalis*), their natural habitats are open steppe areas and grasslands (Purger 1998, Krištín et al. 2017). Besides depending on large lowlands, the presence of some species belonging to the family Corvidae is also essential for them. Like other falcon species, they do not build their own nests, but normally use rookeries of Rooks (*Corvus frugilegus)* or sometime the nests of the solitary Eurasian Magpie (*Pica pica*) or the Hooded Crow (*Corvus cornix*). According to Fehérvári et al. (2008), the distribution of the species (in Hungary) is determined by both the presence of the grasslands and the urbanization of Rooks. Nowadays, the majority breeds in the Hortobágy National Park, while a smaller number in the Kiskunság National Park (Bagyura & Palatitz 2004).

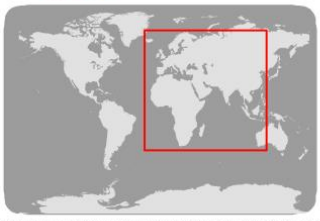


Range

- Extant (breeding)
- Extant (non-breeding)
- Extant (passage)

Compiled by:

BirdLife International and Handbook of the Birds of the World (2018)



The boundaries and names shown and the designations used on this map do not imply any official endorsement, acceptance or opinion by IUCN.

Figure 2. Distribution map of the Ref-footed Falcon © *The IUCN Red List of Threatened Species: Falco vespertinus – published in 2018. http://dx.doi.org/10.2305/IUCN.UK.2018-2.RLTS.T22696432A131939286.en*

RFFs are the only facultative colonial breeders in Hungary and regarded as socially monogamous; they choose one mate for one breeding season. They usually lay 3-4 eggs in the middle of May, and both parents incubate them until hatching on the 28th day (Haraszthy & Bagyura 1993, 1994, Palatitz et al. 2018). During the first 18 days the chicks are fed mainly by males, but later females also participate in hunting. They nurture the chicks until and even a few days after fledging, which happens approximately at the age of 23-27 days (Purger 2001, Palatitz et al. 2018). As for alternative reproductive strategies, we have already had some observations of extra-pair fertilisation, but until the present study there was no clear evidence. Intraspecific brood parasitism was completely unjustifiable without genetic data, since there were also some uncertain cases reported by the field observers from RFF colonies.

Conservation Status and Threats

According to the ICUN Red List (ANNEX I of European Commission's Birds Directive 79/409/EEC), the species is in the "near-threatened" category due to the decreasing population trend; it is predicted to drop by 30% during three generations (Birdlife International 2015, https://www.iucnredlist.org/species/22696432/131939286). The European Commission listed the species in Annex 1 of the Birds Directive as a priority species in the member states. Besides, the Bonn Convention (Convention on the Conservation of Migratory Species of Wild Animals, Appendix I.) and the Berne Convention (Convention on the Conservation of European Wildlife and Natural Habitats, Appendix II.) also cover RFFs.

In Hungary, the species is strictly protected since 1956, and the theoretical natural value of an individual is 500 000 Ft (ca.1450 €). Until the 20th century, the species was widespread in the Carpathian Basin, but the dramatic eradication of Rooks and the intensified agriculture caused severe breeding site shortage and the collapse of the species in Hungary. The mid-century population in Hungary had dropped from 2200-2500 breeding pairs to 500-600 pairs by 2006. Also, at the turn of the millennium 62% of the pairs had to use the nests of the solitary Eurasian Magpie (*Pica pica*) and the Hooded Crow (*Corvus cornix*) exposing themselves and the brood to the bigger risk of falling prey to predators. Most of the remaining colonies were located only in the central and eastern part of the country: 40% between the Danube and the Tisza and 60% in the Trans-Tisza region. In total, the Hungarian population decreased by 30-40% and almost vanished from the Transdanubia region (Keve & Szijj 1957, Fehérvári et al. 2008, Palatitz et al. 2015).

To relieve the dramatic population shrinkage, a nestbox scheme was introduced as part of the first LIFE program for the RFFs between 2006 and 2009[1]. As a result of the conservation efforts, the population stabilized, and by 2009 85% of the Hungarian breeding pairs had returned to nests in colonies (Palatitz et al. 2018). In the last decade, two other programs[2,3] were completed aiming to protect the breeding and feeding areas, prepare action plans for sustainable land use and establish international cooperation for protecting the Slovakian breeding pairs. There are also efforts to reduce other serious local threats to the birds, like electrocution caused by uninsulated power lines, poisoning and road accidents. On a global scale, many individuals die each year due to hunting, unfavourable climatic conditions and stochastic events during migration (https://www.iucnredlist.org/species/22696432/131939286).

Currently, there are 1200-1300 breeding pairs in Hungary, although most of the colonies has switched from natural nests to artificial nestboxes (Kotymán et al. 2015, Palatitz et al. 2015). Considering that ca. 40% of the world population might breed in Europe and 40% of the European population in Hungary, the local protection is well-founded and highly important.

[1] 2006-2009 LIFE05 NAT/H/000122 - Conservation of *Falco vespertinus* in the Pannonian *Region*

[2] 2010-2011: Conservation Management and Animal Health Monitoring of Natura 2000 Bird Species (HU-SRB0901/122/120)

[3] 2012-2018: Conservation of the Red-footed Falcon in the Carpathian Basin (LIFE11/NAT/HU/000926)

Literature Review and Aims

Marker Development

Since the spread of the PCR techniques, microsatellites have become one of the dominant tools of gene mapping. They are also known as STRs (short tandem repeats) or SSRs (simple sequence repeats). In the last decade, only one new type of marker named single nucleotide polymorphism (SNPs) could arise competing with STRs due to their ability of offering tremendous amount of information about the whole genome. Many studies compared their usefulness with microsatellites in genetic studies with controversial results (e. g. Brumfield et al. 2003, Monzón et al. 2014, Kleinman-Ruiz et al. 2017, Putman & Carbone 2014). Still, due to their advantages and their methodological differences from SNPs, microsatellites are useful and convenient, especially in studies when continuous monitoring or the involvement of a few individuals is needed. Keeping in mind their limitations, microsatellites are still used across genetic studies in the field of conservation biology, ecology, evolution biology, behavioural ecology and even wildlife forensic sciences (Ellegren 2004, Selkoe & Toonen 2006, De Barbara et al. 2016).

By definition microsatellites are hypervariable sequences composed of short tandem repeats of 1-6 base pairs as a result of mutations and located mainly in non-coding regions of the genome (Tóth et al. 2000, Ellegren 2004). Their variability derives from the number of repeats (length) not from the base sequence. The exact mechanism is still not clearly understood, though basically the polymerase enzyme's fault is responsible for their development (Ellegren 2004). Mutation events that generate microsatellites presumably vary among different taxa, loci, and repeat motifs. Besides, repeat mechanisms also vary among different species and even alleles of the same loci evolve with different pace as longer alleles tend to mutate faster than shorter ones (Jin et al. 1996, Wierdl et al. 1997, Schlötterer et al. 1998, Ellegren 2000).

The frequency of microsatellites is also closely related to genome size, but in birds and plants generally less microsatellites are present, while mammals and snakes have about twice as much (Neff & Gross 2001, Morgante et al. 2002, Ellegren 2004, Castoe et al. 2012). There might be two reasons in the case of birds that can explain the lower number of microsatellites. On the one hand, bird genomes are rich in coding regions and the majority of microsatellites are situated in non-coding regions regardless of species (Primmer et al. 1996). On the other hand, bird genomes contain less short and long interspersed nuclear elements (SINE, LINE)

(Deininger 1989), and most of them do not end in a poly-A tail, which can serve as a precursor for evolving microsatellites (Arcot et al. 1995, Primmer et al. 1996).

Recently many uncertainties have been raised about the usage of microsatellites in genetic studies. They are criticized because of costly development, reading problems, presence of null alleles and homoplasy (e. g. Putman & Carbone 2014, Hodel et al. 2016, Flanagan & Jones 2018). As it was mentioned above, the mutational mechanisms of microsatellites are still not fully known, and many models exist to explain the process of creating variability. These models assume that each locus is affected by mutation only once, creating a new allele every time. In this way, considering only the fragment lengths homoplasy can hide independent events and technically, we can detect only the differences in allele frequencies or in 'families'. This may lead to false conclusions, especially when using cross-species markers (Putman & Carbone 2014).

In the case of null alleles, if there is homozygote prevalence on a locus, it is possible that alleles cannot amplify during the PCR (Paetkau & Strobeck 1995). This might be due to a mutation affecting the primer binding region or preference for short amplicons or slippage during PCR (Paetkau & Strobeck 1995, Gagneux et al. 1997, Chapuis & Estoup 2007). In many cases, null alleles do not cause any serious problems, although in kinship analyses, it is advisable to exclude them, otherwise they might lead to false parentage exclusions (Dakin & Avise 2004). In the case of population structure, null alleles can cause apparently low genetic diversity, which hides differentiation in calculation of population comparison (Paetkau & Strobeck 1995, Slatkin 1995, Chapuis & Estoup 2007).

In addition, in some cases the neutrality of microsatellites has been disproved as well (Kashi and King 2006; Gemayel et al. 2010, Bagshaw 2017). Since the majority of microsatellites occur in the non-coding regions (introns), previously it was thought that STRs are not under selective pressure and the polymorphism of each locus is not generated by selection, but fuelled only by their own mutation rate, independently from the environmental changes (Ellegren 2004). Since then, microsatellites are still considered as neutral markers in most studies (Haasl & Payseur 2014).

What makes microsatellites suitable for behavioural ecological studies is that they allow unique identification of individuals. Since their variability comes from the number of the same repeat motifs, many variations of a single sequence can evolve differing only in length, and alleles are distinguishable based on this. As a result, an individual can be described by a series of alleles of a polymorphic locus set (Queller et al. 1993) (Figure 3).

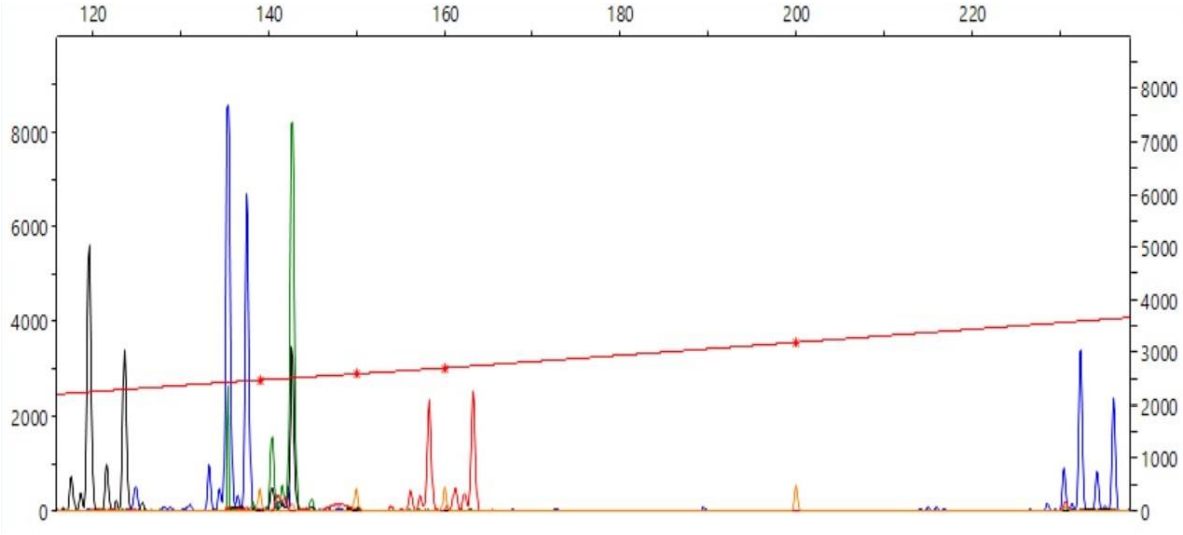


Figure 3. Alleles of five fluorescent dyed microsatellite loci in a Red-footed Falcon individual (Peak ScannerTM Software v.1.0)

In the case of genetic studies where no species-specific markers are available it is common practice to use loci that were already described for closely related species (e. g. Dawson et al. 2010). Primmer et al. (1996, 2005) found that the success of cross-species amplification strongly depends on the time elapsed since the divergence of the species. In case of non-passerines, there is a 50% probability of finding successfully cross-amplifiable microsatellites for species which diverged within 23 million years. Dawson et al. (2010) described several polymorphic loci which are applicable for many species due to their conservative primer binding region. This method is widely accepted, fast and cost-efficient; however, in many cases, only a few markers provide promising results for the target species.

In a previous genetic study of the Red-footed Falcon (Bertók 2017) a marker set of nine microsatellites developed for related species was used (Table 1).

Primer	**Species**	**Reference**
Fnd1.7 **Fnd2.3**	Lesser Kestrel (*Falco naumanni*)	Padilla et al. 2009
Fr34	Gyrfalcon (*Falco rusticolus*)	Nesje & Roed 2000
Fp31 **Fp54** **Fp82-2** **Fp89** **Fp92-1**	Peregrine Falcon (*Falco peregrinus*)	Nesje et al. 2000 a
Fp347	Saker Falcon (*Falco cherrug*)	Nittinger et al. 2007

Table 1. Potential cross-species marker candidates for the Red-footed Falcon

Even though these species are situated relatively close on the phylogenetic tree (Fuchs et al. 2015) and their markers were expected to give interpretable results, their reliability proved to be ambiguous in the first analysis. Consequently, the additional usage of species-specific markers would strengthen the marker set.
Regarding the fact that the genome of birds contains less microsatellites, finding these sequences and primer binding regions is usually difficult. Therefore, it is common practice to use the service of commercial institutions specialised in marker development. However, after getting a list of the potential candidate markers, these still need to be optimized and tested to determine their polymorphism (Selkoe & Toonen 2006).

First, repeat motifs and numbers need to be considered. In general, dinucleotides dominate the genome, but tetranucleotides are also frequent. However, trinucleotides are the most secured against frame shift mutations, still they are less common (Ellegren 2004). Dinucleotides tend to evolve faster than tetranucleotides, and thus their possible larger repeat numbers might indicate the existence of a larger number of alleles, as well (Jin et al. 1996, Wierdl et al. 1997, Schlötterer et al. 1998, Ellegren 2004, Primmer et al 1998). The disadvantage is that dinucleotides arise from the repetition of only two base pairs, and the polymerase enzyme can make in vitro (during the PCR, similarly to the process in vivo) mistakes in the number of repeat motifs, making it harder to distinguish the alleles based on their length (Ellegren 2004).

After selecting the most promising candidates, it is necessary to test them via PCR. Following the initial PCR and electrophoresis, PCR optimisation is needed when there are too many shadow bands on the gel. There are several methods to enhance specificity like raising the annealing temperature, using touchdown PCR, or changing the concentration of $MgCl_2$, primers and DNA in different combinations (http://www.bio-rad.com/en-hu/applications-technologies/pcr-troubleshooting). To increase efficiency, it is possible to put microsatellites requiring the same PCR protocol but differing in length into the same reaction mix creating a multiplex. Also, the usage of fluorescent dyes in the case of microsatellites with similar length range allows analysing them at same time during capillary electrophoresis, which can significantly reduce the cost and time of such studies (Wink 2006).

In the following steps, the most important criteria to develop a proper marker set are high polymorphism and alleles that amplify reliably. As far as the Hardy-Weinberg equilibrium (HWE) is concerned, the lack of HWE can wrongly indicate separated populations, demographic stochasticity or can be related to genetic phenomena like microsatellites coupled

with genes under selection, location in the recombination centre or gender-specific association. When a microsatellite is situated on a sex-chromosome, it might cause homozygote excess in one gender (Wilson et al 1997). However, loci which lack HWE are not necessarily excluded from the analyses, since they must be tested together with all factors before interpreting the results biologically.
Another difficulty of marker development is the previously mentioned presence of null alleles, although several software were invented especially for detecting null alleles, for example Micro Checker v.2.2 (van Oosterhout et al. 2004), and others also have a built-in function like Cervus 3.0 (Kalinowski et al. 2007) or ML-relate (Kalinowski et al. 2006).

As a first step of the genetic analyses of the RFFs, we aimed at developing a species-specific marker set to use them with cross-species markers in further analyses of population structure and alternative reproductive strategies.

Population Structure

Between the populations of a species, there is at least a slight differentiation due to several behavioural and environmental factors like dispersal ability, migration, mating system, spatial barriers or breeding habits (e. g. Peterson & Denno 1998, Hendry & Day 2005, Rutkowsky et al. 2010). Population structure is an important aspect in ecology and evolutionary biology of a species just like in conservation biology. To maintain the evolutionary potential, genetic variability and diversity between different spatial units of a species are important. They allow the identification of populations with high conservation value and prioritise accordingly. Consequently, even after many years of unsuccessful attempts to create a proper population concept, species conservation is usually implemented on the population level. Species are categorised into groups with different status of protection (see IUCN categories: critically endangered, endangered, threatened, etc., IUCN 2001) based mainly on their population size especially because the factors responsible for extinction hit small populations more seriously. In this way, species must also face issues related to genetic factors in addition to the exponentially growing human population, stochasticity in demographics and environmental events.

Recognizing the importance of the genetic variability of populations started in the ‘70s by Frankel (1970, 1974) and has raised several doubts (see Lande 1988). Later, following the publication of some supporting studies (Spielman et al. 2004, Frankham 2005), it became one

of the most important key components in species conservation. In normally evolving populations, where the conditions of the Hardy-Weinberg equilibrium (there is no mutation, migration, selection, random-mating and they are infinitely large) are not met, genetic variability as the basis of evolutionary potential is directly affected. Accordingly, in small populations genetic problems are more likely to appear with stronger effects, such as genetic drift caused by chance or inbreeding depression due to non-random mating, causing the accumulation of deleterious mutations (Frankham 2005).
Otherwise, there are also reports of low genetic variability in species with large populations, such as the albatrosses (*Diomedea exulans* and *Diomedea amsterdamensis*), which do not suffer from any negative symptoms (Milot et al. 2007). Probably even the common ancestor had low variability highlighting that different species can react in different ways to a demographic decline. It is important to understand that this is no reason to question or underestimate the role of low genetic diversity.

Thus, genetic variability is important in every case of conservation biology, and molecular genetic tools like microsatellites provide useful means to study it. They can be used to characterize populations; the differences of allele frequencies across populations allow us to track down demographic events and detect source, sink or isolated populations which might need to be prioritised in protection efforts (Selkoe & Toonen 2006, Wink 2006, Doyle et al. 2018).

Considering Falco species, Nesje et al. (2000 a) developed and published a species-specific marker set of 12 loci for the Peregrine Falcon (*Falco pereginus*), in order to conduct population analyses and study nest fidelity. Knowledge about the area where the individuals return to breed after migration is essential in understanding population structure. The species is cosmopolitan and widespread all over the world creating several subspecies and populations with genetic differentiation (Longmire et al. 1988). It was presumed that the peregrine populations of Norway and Sweden become isolated after a dramatic population decline as they exhibit different nesting habits. The genetic analyses contradicted the expectations and showed that there was no genetic isolation. In a later study Nesje et al. (2000 b) also confirmed that there is no correlation between the detected nesting habitats and genetic differentiation.
In the paper of Rutkowski et al. (2010), rural and urban populations of Polish Common Kestrels (*Falco tinnunculus*) were compared based on microsatellites. They found that one of the urban populations genetically differ from the rural populations. This might be the result of a different

source population of one of the urban populations. The study confirmed the potential importance of source and sink populations beside the effects of urbanization in conservation.
The case of the Prairie Falcon (*Falco mexicanus*) is similar to that of the Peregrine Falcon. Its range runs from Canada to Mexico in North Western America, occupying several types of habitats. Analysis made with SNPs shed light on the population structure throughout their range showing no differentiation. The study was not conducted with microsatellites, but the results highlight the fact that local conservation actions may affect a whole population (Doyle et al. 2018).
In terms of migration, a study (Miller et al. 2012) about the American Kestrel (*Falco sparverius*) confirms that species with wide breeding areas exhibit variability in migratory behaviour as well. The American Kestrel is widespread both in North and South America and partially migratory. Although, the authors could neither confirm nor refute the differentiation of the suspected two subspecies (*F. s. sparverius* and *F. s. paulus*), they found that in general, genetic diversity is lower in non-migratory populations causing more robust differentiation in population structure.

Similarly, RFFs exhibit several characteristics that can have an impact on their population structure. First, they have a wide breeding area spanning from North-Central China in the East, to Kazakhstan in the South and Hungary in the West. Second, they suffered a population decline of 30-40% happening in the 20th century, caused by habitat destruction and breeding site shortage. Third, their migratory behaviour involves the mixing of large numbers of individuals (in the pre-migration sites and the wintering area) which might also contribute to a specific population structure (Esler 2000).

Prior to genetic analyses, we did not have any information about the relationship and possible connection between the Hungarian colonies with those beyond the Carpathians except from tracking data of geolocators (Katzner et al. 2016), satellite telemetry (Fehérvári et al. 2014, Figure 4) and field observations. These kinds of tracking systems give information only about the movement patterns and are usually underrepresented in sample size (Frei et al. 2016). During the last decade, conservationists collected several hundreds of blood and feather samples not just from Hungary but also from Romania to conduct population comparison of the colonies within and beyond the Carpathians.

Figure 4. Satellite telemetry on a Red-footed Falcon (Photo by Péter Fehérvári)

Since the species is protected by international conventions both in Hungary and the neighbouring countries, information about the population structure of the Carpathian Basin is essential. From the viewpoint of conservation, it is also important to study whether the Hungarian population − seemingly separated by the Carpathians − requires further specific measures after recovering from a serious decline.

Alternative Reproductive Strategies

Until the end of the 20th century, it was generally accepted that the overwhelming majority of bird species were monogamous (Lack 1968). Nowadays, only a few of them are regarded as genetically monogamous. Since the spread of genetic tools, in the case of more than 75% of socially monogamous species, extra-pair offspring have been detected, and both males and females are characterised by mixed reproductive strategies such as Extra-Pair Paternity (EPP), Intraspecific Brood Parasitism (IBP) and Quasi-Parasitism (QP) (Griffith et al. 2002). Although the genetic mating system may be hidden, it has an essential role in forming the genetic structure of populations and the planning of species conservation. Genetics brought a revolution in studying avian mating systems as genetic markers can reflect the underlying mechanisms better than field observations.

For a long time, the analyses of alternative reproductive strategies were strongly dominated by EPP studies and scientists were focusing only on the advantages of males, while IBP and others like QP have remained less understood, possibly because they were considered as rare events, and before using molecular techniques they were difficult to observe and detect (Moller 1989, Lombardo et al. 1989, Burkhead et al. 1990). Even after the spread of the new methods, conducting research about IBP or polygamy etc. was still complicated as greater effort is required in collecting genetic samples from both parents, demanding bigger financial resources, too.

There are several hypotheses about the advantages of seeking extra-pair partners, but to date there is no comprehensive explanation for its function. The EPP shows variation at different taxonomic levels, not just between species but also between populations of the same species. It is considered that there is a hierarchical explanation based on the complexity of phylogenetic, social and ecological constraints (Griffith et al. 2002). There are several aspects with impact on EPP and IBP, like breeding density and other breeding habits (Siegel-Causey & Kharitonov 1990), evolutionary lineage (Arnold & Owens 2002), genetic variation (Petrie et at. 1998), environmental effects and even food abundance in different years (Korpimaki et al. 1996). In the case of males, it seems that they have direct advantages and the most important one is increasing the reproductive success without investing more energy in parental care (Griffith et al. 2002). In contrast to males, the potential benefits of females coming from IBP are still unclear. While previous explanations weighed the costs and benefits based on the trade-off between genetic and energetic traits, recent studies are more concerned about benefits expressed in behaviour (Jennions & Petrie 2002, Lyon & Eadie 2008, Eliassen & Jorgensen 2014, Forstmeier et al. 2014). In agreement with these studies, the advantages of females might be indirect, and IBP as an evolutionary strategy might also cause a beneficial behavioural response of males through e. g. cooperation against predators and/or preventing infanticide (Forstmeier et al. 2014).

Besides, since IBP affects the individual's fitness, it must be considered in terms of conservation, too (Lyon & Eadie 2008). In the conservation of high priority species, life history traits and mating system are key factors of the viability (Sutherland 1998, Caro 2007, Pryke et al. 2012, Garnier et al. 2012). Without understanding their consequences, we cannot create and implement successful conservation measures. In a study (Ducatez & Shine 2019) about intentional translocation of several bird species, it was found that life history traits (e. g. clutch size, clutches/year etc.) directly affect long-term survival, reproduction rate, and thus the

successful establishment of a new population. More importantly, species in new environments and modified habitats are forced to adapt to the new conditions which might trigger the evolution of different types of mating strategies and life history traits (Ducatez & Shine 2019). Accordingly, in the case of threatened species and/or bird models, mate choice and parental care are also frequently studied key components.

Raptor species are also considered typically monogamous, but there are some reports about mixed reproductive strategies among falcons (Negro et al. 1996, Rosenfield et al. 2015, Table 8). It is known that they are characterized by a low rate of EPP (3.8%-9.67%), and the only detected case of IBP occurred in the colonial Lesser Kestrel (*Falco naumanni*), where a low rate was found (7.4%, Negro et al. 1996) even after re-evaluating the previous results (6.4%, Alcaide et al. 2005). Polygyny was detected in the Lesser Kestrel (Tella et al. 1996) and polygamy in the Common Kestrel (Charter et al. 2008, Wang et al. 2019). There is no evidence of quasi-parasitism for any Falco species.

In the case of RFFs, the densities of rookeries or the remoteness of the solitary pairs made the observations and sampling of individuals difficult, leaving this facultative colonial breeder unstudied in term of genetics. Launching the nestbox scheme provided better circumstances to conduct research (Figure 5).

Figure 5. A natural rookery and an artificial colony of the Red-footed Falcons (Photo on the right by the author)

In this way, it was possible to detect that compared to other species RFFs maximize their clutch size in 3-4 laid eggs, which seems to be unaffected by food abundance (Palatitz et al. 2018). While under colonial circumstances most chicks can fledge, on average one chick or less does so from a nest of a solitary pair. This observation was explained by Haraszthy and Bagyura

(1993) with combined defence of individuals against predators. In the case of RFFs, both parents participate in parental care (Haraszthy & Bagyura 1993, 1994, Palatitz et al. 2018), incubating and feeding the nestlings. The copulatory behaviour of RFFs was observed by Ile et al. (2002), and they found that RFF males and females are usually closer to the nest in terms of time and distance, but still left it unguarded in the 19.6% of the observation time. RFF females spent around half the time of the fertile period unguarded having the chance to copulate with extra-pair males. Hoi et al. (2011) suggested that in the case of species which breed both colonially and solitary, males adjust their paternity assurance strategy according to the breeding density and nest-site quality. In the Hungarian artificial nestbox colonies of RFFs, field observations showed an infrequent occurrence of extra-pair copulations, suggesting that the rate of EPP, IBP and the possible QP was low (Palatitz et al. 2018).

In the last decades, development of molecular genetic tools brought a revolution in studying the avian mating system (Burke 1989, Selkoe & Toonen 2006). In our study data deriving from observations (social relationships between colour ringed individuals) was combined with genetic data coming from cross-species and species-specific microsatellites. As mentioned above, STRs are convenient in kinship analyses due to their ability of identifying individuals and detect parent-offspring relationship, which are essential in confirmation of extra-pair paternity, intraspecific brood parasitism, quasi-parasitism or polygamy. These characteristics of the RFFs might help to understand the complexity of reproductive behaviour, conservation actions and the possible underlaying mechanisms of the avian mating system.

The aims of the present dissertation are the following:

1. Marker development

    1.1. To create a powerful marker set of new species-specific microsatellites for the RFF

2. Population structure

    2.1. To describe the genetic structure of the Hungarian breeding sites
    2.2. To compare the genetic structure of colonies in the Carpathian Basin and beyond
    2.3. To conduct a population assignment of individuals from a Romanian pre-migration site

3. Alternative reproductive strategies

    3.1. The confirmation of extra-pair paternity (EPP)
    3.2. The confirmation of intraspecific brood parasitism (IBP)
    3.3. To detect other types of alternative reproductive strategies

Materials and Methods

All samples used in the present study were collected by the members of the Red-footed Falcon Conservation Workgroup of MME/Birdlife Hungary between 2008 and 2017. Samples of the marker development and the analyses of alternative reproductive strategies (EPP, QP, IBP and polygamy) did not overlap with the ones used in the population genetic analysis. All the necessary permits have been assured by the Hungarian authorities[4].

Samples

The marker development tests were conducted on 29 RFFs and 24 individuals of six closely related species using three types of samples. In the case of RFFs, blood samples were collected from unrelated individuals (15 males and 14 females) between 2008 and 2015. Sampling sites were selected based on the former LIFE program: Conservation of *Falco vespertinus* in the Pannonian Region (LIFE05 NAT/H/000122).

To test the efficacy of cross-species amplification, DNA was isolated from individuals of Peregrine Falcon (*F. peregrinus*, n=10), Common Kestrel (*F. tinnunculus*, n=8), Gyrfalcon (*F. rusticolus*, n=3) and samples were taken from each one Merlin (*F. columbarius*), Eurasian Hobby (*F. subbuteo*) and Saker Falcon (*F. cherrug*). All *F. cherrug*, *F. subbuteo*, *F. columbarius* DNA samples and a single *F. peregrinus* sample were extracted from toe pads of frozen museum specimens provided by the Hungarian Natural History Museum. Additionally, the *F. peregrinus* and *F. tinnunculus* samples were moulted feathers of unrelated individuals originating from independent geographical regions in Hungary. These samples were provided by the MME Birdlife Hungary and the Kecskemét Zoo (Appendix Table 1).

Blood and/or feather samples of the population genetic analyses were collected from 120 RFF individuals (N_{female}=62 and N_{male}= 58) between 2015 and 2017. In total 21 adults, 99 chicks and juveniles were involved in the analyses. They were derived from 41 Hungarian colonies, five Romanian colonies and three pre-migration roost sites in Romania. They were categorized into eight major groups in Hungary and three in Romania according to their geographical location (Figure 6). Hungary: Borsodi mezőség (n=10), Csanádi-puszták (n=10), Cserebökény (n=10), Hevesi Füves Puszták (n=10), Hortobágy (n=10), Jászság (n=10), Kiskunság (n=10), Vásárhelyi-puszták (n=10). Romania: West region colonies (n=10) close to the Hungarian

[4] Permit numbers: OKTF-KP/56-26/2015, PE-KTFO/1867-10/2018, PE-KTFO/1867-11/2018, PE-KTFO/1867-9/2018.

border, Southeast region colonies (n=10) over the Carpathians and Southeast Region pre-migration site close to the Black Sea (n=20).

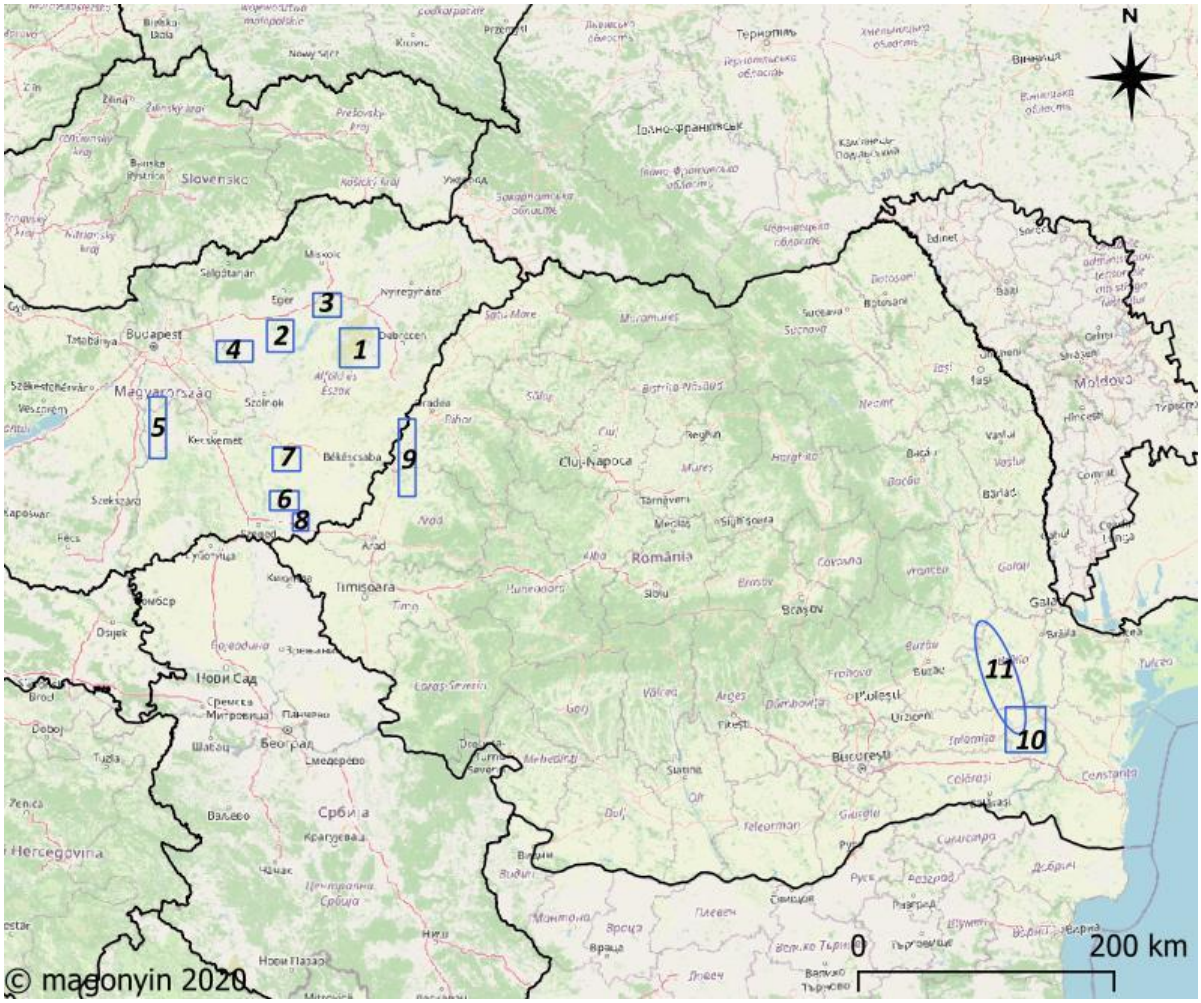


Figure 6. Map of sample sites created with QGIS. 1. Hortobágy, 2. Hevesi füves puszták, 3. Borsodi-mezőség, 4. Jászság, 5. Kiskunság, 6. Vásárhelyi-puszták, 7. Cserebökény, 8. Csanádi-puszták, 9. West region colonies, 10. Southeast region colonies, 11. Pre-migration sites in Southeast region

For the study of alternative reproductive strategies, samples were collected from 128 chicks and 82 adults from 48 RFF families between 2008 and 2015. 12 adults were captured and re-sampled in multiple years. After excluding multiple samplings 97 chicks and 74 adults from 37 families were used in the analyses. We report each individual in the summarised data only one time and other findings separately. The sampling site covered the Vásárhelyi-Grasslands in the Körös-Maros National Park.

During each sampling adults were captured by a net placed right in front of the nest. Samples were taken from each social parent and nestling of the studied broods. Feather samples were

plucked from the back and blood was taken from the brachial vein. Adults were identified using colour-rings. They were regarded as social (putative) parents if they regularly participated in incubation (including males), fed the chicks, behaved like a social pair and they were identified at least two independent occasions during the incubation and rearing period. This was determined by field observation using telescope or camera traps in the case of densely covered nests.

Except of the feathers provided by the MME Birdlife Hungary and the Kecskemét Zoo, all samples were stored in cryotubes in ethyl-alcohol (96%) at -20°C in order to avoid degradation. The mentioned moulted feathers of *F. peregrinus* and *F. tinnunculus* individuals were also stored at -20°C.

DNA Extraction

DNA was extracted with the Genomic DNA Mini Kit (Geneaid®, New Taipei City, Taiwan) using the standard protocol provided by the manufacturer. To facilitate the digestion of feathers and toe pads, 10µl DTT (1,4-dithiothreitol, 1M) was added (Weigmann, 1968), and samples were incubated at 60°C overnight and 24 hours, respectively. Blood samples were incubated for 1.5 hours also at 60°C. In the end, DNA was eluted in 100 µl of elution buffer and stored at -20°C. When both blood and feather samples were available, the usage of blood was preferred for its easier and faster DNA isolation process.

Microsatellite Markers

In the marker development microsatellite candidates of the RFFs were designed by Ecogenics GmbH based on the samples of four *F. vespertinus* individuals. The library was analysed on an Illumina MiSeq platform using the Nano 2x250 v2 format (Balgach Switzerland). After the assembly, 868 contigs or singlets contained microsatellite inserts with a tetra- or a trinucleotide of at least six repeat units or a dinucleotide of at least ten repeat units. Primer design was possible for 580 microsatellite candidates out of which 44 were selected based on repeat motif and repeat length, which features might indicate polymorphism.

Annealing temperatures were determined based on the melting temperature of the primers and did not deviate from them by more than 5°C. At first, a general touchdown PCR program was set, and the same recipe was used for the amplification of all the 44 candidate loci. After visualising the results via agarose gel electrophoresis, many markers failed to amplify due to

the general settings. In order to increase the specificity of primer pairs, the PCR program and the composition of the reaction mixture (primer and $MgCl_2$ concentration) was altered. Before ordering dyed primers, possible polymorphism was tested on agarose gel to exclude monomorphic markers: five independent individuals were selected and tested for the primers previously well-optimised (Figure 7).

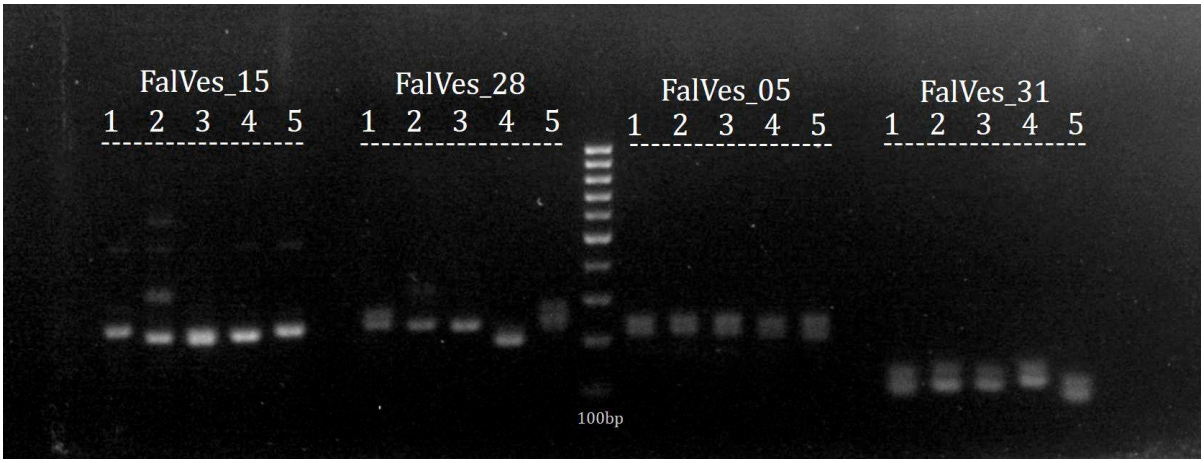


Figure 7. Polymorphism test on 2% agarose gel; four loci tested on the same five individuals deriving from different geographical locations

Based on the preliminary tests the following 12 primer pairs were chosen to be subjected to fragment length analysis:

- FalVes_03 (TET),
- FalVes_04 (PET),
- FalVes_05 (6-FAM),
- FalVes_13 (6-FAM),
- FalVes_15 (PET),
- FalVes_26 (NED),
- FalVes_28 (6-FAM),
- FalVes_30 (NED),
- FalVes_31 (6-FAM),
- FalVes_34 (NED),
- FalVes_38 (HEX)
- FalVes_43 (HEX).

For the population genetic analyses four of these newly developed species-specific markers (FalVes_15, FalVes_26, FalVes_28 and FalVes_31) were selected based on their well-divisible allele ladders. To strengthen the resolution power of our marker set seven more microsatellite loci were added which were described previously for related species: Fnd2.3, Fr34, Fp82-2, Fp89, Fp54, Fp92-1, Fp347 (Table 2).

In the case of the alternative reproductive strategies both types of markers were used, too. Six cross species markers (Fnd2.3, Fr34, Fp82-2, Fp89, Fp54, Fp347) were selected in combination

with all the species-specific markers except FalVes_03 and FalVes_34 (Table 2).

Marker	Analyses	Species	Citation
Fnd2.3	PG & ARS	Lesser Kestrel (*Falco naumanni*)	Padilla et al. (2009)
Fr34	PG & ARS	Gyrfalcon (*Falco rusticolus*)	Nesje & Roed (2000)
Fp82-2	PG & ARS	Peregrine Falcon (*Falco peregrinus*)	Nesje et al. (2000a)
Fp89	PG & ARS		
Fp54	PG & ARS		
Fp92-1	PG		
Fp347	PG & ARS	Saker Falcon (*Falco cherrug*)	Nittinger et al. (2007)
FalVes_04	ARS	Red-footed Falcon (*Falco vespertinus*)	Magonyi et al. (2019)
FalVes_05	ARS		
FalVes_13	ARS		
FalVes_15	PG & ARS		
FalVes_26	PG & ARS		
FalVes_28	PG & ARS		
FalVes_30	ARS		
FalVes_31	PG & ARS		
FalVes_38	ARS		
FalVes_43	ARS		

Table 2. Data of STR markers used in the analyses (PG = Population genetics; ARS= Alternative reproductive strategies

PCR Analyses and Conditions

All loci of the marker development were amplified in 17 µl of simplex PCR reactions, with slight differences in $MgCl_2$ and primer concentrations:

For FalVes_13, FalVes_26, FalVes_31, FalVes_38 and FalVes_43 the mix contained 0.065 µl enzyme (DreamTaq DNA polymerase, 5 U/µl, Thermo Scientific®), 1.7 µl 10X DreamTaq Green Buffer (Thermo Scientific®, includes 20 mM $MgCl_2$), 0.0165 µM $MgCl_2$

(25 mM, Thermo Scientific®), 0.00132 µM dNTP (2 mM, Thermo Scientific®) and 3.75 pmol forward and reverse primer respectively.

For FalVes_15, FalVes_28 and FalVes_30 $MgCl_2$ and primer quantities were reduced to 0.0125 µM $MgCl_2$ and 2.5 pmol primer (forward and reverse separately).

For FalVes_03, FalVes_04, FalVes_05 and FalVes_34 primer quantity was reduced to 2.5 pmol (forward and reverse separately).

A touch down PCR programs were used to ensure the amplification of the correct fragments of the species-specific markers. For all markers except FalVes_13, the initial denaturation was 2 min at 95°C, followed by 11 cycles of 30 s at 95°C, 30 s at 60°C, 30 s at 72°C and 26 another cycles of 30 s at 95°C, 45 s at 55°C, 45 s at 72°C, followed by a final extension of 7 min at 72°C. In the case of FalVes_13, annealing started at 57°C and the temperature was decreased to 52°C.

In the case of population genetic analysis, the amplification of cross-species markers was performed in two different mixes containing multiple markers. *Mix1* contained Fp89, Fr34 and Fp54. *Mix2* contained Fp92-1, Fp347 and Fnd2.3 markers, while the Fp82-2 marker was amplified on its own (Appendix Table 2.)

In both *Mix1* and *Mix2*, DNA was amplified in a 22.81µl reaction mix which contained 0.16µl enzyme (DreamTaq DNA polymerase, 5 U/µl, Thermo Scientific®), 4.53µl 10X DreamTaq Green Buffer (Thermo Scientific®, includes 20 mM $MgCl_2$), 0.04325 µM $MgCl_2$ (25 mM, Thermo Scientific®), 3.46 x 10^{-3} µM dNTP (2 mM, Thermo Scientific®) and 3.325 pmol from each primer. For Fp82-2 the same recipe was used for it as for FalVes_13, FalVes_26, FalVes_31, FalVes_38 and FalVes_43.

The species-specific markers, Falves_15 and Falves_26 were amplified in simplex reactions, as it was described above, while FalVes_31 and FalVes_28 could be amplified in a duplex reaction. In this latter case the reaction mixture was changed as follows: the mix contained 0.01µl enzyme (DreamTaq DNA polymerase, 5 U/µl, Thermo Scientific®), 2.55µl 10X DreamTaq Green Buffer (Thermo Scientific®, includes 20 mM $MgCl_2$), 0.01875 µM $MgCl_2$ (25 mM, Thermo Scientific®), 2x10^{-3} µM dNTP (2 mM, Thermo Scientific®) and 2.5 pmol from each primer pair, respectively.

Both reaction mixes of the cross-species markers were amplifiable under the same PCR conditions differing from the one used for the species-specific marker, which was described above. For cross-species markers, the initial denaturation was 2 min at 95°C, followed by 37 cycles of 30 s at 95°C, 45 s at 55°C, 45 s at 72°C and then 7 mint at 72°C.

In the case of kinship analysis, three different PCR protocols were used, and all loci were amplified separately in a 17 µl reaction mix, differing only in the concentrations of $MgCl_2$ and primer. All basic data of species-specific markers (PCR protocols and conditions) are described in Magonyi et al. (2019). The protocol and conditions for the cross-species markers (Fp89, Fp82-2, Fnd2.3, Fr34, Fp347 and Fp54) was identical to the one used for the first group of species-specific markers (FalVes_13, FalVes_26, FalVes_31 and FalVes_38) and they were amplified in simplexes.

Molecular sex determination was performed using the CHD1 gene (chromodomain helicase DNA-binding) intron 16 to amplify its two types differing in length, the CHD-W and the CHD-Z genes (Fridolfsson & Ellegren 1999, Suh et al. 2011). The marker was amplified in a 10 µl reaction mix which contained 0.04 µl enzyme (DreamTaq DNA polymerase, 5 U/µl, Thermo Scientific®), 1 µl 10X DreamTaq Green Buffer (Thermo Scientific®, includes 20 mM $MgCl_2$), 0.01 µM $MgCl_2$ (25 mM, Thermo Scientific®), 8 x 10^{-4} µM dNTP (2 mM each, Thermo Scientific®) and 2.5 pmol forward and reverse primer, respectively.
During PCR, the initial denaturation was 2 min at 95°C, followed by 9 cycles of 30 s at 95°C, 45 s at 60°C, 45 s at 72°C and by 28 cycles of 30 s at 95°C, 45 s at 52°C, 45 s at 72°C, followed by a final extension of 5 min at 72°C.
As a final step, PCR products were screened on 2% agarose gel by electrophoresis. Using fluorescent dyes, females and males were distinguishable based on double (ZW) and single (ZZ) bands, respectively. The expected range (Ágh et al. 2018) of 'Z' is located between 500 and 750 bp (~600) and 'W' is between 300 and 500 bp (~450) with using Thermo Scientific O'GeneRuler Express DNA Ladder (2012 Thermo Fisher Scientific Inc.).

All PCR protocols and conditions can be found in the Appendix: Table 3 and Table 4.

Software and Statistics

The candidate sequences of potential markers were checked with MEGA 7 (Kumar et al. 2016) to identify misleading complex motifs and avoid potential mistakes.

PCR products were analysed by capillary electrophoresis using the internal size standard GS500LIZ (Applied Biosystems, USA). Fragment lengths were determined using Peak ScannerTM Software v.1.0 (Applied Biosystems, Foster City, CA). Validation of peak reads in the electropherograms performed by re-genotyping of 30 RFF individuals on a different plate and scoring was done by two persons independently for all samples.

The presence of null alleles was estimated with the Micro Checker v.2.2.3 program (van Oosterhout et al. 2004). Basic statistics (N_a, H_e, H_o and PI) were calculated with GenAlEx v.6 (Peakall & Smouse, 2012). Deviation from the Hardy-Weinberg equilibrium (HWE) and possible linkage disequilibrium between loci were tested by Arlequin 3.5.2.2 (Excoffier & Lischer 2010).

For the estimation of genetic differentiation between breeding sites F_{ST} (Weir & Cockerham, 1984) were calculated also by Arlequin 3.5.2.2 (Excoffier & Lischer 2010). Genetic structure was illustrated by Structure v.2.3.4 (Stanford University, USA, 2012). Map of samples was created using the Free and Open Source QGIS 3.4.15 (http://qgis.org). Parentage analysis was performed manually and with using Cervus 3.0 (Kalinowski et al. 2007). The program is based on the calculation of LOD (log of overall likelihood ratio) scores for construction of statistical tests in parentage analyses.

Results

Although the Red-footed Falcon is a well-studied species, to date there was no genetic knowledge available about it. In order to conduct population analysis about the Hungarian colonies and study the reproductive strategies of the species we assembled a microsatellite panel of cross-species markers and completed it with newly developed species-specific markers.

Marker Development

In the analysis samples taken from 29 independent RFF individuals (15 males and 14 females) were involved. At the end of the optimization process ten out of the 12 loci were characterized by polymorphism and distinguishable alleles creating a clear allele ladder. The expected sequences were reliably amplifiable without the excessive presence of confusing shadow-bands on agarose gel. The two remaining markers (FalVes_03 and FalVes_34) were excluded from further population genetic and extra-pair analyses due to uncertain amplification or incomprehensible results. The number of alleles per locus ranged from 6 to 26 (mean 13.4). The mean expected heterozygosity (H_e) was 0.82 (0.59-0.93) and the mean observed heterozygosity (H_o) was 0.69 (0.31-0.93) (Table 3). Significant linkage disequilibrium was found between FalVes_13 and FalVes_26 (p=0.0064), although it was not significant after Bonferroni correction (corrected alpha=0.0011). FalVes_04, FalVes_30, and FalVes_43 deviated significantly from Hardy-Weinberg equilibrium and Micro Checker also detected possible null alleles at these loci (F_{null}= 0.177-0.232, Table 3). The combined probability of identity (PI) was 8.2×10^{-15} and the probability of identity between siblings (PI_{SIBS}) was 2.8×10^{-5}.

Loci name	GenBank accession no.		Primer Sequence	Repeat motif	Range (bp)	N_a	H_o	H_e	F_{null}
FalVes_03	MH981228	F	TCTGTGCAGAGTGTTTACGG	$(TTA)_{29}$	160-212	7	1.000	0.805	-0.1082
		R	TCCTTTCCCACATTTTCTGACTG						
FalVes_04	MH981227	F	TCTCAGGCACTGAAGATAGC	$(TTA)_{26}$	156-260	21	0.483	0.932	0.2326
		R	ACACCAACACAGCATTTACAC						
FalVes_05	MH981218	F	TCACAATGCCTTTAGACCTCTG	$(GATG)_{23}$	181-249	12	0.793	0.853	0.0321
		R	AGGATGCAACTTTGACATTTTTGG						
FalVes_13	MH981219	F	AACAAGTGCTGTTCCTGATG	$(ATT)_{19}$	97-166	13	0.828	0.81	-0.0095
		R	TGTGCACTTCTAATGCTGGTC						
FalVes_15	MH981220	F	GGATCTGTTTGAAGCACAGGG	$(AC)_{18}$	214-330	12	0.793	0.888	0.0501
		R	CACAGCACACGATTCCAGAC						
FalVes_26	MH981221	F	TCCTGAGAGGCATAAACATTTTGG	$(AC)_{17}$	189-215	10	0.724	0.825	0.0551
		R	TATGCAGGAACCAACTCACG						
FalVes_28	MH981222	F	CACATTCCTCGAGCAGACAC	$(TATC)_{22}$	199-325	26	0.931	0.911	-0.0106
		R	AGCAGGACTCTTTCCAGTGAG						
FalVes_30	MH981223	F	CCCTTTGGTTTACAGAAGTCCC	$(AATA)_{7}$	223-239	6	0.31	0.636	0.1988
		R	CAAAGAGATGGTGCGAGGTG						
FalVes_31	MH981224	F	CCTCAGGAAACAAGTCTGGG	$(GAAT)_{10}$	108-144	10	0.793	0.839	0.0249
		R	TGTTAGCTGATGGCCACTTTTC						
FalVes_38	MH981225	F	ACAAGCCGAAATGAAGCGAG	$(GAAA)_9$ & $(AG)_{10}$	216-289	18	0.897	0.906	0.005
		R	GACAGTAGCGGCTGGTTTTC						
FalVes_43	MH981226	F	TGTGGCTTTCGCATTTCTGG	$(TATT)_{10}$	195-225	6	0.31	0.593	0.1773
		R	GTCATTTAGGCATTTCACTGCTG						

Table 3. Characteristics and basic population genetic parameters of the new microsatellite loci of Red-footed Falcon (n=29). N_a=Number of alleles, H_o=Observed heterozygosity, H_e=Expected heterozygosity, F_{null}=Estimated null allele suspicion (e Brookfield 1 method)

All ten new markers and FalVes_03 were tested for cross-amplification on six closely related species: *F. peregrinus*, *F. tinnunculus*, *F. rusticolus*, *F. columbarius*, *F. subbuteo*, and *F. cherrug*.

For *F. peregrinus* (n=10) FalVes_28 failed to amplify in the case of nine individuals and FalVes_04 also worked only on half of the individuals. In the other nine loci, allele number per loci ranged from 2 to 8 (mean 3.9). FalVes_26 and FalVes_43 showed signs of null alleles (F_{null}=0.197; 0.203), and the probability of identity was 1.1×10^{-7}. Deviation from Hardy-Weinberg equilibrium was detected in the case of FalVes_04, FalVes_05, FalVes_13, FalVes_26, FalVes_38, and FalVes_43 (p=0-0.44).

For *F. tinnunculus* (n=8), FalVes_04 and FalVes_43 were monomorphic and FalVes_28 did not yield any product. The numbers of allele per locus ranged from 1 to 11 (mean 5.36).

The results of FalVes_03 are based on the *Falco tinnunculus* (n=8). There were no signs of null alleles at any locus, probability of identity was 8.3×10^{-10} and only FalVes_31 deviated significantly from Hardy-Weinberg equilibrium (p=0.04). In the case of other related species, *F. rusticolus* (n=3), *F. cherrug* (n=1), *F. columbarius* (n=1) and *F. subbuteo* (n=1), the markers were tested on small sample sizes; therefore, we report only the presence of detectable amplicons for all loci in all species (Tables 4a and 4b).

Sequences of the new markers are in Appendix, Table 5.

	Falco pereginus (n=10)				Falco tinnunculus (n=8)			
Loci name	Range (bp)	N_a	H_o	H_e	Range (bp)	N_a	H_o	H_e
FalVes_03	122-186	4	0.400	0.610	120-156	9	0.625	0.766
FalVes_04	150-159	2	0.000	0.320	156	1	–	–
FalVes_05	188-286	6	0.300	0.425	184-212	8	1.000	0.859
FalVes_13	96-135	3	0.444	0.494	93-102	3	0.625	0.539
FalVes_15	216-218	2	0.111	0.105	212-234	7	0.625	0.773
FalVes_26	147-203	4	0.333	0.660	187-207	6	0.875	0.813
FalVes_28	158-362	2	1.000	0.500	–	–		
FalVes_30	350-434	8	1.000	0.860	237-345	11	0.875	0.875
FalVes_31	120-136	4	0.556	0.710	120-162	6	0.625	0.688
FalVes_38	171-213	3	0.444	0.568	207-219	7	0.875	0.813
FalVes_43	190-256	5	0.333	0.673	195	1	–	–

Table 4a. Results of the cross-species amplifications. N_a= Number of alleles, H_o= Observed heterozygosity, H_e=Expected heterozygosity

	Falco rusticolus (n=3)		Falco columbarius (n=1)		Falco subbuteo (n=1)		Falco cherrug (n=1)	
Loci name	Range (bp)	N_a	Range (bp)	N_a	Range (bp)	N_a	Range (bp)	N_a
FalVes_03	163-187	3	141-158	2	170-182	2	163	1
FalVes_04	159	1	165	1	165-195	2	159	1
FalVes_05	216-220	2	183	1	175	1	212-228	2
FalVes_13	97-136	2	94-133	2	100-120	2	97	1
FalVes_15	218	1	220-222	2	214-232	2	218	1
FalVes_26	189	1	185	1	128	1	183-197	2
FalVes_28	176-196	3	236-242	2	176-184	2	238	1
FalVes_30	325-461	4	271-275	2	262	1	309-370	2
FalVes_31	120-134	4	100-108	2	116	1	149-154	2
FalVes_38	211-215	3	205	1	205-215	2	211-215	2
FalVes_43	227-251	2	227-235	2	215-219	2	227	1

Table 4b. Results of the cross-species amplifications. N_a= Number of alleles, H_o= Observed heterozygosity, H_e=Expected heterozygosity

Population Structure

Regarding the global distribution of the species, the Hungarian Red-footed Falcon colonies are located relatively close to each other, while the studied Romanian breeding site is separated by the Carpathian Mountains. In the analysis 100 individuals were genotyped with ten microsatellite markers (six cross-species and four species-specific markers, Table 2) from ten locations.

In the case of cross-species markers from the initial marker set of seven, Fp54 was excluded due to high null allele frequency (F_{null}=0.153) and low polymorphism (N_a=4). In this case the alleles found per locus ranged from 5 to 16 (mean 9 ± 4.05) and for species-specific markers from 11 to 39 (mean 21.25 ± 12.23). In total the mean was 13.9 ± 9.95 and the mean of the average alleles found per locations was 6.56 ± 0.4. The allele frequencies for the ten loci and ten geographical locations are given in Table 6 in the Appendix. The expected heterozygosities (H_e) and observed heterozygosities (H_o) for each location are given in Table 5. The highest observed heterozygosity (H_o) was 0.71 in Hortobágy and the highest allelic diversity (A) was 7.2 in Csanádi-puszták. Significant linkage disequilibrium was found by Arlequin 3.5.2.2 between several pairs of loci in several locations but without any consistency. Fp92-1 (p= 0.0004286), FalVes_15 (p=0.00001199), FalVes_26 (p=0.00034711) and FalVes_28 (p=0.00227) deviated significantly from Hardy-Weinberg equilibrium. Micro Checker v.2.3.3 also detected possible null alleles at several loci: Fp92- 1 (F_{null}=0.2097), Fp89 (F_{null}=0.086), FalVes_15 (F_{null}=0.142) FalVes_26 (F_{null}=0.099), FalVes_28 (F_{null}=0.065).

The probability of identity (PI) value of the marker set was between 2.3×10^{-11} and 3.6×10^{-10} for increasing locus combinations and probability of identity between siblings (PI_{SIBS}) was between 8.5×10^{-5} and 2.7×10^{-4} also for increasing locus combinations.

Locations	N_a (Mean)	H_o (Mean)	H_e (Mean)
Borsodi-mezőség	6.400	0.618	0.677
Csanádi-puszták	7.200	0.690	0.713
Cserebökény	6.900	0.570	0.738
Heves	6.700	0.670	0.728
Hortobágy	6.600	0.710	0.724
Jászság	5.900	0.636	0.710
Kiskunság	6.900	0.659	0.743
Vásárhelyi-puszták	6.000	0.647	0.700
West region colonies in Romania	6.500	0.630	0.761
Southeast region colony in Romania	6.500	0.630	0.705

Table 5. Number of alleles (N_a), Expected (H_e) and Observed Heterozygosity (H_o) of each location

According to the AMOVA test done by Arlequin 3.5.2.2 the molecular variance among individuals is 15.44%, within individuals 84.31% and among populations 0.24%. Accordingly, the F_{ST} value was 0.00247 (p=1), while the F_{IS}=0.1548 (p=0). Among the pairwise F_{ST} values none was statistically significant (Table 6). The Mantel test showed no correlation between pairwise F_{ST} values and pairwise geographical distances r = -0.188313 (p=0.795).

	BOR	CSAN	CSER	HEV	HOR	JAS	KISK	RO1	RO2	VAS
BOR	0.00000	0.05405 ±0.0201	0.12613 ±0.0278	0.40541 ±0.0245	0.21622 ±0.0243	0.15315 ±0.0273	0.06306 ±0.0237	0.56757 ±0.0360	0.14414 ±0.0242	0.08108 ±0.0316
CSAN	0.03032	0.00000	0.18919 ±0.0394	0.46847 ±0.0379	0.55856 ±0.0379	0.13514 ±0.0311	0.10811 ±0.0264	0.17117 ±0.0438	0.31532 ±0.0493	0.27928 ±0.0438
CSER	0.02770	0.01968	0.00000	0.85586 ±0.0390	0.37838 ±0.0485	0.78378 ±0.0490	0.66667 ±0.0433	0.70270 ±0.0327	0.45045 ±0.0429	0.82883 ±0.0298
HEV	0.00575	0.00341	-0.00463	0.00000	0.90991 ±0.0287	0.79279 ±0.0327	0.75676 ±0.0338	0.72072 ±0.0304	0.53153 ±0.0286	0.45946 ±0.0550
HOR	0.01662	0.00255	0.01288	-0.00918	0.00000	0.36937 ±0.0438	0.40541 ±0.0579	0.36937 ±0.0394	0.27928 ±0.0533	0.33333 ±0.0692
JAS	0.02101	0.01845	-0.00161	-0.00328	0.00914	0.00000	0.08108 ±0.0212	0.60360 ±0.0508	0.23423 ±0.0411	0.64865 ±0.0504
KISK	0.02729	0.02049	0.00254	0.00062	0.00852	0.02579	0.00000	0.30631 ±0.0364	0.64865 ±0.0265	0.20721 ±0.0430
RO1	0.00332	0.01832	0.00044	-0.00156	0.00661	0.00025	0.01375	0.00000	0.45946 ±0.0344	0.61261 ±0.0485
RO2	0.02236	0.01018	0.01478	0.00583	0.01380	0.01880	0.00427	0.00876	0.00000	0.18018 ±0.0332
VAS	0.02156	0.01087	-0.00059	0.00896	0.01171	0.00292	0.01802	0.00266	0.01870	0.00000

Table 6. Pairwise F_{ST} values below diagonal and their P values above diagonal *P≤0.05 (BOR − Borsodi-mezőség, CSAN – Csanádi-puszták, CSER – Cserebökény, HEV – Heves, HOR – Hortobágy, JAS – Jászság, KISK – Kiskunság, RO1 – Southeast region colonies of Romania, RO2 – West region colonies of Romania, VAS – Vásárhelyi-puszták

Similarly, the Structure v.2.3.4 (Stanford University, USA, 2012) analysis based on F_{ST} values could not differentiate the breeding sites (Figure 8).

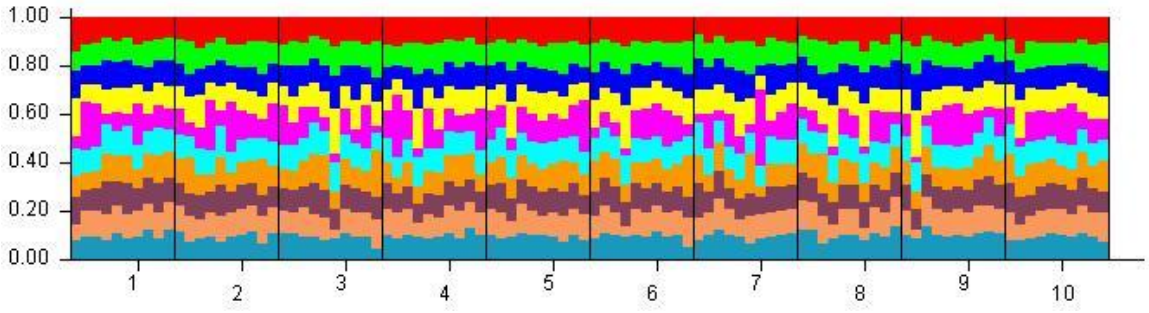


Figure 8. Comparison of the Hungarian and the Romanian breeding sites. Number eight is the colony beyond the Carpathians. There is no detachment of any colour.

In the analysis K (number of possible populations) was set from 1 to 10 and the highest likelihood scores were given in case K=1 (Figure 9).

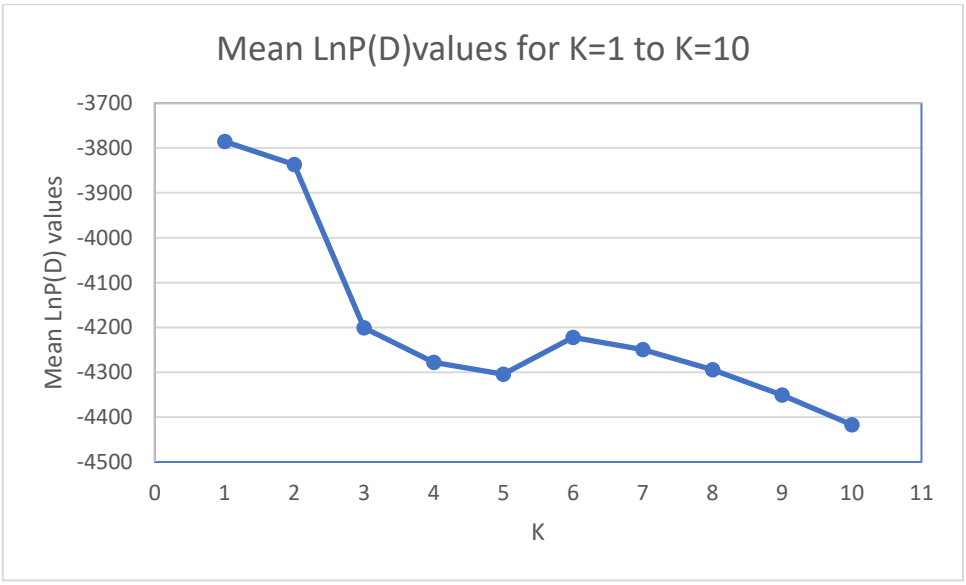


Figure 9. Mean LnP(D) values for K = 1 to K = 10 calculated by STRUCTURE 2.3.4.

Population assignment was not feasible due to the lack of genetic differentiation between the studied colonies of the Carpathian Basin and the Southeast region colony over the Carpathians.

During sex determination, adults were well-distinguishable in the field based on their plumage, but in the case of chicks and juveniles molecular analysis of intron 16 of the CHD1 gene was performed. In total 62 females and 58 males were identified.

Alternative Reproductive Strategies

Results of the extra-pair fertilisation analysis are based on genotyping 171 individuals (74 adults and 97 offspring) from 37 RFF families with seven species-specific and five additional cross-species microsatellite markers (Table 2).

After manually checking the families' data, from the original 16 markers FalVes_04 and FalVes_15 were excluded because reading of peaks failed to give comprehensible and clear results in several cases. FalVes_43 was also excluded from analysis due to the excessive presence of homozygotes. Regarding cross-species markers, Fp54 was similarly excluded due to high null allele frequency (F_{null}=0.1442) and low polymorphism (N_a=4).

The probability of identity (PI) value of the marker set of the remaining 12 loci was PI=9.8×10^{-15} and for siblings $PI_{SIBS}=1.4 \times 10^{-5}$. The exclusion power (EP) value when both parents were known was EP1 = 9×10^{-9}, with only one parent EP2 = 9×10^{-6} and with no parent known EP3 = 9.3×10^{-4} (Calculated with GenAlEx 6.4, Peakall & Smouse 2012). The observed heterozygosity (H_o) was 0.738 (0.415-0.915) and the expected heterozygosity (H_e) was 0.745 (0.445-0.922). In total 170 alleles were found (179 including the chicks), the mean was 14.167. The Micro Checker found one locus (FalVes_26 – F_{null}=0.1058) affected by null alleles because of homozygote excess. Cervus 3.0 (Kalinowski et al. 2007) software also found the social parents of every offspring to be identical with the genetic parents, except for the cases which were identified as EPP, QP or IBP manually as well.

In manual analysis of EPP we considered a chick extra-pair if its genotype differed from the putative father's at least on two loci and the social mother proved to be identical with the genetic mother with no mismatches on the maternal alleles. We detected two extra-pair offspring in one nest of three chicks out of 37 families (2.7%). Altogether 2.06% of the chicks (2/97) were the results of extra-pair paternity.

In the case of IBP, chicks also had to differ at least on two loci from both putative parents in manual analysis to be regarded as IBP. One out of 37 families (2.7%) contained one offspring of IBP. In total 1.03% of chicks (1/97) derived from IBP.

In one case none of the chicks were related to the female, while the social male proved to be the genetic father. This case appeared to be quasi-parasitism (QP) (Table 7).

In another case, we found a male feeding the chicks in two different nests parallelly, at the same time (2014). According to the genetic results this male was the genetic father of all chicks in both nests, representing polygamy.

In total 4.12% of the chicks (4 of 97) did not match with at least one of the parents at least on two loci and seemed to be extra-pair offspring. In each case one nest was affected due either to EPP, QP or IBP (in total three out of 37, 8.11%) (Table 7). Among the studied adults we could not identify the genetic parents of the mismatching offspring neither manually, nor with Cervus 3.0.

	No. of nestlings	Frequency of nestlings (%)	No. of families	Frequency of families (%)
EPP	2	2.06	1	2.70
IBP	1	1.03	1	2.70
QP	1	1.03	1	2.70
Total	**4**	**4.12**	**3**	**8.11**

Table 7. Data of extra-pair paternity (EPP), intraspecific brood parasitism (IBP) and quasi-parasitism (QP) in the Hungarian Red-footed Falcon colonies

From the results of multiple samplings, out of the six resampled males only one raised an EPP chick and later was involved in an IBP case. This male was resampled a third time when it was the genetic father of all three chicks. In the case of females, six were resampled, and only one had EPP chicks, but this one in successive years with different males as social fathers. The female of the one case of QP raised four chicks in 2014, all belonging to the putative father, but in 2015 one out of three was the result of QP. One of the female partners of the polygamous male of 2014 was resampled a year later, and it was raising two chicks in a monogamous relationship with another male. The other female involved in the 2014 polygamy case could not be resampled.

Discussion

Present study is the first to introduce new microsatellite markers for conservation genetic analyses of the Red-footed Falcon (*Falco vespertinus*). Here we present the evaluation of the combined marker set in the analysis of population genetic structure and alternative reproduction strategies of the species.

Marker Development

Prior to the launch of the intensive species conservation programs, collecting reliable data about population structure and mating system was complicated. The usage of field monitoring data combined with cross-species genetic markers proved to be not powerful enough to conduct population structure and kinship analyses. To overcome the problem, we developed a new marker set of ten species-specific microsatellites.

In the analyses of population structure and alternative reproductive strategies, except for FalVes_03 and FalVes_04 all the markers were involved in different combinations with cross-species markers.

Firstly, four of them (FalVes_15, FalVes_26, FalVes_28 and FalVes_31) were used for genotyping 100 individuals in the population structure analyses, although except of FalVes_31 they were not in Hardy-Weinberg equilibrium (HWE) and showed the presence of null alleles. To confirm the results, the analysis was repeated after the exclusion of the most problematic loci, FalVes_15 and Fp92-1 ($P \geq 0.1$) from the cross-species markers giving the same findings.

In the analyses of alternative reproductive strategies all ten markers were tested on 210 individuals. Manual checking of families led to the exclusion of FalVes_04, FalVes_15 and FalVes_43 due to the presence of null alleles and unclear peaks in the electropherograms. Both FalVes_04 and FalVes_43 showed null alleles based on the analyses with Micro Checker and showed divergence from the HWE during the first tests of marker development, too. Regarding FalVes_26 and FalVes_28, both seemed to be slightly problematic in the analyses of population structure, still the high polymorphism they showed made them useful for unique identification of individuals. In our view, by excluding these markers the power of the marker set would have been greatly reduced. The signs of null alleles at these loci were also considered, as two mismatches were needed in the manual analysis. We used the

software Cervus, to check our results statistically allowing only one mismatch. This analysis provided the same results, i.e. both excluded the offspring.

The marker set was also cross-tested on closely related species (*F. peregrinus* (n=10), *F. tinnunculus* (n=8), *F. rusticolus* (n=3), *F. columbarius* (n=1), *F. subbuteo* (n=1) and *F. cherrug* (n=1) and proved to be applicable for all tested species with more than one tested individual especially for *F. peregrinus* and *F. tinnunculus*. These species are usually used in falconry and frequently concerned in forensic affairs. Trading with falcons is allowed if individuals derive from captive breeds but taking chicks from natural nests is prohibited in Hungary. Although some breeders plunder natural nests and put closed rings on the chicks in order to refresh the genetic diversity of their birds. In this case genetic analyses are the only possible way to detect the crime. Our markers also proved to be useful in one case of the Peregrine Falcon, as they were applied in an unpublished forensic case reported by the Hungarian authorities.

In summary, these new markers served as a base for studies to support the conservation management of RFFs making the species a potentially good candidate model of parental care, mate choice and avian coloniality. Even with their limitations presented in the results, the markers could facilitate analyses of population structure, mating system and in the future any further studies where it is unquestionably important to genotype every single individual.

Population Structure

Population structure and genetic variability are highly important in conservation biology to determine the level of protection and conduct effective population management. Since RFFs are also protected in the neighbouring countries, to get more information about the colonies in the Carpathian Basin, a LIFE project (Conservation of the Red-footed Falcon in the Carpathian Basin, LIFE11/NAT/HU/000926) initiated population structure analyses and assignment of individuals deriving from a pre-migration site. Our samples originated from ten breeding sites (including one beyond the Carpathians) and none of them showed differentiation based on the analyses with microsatellites. Since there was no detectable genetic differentiation between the studied colonies the population assignment of the 20 individuals deriving from the Romanian pre-migration site was not feasible.

In the study the basic statistics conducted on 100 individuals showed no Hardy-Weinberg equilibrium on several loci due to homozygote excess, which was also confirmed by the

positive F_{IS} value (0.1548). The larger sample size shed light on the presence of null alleles both in cross-species and species-specific markers. In order to overcome this limitation of the marker set, results were verified with excluding the most problematic markers (Fp92-1 and FalVes_15, P≥0.1) providing the same outcome. This time, the AMOVA showed a slight differentiation (2.7% - 3.5%) between three Hungarian colonies (Appendix Table 7), while the Mantel test showed no effect of distance again (r=-0.199337, p=0.759). Structure still showed no differentiation, and the highest likelihood scores were given in case K=1 again (Appendix Figure 2).

According to the AMOVA the variance deriving from population differentiation is only 0.24% suggesting that the allele frequencies are similar in the subpopulations and we could not detect differences in genetic structure of the sampled locations. This finding was supported by the pairwise F_{ST} values as well: the highest allele frequency divergence was 0.03 detected between the Borsodi-mezőség and Csanádi-puszták which are not particularly far from each other (150.6 km). The results of the Structure software showed no differentiation between the breeding sites including the Romanian Southeast region colonies. Although there is a steep fall between the K=2 and K=3 scenarios (Figure 9), the highest likelihood scores were given for the K=1. Regarding all the other findings, the probability that the colonies in the Carpathian Basin and the studied one beyond the Carpathians belong to the same random mating population is the highest. Besides, since there was no correlation between the pairwise F_{ST} values and geographical distances, Mantel test supported that the Carpathians − as a potential barrier − do not obstruct gene flow, nor do the geographical distances within the sampling area.

These findings could be explained by the ability of RFFs to make long journeys. The species has high dispersal ability; adults explore new areas within moderate distances, and juveniles can fly several thousands of kilometres even before migrating. There are recapture data even from Sweden and Norway (Palatitz et al. 2018).

As a long-distance migrant, the global RFF population tends to flock together on pre-migration sites composed of several thousands of individuals to prepare for the annual trip to Africa. They gather mostly in the Eastern European region close to the coast of the Black Sea and there are usually smaller groups (1000-2000 individuals) in the Carpathian Basin as well. In Hungary ~3500 individuals were estimated on the biggest reported pre-migration site (Borbáth & Zalai 2005) and in 2014 approximately 11 600 individuals were counted in the Carpathian Basin during the same period of autumn migration (Palatitz et al. 2015).

Considering the current number of breeding pairs of Hungary (1200-1300) and slightly higher numbers in Romania (1300-1600 individuals), Hungary is presumably part of the autumn migration route of individuals living in the remote parts of their range (Palatitz et al. 2018). Fehérvári et al. (2014) also found that two of eight experienced females from Hungarian colonies choose to start their migration from Southern Ukraine, even crossing the mountain ranges of the Carpathians. When they finally start the migration, RFFs are able to fly several thousand of kilometres continuously. Based on data of adults equipped with satellite transmitters, RFFs cross the Mediterranean Sea quickly in 2-3 days using the Tradewinds and fly through the Sahara region, sometimes even without rest (Palatitz et al. 2018). Since they fly back through Western Africa during the spring migration, presumably more individuals cross the Carpathian Basin again than only the resident breeding pairs. These data can support our genetic results, highlighting the conservation biological importance of the region (Figure 10).

Figure 10. Satellite data of a Red-footed Falcon between 2016 and 2020 (see more: https://www.satellitetracking.eu/inds/showmap/?check_306=306)

The genetic consistency of the Carpathian Basin also correlates with an observation about philopatry since continuous monitoring (P. Fehérvári unpublished) showed no specific colonies where RFFs prefer to return after migration. This mixing after migration might contribute to maintaining or, conversely, recovering the genetic variability which was (presumably) lost during the population decline in the 20th century.

In conclusion the results of microsatellite analyses showed no genetic differentiation at present, however different approaches might also serve with additional information about the past. Identification of possible bottleneck effects and understanding the demographic history are also important in species conservation and different types of markers may provide different types of information. The mtDNA is frequently used in population genetic studies due to several beneficial characteristics, including its sensitivity to detect bottleneck effects. Although a study (Ludányi 2017) about the RFFs mtDNA D- loop region could distinguish six haplotypes in the same sampling area of our study (except of the Romanian West region colonies), it also showed no particular genetic structure. However, they found a slight differentiation between the Hungarian and the Romanian breeding sites statistically (Φ_{PT} = 0.252, P = 0.002) and Tajima's D values might indicate a recent bottleneck effect.

Our dataset is also expandable with other types of markers in the future – for example with SNPs – and it could be re-evaluated with different resolution power. Although some studies (Väli et al. 2010, Peery et al. 2012, Granevitze et al. 2013, Hodel et al. 2016) debate the higher efficiency of SNPs in detecting the effects of a recent bottleneck, other studies say that SNPs perform better in reflecting demographic history, informing about functional variability and thus the condition of the population (Brumfield et al. 2003, Eckert el al. 2010, García et al. 2018, McCoy et al. 2018). The combination of different types of markers presumably provides more information to understand the recent genetic history of the species and overcome the limitations of microsatellites appearing in F statistics.

In summary, putting aside all uncertainties of microsatellites, the RFF colonies in the Carpathian Basin seem to be genetically connected with individuals breeding in Romania beyond the Carpathians and possibly connected with individuals living in remote areas of the distribution range due to the migration route. As it was the case for the Prairie Falcon (Doyle et al. 2018), we can also support that the Red-footed Falcons form a random mating population within our sampling area, thus conducting international conservation programs to manage them together is advised. The results of the study could be further strengthened by the inclusion of additional genetic markers and samples from the eastern part of the breeding area in a subsequent study. If genetic structure were to be detected, the population assignment could also be conducted.

Alternative Reproductive Strategies

Life history traits and mating system are important key factors in conservation (Sutherland 1998, Caro 2007, Pryke et al. 2012, Garnier et al. 2012). To implement successful conservation measures it is highly recommended to study mate choice and parental care of the target species. However, raptors are seemingly monogamous species many of them use alternative reproductive strategies and based on observations, RFFs do so as well (Negro et al. 1996, Rosenfield et al. 2015).

In our analysis we genotyped 48 RFF families (210 individuals) in total to study the alternative reproductive strategies they might exhibit. Here we present a relatively low rate of EPP (2.7% of nests and 2.06% of chicks) fitting into the trend of other Falco species (Table 8), a low rate of IBP (3.7% of nests and 1.03% of chicks), the first case of QP in a Falco species and polygamy in the RFF.

In the case of Falcons, the rate of EPP is usually between 0 and 9.67%, but there are only a few results so far about IBP. There was no sign of it in either the American Kestrel (*Falco sparverius*, Villarroel et al. 1998) nor in the Eleonora's Falcon (*Falco eleonorae*, Swatschek et al. 1993), although the latter is a colonial species, too. The single detected species with IBP in Falcons was the colonial Lesser Kestrel (*Falco naumanni*) with 7.4% (Negro et al. 1996). The study was re-evaluated several years later with microsatellites and after excluding the false results the IBP rate was reduced to 6.4% (Alcaide et al. 2005) (Table 1). Polygamy was described in the Lesser Kestrel (Tella et al. 1996) and the Common Kestrel (Charter et al. 2008, Wang et al. 2019), but there was no detected case of quasi-parasitism.

Species	EPP % (nest)	EPP% (offspring)	No. of nests	IBP% (nest)	Colonial/ Solitary	Citation
American Kestrel (*Falco sparverius*)	9.5%	11.2%	21	0%	Solitary	Villarroel et al. (1998)
Common Kestrel (*Falco tinnunculus*)	2.7%	1.9% (0-0-5.4%)*	75	No data	No data	Korpimaki et al. (1996)
Lesser Kestrel (*Falco naumanni*)	3.8%	3.4%	26	7.4%	Colonial	Negro et al. (1996)
	9.67%	7.25% (4.2-8.3%)	31	6.4%	Colonial	Alcaide et al. (2005)
Eleonora's Falcon (*Falco eleonorae*)	0%	0%	17	0%	Colonial	Swatschek et al. (1993)
Merlin (*Falco columbarius*)	0%	0%	20	No data	Solitary	Warkentin et al. (1996)
Red-footed Falcon (*Falco vespertinus*)	2.7%	2.06%	37	2.7%	Colonial	Magonyi et al. (2021)

Table 8. Comparison of frequencies of EPP and IBP with other Falco species, *data of three years

According to Ile et al. (2002) it seems like nest and mate guarding or performing agonistic behaviour against conspecific intruders are not common strategies for RFFs. They also studied the copulatory behaviour of RFF pairs, finding that they spent less time together, females left the nest for longer time and the copulatory frequency and duration was also low. Shortly, RFF males allocate not many resources to guard their mates. Besides, since both parents are involved in parental care females do not depend entirely on males and even if the male deserts the nest, the safety provided by the coloniality might give chance to raise the offspring successfully. Haraszthy and Bagyura (1993) also highlighted the higher reproductive success of colonially breeding pairs explaining it with combined defence of the individuals against predators. Furthermore, to avoid raising extra-pair offspring, strategies can be desertion of the nest or exclusion of eggs from incubation with burying it under the nest material or rolling them out of the nest (Stouffer et al. 1987, Arnold & Owens 2002). Regarding the characteristics of RFFs mentioned above, the observed unsuccessful breeding attempts and excluded eggs in RFF colonies (Palatitz et al. 2018), the cases of EPP, IBP and QP reported here can be parts of their breeding strategy.

Additionally, the phenomenon of fixed clutch size may also refer to IBP. The clutch size of raptors and other similar sympatric species is usually regulated by the seasonal and inter-annual gradations of the Common Voles (*Microtus arvalis*) and other supplementary food as a functional response (Van Zyl 1994, Salamolard et al. 2000, Bondí et el. 2014). However, RFFs breed only once in a season and the unexperienced juveniles probably have a high mortality rate during their first migration, they still lay 3-4 eggs. Possibly, there is a strong selective pressure that counterbalances the profit gained from the adaptive clutch size and it may be related to the mating system by the presence of IBP attempts. The recognition of parasitic eggs may lead to the reduction of the clutch size in the host nests, which also happened in the case of the American Coot (*Fulica americana*), but it is still unclear whether they are adaptive and functional responses regardless of other factors (Lyon 2003, Lyon & Eadie 2008). However, in our study we found a low frequency of IBP, this assumption could be tested by experimental nest manipulation and by studying natural colonies.

Besides, the characteristics triggering IBP are also still uncertain, we do not know why this strategy has already emerged in some species and taxonomic families, but not – or not yet – in others. In 2001 Yom-Tov published a list of bird families with IBP showing that it is relatively common in some families such as Anatidae and Phasianidae, but it is unknown or

rare in others. Yom-Tov also described, that IBP is associated with precocial birds and coloniality potentially predicts its presence. According to other studies, IBP is related to high fecundity rates (Arnold & Owens 2002, Lyon & Eadie 2008) and human impact was also introduced as a factor shaping the mating system; for instance, in the case of the cavity-nesting Wood Duck (*Aix sponsa*) (Semel & Sherman 1995). This species originally breeds in tree cavities but placing out more visible boxes caused more frequent IBP. Later the authors found that nest fidelity and philopatry of young females also play an important role (Semel & Sherman 2001). In this case, IBP is a maladaptive consequence of the nestboxes affecting the population dynamics in a negative way.

Similarly, in the case of the RFF, the nestbox scheme is one of the most successful part of the conservation measures. Between 1997 and 2009 the number of Hungarian breeding pairs nesting in colonies increased from 40% to 85% contributing significantly to the recovery of the species in Hungary (Palatitz et al. 2018). Natural nests and nestboxes differ considerably; in contrast with natural nests, boxes are almost entirely closed with only one opening preventing the free view and facilitating EPP and IBP (Figure 11). The angle of view may have a profound impact on alternative reproductive strategies and the detected case of polygamy might indicate it, as well. The same male was feeding in two nestboxes at the same time on the same tree with the entrance holes in the opposite direction. According to the genetic analyses this male proved to be the genetic father in both nests. Despite the fact there is no more genetic information about other nestboxes placed similarly, we consider this information might be useful for later studies. Especially, since there are already proven effects of the nestboxes in RFF colonies. Bragin et al. (2017) found earlier egg laying and − in the case of nestboxes which were placed on forest edges − higher offspring loss, too.

Figure 11. Natural nest of a crow with an RFF chick and artificial nestbox with an adult male RFF sitting in. (Photos by Antal Széll)

We did not find any mention of QP in Falco species, so we consider this case as the first described in the genus. Compared to the EPP, in IBP both parents pay the costs of being parasitized by conspecifics except in the case of ‘quasi-parasitism’ (Petrie & Moller 1991). Although, this phenomenon is quite rare in birds and the alternative explanations have been little studied (Griffith et al. 2004). In our case we can also only report it based entirely on genetic data, although field observations would have been needed to exclude e. g. rapid changing of mates.

All things considered, it is essential to implement further studies about the natural colonies because at this point we could only detect the presence of IBP but not confirm that it is a hidden natural strategy or the side effect of a successful conservation measure of the nestboxes. In the latter case, mate choice and parental care system of the species might be altered by human interventions resulting in long-term consequences on the mating system. Another possible outcome is the increased rate of desertion by females and the reduction of clutch size lowering the reproductive success affecting the population dynamics as well.

Regardless of the underlying mechanism, our current results are the first about EPP, IBP, polygamy and quasi-parasitism in the Red-footed Falcon, increasing the number of bird species where these phenomena were documented. Further research based on these new results about this colonial raptor might also help to understand how the mating system, life history traits and conservation management interact.

Summary of the Results Achieved:

1. Marker development
   1.1. A marker set of new species-specific microsatellites was developed for the Red-footed Falcon and successfully applied in later analyses.
2. Population structure
   2.1. No genetic differentiation could be detected between the Hungarian colonies.
   2.2. Confirmation that the colonies in the Carpathian Basin and beyond could belong to the same random mating population in our sampling area.
   2.3. Population assignment of individuals from a Romanian pre-migration site was not feasible due to the lack of genetic structure of the sampled breeding sites.
3. Alternative reproductive strategies
   3.1. The presence of extra-pair paternity (EPP) was proved.
   3.2. The presence of intraspecific brood parasitism (IBP) was proved.
   3.3. Other types of alternative reproductive strategies were detected:
      3.3.1. Quasi-parasitism (QP) for the first time in a Falco species.
      3.3.2. Polygamy for the first time in the Red-footed Falcon.

Summary

The facultative colonial breeder and unique raptor species of the Hungarian plains, the Red-footed Falcon (*Falco vespertinus*) has been in the focus of conservation efforts for many years. In the last decades, there was successful cooperation between many local and international projects in order to relieve the dramatic population decline caused by the expanding agriculture and breeding site destruction in the last century. The results of habitat protection and artificial nestbox colonies met the expectations and the current 1.200 breeding pairs in Hungary – although not reaching the population size of the last century – is considered stable. Furthermore, by setting up nestboxes the traceability of nesting improved significantly, thus raising further questions and offering new insights into the study of the species. Prior to this study, due to the lack of species-specific genetic markers and accurate sampling, only mark-recapture and satellite tracking served as a source of information. We had no reliable data neither about the relationship between the Hungarian colonies and the colonies beyond the Carpathians, nor about the potential alternative reproductive strategies this species might use. These data are fundamental to successfully enhance the development of the current species conservation measures and deepen our knowledge about the species.

In the present study, our first goal was the development of species-specific markers which in combination with cross-species markers can answer our research questions related to the topics mentioned. In the case of the population structure, we investigated whether there is genetic differentiation between the colonies of breeding sites within the Carpathians Basin and another one beyond the Carpathians in Romania. Besides, we also aimed to analyse the individuals of a pre-migration site beyond the Carpathians in order to implement population assignment to the colonies within the Carpathian Basin or beyond.

Regarding the behavioural ecology study, we aimed to find genetic proof for the extra-pair fertilisation previously observed only in the field, and detect other types of alternative reproductive strategies, especially the intraspecific brood parasitism, which might be the first step in studying the fixed clutch size and the potential consequences of the artificial colonies created from nestboxes on the reproductive system of the species.

Based on 29 RFFs and 24 individuals from six related species, we developed a marker set which highly exceed the resolution power of the set containing markers previously developed for closely related species but also applicable to RFF. In total, we described 10

new markers which in combination with cross-species markers were successfully used in the analyses of population genetics and alternative reproductive strategies.

In the population genetic analyses, the allele frequencies of nine colonies in the Carpathian Basin and a colony beyond the Carpathians were compared with use of ten microsatellite loci. Since the species has a good dispersion ability and it is a long-term migrant, there was no genetic differentiation neither between the closely situated colonies, nor the colony beyond the Carpathians (F_{ST}=0.00247). Accordingly, population assignment of the pre-migration site's individuals to the colonies within the Carpathian Basin and beyond was not possible. It is likely that the Carpathian colonies − despite their geographical separation – are either not threatened by the risk of decreased genetic variability and isolation after the dramatic population decline or no longer threatened due to mixing during migration.

In case of alternative reproductive strategies, we genotyped 171 individuals from 37 RFF families with 12 microsatellites. After manual and Cervus software testing, we found that the number of nests affected by EPP (Extra-Pair Paternity) and IBP (Intraspecific Brood Parasitism) is quite low (2.7%), and the number of chicks affected is 2.06% and 1.03%, respectively. In total, 4.12% of the chicks were extra-pair offspring (four out of 97). In addition, one case of quasi-parasitism and one case of polygamy were found. We examined the phenomenon of IBP thoroughly. It is possible that the IBP is a common strategy for RFFs, which may be indicated by the fixed clutch size; nevertheless, it cannot be excluded at this point that this is the consequence of the use of artificial nestboxes. If the reproductive biology of the species has changed due to human intervention, the modification of the nextboxes must be considered. In summary, the genetic results confirmed the field observations, and serve as a basis for further analyses about natural colonies.

Összefoglalás

A magyar puszták különleges ragadozó madara, a főként telepesen költő kék vércse (*Falco vespertinus*) évek óta kiemelt szerepet kap a természetvédelemben. Az utóbbi évtizedekben több hazai és nemzetközi program is sikeresen működött együtt annak érdekében, hogy enyhítsék a múlt század mezőgazdasági terjeszkedését és költőhely pusztítását követő drámai egyedszámcsökkenést. Az élőhelyvédelem és a költőládákból kialakított mesterséges kolóniák meghozták eredményüket és bár a jelenlegi 1200 párt számláló hazai egyedszám nem éri el a múlt század elejit, a faj jelenléte hazánkban ismét stabilnak tekinthető. A zárt költőládák kihelyezésével jelentősen javult a költések nyomon követhetősége is, ezáltal új kérdéseket feltéve, további távlatokat nyitva a faj vizsgálatában. Pontos mintázhatóság, illetve fajspecifikus genetikai markerek hiányában ezidáig csupán jelölés-visszafogásból és jeladós nyomkövetésből származó információk álltak rendelkezésünkre. Megbízható adataink a hazai, illetve a Kárpátokon túli kolóniák közötti kapcsolatokról és a lehetséges alternatív reprodukciós stratégiákról nem voltak. Mindezek ismerete azonban alapvetően járulhat hozzá a fajhoz köthető tudásunk bővítéséhez és a jelenlegi fajvédelmi tevékenység hatékony fejlesztéséhez.

Kutatásunk során elsőként olyan fajspecifikus mikroszatellita markerek fejlesztését tűztük ki célul, amelyek más rokonfajokra kifejlesztett markerekkel kiegészítve megválaszolhatják feltett kérdéseinket az érintett témákban. A populáció struktúra szempontjából azt vizsgáltuk, van-e genetikai elkülönülés a nagyobb, Kárpát-medencén belül található tájegységek költőtelepei között, valamint a Kárpát-medencén belüli és egy azon túli, romániai költőterület között. A vizsgálatokba továbbá bevontunk egy szintén Kárpátokon túli terület gyülekezőiről befogott egyedek mintáit is, hogy megtudjuk, mekkora valószínűséggel sorolhatóak inkább Kárpát-medencén belüli vagy a hegyláncon túli kolóniákhoz.

A viselkedésökológiai vizsgálatokat tekintve az volt a célunk, hogy a terepi megfigyelések során észlelt extra-pár fertilizációt genetikailag is igazoljuk és kimutassunk egyéb alternatív reprodukciós stratégiákat, különös tekintettel az intraspecifikus fészekparazitizmus jelenlétére, mely kezdeti lépés lehet a fixált fészekaljméret jelenségének megválaszolásában, illetve a költőládákból kialakított mesterséges telepek szaporodási rendszerre gyakorolt hatásának tanulmányozásában.

Összesen 29 független kék vércse egyedet és további hat rokonfaj együttesen 24 példányát alapul véve olyan marker készletet fejlesztettünk, mely jelentősen meghaladta a csupán rokonfajokra fejlesztett, de kék vércsére is alkalmazható potenciális készlet erejét. Összesen 10 új markert írtunk le, melyek a cross-species markerekkel kombinálva mind a populáció genetikai, mind az alternatív reprodukciós stratégiák vizsgálataiban sikeresen alkalmaztunk.

A populációgenetikai vizsgálatok során tíz mikroszatellita lokusz segítségével vetettük össze kilenc kárpát-medencei tájegységhez tartozó kolónia, valamint egy Kárpátokon túli költőterület allélfrekvenciáit. Jó diszperziós képességgel rendelkező, hosszútávú vonuló fajról lévén szó, a közeli területek telepei nem mutattak genetikai elkülönülést, sőt a Kárpátokon túli költőterület sem (F_{ST}=0,00247). Következésképpen a gyülekező egyedeinek genetika alapon történő besorolása a Kárpát-medencén belüli, illetve azon túli költőterületekhez nem volt lehetséges. Valószínűsíthető tehát, hogy a Kárpát-medencei kolóniákat − földrajzi elkülönülésük és az egyedszám csökkenés ellenére − nem, vagy a vonulás során bekövetkező keveredésnek köszönhetően már nem fenyegeti a genetikai variabilitás csökkenése és az izolálódás. Így a nemzetközi fajvédelmi program genetikailag is megalapozottnak tekinthető, hangsúlyozva a kárpát-medencei régió fontosságát a fajvédelemben.

Az alternatív szaporodási stratégiák elemzéseiben összesen 37 kék vércse család 171 egyedének genotípusát vizsgáltuk 12 mikroszatellita marker segítségével. A manuálisan és a Cervus szoftver segítségével végzett elemzéseket követően azt találtuk, hogy az EPP (Extra-Pair Paternity) és IBP (Intraspecific Brood Parasitism) mindössze egy-egy fészket (2,7%), a fiókákat illetőleg pedig 2,06% és 1,03%-ot érintett. Összesen a fiókák 4,12%-a (négy fióka a 97-ből) származott extra pár fertilizációból. Továbbá egy-egy esetben találtunk kvázi parazitizmust és poligámiát is. Közülük az IBP jelenségével foglalkoztunk részletesebben. Lehetséges, hogy az IBP természetes stratégia a kék vércséknél, melyet jelezhet a fixált fészekalj jelensége is, mindazonáltal egyelőre az sem zárható ki, hogy a zárt költőládák használata következtében jelentkezett. Amennyiben az emberi beavatkozás eredményeképpen változott meg a faj szaporodásbiológiája, úgy megfontolandó az eltérő szerkezetű műfészkek kihelyezése. A genetikai eredmények tehát alátámasztották a terepi megfigyeléseket és ösztönözhetnek további, természetes költőtelepek vizsgálatára is.

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References

Ágh, N., Kovács, Sz., Nemesházi, E., & Szabó, K. (2018). Univerzális, ivarhatározáshoz használt CHD1 markerek alkalmazhatósága küldnbözö madárrendekben. *Magyar Allatorvosok Lapja*, *140*(1), 47–59.

Alcaide, M., Negro, J. J., Serrano, D., Tella, J. L., & Rodríguez, C. (2005). Extra-pair paternity in the Lesser Kestrel *Falco naumanni*: A re-evaluation using microsatellite markers. *Ibis*, *147*(3), 608–611. https://doi.org/10.1111/j.1474-919x.2005.00429.x

Arcot, S. S., Wang, Z., Weber, J. L., Deininger, P. L., & Batzer, M. A. (1995). Alu repeats: A source for the genesis of primate microsatellites. *Genomics*, *29*(1), 136–144. https://doi.org/10.1006/geno.1995.1224

Arnold, K. E., & Owens, I. P. F. (2002). Extra-pair paternity and egg dumping in birds: Life history, parental care and the risk of retaliation. *Proceedings of the Royal Society B: Biological Sciences*, *269*(1497), 1263–1269. https://doi.org/10.1098/rspb.2002.2013

Bagyura, J. & Palatitz, P. (2004). Fajmegőrzési tervek: Kék vércse (*Falco vespertinus*). *KvVM TVH.*

Bagshaw, A. T. M. (2017). Functional Mechanisms of Microsatellite DNA in Eukaryotic Genomes, *Genome Biology and Evolution*, *9*(9) 2428- 2443. https://doi.org/10.1093/gbe/evx164

Bertók, P. (2017). Extra-pár fertilitás vizsgálata egy telepesen költő ragadozó madárnál (Study of extra-pair fertility in a colonial bird of prey). *University of Veterinary Medicine, Budapest,* Master's Thesis.

BirdLife International (2015) European Red List of Birds. Luxembourg: Office for Official Publications of the European Communities.

Birkhead, T. R., Burke, T., Zann, R., Hunter, F. M., & Krupa, A. P. (1990). Extra-pair paternity and intraspecific brood parasitism in wild zebra finches *Taeniopygia guttata*, revealed by DNA fingerprinting. *Behavioral Ecology and Sociobiology*, *27*(5), 315–324. https://doi.org/10.1007/BF00164002

Bondì, S., Prommer, M., & Sarà, M. (2014). The diet of Saker Falcon *Falco cherrug* overwintering in the Mediterranean (Sicily). *Avocetta*, *38*(2), 53–58.

Borbáth P. & Zalai T. (2005). Kék vércsék (*Falco vespertinus*) őszi gyülekezése a Hevesi-síkon. *Aquila*, *112*, 39–44.

Bragin, A. E. & Katzner T. E. (2017). Demographic consequences of nestbox use for Red-footed Falcons *Falco vespertinus* in Central Asia. *Ibis*, *159*, 841-853. https://doi: 10.1111/ibi.12503

Brumfield, R. T., Beerli, P., Nickerson, D. A., & Edwards, S. V. (2003). The utility of single nucleotide polymorphisms in inferences of population history. *Trends in Ecology and Evolution*, *18*(5), 249–256. https://doi.org/10.1016/S0169-5347(03)00018-1

Burke, T. (1989). DNA fingerprinting and other methods for the study of mating success. *Trends in Ecology and Evolution*, *4*(5), 139–144. https://doi.org/10.1016/0169-5347(89)90213-9

Caro, T. (2007). Behavior and conservation: a bridge too far? *Trends in Ecology and Evolution*, *22*(8), 394–400. https://doi.org/10.1016/j.tree.2007.06.003

Castoe, T. A., Poole, A. W., de Koning, A. P. J., Jones, K. L., Tomback, D. F., Oyler-McCance, S. J., Fike, J. A., Lance, S. L., Streicher, J. W., Smith, E. N., & Pollock, D. D. (2012). Rapid microsatellite identification from illumina paired-end genomic sequencing in two birds and a snake. *PLoS ONE*, *7*(2). https://doi.org/10.1371/journal.pone.0030953

Chapuis, M. P., & Estoup, A. (2007). Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution*, *24*(3), 621–631. https://doi.org/10.1093/molbev/msl191

Charter, M., Leshem, Y., Izhaki, I., & Halevi, S. (2008). A case of polygamy or co-operative breeding in the Common Kestrel *Falco tinnunculus* in Israel. *Sandgrouse*, *30*, 164–165.

CMS, Convention on the Conservation of Migratoy Spsecies of Wild Animals. (2012). *Appendices I and II of the Convention on the Conservation of Migratory Species of Wild Animals (CMS)*. *February*, 1–10.

Costello, M. J., May, R. M., & Stork, N. E. (2013). Can we name earth's species before they go extinct? *Science, 339*(6118), 413–416. https://doi.org/10.1126/science.1230318

Council of Europe. (2018). Convention on the Conservation of European Wildlife and Natural Habitats. Appendix II – STRICTLY PROTECTED FAUNA SPECIES. In *Convention de Berne relative àla conservation de la vie sauvage et du milieu naturel de l'Europe* (Issue 104).

Dakin, EE; Avise, J. (2004). Microsatellite null alleles in parentage analysis. *Heredity*, *93*, 504–509. https://doi.org/10.1038/sj.hdy.6800545.

Cooper, D. H., & Noonan-Mooney, K. (2013). Convention on Biological Diversity. In *Encyclopedia of Biodiversity: Second Edition* (pp. 306–319). https://doi.org/10.1016/B978-0-12-384719-5.00418-4

Dawson, D. A., Horsburgh, G. J., Ku, C., Ball, A. D., Durrant, K. L., Hansson, B., Bacon, I. D. A., Bird, S., Klein, K. O. S., Krupa, A. P., Lee, J., Marti, D., & Stewart, I. A. N. R. K. (2010). New methods to identify conserved microsatellite loci and develop primer sets of high cross-species utility – as demonstrated for birds. *Molecular Ecology Notes*, *10*, 475–494. https://doi.org/10.1111/j.1755-0998.2009.02775.x

De Barbara, M., Miquel, C., Lobréaux, S., Quenette, P. Y., Swenson, J. E. & Taberlet, P. (2017) High-throughput microsatellite genotyping in ecology: improved accuracy, efficiency, standardization and success with low-quantity and degraded DNA. *Molecular Ecology Resources*, *17*, 492-507. https://doi.org/10.1111/1755-0998.12594

Deininger, P. L. (1989). SINEs:Short interspersed repeated DNA elements in higher eucaryotes. In M. M. Berg, D. E. & Howe (Ed.), *Mobile DNA* (pp. 619–648). American Society for Microbiology.

del Hoyo, J., Elliott, A., Sargatal, J. (1994). *Handbook of the birds of the world* (J. de Hoyo, J., Elliott, A., Sargatal (ed.); Vol. 2. Lynx Edicions Barcelona.

Doyle, J. M., Bell, D. A., Bloom, P. H., Emmons, G., Fesnock, A., Katzner, T. E., LaPré, L., Leonard, K., SanMiguel, P., Westerman, R. & J. DeWoody, A. (2018). New insights into the phylogenetics and population structure of the prairie falcon (*Falco mexicanus*).

BMC Genomics, *19*(233), 1–14. https://doi.org/10.1186/s12864-018-4615-z

Ducatez, S., & Shine, R. (2019). Life-history traits and the fate of translocated populations. *Conservation Biology*, *33*(4), 853–860. https://doi.org/10.1111/cobi.13281

Eckert, A. J., Bower, A. D., GonzÁlez-MartÍnez, S. C., Wegrzyn, J. L., Coop, G., & Neale, D. B. (2010). Back to nature: Ecological genomics of loblolly pine (*Pinus taeda*, Pinaceae). *Molecular Ecology*, *19*(17), 3789–3805. https://doi.org/10.1111/j.1365-294X.2010.04698.x

Eliassen, S., & Jørgensen, C. (2014). Extra-pair mating and evolution of cooperative neighbourhoods. *PLoS ONE*, *9*(7). https://doi.org/10.1371/journal.pone.0099878

Ellegren, H. (2000). Microsatellite mutations in the germline: Implications for evolutionary inference. *Trends in Genetics*, *16*(12), 551–558. https://doi.org/10.1016/S0168-9525(00)02139-9

Ellegren, H. (2004). Microsatellites: simple sequences with complex evolution. *Nature Reviews Genetics*, *5*(6), 435–445. https://doi.org/10.1038/nrg1348

Esler, D. (2000). Applying metapopulation theory to conservation of migratory birds. *Conservation Biology*, *14*(2), 366–372. https://doi.org/10.1046/j.1523-1739.2000.98147.x

Ewing, S. R., Nager, R. G., Nicoll, M. A. C., Aumjaud, A., Jones, C. G., & Keller, L. F. (2008). Inbreeding and loss of genetic variation in a reintroduced population of Mauritius Kestrel. *Conservation Biology*, *22*(2), 395–404. https://doi.org/10.1111/j.1523-1739.2008.00884.x

Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, *10*(3), 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Feest, A., Aldred, T. D., & Jedamzik, K. (2010). Biodiversity quality: A paradigm for biodiversity. *Ecological Indicators*, *10*(6), 1077–1082. https://doi.org/10.1016/j.ecolind.2010.04.002

Fehérvári, P., Harnos, A., Neidert, D., Solt, S. Z., & Palatitz, P. (2008). Modeling habitat selection of the red-footed falcon (*Falco vespertinus*): A possible explanation of recent changes in breeding range within Hungary. *Applied Ecology and Environmental Research*, *7*(1), 59–69.

Fehérvári, Péter, Lázár, B., Palatitz, P., Solt, S., Nagy, A., Prommer, M., Nagy, K., & Harnos, A. (2014). Pre-migration roost site use and timing of post-nuptial migration of red-footed falcons (*Falco vespertinus*) revealed by satellite tracking. *Ornis Hungarica*, *22*(1), 36–47. https://doi.org/10.2478/orhu-2014-0009

Ferguson-Lees, J. & Christie, D. A. (2001). *Raptors of the world*. Houghton Mifflin Harcourt.

Flanagan, S. P. & Jones A. G. (2018) The future of parentage analysis: From microsatellites to SNPs and beyond. *Molecular Ecology*, *28*(3) 544−567. https://doi.org/10.1111/mec.14988

Fridolfsson, A. & Ellegren, H. (1999). A Simple and Universal Method for Molecular Sexing of Non-Ratite Birds. *Journal of Avian Biology*, *30*(1), 116–121.

https://doi.org/10.2307/3677252

Forstmeier, W., Nakagawa, S., Griffith, S. C., & Kempenaers, B. (2014). Female extra-pair mating: Adaptation or genetic constraint? *Trends in Ecology and Evolution*, *29*(8), 456–464. https://doi.org/10.1016/j.tree.2014.05.005

Frankel, O. H. (1970). Variation - the essence of life. *Proceedings of the Linnean Society, New South Wales*, *95*(2), 158–169.

Frankel, O. H. (1974). Genetic conservation: our evolutionary responsibility. *Genetics*, *78*(1), 53–65. https://doi.org/10.1093/sysbio/43.3.387

Frankham, R., Ballou, J. D. & Briscoe, D. A. (2004). *A Primer of Conservation Genetics* (First edit). Cambridge.

Frankham, R. (1996). Relationship of Genetic Variation to Population Size in Wildlife. *Conservation Biology*, *10*(6), 1500–1508. https://doi.org/10.1046/j.1523-1739.1996.10061500.x

Frankham R. (2003). Genetics and conservation biology. *C. R. Biologies*, *326*, S22–S29. https://doi.org/doi:10.1016/S1631-0691(03)00023-4

Frankham, R. (2005). Genetics and extinction. *Biological Conservation*, *126*, 131–140. https://doi.org/10.1016/j.biocon.2005.05.002

Frei, M., Csencsics, D., Brodbeck, S., Schweizer, E., Bühler, C., Gugerli, F. & Bolliger, J. (2016). Combining landscape genetics, radio-tracking and long-term monitoring to derive management implications for Natterjack toads (Epidalea calamita) in agricultural landscapes Manuel. *Journal for Nature Conservation*, *32*, 22–34. http://dx.doi.org/10.1016/j.jnc.2016.04.002

Fuchs, J., Johnson, J. A., & Mindell, D. P. (2015). Rapid diversification of falcons (Aves: Falconidae) due to expansion of open habitats in the Late Miocene. *Molecular Phylogenetics and Evolution*, *82*(PA), 166–182. https://doi.org/10.1016/j.ympev.2014.08.010

Gagneux, P., Boesch, C., & Woodruff, D. S. (1997). Microsatellite scoring errors associated with noninvasive genotyping based on nuclear DNA amplified from shed hair. *Molecular Ecology*, *6*(9), 861–868. https://doi.org/10.1111/j.1365-294X.1997.tb00140.x

García, C., Guichoux, E., & Hampe, A. (2018). A comparative analysis between SNPs and SSRs to investigate genetic variation in a juniper species *(Juniperus phoenicea* ssp. *turbinata*). *Tree Genetics and Genomes*, *14*(6). https://doi.org/10.1007/s11295-018-1301-x

Garnier, R., Ramos, R., Staszewski, V., Militão, T., Lobato, E., González-Solís J., & Boulinier, T. (2012). Maternal antibody persistence: a neglected life-history trait with implications from albatross conservation to comparative immunology. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1735), 2033–2041. doi:10.1098/rspb.2011.2277

Gemayel, R., Vinces, M. D., Legendre, M. & Verstrepen K. J. Variable tandem repearts accelerate evolution of coding regulatory sequences. *Annual Review of Genetics, 44*, 445-477. https://doi.org/10.1146/annurev-genet-072610-155046.

Granevitze, Z., David, L., Twito, T., Weigend, S., Feldman, M., & Hillel, J. (2014). Phylogenetic resolution power of microsatellites and various single-nucleotide polymorphism types assessed in 10 divergent chicken populations. *Animal Genetics*, *45*(1), 87–95. https://doi.org/10.1111/age.12088

Griffith, S. C., Owens, I. P. F., & Thuman, K. A. (2002). Extra pair paternity in birds: A review of interspecific variation and adaptive function. *Molecular Ecology*, *11*(11), 2195–2212. https://doi.org/10.1046/j.1365-294X.2002.01613.x

Griffiths, C. S., Barrowclough, G. F., Groth, J. G., & Mertz, L. (2004). Phylogeny of the Falconidae (Aves): A comparison of the efficacy of morphological, mitochondrial, and nuclear data. *Molecular Phylogenetics and Evolution*, *32*(1), 101–109. https://doi.org/10.1016/j.ympev.2003.11.019

Haraszthy L. & Bagyura J. (1994). A kék és vörös vércse állományának vizsgálata Apajpuszta térségében. *Heliaca*, *1*, 14–16.

Haraszthy, L. & J. B. (1993). Comparison of the nesting habits of the Red-footed Falcon (*Falco vespertinus*) in Colonies and Solitary Pairs. *The Hawk and Owl Trust.*

Hasl, R. J. & Payseur, B. A. (2014) Micorsatellites as Targets of Natural Selection. *Molecular Biology and Evolution*, *30*(2), 285-298. https://doi.org/10.1093/molbev/mss247

Hendry, A. P., & Day, T. (2005). Population structure attributable to reproductive time: Isolation by time and adaptation by time. *Molecular Ecology*, *14*(4), 901–916. https://doi.org/10.1111/j.1365-294X.2005.02480.x

Hodel, R. G. J., Segovia-Salcedo, M.C., Landis, J. B., Crowl, A. A., Sun, M., Liu, X., Gitzendanner, M. A., Douglas, N. A., Germain-Aubrey, C. C., Chen, S.,Soltis, D. E., & Soltis, P. S. (2016). The report of my death was exaggeration: A review for researchers uing microsatellites in te 21st century. *Applications in Plant Sciences, 4*(6):1600025. https://doi.org/10.3732/apps.1600025

Hoi, H., Tost, H., & Griggio, M. (2011). The effect of breeding density and male quality on paternity-assurance behaviours in the house sparrow, *Passer domesticus*. In *Journal of Ethology*, *29*(1), 31–38. https://doi.org/10.1007/s10164-010-0217-1

Ieronymidou, C., Ramirez, I., Burfield, I. J., & Pople, R. G. (2015). The European Red List of birds 2015. *Bird Census News*, *28*(1), 3–19.

Ille, R., Hoi, H., Grinschgl, F., & Zink, R. (2002). Paternity assurance in two species of colonially breeding falcon: The kestrel *Falco tinnunculus* and the red-footed falcon *Falco vespertinus*. *Etologia*, *10*, 11–15.

IUCN. (2001). IUCN Red List Categories and Criteria: Version 3.1. IUCN Species Survival Commission. IUCN, Gland, Switzerland and Cambridge, UK. ii + 30 pp.

Jennions, M. D., & Petrie, M. (2000). Why do females mate multiply? A review of the genetic benefits. *Biological Reviews*, *75*(1), 21–64. https://doi.org/10.1111/j.1469-185X.1999.tb00040.x

Jin, L., Macaubas, C., Hallmayer, J., Kimura, A. & Mignot, E. (1996). Mutation rate varies among alleles at a microstaellite locus: Phylogenetic evidence. *Proceeding of the National Academy of Sciences of the USA*, *75*, 15285–15288. https://doi.org/10.1073/pnas.93.26.15285

Kalinowski, Steven T., Wagner P. Aaron, T. M. L. (2006). ML - relate : a computer program for maximum likelihood estimation of relatedness and relationship. *Molecular Ecology Notes*, *6*, 576–579. doi: 10.1111/j.1471-8286.2006.01256.x

Kalinowski, S. T., Taper, M. L., & Marshall, T. C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, *16*(5), 1099–1106. https://doi.org/10.1111/j.1365-294X.2007.03089.x

Kashi, Y. & King, D. (2006) Simple sequence repeats as advantageous mutators in evolution. *Trends in Genetics*, *22*(5), 253-259. https://doi.org/10.1016/j.tig.2006.03.005

Katzner, T. E., Bragin, E. A., Bragin, A. E., McGrady, M., Miller, T. A., & Bildstein, K. L. (2016). Unusual clockwise loop migration lengthens travel distances and increases potential risks for a central Asian, long distance, trans-equatorial migrant, the Red-footed Falcon *Falco vespertinus*. *Bird Study*, *63*(3), 406–412. https://doi.org/10.1080/00063657.2016.1214107

Keller, L. F., & Waller, D. M. (2002). Inbreeding effects in wild populations. *Trends in Ecology & Evolution*, *17*(5), 230–241. https://doi.org/10.1016/S0169-5347(02)02489-8

Keve, A. & Szijj, J. (1957). Distribution, biologie et alimentation du Facon kobez Falco vespertinus L. en Hongrie. *Alauda*, *25*, 1–23.

Kleinman-Ruiz, D., Martínez-Cruz, B., Soriano, L., Lucena-Perez, M., Cruz, F., Villanueva, B., Fernández, J., & Godoy, J. A. (2017). Novel efficient genome-wide SNP panels for the conservation of the highly endangered *Iberian lynx*. *BMC Genomics*, *18*(1), 1–12. https://doi.org/10.1186/s12864-017-3946-5

Korpimäki, E., Lahti, K., May, C. A., Parkin, D. T., Powell, G. B., Tolonen, P., & Wetton, J. H. (1996). Copulatory behaviour and paternity determined by DNA fingerprinting in kestrels: Effects of cyclic food abundance. *Animal Behaviour*, *51*(4), 945–955. https://doi.org/10.1006/anbe.1996.0098

Kotymán, L., Solt, S., Horváth, É., Palatitz, P., & Fehérvári, P. (2015). Demography, breeding success and effects of nest type in artificial colonies of red-footed falcons and allies. *Ornis Hungarica*, *23*(1), 1–21. https://doi.org/10.1515/orhu-2015-0001

Krištín, A., Tulis, F., Klimant, P., Bacsa, K., & Ambros, M. (2017). Food supply (Orthoptera, Mantodea, Rodentia and Eulipotyphla) and food preferences of the red-footed falcon (*Falco vespertinus*) in Slovakia. *Slovak Raptor Journal*, *11*(1), 1–14. https://doi.org/10.1515/srj-2017-0005

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution*, *33*(7), 1870–1874. https://doi.org/10.1093/molbev/msw054

Lack, D. (1968). Ecological adaptations for breeding in birds. Methuen, 84s. New York, Barnes & Nobel. https://doi.org/10.1017/S003060530000750X

Lacy, R. C. (1997). Importance of genetic variation to the viability of mammalian populations. In *Journal of Mammalogy*, *78*(2), 320–335. https://doi.org/10.2307/1382885

Lande, R. (1988). Genetics and demography in biological conservation. *Science*, *241*(4872),

1455–1460. https://doi.org/10.1126/science.3420403

Larsen B. B., Miller C. E., Rhodes K. M., Wiens J. J. (2017). Inordinate fondness multiplied and redistributed: the number of species on Earth and the new pie of life. *The Quarterly Riview of Biology*, *92*(3), 229–265. https://doi.org/10.1086/693564

Lombardo, M. P., Power, H. W., Stouffer, P. C., Romagnano, L. C., & Hoffenberg, A. S. (1989). Egg removal and intraspecific brood parasitism in the European starling (*Sturnus vulgaris*). *Behavioral Ecology and Sociobiology*, *24*(4), 217–223. https://doi.org/10.1007/BF00295201

Longmire, J. L., Lewis, A. K., Brown, N. C., Buckingham, J. M., Clark, L. M., Jones, M. D., Meincke, L. J., Meyne, J., Ratliff, R. L., Ray, F. A., Wagner, R. P., & Moyzis, R. K. (1988). Isolation and molecular characterization of a highly polymorphic centromeric tandem repeat in the family falconidae. *Genomics*, *2*(1), 14–24. https://doi.org/10.1016/0888-7543(88)90104-8

Ludányi, A. M. (2017). Kék vércse (*Falco vespertinus*) populációk genetikai elkülönítése. *University of Veterinary Medicine, Budapest*, Master's Thesis.

Luikart, G., England, P. R., Tallmon, D., Jordan, S., & Taberlet, P. (2003). The power and promise of population genomics: From genotyping to genome typing. *Nature Reviews Genetics*, *4*(12), 981–994. https://doi.org/10.1038/nrg1226

Lyon, B. E. (2003). Egg recognition and counting reduce costs of avian conspecific brood parasitism. *Nature*, *422*, 495–499. https://doi.org/10.1038/nature01505

Lyon, B. E., & Eadie, J. M. (2008). Conspecific Brood Parasitism in Birds: A Life-History Perspective. *Annual Review of Ecology, Evolution, and Systematics*, *39*(1), 343–363. https://doi.org/10.1146/annurev.ecolsys.39.110707.173354

Mace, M. (2014). *California Condor North American Studbook*. San Diego Zoo Global.

Magonyi, N. M., Mátics, R., Szabó, K., Fehérvári, P., Solt, S., Palatitz, P., & Vili, N. (2019). Characterization of novel microsatellite markers for the red-footed falcon (*Falco vespertinus*) and cross-species amplification in other Falco species. *European Journal of Wildlife Research*, *65*(4). https://doi.org/10.1007/s10344-019-1300-8

Magonyi, N. M., Szabó, K., Bertók, P., Fehérvári, P., Solt, S., Palatitz, P., Vili, N., & Mátics, R. (2021). Extra-pair paternity, intraspecific brood parasitism, quasi-parasitism and polygamy in the Red-footed Falcon (*Falco vespertinus*). *Ibis: international journal of avian science.* Article DOI: 10.1111/ibi.12932

McCoy, R. C., Garud, N. R., Kelley, J. L., Boggs, C. L., & Petrov, D. A. (2014). Genomic inference accurately predicts the timing and severity of a recent bottleneck in a nonmodel insect population. *Molecular Ecology*, *23*(1), 136–150. https://doi.org/10.1111/mec.12591

Miller, M. P., Mullins, T. D., Parrish, J. W., Walters, J. R., & Haig, S. M. (2012). Variation in migratory behavior influences regional genetic diversity and structure among American Kestrel populations (*Falco sparverius*) in North America. *Journal of Heredity*, *103*(4), 503–514. https://doi.org/10.1093/jhered/ess024

Milot, E., Weimerskirch, H., Duchesne, P., & Bernatchez, L. (2007). Surviving with low genetic diversity: The case of albatrosses. *Proceedings of the Royal Society B:*

Biological Sciences, *274*(1611), 779–787. https://doi.org/10.1098/rspb.2006.0221

Møller, A. P. (1989). Intraspecific nest parasitism in the swallow *Hirundo rustica*: the importance of neighbors. *Behavioral Ecology and Sociobiology*, *25*(1), 33–38. https://doi.org/10.1007/BF00299708

Monzõn, J., Kays, R., & Dykhuizen, D. E. (2014). Assessment of coyote-wolf-dog admixture using ancestry-informative diagnostic SNPs. *Molecular Ecology*, *23*(1), 182–197. https://doi.org/10.1111/mec.12570

Mora, C., Tittensor, D. P., Adl, S., Simpson, A. G. B., & Worm, B. (2011). How many species are there on earth and in the ocean? *PLoS Biology*, *9*(8), 1–8. https://doi.org/10.1371/journal.pbio.1001127

Morgante, M., Hanafey, M., & Powell, W. (2002). Microsatellites are preferentially associated with nonrepetitive DNA in plant genomes. *Nature Genetics*, *30*(2), 194–200. https://doi.org/10.1038/ng822

Neff, B. D., & Gross, M. R. (2001). Microsatellite evolution in vertebrates: Inference from AC dinucleotide repeats. *Evolution*, *55*(9), 1717–1733. https://doi.org/10.1111/j.0014-3820.2001.tb00822.x

Negro, J. J., Villarroel, M., Tella, J. L., Kuhnlein, U., Hiraldo, F., Donazar, J. A., & Bird, D. M. (1996). DNA fingerprinting reveals a low incidence of extra-pair fertilizations in the lesser kestrel. *Animal Behaviour*, *51*(4), 935–943. https://doi.org/10.1006/anbe.1996.0097

Nesje, M., RØed, K. H., Lifjeld, J. T., Lindberg, P., & Steen, O. F. (2000 a). Genetic relationships in the peregrine falcon (*Falco peregrinus*) analysed by microsatellite DNA markers. *Molecular Ecology*, *9*(1), 53–60. https://doi.org/10.1046/j.1365-294X.2000.00834.x

Nesje, Marit, & Røed, K. H. (2000). Microsatellite DNA markers from the gyrfalcon (Falco rusticolus) and their use in other raptor species. *Molecular Ecology*, *9*(9), 1438–1440. https://doi.org/10.1046/j.1365-294X.2000.00999-4.x

Nesje, Marit, Røed, K. H., Bell, D. A., Lindberg, P., & Lifjeld, J. T. (2000 b). Microsatellite analysis of population structure and genetic variability in peregrine falcons (*Falco peregrinus*). *Animal Conservation*, *3*(3), 267–275. https://doi.org/10.1017/S1367943000000998

Nittinger, F., Gamauf, A., Pinsker, W., Wink, M., & Haring, E. (2007). Phylogeography and population structure of the saker falcon (*Falco cherrug*) and the influence of hybridization: Mitochondrial and microsatellite data. *Molecular Ecology*, *16*(7), 1497–1517. https://doi.org/10.1111/j.1365-294X.2007.03245.x

O'Grady, J. J., Brook, B. W., Reed, D. H., Ballou, J. D., Tonkyn, D. W., & Frankham, R. (2006). Realistic levels of inbreeding depression strongly affect extinction risk in wild populations. *Biological Conservation*, *133*(1), 42–51. https://doi.org/10.1016/j.biocon.2006.05.016

Official Journal of the European Union EN IV. (2010). Directive 2009/147/EC of the european parliament and of the council. *Review of European Community and International Environmental Law*, *6*(1). https://doi.org/10.1111/1467-9388.00079

Padilla, J. A., Parejo, J. C., Salazar, J., Martínez-Trancón, M., Rabasco, A., Sansinforiano,

E., & Quesada, A. (2009). Isolation and characterization of polymorphic microsatellite markers in lesser kestrel (*Falco naumanni*) and cross-amplification in common kestrel (*Falco tinnunculus*). *Conservation Genetics*, *10*(5), 1357–1360. https://doi.org/10.1007/s10592-008-9711-1

Paetkau, D., & Strobeck, C. (1995). The molecular basis and evolutionary history of a microsatellite null allele in bears. *Molecular Ecology*, *4*(4), 519–520. https://doi.org/10.1111/j.1365-294X.1995.tb00248.x

Palatitz, P., Fehérvári, P., Solt, S., & Horváth, É. (2015). Breeding population trends and pre-migration roost site survey of the red-footed falcon in Hungary. *Ornis Hungarica*, *23*(1), 77–93. https://doi.org/10.1515/orhu-2015-0007

Palatitz, P., Solt, Sz. & Fehérvári, P. (2018). *The Blue Vesper - Ecology and Conservation of the Red-footed Falcon.* (MME 2018). MME BirdLife Hungary.

Peakall, R., & Smouse, P. E. (2012). GenAlEx 6.5: genetic analysis in excel. Population genetic software for teaching and research-an update. *Bioinformatics*, *28*, 2537–2539. https://doi.org/10.1093/bioinformatics/bts460

Peery, Z. M., Kirby, R., Reid, B. N., Stoelting, R., Doucet-Bëer, E., Robinson, S., Vásquez-Carrillo, C., , Pauli, J. N., & Palsbøll, P. J. (2012). Reliability of genetic bottleneck tests for detecting recent population declines. *Molecular Ecology*, *21*(14), 3403–3418. https://doi.org/10.1111/j.1365-294X.2012.05635.x

Peterson, A. M., Denno, R. F. (1998). The Influence of Dispersal and Diet Breadth on Patterns of Genetic Isolation by Distance in Phytophagous Insects. *The American Naturalist*, *152*, 428–446. https://doi.org/10.1086/286180

Petrie, M., & Møller, A. P. (1991). Laying eggs in others' nests: Intraspecific brood parasitism in birds. *Trends in Ecology and Evolution*, 6(10), 315–320. https://doi.org/10.1016/0169-5347(91)90038-Y

Petrie, M, & Kempenaers, B. (1998). Extra-pair paternity in birds: Explaining variation between species and populations. *Trends in Ecology and Evolution*, *13*(2), 52–58. https://doi.org/10.1016/S0169-5347(97)01232-9

Pimm, S. L., Jones, H. L., Diamond, J., Naturalist, T. A., & Dec, N. (1988). On the Risk of Extinction. *The American Naturalist*, *132*(6), 757–785. https://doi.org/doi:10.1086/284889

Primmer, C., R., Møller, A. P., & Ellegren, H. (1996). A wide-range survey of cross-species microsatellite. *Molecular Ecology*, *5*, 365–378. https://doi.org/10.1046/j.1365-294X.1996.00092.x

Primmer, C. R., Painter, J. N., Koskinen, M. T., Palo, J. U., Meril, xe, & Juha. (2005). Factors Affecting Avian Cross-Species Microsatellite Amplification. *Journal of Avian Biology*, *36*(4), 348–360. https://doi.org/10.1111/j.0908-8857.2005.03465.x

Pryke, S. R., Astheimer, L. B., Griffith, S. C., & Buttemer, W. A. (2012). Covariation in life-history traits: Differential effects of diet on condition, hormones, behavior, and reproduction in genetic finch morphs. *American Naturalist*, *179*(3), 375–390. https://doi.org/10.1086/664078

Purger, J. J. (1998). Diet of Red-footed Falcon *Falco vespertinus* nestlings from hatching to fledging. *Ornis Fennica*, *75*(4),185–191. ISSN 0030-5685

Purger, J. J. (2001). Defendence behaviour of Red-footed Falcons *Falco vespertinus* in the breeding period and the effects of disturbance on breeding success. *Ornis Fennica* 78(1), 3–21.

Putman, A. I. & Carbone I. (2014) Challenges in analysis and interpretation of microsatellite data for population genetic studies. *Ecology and Evolution*, *4*(22), 4399−4428. https://doi.org/10.1002/ece3.1305.

Queller, D. C., Strassmann, J. E., & Hughes, C. R. (1993). Microsatellites and kinship: Trends in Ecology and Evolution. *TREE*, *8*(8), 285–288. https://doi.org/10.1016/0169-5347(93)90256-O

Reed, D. H., & Frankham, R. (2003). Correlation between fitness and genetic diversity. *Conservation Biology*, *17*(1), 230–237. https://doi.org/10.1046/j.1523-1739.2003.01236.x

Rosenfield, R. N., Sonsthagen, S. A., Stout, W. E., & Talbot, S. L. (2015). High frequency of extra-pair paternity in an urban population of Cooper's Hawks. *Journal of Field Ornithology*, *86*(2), 144–152. https://doi.org/10.1111/jofo.12097

Rutkowski, R., Rejt, Ł., Tereba, A., Gryczyńska-Siemiątkowska, A., & Janic, B. (2010). Population genetic structure of the European kestrel Falco tinnunculus in Central Poland. *European Journal of Wildlife Research*, *56*(3), 297–305. https://doi.org/10.1007/s10344-009-0320-1

Salamolard, M., Butet, A., Leroux, A., & Bretagnolle, V. (2000). Responses of an avian predator to variations in prey density at a temperate latitude. *Ecology, 81*(9), 2428–2441. https://doi.org/10.2307/177465

Semel, B. & Sherman, P. W. (1995). Alternative Placement Strategies for Wood Duck Nest Boxes. *Wildlife Society Bulletin*, *23*(5), 463–471. http://www.jstor.org/stable/3782956

Schlötterer, C. (2000). Evolutionary dynamics of microsatellite DNA. In *Chromosoma* (Vol. 109, Issue 6, pp. 365–371). https://doi.org/10.1007/s004120000089

Schlötterer, C., Ritter, R., Harr, B., & Brem, G. (1998). High mutation rate of a long microsatellite allele in Drosophila melanogaster provides evidence for allele-specific mutation rates. *Molecular Biology and Evolution*, *15*(10), 1269–1274. https://doi.org/10.1093/oxfordjournals.molbev.a025855

Şekercioğlu, Ç. H., Daily, G. C., & Ehrlich, P. R. (2004). Ecosystem consequences of bird declines. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(52), 18042–18047. https://doi.org/10.1073/pnas.0408049101

Selkoe, K. A., & Toonen, R. J. (2006). Microsatellites for ecologists: A practical guide to using and evaluating microsatellite markers. *Ecology Letters*, *9*(5), 615–629. https://doi.org/10.1111/j.1461-0248.2006.00889.x

Semel, B., & Sherman, P. W. (2001). Intraspecific parasitism and nest-site competition in wood ducks. *Animal Behaviour*, *61*(4), 787–803. https://doi.org/10.1006/anbe.2000.1657

Siegel-Causey, D., & Kharitonov, S. P. (1990). The evolution of coloniality in birds. *Curr.Ornithol.*, *7*, 285–330.

Slatkin, M. (1995). A measure of population subdivision based on microsatellite allele

frequencies. *Genetics*, *139*(1), 457–462.

Spielman, D., Brook, B. W., & Frankham, R. (2004). Most species are not driven to extinction before genetic factors impact them. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(42), 15261–15264. https://doi.org/10.1073/pnas.0403809101

Stouffer, P. C., Kennedy, E. D., & Power, H. W. (1987). Recognition and removal of intraspecific parasite eggs by starlings. *Animal Behaviour*, *35*(5), 583–1584. https://doi.org/https://doi.org/10.1016/S0003-3472(87)80040-4

Suh, A., Kriegs, J. O., Brosius, J., & Schmitz, J. (2011). Retroposon insertions and the chronology of avian sex chromosome evolution. *Molecular Biology and Evolution*, *28*(11), 2993–2997. https://doi.org/10.1093/molbev/msr147

Sutherland, W. J. (1998). The importance of behavioral studies in conservation biology. *Animal Behaviour*, *56*(4), 801–809. https://doi.org/10.1006/anbe.1998.0896

Swatschek I., Ristow D., Scharlau W., W. C. and W. M. (1993). Populationsgenetik beim Eleonorenfalken (*Falco eleonorae*). *Journal of O*, *134*, 137–143. https://doi.org/DOI: 10.1007/BF01640082

Tella, J. L.Negro, J. J., Villarroel, M., Kuhnlein, U., Hiraldo, F., Donazar, J. A., & Bird, D. M. (1996). DNA Fingerprinting Reveals Polygyny in the Lesser kestrel (*Falco naumanni*). *The Auk*, *113*(41), 262–265.

Väli, Ü., Saag, P., Dombrovski, V., Meyburg, B. U., Maciorowski, G., Mizera, T., Treinys, R., & Fagerberg, S. (2010). Microsatellites and single nucleotide polymorphisms in avian hybrid identification: A comparative case study. *Journal of Avian Biology*, *41*(1), 34–49. https://doi.org/10.1111/j.1600-048X.2009.04730.x

Van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M., & Shipley, P. (2004). MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, *4*(3), 535–538. https://doi.org/10.1111/j.1471-8286.2004.00684.x

Van Zyl, A. J. (1994). A comparison of the diet of the common kestrel falco tinnunculus in south africa and europe. *Bird Study*, *41*(2), 124–130. https://doi.org/10.1080/00063659409477208

Villarroel, M., Bird, D. M., & Kuhnlein, U. (1998). Copulatory behaviour and paternity in the American kestrel: The adaptive significance of frequent copulations. *Animal Behaviour*, *56*(2), 289–299. https://doi.org/10.1006/anbe.1998.0788

Wang, Y., Liu, H., Wang, H., Ma, L., & Yi, G. (2019). Polygyny in the Eurasian Kestrel (*Falco tinnunculus*): Behavior, Morphology, Age, Heterozygosity, and Relatedness. *Journal of Raptor Research*, *53*(2), 202–206. https://doi.org/10.3356/JRR-17-89

Warkentin, I. G., D.-Curzon, A., Carter, R. E., Wetton, J. H., James, P. C., Oliphant, L. W., & Parkin, D. T. (1994). No evidence for extrapair fertilizations in the merlin revealed by DNA fingerprinting. *Molecular Ecology*, *3*(3), 229–234. https://doi.org/10.1111/j.1365-294X.1994.tb00056.x

Weigmann, H. (1968). Reduction of disulfide bonds in keratin with 1,4-dithiothreitol. I. Kinetic investigation. *Journal of Polymer Science Part A-1: Polymer Chemistry*, *6*(8), 2237–2253. https://doi.org/https://doi.org/10.1002/pol.1968.150060819

Weir, B.S. & Cockerham, C. C. (1984). Estimating F-Statistics for the Analysis of Population- Structure. *Evolution*, *38*, 1358–1370.

Wierdl, M., Dominska, M. & Petes, T. D. (1997). Microsatellite Instability in Yeast: Dependence on the Length of the Microsatellite. *Genetics Society of America*, *146*, 769–779.

Wilson, A., Sunnucks, P., & Hales, D. (1997). Random loss of X chromosome at male determination in an aphid , Sitobion near fragariae , detected using an X-linked polymorphic microsatellite marker. *Genetical Research*, *69*(3), 233–236. https://doi.org/10.1017/S0016672397002747

Wink, M. & Siebold, I. (1996). Molecular Phylogeny of Mediterranean Raptors (Families Accipitridae and Falconidae. Muntaner, J. & Mayol, J. editors of Biología y Conservación de las Rapaces Mediterráneas, 1994. 335–344

Wink, M. (2006). Use of DNA markers to study bird migration. *Journal of Ornithology*, *147*(2), 234–244. https://doi.org/10.1007/s10336-006-0065-5

Yi Wang, Hongxia Liu, Haitao Wang, Yi Wang, Lianjie Ma, Guodong Yi. (2019). Polygyny in the Eurasian Kestrel (*Falco tinnunculus*): Behavior, Morphology, Age, Heterozygosity, and Relatedness. *Journal of Raptor Research*, *53*(2), 202–206. https://doi.org/https://doi.org/10.3356/JRR-17-89

Yom-Tov, Y. (2001). An updated list and some comments on the occurrence of intraspecific nest parasitism in birds. *Ibis*, *143*(1), 133–143. https://doi.org/10.1111/j.1474-919x.2001.tb04177.x

Online references:

https://www.iucnredlist.org/search?query=Birds&searchType=species (Downloaded: 10.08.2020)

http://falcoproject.eu/hu (Downloaded: 10.08.2020)

http://datazone.birdlife.org/species/factsheet/red-footed-falcon-falco-vespertinus/text (Downloaded: 01.08.2020)

https://www.iucnredlist.org/species/22696432/131939286 (Downloaded: 01.08.2020)

http://www.bio-rad.com/en-hu/applications-technologies/pcr-troubleshooting (Downloaded: 15.08.2020)

http://qgis.org (Downloaded: 10.11.2020)

https://www.satellitetracking.eu/inds/showmap/?check_306=306 (Downloaded: 10.11.2020)

Appendix

Table 1. Information of museum specimens and other related species used in marker development for cross-species testing

Species	Specimens ID	Source
Gyrfalcon (Falco rusticolus)	Falrus_17/745	Samples originally for molecular sex determination
Gyrfalcon (Falco rusticolus)	Falrus_17/828	Samples originally for molecular sex determination
Gyrfalcon (Falco rusticolus)	Falrus_2016/036	Samples originally for molecular sex determination
Eurasian hobby (Falco subbuteo)	Falsub_gy/1341	Museum specimen (Hungarian Natural History Museum)
Saker falcon (Falco cherrug)	Falcher_001	Museum specimen (Hungarian Natural History Museum)
Merlin (Falco columbarius)	Falcol_gy/1115	Museum specimen (Hungarian Natural History Museum)
Common kestrel (Falco tinnunculus)	Ft_001	Kecskemét Zoo
Common kestrel (Falco tinnunculus)	Ft227	MME Birdlife Hungary
Common kestrel (Falco tinnunculus)	Ft233	MME Birdlife Hungary
Common kestrel (Falco tinnunculus)	Ft235	MME Birdlife Hungary
Common kestrel (Falco tinnunculus)	Ft239	MME Birdlife Hungary
Common kestrel (Falco tinnunculus)	Ft256	MME Birdlife Hungary
Common kestrel (Falco tinnunculus)	Ft267	MME Birdlife Hungary
Common kestrel (Falco tinnunculus)	Ft272	MME Birdlife Hungary
Peregrine falcon (Falco peregrinus)	Fp_gy/1231	Museum specimen (Hungarian Natural History Museum)
Peregrine falcon (Falco peregrinus)	Fp001	MME Birdlife Hungary
Peregrine falcon (Falco peregrinus)	Fp002	MME Birdlife Hungary
Peregrine falcon (Falco peregrinus)	Fp003	MME Birdlife Hungary
Peregrine falcon (Falco peregrinus)	Fp004	MME Birdlife Hungary
Peregrine falcon (Falco peregrinus)	Fp005	MME Birdlife Hungary

Peregrine falcon (Falco peregrinus)	Fp006	MME Birdlife Hungary
Peregrine falcon (Falco peregrinus)	Fp007	MME Birdlife Hungary
Peregrine falcon (Falco peregrinus)	Fp008	MME Birdlife Hungary
Peregrine falcon (Falco peregrinus)	Fp009	MME Birdlife Hungary

Table 2. Mixes and dyes of the cross-species markers in the population genetic analysis

	Marker	**Species**	**Repeat motif**	**Range (bp)**	**Dye**
Mix1	Fp54	*F. pererginus*	dinucleotide	99-109	TET
	Fp89	*F. pererginus*	dinucleotide	118-170	TET
	Fr34	*F. rusticolus*	dinucleotide	158-167	PET
Mix2	Fp347	*F. pererginus*	dinucleotide	136-156	TET
	Fp92-1	*F. pererginus*	dinucleotide	105-117	6-FAM
	Fnd2.3	*F. naumanni*	dinucleotide	203-236	6-FAM
Simplex	Fp82-2	*F. pererginus*	dinucleotide	129-140	6-FAM

Table 3. PCR components and markers used in the analyses

PCR components / Markers	**FalVes_13, FalVes_26, FalVes_31, FalVes_38 and FalVes_43 and cross species markers in the kinship analysis**	**FalVes_03, FalVes_04, FalVes_05 and FalVes_34**	**FalVes_15, FalVes_28 and FalVes_30**	**MIX1 (Fp54, Fp89, Fp34)**	**MIX2 (Fp347, Fp92-2, Fnd2.3)**
DreamTaq DNA polymerase	0.065 µl	0.065 µl	0.065 µl	0.16 µl	0.16 µl
DreamTaq Green Buffer	1.7 µl	1.7 µl	1.7 µl	4.53 µl	4.53 µl
MgCl2	0.66 µl (0.0165 µM)	0.66 µl (0.0165 µM)	0.5 µl (0.0125 µM)	1.73 µl (0.04325 µM)	1.73 µl (0.04325 µM)
dNTP	0.66 µl (0.00132 µM)	0.66 µl (0.00132 µM)	0.66 µl (0.00132 µM)	1.73 µl (3.46×10^{-3} µM)	1.73 µl (3.46×10^{-3} µM)
Forward primer	0.75 µl (3.75 pmol)	0.5 µl (2.5 pmol)	0.5 µl (2.5 pmol)	1.33 µl (3.325 pmol)	1.33 µl (3.325 pmol)
Reverse primer	0.75 µl (3.75 pmol)	0.5 µl (2.5 pmol)	0.5 µl (2.5 pmol)	1.33 µl (3.325 pmol)	1.33 µl (3.325 pmol)
dH2O	11 µl	11.5 µl	11.6 µl	6.67 µl	6.67 µl
Aliquot	15.585 µl	15.585 µl	15.525 µl	18.81 µl	18.81 µl
DNA	1.5 µl	1.5 µl	1.5 µl	4 µl	4 µl
End volume	**17 µl**	**17 µl**	**17 µl**	**22.81 µl**	**22.81 µl**

Table 4. PCR conditions for all markers

PCR Steps/ Marker types	60-55°C TD (All species-specific markers except for FalVes_13)			57-52°C TD (FalVes_13)			Cross-species markers			60-52°C TD (CHD-1 for Molecular Sexing)		
Initial denaturation	95 °C	2 min		95 °C	2 min		95 °C	2 min		95 °C	2 min	
Denaturation	95 °C	30 sec	11 cycles	95 °C	30 sec	11 cycles	95 °C	30 sec	11 cycles	95 °C	30 sec	9 cycles
Primer annealing	60 °C	30 sec		57 °C	30 sec		55 °C	45 sec		60 °C	45 sec	
Extension	72 °C	30 sec		72 °C	30 sec		72 °C	45 sec		72 °C	45 sec	
Denaturation	95 °C	30 sec	26 cycles	95 °C	30 sec	26 cycles	x	x		95 °C	30 sec	28 cycles
Primer annealing	55 °C	45 sec		52 °C	45 sec		x	x		52 °C	45 sec	
Extension	72 °C	45 sec		72 °C	45 sec		x	x		72 °C	45 sec	
Final extension	72 °C	7 min		72 °C	7 min		72 °C	7 min		72 °C	7 min	

Table 5. Sequences of the newly developed microsatellites for the Ref-footed Falcon

Loci name	Sequence
FalVes_04	ATTTCTCAGGCACTGAAGATAGCTGAATGGGAGGTTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTGTTA AATCAATGCACATTTAGTGGCACTGCTTGTCATTTAAGTATTTGTATGGAATTAACAGAAAGTGCTACAGCAAAATGTGTAAATGCTGTGTTGGTGTT
FalVes_05	TCACAATGCCTTTAGACCTCTGTTATACTACACAAAGATGTCATCTTTAATATTAATTTGTGGTCCCTAATTTTTCCATATGATACATCACTTTTAAAATGGGAGATGGATGGATG GATGGATGGATGGATGGATGGATGGATGGATGGATGGATGGATGGATGGATGGATGGATGGATGGATGGATGGATGGATATATTTATTTCCACAAAACCTTTTTCCAAA AATGTCAAAGTTGCATCCTGTAA
FalVes_13	ATTAACAAGTGCTGTTCCTGATGTTATCCTATAAAACTTTGTGGATCATCAAGGCAAATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTACT ATTATTGACCAGCATTAGAAGTGCACAG
FalVes_15	CCTGGATCTGTTTGAAGCACAGGGATCAAATACACACACACACACACACACACACACACACACACACACACCCTGCTATGGAACAAAGCATCTTTTTGCCATTAAAATACACTATGCC TAAATAAATGCTTGGGAACAAAAATAAATCAGTATCACATTTAAATTGCCGACTGGAGAGCAGATAGGTGCACGTCACCCATCCTGGTTGCTAGAAAGGTTTGTCTGGAATCGT GTGCTGTGT
FalVes_26	TGTTCCTGAGAGGCATAAACATTTTGGATTATATGTAAGTAACAAAAAAACCCCAACCAACACACACACACACACACACACACACACACACACACAAACCCAAACAAAAAAACTT TGCAACAGGTTGGTATTAAATGAGTATTTCCAGCCTAATGGACTGATGCAAAATGTGTGTTAATGCTGAGCACATCTCACGTGAGTTGGTTCCTGCATAT
FalVes_28	CCCCACATTCCTCGAGCAGACACATACAATCACAATCGATGTCCATTGTCCCCATTTCACACTATTTCTCCATATCATATCTATCTATCTATCTATCTATCTATCTATC TATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTCTGACCATATCAACTATAAGACATTTTACTGCAAACTTTGAAAAGAGACATTTTTTTCACCTCACTGG AAAGAGTCCTGCTC
FalVes_30	TCTCCCTTTGGTTTACAGAAGTCCCACTCTATTTTCAAAATAAAGTTGTATTCTCTATAATAAATAAATAATAAATAAATAAATAAATAAATAAATAAATGAATTAATAAATAT TTTTATATATAATTTTAAAATTCATATAATTATATATAATTTATAATTAATATTATGTATTAATTTAAAACATCATATAAATTAAATAATAATTATAATAAAGCAGCACCTCGCAC CATCTCTTTGC
FalVes_31	GTTCCTCAGGAAACAAGTCTGGGACTGATCTTGTTTTTATTATTTACCAATGCAAAAATAGGTGTAAGAATGAATGAATGAATGAATGAATGAATGAATGAATGAATGAAAGA AAAGTGGCCATCAGCTAACAC
FalVes_38	ACAACAAGCCGAAATGAAGCGAGACGAAAGGATGGGACGAAAGCACGGCTACGGGGTCTGAGGTGCTGTGAAAGACTGAGAGAAAGAGGAGAAATACAGAGAGAGAGAGA GAGAGAGAAGGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAGGAAGAGAAAGAAAGAGAGACGCTTTTTGCCTGGAGCAACCCTGGTACCTGGAAAACCAGCC GCTACTGTCT
FalVes_43	ATTTGTGGCTTTCGCATTTCTGGATGTAAATATTAGTTACACTGGTGTTGGCAACATTATCTTGCATTGAGTGAACTGAGGAAAATTACTAGTTTTTCAGTTCCAAACATAAACA AATACTCTATTTATTTATTTATTTATTTATTTATTTATTTATTTTACATGAACCTTTCTGTAAAGGGTTGATATACAGCAGTGAAATGCCTAAATGACT

Table 6. Allele frequencies and sample sizes by populations (BOR − Borsodi-mezőség, CSAN – Csanádi-puszták, CSER – Cserebökény, HEV – Heves, HOR – Hortobágy, JAS – Jászság, KISK – Kiskunság, RO1 – Southeast region colonies of Romania, RO2 – West region colonies of Romania, VAS – Vásárhelyi-puszták)

Locus	Allele/N	BOR	CSAN	CSER	HEV	HOR	JAS	KISK	RO1	RO2	VAS
fp92-1	**N**	10	10	10	10	10	10	10	10	10	10
	105	0,100	0,350	0,200	0,200	0,300	0,100	0,150	0,100	0,150	0,200
	107	0,050	0,100	0,000	0,100	0,150	0,150	0,000	0,250	0,050	0,100
	109	0,600	0,200	0,500	0,300	0,250	0,400	0,300	0,400	0,300	0,550
	111	0,100	0,050	0,200	0,150	0,200	0,200	0,350	0,200	0,150	0,000
	113	0,000	0,100	0,100	0,100	0,000	0,000	0,050	0,000	0,150	0,000
	115	0,100	0,200	0,000	0,150	0,100	0,150	0,150	0,050	0,200	0,150
	117	0,050	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
fp89	**N**	10	10	10	10	10	10	10	10	10	10
	118	0,000	0,000	0,000	0,000	0,000	0,000	0,050	0,000	0,000	0,000
	120	0,050	0,050	0,000	0,000	0,000	0,000	0,300	0,050	0,100	0,050
	122	0,100	0,400	0,200	0,050	0,150	0,200	0,100	0,250	0,400	0,250
	124	0,050	0,100	0,050	0,100	0,100	0,050	0,000	0,100	0,100	0,000
	126	0,300	0,150	0,300	0,300	0,150	0,500	0,150	0,150	0,050	0,300
	128	0,100	0,150	0,050	0,000	0,050	0,150	0,000	0,100	0,050	0,200
	130	0,050	0,050	0,100	0,250	0,300	0,050	0,150	0,150	0,150	0,100
	132	0,100	0,050	0,100	0,100	0,000	0,000	0,150	0,050	0,150	0,100
	134	0,100	0,000	0,100	0,100	0,050	0,050	0,050	0,150	0,000	0,000
	136	0,000	0,000	0,050	0,000	0,050	0,000	0,000	0,000	0,000	0,000
	140	0,000	0,000	0,000	0,050	0,000	0,000	0,000	0,000	0,000	0,000
	142	0,050	0,000	0,000	0,000	0,050	0,000	0,000	0,000	0,000	0,000
	151	0,000	0,000	0,000	0,050	0,000	0,000	0,000	0,000	0,000	0,000
	160	0,050	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	166	0,000	0,050	0,000	0,000	0,000	0,000	0,050	0,000	0,000	0,000
	170	0,050	0,000	0,050	0,000	0,100	0,000	0,000	0,000	0,000	0,000
fp-82-2	**N**	10	10	10	10	10	10	10	10	10	10
	129	0,000	0,200	0,150	0,000	0,150	0,250	0,050	0,050	0,100	0,100
	131	0,000	0,000	0,000	0,000	0,000	0,000	0,050	0,100	0,000	0,000
	133	0,000	0,000	0,000	0,050	0,050	0,000	0,050	0,000	0,000	0,000
	136	0,300	0,100	0,400	0,300	0,300	0,400	0,350	0,350	0,350	0,250
	138	0,700	0,700	0,450	0,600	0,500	0,350	0,500	0,500	0,450	0,600
	140	0,000	0,000	0,000	0,050	0,000	0,000	0,000	0,000	0,100	0,050
Fp347	**N**	10	10	10	10	10	10	10	10	10	10
	136	0,750	0,750	0,550	0,750	0,750	0,800	0,500	0,700	0,500	0,700
	138	0,000	0,000	0,100	0,000	0,000	0,000	0,050	0,000	0,050	0,000
	140	0,050	0,050	0,100	0,000	0,100	0,050	0,050	0,050	0,050	0,050
	143	0,200	0,200	0,050	0,250	0,150	0,100	0,150	0,150	0,300	0,000
	145	0,000	0,000	0,200	0,000	0,000	0,050	0,150	0,100	0,050	0,200
	147	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,050	0,000

	149	0,000	0,000	0,000	0,000	0,000	0,000	0,050	0,000	0,000	0,000
	154	0,000	0,000	0,000	0,000	0,000	0,000	0,050	0,000	0,000	0,000
	156	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,050
fr34	**N**	10	10	10	10	10	10	10	10	10	10
	158	0,350	0,300	0,300	0,350	0,300	0,200	0,300	0,150	0,300	0,200
	161	0,100	0,000	0,100	0,000	0,000	0,000	0,100	0,000	0,100	0,050
	163	0,450	0,600	0,600	0,450	0,600	0,500	0,550	0,750	0,400	0,650
	165	0,100	0,100	0,000	0,200	0,100	0,250	0,050	0,100	0,200	0,050
	167	0,000	0,000	0,000	0,000	0,000	0,050	0,000	0,000	0,000	0,050
Fnd2,3	**N**	10	10	10	10	10	10	10	10	10	10
	203	0,200	0,250	0,350	0,300	0,150	0,300	0,150	0,300	0,300	0,150
	205	0,000	0,000	0,050	0,050	0,000	0,050	0,000	0,000	0,000	0,050
	208	0,000	0,000	0,050	0,000	0,000	0,000	0,050	0,000	0,000	0,000
	211	0,250	0,150	0,050	0,100	0,150	0,050	0,100	0,150	0,050	0,150
	213	0,000	0,050	0,000	0,000	0,000	0,100	0,000	0,000	0,200	0,000
	215	0,050	0,000	0,050	0,000	0,000	0,000	0,000	0,000	0,000	0,050
	227	0,150	0,000	0,100	0,050	0,050	0,000	0,050	0,050	0,100	0,100
	230	0,000	0,100	0,050	0,050	0,000	0,100	0,050	0,200	0,000	0,050
	232	0,250	0,250	0,300	0,350	0,400	0,200	0,550	0,250	0,250	0,150
	234	0,050	0,000	0,000	0,000	0,000	0,050	0,000	0,000	0,000	0,000
	236	0,050	0,200	0,000	0,100	0,250	0,150	0,050	0,050	0,100	0,300
FalVes_31	**N**	10	10	10	10	10	10	10	10	10	10
	108	0,200	0,050	0,200	0,200	0,100	0,150	0,000	0,250	0,050	0,050
	112	0,300	0,400	0,150	0,200	0,200	0,100	0,350	0,200	0,250	0,100
	116	0,100	0,000	0,150	0,050	0,150	0,200	0,100	0,050	0,050	0,200
	120	0,000	0,050	0,100	0,100	0,050	0,100	0,100	0,150	0,000	0,100
	124	0,200	0,050	0,100	0,100	0,200	0,100	0,150	0,200	0,250	0,250
	128	0,000	0,150	0,000	0,050	0,000	0,150	0,050	0,000	0,000	0,000
	132	0,050	0,050	0,100	0,200	0,200	0,050	0,100	0,050	0,200	0,150
	136	0,050	0,150	0,150	0,050	0,000	0,150	0,150	0,100	0,050	0,100
	140	0,000	0,050	0,000	0,050	0,000	0,000	0,000	0,000	0,000	0,000
	145	0,100	0,050	0,050	0,000	0,050	0,000	0,000	0,000	0,150	0,050
	153	0,000	0,000	0,000	0,000	0,050	0,000	0,000	0,000	0,000	0,000
FalVes_26	**N**	10	10	10	10	10	10	10	9	10	10
	188	0,000	0,000	0,000	0,000	0,000	0,000	0,050	0,000	0,000	0,000
	190	0,000	0,050	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	192	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,056	0,000	0,000
	193	0,150	0,000	0,000	0,000	0,200	0,000	0,050	0,000	0,000	0,050
	195	0,000	0,050	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	197	0,300	0,300	0,300	0,300	0,400	0,350	0,150	0,167	0,050	0,150
	199	0,150	0,250	0,350	0,350	0,100	0,300	0,300	0,333	0,200	0,400
	201	0,100	0,000	0,000	0,000	0,000	0,000	0,000	0,056	0,050	0,050
	204	0,000	0,050	0,050	0,100	0,150	0,050	0,250	0,167	0,150	0,200
	206	0,150	0,200	0,150	0,100	0,050	0,200	0,150	0,111	0,300	0,150
	208	0,150	0,000	0,050	0,050	0,050	0,000	0,050	0,000	0,050	0,000
	210	0,000	0,000	0,000	0,050	0,050	0,000	0,000	0,000	0,150	0,000

	212	0,000	0,000	0,050	0,000	0,000	0,100	0,000	0,000	0,000	0,000
	214	0,000	0,050	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	216	0,000	0,000	0,000	0,050	0,000	0,000	0,000	0,000	0,050	0,000
	218	0,000	0,050	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	220	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,111	0,000	0,000
	231	0,000	0,000	0,050	0,000	0,000	0,000	0,000	0,000	0,000	0,000
FalVes_15	**N**	9	10	10	10	10	9	10	10	10	10
	218	0,056	0,050	0,000	0,000	0,000	0,000	0,100	0,000	0,050	0,000
	220	0,278	0,150	0,300	0,200	0,150	0,333	0,150	0,400	0,350	0,400
	222	0,111	0,100	0,050	0,000	0,050	0,000	0,050	0,050	0,000	0,000
	224	0,000	0,100	0,050	0,150	0,200	0,278	0,250	0,000	0,150	0,200
	226	0,111	0,100	0,050	0,000	0,100	0,000	0,100	0,000	0,000	0,150
	228	0,111	0,150	0,050	0,050	0,300	0,111	0,000	0,150	0,200	0,000
	230	0,056	0,150	0,000	0,050	0,000	0,000	0,000	0,050	0,050	0,150
	232	0,056	0,050	0,150	0,200	0,050	0,167	0,050	0,100	0,150	0,000
	234	0,056	0,050	0,250	0,200	0,050	0,056	0,150	0,100	0,050	0,100
	236	0,111	0,000	0,000	0,150	0,050	0,000	0,000	0,100	0,000	0,000
	238	0,056	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	240	0,000	0,050	0,100	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	243	0,000	0,000	0,000	0,000	0,050	0,000	0,000	0,050	0,000	0,000
	320	0,000	0,000	0,000	0,000	0,000	0,000	0,050	0,000	0,000	0,000
	322	0,000	0,000	0,000	0,000	0,000	0,000	0,100	0,000	0,000	0,000
	326	0,000	0,050	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	337	0,000	0,000	0,000	0,000	0,000	0,056	0,000	0,000	0,000	0,000
Falves_28	**N**	10	10	10	10	10	10	9	10	10	10
	200	0,000	0,100	0,000	0,050	0,000	0,000	0,000	0,000	0,000	0,000
	204	0,000	0,150	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	208	0,000	0,000	0,100	0,000	0,000	0,000	0,167	0,000	0,000	0,000
	212	0,000	0,000	0,050	0,050	0,000	0,000	0,056	0,000	0,000	0,000
	216	0,000	0,050	0,050	0,000	0,050	0,000	0,000	0,000	0,100	0,000
	220	0,000	0,050	0,050	0,100	0,000	0,000	0,000	0,000	0,000	0,050
	224	0,000	0,000	0,000	0,000	0,050	0,000	0,000	0,000	0,000	0,000
	226	0,000	0,000	0,000	0,050	0,000	0,000	0,056	0,000	0,000	0,000
	228	0,050	0,050	0,000	0,050	0,100	0,000	0,000	0,100	0,050	0,050
	232	0,000	0,100	0,000	0,000	0,050	0,050	0,056	0,000	0,050	0,050
	233	0,050	0,050	0,000	0,000	0,050	0,100	0,000	0,000	0,000	0,050
	234	0,000	0,050	0,050	0,000	0,000	0,000	0,111	0,050	0,150	0,050
	236	0,000	0,050	0,050	0,100	0,000	0,050	0,056	0,000	0,000	0,100
	238	0,650	0,100	0,000	0,200	0,200	0,300	0,278	0,450	0,300	0,150
	240	0,000	0,050	0,150	0,050	0,150	0,100	0,056	0,100	0,000	0,150
	242	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,050
	244	0,050	0,050	0,100	0,000	0,000	0,050	0,056	0,000	0,150	0,000
	246	0,000	0,000	0,000	0,100	0,050	0,000	0,000	0,000	0,000	0,050
	248	0,000	0,000	0,150	0,000	0,050	0,100	0,000	0,100	0,000	0,000
	250	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,050	0,050
	251	0,000	0,000	0,000	0,050	0,000	0,100	0,000	0,050	0,000	0,000

	252	0,050	0,000	0,000	0,050	0,100	0,000	0,000	0,000	0,000	0,050
	256	0,000	0,000	0,050	0,000	0,000	0,000	0,000	0,100	0,000	0,000
	260	0,050	0,050	0,000	0,050	0,000	0,000	0,000	0,000	0,000	0,050
	262	0,000	0,000	0,050	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	264	0,000	0,050	0,050	0,000	0,050	0,000	0,000	0,050	0,000	0,050
	268	0,050	0,000	0,000	0,000	0,000	0,100	0,000	0,000	0,000	0,000
	270	0,000	0,000	0,000	0,000	0,050	0,000	0,000	0,000	0,000	0,000
	272	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,050	0,000
	278	0,000	0,000	0,000	0,000	0,000	0,050	0,000	0,000	0,000	0,000
	281	0,000	0,000	0,000	0,050	0,000	0,000	0,000	0,000	0,000	0,000
	286	0,000	0,000	0,050	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	296	0,050	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	304	0,000	0,000	0,000	0,000	0,000	0,000	0,111	0,000	0,050	0,050
	313	0,000	0,000	0,050	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	321	0,000	0,050	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	325	0,000	0,000	0,000	0,000	0,050	0,000	0,000	0,000	0,000	0,000
	338	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,050	0,000
	368	0,000	0,000	0,000	0,050	0,000	0,000	0,000	0,000	0,000	0,000

Figure 1. Principal Coordinates Analysis (PCoA) based the NEI distances (BOR − Borsodi-mezőség, CSAN – Csanádi-puszták, CSER – Cserebökény, HEV – Heves, HOR – Hortobágy, JAS – Jászság, KISK – Kiskunság, RO1 – Southeast region colonies of Romania, RO2 – West region colonies of Romania, VAS – Vásárhelyi-puszták)

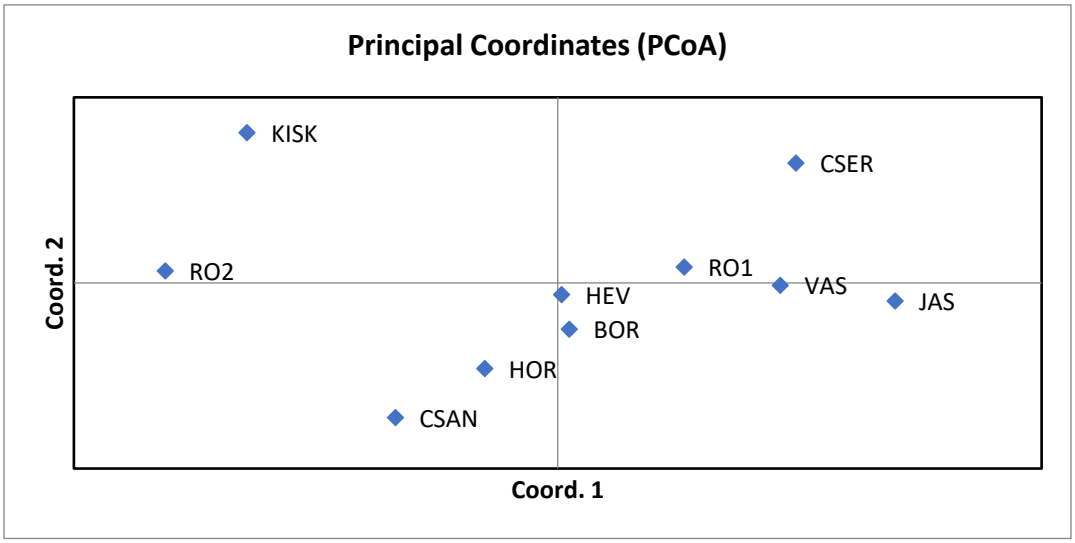
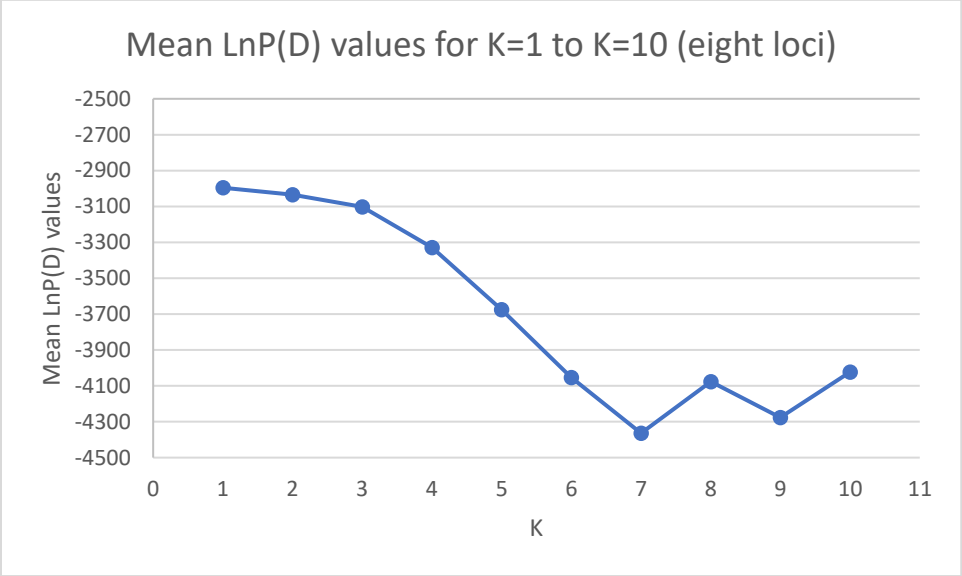


Table 7. Pairwise F_{ST} values based on eight loci (below diagonal) and their P values (above diagonal) *P≤0.05 (BOR − Borsodi-mezőség, CSAN – Csanádi-puszták, CSER – Cserebökény, HEV – Heves, HOR – Hortobágy, JAS – Jászság, KISK – Kiskunság, RO1 – Southeast region colonies of Romania, RO2 – West region colonies of Romania, VAS – Vásárhelyi-puszták)

	BOR	CSAN	CSER	HEV	HOR	JAS	KISK	RO1	RO2	VAS
BOR	0.00000	0.08108 ±0.0286	0.01802 ±0.0121 *	0.60360 ±0.0385	0.23423 ±0.0454	0.07207 ±0.0297	0.07207 ±0.0227	0.35135 ±0.0497	0.16216 ±0.0424	0.01802 ±0.0121*
CSAN	0.02730	0.00000	0.27928 ±0.0370	0.37838 ±0.0264	0.38739 ±0.0334	0.16216 ±0.0503	0.18018 ±0.0449	0.18018 ±0.0235	0.24324 ±0.0273	0.43243 ±0.0504
CSER	0.03512 *	0.01370	0.00000	0.78378 ±0.0430	0.40541 ±0.0365	0.75676 ±0.0579	0.71171 ±0.0287	0.63964 ±0.0497	0.22523 ±0.0365	0.81982 ±0.0459
HEV	0.00251	0.00515	-0.00395	0.00000	0.89189 ±0.0165	0.54955 ±0.0417	0.56757 ±0.0508	0.58559 ±0.0413	0.27928 ±0.0252	0.36937 ±0.0394
HOR	0.01022	0.00768	0.00590	-0.01182	0.00000	0.25225 ±0.0379	0.33333 ±0.0430	0.39640 ±0.0454	0.20721 ±0.0305	0.43243 ±0.0354
JAS	0.02272	0.01674	-0.00334	0.00076	0.01203	0.00000	0.00901 ±0.0091*	0.48649 ±0.0433	0.10811 ±0.0264	0.45946 ±0.0370
KISK	0.02383	0.01954	-0.00080	0.00205	0.00787	0.03359 *	0.00000	0.43243 ±0.0297	0.69369 ±0.0543	0.32432 ±0.0473
RO1	0.00530	0.01227	-0.00060	-0.00198	0.00363	0.00387	0.00517	0.00000	0.37838 ±0.0539	0.62162 ±0.0213
RO2	0.02366	0.01431	0.01661	0.01258	0.01850	0.03008	0.00320	0.01215	0.00000	0.20721 ±0.0385
VAS	0.02711 *	0.00371	-0.00575	0.00414	-0.00004	0.00228	0.00934	-0.00300	0.01830	0.00000

Figure 2. Mean LnP(D) values for K = 1 to K = 10 calculated by STRUCTURE 2.3.4 for eight loci



Publication List

Publications

Magonyi, N. M., Mátics, R., Szabó, K., Fehérvári, P., Solt, S., Palatitz, P., & Vili, N. (2019). Characterization of novel microsatellite markers for the red-footed falcon (*Falco vespertinus*) and cross-species amplification in other Falco species. *European Journal of Wildlife Research, 65*(4). https://doi.org/10.1007/s10344-019-1300-8

Piross, I. S., Siliwal, M., Kumar, R. S., Palatitz, P., Solt, S., Borbáth, P., Vili, N., Magonyi, N., Vas, Z., Rózsa, L., Harnos, A., & Fehérvári, P. (2020). Sex interacts with age-dependent change in the abundance of lice-infesting Amur Falcons (*Falco amurensis*). In *Parasitology Research* (Vol. 119, Issue 8, pp. 2579–2585). https://doi.org/10.1007/s00436-020-06753-w

Magonyi, N. M., Szabó, K., Bertók, P., Fehérvári, P., Solt, S., Palatitz, P., Vili, N., & Mátics, R. (2021). Extra-pair paternity, intraspecific brood parasitism, quasi-parasitism and polygamy in the Red-footed Falcon (*Falco vespertinus*). *Ibis: international journal of avian scienc*e. https://doi.org/10.1111/ibi.12932

Conferences

1. Magonyi, N. M., Vili, V,, Szabó, K., Fehérvári, P., Kumar, S. R. & Mátics, R. (2018) **New microsatellite markers for a protected raptor species, *Falco vespertinus.*** Student Conference on Conservation Science (SCCS) , Centre for Ecological Research – (Poster presentation)

2. Magonyi (2019) **Fifty Shades of Blue.** Student Conference on Conservation Science: SCCS 2019, Centre for Ecological Research − (Presentation)

3. Magonyi (2019) **Extra-pair paternity and intraspecific brood parasitism in the Red-footed Falcon (*Falco vespertinus*) using species-specific and cross-species microsatellite markers**. International Symposium on Reproductive strategies - Reproductive strategies from genes to societies 2019 − (Presentation)