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**Population viability analysis and dispersal characteristics
of the eastern imperial eagle (*Aquila heliaca*)
in the Carpathian Basin**

PhD thesis

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1 SUMMARY

The recovery of the Pannonian (Carpathian Basin) eastern imperial eagle population following a bottleneck in the 20th century has been well documented since 1980 in terms of the number of nesting pairs and productivity. However, knowledge on survival rates and dispersal characteristics, including natal dispersal distances and migration rates, was limited. This dissertation focuses on estimating these missing demographic parameters, and gives predictions for future population growth in the context of a population viability analysis or PVA.

Throughout the dissertation, I will use ‘we’ and ‘our’ when referring to the conducted work and results: although I carried out the vast majority of the laboratory and statistical analyses and also participated in sample collection, this study is the result of the combined efforts of several experts.

In addition to the conventional marking methods of ringing and GPS tracking, we also used genetic monitoring to follow the survival and movements of individuals. This included DNA profiling chicks from plucked feathers and breeding individuals from shed feathers collected at the nest sites. To ensure reliable individual identification even in this growing population, we improved the microsatellite marker set previously used for the imperial eagle. We tested 26 cross-species markers and assembled a final set of 17 loci, which provides eight orders of magnitude higher resolution than the former set (reducing the probability of identity from 10^{-6} to 10^{-14}).

Adult survival is often considered the most important demographic parameter in long-lived species. To estimate the survival of breeding males and females, we carried out mark-recapture analyses using mainly shed feathers from adults. In some cases, we also used plucked feathers from chicks to infer the presence of their parents via parentage analysis. Based on capture histories of 208 males and 411 females from 2011–2022, we estimated a $91.6\% \pm 0.8\%$ SE survival probability, typical for large-sized raptors, but much higher than previous estimates for the same population. We also found moderate evidence that poisoning (i.e. the leading known mortality cause at the time) has a stronger negative impact on male than on female survival.

Combining ringing, GPS and DNA data, we determined the natal dispersal distances of 43 males and 72 females hatched in Hungary between 2012 and 2020. We found that females disperse longer (median 57.6 km) than males (median 35.9 km). High natal philopatry—with only limited dispersal between the subpopulations and no emigration to other populations detected—is possibly governed by conspecific attraction. These

results suggest that the Pannonian population is isolated from other European populations, which implies its genetic uniqueness.

Building on these results, the population viability analysis in VORTEX confirmed that the Pannonian population is self-sustaining and could have recovered from the severe bottleneck of the last century without any significant immigration from other populations. Future projections indicate a viable population in East Hungary, which increases by 11.5% each year and is expected to reach the carrying capacity by 2038, unless poisoning rates were to increase significantly. The results indicate that the conservation measures implemented by Helicon LIFE (2012–2016) and PannonEagle LIFE (2017–2023) projects were highly successful.

2 ÖSSZEFOGLALÁS

A parlagi sas pannon (Kárpát-medencei) populációjának 20. századi drasztikus csökkenését követő regenerálódása jól dokumentált 1980 óta, legalábbis a fészkelő párok száma és a termékenység tekintetében. Azonban a túlélési rátákról és diszperziós jellemzőkről, beleértve a kelési diszperziós távolságokat és migrációs rátákat, korlátozott ismeretek álltak csak rendelkezésre. Ezen disszertáció célja az ismeretlen demográfiai paraméterek becslése, és ezáltal a jövőbeni populációnövekedés előrejelzése egy populáció-életképességi elemzés (PVA) keretében.

A hagyományos jelölési módszerek, mint a gyűrűzés és a GPS-jeladózás mellett genetikai monitorozást is alkalmaztunk az egyedek túlélésének és mozgásának nyomon követésére. Ez a fiókák esetében tokos tollakból, a költő egyedek esetében a fészek alól gyűjtött vedlett tollakból történő DNS-profilozást jelentette. Annak érdekében, hogy ebben a növekvő populációban is megbízható egyedazonosítást tudjunk végezni, továbbfejlesztettük a korábban használt mikroszatellita marker-készletet. Összesen 26 db, rokon fajokban leírt markert teszteltünk, melynek végeredményeképpen egy 17 lókuszból álló markeszettet állítottunk össze, amely nyolc nagyságrenddel nagyobb felbontást biztosít, mint az előző készlet (az identitási valószínűség 10^{-6} -ról 10^{-14} -re csökkent).

A felnőtt egyedek túlélését gyakran tekintik a hosszú életű fajok legfontosabb demográfiai paraméterének. A költő hímek és tojók túlélését jelölés-visszafogásos módszerrel becsültük, főként a vedlett tollaiból állapítva meg jelenlétüket. Bizonyos esetekben a fiókák DNS-profiljából, szülő-utód elemzés során következtettünk a szülők jelenlétére. 208 hím és 411 tojó fogástörténete alapján 91,6% ($\pm 0,8\%$ SE) túlélési valószínűséget becsültünk a 2011–2022 időszakra. Ez a túlélési valószínűség jellemző a nagyméretű ragadozó madarakra, és jóval magasabb, mint az ugyanerre a populációra vonatkozó korábbi becslés. Mérsékelt bizonyítékokat találtunk arra is, hogy a mérgezés (azaz az akkoriban legjelentősebb ismert mortalitási ok) erősebb negatív hatással van a hímek túlélésére, mint a tojókéra.

Gyűrűzési, jeladós és DNS-adatok kombinálásával sikerült meghatároznunk 2012 és 2020 között Magyarországon kikelt 43 hím és 72 tojó kelési diszperziós távolságát. Megállapítottuk, hogy a tojók medián diszperziós távolsága nagyobb (57,6 km), mint a hímeké (35,9 km). Az erős filopatria—a szubpopulációk között csak korlátozott diszperziót figyeltünk meg és nem detektáltunk más populációkba történő elvándorlást—valószínűleg a fajtársakhoz való kötődéssel is magyarázható. Ezek az eredmények

azt jelzik, hogy a pannon populáció elszigetelt más európai populációktól, ami genetikai egyediségére is utal.

A fenti eredményekre alapozva, a VORTEX programban elvégzett populáció-életképességi elemzés megerősítette, hogy a pannon populáció önfenntartó, és a múlt század súlyos palacknyak-hatása után más populációkból történő jelentős bevándorlás nélkül is regenerálódhatott. A jövőre vonatkozó predikciók szerint a kelet-magyarországi állomány életképes, évente 11,5%-kal növekszik, és várhatóan 2038-ra eléri az eltartóképességet, kivéve, ha a mérgezési ráta jelentősen megnövekszik. Az eredmények alátámasztják, hogy a Helicon LIFE (2012–2016) és a PannonEagle LIFE (2017–2023) projektek által végrehajtott természetvédelmi intézkedések rendkívül sikeresek voltak.

3 ABBREVIATIONS

AIC _c	Akaike Information Criterion (corrected for small sample size)
CI	confidence interval
CJS	Cormack-Jolly-Seber (model)
cy	calendar year, used in the context of aging birds; refers to the period of January 1 st to December 31 st of a specific year, where the first calendar year is the year of hatching
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
DTT	1,4-dithiothreitol
H _E	expected heterozygosity
H _O	observed heterozygosity
NDD	natal dispersal distance
PCR	polymerase chain reaction
PI	probability of identity
PI _{SIB}	probability of identity (corrected for the presence of siblings)
PVA	population viability analysis
QAIC _c	Quasi Akaike Information Criterion (corrected for small sample size)
SE	standard error
SD	standard deviation

4 GENERAL INTRODUCTION

4.1 Status of the eastern imperial eagle

The eastern imperial eagle (*Aquila heliaca*, SAVIGNY, 1823; hereafter referred to as 'imperial eagle') is a large-sized, long-lived raptor species of the forest steppe-zone of Eurasia. It belongs to the family Accipitridae, in the order Accipitriformes. It is listed as Vulnerable by IUCN, with an estimated maximum of 10,000 mature individuals globally [1]. The species is threatened by numerous anthropogenic factors across its range, including habitat destruction and alteration, electrocution, poisoning and illegal hunting [2–4]. As it is an apex predator, its conservation is crucial for maintaining ecosystem health [5] (Figure 4.1).

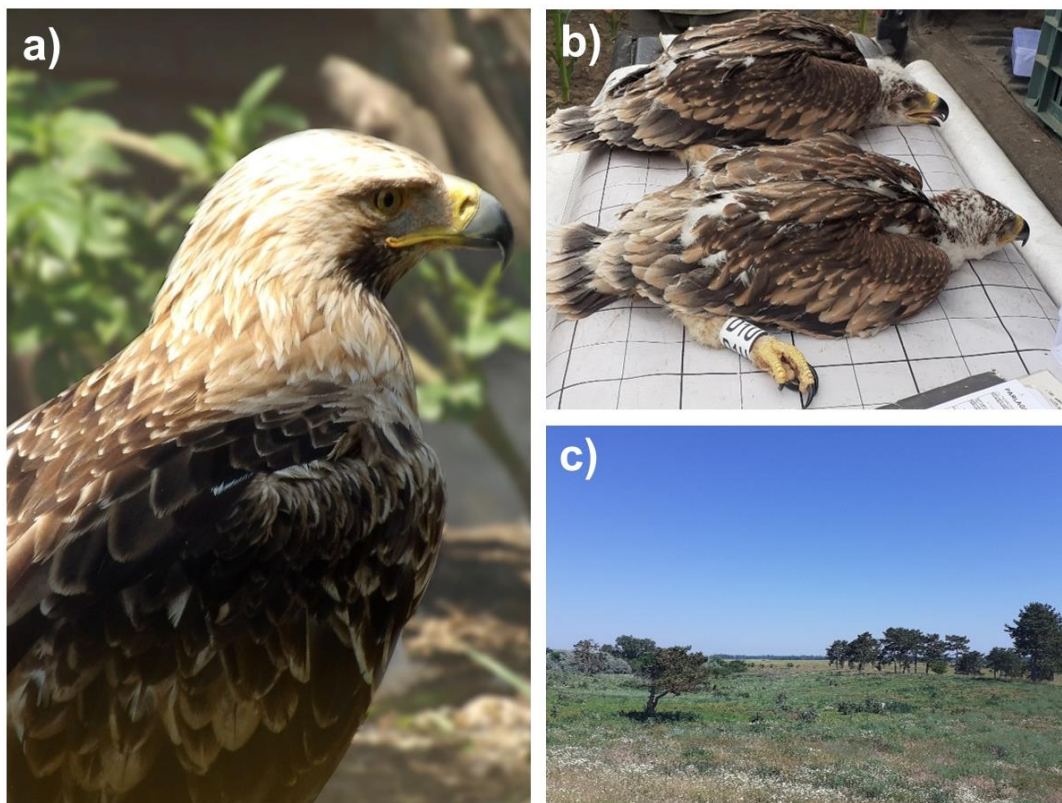


Figure 4.1. Imperial eagle recovering from a shot injury at the Hortobágy Bird Clinic, Hungary (a). 6.5-week-old imperial eagle chicks being ringed for the purpose of studying population dynamics (b). Imperial eagle habitat in Hungary (c).

(Photos: Bernadett Zsinka)

The imperial eagle's distribution spans from Eastern Austria to Russia's Trans Baikal region and from the Southern-Ural Mountains to Turkey [6–8]. About two-thirds of the global population is found in Russia and Kazakhstan [8]. These large eastern populations are migrants, wintering in the Middle East, South-East Asia, and North-East Africa, while its smaller, isolated western populations in Central Europe, the Balkans, Turkey and South Causasus are mainly sedentary [6, 7].

While its global population is considered decreasing due to likely declines in Russia and Kazakhstan [1, 9], its European populations show increasing trends, and the species has been recently categorised as 'Least Concern' in Europe [10]. The largest of these European populations is found at the western edge of the species' range, in the Pannonian Region, more frequently referred to as Carpathian Basin in the Hungarian literature [7, 8, 11]. This Pannonian population numbered about 400 nesting pairs in 2019 [8]. It can be divided into a larger Eastern and a smaller Western subpopulation, separated by about 100 km [12]. The Eastern subpopulation inhabits East Hungary, East Slovakia, North Serbia and West Romania, while the Western subpopulation includes areas of West Hungary, West Slovakia, East Austria and the Southern Czech Republic.

Our main goal was to study the demographic parameters and population dynamics of this Pannonian population to support the species' successful conservation in the future.

4.2 Population viability analysis (PVA)

Population viability analysis (PVA) is a valuable tool for gaining a better understanding of a species' population dynamics [13, 14]. It is used for examining the potential fates of a population, by projecting future population growth and estimating extinction risks under different demographic scenarios [13]. By means of perturbation analyses, PVAs help determine which demographic parameters have the highest impact on population growth rate and viability, thus providing crucial information for conservation management decisions [13, 15–19].

PVA involves the construction of a simulation model which aims to represent real-world population dynamics as accurately as possible, utilising estimates of demographic parameters and the synthesised knowledge on the species and its environment [13]. PVA models are usually not just deterministic (i.e. predicting population growth based on constant birth or death rates) but also account for stochastic effects that cause random variations in these demographic rates, thereby providing a more accurate assessment of population viability [13]. Stochastic effects include demographic stochasticity (random fluctuations of demographic rates across years caused by individual-level chance

events), environmental variation (fluctuations in demographic rates due to environmental changes), catastrophes (extreme cases of environmental variation) and genetic drift and inbreeding [20]. These stochastic effects are incorporated into the model of VORTEX, the most often used software for PVA [21]. Its highly flexible individual-based model also provides options for including subpopulation structure and allows for demographic parameters to be modelled as functions of other parameters. To conduct a PVA analysis for the imperial eagle in VORTEX, we first needed to obtain estimates on previously unknown demographic parameters of reproduction, survival and dispersal.

4.3 Biology and demographic parameters of the imperial eagle

The imperial eagle's lifespan in the wild has been recorded to exceed even 30 years [22]. Similarly to other eagles, its plumage undergoes gradual changes over several years until reaching adult colouration [23]. Based on plumage characteristics, four age categories can be distinguished reliably in the field: juveniles (birds in their first calendar year or 1cy), younger immatures (2cy-3cy), older immatures (4cy-5cy) and adult birds (6cy+).

Imperial eagles are territorial [24]. They usually start breeding at the age of 4cy [22]. Before settling, they are considered floaters, often exploring areas hundreds or thousands of kilometres from their natal place [22, 25]. Despite this high mobility, most of their movements are restricted to their natal population, suggesting high natal philopatry [25, 26]. However, there is only scarce data published on natal dispersal distances, i.e. the distance between the hatching and the first breeding site [25, 27]. Once they settle, they display high breeding philopatry and are considered genetically monogamous [28, 29]. This implies that territory and mate choice have high consequences for the lifetime fitness of the imperial eagle. However, the factors shaping natal dispersal and settlement choices have not been studied to date.

Females produce a single clutch each year in March-April, consisting of 1-3 eggs [22]. The Pannonian population has shown increased reproductive output since 1980, with a growing frequency of three-egg clutches and a decline in single-egg clutches [11, 30, 31]. Between 2021 and 2024, four-egg broods were also recorded on five occasions in Hungary [32]. While the sexes display only minor dimorphism in morphology—females are slightly larger than males—, they practice markedly different roles in parental care. Females do most of the incubation, while males play the major role in food provisioning [22, 33].

In the Pannonian Region, the imperial eagle prefers grasslands and lowland agricultural areas where patches of trees provide nesting sites and open plains foraging opportunities [24, 34]. Its diet mainly consists of small and medium-sized mammals and birds, but diet composition shows high flexibility and follows the abundance of prey species [34, 35]. In Hungary, it mainly preys on the brown hare (*Lepus europaeus*), the common pheasant (*Phasianus colchicus*) and corvid species [35]. Across its range, the imperial eagle also occurs in mountainous or lowland forests, but typically only in areas where human disturbance and persecution (e.g. poisoning or poaching) are significant in open habitats. [22].

This was also the case in the Pannonian Region during the previous century, when the population experienced a drastic decline due to persecution and agricultural intensification [36]. By the 1970s, the few remaining pairs occupied almost exclusively mountainous forest habitats [36]. Following the decrease in persecution due to conservation efforts, the population recolonised lowland areas and has shown an increasing trend ever since [7, 22, 30]. However, the Eastern subpopulation has increased much more rapidly than the Western, despite similar initial population sizes in 1980 [11, 22, 37–39]. This suggests that the two subpopulations differ in their demographic parameters.

In long-lived species, population growth is generally most influenced by survival rates, particularly adult survival [40, 41]. For eagles living in populations largely unaffected by human-caused mortality, annual adult survival typically exceeds 90% [42]. In Hungary, however, a surprisingly low survival (72.3%) based on annual turnover rate was estimated for breeding females between 1997 and 2006 [29], which was later suspected to reflect previously undetected high poisoning activity during that period. Poisoning—both intentional (directly aimed at predators) or accidental (resulting from improper pest control practices)—was reported as the leading known mortality cause between 2005 and 2019 [2, 11]. Its highest rates occurred in 2011–2014, coinciding with a stagnation in population growth [22, 43]. Nonetheless, survival rates and their relationship to poisoning remained poorly understood.

4.4 Using DNA profiling to monitor the imperial eagle

Studying the demographic parameters of long-lived, large-sized raptors is particularly challenging [42, 44, 45]. Usually, to estimate survival or dispersal rates, birds need to be individually identified, and their fate monitored over an extended period of time. To apply conventional markings, such as rings, wing-tags or GPS transmitters, birds need to be captured, which is usually not feasible for large-sized raptors after fledging. Therefore, these techniques can only be used to mark chicks in adequate numbers. DNA profiling of the shed feathers collected from the territories provides a non-invasive alternative for identifying and monitoring adult birds [28, 29, 46–48]. This method requires a set of genetic markers (e.g. microsatellites) that provide sufficient allelic variation to distinguish individuals reliably [49]. Microsatellite markers have previously been used to study population dynamics in the imperial eagle [28, 29, 50]. However, the resolution of these marker sets was adequate only for individual identification in smaller populations and needed to be improved to enable effective individual monitoring in the Pannonian Region as well.

4.5 Aims

The aims of this dissertation were:

1. To **assemble a microsatellite marker set** that ensures the reliable DNA-based individual identification of imperial eagles in the Pannonian population, thereby supporting the study of demographic parameters ([SECTION 5](#)).
2. To **estimate annual survival rates for breeding imperial eagles** in 2011–2022 in East Hungary, using a mark-recapture method based on DNA profiling. We aimed to explore possible sex differences in survival and investigate the relationship between poisoning rates and annual survival probabilities ([SECTION 6](#)).
3. To study **natal dispersal behaviour** in the Pannonian population in 2011–2024 using colour-ringing, GPS tracking and DNA profiling methods. We aimed to study natal dispersal patterns in relation to sex, local density and dispersal direction ([SECTION 7](#)).
4. To **conduct a population viability analysis (PVA)** for the Pannonian population, employing the previously estimated survival and dispersal rates and published fecundity parameters. We aimed to give predictions for future population growth in East Hungary, assess the possible difference in the demographic parameters of the Eastern and Western subpopulations, and investigate which parameters have the highest impact on population growth using elasticity analysis ([SECTION 8](#)).

5 CROSS-SPECIES TESTING OF MICROSATELLITE MARKERS FOR THE RELIABLE INDIVIDUAL IDENTIFICATION OF THE EASTERN IMPERIAL EAGLE

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5.1 Introduction

Individual identification is often required for the study of wild populations [51]. It enables the estimation of survival probability, the monitoring of dispersal behaviour and the study of mate choice and parental care strategies [52–55]. DNA based individual identification (i.e. DNA profiling) further supports the study of relatedness, inbreeding and population structure [28, 56–58]. Moreover, it plays an important role in wildlife forensic cases (e.g. poaching or illegal trade) when comparing samples collected at the crime scene with those linked to a suspect [59, 60].

In the case of protected species, these studies are especially important: investigating the genetic state of a population and uncovering the factors affecting survival and breeding success facilitate the successful conservation of the species [58, 61–63]. Protected species are often elusive, meaning that individual monitoring may only be possible by DNA profiling the biological traces left behind by the individuals, such as shed feathers, hair or faeces [64, 65]. However, such non-invasively collected samples usually contain low-quality, fragmented or contaminated DNA, which must be taken into account when selecting genetic markers for their analysis [66].

5.1.1 *Development of microsatellite marker sets for individual identification*

Microsatellites, also known as STRs (Short Tandem Repeats), are widely used markers for individual identification. They are characterised by a high degree of length polymorphism, where short sequence motifs of 2–6 base pairs repeat in variable numbers. DNA profiles constructed from a sufficiently large number of highly polymorphic microsatellite markers enable not only individual identification, but also the estimation of relatedness between individuals [67, 68].

Microsatellite marker sets for a specific target species can be assembled in two ways: either by screening the genome of the target species for microsatellite markers via sequencing (*de novo* method), or by using markers previously described in related species (cross-species method) [69]. While the latter is cheaper, the genetic distance

between the source species and the target species largely determines the suitability of the markers. The closer the source and the target species are related, the higher the chance that the marker of the source species will also show polymorphism in the target species, and the primers designed for the source species can be utilised in the target species as well [70, 71].

The repeat motif of the markers is another important factor to consider when assembling a marker set. Microsatellites consisting of longer, tetranucleotide repeats generally have more clearly identifiable alleles than those with shorter, di- or trinucleotide motifs, which typically have a greater degree of 'stuttering', i.e. PCR products that are a few repeats shorter or longer than the true allele are often produced due to amplification error by the PCR polymerase enzyme [72]. However, the lower susceptibility of tetranucleotide loci to amplification errors also reflects their reduced mutation rate during cellular DNA replication, meaning they typically have fewer alleles compared to di- or trinucleotide loci. Apart from its length, the sequence of the repeat motif can also influence the degree of polymorphism [73, 74].

Prior to application, candidate markers should be first tested on the target population, to select those polymorphic markers that generate PCR products of adequate quantity and quality, so their alleles can be reliably detected during capillary electrophoresis. In addition, the assembled marker set should have the appropriate resolution for the purpose of the study. One of the most important indicators of resolution is the probability of identity (PI), i.e. the probability that two randomly selected individuals from the population share the same DNA profile [49]. This probability is higher in the case of relatives, so the value of PI corrected for the presence of siblings in the population, PI_{SIB} [49], is a more accurate measurement of marker set reliability. Time- and cost-efficiency are also important aspects of marker set development; multiplex PCR, which enables the joint amplification of multiple markers, facilitate both [75, 76]. Simultaneous allele detection of several loci during capillary electrophoresis also contributes to cost-effectiveness. For this, PCR products of loci in similar length ranges should be marked with fluorescent dyes of different colour [77].

5.1.2 DNA-based individual identification of the protected eastern imperial eagle

The eastern imperial eagle (hereafter 'imperial eagle', *Aquila heliaca*, SAVIGNY 1809) is a Vulnerable (IUCN) species which is highly protected in Hungary. Its global population is estimated at a maximum of 5,000 pairs [1]. The edge of its western distribution is the Pannonian Region, where 356–381 pairs nested in 2019 [8], of which 287 pairs were in

Hungary [31]. Its European populations, including the one in the Pannonian Region, show an increasing trend [1, 22].

Since breeding imperial eagles are difficult to capture due to their large body size, their individual identification is only possible by DNA profiling via non-invasive sampling. Due to their territorial behaviour, they can be reliably sampled from their shed feathers collected around their nests. This method has previously been used to estimate the return rate of breeding birds [29] and to study the population structure of the Slovakian population [12] and kin avoidance, inbreeding, recruitment rate and breeding system in the Kazakh population [28, 50]. The microsatellite marker sets used in the above studies were partly comprised of loci described for the imperial eagle [78] but also contained several markers originally published for the imperial eagle's sister species, the Spanish imperial eagle (*Aquila adalberti*) [79].

These marker sets were suitable for the reliable identification of individuals in the studied populations based on their PI values, but the PI_{SIB} values, which can be orders of magnitude higher than PI, are not available for these marker sets. In the eastern imperial eagle, PI_{SIB} is a better indicator of the reliability of the markers since due to the high territorial fidelity, longevity and monogamous mating system of the species, there are many siblings in the populations [28, 29].

Given the exponential growth of the Hungarian population, our goal was to compile a set of microsatellite markers that has a higher resolution than the previously used marker sets [29] to ensure the reliable individual identification of imperial eagles in the future [49] and to enable the more reliable estimation of relatedness and the detailed study of population structure [80]. In this new set, we included markers already successfully applied in previous studies and new markers described in related species.

5.2 Methods

5.2.1 Sampling and DNA extraction

Shed feathers of imperial eagles breeding in East Hungary were collected between 2011 and 2022 by MME BirdLife Hungary and the relevant National Park Directorates within the framework of the Helicon LIFE (2012–2016, LIFE10/ NATHU/019) and PannonEagle LIFE (2017–2023, LIFE15/NATHU/000902) projects. To preserve DNA quality, the feathers were stored in resealable bags in a dry, dark environment until processing [81]. DNA was extracted from the blood clot in the superior umbilicus region of the feathers [46] using commercial DNA extraction kits (Geneaid™ DNA Isolation Kit Tissue, Omega E.Z.N.A.® Tissue DNA Kit) following the manufacturer's protocol. In addition, 10 µl of

DTT (1,4-dithiothreitol) was also added to the samples to aid in the digestion of keratin [82].

5.2.2 Selection of candidate markers

We selected microsatellite markers for testing based on (i) the phylogenetic distance between the source species and the imperial eagle, (ii) our previous experience with the markers in the source species and (iii) the characteristics of the marker in the source species (repeat motif, polymorphism, allele length).

Of the 26 microsatellites selected for testing, six (Aa26, Aa41, Aa49, Aa53, Aa56, Aa57) were described in the Spanish imperial eagle (*A. adalberti*) [79], thirteen (AQJ10, AQJ22, AQJ36, AQJ52, AQJ62, AQJ71, AQJ72, AQJ79, AQJ84, AQJ88, AQJ91, AQJ118, AQJ120) were published for the Japanese golden eagle (*Aquila chrysaetos japonica*) [83, 84] and seven (Hal01, Hal03, Hal04, Hal09, Hal10, Hal13, Hal14) for the white-tailed eagle (*Haliaeetus albicilla*) [85]. The Spanish imperial eagle markers have been previously used in imperial eagles [28, 29, 50], but they have not yet been tested on the Hungarian population. The Japanese golden eagle loci were chosen because this species also belongs to the genus *Aquila*. Although the white-tailed eagle belongs to the genus *Haliaeetus*, markers of the imperial eagle have been previously used with good results in this species [56, 86], so we assumed that white-tailed eagle markers would be similarly applicable in imperial eagles.

The candidate microsatellite set comprised of 18 dinucleotide (Aa26, Aa49, Aa53, Aa56, Aa57, Hal01, Hal03, Hal04, Hal09, Hal10, Hal13, Hal14, AQJ36, AQJ52, AQJ62, AQJ84, AQJ88, AQJ91), five trinucleotide (AQJ10, AQJ71, AQJ72, AQJ79, AQJ120) and three tetranucleotide (Aa41, AQJ22, AQJ118) loci. Since shed feathers often contain fragmented DNA, we only selected loci that were expected to have alleles shorter than 300 base pairs.

5.2.3 Testing of candidate markers

As a first step, we attempted to amplify the selected loci using their published PCR programs on the samples of 4–15 individuals, that were unrelated based on the previously used marker set [29].

In the case of the Aa and Hal markers, we used forward primers labelled at the 5' end with a fluorescent dye (6-FAM™, HEX™, NED™ or PET™, Applied Biosystems, Waltham, MA, USA). In the case of the AQJ loci, we applied the more cost-effective 'tailing' method in the monoplex testing step by using the universal primer sequence

'Tail A' [87]. Since this technique can introduce difficulties to PCR optimisation [88, 89], we used traditional fluorescent labelling for the AQJ loci as well when testing duplex reactions or optimising the final marker set. To reduce amplification errors ('stuttering' and possible extra adenylation), a GTTT ('pigtail') sequence was added to the 5' end of the reverse primers [90].

For multiplex reactions with labelled forward primers, the PCR mixture contained: 2 μ l 5 \times FIREPol® Master Mix (Solis BioDyne, Tartu, Estonia), 0.5–0.5 μ l 10 pmol/ μ l forward and reverse primers, 5 μ l H₂O, and 2 μ l ca. 50 ng/ μ l concentration of DNA. In the case of tailed primers, 0.25 μ l forward, 0.25 μ l labelled universal primer (Tail A) and 0.5 μ l reverse primer were added to the mixture. In the case of duplex reactions, an equal amount of each primer (0.5 μ l) and 4 μ l of H₂O were added to the mixture.

The results of the PCR reactions were first evaluated by agarose gel electrophoresis on a 2% agarose gel stained with EcoSafe intercalating dye, at a voltage of 100 V and a runtime of 20 minutes. When amplification was unsuccessful, we tried to improve the performance of the markers by reducing the annealing temperature. If artefacts were present, we incorporated a 'touchdown' sequence into the PCR program [91].

Alleles of markers with adequate amplification were separated during capillary electrophoresis using an ABI3130 sequencer (Applied Biosystems, Waltham, MA, USA) and the Gene Scan™ -500LIZ™ size standard (Eurofins-BIOMI Kft., Gödöllő, Hungary). We screened the allele sizes in the OSIRIS v2.16 software [92].

Both previously used and newly tested microsatellites were included in the new marker set. For statistical analysis, 15 unrelated individuals were genotyped with the new set. We used the GenAlEx v.6.503 Microsoft Excel macro to calculate the identity probabilities PI and PI_{SIB} (corrected for the presence of siblings) [93]. Observed heterozygosity (H_o), expected heterozygosity (H_e) and Hardy–Weinberg equilibrium were investigated with Genepop v1.2.2. [94]. The presence of null alleles was examined with MicroChecker v.2.2.2 [95].

5.3 Results

Out of the 26 microsatellites tested, only one Japanese golden eagle locus (AQJ52) failed to amplify. 19 of the 25 successfully amplifying markers were polymorphic: 100% of the loci described in the Spanish imperial eagle (Aa26, Aa41, Aa49, Aa53, Aa56, Aa57), 75% of the markers described in the Japanese golden eagle (AQJ10, AQJ22, AQJ36, AQJ71, AQJ79, AQJ84, AQJ88, AQJ91, AQJ120) and 57% of the white-tailed eagle loci (Hal04, Hal09, Hal10, Hal13). The allele numbers of the polymorphic markers

ranged from two to seven, with a mean of 4.2 alleles for the Spanish imperial eagle, 3.6 alleles for the Japanese golden eagle and 3.8 alleles for the white-tailed eagle loci.

The newly assembled marker set comprised of 17 microsatellites, seven previously used (Aa02, Aa35, Aa36, Aa39, Aa43, IEAAAG09, IEAAAG11) and ten newly tested markers (Aa26, Aa41, Aa49, Aa53, Aa57, AQJ10, AQJ22, AQJ120, Hal04, Hal10) (**Table 5.1**). The markers were selected based on their polymorphism and amplification properties (adequate amount of PCR product, clearly detectable alleles, possibility of multiplex PCR). 14 of the 17 microsatellites could also be successfully amplified in duplex reactions. Capillary electrophoresis of the entire set can be performed in just two panels. Three types of PCR programs were used to amplify the markers: for the Aa and AQJ loci and the IEAAAG loci, modified versions of the PCR program published for the Aa loci were used [79], and for the Hal loci, the modified version of the program with a primer annealing temperature of 57 °C was used [85] (**Table 5.2**).

The mean allele number of the 17 microsatellites based on the genotypes of 15 unrelated individuals was 4.65. Observed heterozygosity ranged from 0.333 to 0.933, while expected heterozygosity ranged from 0.370 to 0.802. All loci were in Hardy-Weinberg equilibrium and no null alleles were detected. The probability of identity of the new marker set was $PI = 7.6 \times 10^{-14}$, the probability corrected for the presence of siblings was $PI_{SIB} = 2.8 \times 10^{-6}$.

Table 5.1. The new microsatellite marker set for the individual identification of the eastern imperial eagle (*Aquila heliaca*) and the attributes of the markers based on 15 individuals from the East Hungarian population: marker name, repeat motif, fluorescent dye, PCR multiplex, range (base pairs), number of alleles, observed heterozygosity (H_o), expected heterozygosity (H_e) and probability of identity values PI and PI_{SIB} for the full marker set.

Marker	Motif	Dye	PCR multiplex	Range (bp)	Alleles	H_o	H_e
<i>Panel 1</i>							
Aa02	di	6-FAM	–	145–161	6	0.933	0.762
Aa35	di	HEX	Aa35–Aa43	252–278	8	0.800	0.802
Aa36	di	HEX	Aa36–Aa39	114–128	5	0.667	0.758
Aa39	di	6-FAM	Aa36–Aa39	192–206	6	0.667	0.742
Aa43	di	6-FAM	Aa35–Aa43	106–116	4	0.400	0.393
IEAAAG09	tetra	6-FAM	IEAAAG09–IEAAAG11	477–489	4	0.600	0.611
IEAAAG11	tetra	6-FAM	IEAAAG09–IEAAAG11	327–339	4	0.667	0.611
Hal04	di	NED	Hal04–Hal10	152–160	5	0.800	0.709
Hal10	di	NED	Hal0–Hal10	231–245	5	0.867	0.696
<i>Panel 2</i>							
Aa26	di	PET	–	155–157	2	0.467	0.370
Aa41	di	HEX	Aa41–Aa57	156–160	2	0.333	0.480
Aa49	di	6-FAM	Aa49–Aa53	151–159	4	0.467	0.561
Aa53	di	NED	Aa49–Aa53	130–136	4	0.667	0.687
Aa57	di	FAM	Aa41–Aa57	114–124	5	0.667	0.687
AQJ10	tri	HEX	AQJ10–AQJ22	182–191	4	0.933	0.743
AQJ22	tetra	PET	AQJ10–AQJ22	189–206	5	0.600	0.756
AQJ120	tri	6-FAM	–	203–218	6	0.800	0.685

$$PI = 7.6 \times 10^{-14}, PI_{SIB} = 2.8 \times 10^{-6}$$

Table 5.2. PCR programs for the loci of the new marker set. The Aa, AQJ and IEAAAG markers were run on the modified versions of the PCR program published for the Aa loci in [79], while the Hal loci were run on the modified version of the program published for Hal loci in [85].

Markers	Aa and AQJ loci	IEAAAG loci	Hal loci
Initial denaturation	95°C (2 min)		
Denaturation	95°C (30 s)		
Annealing	Touchdown (-1°C / cycle) 66-50°C (30 s) 17 cycles 50°C (30 s) 21 cycles	Touchdown (-1°C / cycle) 66-60°C (30 s) 7 cycles 50°C (30 s) 31 cycles	57°C (45 s) 37 cycles
Elongation	72°C (30 s)		72°C (45 s)
Final elongation	72°C (7 min)		

5.4 Discussion

Our cross-species testing of microsatellite markers resulted in ten new loci to be used for the individual identification of the imperial eagle. The probability of identity of the previous marker set was $PI = 3.7 \times 10^{-6}$ [29], compared to which the PI of the 17-marker set reported in this study is eight orders of magnitude lower ($PI = 7.6 \times 10^{-14}$), making it the highest resolution marker set currently published for the imperial eagle [28, 29, 50]. This will ensure the reliable identification of individuals even with the rapid growth of the population [49], contributing to the more accurate estimation of demographic parameters through the genetic tagging of individuals. It also has the potential to serve as an evidentiary tool in cases of poisoning, shooting or illegal trade of imperial eagles. In addition, it enables a more reliable estimation of relatedness and a more detailed examination of population structure, further providing valuable information for conservation programs [80, 96, 97].

Most of the ten newly added microsatellites originate from the imperial eagle's closest relative, the Spanish imperial eagle. Almost all of the tested Spanish imperial eagle markers had adequate amplification properties, reliably detectable alleles and high polymorphism. In contrast, markers of the white-tailed eagle, the most distant relative of the imperial eagle among the three species studied, had the greatest proportion of monomorphic markers. These results correspond to the expectation that a greater degree of polymorphism would be observed for markers of closely related species [70, 71].

A somewhat contradictory result is that the only marker that failed to amplify originated from the *Aquila* Japanese golden eagle, a species phylogenetically closer to the imperial eagle than the white-tailed eagle. This amplification failure could indicate that this microsatellite locus is absent in the imperial eagle, or that the primer binding regions for this locus differ significantly in the two species. Several Japanese golden eagle markers exhibited weak amplification, which did not improve even with reduced annealing temperatures. The one Japanese golden eagle marker which was included in the new marker set (AQJ120) was also characterised by a weaker amplification compared to the Spanish imperial eagle markers, and thus it could only be sufficiently amplified in a monoplex PCR reaction. These results imply the reduced affinity of golden eagle primers, which may be due to differences in the primer binding sequences between the imperial and golden eagle. However, we cannot exclude the different PCR technique ('tailing' method) used in testing of the Japanese golden eagle markers as explanation. Although this type of issue was not reported previously for this method, there were instances where 'tailing' changed the PCR optimum of primers [88, 89]. Therefore, it is possible

that the weak affinity of some Japanese golden eagle primers and their high amplification error rate ('stuttering') are the result of the 'tailing' technique applied. In this case, the traditional fluorescent labelling of primers would help overcome these issues.

In summary, we found that markers of the Spanish imperial eagle, the Japanese golden eagle and the white-tailed eagle can be successfully used for the individual identification of the eastern imperial eagle. The newly constructed marker set will ensure the reliable, time- and cost-effective study of the growing population of this species in the future.

6 MODERATE EVIDENCE FOR THE SEX-DEPENDENT EFFECT OF POISONING ON ADULT SURVIVAL IN A LONG-LIVED RAPTOR SPECIES

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Minor modifications of the text and figures were implemented for this thesis.

6.1 Introduction

Survival is one of the key life-history traits affecting the population growth rate [98] and, consequently, the viability of a population [99]. Therefore, the accurate estimation of age-, life-stage- or sex-specific survival rates is of great importance in understanding the dynamics of a population [100, 101]. In addition, assessing the changes in these demographic parameters in response to certain human pressures, such as habitat alteration, electrocution, or persecution, can facilitate the effective conservation of endangered species [61, 102–105].

In the case of raptors (Accipitriformes, Falconiformes, Catharthiformes and Strigiformes), survival rate is a critical determinant of population growth: since raptors are generally long-lived species with late maturity and low fecundity rates, their population growth rates are more sensitive to survival than to reproductive parameters [40–42, 106, 107]. Therefore, studies on raptor survival are especially important, considering that approximately 20% of raptor species are threatened, and more than half of all raptor species have a decreasing global population [108].

One of the major threats to raptors is poisoning, which can be intentional (directly aimed at controlling predator species) or accidental (resulting from the misuse of pesticides/rodenticides or from secondary poisoning through consumed poisoned prey) [108]. Even though poisoning is known to have detrimental effects on populations [109], direct evidence linking poisoning to increased mortality and population decline remains scarce [102, 110–113].

Sex differences in survival are generally small in raptors [42, 100, 114, 115], with either males or females having lower survival rates, and the direction of difference can be inconsistent even within a species [116, 117]. Sex differences in survival rates are considered to be driven mainly by differences in selective forces on life-history traits resulting in different trade-off optima between survival and reproduction (dimorphic parental behaviour, defence of territory, and body size), while the genetic hypotheses

(unguarded X, mother's curse) seem to be less important [118–120]. In raptors, females tend to be larger and spend more time at the nest during the breeding season, while the males are responsible for providing food for their mates and chicks and defending the territory [121, 122]. These sex differences may lead to different survival rates in various ways. For example, the larger size of females could promote a higher chance of survival during the winter [123], but also a larger risk of electrocution [124]. Furthermore, the more sedentary behaviour of females during the breeding season could either result in less exposure to anthropogenic mortality factors [100] or a higher risk of mortality if they are targeted at the nest [125].

Estimating the survival of raptors can be challenging because they appear in generally low densities and are often difficult to capture [42]. Hence, the population dynamics of most raptor species are still poorly understood [126, 127]. Survival is often estimated using mark-recapture models, which require marking individuals and monitoring their fate through live encounters or dead recoveries [128, 129]. Conventional marking techniques (e.g. rings, wing-tags, transmitters) all require the capture of birds, which is often not feasible for breeding adults, especially for large-sized species. In such cases, DNA profiling from non-invasively collected samples (e.g. shed feathers from the nest site) can provide an alternative method for identifying and monitoring individuals [28, 29, 46–48]. Another genetic-based identification used to estimate the turnover of breeding individuals utilises DNA samples from chicks [130, 131]. In this case, the turnover of breeders is estimated based on relatedness between chicks of the same nest from consecutive years. While the plucked feathers and blood obtained from chicks are considered more reliable sources of DNA than shed feathers [132], this technique requires a high-resolution marker set for reliable relatedness estimation.

Here we investigate the survival of breeding individuals in the eastern imperial eagle *Aquila heliaca* (hereafter 'imperial eagle'). As a Vulnerable [1], large-sized, long-lived raptor, the survival rate of breeding individuals is a particularly important demographic parameter for the species. The imperial eagle's distribution is scattered throughout the Palearctic region, with only a few thousand breeding pairs worldwide [1]. Its westernmost population is found in the Pannonian Region [6, 7], and consists of ca. 356–381 nesting pairs as of 2019 [8], making it the largest unified population outside of Russia and Kazakhstan [11]. Most of this Pannonian population (287 pairs as of 2019) reside in Hungary [31]. Imperial eagles exhibit floater behaviour in their first years and usually start breeding in their third or fourth calendar year [22]. The breeding success of these immature birds is lower than that of adults [133]. Following 1–2 years of successful breeding, adult breeders display high territory and mate fidelity [28, 29]. Sexual

dimorphism in imperial eagles only involves differences in size, with females being the larger sex, and behavioural dimorphism during the breeding season. Similarly to its sister species, the Spanish imperial eagle (*Aquila adalberti*) [134], eastern imperial eagle males also generally spend less time around the nest than females since they rarely take part in incubation, and they play the major role in food provisioning during the chick-rearing period [22, 33]. The imperial eagle is threatened by various anthropogenic factors, such as habitat fragmentation and alteration [4], electrocution and persecution, including the illegal poisoning and shooting of birds [2, 3]. Even though the most dangerous pesticides (e.g. carbofuran) were banned in the EU in 2008, poisoning cases due to these substances still occur in imperial eagles, as well as in several other raptors [43, 135]. In Hungary, poisoning was the leading known cause of mortality between 2005 and 2019, representing 25-35% of all detected mortality cases [2, 11]. Most of these were the result of intentional poisoning incidents, where baits (such as chicken eggs, carcasses of smaller prey animals or parts of large animals) poisoned with legally banned pesticides or insecticides were deployed with the aim of eliminating avian or mammalian predators [43]. The other cases resulted from accidental poisoning, which occurs from the misuse of chemicals aimed to control agricultural pests or rodents. These were usually the result of improperly installed bait stations and/or the use of banned pesticides, which raptors encounter by consuming poisoned prey animals or their carcasses [2, 43].

We used a mark-recapture method based on genetic identification to estimate annual apparent survival and encounter probabilities for breeding imperial eagles in East Hungary between 2011–2022. We aimed to explore possible sex differences in survival and investigate the relationship between poisoning and the annual survival probabilities of breeding males and females. Due to the aforementioned behavioural dimorphism, we expected males to have lower survival rates than females, as they spend more time away from the nest during breeding and, consequently, were assumed to have a higher risk of encountering anthropogenic mortality factors. Additionally, we hypothesised that the effect of poisoning on survival will be sex-dependent due to the seasonal patterns of this behavioural dimorphism and that of poisoning activity. Most cases of poisoning during the study years occurred in the first half of the year, with a peak in early spring (February-April), which coincides with the egg-laying and incubation period (March-May) of the imperial eagle [22, 33, 43]. Since this is the period when females rarely leave the nest and only males hunt for food, we expected males to be more exposed to poisoning than females. Besides, males are also significantly smaller in body size than females (even by 20-25% in weight), therefore the same amount of a poisonous chemical in a prey or bait could be more detrimental or even fatal for males. Since breeding imperial eagles

are difficult to capture and mark with conventional methods, we used DNA profiles obtained from shed feathers for the individual identification of breeders. This method has been previously applied with success in two studies estimating survival in imperial eagles [28, 29]. In addition, we also utilised the long-term genetic monogamy of the species to obtain additional presence data of breeding birds through parentage analysis [28].

6.2 Methods

6.2.1 Sample collection

DNA samples for genetic profiling were collected between June and September each year between 2011 and 2022 in East Hungary in the frame of the national monitoring scheme for the imperial eagle in Hungary (**Figure 6.1**). The eastern part of the country holds about 95% of the national population of imperial eagles. On average, 67% of the nests here were sampled yearly (SD: 13%), with the highest coverage in 2017 (86%, 191 nests) and the lowest in 2012 (51%, 77 nests). Armpit feathers were plucked from nestlings during ringing, and breeding individuals were non-invasively sampled by collecting their shed feathers around the 100m radius of the nest. Plucked feathers were stored at -20°C in 2 ml microtubes filled with 96% ethanol, while shed feathers were stored in tagged plastic bags in dark, dry, and cool conditions to preserve DNA [81]. We processed the majority (90%) of the samples within one year and all samples within three years of collection.

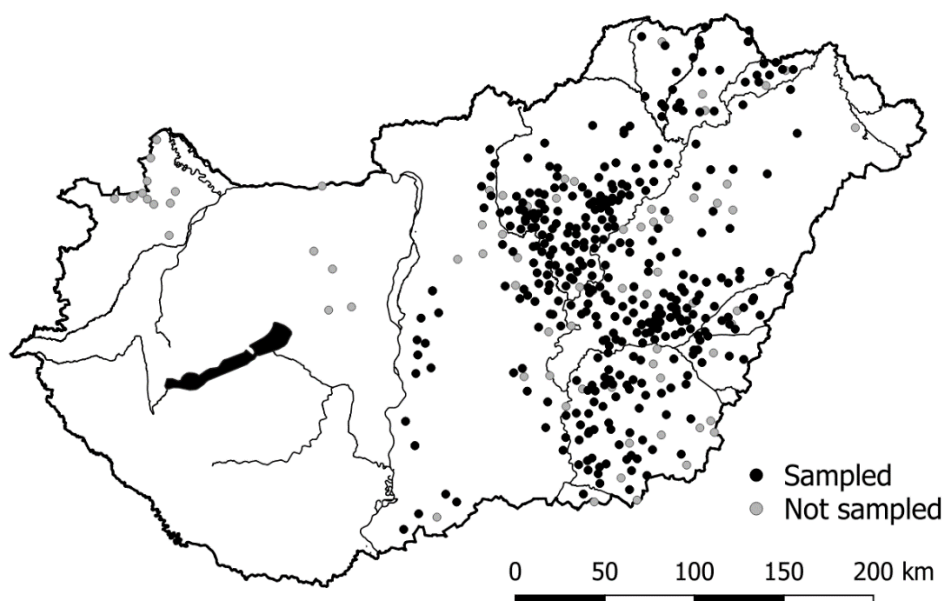


Figure 6.1. Distribution of the eastern imperial eagle in Hungary between 2011 and 2022 with sampled ($n = 369$, black) and not sampled ($n = 73$, grey) nests.

6.2.2 DNA extraction

We extracted the whole genome DNA using the Omega E.Z.N.A.® Tissue DNA Kit (Omega Bio-tek Inc.) following the manufacturer's instructions but using an additional 20 µl of dithiothreitol (1M) to aid in the digestion of keratin [82]. We extracted DNA from the tip of the calamus in plucked nestling feathers and from the superior umbilicus region of shed feathers [46].

6.2.3 Molecular sexing

We conducted molecular sexing on each feather by amplifying introns of the sex chromosome-linked CHD1 gene, using the primers CHD-i16F/CHD-i16R [136]. The PCR reaction included 0.065 µl DreamTaq polymerase (Fermentas), 1.7 µl 10X DreamTaq Green puffer (Fermentas), 0.65 µl 25 mM MgCl₂ (Thermo Scientific), 0.65 µl 2 mM dNTP mix (Thermo Scientific), 1-1 µl 10 pmol/µl forward and reverse primers, 8 µl H₂O and 4 µl ca. 50 ng/µl concentration DNA. The PCR program for molecular sexing constituted of an initial denaturation step at 95°C for 2 minutes, a touchdown section of 9 cycles (denaturation: 95°C for 30s, annealing: temperature lowering by 1°C each cycle from 60-52°C and lasting 45s, elongation: 72°C for 45s), followed by 28 cycles of 95°C for 30s, 52°C for 45s and 72°C for 45s, ending with a final elongation of 7 minutes at 72°C. The PCR products were visualised through gel electrophoresis by UV illumination (2% agarose gel stained with EcoSafe (Pacific Image Electronics Co., Ltd) intercalator, 100 V, 45 minutes); the heterogametic females display two bands while the homogametic males only one.

6.2.4 Individual genotyping

We used nine microsatellite markers for individual identification. Out of these, two tetranucleotide loci (IEAAAG09 and IEAAAG11) [78] were optimised for the imperial eagle, five dinucleotide loci (Aa02, Aa35, Aa36, Aa39 and Aa43) [79] were published for the Spanish imperial eagle (*Aquila adalberti*) and two dinucleotide loci (Hal04 and Hal10) [85] for the white-tailed eagle (*Haliaeetus albicilla*). The 5' end of the forward primers were modified with the following fluorescent dyes (Applied Biosystems™): 6-FAM™ for Aa02, Aa39, Aa43, IEAAAG09, IEAAAG11; HEX™ for Aa35 and Aa36; and NED™ for Hal04 and Hal10. The 5' end of the reverse primer was modified with a pigtail 5'GTTT sequence in the case of Aa02, Aa36 and Aa39.

We performed PCR reactions in a 10 µl volume, containing 2 µl 5xFIREPol® Master Mix (Solis BioDyne), which consists of dNTP-mix, MgCl₂ and Taq DNA-polymerase, 0.5-0.5

µl 10 pmol/µl forward and reverse primers, 5 µl H₂O and 2 µl ca. 50 ng/µl concentration DNA. Aa36 and Aa39, Aa35 and Aa43, Hal04 and Hal10, IEAAAG09 and IEAAAG11 were also amplifiable as duplexes. We used the PCR procedure described in [79] for all Aa loci and applied a modified version of it (touchdown scheme: 66-60°C, annealing at 60°C for 31 cycles) to the IEAAAG loci. For the Hal loci, we used the PCR profile described in [85] with the following modifications: 37 cycles, 45 seconds for both annealing and amplification. Fragment lengths were determined using capillary electrophoresis: PCR products were run on an ABI3130 sequencer (Applied Biosystems, using Gene Scan™ -500LIZ™ Size Standard), and we identified and scored alleles with OSIRIS v2.16 [92]. We performed the fragment analysis similarly to the suggestions of Beja-Pereira et al. [137] by scoring each sample three times independently. Genotypes were assigned blind to the origin of the sample. We checked the deviation from the Hardy-Weinberg equilibrium with Genepop v1.2.2. [94] and investigated the possible occurrence of null alleles and allelic dropouts using MICROCHECKER v.2.2.2 [95]. We calculated probabilities of identity (PI and PI_{SIB}) [49] and exclusion probabilities for parentage analysis (P1X and P2X) with GenAIEx v.6.503 [93].

6.2.5 *Constructing capture histories*

We constructed capture histories (yearly presence-absence data for each individual) from two types of presences (both recorded as '1' in the capture histories): (i) direct presences, when a breeding bird was sampled and genetically profiled directly from its shed feathers and (ii) indirect presences, when the presence of a breeding bird was inferred through parentage analysis due to the lack of shed feathers in that specific year. We could only obtain indirect presences for an individual if both the profile of its mate and at least one of its chicks were known from that specific year and the individual in question had also been profiled in another year (preceding or following the year in focus). We performed parentage analyses for indirect presences manually.

6.2.6 *Survival analysis*

We conducted the survival analysis in the MARK v9.0 software [138] using the RMark v3.0.0 R interface [139] by fitting the Cormack-Jolly-Seber (CJS) open population model [140] to the capture histories. The CJS model estimates annual apparent survival probabilities (ϕ) for each one-year interval and encounter probabilities (p) for each year as parameters of a generalised linear model. As sampling took place in June in each year, these apparent survival probabilities refer to one-year intervals. The estimated survival probability is apparent by definition, as it is the joint probability of surviving and

remaining in the sampling area. However, since imperial eagles display high territory fidelity between years [28, 29], we considered the apparent survival as a good proxy to survival in this case. Therefore, we will refer to 'apparent survival' as 'survival' throughout the article.

We constructed a candidate model set based on previous knowledge on the factors potentially influencing survival and encounter probabilities in this species. Both parameters were assumed to depend on sex (*sex*) based on the difference in behaviour the sexes display during breeding. We expected males to have lower survival and encounter probabilities than females, because we assumed that their more active foraging behaviour during the breeding results in a higher risk of mortality and a lesser probability of finding their shed feathers at the nest site. We investigated annual variation in survival and encounter probabilities with time-dependency (*time*) models. We also assumed that survival probability would show variation across the years not only because of annual variation, e.g. in food supply or weather conditions, but also due to varying levels of poisoning in the last decade. The number of poisoning incidents was taken from the BirdCrime Database of MME BirdLife Hungary, which incorporates all detected cases by the conservation organisations of the country [43]. To investigate the relationship between survival and poisoning, we modelled survival as a function of annual poisoning rate (*poison*), which we calculated as follows: number of poisoned imperial eagles found in East Hungary in each interval (e.g. between the 1st of June 2011 and the 31st of May 2012) / total number of known nesting individuals (twice the number of known nesting pairs) at the beginning of the interval, multiplied by 100 to be expressed as a percentage. Therefore, a 1% poisoning rate means that the observed number of poisoned imperial eagles equals 1% of the known nesting individuals. We presumed that the number of known nesting pairs accounts for at least 95% of the total number of nesting pairs in East Hungary [11].

We emphasise that the number of poisoned imperial eagles includes not only nesting individuals but floaters as well as individuals of unknown age. That is because determining whether the poisoned bird was a breeder or a floater was not possible in a high number of cases. First, there was no available information on age for 34% of the poisoned birds. Second, since imperial eagles can start breeding as early as three calendar years [22], poisoned birds with immature colouration (10%) could have been either floaters or breeders. Only birds in their first or second calendar year (30%) could have been safely excluded as non-breeders. However, due to the high number of poisoned birds with unknown age or unsure status, we decided to include all poisoned individuals when calculating poisoning rates instead of discarding a significant number

of detected cases. Therefore, the poisoning rate used here is only a proxy for the true poisoning rate of breeding individuals. Assuming that the observed poisoning rate of all birds is directly proportional to the poisoning rate of breeding birds, yearly changes in this observed poisoning rate reflect the yearly changes in the true poisoning rate of breeding birds.

Since August 2013, a poison and carcass detection dog (PCDD) unit has also been used to help uncover poisoning incidents and proved to be more successful in both carcass and bait detection than human investigators [43]. Therefore, we corrected the number of poisoned carcasses found before August 2013 for the possibly undetected poisoning events to gain a more accurate estimation of poisoning rates in these early intervals. We did this by assuming the detection probability by the PCDD unit to be 1 and then dividing the number of poisoned imperial eagle carcasses found before August 2013 by the detection probability of human investigators. This probability was estimated to be 0.81 since out of the 42 imperial eagle carcasses detected between August 2013 and August 2020, 34 were recovered by human investigators ([43]; Deák's personal communication).

Lastly, we also modelled encounter probabilities as a function of sampling effort (*effort*), which we calculated for each year as the number of genotyped feathers (shed and chick feathers for parentage analysis included) / total number of known nesting individuals. Controlling for the number of nesting pairs in the case of both *poison* and *effort* was necessary due to the rapid expansion of the population throughout the study [22].

We used the standard CJS model $\{\phi(\text{sex} \times \text{time}), p(\text{sex} \times \text{time})\}$ as the general model, and the candidate model set included the variables mentioned above with both additive (+) and interaction terms (\times). We also considered models with constant (.) survival or encounter probability. We constructed the sex models with males as the reference category. Continuous variables were left untransformed.

Assumptions of the CJS model include the homogeneity of survival and encounter probabilities among groups of marked individuals [140]. Therefore, we used goodness-of-fit tests to assess whether these conditions are met in our dataset. We investigated the goodness-of-fit of our general model to the capture histories with the χ^2 tests of program RELEASE v.3.0 [141]. We estimated the overdispersion parameter (\hat{c}) by dividing the overall χ^2 of the component tests by the overall degrees of freedom. Then, we used this \hat{c} estimate to adjust the AIC_c (Akaike Information Criterion corrected for small sample size) values of models, resulting in a $QAIC_c$ -based model selection. We also adjusted AIC_c values by matching the parameter counts (K) to the model structure and by considering confounded parameters, as recommended by [142]. We ranked the

models using their QAIC_c values and considered them equally parsimonious if the difference in their QAIC_c values (ΔQAIC_c) was less than two [143]. We used the Akaike weights (w_i) to evaluate the relative support of the competing models. Since multiple models gained similar support (see 6.3) we also report the more robust, model-averaged estimates of apparent survival beyond the estimates of individual models. In the model averaging, we only included models with $p \sim \text{sex} \times \text{time}$ (the most-supported function of p , see 6.3). Additionally, we conducted the model selection using multiple values of \hat{c} to investigate how changes in the overdispersion parameter would affect model selection results (\hat{c} sensitivity test).

We used R v.4.3.1. [144] to run RMark and calculate descriptive statistics of genotype data. To create figures, we used the *ggplot2* v.3.4.4. R package [145] and QGIS v.3.28.1. [146].

6.3 Results

6.3.1 Individual identification

During the study, we genotyped 1730 shed feathers, which belonged to 619 breeding individuals (208 males and 411 females) with an average of 2.8 samples per individual. Out of the 15 570 genotyped loci, 1172 (7.53%) failed to amplify adequately, and we detected 106 genotyping errors (0.74%) by comparing multiple samples of the same individual. In total, we found 58 alleles at the nine microsatellite loci, ranging from four (IEAAAG09) to ten (Aa35), with an average of 6.4 alleles per locus. PI and PI_{SIB} values for the complete marker set were 9.5×10^{-9} and 5.7×10^{-4} , respectively. Exclusion probabilities P1X and P2X were 0.997 and 0.963, respectively. We found deviations from the Hardy-Weinberg equilibrium ($p < 0.05$) in three loci (Aa35, Aa36 and Aa43), with the possibility of null alleles on locus Aa36 (Table A1). As null alleles do not amplify during PCR, their presence on a locus leads to falsely identifying heterozygotes as homozygotes, which can result in false mismatches during the comparison of true parent-offspring genotypes, i.e. falsely excluding breeders as parents of a chick. That is because the chick with the null allele appears to be homozygous, while it is expected to be heterozygous based on the putative parents' genotypes. To avoid such false exclusions, we allowed for such mismatches on locus Aa36 during parentage analysis if all other loci indicated a match between the breeder and the chick.

6.3.2 *Survival analysis*

Capture histories of the 208 males and 411 females consisted of a total of 1712 presences (436 presences for males and 1276 presences for females), out of which 181 were indirect presences, i.e. encounter data obtained via parentage analysis (111 and 70 for males and females, respectively). 3% of the males and 20% of the females encountered on the first occasion were also encountered on the last occasion of the 12-year study (one male and 17 females).

Based on the RELEASE goodness-of-fit test results, our dataset satisfied the condition of homogeneous encounter probabilities for both males and females. However, we found minor violations of the assumption of homogeneous survival probabilities for females ($p = 0.023$). This could be mainly attributed to the results of females encountered in 2012, 2014 and 2017. In all three cases, individuals encountered for the first time in the given year were indicated to have lower survival probabilities than those that had been encountered before. However, this heterogeneity is considered low as indicated by our low estimate of overdispersion ($\hat{c} = 1.148$), and so we carried out the model selection procedure after adjusting the AIC_c values with this \hat{c} estimate.

In each of the four most supported models ($\Delta QAIC_c < 2$, $\Sigma w_i = 0.746$), the encounter probability was a function of $sex \times time$ (Table 6.1). Regarding survival, several different models received similar support, as detailed below.

The minimum $QAIC_c$ model $\{\phi(\cdot), p(sex \times time)\}$ estimated survival as a constant probability over time with a 0.916 ± 0.008 SE probability of survival independent of sex. Models in which survival was a function of *poison*, $sex \times poison$, and *sex* were equally parsimonious ($\Delta QAIC_c < 2$, Table 6.1).

Table 6.1. Model selection results for Cormack-Jolly-Seber models estimating annual apparent survival (ϕ) and encounter probability (p) as a function of sex, time and poisoning rate for breeding eastern imperial eagles in East Hungary, 2011–2022. Poisoning rate was calculated as the number of poisoned imperial eagles found / number of nesting individuals \times 100. QAICc: Quasi-Akaike Information Criterion corrected for small sample size and adjusted with $\hat{c} = 1.148$, Δ QAICc: difference in the QAICc values of the model and the most-supported model, w_i : model weight, K : model parameter count. Models receiving little support ($w_i < 0.01$) are not shown, except for the general model $\{\phi(\text{sex} \times \text{time}), p(\text{sex} \times \text{time})\}$.

Model	QAIC _c	Δ QAIC _c	w_i	K	Deviance
$\{\phi(\cdot), p(\text{sex} \times \text{time})\}$	3250.20	0.00	0.268	23	1042.56
$\{\phi(\text{poison}), p(\text{sex} \times \text{time})\}$	3250.90	0.70	0.188	24	1041.19
$\{\phi(\text{sex} \times \text{poison}), p(\text{sex} \times \text{time})\}$	3250.92	0.72	0.187	26	1037.07
$\{\phi(\text{sex}), p(\text{sex} \times \text{time})\}$	3252.09	1.89	0.104	24	1042.39
$\{\phi(\text{sex} + \text{poison}), p(\text{sex} \times \text{time})\}$	3252.77	2.57	0.074	25	1041.00
$\{\phi(\cdot), p(\text{sex} + \text{time})\}$	3253.11	2.91	0.062	13	1065.97
$\{\phi(\text{poison}), p(\text{sex} + \text{time})\}$	3254.15	3.96	0.037	14	1064.97
$\{\phi(\text{sex}), p(\text{sex} + \text{time})\}$	3254.38	4.18	0.033	14	1065.19
$\{\phi(\text{sex} + \text{poison}), p(\text{sex} + \text{time})\}$	3255.42	5.22	0.020	15	1064.19
$\{\phi(\text{sex} \times \text{poison}), p(\text{sex} + \text{time})\}$	3256.21	6.00	0.013	16	1062.94
$\{\phi(\text{sex} \times \text{time}), p(\text{sex} \times \text{time})\}$	3273.28	23.07	0.000	42	1025.93

The poisoning rate (number of poisoned imperial eagles found / number of nesting individuals \times 100) showed two peaks during the studied period, in intervals 2011–2012 and 2018–2019 (**Figure 6.2a**). Based on model $\{\phi(\text{poison}), p(\text{sex} \times \text{time})\}$, poisoning had a negative relationship with survival, resulting in a 0.929 ± 0.013 SE estimated survival for the interval with the lowest poisoning rate (0.46%, 2017–2018) and a 0.884 ± 0.031 SE survival probability for the interval with the highest poisoning rate (4.68%, 2011–2012) (**Figure 6.2b**, black). However, the CI for the coefficient of the poisoning effect overlapped zero ($\beta = -0.127$, 95%CI = -0.333 to +0.080).

Males were estimated to have a lower survival probability than females when survival was assumed to be sex-dependent but constant in time (model $\{\phi(\text{sex}), p(\text{sex} \times \text{time})\}$). However, this difference was small, and the CI for the coefficient of the sex effect overlapped zero (males: 0.909 ± 0.018 SE, females: 0.918 ± 0.009 SE, $\beta = +0.106$, 95%CI = -0.392 to 0.605). This model received somewhat less support ($w_i = 0.104$) than model $\{\phi(\text{sex} \times \text{poison}), p(\text{sex} \times \text{time})\}$ ($w_i = 0.187$), indicating that the difference in the survival of males and females may be attributed to their survival being affected by poisoning to a different degree. Based on the estimates from this model, only male survival probability was affected significantly by poisoning ($\beta = -0.507$, 95%CI = -0.927 to -0.087) (**Figure 6.2b**, blue), implying that poisoning may have a greater effect on the survival of males compared to females.

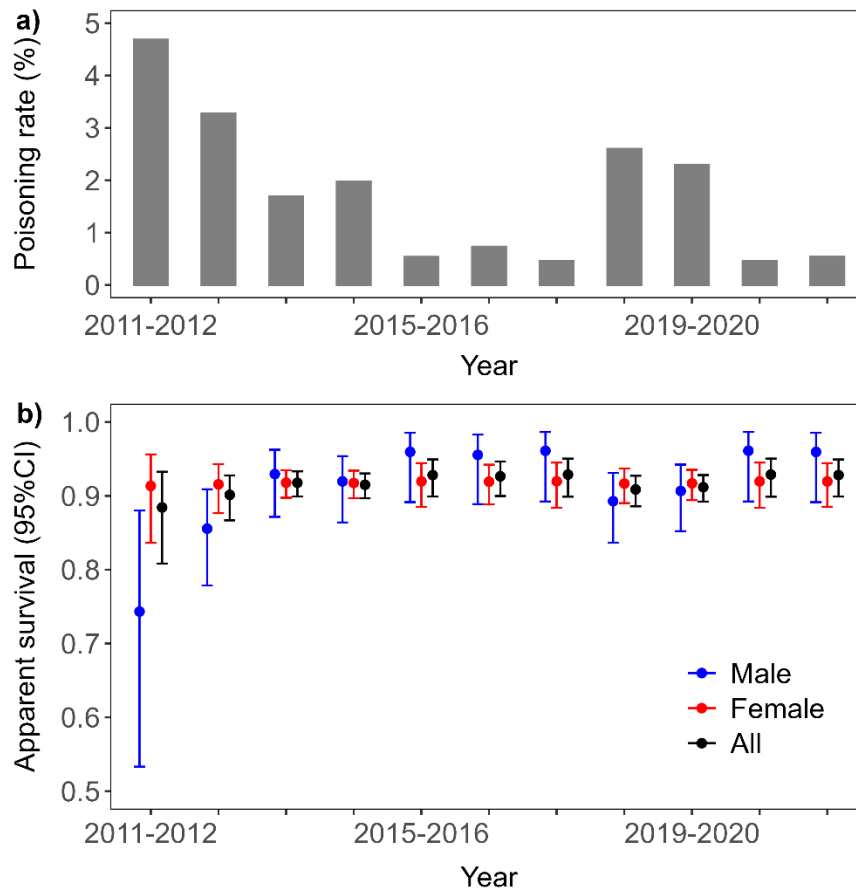


Figure 6.2. Poisoning rates (number of poisoned eastern imperial eagles found / number of nesting eastern imperial eagles \times 100) in East Hungary between 2011 and 2022. Source: BirdCrime Database of MME BirdLife Hungary (a). Estimated apparent survival probabilities ($\phi \pm 95\%$ CI) of breeding eastern imperial eagles from models $\{\phi(\text{sex} \times \text{poison}), p(\text{sex} \times \text{time})\}$ (blue: male, red: female) and $\{\phi(\text{poison}), p(\text{sex} \times \text{time})\}$ (black: all) (b).

Regarding the unconstrained time model $\{\phi(\text{sex} \times \text{time}), p(\text{sex} \times \text{time})\}$, some of the survival parameters were not estimable (characterised by large SEs or SE = 0.000) due to the high number of parameters compared to the amount of data available. Those that were estimable were mostly in agreement with the estimates from the $\phi(\text{sex} \times \text{poison})$ model, as the estimated female survivals showed no clear relationship with poisoning and the male estimates were lower in the two years with the highest poisoning rates. The only interval when the estimates of the two models were clearly different is 2017–2018, when the unconstrained model estimated low male survival despite a low poisoning rate (Table A2).

Model-averaged estimates of apparent survival were 0.929 ± 0.024 SE for males and 0.921 ± 0.014 SE for females in the interval with the lowest poisoning rate (0.46%, 2017–2018), and were 0.865 ± 0.083 SE for males and 0.906 ± 0.027 SE for females in the interval with the highest poisoning rate (4.68%, 2011–2012) (Table A3).

During the \hat{c} sensitivity test, changes in the overdispersion parameter introduced only minor changes to the model selection results which did not affect the main conclusions drawn (Table A4). With no adjustments made for overdispersion ($\hat{c} = 1$), model-averaged estimates of apparent survival at the highest poisoning rate were 0.853 ± 0.088 SE for males and 0.906 ± 0.027 SE for females (Table A5). In the case of $\hat{c} = 1.3$, these estimates were 0.873 ± 0.078 SE for males and 0.906 ± 0.028 SE for females (Table A6).

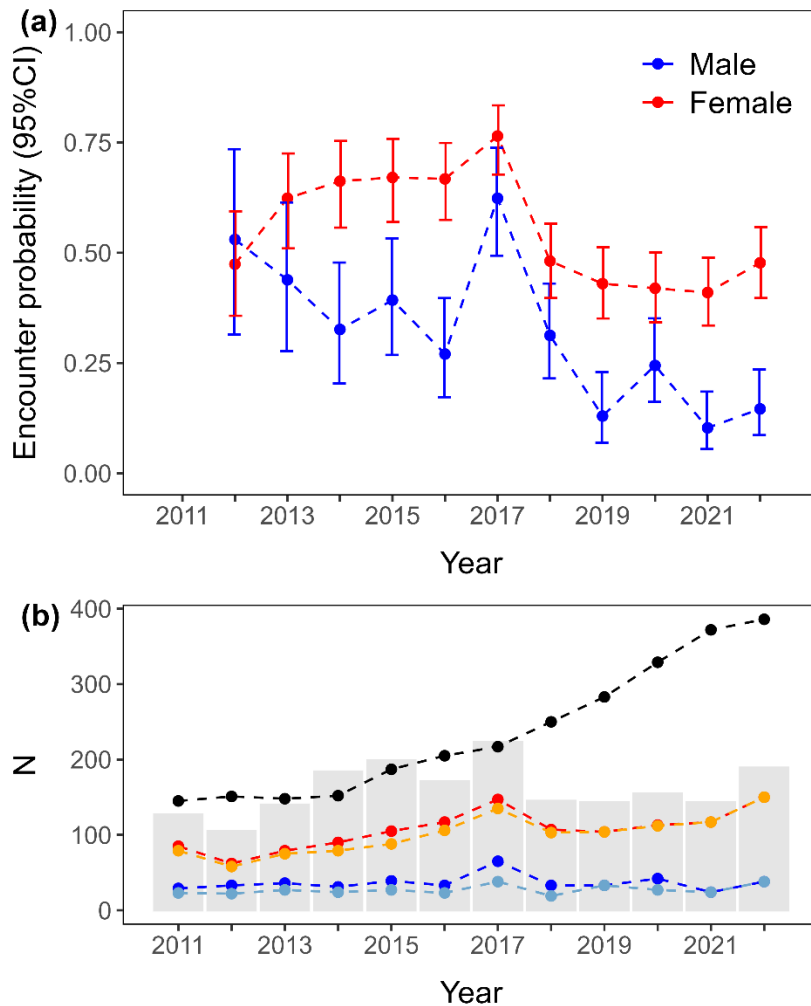


Figure 6.3. Estimates of yearly encounter probabilities (p) from model $\{\phi(\cdot), p(\text{sex} \times \text{time})\}$ for breeding male (blue) and female (red) eastern imperial eagles in East Hungary, 2011–2022 (a).

Sample sizes of the mark-recapture analysis on breeding eastern imperial eagles in East Hungary, 2011–2022 (b): number of nesting pairs (black), number of males identified (all presences: dark blue; only direct presences from shed feathers: light blue), number of females identified (all presences: red; only direct presences from shed feathers: orange) and number of feathers (shed and chick feathers) genotyped (grey bars).

Estimated encounter probabilities were lower for males than females in all models where encounter probability was a function of sex. Estimates of encounter probabilities from the most-supported model $\{\phi(\cdot), p(\text{sex} \times \text{time})\}$ ranged from 0.103 ± 0.032 SE (in 2021) to 0.624 ± 0.064 SE (in 2017) for males and from 0.410 ± 0.040 SE (in 2021) to 0.765 ± 0.040 SE (in 2017) for females (Figure 6.3a). This result generally coincides with the percentage of nesting individuals identified each year, which were lower for males compared to females in all years and showed variation across the years, ranging from 6.50% (in 2021) to 30.0% (in 2017) for males and from 31.5% (in 2021) to 67.7% (in 2017) for females (Figure 6.3b). Values of yearly sampling effort (number of genotyped feathers / number of nesting individuals) showed an average of 0.379 ± 0.136 SD and ranged from 0.192 (in 2021) to 0.605 (in 2015) (Figure 6.3b). Models where encounter probability was constrained as a function of this effort covariate, despite having fewer parameters, received substantially less support than fully time-dependent models ($\Sigma W_{\text{time}} / \Sigma W_{\text{effort}} = 0.987 / 0.007 = 141$), which implies that the number of genotyped feathers was not an adequate predictor of encounter probability.

6.4 Discussion

We estimated annual apparent survival probabilities for breeding eastern imperial eagles in the East Hungarian population using a mark-recapture method based on genetic identification. Although the estimated survival probabilities are only apparent by definition, as the CJS model does not separate actual survival and emigration from the sampling area [140], we presume that since breeding imperial eagles exhibit high territory fidelity [28, 29], the estimated apparent survivals are good proxies to the actual survival probabilities. The previously reported high territory fidelity of breeding imperial eagles is also supported by our data, as over the 12 years of our study, only 9 males (4% of all males studied) and 44 females (9% of all females studied) were identified from more than one territory. Such encounters correspond to less than 4% of all the 1276 presences detected and, in most cases, birds moved only a short distance to the neighbouring territory, similarly to the observations of Vili et al. [29].

The East Hungarian population we studied is the largest in the western range of the imperial eagle's distribution, and therefore, investigating its demography plays a crucial role in preserving this species in Europe. Adult survival is often considered the most important demographic parameter for long-lived species [40, 41, 106], thus, the results of this study are essential for a future viability analysis for this population [13]. Our estimates refer to the period 2011–2022, which was characterised by variable levels of poisoning and the intensive conservation of the species in the context of the Helicon

LIFE (2012–2016, LIFE10NAT/HU/019) and PannonEagle LIFE (2017–2023, LIFE15 NAT/HU/000902) projects. We estimated >90% annual survival on average, a typical estimate for a large raptor such as the imperial eagle [42]. However, our estimates were lower for the first years of the study when poisoning rates were higher. These results agreed with the pattern in the population growth curve of imperial eagles during this period: a plateau was detected in the number of nesting pairs between 2011 and 2014 instead of the exponential growth observed before and after this period ([22], also seen in [Figure 6.3b](#)), implying that the high poisoning rates in 2011–2013 had major effects on population growth. Moreover, our data also provided a moderate evidence for the sex-dependent effect of poisoning on adult survival. The estimated negative effect of poisoning on survival was stronger in males than in females. Therefore, poisoning may lead to a female-biased adult sex ratio (ASR) in this population. This result implies that due to the strict monogamy in this species [28], the number of males may be a limiting factor to the growth of this population. A female-biased ASR can also affect the evolution of reproductive traits like age-to-maturation [147] and may result in increased competition and therefore elevated mortality also in females, which in turn could have adverse effects on population viability [148, 149]. To our knowledge, no direct evidence of sex-difference in age-to-maturation is available in the imperial eagle, but observations of a higher number of immature males than females in the breeding populations suggest that males may reach maturity at an earlier age than females ([150, 151], Horváth's personal communication). Similar observations were also made for the Spanish imperial eagle [152, 153]. An earlier maturation of males could mitigate the negative effect of male-biased mortality on population growth. However, it is important to note, that a higher proportion of immature males in the breeding population may not necessarily mean that males reach maturity sooner than females but could also be the outcome of male-biased adult mortality.

The annual survival of breeding imperial eagles was estimated to be 91.6% on average. This survival estimate is close to the one estimated for the Bulgarian population based on an integrated population model (92.4%, [104]). However, previous turnover-based survival estimates for the imperial eagle were considerably lower: 84% for the population in Kazakhstan [28] and a maximum of 72.3% annual survival rate for females in this East Hungarian population between 1997–2006, with no estimate for males [29]. The 72.3% estimate of Vili et al. [29] is exceptionally low even compared to estimates obtained for the imperial eagle's sister species, the Spanish imperial eagle (92.7–95.3%, [154]; 91.5%, [111]) and for other *Aquila* species (Bonelli's eagle *Aquila fasciata*: 83.9–96.1%,

[155]; 87.0%, [100]; golden eagle *Aquila chrysaetos*: 91.0%, [102]; 90.0%, [156]; 90.5% [157]; 93.0%, [158]).

In the following, we discuss whether poisoning could explain the previously estimated exceptionally low breeder survival rate for the Hungarian population or if some other factors might be responsible. In Hungary, the first two carcasses of illegally poisoned imperial eagles were recovered in 2005, and poisoning has been considered the leading anthropogenic mortality cause of imperial eagles ever since [11]. However, we suppose that poisoning was also present in earlier years, as suggested by the two reported cases of alleged poisoning in 1980–2000 [11]. Only one poisoning rate estimate is available for the time range of the study of Vili et al. [29]. This poisoning rate calculated for 2005–2006 is only 2.74%, which is very low for the estimated female survival rate of 72.3%. Our models predicted much higher survival rates for this relatively low poisoning rate: a 91.6% annual survival for females from the $\phi(\text{sex} \times \text{poison})$ model and a 90.7% annual survival on average for both sexes from the $\phi(\text{poison})$ model.

Reasons behind these seemingly contradicting results can include: (1) actual poisoning rates may have been higher than detected for the previous period, with a more substantial effect on the survival of females than the one reported here; (2) other factors than poisoning may have contributed to this very low survival rate; or (3) methodological differences between the two studies.

Firstly, many poisoning incidents could have remained undiscovered between 1997 and 2006 since no organised poison searches were conducted at the time, and carcasses were rarely tested for poison compounds (Deák's personal communication). Therefore, actual poisoning rates may have been much higher than detected. This is also implied by the high poisoning rates observed in the following years when the monitoring of poisoning was already in focus after the recovery of two poisoned imperial eagles in 2005 [11]. In addition, the survival of females may react to poisoning detectably only above a specific rate. One plausible explanation is that the frequent turnover of males at higher levels of poisoning could introduce a striking effect on the survival of females. This hypothesis is based on studies conducted on other monogamous bird species, which revealed that mate change can lead to a lower apparent survival [159, 160], even in long-lived species [161].

Secondly, factors other than poisoning may have also caused the previously low survival of females. The frequency of electrocution, the second most important mortality factor of imperial eagles, remained more or less constant over the years [2] and thus cannot explain the differences in survival. Another possible factor could be the change in habitat.

During the 1970s, imperial eagles in Hungary resided almost exclusively in the North Hungarian Mountains [36]. These lower-quality mountainous habitats served as a refuge for the species since lower human population density and closed vegetation in these areas ensured a lower risk of persecution [11]. However, the population has expanded significantly over the decades [22], and a larger proportion now resides in lowland areas. These lowland areas support higher fledging success due to better foraging opportunities than the previously occupied mountainous areas [133] and may also promote higher survival in breeders.

Lastly, methodological differences may have also contributed to the difference between our estimate and that of Vili et al. [29]. The latter was based on turnover rate (annual % of breeding individuals replaced by new breeding individuals), which does not account for imperfect detectability, while mark-recapture does. Consequently, breeding dispersal could have introduced a negative bias in the survival estimate of Vili et al. [29]. However, considering the high territory fidelity exhibited by this species [28, 29], dispersal only is unlikely to have caused the large difference in the estimates. Additionally, the estimate of Vili et al. [29] was based on four times fewer individuals than our study and, therefore, carries more uncertainty.

Since Vili et al. [29] only gave estimates for females, our study is the first to explore sex differences in survival in this population of imperial eagles. Models featuring the sex-dependence of survival indicated lower male survival compared to females mainly attributable to the lower survival probabilities of males in years of high poisoning, especially in the first interval. There was only one interval where male survival, in contrast to the low poisoning rate, was estimated to be low by the unconstrained time model. This contradiction may be explained by the imperfect detection of poisoning events or by the increase of another mortality factor in this interval.

A possible mortality factor that may show yearly fluctuations similarly to the poisoning rate and could, therefore, interact with the effect of poisoning on breeder survival, is food supply. Due to their body size dimorphism, food shortages may affect males and females differently [123]. Therefore, prey availability may be enhancing or counteracting the possible sex-dependent effect of poisoning. The latter might explain why models with the sex-dependent effect of poisoning were not better supported.

Another reason why the model selection may not have strongly supported the relationship between poisoning and survival is that in most years of the study, only a relatively low level of poisoning was observed. The negative effect of poisoning on survival may only be prominent at higher rates of poisoning. This is also suggested by

the estimated survival probabilities (**Figure 6.2b**), as in years with low poisoning rates, the CIs of male and female estimates largely overlap, and the sex difference in survival only appears to be relevant in the first intervals, when poisoning rates were much higher.

In contrast to our results of lower male survival, a previous study on imperial eagles implied higher survival for males in Kazakhstan [28] and in the case of the Spanish imperial eagle, both female-biased mortality [124] and no difference in the survival of the sexes have been reported [111]. In raptors, sex-dependent survival can be expected because of behavioural dimorphism [42], through which different environmental factors, including human persecution, influence the sexes differently [100, 125]. Our results suggest this may also be true for the imperial eagle. In spring, females spend most of their time at the nest incubating and males are the ones foraging for food [22, 33]. This period of primarily male foraging coincides with the reported annual peak of poisoning activity from February to April [43]. This was also true in the first interval of our study, when the difference between male and female survival was estimated to be the highest and 64% of the poisoned imperial eagles were recovered between the end of February and the end of April. We hypothesise that this temporal pattern of poisoning activity, coupled with the mentioned behavioural dimorphism, could explain why males may be more at risk from poisoning than females. Moreover, although males could bring back poisoned food to the nest for the females, their quick death after exposure to the poison might prevent them from doing so. During our study, the most commonly used pesticide for the intentional killing of raptors in Hungary was carbofuran [43], which can lead to death in minutes following its ingestion [162, 163]. Therefore, foraging males have only a slight chance of bringing poisoned baits back to the nest as they are very likely to die on site, explaining why they may fall victim to poisoning more frequently than their mates. To further investigate whether breeding males are more threatened by poisoning than females, assessing the age and sex of poisoned imperial eagles would be required.

This previously mentioned behavioural dimorphism also resulted in a smaller sample size and smaller encounter probabilities for males compared to females, since the shed feathers of males could be recovered with substantially less success around the nest. Due to this, our survival estimates for males are less precise than for females. This was especially true for 2011–2012, which was not only the first interval of the study but also had a much higher poisoning rate than the average. Both factors contributed to the fact that less data was available for the estimates for this period. Thus, the uncertainty of these estimates was greater than that of later years.

To obtain more encounter data, especially on males, we also used parentage analysis to infer the presence of breeders. Supplementing our dataset with these indirect presences was made possible by the high territory and mate fidelity of the species. However, this method could have introduced heterogeneity to encounter probabilities since indirect presences could only be obtained of those birds that had been identified previously at least once and whose mate was also identified in the year in question. Additionally, indirect presences could have also introduced upward bias to our survival estimates. This would be the case if the probability of obtaining an indirect presence of an individual was positively correlated with its survival. This can happen in two ways: (i) since indirect presences can only be obtained of birds which are identified in at least one other year, there is a higher chance to obtain indirect presences of individuals which live longer; (ii) since indirect presences are determined through parentage analysis, more data could be obtained on those birds that successfully produced offspring over several years, meaning that if breeding success is positively correlated with survival probability (see for example [164]), the probability of obtaining an indirect presence was higher for birds with better survival. In both cases, birds with better survival would be overrepresented in our dataset. This would be especially true for males, considering that about 25% of the male encounter data were indirect presences. Nevertheless, we found no evidence of indirect presences violating the assumptions of the CJS model based on the goodness-of-fit tests: overdispersion was estimated to be of an acceptable value ($\hat{c} = 1.148$), which was even smaller than the one estimated for the dataset without indirect presences ($\hat{c} = 1.200$). Thus, indirect presences could serve as a valuable tool in cases when encounter data is otherwise scarce, a situation typical for raptors [42].

RELEASE goodness-of-fit tests revealed a slight violation of the assumption of homogeneous survival probabilities in the case of females, both with and without indirect presences: females identified for the first time in 2012, 2014 and 2017 were less likely to be encountered later than females that have been encountered before. Assuming that birds that are identified for the first time in a specific year are mainly young (3-4 calendar year) birds entering the breeding stage, an explanation for this pattern could be the lower apparent survival of younger breeders. Reasons behind this could include a lower actual survival for younger individuals or a lower territory fidelity, leading to their dispersal to another breeding site outside of our study area after unsuccessful breeding attempts [165, 166]. There are two options to investigate if the survival of younger breeders is lower compared to adults. The first is to apply a time-since-marking model, but it requires that all the birds are young when identified for the first time, which is not a valid assumption in this case. The second option is the age-dependent model; however, we

know the age of only a few birds in this study. Continued observation of breeding individuals and incorporation of ringing and satellite-tracking data of young breeders could facilitate answering this question in the future.

Our study provided the first estimates of sex-dependent survival for imperial eagles in Hungary, a population of high conservation value due to its large size and peripheral placement in the distribution of the species. We also investigated the relationship between survival and poisoning rates in imperial eagles for the first time. In conclusion, the annual survival probability of breeding imperial eagles in East Hungary is high despite current anthropogenic threats in the country. The 26% increase in the survival estimate compared to the period of 1997–2006 implies that the conservation measures have contributed to the increase in survival of breeding birds. However, we cannot exclude methodological differences as explanations for the difference between previous and current estimates. Furthermore, while we found no definite evidence for the effect of poisoning and sex on adult survival, our results alert that male survival might be lower and more sensitive to poisoning than female survival. If that is the case, it would be important from both a conservational and evolutionary perspective, as sex-biased adult mortality is considered the main driver of biased adult sex ratios in birds, which in turn influence parental roles and mating competition and can affect population viability [147–149]. Furthermore, in a species, such as the imperial eagle, where food provisioning during breeding is mostly dependent on the male and experienced adults have better hunting capacity [153], male-biased mortality can also have a direct impact on productivity. While the scarcity of male data makes estimates of male survival less precise than that of females, our method of indirectly assessing the presence of breeders through parentage analysis greatly contributed to the data on males. To obtain a deeper understanding of the demography of the population, more precise estimates of breeding male survival would be beneficial in the future. Furthermore, the survival rates of the floater age groups and the young breeders are unknown; a future task is to estimate these from ringing and satellite-tracking data. Moreover, incorporating these results into a population viability analysis could further facilitate our understanding of the effect of poisoning on survival and its consequences on population growth in this protected, long-lived raptor.

7 SEX-BIASED AND DENSITY-DEPENDENT NATAL DISPERSAL IN A HIGHLY MOBILE BUT PHILOPATRIC RAPTOR

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Minor modifications of the text and figures were implemented for this thesis.

7.1 Introduction

Natal dispersal is defined as the movement of an individual from its place of birth to the site where it first attempts to breed [167]. Since it directly influences the distribution patterns of individuals, it has major implications for population dynamics, genetic structure, and colonisation potential, making its study important from both an ecological and a conservation perspective [44, 45, 168–170].

Individual natal dispersal strategies are influenced by numerous external or ‘condition-dependent’ factors (such as conspecific density or habitat quality), as well as internal or ‘phenotype-dependent’ factors (including sex, age, and body condition) [171–176]. These factors may show varying impacts across dispersal stages and influence different dispersal metrics (propensity, distance, timing, and duration) in distinct ways [173, 177–180]. Consequently, natal dispersal strategy is a complex, plastic trait that is difficult to study [171, 173, 178].

Sex bias in dispersal arises when the selective forces operating on dispersal traits are unbalanced between the sexes [181]. Classical hypotheses suggest that the mating system is the main determinant of the direction of sex bias in dispersal [167, 181–183]. However, it has been proposed that rather than the mating system itself, associated traits like sexual dimorphism in morphology, territoriality, and parental care drive the evolution of sex-biased dispersal [184], and social traits like kin cooperation can also influence dispersal patterns [185]. In raptors and birds in general, females are more likely to disperse from the natal population and typically exhibit longer dispersal distances than males [167, 182, 184, 186]. This pattern has been linked to their typically monogamous mating system, where males acquire and defend the territory [167]. Since these tasks may be accomplished more successfully by being familiar with the area, males may benefit more from dispersing shorter distances [167]. As a response, females would have to disperse further to avoid inbreeding [181, 183]. While the classical theories are widely recognised as explanations for sex-biased natal dispersal patterns, recent studies

emphasise that the mechanisms underlying the sex bias in natal dispersal may be more complex and are still not well understood [184, 187, 188].

The relationship between dispersal and conspecific density is even less understood, with the driving factors appearing complex and studies providing inconsistent results on both the direction and the magnitude of density dependence, even within taxonomic groups [189]. For instance, in raptors, both positive and negative density dependence, as well as no density dependence, have been observed [174, 190, 191]. In the case of positive density dependence, individuals are more likely to disperse or disperse longer distances with increasing density, most probably to avoid increased intraspecific competition [189, 192]. On the other hand, negative density dependence occurs when increasing density results in a lower frequency of dispersal or shorter dispersal distances. This may arise if the presence of conspecifics indicates an increased opportunity for mating or serves as a cue for high-quality habitats [192–194]. The results on the density dependence of dispersal are shown to be significantly affected by the way density is measured and the dispersal metrics examined [189, 195]. Furthermore, most studies investigate the relationship between dispersal and density on a population level, as in migration between populations with different densities, and few examine within-population dispersal in regard to local density patterns [189, 192]. Studies also generally focus on how natal site density affects dispersal metrics, whereas the correlation between natal and breeding site densities is rarely examined (but see [53]).

Studying natal dispersal is particularly challenging in the case of raptors [44, 45]. Firstly, they are typically elusive species with small population sizes, making them difficult to capture and mark in adequate numbers by conventional techniques of ringing, wing tags, or GPS tracking [42]. An alternative marking method is genetic identification, when individuals are first DNA profiled as chicks, and later their genotypes are matched to the genotypes of breeding individuals to detect dispersal movements [53, 131]. While the chicks need to be handled to obtain a DNA sample (usually blood or plucked feathers), birds in the breeding stage can be identified noninvasively by DNA profiling their shed feathers collected at their nest site, allowing for difficult-to-capture species to be sampled in great numbers [29, 46, 53]. Moreover, individuals can be sexed with high certainty from their DNA, which is an important aspect for raptors that usually display no plumage dimorphism, and size dimorphism is often not large enough to reliably differentiate the sexes [121]. Another issue is that raptors are highly mobile, meaning that long-distance dispersal events can often remain undetected if the study area is limited or detection probability decreases with distance from the core study area, which is usually the case when birds are marked with rings or wing tags [196]. GPS tracking overcomes this issue,

but it is a more costly method, usually allowing for much smaller sample sizes [197]. The difficulty of studying raptors is further compounded by the fact that many species have delayed maturity, with a floater period of several years [121]. This necessitates long-term studies to investigate natal dispersal, which require a significant amount of time, finances, and human effort. Delayed maturity also means that equipment failure of GPS trackers or the loss of colour rings and wing tags may happen sooner than the birds settle for breeding, further decreasing the number of individuals for which natal dispersal distances (NDDs) can be recorded [197]. Consequently, using multiple marking methods may be beneficial when studying natal dispersal in raptors.

Here we studied natal dispersal in the eastern imperial eagle (*Aquila heliaca*, hereafter: imperial eagle), in the Pannonian breeding population. This large-sized, long-lived raptor species prefers the forest-steppe habitats of Eurasia, where patches of trees provide nesting places next to open grasslands suitable for foraging [24, 34]. Its distribution spans from Austria in the west to the Trans Baikal region of Russia in the east and from the Southern-Ural Mountains of Russia in the north to Turkey in the south [6–8]. The small, isolated, western populations in Central Europe, the Balkans, Turkey and South Caucasus are mostly sedentary, while the large eastern populations are migrants, wintering in the Middle East, South-East Asia, and North-East Africa [6, 7]. The global population of the imperial eagle is estimated at less than 10,000 mature individuals, making it a globally vulnerable species [1]. About two-thirds of its global population is found in Russia and Kazakhstan [8]. A significant number of breeding pairs form a compact, geographically isolated population in the Pannonian Region, in the westernmost part of the species' distribution [7, 8].

There is currently only scarce published data on NDDs in the imperial eagle [25, 27], with no substantial data on sex-specific, nor any data on density-dependent patterns. Improved knowledge of these topics would greatly benefit the conservation of this species by offering insights into its colonisation potential [198] and the extent of genetic exchange among its geographically scattered populations. Exploring the relationship between dispersal and density could facilitate our understanding of previously observed colonisation patterns. Furthermore, identifying any sex-specific patterns could shed light on potential spatial variation in sex ratios, an important aspect considering previous evidence on sex-dependent survival rates [199]. Additionally, knowledge of natal dispersal and associated migration rates would enhance the accuracy of models predicting future population trajectories for this vulnerable species [200].

We investigated natal dispersal patterns using recapture data obtained through colour-ringing, GPS tracking, and DNA profiling. We studied the NDD (i.e. the distance between the natal nest and the first documented breeding location) of birds in relation to their sex and density at the natal and breeding sites. We hypothesised that females would disperse further, as this aligns with the general pattern observed in birds. Furthermore, we examined the relationship between NDD and natal density and investigated whether the eagles dispersed to higher or lower density areas compared to their natal site. We also examined the directional patterns of natal dispersal movements.

7.2 Methods

7.2.1 Study population

We studied the natal dispersal of imperial eagles in the Pannonian Region between 2011 and 2024. The size of this nonmigratory population was estimated at 356–381 nesting pairs as of 2019 [8], with 276 pairs nesting in East Hungary and 11 in West Hungary [31], 45–50 in East Slovakia [201], 21 in West Slovakia [202], 20–23 in East Austria [39] and 6–8 in the Southern Czech Republic [39], 3 pairs in North Serbia and one in West Romania [22]. The Eastern (East Hungary, East Slovakia, West Romania and North Serbia) and Western (West Hungary, West Slovakia, East Austria and the Southern Czech Republic) breeding nuclei of the population are separated by 100 km, with mild genetic differentiation between these two subpopulations [12].

In the 1980s, the imperial eagle occupied almost exclusively mountainous forest habitats (200–700 m a.s.l.) in the Pannonian Region, where it could find refuge from the intensive persecution occurring in the agricultural lowlands [34, 36]. Following the decrease in persecution levels, the number of imperial eagles has increased exponentially, and their distribution has expanded first to the foothill plains, then to the nearby lowlands [11]. In Hungary, this population expansion occurred mainly in a southern direction, from the North Hungarian Mountains towards the Hungarian Great Plain [11]. Now, the vast majority of pairs in the Pannonian population breed in lowland agricultural areas (80–120 m a.s.l.) [22, 39, 202]. In the lowlands, birds usually forage within a 3–8 km radius of the nest on agricultural fields and grasslands while in the mountainous habitats, foraging sites can be as far as 10–15 km from the nesting site [34]. Between 2011 and 2014, the population in Hungary went through a stagnating phase, most probably due to the high rates of poisoning observed during this time [22, 199]. From 2015, after poisoning levels have decreased, the population has again started growing exponentially and has tripled its size by 2022 [22, 43, 199].

Most imperial eagles start breeding in their third or fourth calendar year (abbreviated as 'cy', where the first calendar year is the year of hatching) until which they act as floaters, often exploring areas hundreds or thousands of kilometres from their natal place [22, 25]. Despite their high mobility, most floater movements are restricted to their natal population, suggesting high natal philopatry [25, 26]. Previous studies based on genetic identification also indicate high breeding philopatry [28, 29, 199]. Adult birds display only minor sexual dimorphism in morphology: females are slightly larger than males. However, they have markedly different sex roles during the breeding, as females do most of the incubation while males forage for food [22, 33].

Imperial eagle nesting sites in Hungary have been monitored annually by the Hungarian Imperial Eagle Working Group, which has been operated by MME BirdLife Hungary and the Hungarian national park directorates since 1980. Each year, the locations of nests are recorded (WGS84 coordinate system, five decimal accuracy), along with the breeding success and additional observations on the age and identity of the breeding pair. The monitoring programs were well-organised and had wide coverage during the study period; therefore, the number of known nests is estimated to account for 95% of the total number of nests in the country [11, 31].

7.2.2 Monitoring of natal dispersal

We monitored the natal dispersal of imperial eagle chicks hatched and marked in Hungary between 2011 and 2022 in the frame of the Helicon LIFE (2012–2016, LIFE10NAT/HU/019) and PannonEagle LIFE (2017–2023, LIFE15 NAT/HU/000902) projects. Three types of marking were used for individual identification: ringing (metal ornithological and plastic colour-rings), GPS tracking devices, and DNA profiling. Chicks were either (i) only ringed, (ii) ringed and fitted with a GPS tracker, (iii) ringed and DNA profiled, or (iv) all three types of marking were applied. For molecular sexing, DNA samples were also collected from ringed and GPS-tracked chicks, which were not DNA profiled.

We defined an individual's NDD as the great circle distance in km between its natal nest and the nest where it had its first recorded breeding attempt with successful egg-laying [198]. The first recorded breeding site is most likely the same as or close to the actual first breeding site since breeding dispersal is rare and usually short distance [28, 29, 199].

7.2.3 *Colour-ringing*

Altogether 1660 imperial eagle chicks were ringed between 2011 and 2022 at the age of 4–9 weeks and were tagged with both a metal ornithological ring and a coloured plastic ring (black code on white background).

All ringing and encounter data were obtained between June 2011 and April 2024 from the Hungarian Bird Ringing Databank operated by the Hungarian Bird Ringing Centre of MME BirdLife Hungary. The coordinates of hatching and encounter locations were recorded in the WGS84 projection system with a five-decimal-place accuracy.

To differentiate actual natal dispersal movement (when the encounter location designates a breeding site) from floater movement (explorative movement of immature birds between areas with no breeding attempt), we only considered movements where (i) the encountered bird was at least the age of 3cy (earliest recorded age of breeding in imperial eagles, [39]), (ii) the encounter location could be assigned to a known nesting site, (iii) observations on the age or identity of breeding birds at the nest site confirmed the breeding status, and (iv) egg-laying occurred at the designated breeding site in the year of encounter.

7.2.4 *GPS tracking*

In addition to ringing, between 2011 and 2022, 71 chicks at the age of 7–10 weeks were also equipped with solar-powered GPS backpack transmitters (Microwave Telemetry, Ecotone Telemetry or Ornitela Telemetry). The mean body weight of the tagged chicks was 2.9 kg, and the weight of the transmitter applied (29–70 g) was less than 2% of the birds' body weight [203]. Data registered by the transmitters were stored in the Movebank.org online database, within different studies of MME BirdLife Hungary.

We used GPS tracking data recorded until 30 September 2024. To decide whether a GPS-tracked bird has entered the breeding stage, we examined the movement patterns of birds during each breeding season starting from their third calendar year. Incubating females exhibit a specific movement pattern, where most of their movements are restricted to the nest site, with occasional movements to other parts of the territory. Males also display restricted movement concentrated around the nest site when breeding. Besides, the breeding attempts of GPS-tracked birds were monitored in the field by the Hungarian Imperial Eagle Working Group. Similarly to ringed birds, we only recorded the movement of tracked birds as natal dispersal if at least egg-laying occurred at the breeding site.

7.2.5 DNA profiling

We used genetic monitoring as a third method of studying natal dispersal: we searched for breeding birds and chicks with matching microsatellite genotypes. We analysed the DNA samples (armpit feathers) of 631 chicks ringed between 2011 and 2018, along with the DNA samples of breeding birds (shed feathers found under the nests of breeding pairs) collected from 336 territories between 2013 and 2022. Chicks and breeding adults were sampled in different timeframes, as we expected the chicks hatched in 2011 to start breeding in 2013 (their third calendar year) at the earliest. While we could not verify in all cases through parentage analysis that the shed feathers collected in a given year belonged to the breeding pair, feathers at the nest site almost exclusively originate from the resident pair (Pásztor-Kovács's personal communication), and only feathers shed in the same year when collected are in a suitable condition for DNA analysis [81].

Laboratory procedures of sample preparation, DNA extraction, molecular sexing, and individual identification using the 9-loci microsatellite set are described in detail in [199] ([SECTION 6](#)). We used a two-step method to find matches between breeding adults and chicks and applied strict rules of identity to reduce the probability of false matches. In the first screening, we sexed both the chicks and the breeding birds (primers CHD-i16F/CHD-i16R, [136]) and individually identified them using nine microsatellite loci: Aa02, Aa35, Aa36, Aa39, Aa43 [79]; Hal04, Hal10 [85]; IEAAG09, IEAAG11 [78]. During this first screening, we only accepted a chick and a breeding bird as the same individual if they had matching genotypes on all nine loci and matching sex. To increase the sample size without compromising the reliability of matches, we did a second screening for those pairs of chicks and breeding adults that were genotyped successfully only on eight or seven loci but showed a full match on these loci along with matching sex. These individuals were genotyped on an additional eight loci: Aa26, Aa41, Aa43, Aa53, Aa57 [79]; AQJ10, AQJ22 [83]; AQJ120 [84]. For these loci, we used the same PCR mix described in [199] and a modified version of the PCR program published in [79]: 95°C for 2 min; 17 cycles with a touchdown scheme: denaturation at 95°C for 30 s, annealing at 66°C–50°C for 30 s (–1°C/cycle), elongation at 72°C for 30 s; 21 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 30 s, and elongation at 72°C for 30 s; final elongation at 72°C for 7 min [204]. Similarly to the first screening, we only accepted pairs of chicks and breeding adults as identical if they showed a full match on all the genotyped loci.

To assess the reliability of the marker sets for individual identification, we calculated the probability of identity values corrected for the presence of siblings in the population (PI_{SIB} , [49]) for both the 9- and 17-loci sets using GenAlEx v.6.503 [93].

7.2.6 *Variables of natal dispersal*

We carried out spatial computations in R v.4.2.1. [144] with the *sf* package (v. 1.0-12, [205]).

We aimed to define density as a continuous variable which describes the local density of active nests. As a measure, we used the reciprocal area ($1/\text{km}^2$) of the truncated Thiessen (Voronoi) polygon [206, 207] created around each active nest. Polygons were truncated at a maximum radius of 12.7 km, the average nearest neighbour distance value calculated for the Hungarian population at a much lower density, with no observable saturation or density-dependent effect (1989–2006, [133]). We only calculated local density for 2011–2022, the period of highly intensive nest monitoring in the frame of the Helicon LIFE and PannonEagle LIFE projects. While we only calculated local density for nests in Hungary, we also accounted for the presence of nests in the neighbouring countries (where monitoring was similarly intensive as in Hungary). When a nest had a Thiessen polygon intersecting the country border, either we corrected its polygon for nests in the neighbouring country (Austrian, Romanian, and Serbian border: nest coordinates provided by M. Schmidt, Z. Hegyeli, and M. Ružić, respectively), or we excluded the nest from density calculations (Slovakian border).

For each individual, we defined breeding density as the local density at their first documented breeding nest in their expected year of recruitment. The definition of the expected year of recruitment was based on the median age when imperial eagles started breeding (4cy, calculated from the GPS-tracked birds in this study). For birds that were first observed breeding at the age of 4cy or younger, the expected year of recruitment was the year when they were first observed breeding. For birds that were first observed breeding older than 4cy, the expected year of recruitment was the first year when the bird was at least 4cy old, the territory had already existed, and it was not known to be occupied by another bird of the same sex.

We defined natal density for each individual as the local density at their natal nest in their expected year of recruitment (current density at the natal site, [53, 193]). We used the same year for both natal and breeding density to account for the changes in population density over the years.

7.2.7 *Statistical analysis*

We used R v.4.2.1. [144] for all statistical analyses. We calculated the 95% confidence intervals for the median NDDs of males and females by inverting the sign test [208] using package *BSDA* (v.1.2.2, [209]).

We used package *lmerTest* (v. 3.1-3, [210]) to fit general linear mixed models.

We tested the change in median local density over the years in a model with *density* (log-transformed) as the response variable, *year* as the explanatory variable (continuous) and *territory ID* as a random intercept.

To investigate the relationships between the natal dispersal variables, we constructed two sets of general linear mixed models.

In the first set of models, *NDD* was the response variable (log-transformed), while the explanatory variables were *sex* (male as the reference category), *natal density* (log-transformed) and its quadratic term (describing a U-shaped pattern, which may occur with simultaneous positive and negative density-dependence, [193]) and *natal period* (two-level factor, where natal years 2012–2014 mark a period of population stagnation and natal years 2015–2018 correspond to population increase). *Natal territory ID* and *natal year* were included in the models as crossed random intercepts to account for the correlation between individuals originating from the same territory and hatching in the same year.

We constructed another set of models with *density difference* ($\log(\text{breeding density}) - \log(\text{natal density})$) as the response variable, *sex* (male as the reference category), *NDD* (log-transformed) and its quadratic term, *natal density* (log-transformed) and its quadratic term, and *natal period* (two-level factor, 2012–2014 as reference) as explanatory variables, and *natal territory ID* and *natal year* as crossed random intercepts. Additionally, we tested the Pearson correlation between *breeding density* and *natal density* for males and females (p-values adjusted with the Bonferroni–Holm method). We also calculated the mode of the breeding density distribution based on kernel density estimates.

Exploratory analysis and model diagnostics revealed that the best fit to the linear models and homogeneity of variance were achieved by log transforming both the response and the explanatory variables (*NDD* and *natal density*). This also allowed for keeping variables consistent among models of *NDD* and *density difference*. We also scaled all continuous explanatory variables. In all cases, we initially built models with two-way interactions between the main effects and excluded insignificant interactions and quadratic terms ($p \geq 0.05$) from the reduced models but retained all main effects irrespective of their significance. We investigated model fit based on visual inspection of residuals, outliers and the normality of the random effects. Multicollinearity between explanatory variables was checked using VIF values (package *car* v.3.1-3, [211]).

We also calculated the direction of natal dispersal movements using the *lwgeom* package (v.0.2-14, [212]). To explore any heterogeneity in the direction of natal dispersal movements, we used Rayleigh tests (package *circular* v.0.5-1, [213]) for both male and female movements and movements in the two periods (natal years 2012–2014 and 2015–2020).

We made the figures using *ggplot2* (v.3.5.1, [145]), *sjPlot* (v.2.8.17, [214]), *ggspatial* (v.1.1.9, [215]), *RColourBrewer* (v.1.1-3, [216]) and *gridExtra* (v.2.3, [217]). We obtained the country polygons via package *rnatuarearth* (v.1.0.1, [218]).

7.3 Results

7.3.1 Colour-ringing data

336 of the 1660 ringed chicks were encountered and identified based on their ring ID at least once until April 2024 (**Figure A1**). Altogether, 468 encounters were recorded, of which 137 were recoveries of dead or injured birds, 2 were live recaptures, and 329 were resightings. In the case of 261 resightings, the birds were identified based on their colour-ring ID, while identification based on the metal ring ID occurred only in 67 cases (presumably when the colour-ring became damaged or lost). Most observations came from Hungary (348) and the neighbouring countries of the Pannonian Region (92). Only 28 encounters were recorded outside of the Pannonian Region (one bird observed in Turkey, one in Italy, one in Poland, one in North Macedonia, one in the Netherlands and one both in Finland and the Netherlands). Of these individuals encountered outside the Pannonian population, only two were at least 3cy, and neither of them was observed in a breeding population. In summary, no imperial eagle ringed in Hungary was observed breeding in another population.

7.3.2 GPS tracking data

Of the 71 GPS-tracked birds, 33 were male, 31 were female, and 7 were of unknown sex (**Figure A1**). Only 34 birds reached the 3cy age of maturation, and the oldest age until an individual was followed was 10cy (after which equipment failure occurred).

7.3.3 DNA profiling data

We DNA profiled 631 chicks (298 males, 300 females, and 33 of unknown sex) hatched between 2011 and 2018 (**Figure A1**). Additionally, we genotyped 1786 shed feathers between 2013 and 2022, which belonged to 510 breeding birds (162 males, 334 females, and 14 of unknown sex) (**Figure A1**). The probability of identity corrected for the

presence of siblings in the population (PI_{SIB}) confirmed that the markers used were reliable for individual identification, even in the case of the smaller, 9-loci marker set ($PI_{SIB} = 3.5 \times 10^{-6}$ for the 17-loci set and $PI_{SIB} = 5.8 \times 10^{-4}$ for the 9-loci set).

7.3.4 Recorded natal dispersal events

Using data from 2011 to 2024, we managed to determine the NDD of 116 imperial eagles hatched between 2012 and 2020: 43 males, 72 females, and one with unknown sex. Most of these natal dispersal events were detected via only one of the three identification methods (68 from DNA profiling, 23 from ringing and 13 from GPS tracking), but some events were detected by multiple methods (six by both DNA profiling and ringing, five by both DNA profiling and GPS tracking and one by both ringing and GPS tracking). We found no contradiction between the matches reported by the three methods.

For GPS-tracked birds, the age at first breeding was precisely known, with a median of 4cy for all birds (range 3cy–6cy, $n = 19$), 4cy for males (range 4cy–6cy, $n = 7$) and 4.5cy for females (range 3cy–6cy, $n = 12$). In contrast, the first detected breeding for ringed and DNA-profiled birds may not have been their actual first breeding. Consequently, age at the first detected breeding was higher for birds surveyed using ringing or DNA profiling data (ringing: median 6cy, range 3cy–11cy, $n = 30$; DNA: median 5cy, range 3cy–9cy, $n = 79$).

GPS tracking data revealed three birds (two females and one male, 2.6%), which started breeding in one of the neighbouring countries, in the western part of Slovakia. Concerning the dispersal between the Eastern and Western parts of the Pannonian population, five birds that hatched in East Hungary (4.3%) dispersed to the Western part (one male, one female and one bird of unknown sex to West Hungary; one male and one female to West Slovakia). No dispersal was detected from West Hungary to the Eastern part of the population ([Figure 7.1](#)).

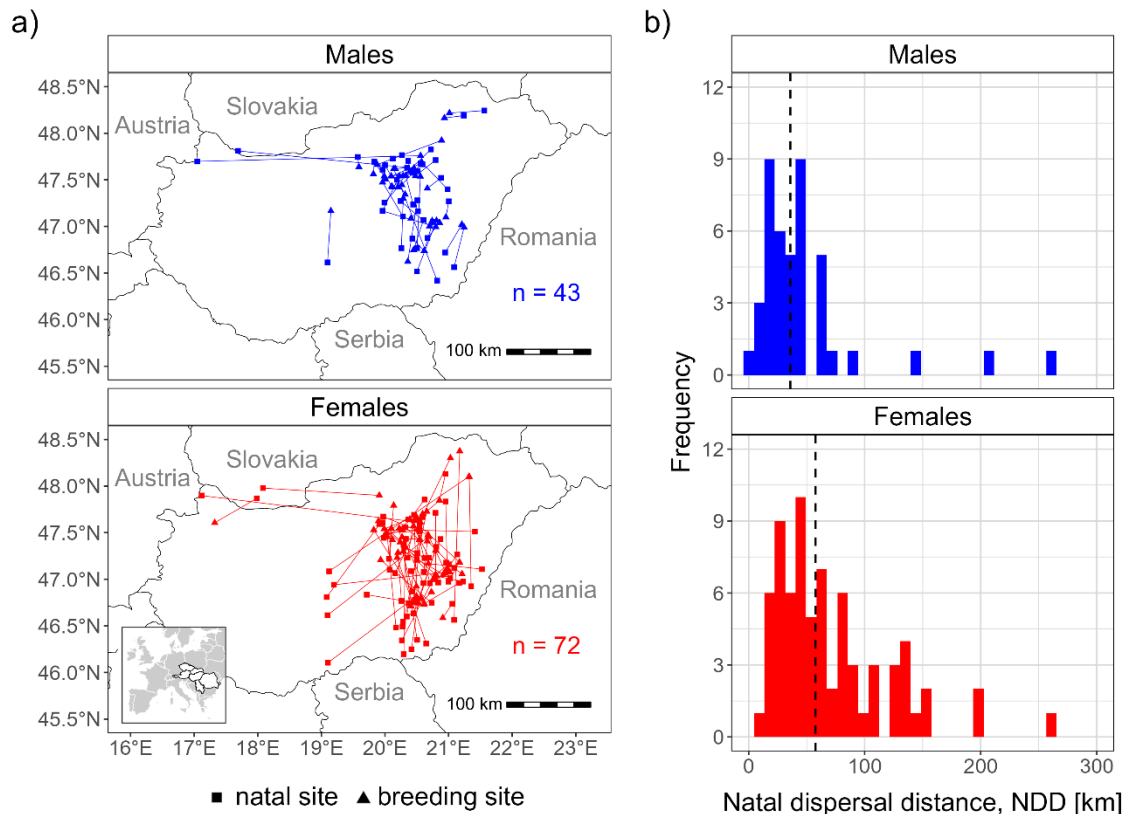


Figure 7.1. Natal dispersal movements (a) and distribution of natal dispersal distances (b) of eastern imperial eagles hatched in Hungary between 2011 and 2022. On (a), squares denote the natal site and triangles the first detected breeding site; on (b) vertical dashed lines indicate the median distances. Inset highlights the countries occupied by the Pannonian population.

7.3.5 Natal dispersal distance in relation to sex, natal density and natal year

The median NDD was 35.9 km for males (95% CI 22.9–45.6 km, range 1.8–262.8 km, $n = 43$) and 57.6 km for females (95% CI 46.4–69.7 km, range 12.3–259.6 km, $n = 72$) (Figure 7.1). Only those birds for which natal density could be determined (39 males and 62 females) were included in the linear mixed models with $\log(NDD)$ as the response variable. Multicollinearity was not a concern ($VIF < 1.5$). NDDs were significantly longer for females than for males (Table 7.1). We found no significant linear or quadratic effect of $\log(natal\ density)$ or $natal\ period$, and none of the two-way interactions were significant.

There was one outlier in the models, a male who settled in the adjacent territory of its natal site (1.8 km) for breeding. We repeated the analysis excluding this record and could draw the same conclusions (Table A7).

Table 7.1. Estimates of the general linear mixed model investigating the relationship of natal dispersal distance (NDD, log-transformed) with sex ('male' as reference), natal density (log-transformed, scaled) and natal period (two-level factor, '2012–2014' as reference) in 39 male and 62 female eastern imperial eagles hatched in Hungary between 2012 and 2018. Natal territory ID and natal year (categorical) were set as crossed random intercepts. Effects in bold were significant ($p < 0.05$).

Response variable: log (NDD)						
Explanatory variables	Estimate	SE	95%CI	df	t-value	p-value
Intercept	3.333	0.165	[3.029, 3.635]	4.87	20.18	< 0.0001
sex (female)	0.546	0.157	[0.224, 0.842]	70.7	3.479	0.0009
log (natal density)	-0.045	0.080	[-0.204, 0.110]	60.1	-0.557	0.5797
natal period (2015 – 2018)	0.274	0.199	[-0.138, 0.642]	3.25	1.379	0.2551
Random effects		SD				
natal territory ID	0.412					
natal year	0.161					
Residual	0.633					

7.3.6 Density difference in relation to sex, natal density and NDD

Median local density calculated for all the active nests increased over the study period from 0.007 km^{-2} in 2011 to 0.014 km^{-2} in 2022 ($\beta = 0.089$, $\text{SE} = 0.002$, $p < 0.001$, [Figure 7.2](#)). The minimum number of recorded territories in a year was 133 in 2011, and the maximum was 380 in 2022.

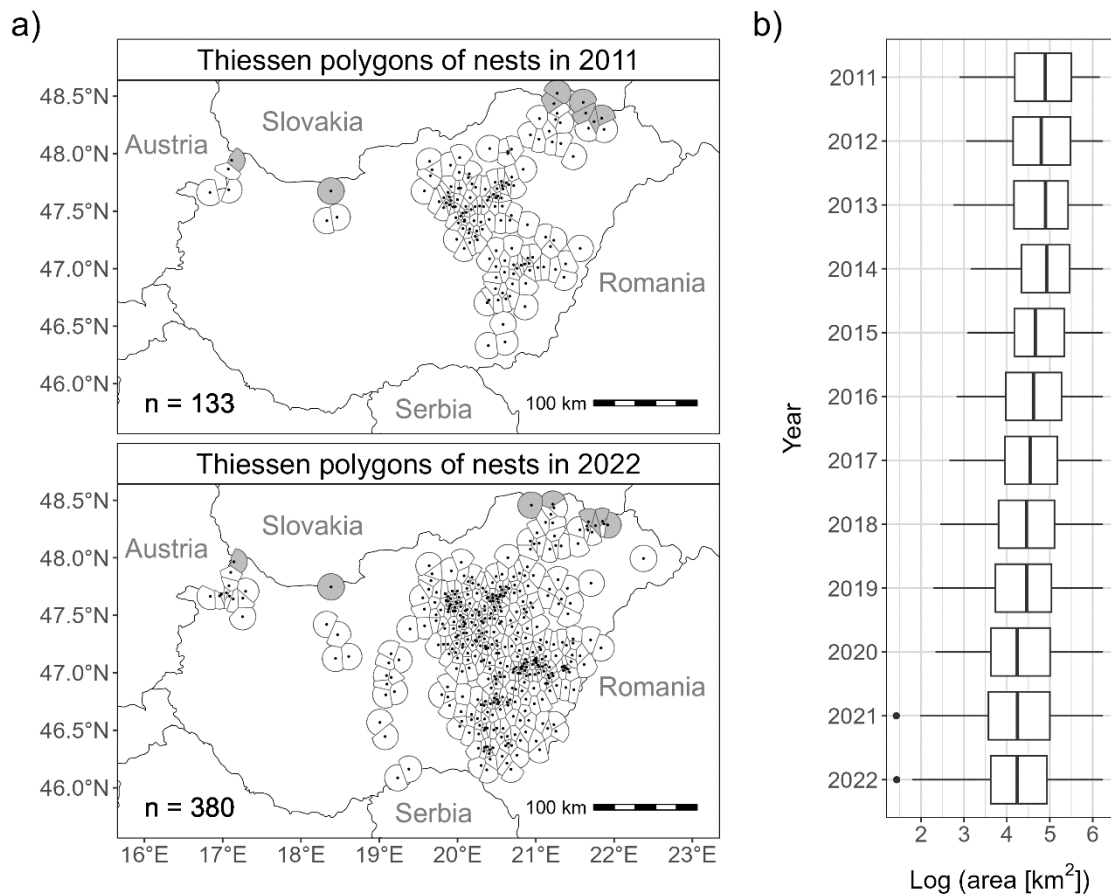


Figure 7.2. Thiessen polygons of eastern imperial eagle nests sampled between 2011 and 2022 in Hungary. Maps show the first and final sampling year, nests are marked with black dots (a). Thiessen polygons were truncated at a maximum radius of 12.7 km. Nests with grey polygons were excluded from the analyses including local density since exact locations of nests in Slovakia were not known. Log transformed area of the Thiessen polygons in each sampled year are shown on (b). The reciprocal of an individual's Thiessen polygon area was used as a measure of local density.

Only those birds for which we could determine both the breeding and the natal density (39 males and 62 females) were included in the Pearson correlation test between natal and breeding density. In the case of males, natal site and breeding site density were significantly positively correlated ($r = 0.455$, $p = 0.0073$), while for females, we found no significant correlation between the two densities ($r = 0.161$, $p = 0.2110$).

We could use the data of the same 39 males and 62 females in the linear mixed models with *density difference* ($\log(\text{breeding density}) - \log(\text{natal density})$) as the response variable. Multicollinearity was not a concern ($VIF < 1.5$). As shown by the intercept of the model, the mean *density difference* was significantly lower than zero (Table 7.2),

indicating that, on average, both males and females dispersed to lower-density territories compared to their natal sites (**Figure 7.3**). Both $\log(\text{natal density})$ and $\log(\text{NDD})$ showed a significant negative relationship with *density difference* (**Table 7.2, Figure 7.3**). We found no significant effect of sex or *natal period*, and neither the quadratic effect of $\log(\text{natal density})$ nor $\log(\text{NDD})$, nor any of the two-way interactions were significant. We repeated the analysis excluding the unusually short dispersal distance of a male, and we obtained similar results (**Table A8**).

The model predicted zero *density difference* at the density value of 0.009 km^{-2} that was very close to the mode of the breeding densities (0.007 km^{-2} , **Figure A2**).

Table 7.2. Estimates of the general linear mixed model investigating the relationship of density difference ($\log(\text{breeding density}) - \log(\text{natal density})$) with sex ('male' as reference), natal density (log-transformed, scaled), natal dispersal distance (NDD, log-transformed, scaled) and natal period (two-level factor, '2012–2014' as reference) in 39 male and 62 female eastern imperial eagles hatched in Hungary between 2012 and 2018. Natal territory ID and natal year (categorical) were set as crossed random intercepts. Effects in bold were significant ($p < 0.05$).

Response variable: density difference ($\log(\text{breeding density}) - \log(\text{natal density})$)						
Explanatory variables	Estimate	SE	95%CI	df	t-value	p-value
Intercept	-0.519	0.143	[-0.796, -0.240]	90.5	-3.637	0.0005
sex (female)	0.062	0.169	[-0.269, 0.388]	93.6	0.364	0.7166
log (natal density)	-0.578	0.079	[-0.730, -0.424]	72.6	-7.345	< 0.0001
log (NDD)	-0.258	0.084	[-0.420, -0.097]	95.7	-3.084	0.0027
natal period (2015 – 2018)	-0.112	0.159	[-0.424, 0.226]	96.0	-0.704	0.4831
Random effects		SD				
natal territory ID	0.236					
natal year	0.000					
Residual	0.729					

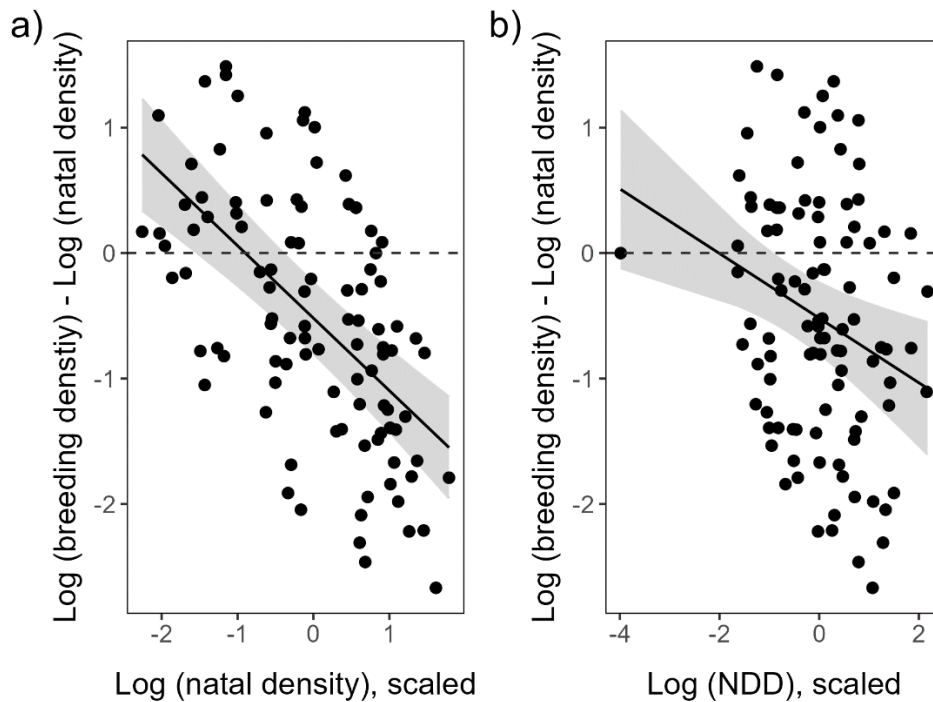


Figure 7.3. Difference between natal site and breeding site density in relation to natal site density (a) and natal dispersal distance (b) in eastern imperial eagles hatched in Hungary between 2012 and 2018. Slopes and 95% confidence intervals were estimated from the linear mixed model in Table 7.2. The horizontal dashed line on both (a) and (b) indicate zero density difference (in such a dispersal event, natal and breeding nest had the same density value).

7.3.7 Direction of natal dispersal movements

We found no deviation from uniformity concerning the direction of natal dispersal movements among birds hatched in the first period of the study, 2012–2014 (Rayleigh tests, males: $r = 0.218$, $p = 0.279$, $n = 27$; females: $r = 0.055$, $p = 0.908$, $n = 32$). However, the natal dispersal directions of females hatched in the second period (2015–2020) showed a significant deviation from uniformity, with most females dispersing towards the south at a mean angle of 183.5° (Rayleigh tests, males: $r = 0.130$, $p = 0.768$, $n = 16$; females: $r = 0.370$, $p = 0.004$, $n = 40$) (Figure 7.4). Even though NDDs seem longer in the second period, the model with $\log(NDD)$ as response variable showed no significant effect of *natal period* (see Table 7.1).

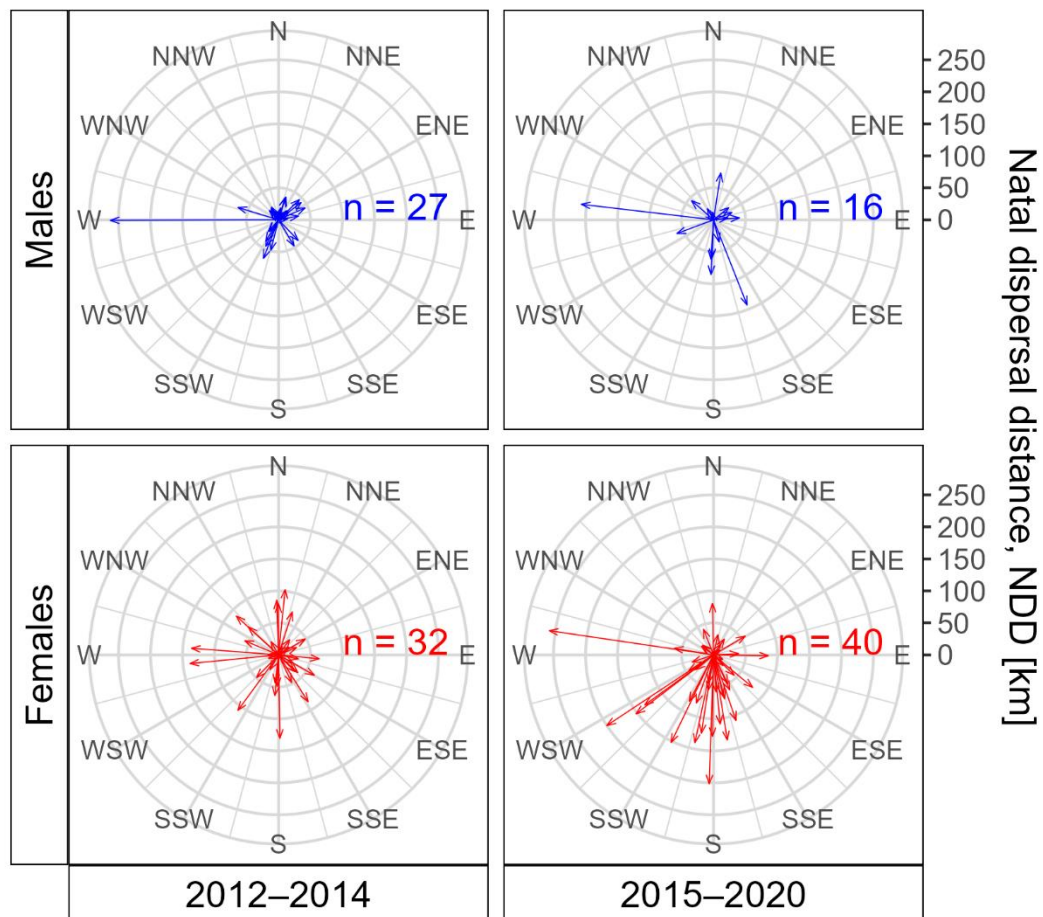


Figure 7.4. Natal dispersal directions and distances of eastern imperial eagles hatched in Hungary in 2012–2014 and 2015–2020. Arrow length indicates distance; abbreviations indicate direction.

7.4 Discussion

Using the combination of colour ringing, GPS tracking, and DNA profiling, we revealed that natal dispersal in the imperial eagle is female-biased, and we also found implications for both positive and negative density dependence: birds generally settled in lower-density sites compared to their natal site but moved to higher-density sites when their natal-site density was low.

7.4.1 Female-biased natal dispersal

We found that imperial eagle females dispersed further than males, as expected from theory and empirical evidence from other birds of prey [53, 86, 167, 184, 188, 219, 220]. The few NDD values previously reported for the imperial eagle [25, 27] are similar to the

values we obtained, but these studies could not make a meaningful comparison between the sexes due to their limited sample sizes.

Sex-biased natal dispersal can be an important mechanism of inbreeding avoidance [188], which is especially crucial in species like the imperial eagle, where small population size and long-term genetic monogamy coupled with high longevity and strong philopatry result in a high number of siblings within a population [28, 29]. We argue that the longer dispersal distances of females helped maintain the Pannonian population's relatively high genetic variability despite the previous bottleneck [12]. On the other hand, the shorter distances of males limit the colonisation potential and speed in this population.

Sex-biased natal dispersal has the potential to introduce spatial variation in sex ratios and, therefore, elevate the extinction risk of small, fragmented populations [221]. Our results suggest that more birds might disperse from the larger Eastern to the smaller Western subpopulation than vice versa (although we marked a significantly larger proportion of chicks in the East than in the West). While we found no evidence of female bias among the individuals dispersing from East to West, the longer NDD of females implies the possibility that most of these long-distance dispersers are females. This could lead to a female-biased sex ratio in the West, which may be further enhanced by male-biased adult mortality due to poisoning [199]. Considering monogamy and the important role of males in providing food for their young, such spatial inhomogeneity in sex ratios is expected to decrease reproductive output at the population level.

7.4.2 Density-dependent natal dispersal

Our results imply that natal dispersal patterns in the imperial eagle are shaped by both competition avoidance and conspecific attraction [53]. In territorial birds, such as the imperial eagle, high conspecific density may not only decrease survival and breeding success through resource depletion, but also via elevated frequencies of territorial intrusions and fights [222]. These effects can lead to a positive relationship between density and dispersive tendency [189, 192]. In line with this, we found that most birds hatched at medium- and high-density areas chose lower density breeding sites compared to their natal site.

On the other hand, we also found indications of negative density dependence, as birds that hatched in low-density areas settled mainly in higher-density areas, showing evidence for conspecific attraction. Conspecific presence can serve as a cue for suitable or high-quality habitats and signal an increased chance for mate acquisition [192–194,

223]. By relying on social cues, imperial eagle floaters may reduce the chance of choosing an unsuitable territory, which would result in a high cost considering their strong breeding philopatry.

Based on our model predictions, birds originating from a local density of 0.009 km^{-2} ($\sim 111 \text{ km}^2$ polygon area) tend to disperse equally towards higher and lower densities; additionally, most birds settle in territories with approximately this density (Figure A2). Thus, we argue that it may represent an optimal trade-off: minimising the risk of settling in a territory with limited resources or mating opportunities while incurring relatively low competition.

Since movements are directed from territories with extreme densities towards those with low-medium density, dispersal is expected to homogenise breeding pair density. On the other hand, most individuals originate from high-density—thus, presumably good-quality—territories and males especially try to settle close to their natal sites, resulting in density heterogeneity. This highlights the two-way relationship between density and dispersal: not only does density influence dispersal, but dispersal also shapes density [189]. Historical reasons may also help explain the highest densities in the core breeding areas at the foothills, as these were occupied first by this exponentially growing population [11].

Even in the high-density, presumably high-quality territories, carrying capacity has not yet been reached since settlement via territory partitioning still occurs. This ongoing partitioning of high-quality territories indicates that territory sizes in the imperial eagle appear to align more closely with an ideal free distribution [224] rather than an ideal despotic one [225]. However, evaluating the relationship between density and fitness remains a task for future research.

The significant positive correlation between breeding site and natal site density in males but not in females complements our results on the sex-biased NDDs in this species; since males disperse shorter distances than females, their breeding and natal site densities are expected to be more similar. Our results also indicated that the longer NDDs belong to individuals moving from high-density breeding territories to low-density peripheral areas. It would be interesting to study whether these longer dispersal distances are associated with lower fitness [226–228].

How density is defined can influence the conclusions drawn on its effect [195]. In this study, we aimed to measure local density on a continuous scale and defined it as the reciprocal area of Thiessen polygons drawn around the nest. It is important to note that the area covered by the Thiessen polygon of a breeding bird's nest is generally a good

indicator but does not necessarily correspond completely to the area of its territory [206, 207]. While it was most probably the case in high-density areas, in low-density areas, actual territory sizes may have been smaller than indicated by the Thiessen polygons.

7.4.3 Temporal changes in dispersal patterns

We found that birds hatched in the first period of the study showed no preferred direction of natal dispersal, whereas females hatched in the second period mainly dispersed in a southern direction. We only detected this directional preference in females; however, we may not have been able to detect any preference in males due to their lower sample size.

We think this shift in dispersal direction resulted from the change in density. In the first period, the population size stagnated at a third of what was observed at the end of the study, meaning that there were still many vacant territories in the northern parts of the Hungarian Great Plain, where most imperial eagles reside [22, 199]. However, in the second period, the population increased exponentially. Since we showed that birds from high-density areas select territories in lower-density habitats, this population growth could have facilitated the range expansion towards the South as low-density areas in the North became increasingly scarce. The preference for southern regions over similarly low-density areas in the West can be explained by the larger contiguous open lowlands in the South, which are the most suitable habitats for the imperial eagle.

7.4.4 High philopatry despite high mobility

Although some individuals have explored areas over 1000 km from their natal site, all birds for which we could determine NDDs have returned to breed in their natal Pannonian population. This population-level philopatry was previously suggested by GPS telemetry studies for the Pannonian and other resident populations as well [25–27, 229, 230].

We must consider the possibility that we failed to detect emigration to other populations in the case of colour-ringed and DNA-profiled birds. The reason is that the detection or reporting probability of colour-ringed birds might have been lower in some areas outside of the Pannonian Region, where the monitoring of imperial eagles was less intensive, and we only used DNA profiling data from Hungary. However, considering that all of the GPS-tracked birds (for which we precisely know breeding locations) settled in the Pannonian population and that no bird after the age of 3cy was recorded in another breeding population, we can conclude that migration to other distant populations is negligible.

Regarding migration between the two subpopulations of the Pannonian population, we detected five movements (~4% of all recorded natal dispersal cases) from the Eastern to the Western subpopulations. This 4% migration rate is consistent with the only low genetic differentiation of the Western and Eastern parts found by Vili et al. [12]. The resulting gene flow is beneficial for maintaining genetic diversity and decreasing inbreeding. However, as we have observed only one-way movement toward the West, further research should give attention to possible source-sink dynamics between the subpopulations.

Assuming 10.9 km as a median territory diameter (based on Thiessen polygon areas), about 50% of males recruit within four, and 50% of females within six territorial units. We interpret this as high philopatry even on a within-population scale, considering that birds may scout areas up to a hundred territorial units away from their natal nest during their floater phase.

Such high philopatry, despite high vagility in birds of prey, is not unusual [53, 231–233]; but see for example [234]) and has important implications for their conservation. For the imperial eagle, it suggests the genetic uniqueness of the Pannonian and similar isolated populations, while also highlighting their elevated risk of extirpation.

7.4.5 Combined marking methods

Our data on NDDs originated from three monitoring methods with different properties. Colour ringing provided presence data with no strict spatial truncation but with a low detection rate compared to the other methods. In contrast, DNA profiling provided a higher detection rate and high certainty of breeding status, but at a higher sample cost along with spatial truncation due to our limited study area. GPS tracking had the benefit of a high detection rate with no spatial truncation, but at a much higher cost, resulting in a low sample size, which by itself would not have been sufficient to study natal dispersal patterns.

Most of our NDD data originates from DNA profiling, where spatial truncation occurred, suggesting that our NDD estimates are downwardly biased, especially for the longer-dispersing females. Excluding the data obtained from DNA profiling, the median NDDs for females and males are 73.1 and 35.9 km, respectively, showing a more pronounced sex bias than the above reported 57.6 and 35.9 km, estimated for all the sampled birds. This implies that we likely underestimated the magnitude of sex bias.

The DNA profiling method of genotype matching chicks and breeding adults has rarely been used in natal dispersal studies [53, 131]. Our study demonstrates that despite its

spatial truncation, it can serve as a valuable, cost-efficient tool for investigating natal dispersal in raptors on a within-population spatial scale. We recommend its use for territorial raptors with low population size, for which GPS tracking of individuals in great numbers is not feasible. For species where chicks are already being ringed on a regular basis, obtaining DNA samples from chicks and collecting shed feathers around the nest site can also be incorporated into the ringing schedule with small extra effort.

7.4.6 Conclusion

We demonstrated that natal dispersal in the imperial eagle is characterised by strong philopatry and longer dispersal distances in females and is likely influenced by both intraspecific competition and conspecific attraction.

Our findings suggest that despite its exponential growth, the Pannonian population is unlikely to come into contact with other populations in the near future, given that the nearest ones—in North Macedonia and Bulgaria—are approximately 600 km away, about three times the maximum NDD we observed. Therefore, these populations should be considered separate conservation units, and management should aim to facilitate gene flow between them to restore a functional metapopulation structure, for example, by reintroducing intermediate stepping-stone populations.

Furthermore, although we only detected movements from the Eastern to the Western subpopulation, implying possible source-sink dynamics between the two with a risk of biased sex ratios, our sample size in the West was very limited. Therefore, we urge natal dispersal studies on chicks from those regions to obtain more reliable estimates of migration rates.

Our results will contribute to developing more accurate population viability models for the species, further supporting its effective conservation.

8 POPULATION VIABILITY ANALYSIS (PVA) OF THE EASTERN IMPERIAL EAGLE IN THE PANNONIAN REGION

8.1 Introduction

Population viability analysis (PVA) is used for investigating population extinction risks and future population growth under different conservation management scenarios [13, 14]. PVA models account for the random variation in demographic rates that can occur due to demographic, environmental or genetic stochasticity [20], allowing for a more robust assessment of population viability [13]. Via perturbation analyses, PVA also helps determine which vital rates are the most influential on population growth rate, thus enabling informed management decisions [13, 15, 17, 18, 235, 236]. Perturbation analyses may use a sensitivity or elasticity approach [237]. Sensitivity examines the impact of absolute changes in demographic rates on the population growth rate, while elasticity measures the effect of proportional changes [17, 237].

PVAs in raptors have examined effects of various anthropogenic threats on population persistence, such as extensive windfarm development [15, 16, 235, 238], poisoning incidents [15, 19, 235], poaching [17], nest plundering [17] and pollutant ingestion [235]. PVA has also been used to assess the impact of different management options for raptors such as supplementary feeding [15, 18, 235], release of captive bred individuals [18] and translocation of juveniles for reintroduction to former breeding sites [15]. Based on perturbation analyses, conservation measures impacting adult or juvenile mortality rates in raptors are found to be more influential on population growth rates than those affecting fecundity [17, 19, 106, 235, 239, 240].

The eastern imperial eagle (*Aquila heliaca*, hereafter 'imperial eagle') is a long-lived, Vulnerable (IUCN) raptor species of the Palearctic [1]. Its global population is estimated at a maximum of 10 000 mature individuals and considered declining on a global scale [1]. On the other hand, its European populations—comprising about 30% of its global distribution—show an increasing trend [10]; however, it continues to be threatened by habitat loss and degradation, electrocution, and persecution, including the poisoning and shooting of birds [2–4, 199].

The Pannonian Region in Europe marks the western edge of the species' distribution, with 356–381 nesting pairs in 2019 [8]. This Pannonian population can be divided into an Eastern and Western subpopulation, separated by about 100 km, with low levels of genetic differentiation between the two [12, 241]. The larger Eastern subpopulation comprises of East Hungary (276 pairs in 2019 [31]), East Slovakia (45-50 pairs [201]),

North Serbia (3 pairs [22]) and West Romania (1 pair [22]). The smaller Western subpopulation includes West Hungary (11 pairs [31]), West Slovakia (21 pairs [202]), East Austria (20–23 pairs [39]) and the Southern Czech Republic (6–8 pairs [39]).

Intensive persecution and agricultural intensification in the previous century led to a drastic decrease in population size; in 1980, only about 14 nesting pairs remained in the Eastern and 6 nesting pairs in the Western subpopulation [22, 36]. During this time, the species occupied almost exclusively mountainous forest habitats, where it could find refuge from intensive persecution, and it only returned to its preferred open grassland and lowland agricultural areas following the decrease in persecution [24, 34, 36]. Despite starting from similar population sizes in 1980, the Eastern subpopulation went through a more than thirty-fold increase until 2022, while the Western subpopulation increased only twenty-fold [11, 22, 37–39].

Using estimates of demographic parameters from 1980–2022, we conducted a PVA for imperial eagles in the Pannonian Region that comprised of the following steps:

- 1) We constructed the basic PVA model for the largest and most extensively studied Hungarian part of the Eastern subpopulation, which we will refer to as East Hungary ('East HU'). We determined the values of uncertain parameters using sensitivity tests and validated the model by comparing the predicted and observed population trajectories between 1980 and 2022.

- 2) Considering that the Eastern ('East') and Western ('West') subpopulations displayed different population trajectories in the last four decades, we aimed to explore the possible demographic differences between the subpopulations using elasticity tests.

- 3) We investigated future population growth under different mortality scenarios for East Hungary.

- 4) Using elasticity analysis, we determined which demographic parameters have the greatest impact on population growth and should therefore be in the focus of conservation.

8.2 Methods

We conducted the PVA in VORTEX (v.10.8.1.0 [21]). The VORTEX population model is individual-based, contains stochastic effects, and allows for demographic parameters to be modelled as functions of other parameters or variables, including density or time [21]. Simulations of population growth over a specified number of years are run multiple times (iterations). Outputs report demographic variables (census data, i.e. number of individuals, nesting pairs, offsprings, etc.) for each year and iteration, extinction

probability (proportion of iterations in which the population went extinct by the end of the simulation) and median time to extinction. We considered the population extinct when only one sex remained. Means and standard deviations of the demographic variables were used to compare models with different parameter settings (scenarios). We carried out these post simulation analyses in R (v.4.4.1 [144]) with *VortexR* (v.1.1.9 [242]) and made figures using *ggplot2* (v.3.5.2 [145]).

8.2.1 PVA model for East Hungary (model 'East HU')

8.2.1.1 Structure

Following the life-history characteristics of the imperial eagle [22], we built a stage-structured PVA model (**Figure 8.1**). Imperial eagles have a floater period of several years [22]. Recruitment into the breeding population normally occurs between ages 3-6cy [22, 39]. During the floater period, birds in the Pannonian Region occasionally disperse between subpopulations (**SECTION 7**). Therefore, we defined the following stages for the model based on age ('cy') and breeding status (floater or nesting): '1cy', '2cy', '3cy floater', '4cy floater', '5cy+ floater' and 'nesting' (including 3cy, 4cy and 5cy+ nesting birds). Within a specific stage, we applied the same demographic parameters to all individuals.

Each simulation year comprised of the following VORTEX steps: *EV* (setting annual demographic rates by applying Environmental Variation), *Breed* (production of offspring based on reproductive rates), *PSUpdate* (Population State Variable Update step: the recalculation of density-dependent parameters based on current density), *rCalc* (calculation of the stochastic growth rate r), *Census*, *Dispersal* (individuals were allowed to emigrate from one subpopulation to the other once in their lifetime – at '2cy' – with probability D), *Mortality* and *ISUpdate* (Individual State Variable Update step: surviving individuals progress between stages, i.e. age and remain floaters or enter the nesting stage). During the *ISUpdate*: (i) '1cy' individuals become '2cy', (ii) '2cy', '3cy floater' and '4cy floater' individuals either become 'nesting' with P_{E3} , P_{E4} and P_{E5} entry probabilities, or they become '3cy floater', '4cy floater' and '5cy+ floater' with $(1-P_{E3})$, $(1-P_{E4})$ and $(1-P_{E5})$ entry probabilities, (iii) '5cy+ floater' individuals either become 'nesting' with P_{E6} entry probability or remain '5cy+ floater', (iv) 'nesting' individuals remain 'nesting'. Therefore, for floaters aged 5cy or older and nesting individuals (of any age), further ageing is not modelled, i.e. fecundity and survival no longer change with age (**Figure 8.1**). Compared to the default VORTEX settings, we included *Census* after *Breed* instead at the end of the simulation year as field census also takes place after the production of offspring, during the chick-rearing period in June. Input parameters are discussed below

in detail and their final values (following sensitivity and elasticity tests) are listed in Appendix tables [Table A9](#), [Table A10](#) and [Table A11](#).

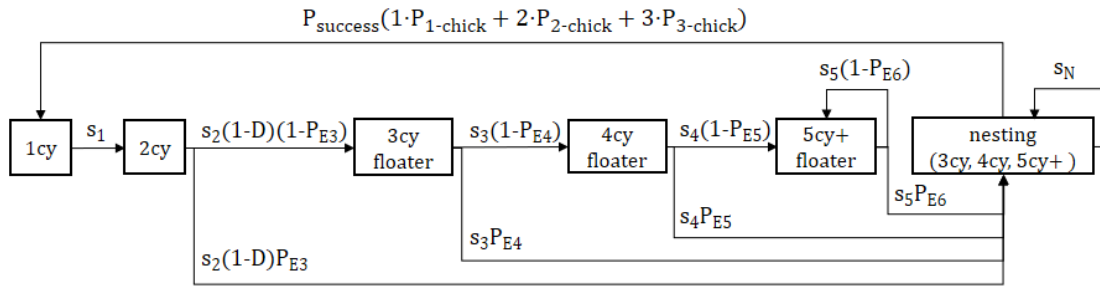


Figure 8.1. Structure of the PVA model describing the population dynamics of imperial eagles in the Pannonian Region. s : survival probability, P_E : probability of entering the nesting stage, D : probability of emigrating from the subpopulation, P_{success} : proportion of successful (producing at least one chick) nesting pairs, $P_{n\text{-chick}}$: proportion of n -chick broods among successful pairs ($n = 1, 2, 3$).

8.2.1.2 Reproductive parameters and mortality of nesting birds

Reproductive system was set to long-term monogamy, as imperial eagles are genetically monogamous and exhibit strong breeding site and mate fidelity throughout their long lifespan [28, 29]. Annual data on the number of nesting pairs and productivity were provided by MME BirdLife Hungary [11, 30, 31]. For each year in the model (1980–2022), we used the observed breeding success (proportion of nesting pairs with at least one chick) and the proportions of 1-chick, 2-chick and 3-chick broods among successful pairs.

Based on 22 GPS tracked birds marked as chicks and followed until their first nesting attempt [241] ([SECTION 7](#)), we estimated the probability P_{Ei} (the probability of entering the nesting stage at age i , conditional on surviving until age i) for calendar years $i = 3\text{cy}$, 4cy , 5cy and 6cy .

We used 8.4% for the average annual mortality rate of nesting birds, estimated for 2011–2022 by the constant-survival Cormack-Jolly-Seber mark-recapture model in Zsinka et al. [199] ([SECTION 6](#)).

To account for the variability in mortality rates between years, we applied an annual standard deviation (SD) of 4.3 percentage points on mortality for both the nesting and non-nesting stages. This SD was calculated from the annual mortality estimates of the

year-dependent mark-recapture model in Zsinka et al. [199] ([Table A2](#)). Unreliable estimates (characterised by large SEs or SE = 0.000) were not included in the SD calculation.

8.2.1.3 *Mortality of non-nesting stages and emigration rate*

Mortality estimates of non-nesting stages originated from a Kaplan-Meier survival model based on 101 GPS tracked birds between 2003 and 2023 [243]. However, since 12 birds for which transmitter failure occurred had unknown fates (it was not known whether they were alive or dead at the time of failure), mortalities could only be estimated as minimum and maximum mortalities: 30.0–35.5% from 1cy to 2cy, 15.0–21.4% from 2cy to 3cy and 7.9–14.8% from 3cy to 4cy, 5.3–9.7% from 4cy to 5cy and 8.3–8.5% from 5cy to 6cy. To reduce the number of mortality parameters to be tested in the sensitivity analysis (see below), for the mortalities 4cy to 5cy and 5cy to 6cy, we used the mortality estimated for nesting birds (8.4%).

Since we only included the East Hungarian part of the population in our baseline model, we modelled dispersal as net emigration rate from East Hungary to other parts of the Pannonian population (we previously found no evidence of emigration to other populations [241] [SECTION 7](#)). From colour-ringing, GPS-tracking and DNA profiling records ($n = 116$), we previously estimated a dispersal rate of 4.3% from East Hungary to the Western subpopulation and no dispersal from West to East [241] ([SECTION 7](#)). This suggests a higher rate of dispersal from East to West, but this result is highly uncertain due to the lack of an adequate sample of marked birds in the West. From GPS tracking only—no spatial truncation, unlike the case of DNA profiling data—, we estimated a higher dispersal rate of 11% from East Hungary to the Western subpopulation, but this was based on a much smaller sample size ($n = 19$) [241] ([SECTION 7](#)). Based on these estimates, we determined a possible range of net emigration rate from 0% to 7.5% (above this value, observed population growth could not be reproduced even with the minimum values of mortalities).

Due to these uncertainties in the mortality rates of non-nesting stages and emigration rate, we conducted sensitivity tests for these parameters to identify the values that best reproduced the observed population growth. We used a factorial design, with four, equally distributed intermittent values between the minimum and maximum rates of emigration and ‘1cy’, ‘2cy’ and ‘3cy floater’ mortalities. Altogether 1296 scenarios were run, with 250 iterations each. To validate our model and choose the most fitting parameter scenario, we visually inspected the observed and simulated population trends and calculated the difference between the observed values and the simulated means of

the number of nesting pairs, successful pairs and chicks for each year. The scenario with the smallest absolute sum difference in the number of nesting pairs was chosen as the best fit.

8.2.1.4 Carrying capacity and density-dependence

We defined carrying capacity (K) as the maximum number of nesting pairs that could occur in East Hungary (extending from the eastern border to the Transdanubian Mountains) based on the amount of potentially suitable habitats available. First, we used the most recent (2018) CORINE Land Cover data [244] to calculate the coverage percentage of potentially suitable areas in each 10 x 10 km UTM grid cell. Since the imperial eagle's preferred habitats in the Pannonian Region are open grasslands and lowland agricultural areas [22, 245], the following CORINE land cover classes were considered potentially suitable: 'Non-irrigated arable land', 'Permanently irrigated land', 'Pastures', 'Annual crops associated with permanent crops', 'Complex cultivation patterns', 'Land principally occupied by agriculture, with significant areas of natural vegetation', 'Natural grassland'. We conducted spatial calculations in R using packages *sf* (v.1.0-12, [205]) and *maturalearth* (v.1.0.1., [218]). To estimate the relationship between the number of nesting pairs in a UTM cell and the percentage coverage of these potentially suitable areas, we fit a zero-inflated Poisson regression model with the *glmmTMB* package (v.1.1.11, [246]). To model the relationship that best describes the population at carrying capacity, we only included data from the most saturated area (Heves county, Hungary). Model fit was investigated with the *DHARMA* package (v.0.4.7, [247]). To estimate K , we took the sum of the number of nesting pairs predicted by the model for all UTM cells.

Generally, density dependence is included in a PVA by applying a term that is a function of density, $f(N)$, as a multiplier for some of the demographic parameters [248]. As yet, no data is available to estimate the form of the functions describing density dependence in survival or fecundity in the Pannonian population, as it is still growing exponentially. So, we used the rescaled version of the theta-logistic function [248] in our PVA, which is the default option in VORTEX [249] and widely used in similar studies (e.g. [250, 251]). We chose the nesting stage, i.e. the number of nesting pairs (shown as *PAIRS* in the formulas), to control the feedback mechanism. Given the territoriality of the species, it is a realistic assumption that the available habitat limits the number of nesting pairs. The carrying capacity means the number of nesting pairs in the saturated population; when it is reached, the P_{E_i} probabilities of entering the nesting stage for different non-nesting stage groups decrease to only a fraction of their density-independent values. In addition

to the probabilities of entry into the nesting population, we applied a density-dependent multiplier to s_1 , the first-year survival probability.

We omitted Allee-effect in modelling density dependence, so the version of the theta-logistic function we use monotonically decreases with $PAIRS$, and has only three parameters: K , the carrying capacity, $f(K)$, the value of the function when the number of breeding pairs equals K , and θ , the shape parameter that determines whether the density dependence occurs already at low densities (e.g. $\theta = 1$, linearly decreasing function) or only at high densities close to K (e.g. $\theta = 4$, nonlinear function modelling minimal effect for low densities but decreasing sharply close to K). The density-dependence functions differ for first-year survival and probability of entry, but for simplicity, we applied the same $f_E(K)$ and θ_E parameters to all the four P_{E_i} entry probabilities:

$$f_{s_1}(PAIRS) = 1 - \left(1 - f_{s_1}(K)\right) \times \left(\frac{PAIRS}{K}\right)^{\theta_{s_1}}$$

$$f_{E_i}(PAIRS) = 1 - \left(1 - f_E(K)\right) \times \left(\frac{PAIRS}{K}\right)^{\theta_E}$$

Before setting the $f_{s_1}(K)$ and $f_E(K)$ parameters of the above density dependence functions, we have calculated R_0 , the net reproductive rate (the expected total number of female offspring of a newborn female, i.e. the per generation growth rate) at low density. For this, we used the ‘East HU’ demographic parameters and the standard formula $R_0 = \sum_{i=1}^{max} l_i m_i$, where l_i is the probability of survival up to age i and m_i is the fecundity at age i [252]. Knowing R_0 for the density-independent case, the task was to set $f_{s_1}(K)$ and $f_E(K)$ so that R_0 decreases to 1 when $PAIRS$ reaches K .

For $f_{s_1}(K)$ in the density dependence function of the first-year survival, we should choose a value that is considerably greater than $1/R_0$, because multiplying s_1 with $1/R_0$ would result in R_0 decreasing to 1. This follows from the above formula of R_0 and from the survivorship up to age i being a product of the annual survival probabilities $l_i = s_1 \times s_2 \times \dots \times s_i$. As s_1 occurs in every term of the R_0 sum formula, it affects the expected number of offspring at all maternal ages the same way (e.g. halving s_1 halves the expected number of offspring at all maternal ages). By setting $f_{s_1}(K) > 1/R_0$, R_0 is still over 1 at $PAIRS = K$, so density dependence can also be applied to the P_{E_i} entry probabilities, further decreasing R_0 to 1.

Infinitely many combinations of $f_{s_1}(K)$ and $f_E(K)$ parameters satisfy the requirement that at $PAIRS = K$ the net reproductive rate R_0 decreases to 1 (and so does λ , the annual

population growth rate). By trial and error, we chose a combination that resulted in approximately a 1:1 breeder to floater ratio, as expected for a healthy saturated raptor population [253]. We calculated the nesting to floater ratio from the first eigenvector of the deterministic stage-based (self-looped) Leslie matrix [252, 254], representing the deterministic matrix projection model (**Table A12**) corresponding to our stochastic PVA model. We chose shape parameters $\theta_{s_1} = \theta_E = 4$ resulting in most density-dependent change occurring close to carrying capacity, while negligible effect at low densities as suggested by Fowler [255] for species with low reproductive rates, long life expectancy, and with populations that are mainly limited by resources.

8.2.1.5 Initial population size and stage distribution

The stage distribution (proportion of different stage groups) of the initial population was not known, so we used the stable stage distribution we estimated from the population's Leslie matrix [252] (**Table A12**), which we constructed from the most fitting mortality and dispersal probabilities determined by the sensitivity tests, the estimated entry probabilities and the mean reproductive output calculated for 1980–2022. Since each simulation year began with the production of chicks (*Breed* step), we did not include chicks ('1cy') in the initial population and adjusted the stage distribution accordingly. The initial population size was calculated from the proportion of nesting birds in the population based on this initial stage distribution and the observed number of nesting pairs in the starting year.

8.2.2 Modelling the Eastern and Western subpopulations (model 'East-West')

To investigate the differences in the Eastern (East Hungary, East Slovakia, West Romania and North Serbia) and Western (West Hungary, West Slovakia, East Austria and the Southern Czech Republic) subpopulation dynamics between 1980–2022, we added a two-population structure to the baseline model validated for East Hungary.

We used annual data on the number of nesting pairs, breeding success, and the proportion of 1-chick, 2-chick and 3-chick broods for both the Eastern and the Western subpopulations [37–39, 202, 256]. Initial subpopulation sizes, initial stage distributions and carrying capacities were estimated similarly to East Hungary. In this model, we used the previously estimated 4.3% dispersal rate for both East-to-West and West-to-East movements [241] (**SECTION 7**). We considered the probabilities of entering the nesting stage to be the same in both subpopulations as in East Hungary.

Running the 'East-West' model with the baseline settings (East Hungarian parameter values) revealed that the predicted growth rates of both the Eastern and the Western subpopulations were greater than observed for 1980–2022 (see [Results](#)). Therefore, we ran elasticity tests to estimate how much higher the mortality rates in the Eastern and Western subpopulations would need to be—compared to those in East Hungary—to best match the observed trends. For the West, mortalities of all stages were increased by either 30%, 35%, 40%, 45%, 50%, 55% or 60%. For the East, we took into account that Hungary holds most of the subpopulation and the remaining Slovakian, Romanian and Serbian parts comprised only about 25% on average over the years; therefore, mortalities of all stages in the East were increased an order of magnitude lower, only by either 3%, 3.5%, 4%, 4.5%, 5%, 5.5% or 6%. Altogether 49 scenarios were created that were run for 250 iterations each. The parameter scenario with the best fit was chosen similarly as described above in 8.2.1.3.

8.2.3 Future population growth under different mortality scenarios (model 'Future')

We investigated the expected population growth for East Hungary in 2022–2065 under three mortality scenarios ('Baseline', 'West-like' and 'Poison'), combined with three productivity scenarios ('High', 'Average', 'Low').

In the 'Baseline' mortality scenario, we used the same mortalities as in the 1980–2022 'East HU' model. In the 'West-like' scenario, we used the mortalities estimated for the Western subpopulation from the East-West model's elasticity test. In the 'Poison' scenario, we applied a 60% increase to the mortalities of all stages, calculated from the mortality estimates of the years with the lowest and highest known rates of poisoning ([SECTION 6](#)).

In the 'High' productivity scenario, breeding parameters were taken from the year with the highest productivity (chick / nesting pair) between 2013 and 2022 (the last 10 years). Similarly, in the 'Low' productivity scenario, parameters of the lowest productivity year were used in each iteration. In the 'Average' scenario, the breeding parameters for each year were randomly selected from those observed during the period 2013–2022. With this method, the interannual variability of productivity resulting from environmental factors was incorporated into the model, and the iterations of the same scenario had somewhat different productivity parameters. For each of the three productivity scenarios, we also investigated the highest mortality increase that would result in a stagnating population.

The initial stage distribution and initial population size was estimated similarly to the 'East HU' model. Emigration rate and entry probabilities were the same as in the 'East HU' model. In the 'Future' model, we applied the theta-logistic density-dependence functions for all the entry probabilities and for the first-year survival.

8.2.4 Elasticity analysis

Using the 'East HU' model structure and parameter settings, we conducted an elasticity analysis to measure how proportional changes in five key demographic parameters (breeding failure, '1cy', '2cy', '3cy floater' or 'nesting' mortality) impact the population growth rate. We examined breeding failure instead of breeding success so modifying the parameter will result in the same directional change in growth rate as for mortalities. We used a single factor perturbation design: in each scenario, we only modified the value of one parameter while keeping the other four parameters at their baseline values. Parameter values were modified by either -30%, -20%, -10%, +10%, +20% or +30% similarly to Katzner et al. [257]. Altogether 31 scenarios were created (30 with modifications and one with all parameters at their baseline values) that were run for 250 iterations and 20 years. To compare the impact of each parameter, we fit a linear regression between the mean stochastic growth rate r calculated by VORTEX (for each scenario, averaged across the 250 iterations and the 20 years) and the proportional change in the parameter.

8.3 Results

8.3.1 PVA model for East Hungary (model 'East HU')

Based on data from MME BirdLife Hungary [11, 30, 31], for the years 1980–2022, the mean percentage of successful nesting pairs in East Hungary was 67.8% and the mean percentages of 1-chick, 2-chick and 3-chick broods among successful pairs were 37.4%, 47.0% and 15.6%, respectively. Productivity has increased over the period, with an average of 57.1% 1-chick, 42.9% 2-chick and 0% 3-chick broods in 1980–1984 to 31.7% 1-chick, 45.7% 2-chick and 22.5% 3-chick broods in 2018–2022.

The probability of entering the nesting stage was 0.09 at the age of 3cy (P_{E3}), 0.65 at 4cy (P_{E4}), 0.71 at 5cy (P_{E5}) and 1 at 6cy (P_{E6}). We estimated a carrying capacity of 1310 nesting pairs for East Hungary. From the Leslie matrix, we calculated a stable stage distribution (excluding '1cy' individuals) of 22% '2cy', 14% '3cy floater', 4% '4cy floater', 1% '5cy floater' and 59% 'nesting' individuals. To achieve a mean initial population size

of 6 nesting pairs (observed size in 1980) across iterations, the initial population size was set at 24 individuals.

Sensitivity tests revealed that the predicted population sizes (numbers of nesting pairs, successful pairs and chicks) showed the best fit to the observed trajectories when '1cy', '2cy' and '3cy floater' mortalities were set at 30%, 15% and 9.3%, respectively, and the net emigration rate was 3% (Figure 8.2).

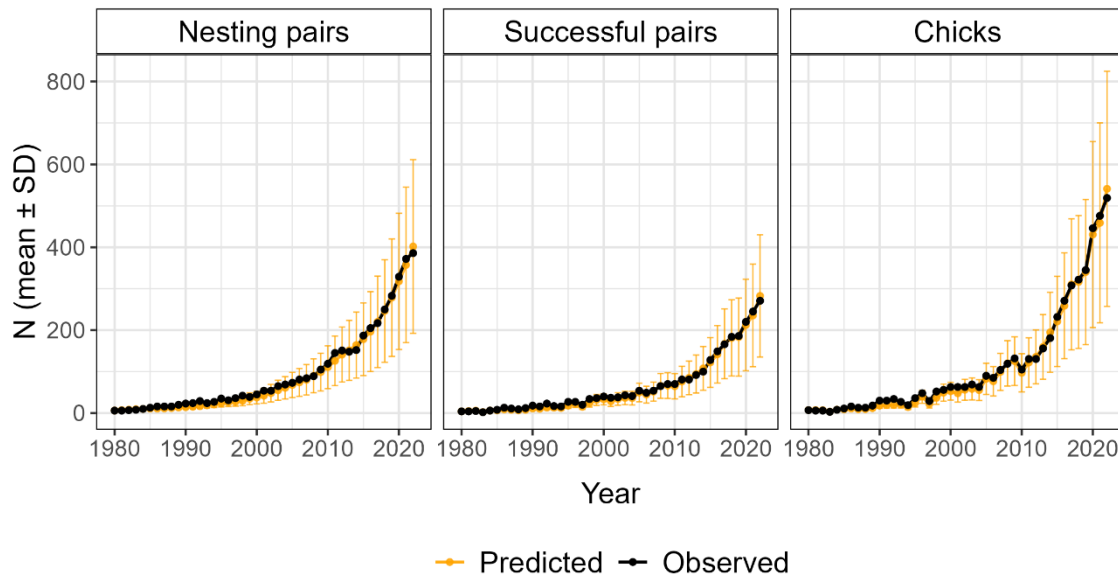


Figure 8.2. Observed and predicted (mean \pm SD from 250 iterations) numbers of nesting pairs, successful pairs and chicks of the imperial eagle in East Hungary 1980–2022. Predicted values are from the scenario with the best fitting parameter settings of '1cy', '2cy' and '3cy floater' mortalities and net emigration rate, determined via sensitivity tests.

8.3.2 Modelling the Eastern and Western subpopulations (model 'East-West')

Based on data published in [37–39, 202, 256], for the years 1980–2022, the mean percentage of successful nesting pairs in the Eastern subpopulation was 68.3% and the mean percentages of 1-chick, 2-chick and 3-chick broods among successful pairs were 39.1%, 46.6% and 14.3%, respectively. In the Western subpopulation, mean breeding success was 70.8% and the mean percentages of 1-chick, 2-chick and 3-chick broods were 37.2%, 49.3% and 13.4%, respectively.

Initial subpopulation sizes were set at 34 in the East and 24 in the West, to achieve mean initial numbers of nesting pairs of 9 and 6, respectively.

Using the mortalities estimated for East Hungary, both the Eastern and the Western subpopulations showed higher population growth rates than observed (Figure 8.3). Based on elasticity tests, the best fit to the observed trajectories were obtained when mortalities were adjusted 5.5% higher for the East (31.5%, 15.8%, 9.7% and 8.8% for '1cy', '2cy', '3cy floater' and all other stages, respectively), and 35% higher for the West (40.5%, 20.3, 12.5% and 11.3% for '1cy', '2cy', '3cy floater' and all other stages, respectively) compared to the East Hungarian estimates (Figure 8.3).

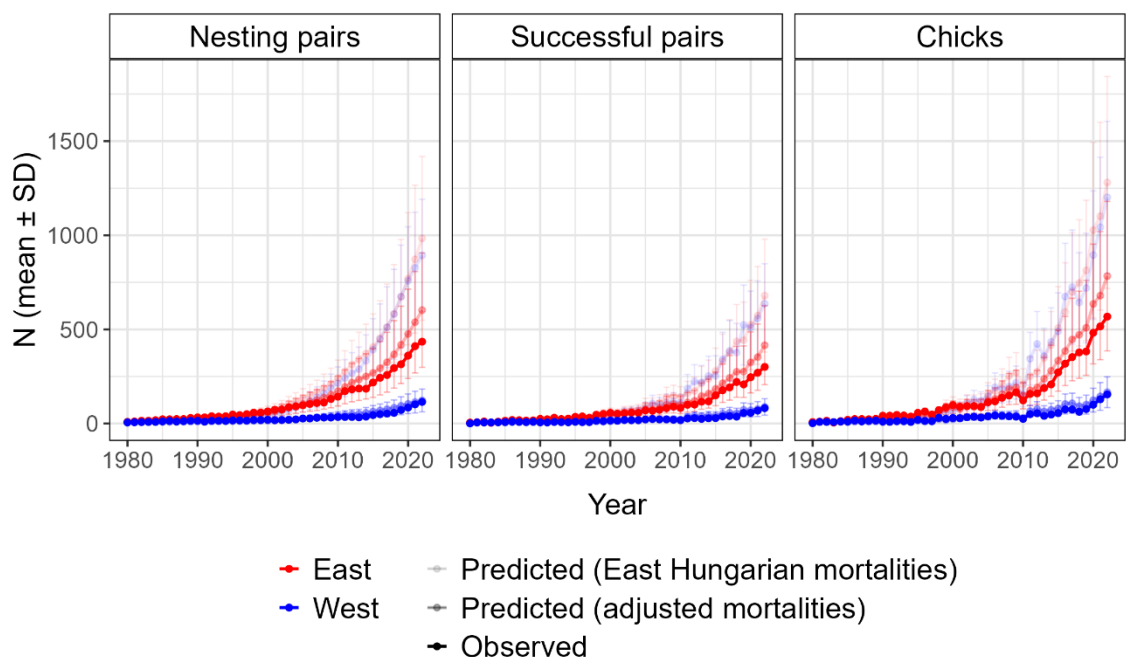


Figure 8.3. Observed and predicted (mean \pm SD from 250 iterations) numbers of nesting pairs, successful pairs and chicks of the imperial eagle in the Eastern (red) and Western (blue) subpopulations of the Pannonian Region in 1980–2022. Predicted values originate from two scenarios: mortalities same as in East Hungary and mortalities adjusted to achieve the best fit to the observed trajectories.

8.3.3 Future population trajectories (model 'Future')

Carrying capacity K for East Hungary was estimated as 1310 nesting pairs. To set the $f_{s_1}(K)$ and $f_E(K)$ parameters of the theta-logistic density dependence functions used in the 'Future' scenarios, first, we made deterministic calculations using the 'East HU' model's demographic parameters, which resulted in $R_0 = 3.36$ and $\lambda = 1.145$. Then we have checked by both deterministic calculations and VORTEX simulations that decreasing all the P_{E_i} entry probabilities to 10% of their original values ($f_E(K) = 0.1$), and halving s_1 , the first-year survival probability ($f_{s_1}(K) = 0.5$) when $PAIRS = K$, the net reproductive rate decreased to close to 1, and the breeder to floater ratio was approximately 1:1.

Figure 8.4 shows the predicted future population trajectories in East Hungary under the combinations of three mortality and three productivity scenarios. The probability of extinction until 2064 was zero for all examined scenarios (**Table 8.1**). Under 'Baseline' mortality (same mortalities as in the 1980–2022 'East HU' model) and 'Average' productivity (randomly sampled breeding parameters from 2013–2022), the population is expected to reach the estimated carrying capacity of 1310 nesting pairs by 2038. With 'High' productivity (maximum value from 2013–2022) carrying capacity was reached by 2035, while in the case of 'Low' productivity (minimum value from 2013–2022), this did not occur until 2056. In the 'West-like' (35% higher mortalities) and 'Poison' (60% higher mortalities: 48%, 24%, 14.8% and 13.4% for '1cy', '2cy', '3cy floater' and all other stages, respectively) scenarios, population growth is reduced, and the population stabilises below the estimated carrying capacity, regardless of productivity. Population stagnation would occur if mortalities for all stage groups were increased by 90% (57%, 28.5%, 17.6% and 16% for '1cy', '2cy', '3cy floater' and all other stages, respectively) assuming 'High' productivity, by 83% (54.9%, 27.5%, 17% and 15.4%) assuming 'Average' and by 70% (51%, 25.5%, 15.8% and 14.3%) assuming 'Low' productivity.

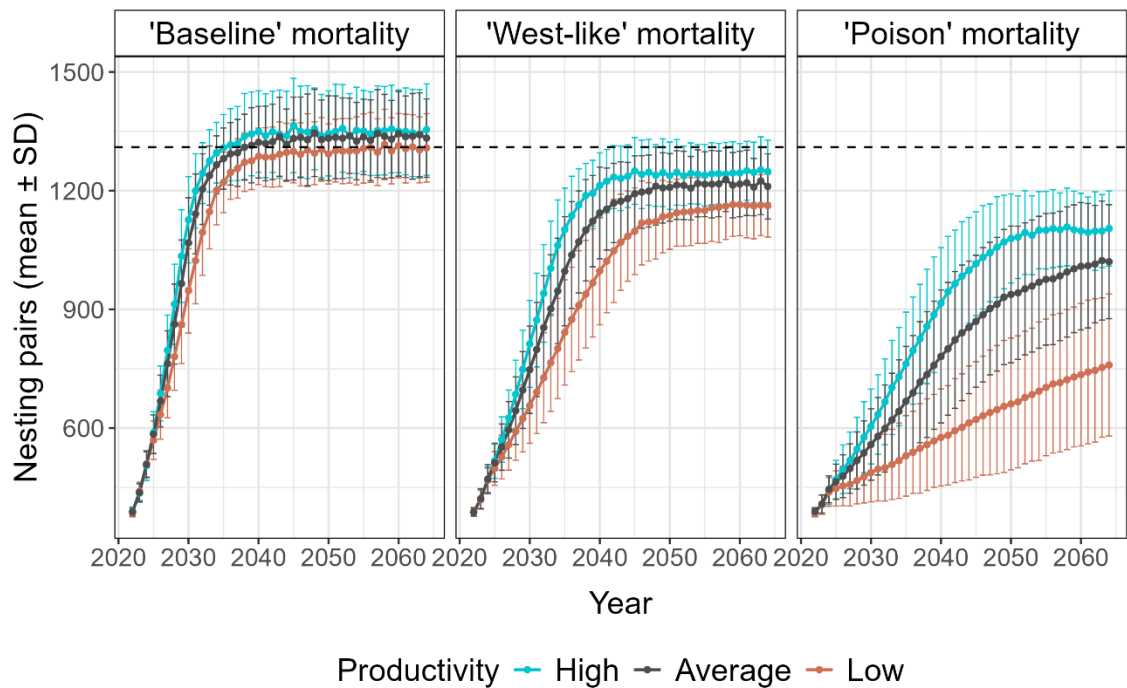


Figure 8.4. Predicted (mean \pm SD from 250 iterations) number of nesting pairs of the imperial eagle in East Hungary for 2022–2064 under three mortality scenarios ('Baseline', 'West-like': 35% higher mortalities, 'Poison': 60% higher mortalities) and three productivity scenarios (High, Average, Low). The horizontal dashed line indicates carrying capacity.

Table 8.1. Predicted (mean \pm SD from 250 iterations) number of nesting pairs of the imperial eagle in East Hungary by the year 2064 under three mortality scenarios ('Baseline', 'West-like': 35% higher mortalities, 'Poison': 60% higher mortalities) and three productivity scenarios (High, Average, Low).

Mortality	Productivity	Nesting pairs (mean \pm SD)
'Baseline'	High	1355 \pm 115
	Average	1333 \pm 99
	Low	1308 \pm 86
'West-like'	High	1248 \pm 79
	Average	1211 \pm 82
	Low	1162 \pm 79
'Poison'	High	1105 \pm 95
	Average	1021 \pm 144
	Low	760 \pm 179

8.3.4 Elasticity analysis

Using the baseline parameter values, the stochastic population growth rate for East Hungary was estimated as $r=0.1092$, meaning a 11.5% annual increase (calculated as $\lambda = \exp(0.1092) = 1.115$). Elasticity analysis showed that breeding failure and mortality of nesting birds have the highest impact on the population growth rate, with a 10% change in the parameter values inducing 0.0067 (breeding failure) and 0.0065 (nesting mortality) negative change in r , followed by the impact of '1cy mortality' ($\Delta r = -0.0059$), '2cy mortality' ($\Delta r = -0.0023$) and '3cy floater mortality' ($\Delta r = -0.0013$) (Figure 8.5). Translating the change in r to a change in the annual growth rate λ , we get that a 10% increase in breeding failure (baseline value = 32.2%)—while holding the other demographic parameters on their baseline values—results in a decrease of λ by 0.7% (calculated as $\exp(-0.0067) = 0.993$). Similarly, a 10% increase in nesting mortality (baseline value = 8.4%) also leads to a 0.7% decrease in λ , and the same proportional increase in '1cy mortality' (baseline value = 30%) causes a 0.6% decline in λ . In comparison, a 10% increase in '2cy mortality' (baseline value = 15%) is expected to decrease λ by only 0.2% and the same proportional increase in '3cy floater' mortality (baseline value = 9.3%) causes only a 0.1% decline in λ .

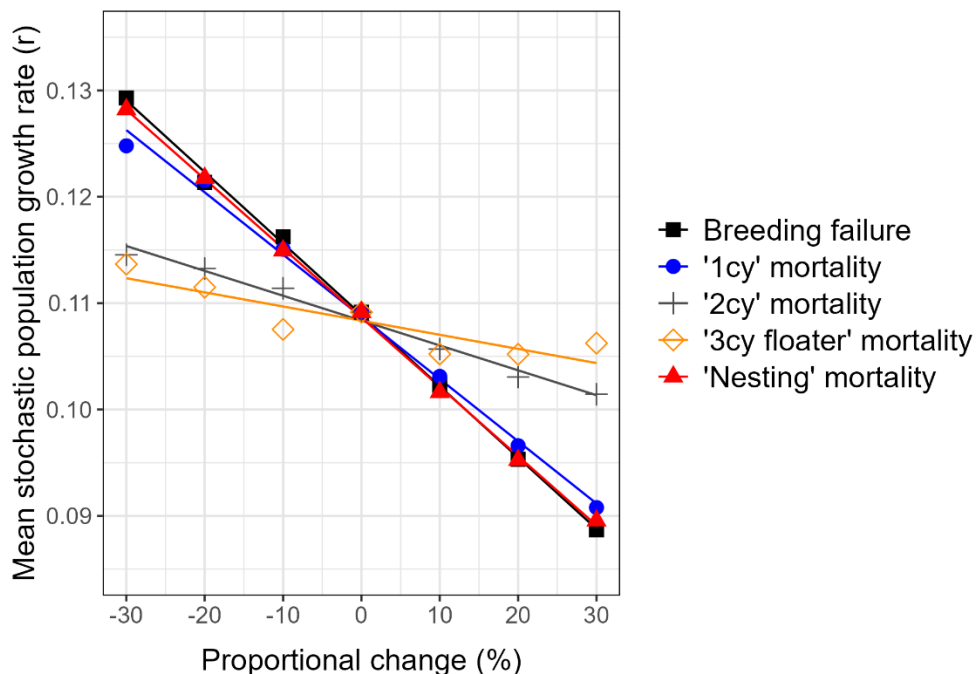


Figure 8.5. Results of the elasticity analysis showing the variation in the mean stochastic population growth rate (r) resulting from the proportional changes in breeding failure, '1cy' mortality, '2cy' mortality, '3cy floater' mortality and 'nesting' mortality in the imperial eagle in East Hungary.

8.4 Discussion

In the context of a PVA, we constructed a population dynamics model for the imperial eagle, which allowed us to explore the possible values of currently uncertain or unknown demographic parameters of the Pannonian population, to investigate the impact of changes in breeding failure and mortality, and to give predictions for future population growth.

In our models, we used the observed values of breeding success and brood sizes and estimates of mortality, dispersal rates and age-specific nesting probabilities based on data from 1980–2022. According to previous results on the high philopatry of the species [25–27, 229, 230, 241] ([SECTION 7](#)), we considered the Pannonian population closed, with no immigration from other distant populations. Considering that we managed to reproduce the observed population growth for this period under such assumptions, we conclude that the Pannonian population is self-sustaining and were able to recover from the severe bottleneck it experienced in the previous century [22, 36] without any significant influx from other populations.

As we had highly uncertain estimates for dispersal rate and the mortalities of immature birds, we conducted sensitivity tests in VORTEX for East Hungary to determine which values of these parameters would most closely reproduce the observed population trends. For dispersal rate, this meant a 3% net emigration from East Hungary into other parts of the Pannonian population. Considering that East Hungary constituted, on average, 60% of the population in the studied years and assuming equal rates of dispersal across the Region, this represents a ~9% dispersal rate between East Hungary and other areas. This rate is plausible, considering our previous minimum and maximum estimates of 4.3% and 11% of dispersal from East Hungary to the West. In the case of mortalities, predicted population growth showed the best fit to the observed trend with 30% 1cy, 15% 2cy and 9.3% 3cy (floater) mortalities. These 1cy and 2cy mortalities are the minimum estimates for the parameters and are considered quite low compared to other estimates for the imperial eagle (50% and 27.7% [104]; 40.9% and 16.7% [258]). However, there are examples of similar estimates from other raptors [42, 112, 156, 259].

An important consideration when interpreting these results is that second-year mortality and dispersal rate cannot be separated, as only 2cy birds were allowed to disperse in this model. This means that there are several other parameter combinations which could produce the same population trend (e.g. increased 2cy mortality can be compensated by decreased net emigration). Additionally, an approximately 4 percentage point annual variation was also applied to all mortality rates to account for environmental stochasticity,

thus the actual mortality rates could be both lower and higher in different years of the simulation. This environmental variation together with demographic stochasticity decreased the stochastic r compared to the deterministic calculations, as expected. Also note that mortality rates were estimated for 2011–2022, a period which included years with the highest rates of poisoning known from the last four decades [43, 199]. This implies that these minimum estimates originate from a period of elevated mortalities and mortality rates in other years of 1980–2022 may have been lower.

Results of the model simulating past population trends of the two subpopulations suggest that in the Western areas and in the Eastern areas outside of Hungary—mainly in East Slovakia, as Romania and Serbia harbours only a few pairs—mortality rates may be higher than in East Hungary. One possible explanation for this would be higher levels of anthropogenic threats in these areas, mainly persecution (poisoning or shooting) or electrocution. This concept is supported by the observation that population growth rates in these parts accelerated at the time of the PannonEagle LIFE conservation project, which mainly focused on the prevention of persecution activities [43]. Another reason behind higher mortality rates in the Western subpopulation and East Slovakia could be lower habitat quality due to the hillier landscape [38]. However, in case these habitats are lower quality, it is not reflected in the reproductive parameters, as the documented breeding success and productivity data are similar across the whole region.

Alternatively, it could occur that mortality rates—primarily immature mortalities—are actually higher in East Hungary than we estimated, and instead, a higher rate of dispersal from other parts of the Pannonian population is responsible for the enhanced growth rate in East Hungary compared to other areas. The Great Plain in East Hungary could be attracting many birds to settle, considering it is the largest contiguous area of suitable habitats in the region. However, current GPS tracking data suggest that birds in the West are also philopatric to their natal areas [25], implying low rates of dispersal between the subpopulations. Overall, we advocate for further research on mortality and dispersal rates in other areas of the Pannonian Region as well.

Our elasticity analysis suggests that breeding failure, mortality of nesting birds and mortality of 1cy birds have the highest impact on population growth rate. Mortality of breeding adults is generally considered the most important parameter in the population dynamics of long-lived birds with deferred maturity and low fecundity, such as raptors [41]. A similar analysis on imperial eagles also found adult survival to be the most influential factor, but noted that even when the survival of breeding birds is high, changes in other demographic parameters are still highly consequential to population growth and

persistence [106]. The annual mortality of nesting birds is an important factor as it applies to the population's reproducing individuals over many years, directly influencing reproductive output. Similarly, breeding failure also directly impacts fecundity, and first-year mortality is another parameter that affects all produced individuals. Changes in breeding failure (or success) usually have lower impact on the population growth rate of raptors compared to changes in mortality [17, 19, 106, 235, 239, 240]. We think that the high importance of breeding failure in this population is the consequence of its high rate (~32%) relative to mortality rates (only 30% for 1cy and ~8% for nesting birds) and the high mean productivity (~1.8 chicks per successful pairs). Based on these results, preventing breeding failure (e.g. by minimising human disturbance during breeding) and reducing anthropogenic mortality risks for nesting and juvenile birds should remain in the focus of imperial eagle conservation.

A potentially important factor which was not considered in the models but may influence the survivorship and reproductive output of nesting birds is senescence i.e. the decline in survival probability and reproductive performance with age [260], attributable to the accumulation of somatic damage and/or mutations over time [261]. Only few studies have explored senescence in raptors [107, 119, 260, 262, 263], as studying age-specific survival and reproduction in long-lived species is a daunting task, especially because the exact age of adult birds is often unknown. Age-related reductions in survival and reproductive success can also be accelerated by chronic lead poisoning, which occurs in raptors that are exposed to non-lethal concentrations of lead for a prolonged time, for example, via scavenging on carcasses that contain ammunition remains [264–268]. Considering the importance of nesting bird mortality for the population dynamics of the imperial eagle, investigating senescence in adult birds could be a key direction for future research.

Our projections for future population growth in East Hungary indicate a viable population. Assuming similar productivity values as observed in 2013–2022, the population may only show a decreasing trend if current mortality rates were increased by more than 83%. This would mean mortalities of approximately 55% in the first year, 27.5% in the second and 15.4% for nesting individuals. With the current mortality rates, the population is expected to reach carrying capacity in the next decade, as long as it maintains the current reproductive output.

As the population approaches carrying capacity, density-dependent effects on demographic parameters such as survival and fertility are expected to come into play [248]. Since no such effect was detectable at the time of the study—neither in the

population growth rate, nor in reproductive output [30]—we tried modelling a realistic density-dependency of mortality and reproduction, by applying a monotonous reduction in entry probabilities (resulting in a prolonged floater period) and first-year survival (which can also be interpreted as density-dependent fertility) with growing population size. Since the population is still expanding, we expect to observe density-dependent effects in the upcoming years, which will enable a more accurate modelling of these effects.

Carrying capacity is another parameter that should be estimated more accurately in the future. Here, we used a relatively simple method, by assessing the relationship between nest density and the coverage of potentially suitable areas, taking the high-density Heves county as a reference for extrapolation to other regions. This may have resulted in an overestimation of carrying capacity: the high density in Heves is probably supported by high quality habitats, whereas in other regions, habitats may be less suitable, either due to differences in prey availability, less nesting opportunities or more anthropogenic threats [133]. For instance, a recent study suggested that about third of the Hungarian Great Plain is likely suboptimal for nesting due to the existing infrastructure network [245]. Therefore, a more complex approach to estimating habitat suitability would be beneficial in the future for more accurate predictions for population size.

For this PVA, we utilised data and knowledge acquired over 40 years of monitoring the imperial eagle in the Pannonian Region. We had the opportunity to use highly accurate data on the number of nesting pairs and productivity, as well as reliable estimates of mortality rates for nesting birds in East Hungary. In conclusion, the Pannonian population appears to be self-sustaining, reinforcing previous evidence which supports its recognition as a separate conservation unit. Overall, the population is considered viable and is expected to increase under the current values of demographic parameters, but maintaining low levels of persecution should remain in the focus of conservation. Additionally, further investigation into mortality and dispersal rates beyond East Hungary would be essential for a more accurate assessment of the population's status and conservation needs.

9 GENERAL DISCUSSION

The Pannonian population holds particular importance for the conservation of the imperial eagle, owing to its positive population trend and peripheral placement in the species' range [1, 11, 25]. Thanks to the continuous and extensive monitoring over the last four decades, we were able to address knowledge gaps on survival rates, natal dispersal behaviour and population dynamics.

Adult survival is a highly influential demographic parameter in raptors [40, 41, 106], as also indicated by our elasticity analysis. We estimated >90% mean annual survival for breeding birds in East Hungary, a value typical of a healthy population of large raptors [42]. However, we also found some evidence for the negative relationship between poisoning rates and adult survival. Mortality rates were estimated to have risen by ~60% at peak poisoning activity compared to years with minimal poisoning—an increase that, if affecting all age classes, could significantly reduce the population growth rate in the future, as indicated by our PVA projections. Poisoning also seemed to have a higher impact on male survival, likely due to their increased foraging activity during the chick-rearing period, when most poisoning incidents were recorded [43]. These results offer valuable insight into the impact of poisoning on survival—an area that, despite its importance for raptor conservation [108, 109, 113], is still insufficiently understood.

Natal dispersal behaviour also plays a key role in population dynamics [45]. Our findings confirmed the expected female-biased natal dispersal, consistent with both theory and empirical evidence from birds [167, 184, 188]. This sex bias could function as a mechanism of inbreeding avoidance [188], potentially contributing to the maintenance of the previously reported high genetic diversity despite the former bottleneck [12]. The males' shorter dispersal underlines their importance in colonisation dynamics, especially if we consider their potential vulnerability to poisoning. The correlation between natal and breeding site densities is rarely examined [53], but here we showed that although imperial eagles generally disperse toward lower density areas compared to their natal site, they are also attracted to the presence of conspecifics.

Conservation relevance

The detected high philopatry and PVA-based evidence of self-sustainability together suggest that the Pannonian population is largely isolated. Such isolation increases the risk of inbreeding and, consequently, inbreeding depression, which may manifest as reduced survival or hatching success, ultimately lowering the average fitness of the population [269]. A study in Kazakhstan found increased genome-wide heterozygosity in adult imperial eagles compared to juveniles, indicating that increased homozygosity—a direct consequence of inbreeding—may reduce the likelihood of surviving to adulthood [270].

However, considering the continued expansion of the Pannonian population, its isolation and the associated risk of inbreeding is expected to lessen over time. The southward expansion towards Serbia promotes future connectivity with the nearest populations in Bulgaria and North Macedonia. Serbia contains extensive potential habitat for imperial eagles, currently mostly unoccupied following the population's extinction in the last century. Recent conservation measures are promising, since as of 2023, five breeding pairs have already appeared in the country. Strengthening habitat protection and species conservation in Serbia is, therefore, crucial for supporting population recovery and interconnectedness. In addition, the establishment of stepping-stone populations in these areas may also accelerate the population's expansion and enhance long-term connectivity.

When connecting these populations, a potential concern is that their genetic isolation may have led to local adaptations, which could cause individuals of mixed origin to have reduced fitness, a phenomenon called outbreeding depression. However, outbreeding depression is generally not a concern in same-species populations from similar environments [271]. Additionally, the species' high flexibility in prey selection suggests a strong capacity to adapt to environmental changes [35].

While the Pannonian population is highly viable at the current demographic parameters, population growth may be hindered by elevated levels of poisoning activity. Thus, we advocate for the continued practice of the successful anti-poisoning measures implemented by Helicon LIFE and PannonEagle LIFE projects in Hungary and in the neighbouring countries as well. In addition, monitoring should focus on any possible source-sink dynamic between the two subpopulations, as highlighted by the potentially unbalanced dispersal rates and the possible mortality differences suggested by the PVA.

Furthermore, as an umbrella species, every conservation action targeting the imperial eagle—such as anti-poisoning initiatives and habitat protection—also benefits a wide range of raptor and prey species, thereby supporting overall ecosystem health.

Future research directions

During the PVA, we assumed that mortality factors (e.g. poisoning) would impose the same proportional change on the mortalities of immature as on breeding birds. However, further research is needed to understand how young survival responds to anthropogenic threats, especially considering the high impact of juvenile mortality on population growth. Weather conditions can also affect survival and reproductive success in raptors [272–274], and adverse weather has been shown to increase chick mortality in the imperial eagle [275]. Studying the effects of extreme weather on demographic rates would be important, as such events are becoming more frequent due to climate change [276].

Further research on what constitutes a suitable habitat for imperial eagles would also be beneficial [245]. Combined with results on natal dispersal patterns, such knowledge could be used for predicting where new territories are likely to be established [245, 277, 278], aiding in the future monitoring of this rapidly growing population.

The next decades' studies should answer the following questions of density dependence: will the age of first breeding show positive density dependence, indicating that the probability of entry to the nesting stage decreases with density? Will the floater-to-breeder ratio grow close to 1:1 as expected for a healthy raptor population at saturation [253]? Will juvenile survival or dispersal probability and distance show density-dependent changes? What is the carrying capacity for the Eastern and Western parts of the Pannonian population?

PVA results for the two subpopulations highlight the importance of further studies on demographic rates in the Western subpopulation and East Slovakia, mainly mortality and dispersal rates. Dispersal studies may also be supported by further population genetic analyses [12], for which our microsatellite marker set may prove valuable.

Although our study focused primarily on the imperial eagle, we hope that our findings will also contribute to the conservation of other raptors and will provide useful information for the fields of population ecology, evolutionary biology and behavioural ecology as well.

10 NEW SCIENTIFIC RESULTS

METHODOLOGICAL RESULTS

1. **Microsatellite markers** of the white-tailed eagle (*Haliaeetus albicilla*) and the Japanese golden eagle (*Aquila chrysaetos japonica*) can be utilised for the individual identification of the imperial eagle ([SECTION 5](#)).
2. We assembled the **highest resolution microsatellite marker set** currently available for the imperial eagle, enabling more reliable individual identification and relatedness estimation in the future ([SECTION 5](#)).

ECOLOGICAL RESULTS

3. Using a mark-recapture method based on genetic identification from shed feathers, we managed to estimate the **annual survival probabilities** of both male and female breeding birds in East Hungary for 2011–2022 ([SECTION 6](#)).
4. We found moderate evidence that **poisoning activity may lead to a male-biased mortality** in breeding birds, probably attributable to the behavioural differences of the sexes ([SECTION 6](#)).
5. We report that **natal dispersal distances** in the imperial eagle are female-biased ([SECTION 7](#)).
6. Our study on the **density dependence of natal dispersal** suggests that both competition avoidance and conspecific attraction influence settlement decisions in the imperial eagle ([SECTION 7](#)).

MODELLING RESULTS

7. We constructed a population model which, using realistic values of demographic parameters, successfully reproduced the population growth observed in the Pannonian Region since 1980. This implies that the **Pannonian population is self-sustaining**, as its recovery following the bottleneck in the 20th century was possible without significant immigration from other distant populations ([SECTION 8](#)).
8. Results of the PVA suggest that **mortality rates** in the Western subpopulation and East Slovakia may be higher than in East Hungary ([SECTION 8](#)).
9. Based on elasticity analyses, proportional changes in breeding failure, nesting mortality and first-year mortality have the **highest impact on population growth rates** ([SECTION 8](#)).
10. The population in East Hungary increases by 11.5% each year and is expected to **reach the carrying capacity by 2038**. Increase in mortality rates due to elevated poisoning activity would hinder population growth rates, but with the current values of reproductive parameters, the population is not expected to decline up to an 83% increase in mortality rates across all age groups ([SECTION 8](#)).

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12 THE AUTHOR'S PUBLICATIONS

12.1 Full-text publications in peer-reviewed journals with an impact factor

Zsinka B, Vili N, Szabó K, Tisza Á, Csonka V, Pásztor-Kovács S (2024) Mikroszatellita-markerkészlet fejlesztése parlagi sasok (*Aquila heliaca*) egyedi azonosításához rokon fajokban leírt markerek segítségével. *Magy Állatorvosok Lapja* 146:357–365.

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12.2 Oral presentations at international and Hungarian conferences

Csonka V, **Zsinka B**, Horváth M, Vili N (2021) The effect of sex and local density on the natal dispersal of eastern imperial eagles in the Carpathian Basin. *Magyar Etológiai Társaság XXIII. Konferenciája*, Budapest, Hungary, 26-27 November 2021.

Zsinka B, Kövér S, Csonka V, Vili N, Szabó K, Fatér I, Juhász T, Horváth M, Pásztor-Kovács S (2023) Genetic monitoring of Imperial Eagles in the Pannon region. *PannonEagle LIFE Closing Conference*, Jászberény, Hungary, 27 January 2023.

Zsinka B, Horváth M, Pásztor-Kovács S, Kövér S (2024) A parlagi sas (*Aquila heliaca*) populációéletképességi elemzése a Pannon régióban. In Lőrinczi G, Tölgyesi C (eds.): 13. Magyar Ökológus Kongresszus – Előadások és poszterek összefoglalói (p. 88.). Szeged, Hungary, 21-23 August 2024.

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12.3 Poster presentations at international and Hungarian conferences

Zsinka B, Kövér S, Pásztory-Kovács S, Vili N, Szabó K, Fatér I, Horváth M (2022) Survival estimation of eastern imperial eagles (*Aquila heliaca*) in Hungary with a genetic-based mark-recapture method. In Zasadil P, Ludvíková V, Báldi A (eds.): Book of Abstracts – 6th European Congress of Conservation Biology. Prague, Czech Republic, 22 – 26 August 2022.

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12.4 Publications not related to the topic of the thesis

Kristensen AU, **Zsinka B**, Lang Z, Hetényi N (2023) Survey of the Husbandry, Health, and Welfare of Norwegian Pet Rabbits. *J. Adv. Vet. Res.* 15:767-775.

Gilián LD, Endrédi A, **Zsinka B**, Neményi A, Nagy JG (2019) Morphological and reproductive trait-variability of a food deceptive orchid, *Cephalanthera rubra* along different altitudes. *Appl. Ecol. Environ. Res.* 17:5619-5639.

Nagy JG, **Zsinka B**, Verebélyi V, Zorkóczy OK, Tyler T (2017) A *Vaccinium microcarpum* (Turcz. ex Rupr.) Schmalh. Magyarországon. *Kitaibelia* 22: 71-76.

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Zsinka B, Báldi A, Vajna F, Balogh E, Palotás B, Mázsa K (eds.) (2023): 8th Student Conference on Conservation Science, Balatonvilágos 2023: SCCS Europe - Connecting Eastern and Western Europe in conservation biology Abstracts. Balatonvilágos, Hungary, 13 – 16 September 2023.

13 APPENDIX

Table A1. (SECTION 6) *Microsatellite marker set used for the individual identification of breeding eastern imperial eagles in East Hungary between 2011 and 2022. Na: number of alleles, Ho: observed heterozygosity, He: expected heterozygosity, p-value of the Hardy-Weinberg equilibrium test and probability of identity values PI and P_{SIB} for the full marker set. We found deviations from the Hardy-Weinberg equilibrium in the case of loci Aa35, Aa36 and Aa43.*

Locus	Source	Na	Ho	He	p-value
Aa02	[79]	6	0.8228	0.7674	0.2370
Aa35	[79]	10	0.8211	0.8184	0.0000
Aa36	[79]	6	0.7023	0.7653	0.0014
Aa39	[79]	9	0.7631	0.7628	0.1013
Aa43	[79]	8	0.5101	0.5164	0.0265
IEAAAG09	[78]	4	0.5565	0.5548	0.0580
IEAAAG11	[78]	5	0.6815	0.6918	0.2591
Hal04	[85]	5	0.7482	0.7291	0.0778
Hal10	[85]	5	0.6415	0.6102	0.3394

PI = 9.5×10^{-9} , P_{SIB} = 5.7×10^{-4}

Table A2. (SECTION 6) Apparent survival probabilities (ϕ) of breeding eastern imperial eagles in East Hungary estimated from the unconstrained time model $\{\phi(\text{sex} \times \text{time}), p(\text{sex} \times \text{time})\}$. Estimates for the last interval of the study (2021–2022) are not provided since they cannot be estimated separately from encounter probabilities in models where both survival and encounter are time-dependent.

Parameter	Sex	Estimate (ϕ)	SE (ϕ)	95% CI (ϕ)
$\phi_{2011-2012}$	Male	0.7802	0.1147	0.4890 – 0.9294
	Female	0.9403	0.0546	0.7009 – 0.9906
$\phi_{2012-2013}$	Male	0.8066	0.1002	0.5420 – 0.9363
	Female	0.8665	0.0532	0.7249 – 0.9412
$\phi_{2013-2014}$	Male	0.8826	0.0824	0.6126 – 0.9728
	Female	0.9554	0.0394	0.7779 – 0.9924
$\phi_{2014-2015}$	Male	1.0000	0.0000	1.0000 – 1.0000
	Female	0.8910	0.0407	0.7825 – 0.9490
$\phi_{2015-2016}$	Male	0.9101	0.0791	0.6036 – 0.9854
	Female	0.9658	0.0348	0.7819 – 0.9955
$\phi_{2016-2017}$	Male	1.0000	0.0000	1.0000 – 1.0000
	Female	0.8937	0.0401	0.7863 – 0.9505
$\phi_{2017-2018}$	Male	0.7716	0.1186	0.4745 – 0.9267
	Female	0.8539	0.0463	0.7384 – 0.9237
$\phi_{2018-2019}$	Male	1.0000	0.0000	1.0000 – 1.0000
	Female	0.9671	0.0616	0.3971 – 0.9992
$\phi_{2019-2020}$	Male	1.0000	0.0000	1.0000 – 1.0000
	Female	0.9175	0.0697	0.6466 – 0.9854
$\phi_{2020-2021}$	Male	1.0000	0.0002	0.9996 – 1.0000
	Female	1.0000	0.0003	0.9995 – 1.0000

Table A3. (SECTION 6) Model-averaged estimates of apparent survival probabilities (ϕ) of breeding eastern imperial eagles in East Hungary. Only models with $p \sim \text{sex} \times \text{time}$ ($n = 8$) were included in the averaging. Models were adjusted for overdispersion with estimated $\hat{c} = 1.148$.

Parameter	Sex	Estimate (ϕ)	SE (ϕ)	95% CI (ϕ)
$\phi_{2011-2012}$	Male	0.8648	0.0828	0.6149 – 0.9625
	Female	0.9057	0.0272	0.8371 – 0.9472
$\phi_{2012-2013}$	Male	0.8959	0.0309	0.8179 – 0.9428
	Female	0.9115	0.0150	0.8774 – 0.9368
$\phi_{2013-2014}$	Male	0.9182	0.0165	0.8794 – 0.9453
	Female	0.9174	0.0091	0.8976 – 0.9336
$\phi_{2014-2015}$	Male	0.9150	0.0155	0.8792 – 0.9409
	Female	0.9164	0.0090	0.8969 – 0.9325
$\phi_{2015-2016}$	Male	0.9284	0.0237	0.8659 – 0.9631
	Female	0.9211	0.0130	0.8916 – 0.9431
$\phi_{2016-2017}$	Male	0.9269	0.0225	0.8688 – 0.9604
	Female	0.9204	0.0120	0.8935 – 0.9410
$\phi_{2017-2018}$	Male	0.9288	0.0243	0.8639 – 0.9641
	Female	0.9212	0.0136	0.8901 – 0.9440
$\phi_{2018-2019}$	Male	0.9069	0.0185	0.8637 – 0.9374
	Female	0.9142	0.0111	0.8899 – 0.9336
$\phi_{2019-2020}$	Male	0.9110	0.0161	0.8741 – 0.9379
	Female	0.9153	0.0099	0.8938 – 0.9327
$\phi_{2020-2021}$	Male	0.9291	0.0242	0.8645 – 0.9641
	Female	0.9214	0.0135	0.8905 – 0.9441
$\phi_{2021-2022}$	Male	0.9279	0.2196	0.0203 – 0.9999
	Female	0.9205	0.2015	0.0497 – 0.9996

Table A4. (SECTION 6) Results of the \hat{c} sensitivity test: model selection results of Cormack-Jolly-Seber models estimating annual apparent survival (ϕ) and encounter probability (p) for breeding eastern imperial eagles in East Hungary, 2011–2022, in the case of $\hat{c} = 1$ (no adjustment for overdispersion) and $\hat{c} = 1.3$. Poisoning rate was calculated as the number of poisoned imperial eagles found / number of nesting individuals $\times 100$. (Q)AICc: (Quasi) Akaike Information Criterion, $\Delta(Q)AICc$: difference in the (Q)AICc values of the model and the most-supported model, w_i : model weight, K : model parameter count. Models receiving little support ($w_i < 0.01$) are not shown, except for the general model $\{\phi(\text{sex} \times \text{time}), p(\text{sex} \times \text{time})\}$. Models in yellow indicate the four most-supported models ($\Delta(Q)AICc < 2$) from the model selection with estimated $\hat{c} = 1.148$.

$\hat{c} = 1$						
Model	AIC _c	ΔAIC_c	w_i	K	Deviance	
$\{\phi(\text{sex} \times \text{poison}), p(\text{sex} \times \text{time})\}$	3724.22	0.00	0.29	26	1190.56	
$\{\phi(\cdot), p(\text{sex} \times \text{time})\}$	3724.31	0.09	0.27	23	1196.86	
$\{\phi(\text{poison}), p(\text{sex} \times \text{time})\}$	3724.81	0.59	0.21	24	1195.29	
$\{\phi(\text{sex}), p(\text{sex} \times \text{time})\}$	3726.18	1.96	0.11	24	1196.66	
$\{\phi(\text{sex} + \text{poison}), p(\text{sex} \times \text{time})\}$	3726.66	2.44	0.08	25	1195.07	
$\{\phi(\cdot), p(\text{sex} + \text{time})\}$	3730.69	6.47	0.01	13	1223.73	
$\{\phi(\text{poison}), p(\text{sex} + \text{time})\}$	3731.58	7.37	0.01	14	1222.59	
$\{\phi(\text{sex}), p(\text{sex} + \text{time})\}$	3731.84	7.62	0.01	14	1222.84	
$\{\phi(\text{sex} \times \text{time}), p(\text{sex} \times \text{time})\}$	3749.16	24.94	0.00	44	1177.76	
$\hat{c} = 1.3$						
Model	QAICc	$\Delta QAICc$	w_i	K	Deviance	
$\{\phi(\cdot), p(\text{sex} \times \text{time})\}$	2875.64	0.00	0.18	23	920.66	
$\{\phi(\cdot), p(\text{sex} + \text{time})\}$	2875.82	0.18	0.16	13	941.33	
$\{\phi(\text{poison}), p(\text{sex} \times \text{time})\}$	2876.50	0.86	0.11	24	919.45	
$\{\phi(\text{poison}), p(\text{sex} + \text{time})\}$	2876.98	1.33	0.09	14	940.45	
$\{\phi(\text{sex} \times \text{poison}), p(\text{sex} \times \text{time})\}$	2877.00	1.36	0.09	26	915.82	
$\{\phi(\text{sex}), p(\text{sex} + \text{time})\}$	2877.17	1.53	0.08	14	940.65	
$\{\phi(\text{sex}), p(\text{sex} \times \text{time})\}$	2877.56	1.91	0.07	24	920.51	
$\{\phi(\text{sex} + \text{poison}), p(\text{sex} + \text{time})\}$	2878.33	2.69	0.05	15	939.76	
$\{\phi(\text{sex} + \text{poison}), p(\text{sex} \times \text{time})\}$	2878.40	2.76	0.04	25	919.28	
$\{\phi(\text{sex} \times \text{poison}), p(\text{sex} + \text{time})\}$	2879.27	3.63	0.03	16	938.66	
$\{\phi(\text{sex} \times \text{time}), p(\text{sex} \times \text{time})\}$	2904.90	29.26	0.00	44	905.97	

Increasing the overdispersion parameter introduced only minor changes to the model selection results. Models with $p(\text{sex} + \text{time})$ gained more support when $\hat{c} = 1.3$ compared to $\hat{c} = 1.148$. This is because model selection favours models with fewer parameters when overdispersion is higher, but based on the proportion of males and females sampled each year, we consider $p(\text{sex} \times \text{time})$ a more realistic assumption than $p(\text{sex} + \text{time})$. Since the relative order of models with $p(\text{sex} \times \text{time})$ did not change, we draw the same conclusions regarding the relationships between survival and the studied variables.

Table A5. (SECTION 6) Results of the \hat{c} sensitivity test: model-averaged estimates of apparent survival probabilities (ϕ) of breeding eastern imperial eagles in East Hungary. Only models with $p \sim \text{sex} \times \text{time}$ ($n = 8$) were included in the averaging. Models were not adjusted for overdispersion ($\hat{c} = 1$).

Parameter	Sex	Estimate (ϕ)	SE (ϕ)	95% CI (ϕ)
$\phi_{2011-2012}$	Male	0.8534	0.0882	0.5939 – 0.9586
	Female	0.9058	0.0266	0.8394 – 0.9465
$\phi_{2012-2013}$	Male	0.8920	0.0322	0.8109 – 0.9408
	Female	0.9116	0.0147	0.8782 – 0.9365
$\phi_{2013-2014}$	Male	0.9193	0.0166	0.8803 – 0.9463
	Female	0.9175	0.0087	0.8988 – 0.9330
$\phi_{2014-2015}$	Male	0.9154	0.0153	0.8801 – 0.9410
	Female	0.9165	0.0086	0.8981 – 0.9318
$\phi_{2015-2016}$	Male	0.9315	0.0244	0.8655 – 0.9663
	Female	0.9212	0.0126	0.8927 – 0.9426
$\phi_{2016-2017}$	Male	0.9296	0.0231	0.8687 – 0.9635
	Female	0.9205	0.0116	0.8947 – 0.9405
$\phi_{2017-2018}$	Male	0.9318	0.0252	0.8626 – 0.9675
	Female	0.9212	0.0134	0.8906 – 0.9438
$\phi_{2018-2019}$	Male	0.9055	0.0187	0.8621 – 0.9363
	Female	0.9144	0.0108	0.8906 – 0.9333
$\phi_{2019-2020}$	Male	0.9106	0.0159	0.8741 – 0.9372
	Female	0.9154	0.0096	0.8947 – 0.9323
$\phi_{2020-2021}$	Male	0.9322	0.0250	0.8637 – 0.9676
	Female	0.9215	0.0133	0.8912 – 0.9440
$\phi_{2021-2022}$	Male	0.9305	0.2687	0.0039 – 1.0000
	Female	0.9203	0.2468	0.0156 – 0.9999

Table A6. (SECTION 6) Results of the \hat{c} sensitivity test: model-averaged estimates of apparent survival probabilities (ϕ) of breeding eastern imperial eagles in East Hungary. Only models with $p \sim \text{sex} \times \text{time}$ ($n = 8$) were included in the averaging. Models were adjusted for overdispersion with $\hat{c} = 1.3$.

Parameter	Sex	Estimate (ϕ)	SE (ϕ)	95% CI (ϕ)
$\phi_{2011-2012}$	Male	0.8726	0.0781	0.6334 – 0.9645
	Female	0.9057	0.0279	0.8351 – 0.9480
$\phi_{2012-2013}$	Male	0.8986	0.0299	0.8232 – 0.9440
	Female	0.9115	0.0154	0.8764 – 0.9374
$\phi_{2013-2014}$	Male	0.9175	0.0167	0.8783 – 0.9449
	Female	0.9173	0.0096	0.8964 – 0.9343
$\phi_{2014-2015}$	Male	0.9147	0.0158	0.8781 – 0.9411
	Female	0.9163	0.0095	0.8956 – 0.9332
$\phi_{2015-2016}$	Male	0.9264	0.0231	0.8662 – 0.9607
	Female	0.9210	0.0134	0.8904 – 0.9435
$\phi_{2016-2017}$	Male	0.9250	0.0220	0.8689 – 0.9583
	Female	0.9203	0.0125	0.8922 – 0.9416
$\phi_{2017-2018}$	Male	0.9267	0.0237	0.8646 – 0.9616
	Female	0.9211	0.0139	0.8892 – 0.9444
$\phi_{2018-2019}$	Male	0.9078	0.0186	0.8644 – 0.9383
	Female	0.9142	0.0114	0.889 – 0.93410
$\phi_{2019-2020}$	Male	0.9113	0.0164	0.8736 – 0.9386
	Female	0.9152	0.0103	0.8928 – 0.9333
$\phi_{2020-2021}$	Male	0.9269	0.0236	0.865 – 0.96170
	Female	0.9212	0.0139	0.8894 – 0.9445
$\phi_{2021-2022}$	Male	0.9260	0.1871	0.056 – 0.99960
	Female	0.9206	0.1715	0.1044 – 0.9991

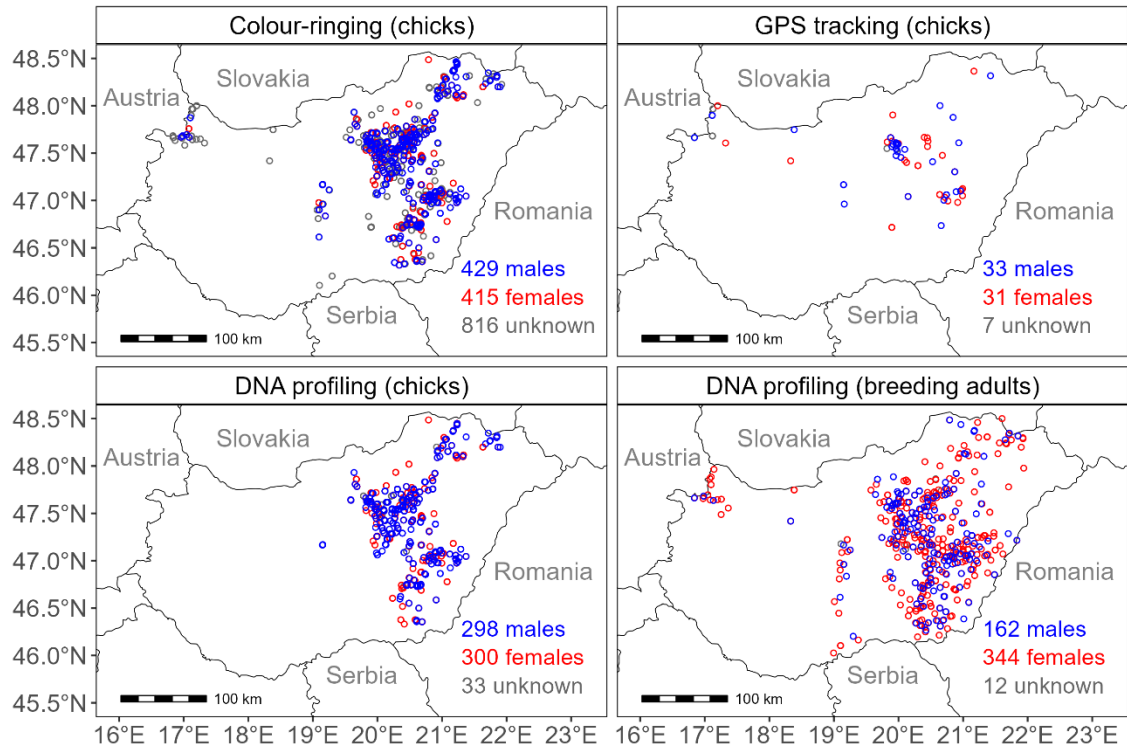


Figure A1. (SECTION 7) Locality of eastern imperial eagle chicks and breeding adults identified in Hungary between 2011 and 2022. Locality indicates natal nest for chicks and breeding site for breeding adults. Chicks were either marked through colour-ringing, GPS tracking, DNA profiling, or the combination of the above. Unknown sex means that molecular sexing was not carried out or was unsuccessful.

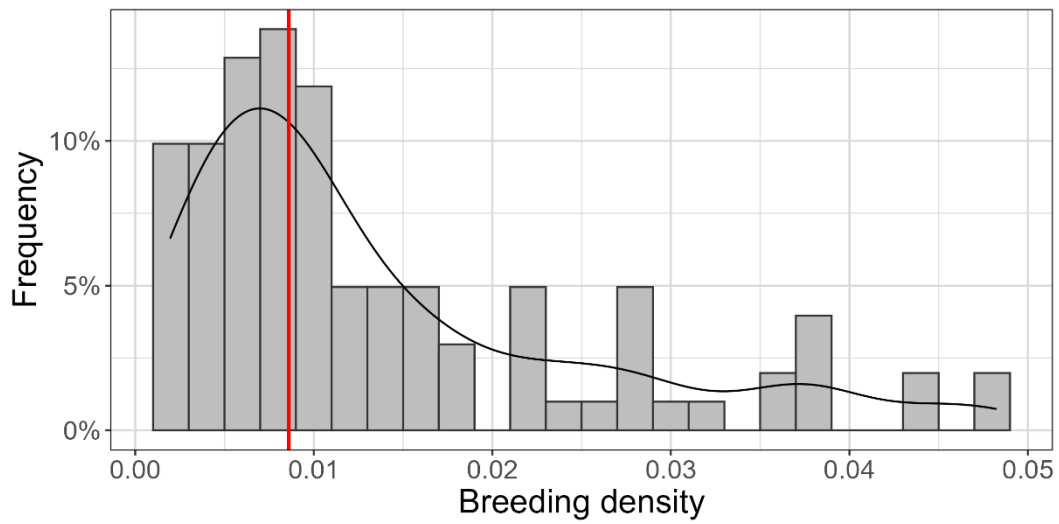


Figure A2. (SECTION 7) Distribution of breeding site densities. Estimated from our model of density difference, the red line represents the natal density value from which birds disperse in equal proportions to lower- and higher-density breeding sites.

Table A7. (SECTION 7) Estimates of the general linear mixed model investigating the relationship of natal dispersal distance (NDD, log-transformed) with sex ('male' as reference), natal density (log-transformed, scaled) and natal period (two-level factor, '2012–2014' as reference) in 38 male (**outlier male with NDD of 1.8 km excluded**) and 62 female eastern imperial eagles hatched in Hungary between 2012 and 2018. Natal territory ID and natal year (categorical) were set as crossed random intercepts. Effects in bold were significant ($p < 0.05$).

Response variable: log (NDD)						
Explanatory variables	Estimate	SE	95%CI	df	t-value	p-value
Intercept	3.395	0.135	[3.141, 3.646]	7.98	25.104	<0.0001
sex (female)	0.440	0.145	[0.145, 0.711]	76.5	3.024	0.0034
log (natal density)	-0.016	0.073	[-0.158, 0.126]	67.0	-0.215	0.8302
natal period (2015 – 2018)	0.383	0.160	[0.065, 0.683]	4.98	2.390	0.0626
Random effects		SD				
natal territory ID	0.336					
natal year	0.093					
Residual	0.605					

Table A8. (SECTION 7) Estimates of the general linear mixed model investigating the relationship of density difference ($\log(\text{breeding density}) - \log(\text{natal density})$) with sex ('male' as reference), natal density (log-transformed, scaled), natal dispersal distance (NDD, log-transformed, scaled) and natal period (two-level factor, '2012–2014' as reference) in 38 male (**outlier male with NDD of 1.8 km excluded**) and 62 female eastern imperial eagles hatched in Hungary between 2012 and 2018. Natal territory ID and natal year (categorical) were set as crossed random intercepts. Effects in bold were significant ($p < 0.05$).

Response variable: density difference ($\log(\text{breeding density}) - \log(\text{natal density})$)						
Explanatory variables	Estimate	SE	95%CI	df	t-value	p-value
Intercept	-0.518	0.144	[-0.798, -0.238]	89.6	-3.609	0.0005
sex (female)	0.062	0.170	[-0.271, 0.390]	92.6	0.366	0.7153
log (natal density)	-0.578	0.079	[-0.732, -0.423]	71.2	-7.289	<0.0001
log (NDD)	-0.253	0.092	[-0.432, -0.075]	94.6	-2.740	0.0073
natal period (2015 – 2018)	-0.116	0.165	[-0.438, 0.234]	95.0	-0.707	0.4810
Random effects		SD				
natal territory ID	0.240					
natal year	0.000					
Residual	0.732					

Table A9. (SECTION 8) Final input parameters (following the estimation of uncertain dispersal mortality rates using sensitivity tests) for the stage-structured PVA model 'East HU'. VORTEX incorporates 'stage-structure' by replacing the default 'age-structure'. Therefore, note that in the case of parameters marked with red, the value of 'age' refers to a 'stage' category, where 0 = '1cy', 1 = '2cy', 2 = '3cy floater', 3 = '4cy floater', 4 = '5cy+ floater' and 5 = 'nesting'.

Menu	Parameter	East Hungary		Comment	
Scenario Settings	No. of iterations	250			
	No. of years	43			
	Duration of years in days	365			
	Extinction definition	Only 1 sex remains		Default setting	
	Number of populations	2 (East Hungary and "Population 2", which served only as a placeholder to model emigration from East Hungary to other parts of the Pannonian Region)			
	Order of events in a Vortex year		EV		
			Breed		
			PSUpdate		
			rCalc		
			Census		
		Disperse			
		Mortality			
Species Description	Inbreeding depression	yes		Default setting	
	Lethal equivalents	6.29		Default setting	
	Percent due to recessive alleles	50		Default setting	
	EV correlation between reproduction and survival	0.5		Default setting	
	Sample EV from distribution, rather than binomial	no		Default setting	
	State Variables	Global State Variables	no		
Population State Variables		yes			
PE3		I:	0.09		
		T:	=0.09		
PE4		I:	0.65		
		T:	=0.65		
PE5		I:	0.71		
		T:	=0.71		
PE6	I:	1			
	T:	=1			

Table A9. (continued)

Menu	Parameter	East Hungary	Comment
State Variables	Individual State Variables	yes	
	RNUM	I: =RAND	
		B: =RAND	
		T: =RAND	
	AGE	I: =IF(RNUM<0.22;1; IF(RNUM<0.36;2; IF(RNUM<0.4;3; IF(RNUM<0.41;4;5)))	
T: =IF([AGE=5];5; IF([AGE=4]AND[RNUM<=PE6];5; IF([AGE=3]AND[RNUM<=PE5];5; IF([AGE=2]AND[RNUM<=PE4];5; IF([AGE=1]AND[RNUM<=PE3];5; IF([AGE=4];4;AGE+1))))			Function for progressing living individuals between stages based on PE entry probabilities
Dispersal	Age range: Youngest	1	'2cy'
	Age range: Oldest	1	'2cy'
	Dispersing sexes	both	
	% Survival of dispersers	100	
	Apply multiplier of	1	
	Fill matrix with	1	
	Percent of individuals in each age class that disperse between each pair of populations each year:		
	<i>from East Hungary to East Hungary</i>	97	
	<i>from East Hungary to "Population 2"</i>	3	
	<i>from "Population 2" to East Hungary</i>	0	
<i>from "Population 2" to "Population 2"</i>	100		
Reproductive System	Reproductive System	Long-term monogamy	
	Age of first offspring females	5	'nesting'
	Maximum age of female reproduction	5	'nesting'
	Age of first offspring males	5	'nesting'
	Maximum age of male reproduction	5	'nesting'
	Maximum lifespan	5	'nesting'
	Maximum no. of broods per year	1	
	Maximum no. of progeny per brood	3	
	Sex ratio at birth - in % males	50	
	Density dependent reproduction	no	

Table A9. (continued)

Menu	Parameter	East Hungary	Comment
Reproductive Rates	% adult female breeding	100	
	SD in % breeding due to EV	0	
	Percentage of 0 broods per year	=TIMESERIES (% of breeding failure each year)	
	Percentage of 1 broods per year	=100-breeding failure%	
	Percentage of 1 offspring per female per brood	=TIMESERIES(% of 1chick broods each year)	
	Percentage of 2 offspring per female per brood	=TIMESERIES(% of 2chick broods each year)	
	Percentage of 3 offspring per female per brood	=100-(1chick broods% + 2chick broods%)	
Mortality Rates	Mortality from age 0 to 1, both sexes	30	'1cy' mortality
	Mortality from age 1 to 2, both sexes	15	'2cy' mortality
	Mortality from age 2 to 3, both sexes	9.3	'3cy floater' mortality
	Mortality from age 3 to 4, both sexes	8.4	'4cy floater' mortality
	Mortality from age 4 to 5, both sexes	8.4	'5cy+ floater' mortality
	Annual mortality after age 5, both sexes	8.4	'nesting' mortality
	SD in mortality (all age groups)	4.3	
	Delay 1st year mortality until all annual mortality is done (rather than in Breed)	yes	
Catastrophes	No. of types of catastrophes	0	
Mate monopolization	Degree of monopolization of breeding opportunities - Males in breeding pool (both populations)	100	
	Initial Population Size	Use stable age distribution	Stable stage distribution was modelled in the Individual State Variable AGE.
Carrying Capacity	Initial population size	24	
	Carrying Capacity (K)	1310	
	SD in K due to EV	0	
	Implement K based on a limit on some other population variable other than N	yes	
	Population variable to be tested against K	=PAIRS	
Harvest	Populations harvested?	no	
Supplement	Populations supplemented?	no	

Table A10. (SECTION 8) Final input parameters (following the estimation of uncertain mortality rates using elasticity tests) for the stage-structured PVA model 'East-West'. The table includes only input parameters that were modified compared to the original 'East HU' model. VORTEX incorporates 'stage-structure' by replacing the default 'age-structure'. Therefore, note that in the case of parameters marked with red, the value of 'age' refers to a 'stage' category, where 0 = '1cy', 1 = '2cy', 2 = '3cy floater', 3 = '4cy floater', 4 = '5cy+ floater' and 5 = 'nesting'.

Menu	Parameter	Both populations	East	West	Comment
Dispersal	Percent of individuals in each age class that disperse between each pair of populations each year:				
	<i>from East to East</i>	95.7			
	<i>from East to West</i>	4.3			
	<i>from West to East</i>	4.3			
	<i>from West to West</i>	95.7			
Reproductive Rates	Percentage of 0 broods per year	=TIMESERIES (% of breeding failure each year)			Based on different datasets for East and West.
	Percentage of 1 broods per year	=100-breeding failure%			
	Percentage of 1 offspring per female per brood	=TIMESERIES(% of 1chick broods each year)			
	Percentage of 2 offspring per female per brood	=TIMESERIES(% of 2chick broods each year)			
	Percentage of 3 offspring per female per brood	=100-(1chick broods% + 2chick broods%)			
Mortality Rates	Mortality from age 0 to 1, both sexes		31.5	40.5	'1cy' mortality
	Mortality from age 1 to 2, both sexes		15.8	20.3	'2cy' mortality
	Mortality from age 2 to 3, both sexes		9.7	12.5	'3cy floater' mortality
	Mortality from age 3 to 4, both sexes		8.8	11.3	'4cy floater' mortality
	Mortality from age 4 to 5, both sexes		8.8	11.3	'5cy+ floater' mortality
	Annual mortality after age 5, both sexes		8.8	11.3	'nesting' mortality
Initial Population Size	Initial population size		34	24	
Carrying Capacity	Carrying Capacity (K)		2830	1500	

Table A11. (SECTION 8) Input parameters for the stage-structured PVA model 'Future', predicting future population growth in East Hungary under the 'Baseline' mortality and 'Average' productivity scenario. The table includes only input parameters that were modified compared to the original 'East HU' model. VORTEX incorporates 'stage-structure' by replacing the default 'age-structure'. Therefore, note that in the case of parameters marked with red, the value of 'age' refers to a 'stage' category, where 0 = '1cy', 1 = '2cy', 2 = '3cy floater', 3 = '4cy floater', 4 = '5cy+ floater' and 5 = 'nesting'.

Menu	Parameter	East Hungary - Future "Baseline" mortality, 'Average' productivity		Comment
State Variables	Global State Variables	yes		
	BREEDYEAR	I:	=DISCRETE(1;1;1;1;1;1;1;1;1;1)	Randomly chosen value between 1 and 10 each year, refers to a year between 2013 and 2022 from which the reproductive parameters are taken (see Reproductive Rates below)
		T:	=DISCRETE(1;1;1;1;1;1;1;1;1;1)	
	Population State Variables	yes		
	PE3	I:	0.09	Function of density dependence for entry probability PE3
		T:	=0.09*(1-0.9*(PAIRS/K)^4)	
	PE4	I:	0.65	Function of density dependence for entry probability PE4
		T:	=0.65*(1-0.9*(PAIRS/K)^4)	
	PE5	I:	0.71	Function of density dependence for entry probability PE5
		T:	=0.71*(1-0.9*(PAIRS/K)^4)	
PE6	I:	1	Function of density dependence for entry probability PE6	
	T:	=1*(1-0.9*(PAIRS/K)^4)		
Reproductive Rates	Percentage of 0 broods per year	= LOOKUP (BREEDYEAR, % of breeding failure 2013–2022)		
	Percentage of 1 broods per year	=100-breeding failure%		
	Percentage of 1 offspring per female per brood	= LOOKUP (BREEDYEAR, % of 1chick broods 2013–2022)		
	Percentage of 2 offspring per female per brood	= LOOKUP (BREEDYEAR, % of 2chick broods 2013–2022)		
	Percentage of 3 offspring per female per brood	=100-(1chick broods% + 2chick broods%)		
Mortality Rates	Mortality from age 0 to 1, both sexes	=0.5*(PAIRS/K)^4*(100-30)+30		Function of density dependence for '1cy' mortality
Initial Population Size	Initial population size	1340		

Table A12. (SECTION 8) Stage-based (self-looped) Leslie matrix representation of the ‘East HU’ PVA model.

	‘1cy’	‘2cy’	‘3cy floater’	‘4cy floater’	‘5cy+ floater’	‘nesting’
‘1cy’	0	$s_2(1 - D)P_{E3} \times m$	$s_3P_{E4} \times m$	$s_4P_{E5} \times m$	$s_5P_{E6} \times m$	$s_N \times m$
‘2cy’	s_1	0	0	0	0	0
‘3cy floater’	0	$s_2(1 - D)(1 - P_{E3})$	0	0	0	0
‘4cy floater’	0	0	$s_3(1 - P_{E4})$	0	0	0
‘5cy+ floater’	0	0	0	$s_4(1 - P_{E5})$	$s_5(1 - P_{E6})$	0
‘nesting’	0	$s_2(1 - D)P_{E3}$	s_3P_{E4}	s_4P_{E5}	s_5P_{E6}	s_N

The rows and columns of the Leslie matrix represent the six stages: ‘1cy’, ‘2cy’, ‘3cy floater’, ‘4cy floater’, ‘5cy+ floater’ and ‘nesting’ (including 3cy, 4cy and 5cy+ nesting birds). $L[i, j]$, the element in row i and column j represents the contribution of stage j females (of year t) to stage i females (of year $t+1$).

The probabilities of the ‘*survival and progress to the next stage*’ changes are found in the subdiagonal elements of L . For instance, $L[3,2]$ represents the contribution of ‘2cy’ individuals (of year t) to the ‘3cy floaters’ (of year $t+1$). To calculate the probability of this stage change, we have to multiply s_2 , the probability of surviving from 2cy to 3cy, $1 - D$, the probability of not emigrating (D is the net emigration rate from East Hungary to other parts of the Pannonian population), and $1 - P_{E3}$, the probability of not entering the breeding stage at 3cy.

The probabilities of the ‘*survival and staying in the current stage*’ changes are found in the last two diagonal (self-looped) elements of L . For instance, $L[5,5]$ is the probability that a 5cy+ floater (of year t) survives (s_5) and does not enter the breeding stage ($1 - P_{E6}$) in the following year. Younger than 5cy floaters cannot remain in the same stage (the diagonal elements are zeros up to $L[4,4]$) because their survival and entry probabilities change (improve) with age.

The ‘*stage j females give birth to stage 1 offsprings (1cy)*’ type contributions occur in the first row of L . For instance, $L[1,2]$ is the expected number of female offspring (in year $t+1$) of a 2cy female (of year t). If she survives with probability s_2 and does not emigrate with probability $1 - D$, she is expected to produce m female chicks the following year. We calculated m , the average number of female offspring of a breeding female per year, as

$$m = P_{success}(1 \times P_{1\ chick} + 2 \times P_{2\ chick} + 3 \times P_{3\ chick})/2,$$

where dividing by 2 means that only female chicks are counted assuming equal sex ratios. As senescence is not included in the model, m applies to breeding stage females of any age.

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