

# **HABITAT- AND PREY-SELECTION OF IMPERIAL EAGLES (*AQUILA HELIACA*)**

**PhD thesis**

**Márton Horváth**

*Department of Ethology, Faculty of Science, Biological Institute, Eötvös Loránd University*

**Supervisors:**

**Dr. Csaba Moskát** (*Animal Ecology Research Group of the Hungarian Academy of Sciences, c/o Hungarian Natural History Museum*)

**Dr. Péter Kabai** (*Szent István University Faculty of Veterinary Science, Department of Ecology*)



**Faculty of Science, Biological Institute, Eötvös Loránd University**

**PhD Programme in Biology** (*Head of Programme: Prof. Anna Erdei*)

**Ethology Programme** (*Head of Programme: Dr. Ádám Miklósi*)

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## CHAPTER 1. INTRODUCTION



*Typical mountainous imperial eagle breeding habitat. Photo: Márton Horváth*

## From individual behaviour to population dynamics

Change in the size of a given animal population is affected by the number of birth, death, immigration and emigration of individuals (Begon et al. 1990). These factors are determined by environmental variables (i.e. availability of breeding and foraging habitats, presence of predators or other risk factors, stochastic events etc.) and also by individual behavioural decisions as responses to them. According to evolutionary theory animals have been selected to make decisions as to maximize their *fitness*, i.e. their contribution to the gene pool in the next generation (Daan & Tinbergen 1997).

Basically two strategies are assumed for maximising fitness at a given period of life, such as by (1) increasing current fecundity, or by (2) increasing chances of survival and thus creating the possibility for future reproduction. As these two strategies are exclusive they are in an antagonistic relationship. Such situations are typically referred as *trade-offs* in ecology, and the study of optimal behaviour (i.e. when fitness is maximized in trade-off situations) is one of the main topics of behavioural ecology (Krebs & Davies 1997).

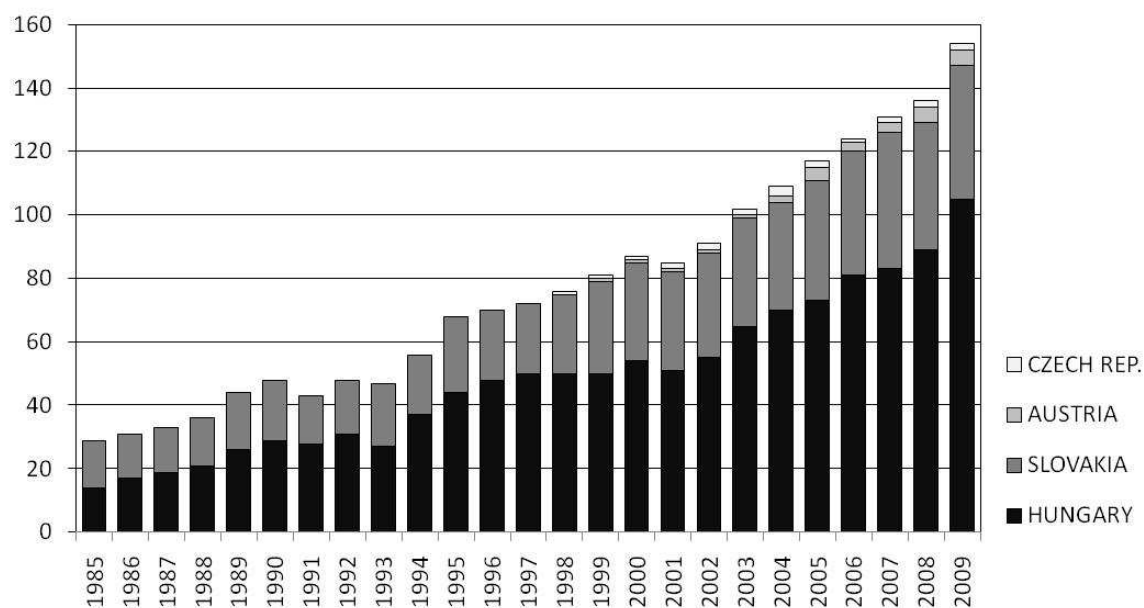
Trade-off situations can be identified between most activities in life, but also within them (Daan & Tinbergen 1997, MacArthur & Pianka 1966). E.g. a breeding bird needs to make choices between a closer, but lower quality food source and a further, but higher quality one during foraging. Such decision affects the time needed for foraging, and consequently, the time and energy left for guarding the nest against predators. The decision in such trade-off situations can be analysed by estimating the *costs* and *benefits* of a given behaviour, which are not only determined by environmental variables, but also depend on decisions of other members of the population (i.e. intra-specific competitors and co-operators). If we examine the behavioural patterns within a population we may find that individuals follow distinct strategies in making behavioural decisions in a given situation. A single behavioural strategy is evolutionary stable (ESS), if it cannot be replaced by any alternative strategy that is initially rare. More than one alternative strategies can be in an evolutionarily stable state at such ratios that their fitness values are equal (Maynard-Smith 1982).

Most studies evaluating behavioural strategies in populations are executed locally (on only a fraction of the total population) and within a short period of time, therefore information is gathered as from a snapshot and stability of the observed strategies is assumed. However, optimality of a behaviour or stability of a strategy is not necessarily constant, as they can change significantly in time and space with changing environmental conditions.

Populations of territorial top-predators, such as raptors, create a unique possibility to study the relationship between behavioural strategies and population dynamics, as these species are often (1) flagship species in conservation, therefore long-term monitored, (2) breed in small numbers, therefore surveys cover the total population, and (3) have large territories, therefore distribution of strategies in large geographical scale can be studied (Newton 1979). Moreover most of the top predators are threatened, therefore understanding the behavioural background of population dynamical processes is inevitable for taking effective conservation measures, and also to model population viability in a long-term (Meffe & Carroll 1997).

### **The case of the eastern imperial eagle (*Aquila heliaca*) in the Carpathian basin**

The eastern imperial eagle (*Aquila heliaca*) is a Vulnerable raptor distributed in the Eurasian forest-steppe zone and reaching the western limit of its range in the Carpathian basin, in Central Europe (BirdLife 2009). Here, the Hungarian, Slovakian, Austrian and Czech breeding pairs are forming the largest unified population of the species outside Russia and Kazakhstan, and they are separated by more than 500 km from the nearest neighbouring populations of the Balkan Peninsula and East Ukraine (Horváth et al. 2002). The population in the Carpathian Basin after a century of decline reached its lowest size by the 1970's, when a population survey and conservation programme started in both Hungary (Bagyura et al. 2002, Haraszthy et al. 1996) and Slovakia (Danko & Chavko 1996). The continuous monitoring has revealed a significant increase in the population size during the last 25 years (Fig. 1), which happened in parallel with the expansion of breeding range. Till the late 1980's the few nesting sites of the species were recorded in



**Figure 1.** Population dynamics of the eastern imperial eagle in the Carpathian Basin between 1985 and 2009. Data are summarized from Horváth et al. (2009a) and Chavko, Wichmann & Horal (unpubl. data).

remote, undisturbed mountainous forests and pairs were foraging on the open habitats of the foothills, sometimes even 10-15 km from their nest sites. Surprisingly, in 1989 two new breeding pairs were discovered approximately thirty kilometres from each other and from the nearest mountainous breeding pairs in a completely different lowland habitat type, where they nested on isolated trees and shelterbelts at open agricultural fields. From this time an exponential increase of the population at this new habitat type has been detected in parallel with the stability, or even slow decrease in some regions, of traditional mountainous breeding pairs. By 2009 most (85%) of the population could be found in the lowland habitats of the Great Hungarian Plain (see details in Chapter 2.1).

### **Nesting habitat selection at first breeding**

The nesting habitat selection of maturing imperial eagles, who are responsible for population growth, is the main process which determine population trends in the two types of habitats. A maturing individual can decide if it starts breeding early at a habitat



of inferior quality or to postpone reproduction until it is able to occupy a territory of high quality. If available nesting sites are abundant, a mixed strategy can be predicted: if cost of settling in a poor territory is low, individuals might start reproduction early in inferior site and move to territories of better quality when mature enough to be able to defend them. It should be noted, however, that the real quality of the habitat as expressed in terms of real fitness gains can be different from the perceived quality territory. It is well known that ecological traps are habitats of inflated perceived quality. Less attention is directed at the opposite case when the perceived quality is lower than the real one. A habitat can be undervalued for several reasons.

In the case of the imperial eagle we have evidence of severe persecution by humans in the past (shooting into the nest, collection of chicks etc.) and eagles probably learned to avoid habitats with visible human presence. Such an avoidance might persist long even after the pressure and real danger diminishes and by slow habituation it may take generations to close the gap between the perceived and real quality. This process is complicated by the fact, that reaction to effects perceived as dangerous, even if no longer risky can still diminish fitness. For example, leaving the nest when humans approach is adaptive in times when the birds are directly persecuted. When persecution is diminished because of conservation efforts leaving the nest will increase the mortality of chicks even when human pose no danger (Ferrer et al. 1990). In such a case the value of a habitat with high human presence can become low, but it is because the perceived threat is greater than the real one. We assume that the recently observable change in the occupancy of lowland habitats is a result of adaptation, as eagles can estimate more accurately the real threat of regular human presence in the close vicinity of their nest sites and less frequently make maladaptive alert reactions.

Recently the Hungarian population of imperial eagles seems to be far from reaching its maximal size, as the exponential growth has not slowed, and there are no signs of decrease in fecundity (see Chapter 3.2), and still only a fraction of suitable habitats are occupied by the species (see Chapter 2.2). Therefore a maturing individual can decide where to settle for breeding, i.e. to choose between two coexisting nesting habitat selection strategy observable simultaneously in the population.

## Differences between mountain and lowland breeding habitats

The mountainous and plain breeding habitats differ greatly in several important aspects, which can have significant effects on the population dynamical parameters.

(1) The forested mountainous nesting sites are relatively far from the nearest potential open foraging habitats ( $2368 \pm 1526$  m,  $n = 261$ , unpubl. data). In the lowlands almost all of the breeding attempts (in 98%,  $n = 383$ , unpubl. data) occurred on isolated trees and shelterbelts less than 20 meter away from potential foraging areas, while in the remaining few cases eagles bred in forest patches, but much smaller than the mountainous ones ( $124 \pm 62$  m from the nearest foraging area,  $n = 8$ , unpubl. data). Moreover, the elevations of mountainous nest sites are 200-500 m higher than the foraging areas, while lowland nests are only 10-20 m above the plain foraging habitats. These can result in better foraging possibilities at lowland sites, as less time and energy should be spent with transferring the food from the hunting sites to the nest.

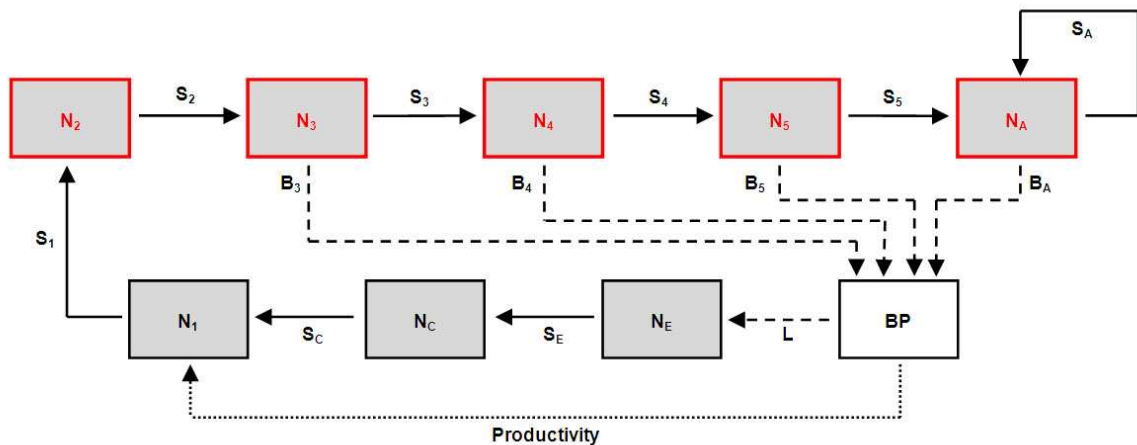
(2) The foraging habitats at foothills and lowlands are different in habitat structure and also in prey density. E.g. the density of brown hare (*Lepus europaeus*), the main prey species of imperial eagles in Hungary (see Chapter 3.1) differed greatly between two sample sites, as it was 6 individuals per ha in the foothills of the Mátra Mountains and reached 22 individuals per ha in a nearby lowland habitat in the Heves Plain (Kovács et al. 2008).

(3) Human disturbance, which is one of the main causes of breeding failures (see Chapter 2.1), also differs between the two habitat types. Regular agricultural activities are undertaken in the lowlands, even in the close vicinity of nest sites, while occasional forestry work and accidental tourism are the only human activities in the mountainous breeding habitats. E.g. human disturbance occurred in  $9.7 \pm 19.3$  % of time in the close (< 500 m) vicinity of nest sites during the incubation period in lowlands ( $n = 13$ , 65 h of observation), while this ratio was only  $0.1 \pm 0.3$  % in the mountains ( $n = 8$ , 34 h of observation) (unpubl. data).

(4) Number of reported cases of persecution (poisoning, shooting) and accidents (electrocution, collision with vehicles) are higher in the freshly occupied lowland habitats (see Chapter 2.1)

## Population dynamical parameters

As the relative size of the mountainous and plain populations reversed during the last decade, the above-mentioned differences could directly affect the reproductive success and mortality rates within the population. For the individual both habitats have costs and benefits in a trade-off relation, and the cumulative effects on fitness of such factors are not obvious. The long-term population monitoring data on the species create a unique background to study the changes in population parameters in parallel with the dynamics in the main habitat types. Nevertheless the demography of an imperial eagle population is a result of several processes (Figure 1), which are difficult or even impossible to assess with traditionally collected field data. In the present study I am discussing some aspects affecting breeding population size ( $BP$ ), reproduction ( $N_e$ ,  $N_c$ ,  $N_1$ ) and mortality of breeding birds ( $N_A$ ), while the demography of early age groups ( $N_{2-5}$ ) remains to be addressed by further studies.



**Figure 2.** Processes affecting population dynamics of eastern imperial eagles. Symbols are:  $N_x$  is the number of individuals in the given age class,  $BP$  is the number of breeding pairs,  $S_x$  is the survival rate of a given age class to the next one,  $B_x$  is the ratio of birds in the given age class, which start breeding,  $L$  is the mean number of laid eggs per breeding pair. Age classes are: 1-5 represents birds in their 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> calendar year respectively,  $A$  represents adult birds,  $E$  represents eggs and  $C$  represents chicks. The capsules in the upper part of the figure represents the age classes of the population at the beginning of a breeding season, while the lower capsules represent the main stages of reproduction. Immigration and emigration were not considered assuming a closed population. Modified after Katzner et al. (2006a).

## **Aims of the thesis**

The studies presented in the thesis investigate the relationship between the recently changed habitat selection strategy (i.e. lowland expansion and abandonment of mountains) and population dynamical parameters of imperial eagles in Hungary. Chapter 2.1 analyses the situation of the whole national population, while the latter ones focus on the East-Hungarian subpopulation, which is the most well-known and largest continuously distributed subpopulation of the Carpathian basin.

*Chapter 2.1* summarizes the detected changes in population dynamics, breeding success and mortality factors of the Hungarian imperial eagle population during the last decade.

*Chapter 2.2* investigates whether the expansion of imperial eagles is not hindered significantly by the well-developed infrastructural network at the lowland habitats.

*Chapter 3.1* compares of prey composition in traditional and freshly occupied breeding habitats and discusses the possible effects of diet on reproductive success.

*Chapter 3.2* investigates how density, age structure and habitat type could affect simultaneously the reproductive success of imperial eagles.

*Chapter 4.1* presents an improved method of DNA extraction from shed feathers, and discusses how the application of this novel method can add to the study of eagles, and other bird species difficult to handle.

*Chapter 4.2* estimates the mortality rates of breeding imperial eagles at the two habitat types, by non-invasive genetic sampling of individuals.

## CHAPTER 2. POPULATION DYNAMICS AND DISTRIBUTION



*Typical lowland imperial eagle breeding habitat. Photo: Márton Horváth*

## 2.1 POPULATION DYNAMICS OF THE IMPERIAL EAGLE (*AQUILA HELIACA*) IN HUNGARY BETWEEN 2001 AND 2009

### ABSTRACT

The globally threatened eastern imperial eagle (*Aquila heliaca*) reaches the western border of its range in the Carpathian Basin, which is the largest known population outside Russia and Kazakhstan. An increasing trend of this population in Hungary and also in the nearby areas of Slovakia has been reported since the 1980's, when the number of breeding pairs supposedly reached the historical minimum. In the present study we evaluated the dynamics of the Hungarian imperial eagle population between 2001 and 2009. As a result of the continuous increase of the population the monitoring program revealed 105 nesting pairs by 2009. While an expansion of the breeding area towards lowland agricultural habitats was observed, the ratio of pairs inhabiting the historical mountainous breeding habitats decreased from 50% to only 15% during the study period. The frequency of the two- and three-chick broods in respect to single-chick broods increased comparing to the 1980-2000 period showing a higher average annual productivity of the population (1.15 fledglings per nesting pair). Besides the favourable changes in population trend and productivity, the area expansion in the recently occupied lowland habitats also raised several new threats to the population, such as the increased number of illegal poisoning incidents and more frequent collisions with vehicles.

*Horváth, M., Szitta, T., Fatér, I., Kovács, A., Demeter, I., Firmánszky, G. & Bagyura, J. 2009. Acta Zoologica Bulgarica (in press).*

## INTRODUCTION

The eastern imperial eagle (*Aquila heliaca*) inhabits a large area of the Palearctic region along the forest-steppe belt, although its distribution is highly scattered (Del Hoyo et al. 1995). World population consists of a few thousand breeding pairs only, and the species is classified as 'Vulnerable' by the IUCN Red List of Threatened Species (BirdLife 2009). In spite of the high conservation priority of this species, only a fraction of the populations is monitored regularly (Horváth et al. 2002), although the quality of available data increased considerably during the last decade, especially for the largest eastern populations (e.g. Belik et al. 2002, Karyakin et al. 2008). Long-term monitoring of eastern imperial eagle populations, which would enable us to evaluate changes in the population size, distribution, breeding success or mortality (Ferrer 2001), are extremely rare and probably only two significant populations have been monitored continuously over decades. One of these populations in the Naurzum Reserve in North-Kazakhstan remained stable since 1978 (Bragin 1999, Katzner et al. 2006a), while the other population in the Carpathian Basin in Central Europe increased significantly since 1977 (Danko & Chavko 1996, Haraszthy et al. 1996, Bagyura et al. 2002).

The Carpathian Basin's population of eastern imperial eagles is the most western population in its distribution area (Danko & Haraszthy 1997), where imperial eagles have continuously occurred and bred since written information was available. The first proved data is from as early as 1811, when an adult pair was collected near Vienna, East-Austria (Natural History Museum of Vienna, unpubl. data). Later during the 19th century the imperial eagle was reported as a breeding species mainly from the southernmost parts of the Carpathian Basin, as Vojvodina (North-Serbia) and Slavonia (Northeast-Croatia) (Vasvári 1938). Nevertheless, we cannot exclude that this species was already present in other regions of the Basin as well, since reliable data were only available in exceptional cases from these early periods. The first published data from the present territory of Hungary is from the Bakony Mountains, where imperial eagles were observed during the breeding season in 1889. The first localised nest sites were reported from the beginning of the 20th century from the Vértes (1912), Zemplén (1913) and Bükk Mountains (1929) (Vasvári 1938, Vásárhelyi 1964). After the World War II nesting of the imperial eagle were

also recorded in almost all other mountainous regions in Hungary, such as the Buda (1950), Mecsek (1951), Gerecse (1954), Börzsöny (1957), Bakony (1959) and Pilis Mountains (1968), most probably as the result of an improved data collection, and not because of the expansion of the species (Bécsy 1972, Cseresnyés 1960, Pátkai 1951, Sággy 1968, Somogyi 1971, Tapfer 1973, Hungarian Bird Ringing Centre unpubl. data, Nádai unpubl. data). Since no specific national surveys were performed until the late 1970's, we are not able to estimate the population size exactly in Hungary prior to these years. Nevertheless, we can conclude that in the middle of the 20th century the imperial eagle was a rare, but a characteristic breeding species in the Hungarian mountains, while the lack of observations and data on breeding from the Hungarian Plain suggest that the species probably did not breed regularly in open lowland areas (Vasvári 1938).

A national species-specific survey was initiated by MME Birdlife Hungary in 1977, and its results revealed that the species disappeared from several previously known breeding areas (i.e. Pilis, Buda and Mecsek Mountains) and also became scarce in the remaining ones (Haraszthy et al. 1996). From the available nesting records Hungarian population seems to be reached its historical minimum by the late 1970's and early 1980's, when probably not more than 15-25 breeding pairs remained in mountainous forests (Bagyura et al. 2002). From 1980 onwards due to favourable environmental changes and to the continuous population monitoring and conservation programme of MME and national park directorates of the Hungarian Ministry of Environment and Water, the known imperial eagle population size was increasing continuously. In the first decade of the programme (1980-1989) the increase in the population size was partly due to the increased area covered by monitoring, but it was also proved that on the most intensively surveyed regions several new pairs of immature birds occupied territories. From 1990 onwards the monitoring has regularly covered the great majority of potential breeding habitats, and the changes in the number of localised nesting pairs have presumably reflected the real changes in the population size. In parallel with the increasing population size the breeding distribution of the species expanded from the mountain forests to the open plain habitats, where the first two breeding pairs were recorded in 1989. By 2000 the Hungarian population consisted of 54 known territorial pairs, from which 50% occupied lowland agricultural habitats (Bagyura et al. 2002).



In the present study our aim was to follow the dynamics of the Hungarian imperial eagle population between 2001 and 2009 and to compare recent population trends with the previously studied period of 1980-2000. We predict that the increasing trend of breeding pairs in the lowlands is maintained in parallel with the expansion of the nesting area, while the stability of the mountainous breeding pairs is expected. We also predict that the average breeding success did not change significantly in comparison with the previous period, while the main causes of mortalities and breeding failures are expected to be different due to the different threatening factors in newly colonized breeding habitats.

## **MATERIAL AND METHODS**

### *Study area*

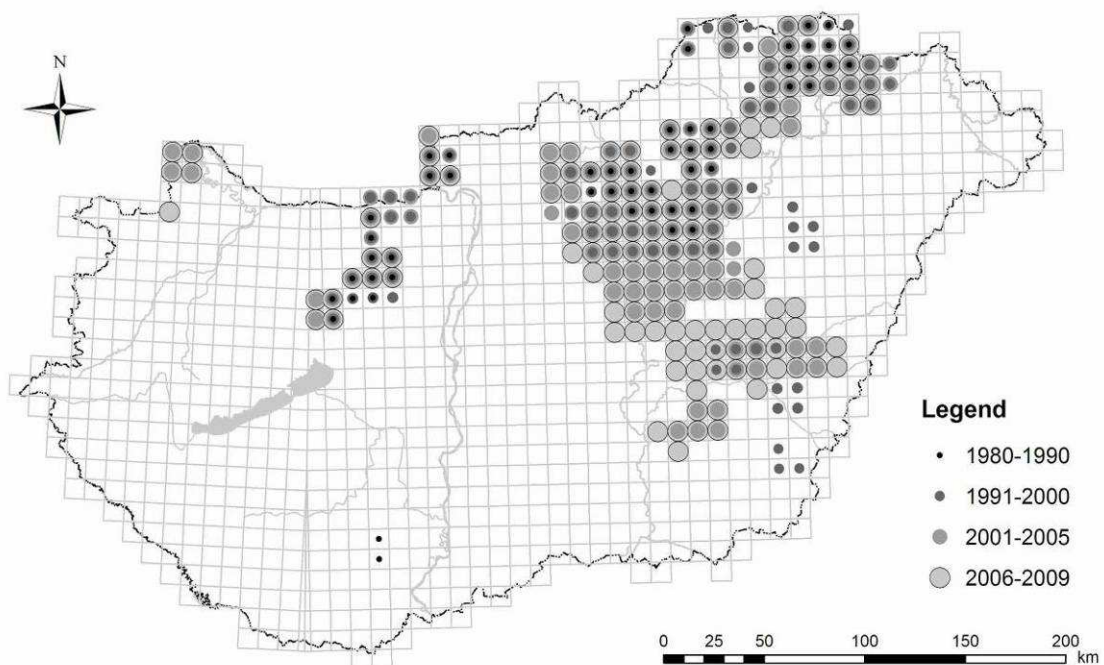
The core breeding area of the eastern imperial eagle in Hungary located in the central part of the Great Hungarian Plain (Jászság, Heves, Borsod, Nagykunság and Békés Plains) and adjacent North Hungarian Mountains (Mátra, Bükk and Zemplén Mts.). Scattered breeding pairs can also be found in the western regions of the country, namely in the Dunántúl Mountains (Vértes and Gerecse Mts.) and most recently in the Little Hungarian Plain at the north-western corner of Hungary (Fig. 1).

In forested mountainous habitats (200-700 m a.s.l.) imperial eagles breed in sessile (*Quercus petraea*), downy (*Q. pubescens*) and turkey oak (*Q. cerris*), beech (*Fagus sylvatica*) and introduced pine (*Pinus nigra*, *P. sylvestris*, *Larix decidua*) forests (Kovács et al. 2005), sometimes more than ten kilometres from the nearest open foraging habitats. Recently a tendency shows that an increasing number of pairs are moving from the inner parts of the mountains toward the edges of the mountain ranges, and some of these pairs already occupied nest sites in the foothill foraging areas. In the lowland plain habitats (80-120 m a.s.l.) the imperial eagle breeds in small groups of poplars (*Populus spp.*) and black locust trees (*Robinia pseudoacacia*). A complex of intensive agricultural fields and remaining grassland areas are the most preferred foraging habitats, usually within a 3-8

km radius around the breeding sites. In contrast, some of the mountainous pairs regularly forage even in a 10-15 km distance from their nests.

### *Population survey*

The active and potential breeding territories of imperial eagles in Hungary were monitored within the framework of the Hungarian Imperial Eagle Working Group during the study period, as a continuation of the comprehensive species-specific survey started in 1980 (Haraszthy et al. 1996, Bagyura et al. 2002). The volunteers and employees of the MME BirdLife Hungary, the rangers of national park directorates and the amateur birdwatchers formed a well-trained national network of approximately 500 observers covering the majority of the potential breeding area of the imperial eagle in Hungary. Therefore we suppose that less than 5% of the breeding imperial eagle pairs could be unnoticed, and the distribution and size of the population were reliably revealed within the study period.



**Figure 1.** Expansion of the breeding range of imperial eagles in Hungary between 1980 and 2009. Data are presented in a 10 km x 10 km UTM grid. GIS processed data derived from BAGYURA et al. (2002) represent the situation prior to 2001.

We have assigned territory coordinators for each breeding pair, who were responsible for the regular check of the given territories according to the suggested monitoring protocol. This included a thorough search for active nesting sites in potential territories at the beginning of the breeding seasons, during the time of displaying and nest building, but before leafing (February-March). Areas were handled as potential imperial eagle territories, if (1) an active nest was localised within a 10 km radius after 1980, or (2) more than one observations of adult or subadult birds were reported during the breeding season. In open lowland areas nest searching was executed by checking all of the potential trees or forest patches. However, in forested mountainous habitats typically daylong observations of the territorial birds' behaviour from vantage points were usually needed to locate the nest or to clarify the status of the territory. Identified nesting sites were checked at least once a month during the breeding period (from April to August) to determine breeding success. Nest observations were carried out by spotting scopes (20-60X) from large distance (0.5-1.5 km) to avoid disturbance of the birds (Gonzalez et al. 2006a). However, one occasion in June we ringed the chicks in almost all of the nests and fixed the coordinates of the nests by GPS.

Altogether 673 nesting events in 127 different territories were followed in Hungary between 2001 and 2009. Data on breeding attempts were processed in a GIS database. In order to compare recent data with those previously reported by Bagyura et al. (2002), we also processed historical data of 473 breeding attempts from the period of 1980 and 2000, and incorporated them in the same GIS database. Breeding success was described by three variables, such as the frequency of breeding attempts with at least one fledged chick (referred to as *success rate*), the mean number of young fledged per successful pairs (referred to as *fledging success*), and the mean number of young fledged per nesting pairs (referred to as *productivity*). In those cases when the number of died eggs (72 cases) or chicks (10 cases) were not known in a failed breeding attempt, we assumed the mortality of two eggs or chicks, as this is the most typical brood size in Hungary (authors own data).

Distribution of the territories was visualized in a 10 km x 10 km UTM grid by using ArcMap© software (ESRI Inc., version 9.0). Grid cells were considered as occupied if they were overlapped with an active breeding territory during the study periods. Hypothetical

territory boundaries were defined as a 3.5 km buffer zones around the active nests. This size is approximately equal to the half of the average nearest neighbour distance in Hungary (Kovács et al. 2005). It is notable, that for the closely related species, the Spanish imperial eagle (*Aquila adalberti*), almost the same value, 3.25 km was defined and applied in a similar study (Gonzalez et al. 1992). Statistical analyses were carried out with the SPSS programme package (SPSS Co., ver. 17). We used the *F* ratio statistics to analyse trends during the study period. We compared the annual averages of breeding parameters to the average values of the 1980-2000 period by one-sample T-tests, as none of the breeding success variables were deviated from the normal approximation.

## RESULTS

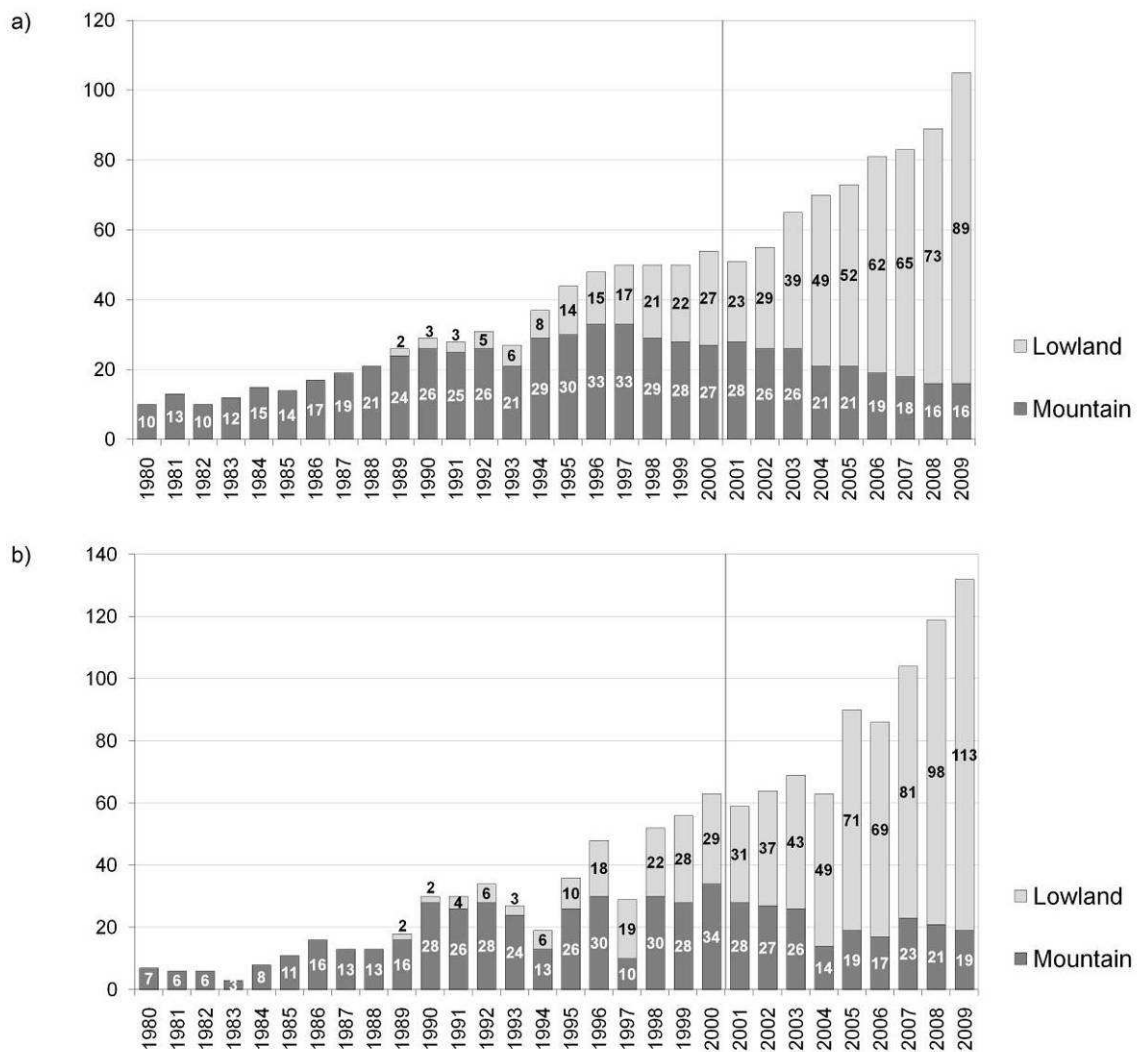
### *Changes in the breeding area*

The breeding area of imperial eagles expanded significantly in the Great Hungarian Plain during the study period (Fig. 1). This species also occupied the Little Hungarian Plain as a result of the expansion of the West-Slovakian and East-Austrian populations (Vácz 2008). On the other hand the shrinking of the breeding range was observed in the western mountainous breeding pairs in the Bakony, Vértes, Gerecse and Börzsöny Mountains (only three pairs remained by 2009 out of the eight in 2001). Similarly, the single breeding pair of the Aggtelek Karst Mountains at the north-eastern part of the country disappeared in 2003.

### *Population trend*

In Hungary the increasing trend of the imperial eagles' breeding population, which started in the 1980's, continued between 2001 and 2009, and with the 10% mean annual increase it has become more intensive than ever since 1980 ( $F = 155.169$ ,  $df = 1, 7$ ,  $P < 0.001$ ; Fig. 2a). The trend of the population differed relevantly between the two main

breeding habitat types, as the number of mountainous breeding pairs slowly decreased (annually by -5% in average,  $F = 135.151, df = 1, 7, P < 0.001$ ), while lowland populations increased remarkably (annually by 15% in average,  $F = 374.502, df = 1, 7, P < 0.001$ ). Altogether, the Hungarian population almost doubled during the nine years of study and reached 105 breeding pairs by 2009, of which as many as 85% bred in lowland agricultural habitats.



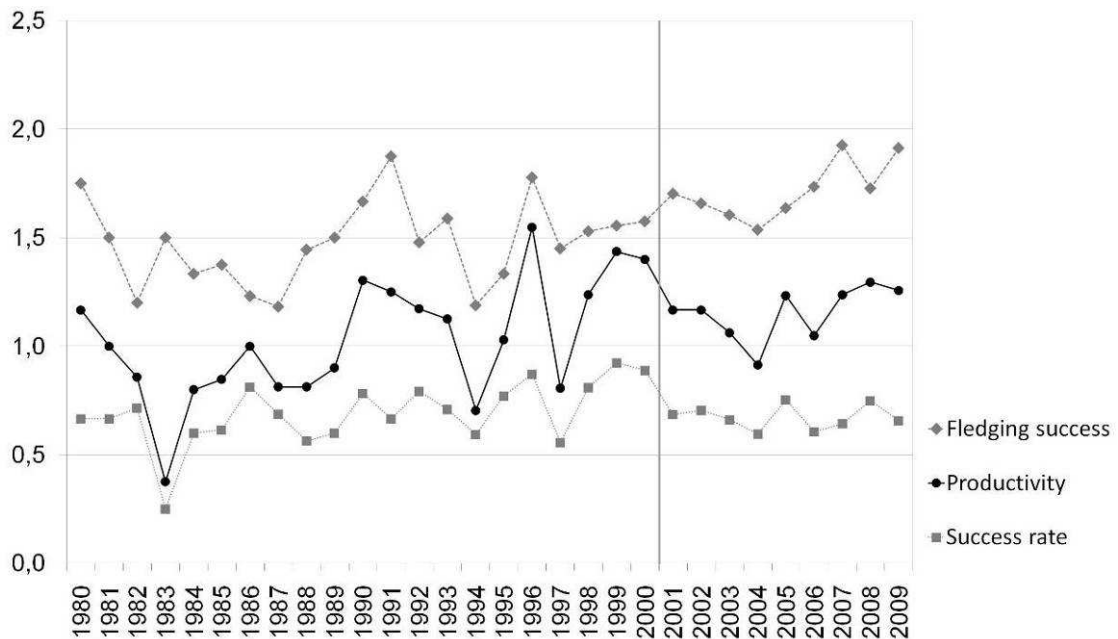
**Figure 2.** Number of known nesting pairs (a) and fledglings (b) of imperial eagles in Hungary between 1980 and 2009. Data prior to 2001 were taken from BAGYURA et al. (2002).

**Table 1.** Causes of breeding failures, mortality of eggs and chicks of imperial eagles in Hungary between 2003 and 2009. Mortality data includes complete failures of 142 broods and also those 60 cases when the incident affected only a part of the brood. The number of failed breeding attempts and the estimated number of dead offsprings are followed by their relative frequencies in brackets.

Cause	Breeding failure			Mortality		
	Incubation	Chick-rearing	Total	Egg	Chick	Total
Storm	18 (16%)	15 (54%)	33 (23%)	37 (15%)	34 (42%)	71 (22%)
Disturbance	15 (13%)	2 (7%)	17 (12%)	27 (11%)	3 (4%)	30 (9%)
Unfertilized eggs	7 (6%)		7 (5%)	23 (10%)		23 (7%)
Poisoning	2 (2%)	1 (4%)	3 (2%)	4 (2%)	3 (4%)	7 (2%)
Cainism					3 (4%)	3 (1%)
Shooting to the nest	2 (2%)		2 (1%)	3 (1%)		3 (1%)
Mortality of parents	1 (1%)		1 (1%)	2 (1%)		2 (1%)
Illegal logging	1 (1%)		1 (1%)	2 (1%)		2 (1%)
Abnormal embryo				1 (0%)		1 (0%)
Haywire coiled on chick					1 (1%)	1 (0%)
Nest-robbing					1 (1%)	1 (0%)
Unknown	68 (60%)	10 (36%)	78 (55%)	139 (58%)	36 (44%)	175 (55%)
Total	114 (100%)	28 (100%)	142 (100%)	239 (100%)	81 (100%)	320 (100%)

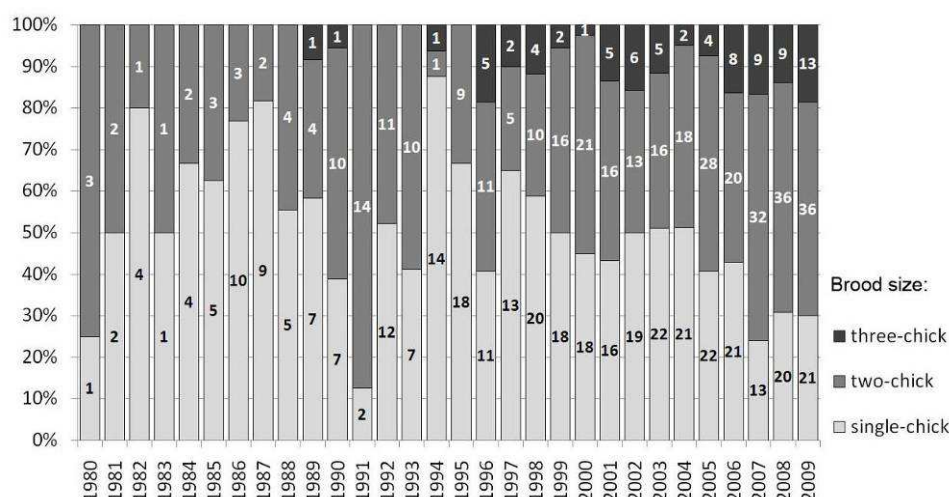
### *Breeding success*

A total of 783 imperial eagle chicks fledged from 452 successful breeding attempts in Hungary between 2001 and 2009. Brood mortality was accurately surveyed between 2003 and 2009, meanwhile 142 breeding failures and further 60 cases of partial loss of broods caused the mortality of approximately 239 eggs and 81 chicks (Table 1). Similarly to the trend of the population size, the number of fledglings also increased significantly in lowland habitats ( $F = 183.121$ ,  $df = 1, 7$ ,  $P < 0.001$ ), but the decrease observable in the mountains was not significant ( $F = 2.914$ ,  $df = 1, 7$ ,  $P = 0.132$ ; Fig. 2b). Annual success rate ranged between 59 and 75%, and fledging success between 1.54 and 1.93, resulting in a 0.91-1.30 annual productivity (Fig. 3).



**Figure 3.** Mean annual productivity (no. of fledglings/no. of nesting pairs), success rate (no. of successful pairs/no. of nesting pairs) and fledging success (no. of fledglings/no. of successful pairs) of imperial eagles in Hungary between 1980 and 2009. Data prior to 2001 were taken from Bagyura et al. (2002).

In the study period the mean annual success rate was similar to the rate of the previous 20-year period (1980-2000: 69% vs. 2001-2009: 67%,  $t = -0.925$ ,  $df = 8$ ,  $P = 0.382$ ). The mean proportion of two-chicks brood was somewhat higher recently, but the difference was not statistically significant (1980-2000: 41% vs. 2001-2009: 48%,  $t = 1.889$ ,  $df = 8$ ,  $P = 0.096$ ). Although the frequency of three-chicks broods was significantly higher in the recent study period than in the previous one (1980-2000: 5% vs. 2001-2009: 14%,  $t = 5.435$ ,  $df = 8$ ,  $P < 0.001$ ; Fig. 4), and in parallel the frequency of one-chick broods decreased (1980-2000: 54% vs. 2001-2009: 40%,  $t = -4.047$ ,  $df = 8$ ,  $P < 0.005$ ). The higher frequency of larger broods resulted in a higher mean of fledging success (1980-2000: 1.48 vs. 2001-2009: 1.72,  $t = 5.381$ ,  $df = 8$ ,  $P < 0.001$ ) and also a higher overall productivity (1980-2000: 1.03 vs. 2001-2009: 1.15,  $t = 3.001$ ,  $df = 8$ ,  $P < 0.05$ ).



**Figure 4.** Frequency of single-, two- and three-chick broods among successful imperial eagle breeding attempts in Hungary between 1980 and 2009. Columns represent the relative frequencies of the different brood sizes by years. The exact numbers of different sized broods are written in the columns. Data prior to 2001 were taken from Bagyura et al. (2002).

### *Causes of mortality*

Identified mortality causes of full-grown imperial eagles are summarized in Table 2. Before 2005 electrocution was the main mortality cause, but poisoning incidents increased significantly between 2005 and 2008, and by now poisoning became the most important human-induced threat to imperial eagles in Hungary. Collision with vehicles has become an important mortality factor, too, which proved to be highly dangerous especially for juvenile eagles fledged from shelterbelts along railways, as already three fatal accidents were reported, out of nine such breeding attempts.

## **DISCUSSION**

### *Population dynamics*

In parallel with the increase of the Hungarian imperial eagle population the numbers of imperial eagle breeding pairs in the neighbouring South-Slovakian, East-Austrian, and



**Table 2.** Mortality factors of full-grown imperial eagles found in Hungary between 1980 and 2009. Data prior 2001 were taken from the database of the Hungarian Bird Ringing Centre, the Raptor Rehabilitation Centre of Hortobágy National Park and from Bagyura & Szitta (unpubl. data). The numbers of specimens are followed by their relative frequency during the given period in brackets. Other causes including single cases of accidental trapping by a pheasant farm's net and by a poacher's loop, drowning in flood just after fledging and a fungal lung infection.

Cause	1980-2000	2001-2009	Total
Poisoning		29 (32%)	29 (26%)
Electrocution	4 (17%)	20 (22%)	24 (21%)
Collision with vehicle		8 (9%)	8 (7%)
Starving		6 (7%)	6 (5%)
Shooting	1 (4%)	4 (4%)	5 (4%)
Collision with electric cable	1 (4%)	1 (1%)	2 (2%)
Other		4 (4%)	4 (4%)
Unknown	17 (74%)	18 (20%)	35 (31%)
Total	23 (100%)	90 (100%)	113 (100%)

Southeast-Czech regions of the Carpathian Basin also increased (Chavko & Wichmann unpubl. data). By 2009 the population of the Carpathian Basin consisted altogether more than 150 known breeding pairs, therefore recently it seems to be the biggest unified population of the eastern imperial eagle outside Russia and Kazakhstan. As more suitable areas in the Carpathian Basin are still available for the species, we predict further expansion of the population in the future.

### *Consequences for conservation*

The sharp increase of illegal poisoning incidents in the recent past bewares us that the favourable conservation status of the species is still vulnerable. The species is highly exposed to poisoning, since wandering immature individuals, but also territorial adult pairs frequently take carcasses, especially wintertime (Del Hoyo et al. 1995). Intentional illegal poisoning against carnivores and corvids started to spread in Hungary after 2005, probably due to changes in authorised hunting methods (Horváth 2008). A good example for this threat that in a four-year period 29 poisoned imperial eagles have been found

recently. It was shown for the Spanish imperial eagle that such a sudden increase in the mortality due to poisoning could negatively influence population dynamics, and even reverse the increasing trend of eagle populations locally (Ferrer & Penteriani 2008). Probably the increased mortality of imperial eagles in Hungary due to poisoning caused that the overall annual increase of the population was smaller in 2007 and 2008 (only 4% in average), just after the peak of the reported poisoning incidents than in other years of the study period (12% annual increase in average). MME BirdLife Hungary started an anti-poisoning campaign in 2007, which should be maintained in the future even if the number of incidents decreased. Fortunately, during the winter of 2008/2009 relatively few poisoning incidents were reported and the previous increasing trend of the population continued with 16 new nest building pairs in 2009 (equal to 19% increase in one year).

Besides poisoning, the other main direct threat to the eastern imperial eagle is electrocution, similarly to the Spanish imperial eagle (Ferrer & Hiraldo 1991, González et al. 2007). In spite of the almost 20-year effort for bird-friendly modification of electric pylons in Hungary (Bagyura et al. 2004), electrocution is still among the most important mortality factors of several raptor species, including the imperial eagle. Although the presumably most dangerous power lines have been located, and the most effective methodology for bird-friendly modifications is known, we may have to wait further decades for a satisfactory solution of the problem because of financial considerations (Horváth et al. 2008).

The third most frequent cause of mortality affecting fledged imperial eagles was the collision with cars and trains. As it was also reported by Danko & Balla (2007), some imperial eagle pairs in the Carpathian Basin are able to tolerate the immediate vicinity of roads and railways when they choose nesting trees. This adaptability to the more disturbed lowland areas increased significantly the available habitats for the species, but these new type of habitats can also affect the mortality rate of eagles, e.g. by increased incidents of collisions with vehicles.

Storms destroying nesting trees were the most common cause of the breeding failures recorded in Hungary. The shortage of suitable nesting trees in many parts of the Hungarian Plain forces the eagles to nest on suboptimal trees as well (e.g. on weaker black locust trees), which are exposed to the damage of windstorms. A similar problem

was reported in a Spanish imperial eagle population, where the alien eucalyptus (*Eucalyptus* sp.) tree became the main nesting tree species, and these nests were more frequently damaged by windstorms than those on native tree species (Calderon et al. 1987).

Summarizing our results, we suppose that the Hungarian population of eastern imperial eagles is in a favourable conservation status, nevertheless the changes in the main breeding habitat types raised several new threats for the population. Nature conservation organizations should be able to address these new problems to promote the further expansion of this globally threatened species, for what the Hungarian Plain still provides suitable unoccupied habitats.

## 2.2 EFFECTS OF INFRASTRUCTURAL NETWORK ON THE DISTRIBUTION OF IMPERIAL EAGLES IN THE HUNGARIAN PLAIN

### ABSTRACT

We analysed the effects of settlement-, road-, railway- and power line network (referred to as infrastructures in the followings) on the present and potential breeding distribution of imperial eagles in the Hungarian Plain with intrinsic Conditional Autoregressive (CAR) models in a Bayesian framework. We found that eagles avoided for nesting a distinct buffer around most types of infrastructures and these buffers covered approximately one third of the study area. Network of settlements was proved to have absolutely the highest effect on distribution, although roads and medium-voltage power lines was also avoided remarkably. A prediction model built for the total potential breeding area of the species in East-Hungary, showed that in spite of the relatively dense infrastructural network and the strong avoidance of them by the eagles, there are still several most probably suitable habitats for the species, which are still not inhabited. The model predicted the location of those areas, which will be most probably avoided, and also those which most probably will be colonized by the eagles during the expansion of the population.

*Horváth, M., Fehérvári, P., Szitta, T., Harnos, A. & Moskát, C. 2009. Manuscript (in prep).*

## Introduction

Expansion of settlements and infrastructures has an overall negative impact on biodiversity directly by the destruction, degradation and fragmentation of natural habitats, and indirectly through increased human presence. Such changes effects have been identified as key threats to many vulnerable bird species in most parts of the world, including the highly populated areas of Europe (BirdLife International 2004a; Heath & Evans 2000). Already more than a quarter of the European Union's (EU) territory has now been directly affected by urban land use and further intensive developments are foreseen, especially in the new Member States of Central and Eastern Europe (EEA 2006). Besides urban sprawl, the developments of the Trans-European Transport (TEN-T) and Energy (TEN-E) Networks are also fragmentising the natural habitats of Europe (Byron & Arnold 2008, Fischer & Waliczky 2001). For instance the 2400 km of express roads and highways, which are planned to be built or developed in Hungary between 2007-2013, will approximately double the national network of these infrastructures (Hungarian Ministry of Economy and Transport 2006). The EU funds caused socio-economic and land-use changes in the new Member States could have a strong impact on Europe's biodiversity, since natural and semi-natural habitats of these countries still hold significant populations of several threatened species, which already disappeared or became rare in the Western-European part of their former range (BirdLife International 2004b). To compromise regionally conservational and economical claims, decision-makers need precise site- and species-specific empirical information, but these are rarely available comprehensively. For the conservation of the most threatened species studies should locate the important habitat patches, and find out what density of or distance from infrastructures affect significantly their distribution and/or productivity.

Habitat selection models of bird populations frequently incorporate infrastructural factors (e.g. settlement, road, railway, power line network) to investigate their effects on species' distribution (López-López et al. 2006; Morán-López et al. 2006a; Osborne et al. 2001; Sergio et al. 2004a; Sergio et al. 2004b; Sergio et al. 2006a; Suárez et al. 2000). Number and quality of such habitat selection studies has increased recently, mainly due to the rapid evolution of geographical information systems (GIS) and powerful statistical

methods (e.g. Engler et al. 2004; Lehmann et al. 2002; Rushton et al. 2004; Sergio et al. 2006a). Such studies typically analyse habitat variables measured in occupied (i.e. breeding or foraging habitats) and unoccupied sites (Cody 1985). Unoccupied sample sites might be selected at random, which contain a representative proportion of the unoccupied, but theoretically suitable parts of the study area (e.g. Brambilla et al. 2006; Gavashelishvili & McGrady 2006; Moskát & Honza 2000; Morán-López et al. 2006b; Sergio et al. 2006a; Suárez et al. 2000). Alternatively sample sites can be occupied and unoccupied quadrats of a grid system, which methodology enables habitat models to analyse the distribution of species and habitat variables for the entire study area (e.g. López-López et al. 2006; López-López et al. 2007; Osborne et al. 2001; Siriwardena et al. 2000; Tapia et al. 2007).

Raptor species are widely used subjects of habitat selection studies, because (1) they usually breed in low densities, therefore large spatial scale habitat preference can be studied within one population (e.g. Carrete et al. 2007; Gavashelishvili & McGrady 2006; Hirzel et al. 2004; Sergio et al. 2006a); (2) large proportion of the raptor species is in unfavourable conservation status (68% in Europe according to BirdLife 2004b), therefore exact information on habitat requirements are needed for planning effective conservation works (Balbontín 2005; Ferrer & Harte 1997; Sará & Di Vittorio 2003; Syartinilia & Tsuyuki 2008; Väli et al. 2004); and (3) raptors, as spectacular top-predators, are frequently used flagship or umbrella species in conservation policy (Caro & O'Doherty 1999), and their presence locally could potentially detect areas with relatively higher level of biodiversity (Sergio et al. 2006b, but see comments and critics in Sergio et al., 2008 and references therein).

The eastern imperial eagle *Aquila heliaca* is a large-sized raptor, distributed along the Eurasian forest-steppe habitats from Central Europe to the Lake Baikal. In spite of its wide range, the breeding areas are scattered, and world population is less than 10 000 breeding pairs (BirdLife International 2009; Ferguson-Lees & Christie 2001), with their great majority (>90%) located in Russia and Kazakhstan (Karyakin et al. 2008). The species' conservation status is treated as 'vulnerable' globally (BirdLife International 2009) and 'rare' in Europe (BirdLife International 2004b), where it disappeared from large parts of its former distribution area during the 19<sup>th</sup> and 20<sup>th</sup> centuries. Recently only two

small isolated populations can be found in the territory of the EU, namely in Bulgaria (20-25 pairs, Stoychev et al. 2004) and in the Carpathian basin, mostly in Hungary and Slovakia (total of 135-140 pairs, M. Horváth & J. Chavko, unpubl. data). The Spanish imperial eagle (*Aquila adalberti*) is the closest relative of the eastern species, and its populations are localised in the Iberian Peninsula, so it is one of the rarest raptors in the world (Ferrer 2001; Gonzalez & Margalida 2008).

A significant negative effect of human disturbance and infrastructural network on habitat selection and behaviour of the Spanish imperial eagle has been demonstrated at different spatial scales within Spain (Bisson et al. 2002; Ferrer & Harte 1997; Gonzalez et al. 1990; Gonzalez et al. 1992; Gonzalez et al. 2006a), but no such data are available for the eastern species, which inhabits different habitat types with different density of infrastructural network in Central and Eastern Europe.

The largest long-term surveyed European population of the eastern imperial eagle inhabits the Hungarian Plain and surrounding low mountains (Haraszthy et al. 1996; Bagyura et al. 2002; Horváth *et. al.* 2009a), which region is also an important target of recent infrastructural developments (Hungarian Ministry of Economy and Transport 2006). The 25 years population monitoring has assumed that this large-sized raptor species is highly sensitive for human disturbance and habitat alteration, and also that most infrastructural factors directly or indirectly (by increasing disturbance and persecution) could affect mortality and/or breeding success of imperial eagles. The 25 years of population monitoring has shown that human related mortality represented 87% of the known death causes in the Hungarian population (n=78 1990-2009, Horváth et al. 2009a), including electrocution (21%), persecution (poisoning 26% and shooting 56%), collision with vehicles (7%) and with electric wires (2%). Human disturbance (26%) and persecution (8%) were also among the most important known causes of recorded breeding failures (n=64 2003-2009, Horváth et al. 2009a).

In the present study our aim was to analyse the effects of settlement-, road-, railway- and power line network (referred to as infrastructures in the followings) on the present and potential breeding distribution of imperial eagles in the Hungarian Plain. We predicted that infrastructures significantly affect the breeding distribution of imperial eagles within the densest and most intensively studied population of Central Europe.

Since this population is recently expanding to south-eastern direction, we also built a predictive model for the other parts of the Hungarian Plain, to locate areas where existing infrastructural network may hinder the settlement of the species, and also to find those potential unaffected habitats, where more intensive field surveys are necessary to confirm the presence or absence of the species.

## Materials and Methods

### *Study area*

Effects of infrastructure on nest site selection was modelled by data obtained in the Core Study Area inhabited by eagles. Using our model we then estimated potentially suitable nest sites for a larger region, the Model Prediction Area.

The Core Study Area is a 2800 km<sup>2</sup> north-western fraction of the Hungarian Plain, at the Jászság and Heves-Borsod Plains (47°23'–47°50'N 19°46'–20°54'E) (Fig. 1). This open plain area (altitude between 90 and 120 m a.s.l.) is bordered by the Tisza and Zagyva rivers from south, and by the foothills of the Mátra and Bükk mountains from north. The predominant habitats are intensively cultivated agricultural fields, which are scattered by shelterbelts of poplar (*Populus* sp.) and black locust tree (*Robinia pseudoacacia*) plantations, and rarely with some remnants of semi-natural grassland and oak (*Quercus robur*) forest fragments. Imperial eagles are nesting almost exclusively on trees in the study area, with only one exceptional case, when a nest was built on a high-voltage electric pylon.

The Model Prediction Area was established in the eastern part of the Hungarian Plain, namely the low elevation parts (78-150 m a.s.l.) of the Tisza River's drainage basin. The 34 400 km<sup>2</sup> area (46°07'–48°32'N 19°30'–22°54'E) is bordered by foothills from north, by the country border from east and south, and by the Kiskunság Sand Ridge from west. We have excluded the forested habitats of the South-Nyírség, so the predicted area included a large coherent open region, predominantly covered by agricultural fields, similarly to the Core Study Area. Suitable nesting trees (> 8 m high *Populus* or *Robinia*



trees) and all three main prey species (*Cricetus cricetus*, *Lepus europeus* and *Phasianus colchicus*) are generally distributed (Bihari 2004; Csányi 2008), therefore we considered the whole area as a potential breeding habitat for the imperial eagle for this large spatial scale analysis.

#### *Population survey*

The entire Core Study Area was surveyed for active imperial eagle nests by the members of the Hungarian Imperial Eagle Working Group at the beginning of each breeding season (February-March) between 1989 and 2008. The sedentary behaviour of the species, the frequently executed display flights and calls, and the usually well visible nest sites greatly facilitated the observation of breeding pairs, therefore, we consider the actual distribution of imperial eagles to be reliably known within the study area. Breeding population increased continuously during the study period from only two breeding pairs in 1989 to 37 by 2008, meanwhile it became the densest subpopulation in Central Europe, representing 42% of the national and 25% of the EU's eastern imperial eagle population.

Nest sites were marked on topographic maps (Hungarian Army Cartographic Service, scale 1:25 000) and were located by GPS (for breeding attempts prior to 2002 the correction with GPS coordinates were executed subsequently). During the 20 years study period all together 319 nesting events in 43 different territories of the Core Study Area were included in a GIS database. Data on further 189 breeding attempts at 38 territories, located in less densely populated areas of the Hungarian Plain, were also monitored in the same period and these data were used for testing the predictions of the model. In 2008 the total study area contained 73% (64 breeding pairs) of the national population.

### *Selection of scales and measurement of habitat variables*

The density of infrastructural network in relation to presence or absence of eagles were analysed in a 2.5 km × 2.5 km UTM grid system, which grid is generally used in national bird monitoring and census techniques (Szép & Gibbons 2000). The scale of the grid system was also close to the half of the average nearest neighbour distance (NND) in the Core Study area in 2008 (NND =  $4.512 \pm 1.405$  km,  $n = 37$ ), which measure is frequently used in habitat selection studies of raptors to determine the scale of territory-level analyses (Bednarz & Dinsmore 1981; Sergio et al. 2006a; Suárez et al. 2000).

Density of each infrastructure was determined for each grid cell, such as the coverage of settlements ( $\text{km}^2/\text{km}^2$ ) and length of linear infrastructures ( $\text{km}/\text{km}^2$ ). Since multivariate studies are facing one of the main trade-offs in statistical analyses, namely to build models complex enough to describe data adequately, but also to keep it simple for clear interpretation and to avoid overparametrization (Agresti 2002), we have also initiated a single explanatory variable. This “cumulative infrastructural effect” (CIE) variable was determined as the coverage of areas, which are closer to an infrastructure than the nearest eagle nest found during the 20 years of the study. The observed minimal distances differed greatly (0-700 m) among the types of infrastructures (see details in Table 1.), therefore the CIE variable is not simply representing the density of overall infrastructural network, but it is also weighting the factors according to the eagles’ observed avoidance from them.

Most infrastructures (settlements, major and minor roads, railways) were measured from the National Geographical Information Database (OTAB©, GraphIT Ltd., version 2003). The detailed GIS databases of local electric companies (ÉMÁSZ Nyrt., E.ON TITÁSZ Zrt. and MAVIR Zrt.) were used to locate medium- and high-voltage power-lines. The measured infrastructural variables did not change significantly (> 5%) during the study period, except the length of major roads, which has increased by 22% (125 km), so here we have used the recent stage for the analyses. Each spatial measure was executed by the ArcMap© software (ESRI Inc., version 9.0) and the Hawth’s Analysis Tools extension (Beyer 2004).

**Table 1** Density of infrastructures in the occupied and unoccupied quadrats of the core study area and their effects on mortality, disturbance and nesting locations of imperial eagles based on the 20 years population monitoring data. In the buffer coverage column the minimal nesting distance was applied as a buffer around the infrastructures, therefore it shows the factors habitat shrinking effect, and also their relative contribution in the CIE variable.

Type	Density (mean $\pm$ SD)		Minimal nesting distance <sup>a</sup>	Buffer coverage <sup>b</sup>
	occupied (n = 121)	unoccupied (n = 327)		
Settlement <sup>b</sup>	0.018 $\pm$ 0.050	0.071 $\pm$ 0.126	700 m	20.74%
Major paved road <sup>c</sup>	0.063 $\pm$ 0.147	0.084 $\pm$ 0.177	100 m	0.75%
Minor paved road <sup>c</sup>	0.124 $\pm$ 0.190	0.205 $\pm$ 0.211	250 m	8.39%
Railway <sup>c</sup>	0.055 $\pm$ 0.129	0.084 $\pm$ 0.186	0 m	-
Medium-voltage (10-35 kV) power line <sup>c</sup>	0.219 $\pm$ 0.294	0.427 $\pm$ 0.425	100 m	6.25%
High-voltage (120-750 kV) power line <sup>c</sup>	0.137 $\pm$ 0.224	0.181 $\pm$ 0.315	100 m	2.71%
Cumulative infrastructural effect (CIE) <sup>b</sup>	0.197 $\pm$ 0.191	0.380 $\pm$ 0.274	-	33.05%

<sup>a</sup> Values were rounded with a 50 m precision. The exceptional nest, which was built on a high-voltage electric pylon, was excluded.

<sup>b</sup> Density represented in km<sup>2</sup> / km<sup>2</sup>.

<sup>c</sup> Density represented in km / km<sup>2</sup>. The sections outside settlement boundaries were measured.

### *Statistical analyses*

We applied intrinsic Conditional Autoregressive (CAR) models in a Bayesian framework (Latimer et al. 2006; Sudipto et al. 2004) to analyse the relationship between infrastructure variables and presence/absence of imperial eagles in the core study area. Using the sample dataset, the prior assumptions on data distribution, and the constructed data likelihood (i.e. model), posterior distributions of the likelihood parameters were sampled via Markov Chain Monte-Carlo methods. The resulted posterior distributions were characterized by descriptive statistics (e.g. mean, median, quantiles) and the 95% credible interval (analogue to the frequentist 95% confidence interval).

Spatial autocorrelation was controlled by adding a global random spatial effect term ( $\rho_i$ ) to the linear predictor:

$$\log\left(\frac{p_i}{1-p_i}\right) = W_i' \beta + \rho_i + \varepsilon_i$$

where  $p_i$  is the probability of the presence imperial eagle nests in a  $i^{\text{th}}$  cell,  $W_i'$  is the matrix of descriptive environmental variables, and  $\beta$  is the matrix of associated coefficients,  $\varepsilon_i$ , and  $\rho_i$  is the spatial uncorrelated and correlated spatial heterogeneity, respectively. The left side of the equation is the logit link function. Formally, the correlated spatial random term  $\rho$  is determined as the conditional distribution depending on its neighbours as follows:

$$\rho_i | \rho_j \approx N\left(\frac{\sum_{j \in \delta} w_{ij} \rho_j}{w_{i+}}, \frac{\sigma_\rho^2}{w_{i+}}\right)$$

where  $w_{i+}$  denotes the pre-defined total number of neighbouring cells of the cell(i), and  $\sigma_\rho^2$  is the conditional variance. Neighbours of any cell were defined as the 8 adjacent cells to it. Uninformative prior settings were applied for all parameters. The posterior distribution was sampled by three initial Markov-chains with 100 000 iterations and a burn-in value of 10 000.

Three separate models were built. In the first model we used all the seven individual infrastructure variables (multivariate model), and estimated the relative importance of each infrastructure. For the second model we only used the previously constructed “cumulative infrastructural effect” (CIE) explanatory variable. In a third model we estimated the effects of infrastructural network on the potential distribution of imperial eagles in the eastern Hungarian Plain (predictive model). The mean value of the posterior distribution of the CIE model parameters’ effects was applied to the CIE values measured in the predicted area. Probabilities were obtained by calculating the inverse logit transformation for each cell:

$$p(y) = \frac{\exp(LP)}{1 + \exp(LP)}$$

where LP is the linear predictor (Faraway 2006). Model fitting and predictive power of both models was tested by calculating Cohen's  $\kappa$  statistics (Cohen 1960) and the receiver operating characteristics (ROC) curve. The R 2.9.0 software (R Development Core Team 2007) and Winbugs 1.4.3 were used for data analysis and modelling.

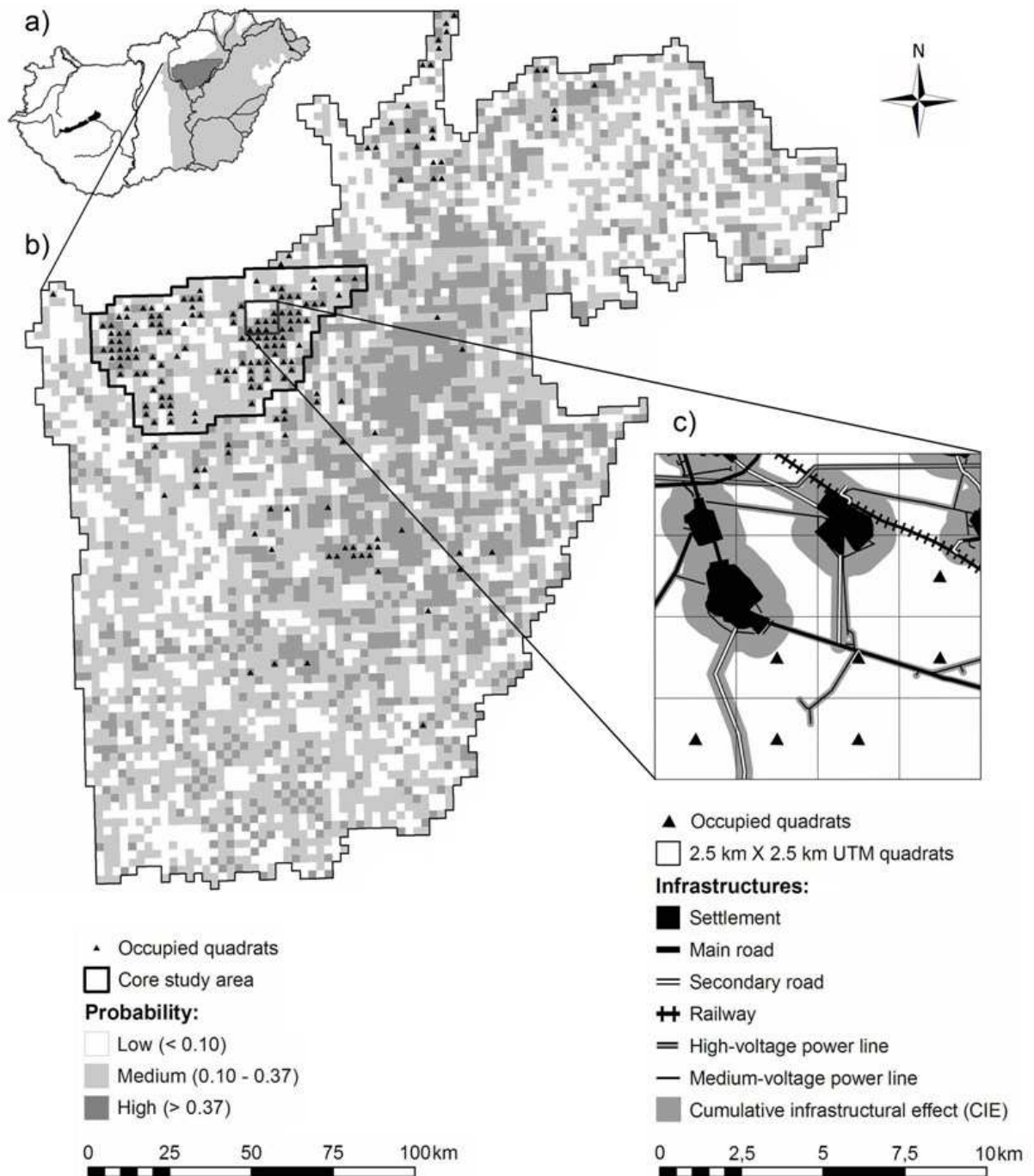
## Results

### *Minimal observed distance of nest sites from infrastructures*

We found that eagles have avoided for nesting a distinct buffer around most types of infrastructures. These buffers (i.e. the CIE variable) covered approximately one third of both the core study area (33.04%) and the whole eastern parts of the Hungarian Plain (32.89%), but nest sites were only exceptionally situated within them (< 1%). Since the observed minimal distances and also the density varied greatly among the types of infrastructures, they participated in a different extent in the CIE variable (Table 1.). The settlements and their buffers had the largest coverage, but minor roads, medium- and high-voltage power lines also assigned significant areas, while the less dense major road network had only localised effects. Nests were built in seven cases just beside (< 10 m from) railways, therefore they were not included in the CIE variable.

### *Infrastructural effects on present distribution within the Core Study Area*

The posterior distributions of the multivariate and CIE models' coefficients are presented in Table 2. Settlements showed the most remarkable negative effect on the presence of eagles in the multivariate model, where the 95% credible intervals of the coefficients' distribution were considerably below zero. Minor roads and high-voltage power lines had much smaller, but also detectably negative influence. The coefficients' median for major roads, railways and medium-voltage power lines also had negative values, but their credible intervals overlapped with zero. The CIE model showed a considerably negative



**Figure 1** Map showing (a) the location of the study area in Hungary, (b) the present breeding distribution and the predicted probability of occurrence of the imperial eagle in the core study area (by the CIE model) and in other parts of the eastern Hungarian Plain (by the prediction model), and (c) the coverage of CIE variable in relation to the distribution of different infrastructures and occupied vs. unoccupied quadrats in a typical section of the study area.

**Table 2** Descriptive statistics of the posterior distributions of parameters of the two models describing Imperial Eagle presence/absence in the core study area

	mean	SD	CI 2.5% <sup>a</sup>	median	CI 97.5% <sup>a</sup>
Multivariate model					
Settlement*	-8,6940	2,7240	-14,5000	-8,5230	-3,7860
Major road	-0,0007	0,0011	-0,0029	-0,0007	0,0014
Minor road*	-0,0022	0,0010	-0,0042	-0,0021	-0,0004
Railway	-0,0014	0,0010	-0,0036	-0,0014	0,0005
Medium-voltage power line	-0,0009	0,0007	-0,0023	-0,0008	0,0004
High-voltage power line*	-0,0014	0,0006	-0,0026	-0,0014	-0,0003
Deviance	365,17	36,90	288,50	368,40	431,30
CIE model					
CIE*	-5.196	1.022	-7.494	-5.113	-3.431
Deviance	371.9	34.37	296.1	375.2	429.8

<sup>a</sup> CI stands for credible intervals. In the bayesian framework the 95% credible intervals allow decision making on the relevance of a certain parameter; in case 0 falls in this intervall the effect of the parameter can be considered irrelevant,

\* Parameters relevant in describing Imperial Eagle presence/absence in a grid cell

univariate distribution of coefficients, corresponding to a strong adverse effect on distribution.

The probability of occurrence was determined for each UTM cell by the models, and the maximum percentage of the cells was classified correctly at 0.40 probability value in the multivariate and at 0.37 in the CIE model (Fig. 2a). If we applied these probability values as a separation limit between predicted and unpredicted cells, we could classify correctly 92% of the cells by the multivariate and 89% by the CIE model (Table 3). Cells with probability values less than 0.10 covered approximately one third of the core study area (30% with the multivariate and 32% with the CIE model), although they contained only two (< 2%) occupied quadrats at both models, therefore we applied this value for predicting the most probably unsuitable areas.

**Table 3** Comparison of the multivariate and CIE models' prediction: results with the probability values that gave the maximum percentage of cells classified correctly in the Core Study Area.

Observed/Predicted	Multivariate model		CIE model	
	Occupied	Unoccupied	Occupied	Unoccupied
Occupied	95	11	94	22
Unoccupied	26	316	27	305

**Table 4** Comparison of the multivariate and CIE models' AUC and Cohen's  $\kappa$  statistics.

Model	AUC	AUC sd	Cohen's $\kappa$	Lower CI	Upper CI
Multivariate	0.935	0.013	0.622	0.54	0.73
CIE	0.932	0.014	0.578	0.5	0.65

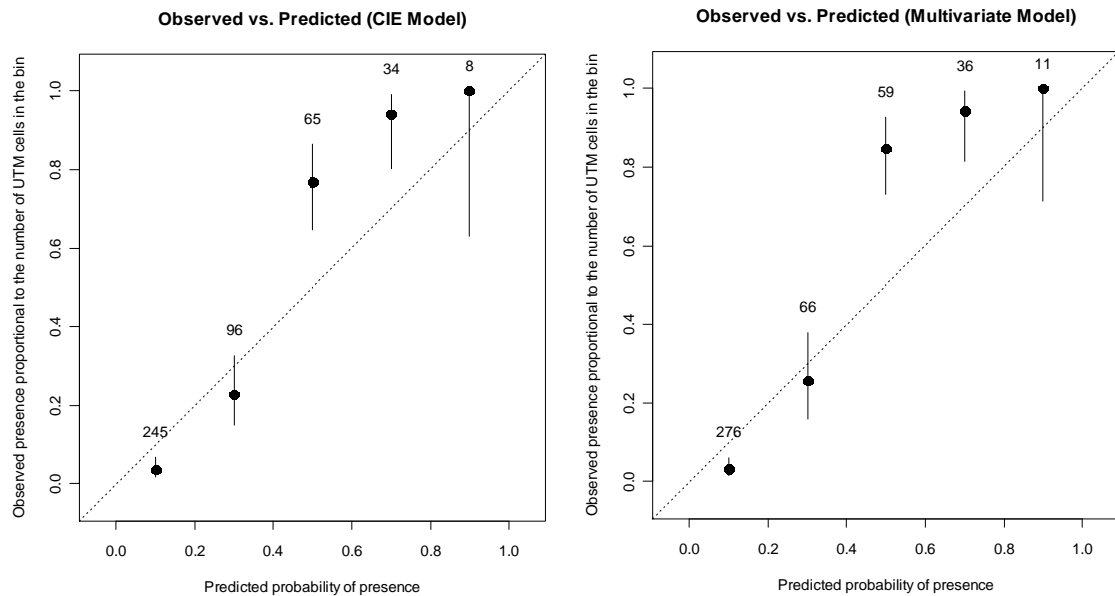
The AUC and Cohen's  $\kappa$  statistic values of the two competing models were similar (Table 4), moreover there was no deviation in the pattern and bin distribution of goodness-of fit plots as well (Fig. 2b). Since the simpler CIE model described imperial eagle presence in the study site equally well, we used this model formula to estimate the probability of presence for the eastern Hungarian Plains.

#### *Infrastructural effects on the potential distribution in the eastern Hungarian Plain*

We divided the UTM cells of the Eastern Hungarian Plain into three (low-medium-high) probability categories based on the values calculated by the prediction model. The 0.37 probability value, which classified the maximum percentage of cells correctly in the CIE model, was used as a lower limit to determine high probability cells, while the 0.10 value were applied as an upper limit for low probability cells (Fig. 1b).

The prediction map showed that large parts (32%) of the Hungarian Plain are classified as low probability nesting areas for the imperial eagle because of the existing infrastructural network. The model also pointed out that approximately two third of the Plain is probably still providing suitable breeding habitats for the species regarding the effects of infrastructures, and it has located those priority habitats (24% of the Plain), where the lack of infrastructures most probably facilitating the future settlement of the species. The recent distribution of imperial eagles was in correspondence with the results of the model, since only one (1%) occupied quadrat was determined as low probability habitat, while 43 (61%) of them were in high probability quadrats.





**Figure 2** Comparison of the multivariate and CIE models' predictions for the core study area by the observed vs. predicted presence of eagles within the UTM cells.

## Discussion

Eastern imperial eagles have switched their main breeding habitats from relatively undisturbed mountain forests to highly populated open agricultural habitats in the Carpathian basin during the last two decades (Bagyura et al. 2002; Kovács et al. 2008). Their increasing population seems to be well adapted to human disturbance and in some cases they can also tolerate the extreme proximity of infrastructures (Danko & Balla 2007). Nevertheless our habitat-selection models have shown that the network of settlements and linear infrastructures is still remarkably affecting the breeding distribution within the densest Central European imperial eagle population. Other European studies also reported that the presence of infrastructures and/or human disturbance are among the first factors which determine the habitat selection of several rare raptor species (Balbontín 2005; Donazar et al. 1993; Gavashelishvili & McGrady 2006; López-López et al. 2007; Morán-López et al. 2006a; Sará & Di Vittorio 2003), including the Spanish imperial eagle as well (Bisson et al. 2002; Gonzalez et al. 1992).

These studies are generally building complex habitat selection models to identify the most important affecting factors out of numerous environmental variables, and they are not quantifying separately the habitat shrinking effect of infrastructures. Here we have focused especially on the effects of the different infrastructural factors and we presumed that other environmental and ecological requirements (i.e. prey abundance, nesting and foraging habitat structure, climatic factors etc.) are suitable for the species within its local distribution area. We have found that the existing infrastructural network significantly shrinks the available nesting habitats, since it hampers the settlement of the species in one third of both the core study area and the whole eastern part of the Hungarian Plain. On the other hand the recently existing gaps among settlements, roads and power lines can still provide enough suitable habitats for the further expansion of the species.

Habitats with low infrastructural effect (i.e. high probability quadrats in Fig. 1) were three times more frequently occupied by imperial eagles than it would be expected from their relative frequency. Some regions containing large coherent potential habitats (like the Hortobágy, Bihar, and Dévaványai-sík) are nationally protected and designated as Special Protection Areas (SPA) within the EU's Natura 2000 network. The valid national obligations for protected areas and SPAs, including bird population monitoring and habitat preservation, can hopefully guarantee the immediate location and long-term conservation of breeding pairs colonizing these regions. However, as it was similarly reported for other wide-ranging European raptors (López-López et al. 2007; Watson & Whitfield 2002), a large proportion of high probability habitats fall outside SPAs (67.63%) and national protected areas (84.07%). Here the intensity of bird population surveys and the lobbying possibilities of nature conservation against other stakeholders (e.g. infrastructure developers) are much lower. Some of these areas are important habitats for other threatened bird species, such as the Saker Falcon (*Falco cherrug*), Red-footed Falcon (*Falco vespertinus*), Great Bustard (*Otis tarda*) and Roller (*Coracias garrulus*) (Heath & Evans 2000). Infrastructural developments which will affect these unprotected priority habitat patches, should consider the presence and tolerance of these globally threatened species, which emphasize the need of single- and multispecies habitat selections studies in this particular region.

The relative tolerance towards infrastructures can vary significantly among species (Blumstein et al. 2005), habitats (Donazar et al. 2002), individuals (Richardson & Miller 1997) and also among the different types of infrastructures. Imperial eagles in our study area showed more tolerance towards major paved roads than towards minor ones, which is just the opposite that one could expect. The probable reason is that eagles are especially sensitive to irregular and unpredictable movements of pedestrians and cyclers, or to cars which stop beside the road, which are frequent events along minor roads. On the other hand they can get used to regularly and predictably moving vehicles (cars, trucks, trains), which are almost the only type of disturbance connected to major roads and railways (Danko & Balla 2007). These results are in accordance with the findings of Gonzalez et al. (2006a), who reported that Spanish imperial eagles almost never showed alert reaction for continuously moving vehicles, while they were sensitive within a distinct buffer to pedestrians, stationary cars and especially to hunters and ecotourists, who could pay direct attention to the birds.

Buffer zones, where human activities are temporary restricted, are frequently used methods in conservation to decrease the effects of disturbance and/or habitat destruction (Whitfield et al. 2008). For the proper conservation of the Spanish imperial eagle Gonzalez et al. (2006a) suggested an inner buffer zone (with a radius of 500 m) around nest sites, where no human activity should be allowed during the breeding season, and an outer buffer zone (with radius between 500-800 m), where some activities, including vehicles passing, may be authorized. Such buffer zones with 100-500 m radiuses around eastern imperial eagle nests were applied also in Hungary from 1980 onwards, but only in such cases, when the authorised experts decided that the given activity (e.g. forestry, hunting, bee-keeping, ecotourism) would probably cause the failure of the breeding attempt (Bagyura et al. 2002; Haraszthy 1996). At the same time imperial eagles several times bred successfully much closer than 500 m to all types of studied linear infrastructures (including public paved roads and railways) in our study area, where the general ban of human activities would be neither possible, nor reasonable. We believe that sedentary and territorial eagles are able to assess the level of human presence according to their tolerance, during the nest site selection period, and they can choose an alternative site if a continuously appearing activity is disturbing them. Hence

we do not recommend any restrictions for imperial eagle conservation purposes in the usage of an existing infrastructure, nor in other human activities, which were already going on with a similar intensity before the beginning of the given breeding season.

### *Conservation implications*

Based on our results and the above mentioned consequences, we propose the following conservation recommendations during the operation or development of infrastructures in active or potential imperial eagle breeding habitats:

- (1) Plans for new linear infrastructures and investments should aspire to minimize further fragmentation of priority habitat patches of imperial eagles and other globally threatened species. Adverse effects could be decreased if new infrastructures are established close to already existing ones and if they do not set apart previously coherent, undisturbed habitat patches.
- (2) Electrocution is the most significant mortality cause, which is connected directly to infrastructures, and probably the only one, which can be reduced effectively by mitigation measures (Ferrer & Hiraldo 1991; Bagyura et al. 2004). According to the 2009 modifications of the Hungarian Nature Conservation Law (53/1996), all newly built or totally renovated power lines sections must be developed in a way, which do not threaten wild bird populations. Besides the strict observance of the new regulation, the bird-friendly modification of the already existing ca. 235 000 dangerous power poles of the Hungarian Plain would be the final solution of the problem. In mid-term the modification of the ca. 14 000 most dangerous pylons, which are situated in highly potential imperial eagle habitats located by our model, would be essential to decrease significantly the frequency of electrocution incidents.
- (3) Not regular human activities connected to infrastructures (i.e. development or maintenance works) in the close vicinity of an active nest site can threaten the successful breeding. If works are initiated during the breeding season and coupled with significant disturbance, they should be restricted between 15 March and 15

August (extreme dates for egg laying and fledging, M. Horváth, unpubl. data) within a 500-800 m radius around the nest site (Gonzalez et al. 2006a).

- (4) Intensive field surveys and monitoring should be focused on high probability areas, especially in those regions where SPA and protected area coverage is low (Nagykunság, Hajdúhát, Békés Plain). Our model will hopefully help the more efficient detection of new breeding pairs, which is the base of conservation measures against habitat destruction and persecution.

### CHAPTER 3. PRODUCTIVITY



### 3.1 EFFECT OF PREY COMPOSITION ON THE REPRODUCTIVE SUCCESS OF IMPERIAL EAGLES (*AQUILA HELIACA*)

#### ABSTRACT

Reproductive success of raptor species is significantly affected by the quantity and/or quality of available prey. In the present study we compared the prey composition and reproductive success of breeding imperial eagles in East-Hungary, where 434 nesting events in 81 different territories had been monitored between 1995 and 2004. We identified 1297 prey items originating from 43 bird and 16 mammal species (532 and 764 specimens, respectively). Three prey species, the brown hare (*Lepus europaeus*), the hamster (*Cricetus cricetus*) and the pheasant (*Phasianus colchicus*), seem to have especially important role in the diet of imperial eagles in Hungary, although their relative frequencies varied greatly among different regions. We found that eagles were less productive in a region where hamster was the dominant prey (West-Zemplén Mts.) compared to a recently colonized region abundant in hare (Heves Plain) suggesting that hares provide better food supply than hamsters. The increase of game species in the diet of imperial eagles could generate hostility in hunters, which conflict should be resolved between nature conservation and small-game management by initiating common projects to improve hare and pheasant habitats and to raise public awareness,

*Horváth, M., Szitta, T, Firmánszky, G., Solti, B., Kovács, A., Kabai, P. & Moskát C. 2009. Acta Zoologica Academiae Scientiarum Hungaricae (in press).*

## INTRODUCTION

The eastern imperial eagle (*Aquila heliaca*) is a large-sized raptor with a broad breeding distribution range along the forest-steppe zone from East-central Europe to the Lake Baikal in Asia (Del Hoyo et al. 1995). In Hungary, the imperial eagle became a rare breeder by the early 1980's, when only 15-25 breeding pairs were estimated in remote mountainous forested areas (Bagyura et al. 2002, Haraszthy et al. 1996). From 1989 the eagles have gradually increased their range of distribution by occupying lowland agricultural habitats in the vicinity of their former breeding range (Bagyura et al. 2002). As a result of such continuous increase during the last two decades, the eagles now inhabit a large part of the Hungarian Great Plain, and their known population size reached 105 breeding pairs in 2009, and 85% of those nest in lowland areas (Horváth et al. 2009a). The breeding pairs in Hungary are part of a population inhabiting the Carpathian Basin, South-Slovakia, East-Austria and the Czech Republic (Horváth et al. 2002).

The diet of the eastern imperial eagle predominantly consists of small and medium sized mammals, birds and reptiles in most part of its range, although their relative frequencies vary greatly among regions (Del Hoyo et al. 1995). Decades ago sousliks (*Spermophilus* spp.), and hamsters (Cricetinae) were considered as the main prey species of imperial eagles in most parts of their breeding range (Del Hoyo et al. 1995). Sousliks are still the most important food items for the largest eastern populations of Russia and Kazakhstan (Belik et al. 2002, Karyakin et al. 2008). However, the severe decline of souslik populations apparently forced the eagles to adapt to other food sources in the western part of their distribution area resulting in significant changes in the diet composition. Rook (*Corvus frugilegus*) was reported to be the main prey in Serbia (Vasic & Misirlic 2002), and in some regions of western Russia (Belik et al. 2002). In the Caucasus high ratio of reptiles (30%) and small rodents (54%) were detected (Abuladze 1996), whereas the European brown hare (*Lepus europaeus*) and chicken (*Gallus domesticus*) were found to be the main prey species (26% and 21 % respectively) in South-Bulgaria (Marin et al. 2004).

In the Carpathian Basin early literatures mentioned souslik (*Spermophilus citellus*) as a main prey of the imperial eagles (Chernel 1899), although hamster (*Cricetus cricetus*)



and occasionally hare and poultry were also noted (Nagy 1943, Pátkay 1947, Szemere 1912, Vasvári 1938). Haraszthy et al. (1996) published the first comprehensive survey on the diet of imperial eagle in Hungary, and they found that during the 1980's and early 1990's the hamster was the main prey species in Hungary, composing 51% of all identified prey items. Hares (12%) and pheasants (*Phasianus colchicus*, 12%) were also frequently taken, and souslik composed already only 7% of the diet at that time. Chavko et al. (2007) studied the diet composition in the Slovakian part of the Carpathian Basin between 1970 and 2005, where hares (35%) were the most frequently identified preys, followed by hamsters (19%) and pheasants (13%), and sousliks composed only 4% of the identified prey items.

Prey composition and density are among the most important factors determining the breeding success of large raptors (Newton 1979, Gonzalez et al. 2006b, Steenhof & Kochert 1988), as well as the settling strategies of dispersing juveniles (Penteriani et al. 2006), however, data on the relationship between diet composition and productivity of imperial eagles are sporadic. Katzner et al. (2005) compared four different breeding areas with different availability of food supplies in a dense imperial eagle population in Kazakhstan. They found that dietary diversity and productivity varied between the regions without any significant relationship between the two variables. The same authors found that eagles nesting near a high-density prey resource used that resource almost exclusively, while in locations with no single high-density prey species, their diet was more diverse (Katzner et al. 2006).

The closest relative of the eastern imperial eagle, the Spanish imperial eagle (*Aquila adalberti*) an endangered raptor confined to the Iberian peninsula is highly dependent on its main prey species, the rabbit (*Oryctolagus cuniculus*) (Ferrer & Negro 2004). Sánchez et al. (2009) found variability in the diet of the Spanish imperial eagle between different regions, and suggested that where their main prey was scarce, eagles were able to change their diet utilizing alternative prey species, such as pigeons (*Columba* spp.) or carrion.

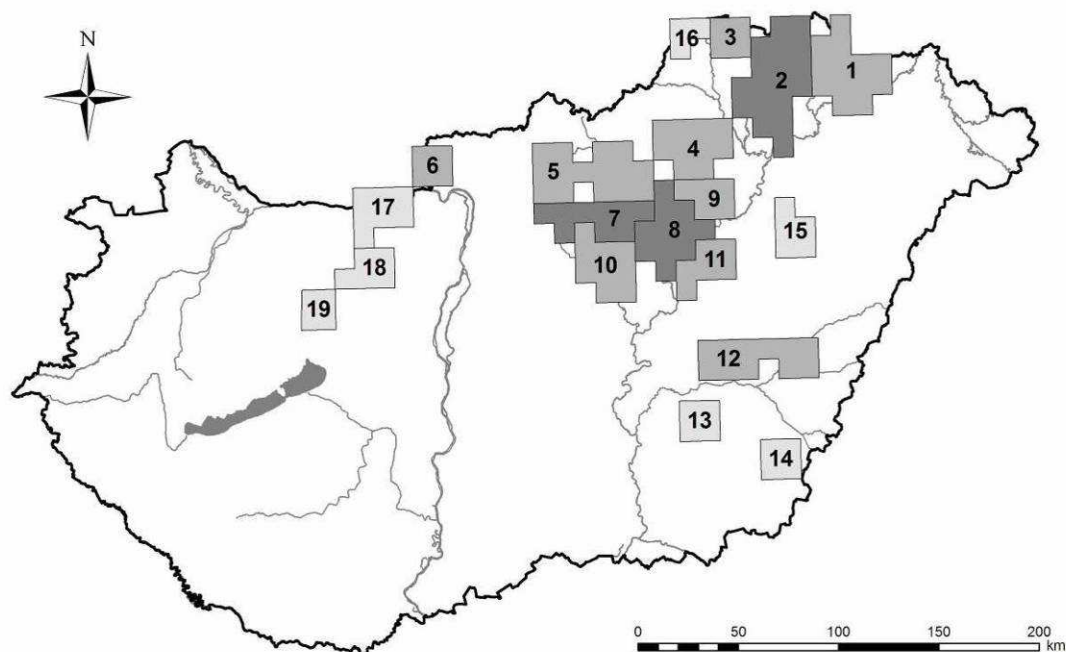
In the present study we compared the prey composition of breeding imperial eagles in different areas of Hungary. We hypothesized that quality of food source affects reproductive success of imperial eagles. We predicted that prey composition and main

prey species vary among geographical regions offering different quality of food supply. We also predicted higher reproductive success in areas where eagles feed their nestlings mainly with hares, one of the largest mammals preyed upon by these birds, compared to those areas, where the main prey is the much smaller-bodied hamster.

## MATERIAL AND METHODS

### *Study area*

Diet composition of imperial eagles was analysed in the Great Hungarian Plain and adjacent low mountains, where the great majority (> 95%) of the eastern imperial eagle breeding population can be found in Hungary (Fig. 1). We compared the diet and reproductive success of eagles in the two most intensively studied regions. The western parts of the Zemplén Mountains (48°00′-48°30′N, 21°00′-21°20′E, 1800 km<sup>2</sup>) is one of the traditional breeding habitats of imperial eagles in Hungary, where their presence has been reported from as early as 1913 (Vasvári 1938). The intensive agricultural fields and the incorporated small-sized pastures of the Hernád River valley (110-160 m a.s.l.) form the most important foraging areas for imperial eagles in this region, and recently some pairs also breed in these open lowland landscape on small groups of poplars (*Populus* spp.) and black locust trees (*Robinia pseudoacacia*). The nearby foothills of the Zemplén mountains (200-700 m a.s.l.) represent traditional breeding habitats, where imperial eagles are nesting in oak (*Quercus petraea*, *Q. cerris*) and beech (*Fagus sylvatica*) forests, or on pine trees (*Pinus silvestris*, *P. nigra*, *Larix decidua*). Seventy kilometres further from the Zemplén mountains in south-west direction lies the Heves Plains (47°30′-47°45′N 19°50′-20°40′E 2000 km<sup>2</sup>), which comprises a mosaic of intensive agricultural fields and grassland habitats (85-100 m a.s.l.). The first recent breeding of the species was recorded here in 1989, and due to an intensive expansion this became the biggest and densest sub-population in Hungary by 2005. Poplar and black locust trees are the main nesting trees in this region.



**Figure. 1.** Breeding distribution of the imperial eagle in Hungary between 1995 and 2004. Data are presented in a 10 km x 10 km UTM grid. Regions: (1) East-Zemplén Mts., (2) West-Zemplén Mts., (3) Cserehát Mts., (4) Bükk Mts., (5) Mátra Mts., (6) Börzsöny Mts., (7) West-Heves Plain, (8) East-Heves Plain, (9) Borsodi Mezőség Plain, (10) Jászság Plain, (11) Nagykunság Plain, (12) Dévaványa Plain, (13) West-Békés Plain, (14) South-Békés Plain, (15) Hortobágy Plain, (16) Aggtelek Mts., (17) Gerecse Mts., (18) Vértes Mts., (19) East-Bakony Mts. Black: two sample areas, where diet composition and reproductive success were compared. Grey: data on prey composition presented. Light grey: no data on prey composition were available.

### *Population and prey survey*

The identified and potential breeding territories of eastern imperial eagles were monitored in the entire study area by the members of the Hungarian Imperial Eagle Working Group between 1995 and 2004. Monitoring protocol included the localization of the active nest sites at the beginning of each breeding season (February-March), and active nests were checked by distant observations for at least once per month during the breeding period (from April to August) to determine breeding success (i.e. number of

fledglings). Observations were carried out from a distance ( $> 1000$  m) to avoid disturbance, except one occasion in June, when the nests were visited in order to mark the chicks with rings.

Food remains were collected twice a year. First, during the ringing procedure remains were collected directly from the nests and its surroundings, while following fledging (August-September) only the ground below the nests and roost sites were checked for food remains. Collected remains, including pellets, feathers, bones, hairs and skins of prey animals were identified in comparison with reference materials. The minimum number of individuals in each collection was determined by the type and quantity of the remains found. It should be noted that the chance of finding remains depends on the size of the prey species (Sánchez et al. 2008), thus data on diet composition of any eagle pair may be biased. Assuming similar bias in the different territories, however, such data are often used for comparing habitats.

Over the 10-year study period a total of 434 nesting events in 81 different territories were observed in the study area, and a total of 1297 prey items in 71 territories were collected and identified. We used territories as sampling units to avoid pseudoreplication that would occur if each breeding attempt of the same pair was handled separately (Katzner et al. 2005). Breeding success was calculated as the average number of fledglings per successful breeding in a territory.

### *Statistical analysis*

Statistical analyses were carried out with the SPSS ver. 17 programs package (SPSS Co.). We used non-parametric tests for comparison of data-sets deviating from the normal approximation. All statistical tests were two-tailed. A hierarchical cluster analysis was carried out by the SYN-TAX 2000 for Windows programs package (Podani 2001). We clustered the different regions on the basis of the average diet composition of imperial eagle territories in the region, choosing the similarity ratio index for the calculation of the procedure matrix and the UPGMA method for fusion strategy. Territories with less than 5 identified prey items were excluded from cluster analysis.

**Table 1.** Summary of bird species found at imperial eagle nests and roost sites between 1995 and 2004 in Hungary.

Species	Prey item		Territory	
	pieces	%	pieces	%
<i>Alauda arvensis</i>	4	0.3%	4	5.6%
<i>Anas crecca</i>	2	0.2%	2	2.8%
<i>Anas domestica</i>	3	0.2%	3	4.2%
<i>Anas platyrhynchos</i>	21	1.6%	15	21.1%
<i>Anas querquedula</i>	2	0.2%	2	2.8%
<i>Anser</i> sp.	1	0.1%	1	1.4%
<i>Anser domestica</i>	1	0.1%	1	1.4%
<i>Ardea cinerea</i>	5	0.4%	5	7.0%
<i>Asio flammeus</i>	2	0.2%	2	2.8%
<i>Asio otus</i>	23	1.8%	18	25.4%
<i>Buteo buteo</i>	11	0.8%	8	11.3%
<i>Ciconia ciconia</i>	1	0.1%	1	1.4%
<i>Circus aeruginosus</i>	1	0.1%	1	1.4%
<i>Columba</i> sp.	11	0.8%	10	14.1%
<i>Columba livia</i> forma <i>domestica</i>	82	6.3%	35	49.3%
<i>Columba oenas</i>	2	0.2%	2	2.8%
<i>Columba palumbus</i>	22	1.7%	16	22.5%
<i>Corvus</i> sp.	8	0.6%	7	9.9%
<i>Corvus corax</i>	8	0.6%	6	8.5%
<i>Corvus cornix</i>	11	0.8%	8	11.3%
<i>Corvus frugilegus</i>	9	0.7%	6	8.5%
<i>Corvus monedula</i>	2	0.2%	2	2.8%
<i>Cuculus canorus</i>	1	0.1%	1	1.4%
<i>Egretta alba</i>	2	0.2%	2	2.8%
<i>Emberiza calandra</i>	1	0.1%	1	1.4%
<i>Falco tinnunculus</i>	7	0.5%	7	9.9%
<i>Fulica atra</i>	3	0.2%	3	4.2%
<i>Galerida cristata</i>	4	0.3%	2	2.8%
<i>Gallus domesticus</i>	19	1.5%	16	22.5%
<i>Garrulus glandarius</i>	2	0.2%	1	1.4%
<i>Larus ridibundus</i>	2	0.2%	2	2.8%
<i>Limosa limosa</i>	1	0.1%	1	1.4%
<i>Numida meleagris</i>	1	0.1%	1	1.4%
<i>Perdix perdix</i>	11	0.8%	9	12.7%
<i>Pernis apivorus</i>	2	0.2%	1	1.4%
<i>Phasianus colchicus</i>	196	15.1%	53	74.6%
<i>Philomachus pugnax</i>	1	0.1%	1	1.4%
<i>Pica pica</i>	22	1.7%	15	21.1%
<i>Picus viridis</i>	1	0.1%	1	1.4%
<i>Platalea leucorodia</i>	1	0.1%	1	1.4%
<i>Scolopax rusticola</i>	1	0.1%	1	1.4%
<i>Streptopelia turtur</i>	1	0.1%	1	1.4%
<i>Strix aluco</i>	2	0.2%	2	2.8%
<i>Sturnus vulgaris</i>	5	0.4%	4	5.6%
<i>Turdus merula</i>	1	0.1%	1	1.4%
<i>Vanellus vanellus</i>	7	0.5%	6	8.5%
Unidentified passerine	1	0.1%	1	1.4%
Unidentified bird	5	0.4%	4	5.6%
Total birds	532	41.0%	61	85.9%

**Table 2.** Summary of mammal species found at imperial eagle nests and roost sites between 1995 and 2004 in Hungary.

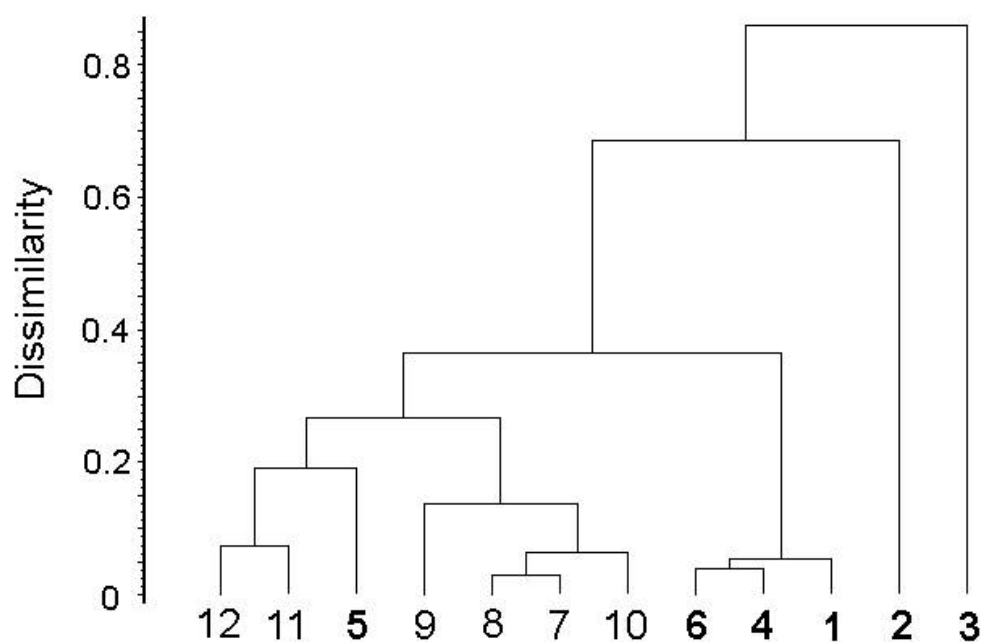
Species	Prey item		Territory	
	pieces	%	pieces	%
<i>Capreolus capreolus</i>	32	2.5%	25	35.2%
<i>Cricetus cricetus</i>	271	20.9%	44	62.0%
<i>Erinaceus concolor</i>	7	0.5%	5	7.0%
<i>Felis</i> sp.	3	0.2%	3	4.2%
<i>Felis catus</i>	3	0.2%	3	4.2%
<i>Lepus europaeus</i>	387	29.8%	61	85.9%
<i>Lutra lutra</i>	1	0.1%	1	1.4%
<i>Microtus</i> sp.	3	0.2%	3	4.2%
<i>Microtus arvalis</i>	24	1.9%	18	25.4%
<i>Mustela</i> sp.	1	0.1%	1	1.4%
<i>Ondatra zibethica</i>	1	0.1%	1	1.4%
<i>Ovis musimon</i>	1	0.1%	1	1.4%
<i>Rattus norvegicus</i>	6	0.5%	5	7.0%
<i>Sciurus vulgaris</i>	1	0.1%	1	1.4%
<i>Spermophilus citellus</i>	11	0.8%	9	12.7%
<i>Sus scrofa</i>	4	0.3%	4	5.6%
<i>Vulpes vulpes</i>	8	0.6%	7	9.9%
Total mammals	764	58.9%	69	97.2%

## RESULTS

### *Diet composition in the Great Hungarian Plain*

Altogether we identified 532 bird specimens belonging to at least 43 species (Table 1) and 764 mammal specimens of 15 species (Table 2) in the diet of imperial eagles in East-Hungary. Besides birds and mammals only a single reptile, remains of an unidentified Colubridae, was found (< 0.1%). We found that hares, hamsters and pheasant were the most frequently taken prey species composing 65.8% of the identified specimens.

The relative frequencies of the three main species in the diet varied greatly among the different regions of the study area (Table 3). Cluster analysis according to the frequency of the three main prey species aggregated all plain regions with a single mountainous site in one cluster at dissimilarity 0.3 (Fig. 2). The single mountainous site in that cluster was the Mátra Mountains where diet contained hares and pheasants with high frequency, similarly to the plain regions. Diet compositions of three mountainous sites (Börzsöny, Bükk and East-Zemplén Mountains) were very similar to each other with



**Figure. 2.** Cluster analyses of imperial eagle breeding areas based on prey composition data. Region codes are presented in Table 1. Codes with bold characters represent mountainous habitats.

**Table 3.** Relative frequencies of the three main prey species in the diet of imperial eagles. Territorial data are averaged according to the twelve regions of the study area (standard deviations in parentheses).

Region	Territory	Prey item	Hare	Hamster	Pheasant
1 East-Zemplén Mts.	6	80	0.198 (0.124)	0.216 (0.210)	0.244 (0.214)
2 West-Zemplén Mts.	12	270	0.069 (0.083)	0.558 (0.270)	0.080 (0.087)
3 Cserehát Mts.	1	9	0.000 (0.000)	0.000 (0.000)	0.111 (0.000)
4 Bükk Mts.	5	34	0.318 (0.419)	0.121 (0.138)	0.121 (0.138)
5 Mátra Mts.	10	104	0.334 (0.280)	0.019 (0.043)	0.212 (0.148)
6 Börzsöny Mts.	3	20	0.205 (0.056)	0.169 (0.150)	0.169 (0.150)
7 West-Heves Plain	8	242	0.465 (0.288)	0.040 (0.056)	0.127 (0.106)
8 East-Heves Plain	12	316	0.454 (0.219)	0.094 (0.069)	0.132 (0.077)
Borsodi Mezőség					
9 Plain	5	155	0.443 (0.320)	0.161 (0.115)	0.101 (0.069)
10 Jászság Plain	4	38	0.444 (0.134)	0.179 (0.155)	0.125 (0.144)
11 Nagykunság Plain	2	13	0.321 (0.253)	0.000 (0.000)	0.226 (0.084)
12 Dévaványa Plain	3	16	0.360 (0.096)	0.000 (0.000)	0.210 (0.214)
Total	71	1297	0.316 (0.260)	0.173 (0.233)	0.147 (0.129)

more balanced occurrence of all three dominant prey species. Two further mountainous sites were found unique, because of the exceptionally high occurrence of hamsters in the diet in one case (West-Zemplén mountains), and in the other case because of the absence of both hares and hamsters in the diet of the single breeding pair inhabiting the given region (Cserehát Mountains).

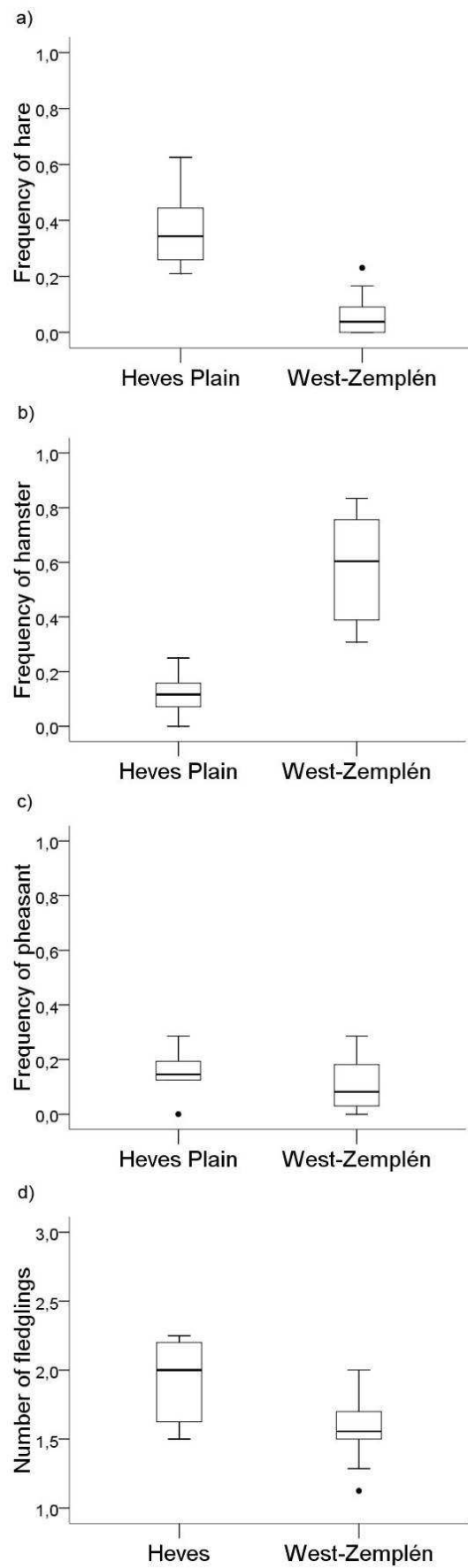
#### *Effects of diet composition on reproductive success*

Diet composition were similar in the East and West Heves Plains (Fig. 3), therefore these two sites were aggregated into a single Heves Plain region for further analysis. Diet composition and reproductive success was compared between the Heves Plain and the West Zemplén Mountains including those territories ( $n = 14$  and  $10$ , respectively) where successful breeding occurred during the study period. We found significant differences between the two regions, i.e. higher frequency of hares and lower frequency of hamsters in Heves as compared to the West Zemplén Mountains (hare: Fig 3a, Mann-Whitney test,  $Z = -3.991$ ,  $P < 0.001$ ; hamster: Fig. 3b, Mann-Whitney test,  $Z = -4.104$ ,  $P < 0.001$ ). The occurrence of pheasants in the diet was intermediate in both regions without any significant difference (Fig. 3c, Mann-Whitney test,  $Z = -1.089$ ,  $P = 0.285$ ). The number of fledglings was significantly higher in the hare-dominated Heves Plain than in the hamster-dominated Zemplén region (Fig. 3d; Mann-Whitney test,  $Z = -2.072$ ,  $P < 0.05$ ).

## **DISCUSSION**

Our data suggest that three prey species, the brown hare, the hamster and the pheasant, have especially important role in the diet of imperial eagles in Hungary. Compared to earlier data from the 1980's, our study indicates dramatic changes in prey composition (Haraszthy et al. 1996). The importance of hamsters decreased, while hares and pheasants became more abundant in the analysed prey remains. The change in the consumption of the souslik is also remarkable, as souslik used to be the 4th most freque-





**Fig. 3.** Frequency of the three main prey species (a-c) and reproductive success (d) of imperial eagles in two East-Hungarian regions. Boxplots presents the 5, 25, 50, 75 and 95 percentiles. Dots are outliers.

ntly identified prey, and now it composes less than 1% of the consumed specimens. However, it should be noted that sousliks are still reported as the main prey in some western Hungarian breeding areas (Vértes and Gerecse Mts.), which were not included in our analyses (Viszló & Csonka unpubl. data).

Changes in diet composition are apparent not only by time, but also by habitat type. Diet of eagles nesting in the plain habitat was dominated by the brown hare, whereas in most mountainous areas the hamster was an important prey type. Such changes in time and space reflect tendencies in population dynamics of prey species on the one hand, and changes in habitat occupied by eagles on the other hand. The European souslik is considered vulnerable and despite efforts to reintroduce them into their traditional range their numbers are currently in serious decline (Vácz 2006). Hamsters with their comparative body size provide a good alternative to souslik for the eagles, and they are still widespread and locally abundant in the agricultural habitats of East Hungary, although their populations have also been shrinking during the last decades (Bihari 2004). On the other hand, the increased prevalence of the brown hare in the diet cannot be explained by its population dynamics, because hare population size has been continuously declining since the 1960's (Báldi & Faragó 2007). Despite of this decline, the brown hare is still one of the most abundant medium-sized mammal in the lowland agricultural habitats currently occupied by imperial eagles, while their density is remarkably lower in the foothills of adjacent mountains, where alternative prey species are dominating in the diet (Kovács et al. 2005). Thus imperial eagles apparently can successfully adapt to changes in the availability of prey species in time or space. Similar adaptations were reported from Spain and Kazakhstan (Katzner et al. 2005, Sánchez et al. 2009).

Although imperial eagles can modify their diet composition as a response to the dynamics of prey populations, quantity and/or quality of available prey species is fundamentally affecting the distribution and reproductive success of imperial eagles, as it was shown for several other raptor species as well (McIntyre 2002, Newton 1979, Steenhof & Kochert 1988). We found that reproductive success in the Zemplén Mountains, where the diet is dominated by hamsters, was significantly lower than in the nearby plain region (Heves Plain) with brown hare in abundance. Hamster is a

predominantly nocturnal mammal with a weight of 140-400 g (Bihari et al. 2007). On the other hand hares are ten times heavier than hamsters on average and more active at daylight, so if hamster availability is not much higher than hare availability, eagles are probably not able to kill and transport enough hamsters to compensate for their smaller body size. Moreover the amplitude of the hamsters population cycle is more pronounced than of hares, probably causing that the food supply cannot be continuously optimal over a longer period in those territories, where hamsters are dominating in the diet. This is in correspondence with the findings of Bihari et al. (2008), who reported that hamsters' frequency in the diet of imperial eagles in the Zemplén Mountains reflected to their population cycles. For a better understanding how food supply affects reproductive success of imperial eagles further research is needed including more breeding areas as well as quantitative analysis of prey availability.

Expansion of the imperial eagle to the plain habitat, where their main prey species are larger and/or more abundant, provides a good prospect for the survival of this globally threatened species, although this novel move creates new challenges for nature conservancy as increasing conflict of interest with small-game management and hunting associations can be predicted. There are already signs of increasing number of illegal poisoning and shooting incidents (Horváth et al. 2009a), which if not stopped can make the eagle population extremely vulnerable, as it was shown for other eagle species with similar foraging habits in Western-Europe (Ferrer & Penteriani 2008, Whitfield et al. 2004). Thus the conflicts of interest should be handled carefully. Nature conservation organizations should establish a more effective communication and cooperation with hunting associations by raising public awareness and by initiating common projects to improve hare and pheasant habitats (Kovács et al. 2005). In parallel more attention should be focused on the decline of traditional prey species, such as the hamster (Bihari 2004) and the souslik (Báldi et al 1995), especially in those regions, where they are still composing the main prey of imperial eagles and/or other globally threatened predators, such as the saker falcon (*Falco cherrug*).

### 3.2 SIMULTANEOUS EFFECT OF HABITAT AND AGE ON REPRODUCTIVE SUCCESS OF IMPERIAL EAGLES (*AQUILA HELIACA*) IN HUNGARY

#### ABSTRACT

In the East-Hungarian study area an unexpected expansion of the eastern imperial eagle population was observed into the Hungarian Great Plain from 1989 onwards, and here the population markedly increased from 2 to 59 breeding pairs by 2006, while the mountainous population remained more or less stable between 12-17 breeding pairs. At the beginning of the expansion process inner-nest distances was lower in the mountains than in the lowlands, but presently they are similar, indicating a saturation process in the lowland areas, but up till now density dependence was still not observable in breeding success. During the study period a higher ratio of non-adult pairs was observed in the lowland territories (49%) than in the mountains (22%). We analysed simultaneously the effect of age and habitat type on breeding success. We found that both age and habitat had significant effect on breeding success variables. We found that age-effect is more significant on success rate (i.e. the ratio of pairs that produce at least one chick), while habitat-effect was more evident on fledging success (i.e. the number of fledglings per productive pair). The overall productivity (i.e. number of fledglings per breeding pair) was affected primarily by the age of the pairs, but the interaction of age and habitat type had also significant effect. We suppose that better feeding possibilities (closer foraging areas and larger prey density) could explain the higher fledging success in the lowlands. We also predicted that pairs inhabiting lowland agricultural areas will have a lower success rate due to higher human disturbance, but even if this trend was observable it had significant effect only in interaction with the age of the pairs, therefore adult pairs probably can get used with higher disturbance even in the close vicinity of their nesting sites.

*Horváth, M., Szitta, T., Bagyura, J., Fatér, I., Firmánszky G. & Moskát C. 2009. Manuscript (in prep.).*

## INTRODUCTION

Biotic and abiotic environmental variables, like topography of the area, structure and composition of the vegetation, prey availability, predators, and competitors, mainly influence habitat selection in birds (Hildén 1965, Cody 1985, Wiens 1989). Habitat selection of larger-sized raptor species typically depends on the availability of nesting sites and feeding areas (Janes 1985, Tapia et al. 2008). If raptors choose an optimal nesting habitat they increase their reproductive success, by decreasing the risk of predation (Sergio et al. 2007), avoiding competitors (Katzner et al. 2003), or human disturbance (López-López et al. 2007). Another important component, which is connected to habitat quality and has main effects on raptors' reproductive success, is prey-availability in the feeding areas, like density and quality of preys (Penteriani et al. 2006, Sergio et al. 2006) and distance of hunting areas from nesting sites. Distance between nests and feeding areas play a key role when birds optimize their energy expenditure for maximizing their fitness (Cowie 1977). If raptors can select for safe nesting sites with nearby high-quality feeding areas, the populations may survive better, or even able to spread into new areas. So the parallel study of the quality of nesting sites and feeding areas seems to be important factors, which mainly affect the viability of raptor populations (Krüger 2002). Besides the direct and indirect effects of habitat quality two another another components, which was proved to affect crucially breeding success of raptor population is the density dependence (Ferrer & Donazar 1996, Ferrer et al. 2006, Ferrer & Penteriani 2008) and age of breeding birds (Balbontín et al. 2003, Ferrer & Bisson 2003, Margalida et al. 2007a, Penteriani et al. 2003, Steenhof et al. 1983), The effect of age can be especially important in instable populations, when the relative frequency of non-adult breeders increases, due to lack of adult breeders in comparison to available habitats (Ferrer et al. 2004).

Many of the large-sized raptors are among the threatened birds in the world (Del Hoyo et al. 1995). The eastern imperial eagle (*Aquila heliaca*) has a wide distribution area in the forest-steppe zone of the Palearctic Region, it breeds from western Austria, Czech Republic, Slovakia and Hungary throughout eastern Europe and Siberia to the Baikal Lake in Russia. Nevertheless its distribution is scattered and the species is globally threatened,

as its world population consists only a few thousand breeding pairs (BirdLife International 2009, Del Hoyo et al. 1995).

Throughout its distribution area imperial eagles are connected to open foraging habitats, where its medium-sized mammal or bird species are available (Del Hoyo et al. 1995). Although in several regions they are breeding in forested mountains, mostly due to persecution or the lack of suitable trees in the nearby open plain habitats (Karyakin et al. 2008, Petrov et al. 1996). We have no reliable data about the breeding distribution of imperial eagles in Hungary, although all early literature since the end of the 19th century mention it as a rare breeder of the Hungarian mountains, and only scarce observations are available from the Great Hungarian Plain (Vasvári 1938). The Hungarian population of the eastern imperial eagle presumably reached its historical minimum with only 15-25 pairs breeding pairs in mountainous forests by the 1980's. Afterwards during the last two decades an unexpected increase of the population was observed and by 2009 the Hungarian population already exceeded 100 pairs (Horváth et al. 2009a). In parallel the species also expanded its breeding range from mountain forests to lowland agricultural areas, and as two-third of the territory of Hungary belongs to such type of areas, probably imperial eagles has got some chance for further expansion in the nearby future.

In the present study we investigated the factors affecting reproductive success of the largest subpopulation of imperial eagles in Central Europe during its area expansion. As the population is still exponentially increasing and still much under the saturation, we predicted that recently there is no significant density dependent effect on productivity. Nevertheless we hypothesized that both age of the breeding birds and habitat type affects the reproductive success of imperial eagles. We predicted that the chance of successful breeding in the lowland areas is lower than in the mountains, due to a higher level of human disturbance connected to intensive agriculture and higher density of urbanized areas and infrastructure. On the other hand we also predicted that that fledging success is higher in lowland areas where brown hares (*Lepus europeus*), the main prey species of imperial eagles, are more abundant and open foraging areas are closer to the nesting sites.

## METHODS

### *Study area*

The study was conducted in the central part of the Great Hungarian Plain and adjacent low mountains (46°30′-48°30′N 19°50′-21°40′E, 20 000 km<sup>2</sup>). Most parts of the study area are lowlands between 80-100 m a.s.l., like the Jászság, Heves, Borsod, Nagykunság and Békés Plains, which lie in the wide valley of the Tisza River and its tributaries. The Plain is mostly covered by intensive agricultural fields and small remnants of grasslands, which are scattered by small groups of poplars (*Populus* spp.) and black locust trees (*Robinia pseudoacacia*). The northern border of the Plain is formed by the Mátra, Bükk and Zemplén Mountains (200-1014 m a.s.l.) which are predominantly covered by oak (*Quercus petraea*, *Q. cerris*, *Q. pubescens*), beech (*Fagus sylvatica*), and introduced pine (*Pinus silvestris*, *P. nigra*, *Larix decidua*) forests.

### *Data collection*

Breeding territories of the eastern imperial eagle were monitored by a well-trained network of observers, organised by MME BirdLife Hungary and Hungarian national park directorates since 1980 (Bagyura et al. 2002, Haraszthy 1996, Horváth et al. 2009a). In the present study we analysed our dataset collected between 1989 and 2006, when monitoring covered all potential breeding territories. At the beginning of each breeding season (February-March) all previously identified territories and their surroundings up to 10 km were visited in order to locate active nests of imperial eagle pairs. Afterwards, during the whole breeding season (from April to August) all active nests were controlled for at least once per month to determine breeding success or failure in each breeding stage (incubation, small-, medium- and large-chick and fledging periods). Observations were carried out by spotting scopes (20–60X) from a distance (0.5–1.5 km) to avoid unnecessary disturbance of the birds (Gonzalez et al. 2006a). We checked nest content

once during the breeding season to control the exact number of alive or dead offspring, when the chicks were 4-7 weeks old (mostly between 10-25 June), or when the breeding was failed. Locations of the nests were identified with GPS and stored in a GIS database together with other breeding parameters.

During this 18-year study 645 breeding attempts were monitored in 89 breeding territories. By definition two nests belonged to the same territory if they were closer than 7.3 km to each other (the average nearest neighbour distance in 2006) and not more than one territorial pair used them within the same year (Ferrer & Bisson 2003). Two different breeding habitats were used by the species in the study area, such as mountainous forests and small patches of trees in open agricultural landscape of the lowlands. The breeding attempts were classified into the two breeding habitat types based on the 200 m contour line. This contour line also separated the open and forested nest sites without any notable overlap, as all (100%) of the 262 “mountainous”, but only eight (2.1%) out of the 383 “lowland” breeding attempts were in forested habitats. Subsequent breeding attempts of the same territory were usually within the same habitat type (15 pure mountainous and 67 pure lowland territories). However, in seven territories the pairs switched between the two habitat types. These mixed territories were also classified into that habitat type where more breeding attempts occurred and only the average of these breeding attempts were used for territorial comparisons.

Birds of the breeding pairs were categorised during the population monitoring as “*adult*” or “*non-adult*” birds, as these two age classes can be distinguished relatively easily in the field based on plumage characteristics. Similarly to the Spanish imperial eagle (Ferrer et al. 2004, Gonzalez et al. 2006b) and other large eagles (Balbontín et al. 2003, Steenhof et al. 1983), eastern imperial eagles regularly breed already in their 3rd–5th calendar year, before they accomplish their moult to the dark brown adult plumage (Katzner et al. 2006a, authors own data). Nonetheless, the exact aging of non-adult eagles needs substantial experience (Forsman 1999). A breeding pair was classified as “*non-adult*” if at least one member of the pair was in non-adult plumage, and as “*adult*” if both members were in adult plumage. In 25 cases (4% of breeding attempts) the age class of both birds could not be identified, therefore these were typically excluded from calculations.



### *Statistical analyses*

To avoid pseudoreplication that would arise if each breeding attempt would be included separately, we used territories as sampling units in the comparative analyses (c.f. Ferrer & Bisson 2003, Margalida et al. 2007a, Penteriani et al. 2003, Sergio & Newton 2003, Steenhof 1987), and annual mean values for analysing temporal trends during the study period (Balbontín et al. 2003, Ferrer & Bisson 2003). Breeding success was measured with three variables (Steenhof 1987, Balbontín et al. 2003), such as the number of fledglings per a nesting pair (referred to as *productivity* in the followings), the frequency of nesting pairs that fledged at least one chick (referred to as *success rate* in the followings), and the number of fledglings per successful nesting pairs (referred to as *fledgling success* in the followings). For the calculation of mean values for territories we controlled for year effect by subtracting annual means from the original breeding success data (Ferrer & Bisson 2003, Penteriani et al. 2003). We excluded those territories from the analyses where less than three breeding attempts occurred. Density dependence was estimated by nearest neighbour distances (NND) (Newton et al. 1977, Penteriani et al. 2003).

Normal distribution of variables was tested prior applying parametric tests, and in case of significant deviation non-parametric tests were used. Generalized Linear Models were used to test simultaneous effect of habitat type and age of birds on breeding success variables, and the interaction of the two factors was removed from the model, if it had no significant effect. All statistical tests were two-tailed and significance level was set at  $P < 0.05$ . Data are presented as mean  $\pm$  SD. Distances were measured by ArcMap® software (ESRI Inc., version 9.0). Analyses were executed by the SPSS programmes package (SPSS Co., ver. 17).

## RESULTS

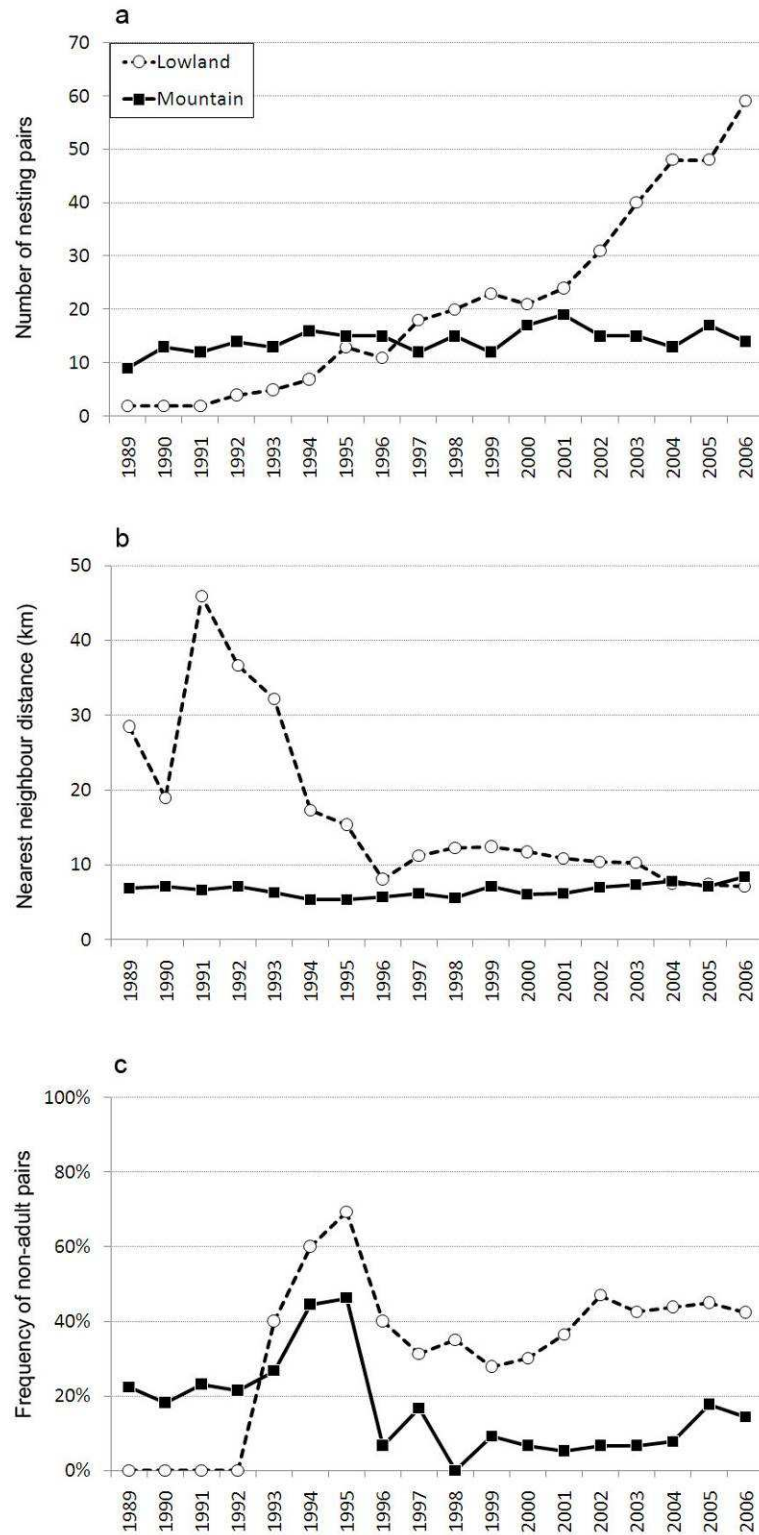
### *Temporal variation of population parameters*

Altogether 79 new territory occupancies (i.e. when a breeding pair appeared in a territory where no breeding attempt was recorded in the previous year) were observed during the study period, that of only 12 (15%) were located in the mountains, while the rest of the new territories (85%) appeared in the lowland. We identified the age of both breeding birds in 70 cases, from which 57 (81%) new territories were occupied by non-adults. This frequency was significantly different from the 24% value of non-adults, which was observed among the other 550 breeding attempts ( $\chi^2 = 123.416$ ,  $P < 0.001$ ).

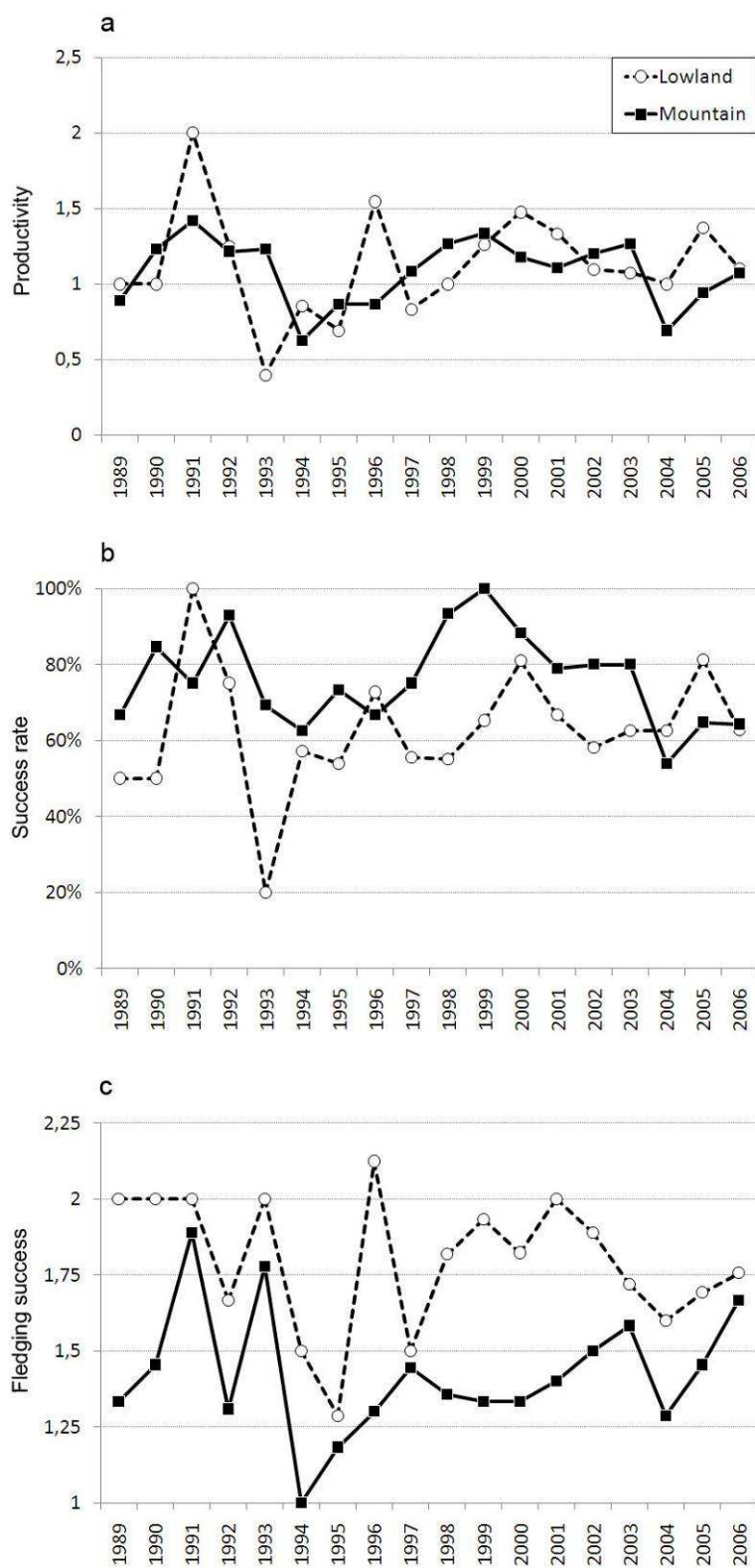
Population size increased markedly in the lowland (annually increased by 25%;  $F = 281.401$ ,  $df = 1,16$ ,  $P < 0.001$ ), and also in the mountains, although here the trend was not so sharp and not constant (annually increased by 5%;  $F = 5.859$ ,  $df = 1,16$ ,  $P = 0.033$ , Fig. 1a). In parallel with the increasing population size the NND decreased in the lowland ( $F = 22.445$ ,  $df = 1,16$ ,  $P < 0.001$ ), but did not change significantly in the mountains ( $F = 2.747$ ,  $df = 1,16$ ,  $P = 0.117$ , Fig. 1b). The frequency of non-adult pairs in the breeding population increased in the lowlands ( $F = 7.401$ ,  $df = 1,16$ ,  $P = 0.015$ ) and decreased in the mountains ( $F = 5.379$ ,  $df = 1,16$ ,  $P = 0.034$ , Fig. 1c). None of the breeding success variables showed significant trends in the two habitat types during the study period (productivity:  $F = 0.148$ ,  $df = 1,16$ ,  $P = 0.705$  for mountains and  $F = 0.108$ ,  $df = 1,16$ ,  $P = 0.747$  for the lowland, Fig. 2a; success rate:  $F = 0.401$ ,  $df = 1,16$ ,  $P = 0.535$  for the mountains and  $F = 0.613$ ,  $df = 1,16$ ,  $P = 0.445$  for the lowland, Fig. 2b; fledgling success:  $F = 0.041$ ,  $df = 1,16$ ,  $P = 0.842$  for the mountains and  $F = 0.718$ ,  $df = 1,16$ ,  $P = 0.409$  for the lowland, Fig. 2c).

### *Density dependence*

Territory averages of NND was significantly lower in mountainous than in lowland territories ( $6.6 \pm 3.2$  km and  $12.7 \pm 14.5$  km, respectively; Mann-Whitney U-test,  $Z = -2.728$ ,  $P = 0.006$ ), although the difference became non-significant, or even reversed by



**Figure 1.** Changes in number of nesting pairs (a), nearest neighbour distance (b) and frequency of non-adult pairs (c) of eastern imperial eagles in lowland and mountain habitats of East-Hungary between 1989 and 2006.



**Figure 2.** Changes in productivity (a), success rate (b) and fledging success (c) of eastern imperial eagles in lowland and mountain habitats of East-Hungary between 1989 and 2006.

the last years of the study period ( $8.4 \pm 4.8$  km and  $7.1 \pm 4.3$  km in 2006, respectively; Mann-Whitney U-test,  $Z = -0.722$ ,  $P = 0.470$ ). We did not find any significant correlation between NND and any of the breeding success variables (productivity:  $r_s = -0.022$ ,  $P = 0.842$ ; success rate:  $r_s = -0.056$ ,  $P = 0.601$ ; fledgling success:  $r_s = 0.113$ ,  $P = 0.338$ ). Moreover, as density increased in the lowland habitats during the study period, no changes were observed in any of the breeding success variables (see above). Therefore we assumed that there was no significant density-dependent effect of the studied parameters in the study period.

### *Simultaneous effect of age and habitat*

Breeding success variables showed some difference both regarding habitat type and age of breeding birds in univariate comparisons without considering the possible simultaneous effects (Table 1 and Table 2). We also found significant correlations between the frequency of non-adult pairs and each of two breeding success variables of the territories (productivity:  $r_s = -0.354$ ,  $P = 0.002$ ; success rate:  $r_s = -0.337$ ,  $P = 0.004$ ), although fledgling success did not correlate significantly ( $r_s = -1.102$ ,  $P = 0.406$ ). Nevertheless, the frequency of non-adult pairs differed significantly between the two habitat types ( $22.1 \pm 26.3$  % in the mountains and  $49.5 \pm 35.1$  % in lowlands; Mann-Whitney U-test,  $Z = -2.964$ ,  $P = 0.003$ ), therefore the possible simultaneous effects were tested in a generalized linear model.

**Table 1.** Comparison of breeding success variables between the territories of the two habitat types. (Productivity = the number of fledglings per a nesting pair; Success rate = the frequency of nesting pairs that fledged at least one chick; Fledging success = the number of fledglings per successful nesting pairs.)

	Mountain		Lowland		<i>t</i>	<i>P</i>
	<i>n</i>	mean $\pm$ SD	<i>n</i>	mean $\pm$ SD		
Productivity	20	$-0.098 \pm 0.371$	51	$-0.005 \pm 0.466$	0.805	0.424
Success rate	20	$0.006 \pm 0.233$	51	$-0.043 \pm 0.210$	-0.851	0.398
Fledging success	19	$-0.162 \pm 0.306$	50	$0.086 \pm 0.390$	2.494	0.015*

\* significant at  $P = 0.05$  level

**Table 2.** Pairwise comparison of breeding success variables of adult and non-adult pairs within the same territories. (Productivity = the number of fledglings per a nesting pair; Success rate = the frequency of nesting pairs that fledged at least one chick; Fledging success = the number of fledglings per successful nesting pairs.)

	Adult		Non-adult		<i>t</i>	<i>P</i>
	<i>n</i>	mean $\pm$ SD	<i>n</i>	mean $\pm$ SD		
Productivity	46	0.107 $\pm$ 0.602	46	-0.162 $\pm$ 0.756	-1.995	0.052
Success rate	46	0.104 $\pm$ 0.279	46	-0.115 $\pm$ 0.387	-3.257	0.002*
Fledging success	35	-0.079 $\pm$ 0.448	35	0.009 $\pm$ 0.435	0.903	0.373

\* significant at *P* = 0.05 level

The linear models showed different results for the three breeding success variables (Table 3). We found that overall productivity was affected primarily by the age of the breeding pairs, but also by the interaction between habitat and age effects. Success rate was also primarily affected by the age of the pairs, although the habitat and interaction between the two effects were also significant. Moreover, habitat showed stronger effect on fledging success, but age of the pairs also had a significant effect (Table 3).

## DISCUSSION

Our results revealed that habitat selection of imperial eagles in Hungary changed in the last two-three decades, causing an unexpected population growth and breeding area expansion. From their mountainous refugees imperial eagles expanded their original breeding areas in Hungary, to the lowlands, which were most probably abandoned for at least one hundred years or even more. Although presently the new breeding area, the Hungarian Great Plain, with its developed agriculture and dense system of settlements, seems to be suffering from high human disturbances, the reproductive success parameters suggested that this new breeding area offer high-quality breeding sites for the eagles.

**Table 3.** Results of the Generalized Linear Models analysing the effects of habitat type and age of the breeding birds on breeding success variables.

	Type III Sum of Squares	Mean Square	<i>F</i>	<i>P</i>
<b>Productivity</b>				
Corrected Model	2.447	0.816	4.895	0.004 *
Intercept	0.617	0.617	3.702	0.059
Habitat	0.002	0.002	0.01	0.922
Age	2.308	2.308	13.853	< 0.001 *
Habitat x Age	0.810	0.81	4.859	0.031 *
<b>Success rate</b>				
Corrected Model	0.767	0.256	6.856	< 0.001 *
Intercept	0.207	0.207	5.547	0.021 *
Habitat	0.152	0.152	4.074	0.048 *
Age	0.725	0.725	19.449	< 0.001 *
Habitat x Age	0.383	0.383	10.275	0.002 *
<b>Fledging success</b>				
Corrected Model	1.379	0.690	5.289	0.007 *
Intercept	0.090	0.090	0.692	0.409
Age	0.531	0.531	4.071	0.048 *
Habitat	1.285	1.285	9.856	0.003 *
Habitat x Age **	-	-	-	-

\* significant at  $P = 0.05$  level

\*\* interaction was not significant, therefore it was removed from the model

Similarly to other studies (Ferrer et al. 2003, Balbintín et al. 2003, Steenhof 1983) we found a strong effect of age of breeding birds on reproductive success variables, as non-adult pairs bred less successfully. Not surprisingly, in the newly-occupied areas non-adult eagles were more frequent than in the traditional mountainous areas and we found that age and habitat type had simultaneous effects on breeding success variables, as it was also shown on Bonelli's eagles (*Aquila fasciata*) in Spain (Penteriani et al. 2003). We found that age-effect is more significant on success rate (i.e. the ratio of pairs that produce at least one chick), while habitat-effect was more evident on fledging success (i.e. the number of fledglings per productive pair). The overall productivity (i.e. number of fledglings per breeding pair) was affected primarily by the age of the pairs, but the interaction of age and habitat type had also significant effect. We suppose that better feeding possibilities (closer foraging areas and larger prey density) could explain the higher fledging success in the lowlands. We also predicted that pairs inhabiting lowland agricultural areas suffer more from human disturbance will result in a lower success rate,

but even if this trend was observable it had significant effect only in interaction with the age of the pairs. This interaction is probably caused by inexperienced non-adult pairs, which are threatened by the higher level of lowland disturbance, while experienced adult pairs can breed with similar success as in the undisturbed mountains.

Although the exact causes, which started the population expansion in the imperial eagles in Hungary is not known, our study revealed that freshly occupied lowland habitats can be more productive than traditional mountainous ones. Such a process is surprising in an expanding population as most studies report that if a population is not saturated the best habitats are occupied first (Newton 1979, Sergio & Newton 2003). Moreover the population size of brown hare (*Lepus europaeus*), the main prey species of imperial eagles in Hungary (Horváth et al. 2009b), has been continuously declining since the 1960's in parallel with the intensification of agriculture (Báldi & Faragó 2007). Therefore the colonization of the lowlands is most probably not caused by any recent increased availability of food supply, but by an increasing population size in the original habitats. After the population in the mountains started to increase and the level of persecution decreased in the lowlands, some eagles tried to settle in these new habitats. The appearance of these first inventory pairs was probably the most important step in the expansion, as the high natal philopatry of the species (Gonzalez et al. 2006b) hinders the sudden colonization habitats far from the original breeding distribution area. The huge and well visible nests and territorial behaviour of conspecifics signed for immatures that lowland agricultural areas can be also suitable habitats for them (Newton 1979). During the last two decades these freshly colonized habitats proved to be even more suitable for the species, than the traditional mountainous forests, and since large agricultural regions of the Hungarian Plain are still not inhabited by the species we expect further expansion in the near future.



## CHAPTER 4. TURNOVER



#### **4.1 AN OVERLOOKED DNA SOURCE FOR NON-INVASIVE GENETIC ANALYSIS IN BIRDS**

##### **ABSTRACT**

Non-invasive sampling is a useful tool for genetic analyses of endangered and/or elusive species, but it is often inapplicable due to the low quality and quantity of the DNA obtained. In this study we show that the blood clot located in the superior umbilicus of the feather shaft is a better source of DNA than the previously used tip samples from moulted feathers. We found that feather clots from museum specimens provided results nearly as good as footpad and better than those from the more commonly used museum skin snips. Feather clots proved to be a good source of DNA for genetic analysis that will significantly facilitate genetic monitoring of wild bird populations.

*Horváth, M.B., Martínez-Cruz, B., Negro, J.J., Kalmár, L. & Godoy, J.A. 2005. Journal of Avian Biology 36(1): 84-88.*

## INTRODUCTION

Non-invasive methods may be the only alternative for the genetic analysis of endangered and/or elusive species that are difficult to sample otherwise. Museum samples, on the other hand, provide a unique opportunity to gain insights into the past that can then be used to better understand current genetic patterns and to test hypotheses about the demographic processes that produced them. However, the use of non-invasive sampling of free ranging animals and museum samples often does not provide reliable results because of low quality and quantity of the extracted DNA that can lead to allelic drop-outs and false alleles. Two strategies have been proposed to overcome these problems: i) a multiple tubes approach, in which each sample is repeatedly genotyped (Taberlet et al. 1996), and ii) the accurate estimation of DNA concentration through quantitative PCR and the rejection of samples containing amounts of DNA lower than a reliability threshold (Morin et al. 2001). The multiple-tube approach significantly increases labour and reagent costs, rendering some projects unpractical, whereas accurate quantification of DNA by PCR requires expensive equipment and the elimination of samples with low amounts of DNA.

Some genetic studies on birds (e.g. Mundy et al. 1997b, c) have successfully used feathers plucked from living birds (i.e. non-destructive sampling according to Taberlet et al. 1999). However, only a couple of studies (e.g. Morin et al. 1994, Srikan and Woodruff 1998, Petersen et al. 2003, Segelbacher et al. 2003) have used moulted feathers collected in the field (i.e. non-invasive sampling according to Taberlet et al. 1999). These studies reported successful mitochondrial DNA amplification (Morin et al. 1994, Srikan and Woodruff 1998, Petersen et al. 2003), but limited success with nuclear DNA amplification (Segelbacher 2002). In several studies using museum bird specimens, skin and footpad samples have also been used, sometimes in conjunction with plucked feathers (Ellegren 1991, Mundy et al. 1997a, Gautschi 2001, Godoy et al. 2004). Although such samples can provide suitable amounts of DNA, the reported success rates are variable and the potential damage to the specimens needs to be considered.

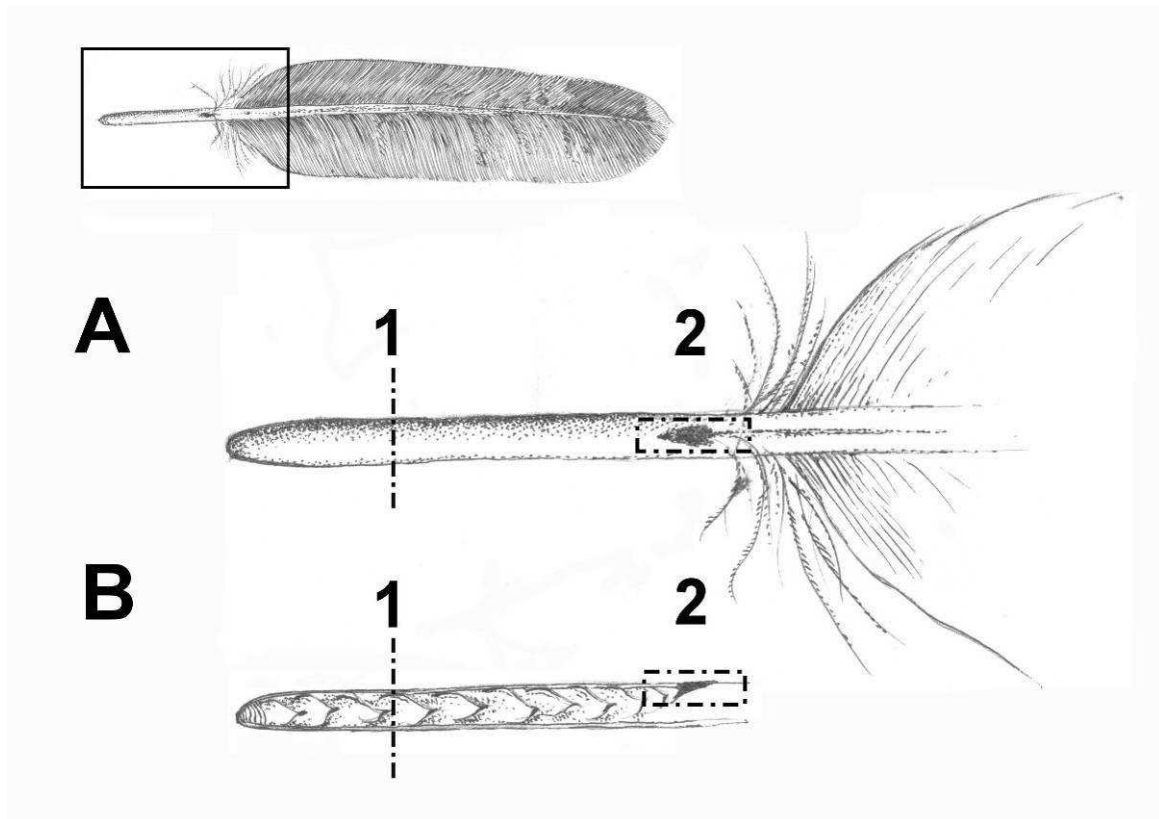
Most previous studies using feather samples for genetic analyses, collected both invasively and non-invasively, reported that the DNA was extracted from the basal tip of

the calamus (e.g. Morin et al. 1994, Mundy et al. 1997b, Srikwan and Woodruff 1998, Segelbacher 2002, Petersen et al. 2003). An alternative and potentially abundant source of DNA from feathers, a blood clot embedded in the shaft (see Fig. 1), has so far remained overlooked. Once the feather is completely developed, the mesenchymal pulp, containing a single axial artery, is completely reabsorbed from the calamus and only keratinised pulp caps remain (Hodges 1974, Fig. 1B). These pulp caps, along with feather follicle cells attached to the outer surface of the calamus, are most likely the main source of DNA when only the tip of the feather is used. However, the superior umbilicus, where the reabsorbing mesenchymal pulp crosses the wall of the calamus in a narrow channel during feather development, typically contains a visible blood clot, which is the remnant of the axial artery (Proctor and Lynch 1993). This clot is likely to be included when the tip of a small feather is sampled, but will be totally excluded from large feathers.

In this study we evaluated the usefulness of umbilical clot samples for genetic analysis of birds and compared it to other commonly used DNA sources, from both field-collected moulted feathers and museum specimens

## **METHODS**

Eight feathers from free-ranging Spanish imperial eagles were collected under nest or perching sites in Doñana National Park, southern Spain (37°N, 6°30'W) during August-September 2001. Feathers may have stayed on the ground for 0-7 months before being collected and stored in plastic bags at room temperature under dry and dark conditions for 7-8 months. DNA was separately extracted from: (i) the 1 cm basal tip of the calamus; (ii) the superior umbilicus containing the blood clot (Fig. 1). Five museum specimens of the Spanish imperial eagle from Doñana Biological Station were also sampled as follows: (i) one feather was cut from the back of the specimen, along with a small piece of skin (approx. 10 mm<sup>2</sup>), around the tip (Gautschi 2001), (ii) a piece of the footpad (approx. 100 mm<sup>3</sup>) was cut out and the outer surface removed in order to avoid contamination (Mundy et al. 1997a), (iii) the umbilical clot of the 10th primary was collected as described above. Clots were collected without removing the feather from the specimen.



**Figure 1.** General view of a typical flight feather: (A) detail of a posterior view of the base of the feather, and (B) longitudinal cross-section through the feather calamus. Two different sampling areas for feathers are shown: (1) basal tip of the calamus, (2) blood clot from the superior umbilicus.

DNA was extracted using the Qiagen DNeasy tissue kit following the manufacturer's instructions for animal tissues. DNA was eluted in a final volume of 120  $\mu$ l. An extraction blank was always included to monitor for foreign DNA contamination. All the extractions were performed in a dedicated "clean" lab, free of modern DNA and PCR products and permanently UV irradiated when not in use. DNA concentration was measured fluorimetrically with a Hoefer-Dyna Quant 200 Fluorimeter, using Hoetsch-33258 dye. Values under 10 ng/ $\mu$ l were considered below the detection limit and recorded as nondetectable. Four  $\mu$ l of each extract were run in a 0.8% agarose gel in order to evaluate the level of DNA degradation.

Three segments of the mitochondrial genome differing in size were targeted in PCR amplifications: (i) a 210 bp fragment from the control region using primers AID1 (Martínez-Cruz et al. 2004) and AIR (5'-GGGAGATTGGAGATATCTAGGC-3'), (ii) a 450 bp

fragment from the control region using primers Thr and Fbox (Godoy et al. 2004), and (iii) a 1026 bp fragment, encompassing the whole cytochrome b gene using primers MT-AB and MT-FB (Seibold et al. 1996). Polymerase chain reactions (PCRs) were performed as previously described (Godoy et al. 2004). Amplification products were sized and visualised on 2% agarose gels containing 0.3 µg/ml ethidium bromide.

Molecular sexing was accomplished through amplification of CHD1 gene fragments (Fridolfsson and Ellegren 1999). Five different PCRs per DNA extract were performed. For the Spanish imperial eagle, fragments amplified with these primers yield a product of around 700 bp in both sexes and another one of around 450 bp in females. Twelve µl of each PCR products were run in 2% agarose gel containing 0.3 µg/ml ethidium bromide and photographed under UV light.

Amplification of three microsatellite markers (Aa36, Aa39, Aa53) was performed with primers and PCR conditions as described by Martínez-Cruz et al. (2002). When using extracts from the basal tip of shed feathers, three parallel PCRs per sample were performed to evaluate the occurrence of genotyping errors. PCR products were visualised and sized in an ABI310 Genetic Analyser and alleles were assigned using Genotyper 2.5 software (Applied Biosystems).

## RESULTS

For field-collected feathers, clot samples yielded higher amounts of DNA than tip samples, as revealed by both gel electrophoresis and fluorimetric quantification. High levels of DNA degradation were observed in most cases, but less so for clot samples (data not shown). Fluorimetric measurements detected a relatively high concentration of DNA in most of the clot samples, while extracted DNA was not detectable in any of the basal tip samples (always less than 10 ng/µl); (Table 1). As expected, the observed general trend was for larger feathers to yield more DNA. The clot and footpad samples of museum specimens contained similar amounts of DNA ( $51.8 \pm 46.6$  ng/µl and  $53.4 \pm 33.1$  ng/µl respectively), while skin samples of the same specimens provided lower DNA concentrations ( $26.7 \pm 16.5$  ng/µl) and in one case, no DNA could be detected.

**Table 1.** Comparison of performance among five different sample types in four different DNA analyses. Moulded feathers included three remiges, four rectrices, and one covert. Museum specimens dated from 1940 1966 1976 1985, and 1992.

DNA analysis	Sampling methods / Number (%) of cases				
	Clot - shed feather	Tip - shed feather	Clot - museum specimen	Footpad - museum specimen	Skin - museum specimen
1. DNA quantity	n = 8	n = 8	n = 5	n = 5	n = 5
DNA visible on gel	8 (100%)	1 (11.1%)	5 (100%)	5 (100%)	4 (80%)
DNA detectable with fluorimeter (>10ng/μl)	7 (87.5%)	0 (0%)	5 (100%)	5 (100%)	4 (80%)
Mean DNA concentration ± SD (ng/μl)	92.1 ± 76.8*	-	51.8 ± 46.6	53.4 ± 33.1	26.7 ± 16.5*
2. mtDNA amplification	n = 8	n = 8	n = 5	n = 5	n = 5
210 bp fragment amplification	8 (100%)	8 (100%)	5 (100%)	5 (100%)	5 (100%)
450 bp fragment amplification	8 (100%)	8 (100%)	4 (80%)	5 (100%)	5 (100%)
1026 bp fragment amplification	7 (87.5%)	7 (87.5%)	3 (60%)	4 (80%)	3 (60%)
3. Molecular sexing	n = 8 x 5	n = 8 x 5	n = 5 x 5	n = 5 x 5	n = 5 x 5
450 bp fragment amplification (in females only)**	25 (100%)	22 (88%)	20 (100%)	20 (100%)	15 (75%)
700 bp fragment amplification	40 (100%)	12 (30%)	20 (80%)	24 (96%)	15 (60%)
Correct sexing after one PCR per sample	40 (100%)	25 (55.6%)	25 (100%)	25 (100%)	20 (80%)
Correct sexing after five PCRs per sample	8 (100%)	7 (87.5%)	5 (100%)	5 (100%)	4 (80%)
4. Microsatellite genotyping	n = 8	n = 8 x 3	n = 5	n = 5	n = 5
Aa36 (109-119 bp) amplification	8 (100%)	24 (100%)	5 (100%)	5 (100%)	4 (80%)
correct genotype	8 (100%)	24 (100%)	5 (100%)	5 (100%)	4 (80%)
Aa39 (191-223 bp) amplification	8 (100%)	24 (100%)	5 (100%)	5 (100%)	4 (80%)
correct genotype	8 (100%)	21*** (87.5%)	5 (100%)	5 (100%)	4 (80%)
Aa53 (123-133 bp) amplification	8 (100%)	24 (100%)	5 (100%)	5 (100%)	4 (80%)
correct genotype	8 (100%)	24 (100%)	5 (100%)	5 (100%)	4 (80%)

\*: Mean for the seven and four samples that could be quantified respectively.

\*\*: n = 5 x 5 for shed feathers from females and n = 4 x 5 for female museum specimens.

\*\*\*: Two allelic dropouts and one false allele occurred.

In mitochondrial DNA amplifications, tip and clot samples of moulted feathers performed equally well, each producing only one failed amplification of the largest fragment. The amplification of the largest (1026 bp) mtDNA fragments from museum samples was less successful, but all three sampling methods were similarly effective (Table 1).

However, a great difference was observed between clot and basal tip samples of shed feathers in the efficiency of nuclear DNA amplification. Clot samples provided more consistent amplification of both CHD1 fragments (Table 1) and stronger bands in all cases (data not shown). Among samples from museum specimens, the worst results were obtained from skin samples, followed by clot (one sample failing to amplify the larger

fragment in all five PCRs tried) and footpad (a single failure of the 700 bp fragment). Since the product usually failing to amplify was the larger common product, this did not lead to sex misidentifications; it would, however, if the larger fragment corresponds to the female-specific product, as observed in some waterfowl (J. A. Godoy, unpubl. data).

All 96 microsatellite amplifications performed from shed feathers yielded product. Two allelic dropouts and one false allele occurred, all of them from tip samples and for the same locus (Aa39). All museum samples yielded product except one skin sample, which did not amplify for any of the three microsatellite markers; no genotyping errors were detected.

## **DISCUSSION**

Our study demonstrates that the blood clot found in the superior umbilicus of moulted feathers provides more and better quality DNA than the previously used basal tip of the calamus. Despite their lower DNA yield, basal tip samples appeared to be as good a source of DNA as clot samples for mitochondrial DNA fragment amplifications (see also Morin et al. 1994, Srikwan and Woodruff 1998). Nonetheless, the lower quantity and quality of DNA obtained from basal tip samples sometimes resulted in failed amplifications of nuclear DNA and genotyping errors. Segelbacher (2002) reported similar problems when using basal tip samples from shed feathers of capercaillie *Tetrao urogallus* and concluded that only approximately 50% of all feathers could be used for reliable genotyping. In contrast, we found that the higher DNA concentration of clot samples consistently resulted in efficient molecular sexing and microsatellite genotyping.

The improved yield and quality of this new feather DNA source might render the expensive procedures of the multiple-tube approach (Taberlet et al. 1996), or quantitative PCR (Morin et al. 2001) unnecessary in some cases. However, since species particularities, feather types and environmental conditions might influence DNA yield and quality, a careful evaluation of performance should be carried out before deciding to rely solely on umbilical clot sampling. As flight feathers (i.e. remiges and rectrices) of all 67 bird species belonging to 30 different families examined contained visible superior



umbilical blood clots (M. Horváth et al. unpubl. data), clots may be a universal source of suitable DNA for birds.

Of the three type of tissues sampled from museum specimens, umbilical clot and footpad provided similarly good results, whereas skin samples performance was slightly inferior. Clot samples may be preferred by some curators as the sampled area remains hidden and multiple samples (one clot per large feather) are usually available from a single specimen. Although our results were obtained with specimens less than 60 years old, and therefore the performance of our method needs to be evaluated in older specimens, we have obtained reliable genotypes from imperial eagle specimens up to 115 years old using clots (B. Martínez-Cruz et al. unpubl. data).

The early promises of non-invasive sampling for field studies of vertebrates have not yet been fulfilled, as reflected by the relative scarcity of published studies (but see Morin et al 1994, Srikwan and Woodruff 1998, Petersen et al. 2003 and Segelbacher et al. 2003 for examples with birds). Extensive application of non-invasive methods has clearly been hampered by the limitations imposed by the low-quality and low-quantity of DNA obtained from most non-invasively collected remains. The discovery of a new suitable source of DNA in shed feathers significantly alleviates these limitations and will hopefully facilitate new exciting studies on bird behaviour, population genetics and conservation based on non-invasively collected feathers.

## 4.2 ESTIMATING TURNOVER RATE OF BREEDING EASTERN IMPERIAL EAGLES BY USING NON-INVASIVE GENETIC ANALYSES

### ABSTRACT

Here we present preliminary data on turnover rate as determined by genetic tagging of imperial eagles in individual territories. Shed feathers were collected non-invasively from June to September each year starting in 2000. DNA was extracted from the superior umbilicus part of the feather shaft. Sex of the individuals was determined by amplifying introns of the sex-chromosome-linked CHD gene. DNA profile was obtained by touchdown PCR using a primer set for six tetranucleotide and two dinucleotide microsatellite loci. So far we have analysed all feathers collected between 2000 and 2005 resulting 143 DNA profiles identifying 61 females and 19 males. Average turnover rate defined as the relative frequency of individuals replaced in the territories from year to year was 24% in our sample. Interestingly, turnover rate was relatively low in the traditional mountainous territories (11%) and much higher in the recently established lowland territories (29%). None of the individuals of known profile changed site within the studied territories, therefore turnover rate might be close to true the mortality rate. However, as the lowland population has been expanding exponentially, movement of eagles from studied nest sites to ones not part of our analysis cannot be excluded at the present time. We need more data to differentiate the effects of mortality and emigration to new sites. At present, the turnover rate can be interpreted as a maximum estimate of mortality rate of breeding birds

*Vili, N., Szabó, K., Kovács, Sz., Kalmár, L. & Horváth, M. 2009. Manuscript (in prep).*

## INTRODUCTION

Accurate estimation of mortality rate is inevitable for understanding the dynamics of populations (e.g. Ferrer & Calderon 1990, Kirk & Hyslop 1998), and is crucial for population viability analyses (PVA) of endangered species (Wakamiya & Roy 2009). As dead animals of most species are rarely seen in the field, indirect methods have been used to estimate mortality. The most important approach relies on the family of mark-recapture methods. However, monitoring the ratio of marked and unmarked animals can yield a reliable estimate only when large numbers of marked individuals are recovered (Seber 1982). Traditional procedures for marking need the capture, handling, tagging and recapturing of individuals, procedures sometimes not implementable in suitable sample size for larger bodied animals or can expose them to serious danger of injuries.

Genetic studies analysing non-invasively collected samples opened up an avenue for studies on species difficult or risky to mark (Taberlet et al. 1997, Sloane et al. 2000, Horváth et al. 2005). This approach is especially important in the case of endangered birds, and indeed, the number of studies capitalizing on DNA extracted from shed feathers or faeces has been increasing (e.g. Banhos et al. 2008, Hailer et al. 2007, Idaghdour et al. 2003, Regnaut et al. 2006, Rudnick et al. 2008). Nevertheless, only a few studies have estimated mortality rates by DNA profiles (Rudnick et al. 2008), most probably because the ratio of identified individuals is often too low. Rapid degradation of shed feathers in the field hampers reliable analysis and diminishes sample size (Taberlet et al. 1999). Recent methodological improvements in DNA extraction (Bayard De Volo et al. 2008, Hogan et al. 2008, Horváth et al. 2005), however, made such studies more feasible, especially in the case of larger species, such as e.g. grouses, cranes, bustards and raptors (Bao et al. 2009, Duriez et al. 2007, Hailer et al. 2007, Idaghdour et al. 2003, Martínez-Cruz et al. 2007).

The eastern imperial eagle (*Aquila heliaca*) is a globally threatened large raptor species distributed along the forest steppe zone of Eurasia (BirdLife International 2009). Its sister species, the Spanish imperial eagle (*Aquila adalberti*) is localised and sedentary in the Iberian peninsula, and it is one the rarest, but most well studied raptor species in

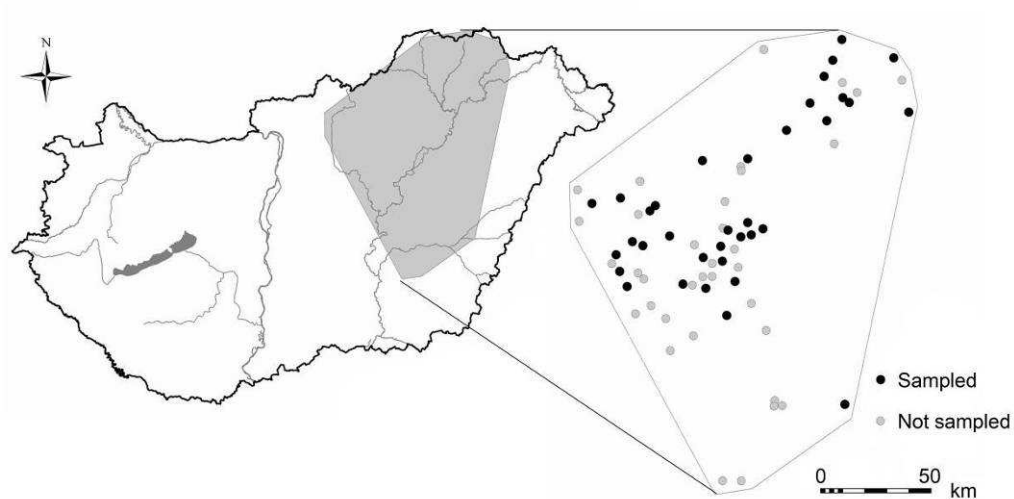
the world (e.g. Ferrer 2001, González & Margalida 2008). Based on field observations, mortality rate of breeding Spanish imperial eagles was estimated at 4.8 – 7.4 % in one particular population (Ferrer & Calderon 1990) and 1.4 – 8.2% when most of the species' range was covered (Ortega et al. 2009). The only study estimating the mortality rate of eastern imperial eagles, reported a much higher (16%) annual mortality rate based on non-invasive genetic sampling of a migratory and long-term stable population in Kazakhstan (Rudnick et al. 2005). This remarkable difference in mortality between the two sister species was accounted for by the differences in their migration habits (sedentary vs. long-distance migrant), in risk factors, but also by the difference in reliability and accuracy of the methods used (molecular identification vs. field observations).

In our genetic studies the long-term aim is to monitor individual eagles and to draw conclusions from such life histories on population estimates. The data we have analysed so far allow us to point at differences in turnover as maximum estimate of mortality between the mountainous and plan habitats and

## **METHODS**

### *Sample collection and DNA extraction*

Shed feathers of adult breeding imperial eagles were collected under nests and roost sites in eastern Hungary between 2000 and 2005 (Figure 1). Each nest was visited at two occasions yearly, in June, when nestlings were ringed as a part of the population monitoring protocol (Horváth et al. 2009a) and following fledging between July and September. For DNA extraction we used feathers with minor or no sign of degradation (Hogan et al. 2008), most probably shed less than 1-3 weeks prior to the collection. Feathers were stored in dry, cool and dark place to minimize further degradation of DNA. The superior umbilicus part of the feather shaft was separated as described by Horváth et al. (2005). DNA was extracted according to a standard salting out procedure followed by chloroform-isoamylalcohol purification (Gemmell & Akiyama 1996). The efficiency of the



**Figure 1.** Map showing the range of the study population of eastern imperial eagles in Hungary, and the location of sampled and not sampled territories.

purification procedure and the degradation of DNA were checked before further analyses by agarose gel electrophoresis (3µl on 0.8% gel).

### *Molecular sexing*

Molecular sexing was performed by amplifying introns of the sex-chromosome-linked CHD gene. Primers (2550F and 2718R) and PCR conditions were used as described by Fridolfsson & Ellegren (1999) except for the difference in touchdown scheme: instead of annealing on 60°C, temperature was decreased from 66°C to 50°C. In imperial eagles, these primers amplify a larger intron sequence (CHD1Z, 700bp) in both sexes and a smaller one (CHD1W, 450bp) only in females (Horváth et al. 2005). 5µl of the PCR product was visualized by ethidium-bromide on 2.5% agarose gel, which enabled us to identify the sex of the sampled specimen.

### *Microsatellite fragment analysis*

Sequences of nine dinucleotide repeats (Martínez-Cruz et al. 2002) and eight tetranucleotide repeats (Busch et al. 2005) have been published for Spanish and eastern imperial eagles. To identify individual DNA-profiles of the breeding eagles, two dinucleotide and six tetranucleotide loci (Aa39, Aa02, IEAAAG04, IEAAAG12, IEAAAG11, IEAAAG15, IEAAAG09 and IEAAAG14) were selected and amplified by the use of fluorescently labelled primers (dye set: HEX yellow, TET green, 6-FAM blue).

A modified version of the PCR protocol described by Martinez-Cruz et al. (2002) was used in the amplification procedure. Touchdown PCR was performed in all loci. Annealing temperature was decreased from 66 to 50 °C for loci Aa39, Aa02, IEAAAG09 and IEAAAG14, and from 60 to 50 °C in the case of IEAAAG04, IEAAAG12, IEAAAG11 and IEAAAG15. The exact fragment lengths of the amplified PCR products were determined by capillary electrophoresis (ABI 310 automated sequencer) with GeneScan 3.7 and PeakScanner 1.0 softwares (Applied Biosystems), using the ILS 600 (Promega) internal lane standard.

**Table 1.** Size and frequency of alleles of the used marker set at the East-Hungarian population of imperial eagles.

Locus	Allele size (allele frequency)										
IEAAG04	228	232	236	240	242						
	(0.061)	(0.087)	(0.83)	(0.018)	(0.004)						
IEAG09	476	480	484	488							
	(0.201)	(0.018)	(0.164)	(0.617)							
IEAAAG11	102	106	114								
	(0.004)	(0.811)	(0.185)								
IEAAAG12	124	128									
	(0.471)	(0.529)									
IEAAAG14	192	196									
	(0.370)	(0.630)									
IEAAAG15	324	328	332	336							
	(0.178)	(0.357)	(0.163)	(0.302)							
Aa02	135	137	139	141	143	145	148	150	152		
	(0.004)	(0.065)	(0.09)	(0.004)	(0.312)	(0.228)	(0.004)	(0.217)	(0.076)		
Aa39	180	182	184	188	190	192	193	194	196	198	200
	(0.036)	(0.022)	(0.274)	(0.066)	(0.131)	(0.274)	(0.011)	(0.007)	(0.117)	(0.047)	(0.015)

**Table 2.** Annual number and changes of female birds sampled in mountainous and lowland territories at the East-Hungarian population of imperial eagles. The number of sampled nests is followed by their ratio compared to the total population size in the study area at the first year of comparison. The number of changes is followed by the ratio of changed individuals compared to the number of sampled nests.

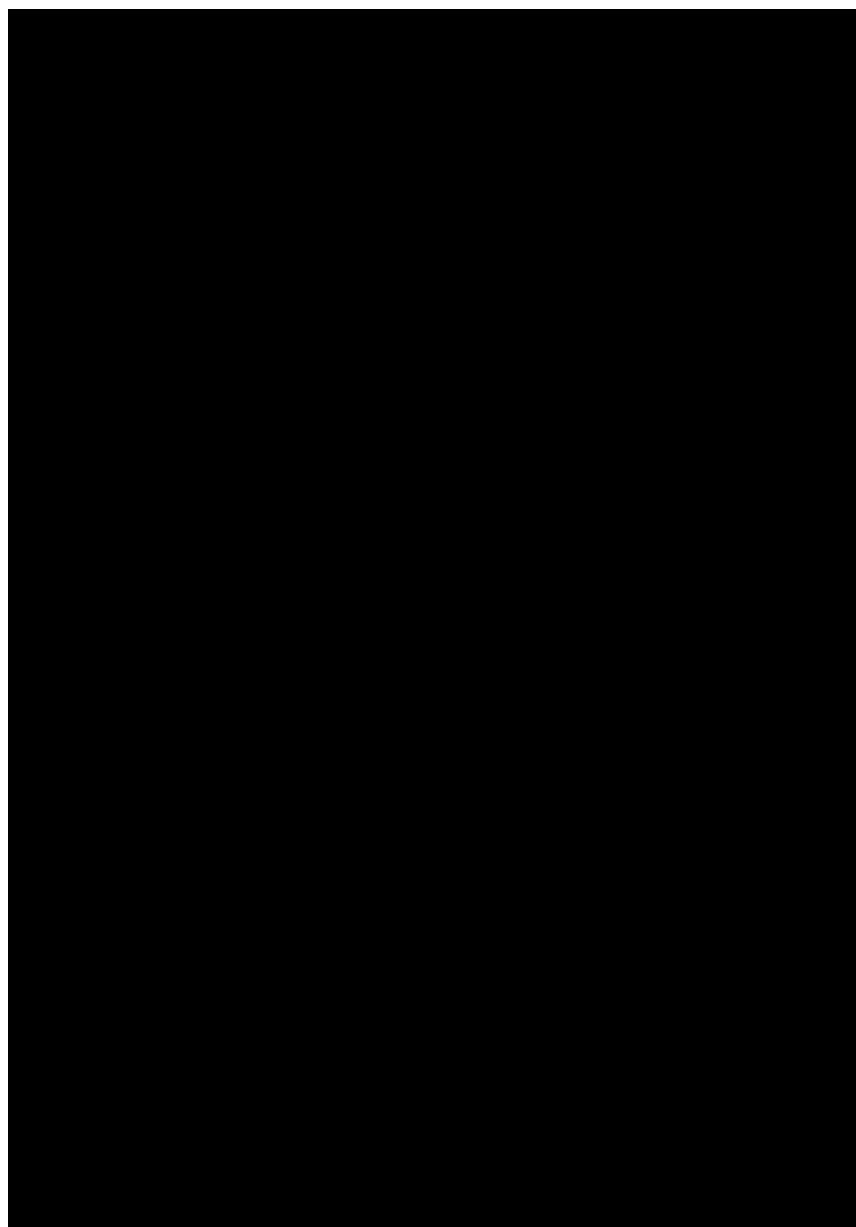
years	Sampled nest						Change					
	Total		Lowland		Mountain		Total		Lowland		Mountain	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
2000-2001	13	34%	8	38%	5	29%	3	23%	3	38%	0	0%
2001-2002	16	37%	11	46%	5	26%	1	6%	1	9%	0	0%
2002-2003	26	57%	19	61%	7	47%	6	23%	5	26%	1	14%
2003-2004	23	42%	18	45%	5	33%	7	30%	6	33%	1	20%
2004-2005	19	31%	13	27%	6	46%	6	32%	5	38%	1	17%
Total	97	40%	69	42%	28	35%	23	24%	20	29%	3	11%

In order to assure that the marker set is appropriate for the individual identification we calculated the probability of identity (PI), an estimate of the average probability that two unrelated individuals, drawn from the same randomly mating population, will by chance have the same multilocus genotype (GenAlEx, Peakall & Smouse 2006). Possible allelic dropouts were taken into account at the genotype determinations. Turnover rate was calculated for each consecutive year between 2000 and 2005, by comparing the genotypes of the sampled females according to Rudnick et al. (2005).

## RESULTS

Molecular sexing revealed significant bias in our sample as 124 feathers originated from females compared to 19 feathers shed by males (significant difference from 1:1 ratio,  $\chi^2 = 77.098$ ,  $P < 0.001$ ). Data on males were excluded from the present analysis due to their low sample size. Sixty one individual females at 35 territories (26 in lowland and 9 in mountainous habitats) were identified. Allele sizes and frequencies are shown in Table 1 and summary of individual identifications are presented in Figure 2. The Probability of Identity (PI) for the breeding females was estimated as  $1.04 \times 10^{-5}$ , and can be considered as appropriate for individual identification. In average we managed to sample 40% of the

breeding females in the study population between 2000 and 2005 (Table 2). Average annual turnover of adult birds estimated as the ratio of new individuals appearing in the territories was 24% during the study period, although it showed remarkable differences between years (Table 2). The turnover rate was remarkably higher in the lowlands (29%) than in the mountains (11%).



**Figure 2.** Summary of analysed samples and identified female specimens according to the breeding territories



## DISCUSSION

The unexpected bias in sex ratio of the feathers collected under the nests was most probably caused by the difference in behaviour between the sexes. Females spend more time on the nest and its immediate vicinity than males (Margalida et al. 2007b, own observations). As life history of males is also important we should find ways to sample males with higher efficiency in the future.

Dynamics of a population is basically determined by the relative rates of mortality, natality, immigration and emigration (Begon et al. 1990). For the imperial eagle population in Hungary the annual changes in the number of breeding pairs and productivity has been estimated by intensive field surveys since 1980 (Bagyura et al. 2002). The population has been increasing exponentially since the early 1990's and the maximal population size has not yet been reached (Horváth et al. 2009a). Therefore we are aware of the limitations of our preliminary data, because variables relevant to population dynamics are difficult to obtain and interpret during exponential expansion.

It is important to note that no movement of females was detected between the territories. As sampled nests represented 40% of all known territories, and the distribution of sampled and not sampled nest sites overlap considerably, random like switching between sites can be ruled out. Until more data is available, turnover rate of 24% can be considered as a maximum rate of mortality. As opposed to our prediction this turnover rate in our sedentary and increasing eagle population was 50% higher than it was reported from Kazakhstan in a long-term migrant and stable population. However, expansion of the population is apparent not only in numbers but in space, therefore we cannot entirely exclude the possibility that some eagles after spending some time in a newly established territory leave for a better habitat not sampled by the present study, therefore observed turnover can significantly overestimate the real mortality.

## CHAPTER 5. CONCLUSIONS



*Immature imperial eagle. Photo: András Kovács*

## **Mountain or lowland? – Individual decisions or population dynamic processes?**

The decision of an immature imperial eagle where to choose its first nesting site depends on several environmental and population variables. During the first three years of life juvenile non-breeding imperial eagles spend most of their time on so called temporary settlement areas (TSAs), where the duration of stay is a function of the availability of food (Ferrer 1993, Penteriani et al. 2006). These TSAs are habitat patches suitable for the birds to survive, but they do not belong to the territory of any breeding pair, because they are too small for a territory (i.e. to supply continuous and plenty food source during the breeding season), or simply because the population size is too small to inhabit all suitable habitats. It was shown in Hungary that the most important TSAs became important breeding habitats for the species during its expansion (Kovács et al. 2005).

Besides switching between TSAs, juveniles also frequently and systematically visit almost all breeding territories of their natal population (Ferrer 1993, González et al. 2006, Kovács et al. 2005). During these explanatory movements young eagles are able to determine the occupancy or vacancy of territories, and probably also to estimate their quality, as in the case of Spanish imperial eagles, because better territories were more frequently intruded by immatures (Ferrer 2001). Therefore a maturing eagle, at the age of 3 or 4 years, already has plenty of information about the possible nesting places in the potential breeding area of the natal population. In the Hungarian population the most obvious decision of an eagle is to choose between an undisturbed mountainous forest, far from the nearest foraging area, or a lowland agricultural habitat with prey in abundance, but also with the presence of humans, the only “predators” of this large raptor. The factors affecting this decision are connected with problem how productivity can be increased if choosing a lowland site, or how survival can be increased if choosing a safer mountainous habitat. In the present thesis I have started the analyses of these complex questions, and some background parameters behind the habitat selection decisions could be pinpointed.

In the first few chapters of the theses I have presented four studies using a 20 years population monitoring database of eastern imperial eagles in Hungary, to describe and explain differences in population dynamic parameters between the traditional

mountainous breeding habitats and freshly occupied lowland agricultural areas. In the last two chapters I have presented a novel method developed for more effective non-invasive genetic sampling of birds and tested it to assess turnover rate at the two breeding habitat types of imperial eagles.

### **Population dynamics of mountainous and lowland imperial eagles**

In *Chapter 2.1* I have shown that the population trends are completely different between the two habitats, as population size is exponentially increasing in lowlands, while slowly decreasing in mountains. The shift of the population towards the completely different habitats of the Hungarian Great Plain caused changes in the relative importance of mortality factors and productivity. In the new habitats eagles were frequently killed by persecution (especially poisoning) any by collision with vehicles. Nevertheless, productivity increased during the expansion, as two- and three-chick broods became more common compared to single-chick broods. Moreover, the increasing trend of the population has not yet diminished, therefore we predict further expansion of the species in the near future, although the novel threatening factors raise challenges for conservation.

In *Chapter 2.2* I have investigated the effects of infrastructural network on the habitat selection of imperial eagles in the lowland habitats. In the densest subpopulation of the species infrastructural network was proved to be a key factor affecting distribution. A prediction model built for the total potential breeding area of the species in East-Hungary showed that in spite of the relatively dense infrastructural network and the strong avoidance of them by the eagles, there are still several suitable habitats for the species, which are still not inhabited. The model predicted the location of those areas, which will be most probably avoided, and also those which most probably will be colonized by the eagles during further expansion of the population.

In *Chapter 3.1* the possible relationship between prey composition and reproductive success was analysed. I found great variability in diet composition of imperial eagles in East-Hungary, but in all regions hares, hamsters or pheasants dominated diet consisting of ca. 60 identified prey species. I have compared the productivity between the two most typically different regions, such as the hare-dominated Heves Plain and the hamster-dominated West-Zemplén region. I have found that productivity was significantly higher in the hare-dominated region, which suggests that this prey species could provide a better quality food supply than hamsters, which are ten times smaller and also their population size is more erratic.

In *Chapter 3.2* I have analysed the simultaneous effects of habitat and age of breeding birds on the reproductive success of imperial eagles. We found that both age and habitat had significant effects on breeding success variables. We found that age-effect was more significant on the ratio of successful pairs, while habitat-effect was more evident on the number of fledglings per productive pair. We suppose that better feeding possibilities (closer foraging areas and larger prey density) could explain the higher fledging success in the lowlands. We also predicted that pairs inhabiting lowland agricultural areas would have a lower success rate due to higher human disturbance, but even if this trend was observable it had significant effect only in interaction with the age of the pairs, therefore adult pairs probably can adapt to higher disturbance levels even in the close vicinity of their nesting sites.

### **Using non-invasive genetic sampling to estimate population parameters**

Although the methodology to extract DNA from samples containing small amount of DNA has been available for almost two decades (Ellegren 1991), non-invasively collected samples were still only exceptionally used in field studies of birds till the past few years, due to methodological problems derived from low quality and quantity of obtainable DNA (Morin et al. 1994, Srikwan & Woodruff 1998, Segelbacher et al. 2003).

In *Chapter 4.1* I have shown a novel methodology developed for improved DNA analysis from non-invasively collected feathers. I proposed that not the basal tip of the feathers should be sampled, as done in all previous studies, but instead, the superior umbilicus part of the feather shaft, where an embedded blood clot provides a significantly more effective source of DNA. Our sampling method have become generally used in genetic analysis of free-ranging birds during the last few years (Banhos et al. 2008, Bantock et al. 2008, Bao et al. 2009, Bayard De Volo et al. 2008, Beja-Pereira et al. 2009, Booms et al. 2008, Bortolotti et al. 2009, Bourke & Dawson 2006, Caetano & Ramos 2008, Duriez et al. 2007, Gebhardt & Waits 2008, Gebhardt et al. 2009, Hailer et al. 2007, Harvey et al. 2006, Hogan et al. 2008, Martínez-Cruz et al. 2007, Martínková & Searle 2006, Mino & Del Lama 2009, Ong & Vellayan 2008, Rawlence et al. 2009, Rudnick et al. 2007, Wandeler et al. 2007, Wilson et al. 2008).

In *Chapter 4.2*, with my co-authors, I estimated the turnover rate of breeding female imperial eagles in lowland and mountainous habitats of Hungary. We found that average turnover (24%) was much higher than expected, and also higher than in other populations. The two habitats also differed in their turnover rate, which was remarkably higher in the lowlands (29%) than in the mountains (11%). We cannot be sure if this high turnover rate is mostly caused by mortality, or by an unexpectedly high shift rate of breeding birds between territories, nevertheless till further research we can use turnover rate as a maximal estimation of mortality.

## **Final remarks**

The results presented here show great differences in many aspects between traditional mountainous and recently colonized lowland habitats of imperial eagles. During the studies we have answered several questions, but as usual, much more new questions were raised. This natural field experiment, with two completely different habitat selection strategies coexisting, serves a great opportunity for further research of this globally threatened raptor.

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## OWN PUBLICATIONS

### *Published papers and manuscripts related to the thesis:*

- Bagyura, J., Szitta, T., Haraszthy, L., Firmánszky, G., Viszló, L., Kovács, A., Demeter, I. & Horváth, M. 2002. Population increase of Imperial Eagle (*Aquila heliaca*) in Hungary between 1980 and 2000. **Aquila**, 107-108, 133-144.
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- Vili, N., Szabó, K., Kovács, Sz., Kalmár, L. & Horváth, M. 2009c. Estimating turnover rate of breeding imperial eagles by using non-invasive genetic sampling. *Manuscript*.

**Books, book chapters and edited publications:**

- Bihari, Z., Horváth, M., Lanszki, J. & Heltai, M. 2008. Role of the Common Hamster (*Cricetus cricetus*) in the diet of natural predators in Hungary. In: Millesi, E., Winkler, H. & Hengsberger, R. (eds.): The Common Hamster (*Cricetus cricetus*): Perspectives on an endangered species. Austrian Academy of Sciences, Wien. Biosystematics and Ecology Series No. 25. pp. 61-68.
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## SUMMARY

The studies presented in the thesis investigate the relationship between the recently changed habitat selection strategy (i.e. lowland expansion and abandonment of mountains) and population dynamical parameters of imperial eagles in Hungary.

In *Chapter 2.1* I showed that the population trends are completely different between the two habitats, as population size is exponentially increasing in lowlands, while slowly decreasing in mountains. In *Chapter 2.2* I found that infrastructural network affects significantly the distribution of imperial eagles in the lowland habitats, although there are still several most probably suitable habitats for the species, which are still not inhabited.

In *Chapter 3.1* I present a great variability in diet composition of imperial eagles in East-Hungary, and I have found that productivity was significantly higher in a typically hare-dominated region, than hamster-dominated one, which could propose that hare could serve better quality of food supply. In *Chapter 3.2* I showed that both age and habitat had significant effect on breeding success variables, although age-effect was more significant on the ratio of successful pairs, while habitat-effect was more evident on the number of fledglings per productive pair.

In *Chapter 4.1* I have shown a novel methodology developed for improved DNA analysis from non-invasively collected feathers, where I proved that the superior umbilicus part of the feather shaft can serve a significantly more effective source of DNA, than the basal tip of the feathers, which was used in all studies did before. In *Chapter 4.2* the annual turnover rate of breeding female imperial eagles was estimated by non-invasive genetic analyses. We found that average annual turnover (24%) was much higher than it was expected, and it was remarkably higher in the lowlands (29%) than in the mountains (11%).

The studies presented here show great differences in many aspects between traditional mountainous and recently colonized lowland habitats of imperial eagles. Based on the results we can suppose further expansion of the species in the near future, although the changes of population parameters raise also challenges for conservation.

## ÖSSZEFOGLALÓ

### A parlagi sas élőhely- és táplálékválasztása

Az itt bemutatott tanulmányok a parlagi sas élőhely-választási stratégiájában közelmúltban bekövetkezett változások (síkvidéki expanzió, illetve hegyvidéki területek elhagyása) és a populációdinamikai paraméterek közötti összefüggéseket vizsgálják.

Az 2.1 fejezetben bemutattam, hogy a két élőhelyen megfigyelhető állományváltozási trendek alapjaiban különböznek, mivel a síkvidéki területeken exponenciálisan nő a költő párok száma, addig a hegyvidéki állományok lassan csökkennek. A 2.2 fejezetben az infrastrukturális hálózat jelentős hatását mutattam ki a parlagi sas jelenlegi és potenciális alföldi elterjedési területére, mindazonáltal jelentős, nagy valószínűséggel alkalmas területek még lakatlanok, megteremtve ezzel a faj expanziójának további lehetőségét.

A 3.1 fejezetben nagy variabilitást mutattam ki a parlagi sasok táplálék összetételében, és azt találtam, hogy egy tipikusan nyúl-dominanciájú területen a költési siker magasabb volt, mint és egy tipikusan hörcsög-dominanciájún, ami azt feltételezi, hogy a nyúl jobb minőségű táplálék a sasok számára. A 3.2 fejezetben kimutattam, hogy az élőhely-típus és a költő madarak kora együttesen befolyásolja a produktivitást, habár a kor-hatás inkább érvényesült a sikeres párok arányában, míg az élőhely a kirepült fiókák számában sikeres költés esetén.

A 4.1 fejezetben egy új genetikai mintavételi módszert mutattam be madártollakon, és bebizonyítottam, hogy a cséve ún. felső köldöke jobb DNS forrásnak bizonyulhat, mint a korábban általánosan használt tollvég. A 4.2 fejezetben a költő madarak éves kicserélődési arányát becsültük a nem-invázív genetikai vizsgálatokkal, és a vártnál jóval magasabb átlagos kicserélődési arányt kaptunk (24%), amely jelentősen különbözött az alföldi (29%) és a hegyvidéki (11%) élőhelyeken.

Az itt bemutatott tanulmányok számos különbözőséget mutattak a parlagi sasok populációs paramétereiben a tradicionális hegyvidéki, és a közelmúltban kolonizált alföldi élőhelyek között. Az eredmények alapján feltételezhetjük, hogy a populáció expanziója tovább fog folytatódni, habár a populáció fő paramétereinek a változása komoly kihívásokat is jelenthet a természetvédelem számára.