Acta Zoologica Academiae Scientiarum Hungaricae 59(3), pp. 279–295, 2013

HIGH TURNOVER RATE REVEALED BY NON-INVASIVE GENETIC ANALYSES IN AN EXPANDING EASTERN IMPERIAL EAGLE POPULATION

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In this study we estimated the annual turnover rates, and so indirectly the possible maximum mortality rates of female Eastern Imperial Eagles (*Aquila heliaca*) in an expanding population. DNA was extracted from shed feathers collected in territories where at least three consecutive years of breeding occurred. As DNA samples could not be obtained at every studied nest in each year, minimum and maximum turnover rates were estimated. The calculated rates, 27.7% (minimum) and 35.5% (maximum), are much higher than what could be expected based on studies of other raptor species. According to previous studies territory switching can occur frequently in an expanding population. However, we found evidence of it in only two of the 46 studied territories, thus we assume that despite the ongoing saturation some level of site fidelity is present in the population. Our findings suggest the high prevalence of mortality, most likely from poisoning and electrocution, but also predict a large number of floater individuals that fill up the vacant territories.

Keywords: *Aquila heliaca*, non-invasive sampling, genetic tagging, turnover rate, bird crime.

INTRODUCTION

Accurate estimation of mortality rate is important for understanding the dynamics of populations (e.g. Ferrer & Calderon 1990, Kirk & Hyslop 1998), and is crucial for population viability analyses of endangered species (Wakamiya & Roy 2009). The monitoring of mortality rate is an important part of species conservation programmes as it helps to estimate the effect of potential or real threats like habitat loss, illegal hunting, trapping, poisoning or egg collecting (Donázar *et al.* 2002, Smart *et al.* 2010) as well as the efficiency of particular conservation measures (López-López *et al.* 2011).

As dead animals of most species are rarely found in the field, indirect methods are usually 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). When using

these traditional methods, capture, handling, tagging and recapture of individuals are necessary. These procedures are sometimes not implementable if there is potential risk of exposing an animal to serious injury or for larger bodied animals. Therefore, genetic studies are used to analyse non-invasively collected samples, allowing studies on less robust or "difficult to tag" species (Taberlet *et al.* 1997, Sloane *et al.* 2000, Horváth *et al.* 2005). This approach is especially important for endangered birds, the number of studies based on or applying genetic data from DNA extracted from shed feathers has increased in the last decade (e.g. Bourke *et al.* 2006, Martínkovà & Searle 2006, Seki 2006, Rudnick *et al.* 2007, Banhos *et al.* 2008, Guerrini & Barbanera 2009, Alcaide *et al.* 2010, Jacob *et al.* 2010, Väli *et al.* 2010, Miller *et al.* 2011). Nevertheless, only a few studies have estimated mortality rates by DNA profiles (Kohn *et al.* 1999, Rudnick *et al.* 2008), most likely because the ratio of re-identified individuals is often too low.

As the rapid degradation of shed feathers in the field can hamper reliable analysis and reduce sample size (Taberlet *et al.* 1999) methodological improvements were made in DNA extraction (Horváth *et al.* 2005, Bayard De Volo *et al.* 2008, Hogan *et al.* 2008, Beja-Pereira *et al.* 2009) to make such studies more feasible. However, this kind of sampling is typically used for larger species, such as grouse, cranes, bustards and raptors (Idaghdour *et al.* 2003, Duriez *et al.* 2007, Hailer *et al.* 2007, Martinez-Cruz *et al.* 2007, Bao *et al.* 2009).

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 of the rarest, but most well studied raptor species in the world (e.g. Ferrer 2001, Gonzalez & 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 turnover rate of Eastern Imperial Eagles, reported a much higher annual loss from the population (16%) based on non-invasive genetic sampling of a migratory and long-term stable population in Kazakhstan (Rudnick *et al.* 2005). This remarkable difference in estimated mortality between these populations of the two sister species may be explained by the differences in their migration habits (sedentary vs long-distance migrant), risk factors, in additions to the difference in reliability and accuracy of the analyitical methods used (molecular identification vs. field observations).

The relative frequency of different mortality causes is quite well-known in some Imperial Eagle populations based on the analyses of accidentally

found dead birds (Gonzalez *et al.* 2007, Horváth *et al.* 2011). It is revealed that poisoning and electrocution are the two main mortality factors in most of the species range (e.g. representing 26% and 21% respectively of recorded mortality in Hungary), although their possible effect on the population cannot be estimated unless the overall mortality rate is known. In this study we estimated the annual turnover and so indirectly the possible maximum mortality rate of breeding Eastern Imperial Eagles in an expanding Central European population with the use of non-invasive genetic techniques in order to enable future studies to estimate the effect of human-induced mortality factors and conservation measures on the population.

MATERIALS AND METHODS

Study area

The 21,600 km^2 study area covers the north-eastern part of the Hungarian Plain and nearby the mountains of Mátra, Bükk, and Zemplén (46°35′–48°30′N 19°35′–21°50′E), a region holding more than 90% of the Hungarian, and more than 60% of the EU population of Imperial Eagles. The lowland plain habitats (90–120 m a.s.l.) are mainly intensive agricultural fields with small pastures and groups of poplar (*Populus* spp.) and black locust trees (*Robinia pseudoacacia*). The mountain habitats (200–700 m a.s.l.) are mainly forested by native oak (*Quercus petrea*, *Q. cerris*) and beech (*Fagus sylvatica*) or by introduced pine trees (*Pinus silvestris, P. nigra, Larix decidua*).

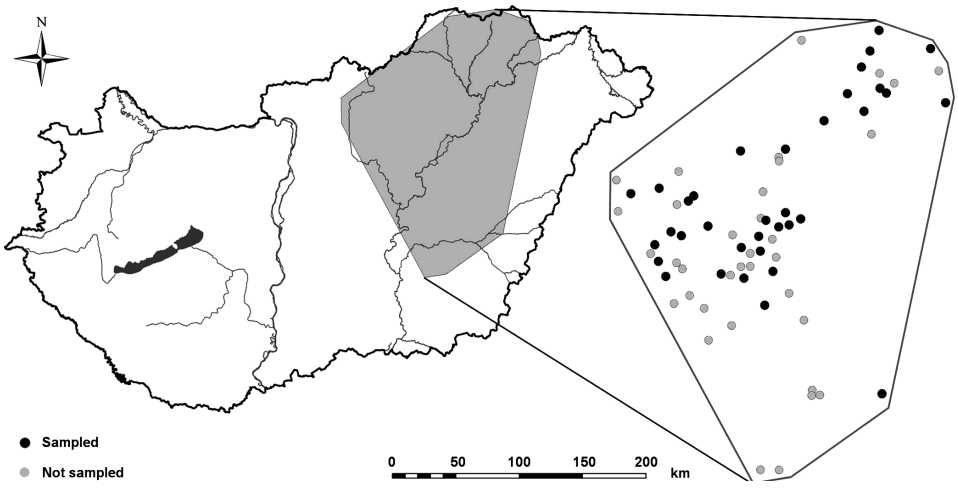

Fig. 1 Map showing the range of the study population of Eastern Imperial Eagles in Hungary and the location of sampled and not sampled territories in 2003 (35 of the 61 nesting sites in that year).

Sample collection and DNA extraction

Altogether 3771 shed feathers of adult breeding eagles were collected under nests and roost sites in Eastern Hungary between 1997 and 2006 (Fig. 1). Most nests and nearby roosting trees were visited twice per year, first in June, when nestlings were ringed as part of the population monitoring protocol (Horváth *et al.* 2011) and between July and September after fledging, in order to reduce disturbance. As at this time chicks' flight feathers are only growing and the breeding pair is extremely territorial all feathers can be assumed to belong to the resident pair. Also it is unlikely that feathers from the year before sampling could remain in a condition good enough to be suitable for DNA analyses (Vili *et al.* 2013).

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 a dry, cool and dark place to minimize further degradation of the DNA. The superior umbilicus part of the feather shaft was separated as described by Horváth *et al.* (2005).

Altogether 46 territories, where at least three years of consecutive breeding was observed were selected. As we found strong bias toward females in a previous study of Imperial Eagles where all available feathers were submitted to molecular sexing (Vili *et al.* 2009*b*) we primarily searched for the most suitable female samples. Because of this, one to three of best quality feathers of a given territory in a given year were selected for DNA extraction and molecular sexing. DNA was extracted according to the standard salting out method as described by Gemmell and Akiyama (1996). To facilitate the digestion process by reducing disulphide bonds in the keratin tissue, 10 µl 1M Dithiothreitol (DTT) was added to each sample (Weigmann 1968). The efficiency of the purification procedure and the degradation of DNA were checked before further analyses by agarose gel electrophoresis (3µl on 0.8% gel).

Molecular sexing

Each individual feather was sexed by amplifying introns of the sex chromosome-linked CHD1 gene, using the primers (2550F and 2718R) and PCR conditions published by Fridolfsson and Ellegren (1999). In Imperial Eagles, these primers amplify a larger sequence (CHD1Z, 700bp) in both sexes and a smaller one (CHD1W, 450bp) in females only (Horváth *et al.* 2005, Vili *et al.* 2009*a*). To identify the sex of the sampled specimen 5µl of the PCR product was visualized by ethidium-bromide on 2.5% agarose gel.

Microsatellite fragment analysis

Sequences of nine dinucleotide repeats (Martinez-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 breeding eagles, two dinucleotide (Aa02 and Aa39) and six tetranucleotide loci (IEAAAG04, IEAAAG12, IEAAAG11, IEAAAG15, IEAAAG09 and IEAAAG14) were selected and amplified with the use of fluorescently labelled primers. A modified version of the PCR protocol described by Martinez-Cruz *et al.* (2002) was used in the amplification procedure in which a touchdown PCR was performed in all loci. For loci Aa39, Aa02, IEAAAG09 and IEAAAG14 the annealing temperature was decreased from 66 to 50°C and for IEAAAG04, IEAAAG12, IEAAAG11 and IEAAAG15 from 60 to 50°C. The exact fragment lengths of the amplified PCR products

Table 1. Observed patterns of re-identification and replacement events of females with incomplete sampling. A and B represent the different genotypes, question marks represent the years without suitable samples for individual identification. When a different female was found after the missing years, minimum and maximum number of changes was calculated. E.g. in rows a and b no change was assumed, because the same female resided at the nest site both times; in row d the minimum is one that was detected by the genetic tagging and the maximum is three, because there were two years without data.

		Number of studied years						Number of changes	
		1	2	3	4	5	6	minimum	maximum
Observed patterns	a	A	?	A	–	–	–	0	0
	b	A	?	?	A	–	–	0	0
	c	A	?	B	–	–	–	1	2
	d	A	?	?	B	–	–	1	3
	e	A	?	?	?	B	–	1	4
	f	A	?	?	?	?	B	1	5

were determined by capillary electrophoresis (ABI PRISM® 310 Genetic Analyzer, Applied Biosystems) with GeneScan 3.7 and PeakScanner 1.0 softwares (Applied Biosystems), using the ILS600 (Promega) internal lane standard. Each sample was scored three times independently and genotypes were assigned without the knowledge of the sample's origin. Allele frequencies and probability of identity (PI, the probability that two randomly chosen individuals in a population will have the same genotype at multiple loci, Waits *et al.* 2001) were calculated with GenAlEx 6.4 (Peakall & Smouse 2006), deviation from the Hardy-Weinberg equilibrium and the probability of null alleles were calculated with Micro-checker 2.2.3 (Van Oosterhout *et al.* 2004).

Turnover rate

Genetic tagging allowed us to estimate the turnover rate of only females in the population. As we could not obtain DNA sample at every studied nest in each year, minimum and maximum turnover rates were estimated. According to the data from nest site ranges where suitable samples were available from each studied year, when a female was identified in any two years at a certain nest site no other female was found between those dates at the same site. Any female lost from a nest site range did not reappear later in the same range. These data suggest that when one individual was identified at least twice as a resident female (first at the start of the studied period and second after the years without samples), the same individual was using the nest site range in the missing years as well (Table 1, rows a–b).

When data was missing between different individual residents, the minimum turnover rate was estimated by assuming a single change that was actually detected by genotyping, and the maximum rate was estimated by assuming changes for every missing year in addition to the one detected (Table 1, rows c–f). Both minimum and maximum rates were determined by averaging the number of turnover events in each territory, in order to avoid

pseudo-replication, which could happen if using the data of the same individuals or the same territories as independent.

Differences between the turnover rates in mountain and lowland territories were tested with Brunner-Munzel test using the "lawstat" package (Hui *et al.* 2008) implemented in the R statistical computing environment (version 2.12.1; R Development Core Team 2011).

RESULTS

Sampling success

Altogether 497 selected feathers were sexed of which 450 (90.5%) derived from females and only 47 (9.5%) from males. Therefore, due to their low sample size males were excluded from the present analysis.

For the fragment analyses we selected the best quality female feather of a given breeding attempt. Out of these 166 samples 153 (92.1%) amplifications were successful, determining altogether 77 different female genotypes in 46 territories. Therefore indicating that 28.8% of all breeding attempts (530) and 51.1% of all territories (90) was successfully sampled in the study area during the 10 year period.

Individual identification

Two of the eight loci were assumed to contain null alleles (Aa39 and IEAAAG09). In order to avoid the genotyping bias all homozygous genotypes were excluded from the analyses on these loci. On the other six loci there was no significant difference from the Hardy-Weinberg equilibrium and there was no evidence for linkage disequilibrium (Table 2). The PI value of the marker set was 3.7×10^{-6}. Genotyping errors were calculated as described by Rudnick *et al.* (2005) because of the similarity of the sampling and processing methods. The 153 analysed female feathers represented 1224 genotyped loci out of which 25 (2.04%) were missing after multiple PCRs (1.22%) or could not be clearly identified (0.82%) and hence were treated as missing data.

In the first sampled year of all the 46 territories we identified 46 different females. In the subsequent years 107 individuals were identified, out of which 74 (69.2%) were the same as in the previous year, meaning they were re-found in the territory. In 31 cases (28.9%) new residents were found and only two birds (1.9%) were detected in a different territory as before (see details below).

Out of the 31 replacement events, we had data on the age category of the new and replaced females in 20 cases based on their plumage identified in the field. In the majority (14) of these, adults were replaced by adult birds. In four cases females in immature plumage were replaced by immature ones and in only two cases were immature birds replaced by adults.

Table 2. Size and frequency of alleles and the deviation from Hardy-Weinberg equilibrium (dev HWE) of the loci used at the East-Hungarian population of Imperial Eagles.

Locus	Allele size (allele frequency)									dev HWE	
										prob	S.E.
IEAAAG04	218	228	232	236	240					0.079	0.012
	(0.007)	(0.073)	(0.069)	(0.818)	(0.033)						
IEAAAG09*	476	480	484	488						–	–
	(0.295)	(0.036)	(0.241)	(0.429)							
IEAAAG11	306	324	328	332	336	344				0.078	0.01
	(0.008)	(0.185)	(0.359)	(0.149)	(0.294)	(0.004)					
IEAAAG12	124	128								0.731	0.003
	(0.485)	(0.515)									
IEAAAG14	192	196								0.722	0.003
	(0.380)	(0.620)									
IEAAAG15	102	106	114							0.815	0.005
	(0.004)	(0.814)	(0.182)								
Aa02	137	139	141	143	145	150	152			0.095	0.008
	(0.065)	(0.088)	(0.004)	(0.302)	(0.248)	(0.218)	(0.076)				
Aa39*	180	184	188	190	192	194	196	198	200	–	–
	(0.046)	(0.289)	(0.053)	(0.158)	(0.243)	(0.033)	(0.092)	(0.072)	(0.013)		

* Because of the presence of null alleles only the heterozygous genotypes were involved in the analyses and no test on the Hardy-Weinberg equilibrium and linkage disequilibrium was performed on these loci.

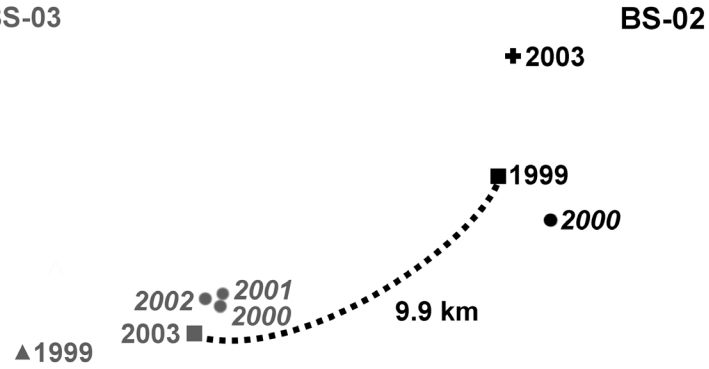

Fig. 2. First identification (1999, territory code BS-02) and re-identification (2003, BS-03) of a female. The territories were approximately 10 km away from each other and the original BS-02 territory was vacant in 2001-2002, but it was occupied by a pair with a new female in 2003; different markings represent different genetically tagged females, black markings represent the nests from the BS-02 territory , grey markings represent nests from the BS-03 territory; years in italic (nests marked by circles) represent nesting sites without samples.

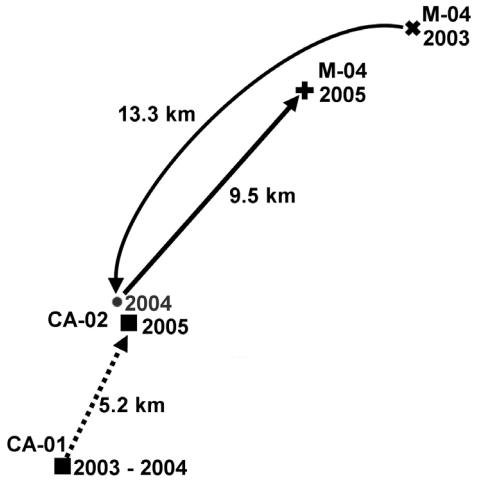

Fig. 3. Changes of territory boundaries of two pairs (CA-01 and M-04) between 2003 and 2005; different black markings represent different genetically tagged females, the circle represents a non-sampled nesting site. In 2003 both females were genotyped and their nests were 18.5 km away from each other with no further territories between them. In 2004 one pair (presumably the birds previously identified in territory M-4) appeared in a new territory (CA-02) that was founded 5.2 km away from the active CA-01 nest and 13.3 km from the inactive M-04 nest of 2003. Unfortunately, none of the birds were sampled that year. In 2005 the M-04 pair was replaced by a new one and the pair moved back approximately 9.5 km towards the original M-04 nest. There was no change in the CA-01 pair which stayed in place in 2004 and moved into the CA-02 territory in 2005.

Birds re-identified in different territories

Birds were re-identified in different territories in two cases. In the first one, a breeding female was found again three years later in a nest which was assigned to another territory (Fig. 2). In the other case, according to field observation, two breeding pairs were assumed to have changed their territory boundaries several times in a three year period. This was later partly confirmed by genetic data (Fig. 3).

Turnover rate

Turnovers were calculated in each territory then averaged to obtain the minimum and maximum average turnover rates. Minimum average yearly turnover rate of female resident eagles (replacement of a genetically tagged resident by a genetically tagged new female) was 27.7%, while maximum rate (interpreting all missing years between two different females on the same territory as replacement events) was 35.5 % (see Materials and methods and Table 1).

Turnover rate tends to be smaller in the mountain territories than in the lowlands (21.2–28.3% vs. 29.3–38.0% respectively, Table 3*a* and *b*) although the difference was not statistically significant (minimum rate: Brunner-Munzel Test Statistic = -0.8035, df = 24.409, p = 0.429; maximum rate: Brunner-Munzel Test Statistic = -0.764, df = 24.237, p = 0.452).

Table 3a. Turnover events in the sampled lowland territories. Capital letters (A-C) represents genetically identified female individuals in the given territory in alphabetic order, while lower cases (a-c) are the probable individuals, which were not genetically identified in the given year but according to our observations the double replacement (e.g. A – B – A) is unlikely. Abbreviations: terr – territory code; pss – possible changes in the studied period, it is equal to the number of years studied minus one, min – minimum changes, i.e. observed changes, max – maximum, i.e. the sum of observed and possible changes considering the results of genetic tagging; * marks the female that was first identified in 1999 (BS-02) than re-identified in 2003 (BS-03).

	Studied years (1997–2006)										No. of changes		
terr	'97	'98	'99	'00	'01	'02	'03	'04	'05	'06	pss	min	max
HV-04	A	?	?	?	?	B	b	B			7	1	5
HV-01	A	a	A	a	a	A	A	A			7	0	0
HV-03		A	A	B	?	C					4	2	3
BS-03			A	?	?	?	B*	B*	?	C	7	2	6
BS-02			A*	?	?	?	B				4	1	4
HS-06				A	a	A	A	A	A		5	0	0

Table 3a (continued)

	Studied years (1997–2006)										No. of changes		
terr	'97	'98	'99	'00	'01	'02	'03	'04	'05	'06	pss	min	max
HV-02				A	a	A	A	A			4	0	0
HS-07				A	A	A	A				3	0	0
HV-06				A	A	A	A				3	0	0
HS-01				A	B	B	B	B	B		5	1	1
BK-02				A	B	C	C	C			4	2	2
HS-05					A	?	B	B			3	1	2
BS-01					A	A	A				2	0	0
HS-21					A	A	A				2	0	0
BS-05					A	B	C	c	c	C	5	2	2
HS-03						A	A	A	A		3	0	0
HS-15						A	A	A	B		3	1	1
J-02						A	a	A			2	0	0
HS-14						A	B	B			2	1	1
SZ-01							A	?	B		2	1	2
BE-07							A	A	A		2	0	0
CA-01							A	a	A		2	0	0
HS-17							A	a	A	A	3	0	0
HS-13							A	B	B		2	1	1
NK-01							A	B	B		2	1	1
HS-16							A	B	B	B	3	1	1
HS-18							A	B	B	C	3	2	2
HS-08							A	B			1	1	1
BS-06								A	A		1	0	0
NK-02								A	A		1	0	0
HS-19								A	A	A	2	0	0
BE-06								A	B		1	1	1
HS-04								A	B		1	1	1
J-07									A	B	1	1	1
sum											102	24	38

Table 3b. Turnover events in the sampled mountain territories.

	Studied years (1997–2006)										No. of changes		
terr	'97	'98	'99	'00	'01	'02	'03	'04	'05	'06	pss	min	max
Z-01	A	A	a	A	A	A	A	A	A		8	0	0
Z-02			A	A							1	0	0
M-01				A	?	B	C	c	C		5	2	3
M-03				A	A	?	?	B	C		5	2	4
B-01				A	a	a	A	a	A		5	0	0
M-06				A	a	A	A	A	A		5	0	0
M-04					A	A	A	?	B		4	1	2
B-04					A	B	C				2	2	2
Z-03						A	A				1	0	0
Z-05						A	A				1	0	0
B-05							A	a	A		2	0	0
Z-04							A	B	B		2	1	1
sum											41	8	12

DISCUSSION

Dynamics of a population are determined by the relative rates of natality and mortality, immigration and emigration (Begon *et al.* 1990). Annual changes in the number of breeding territories and productivity of the Eastern Imperial Eagle population in Hungary have been estimated based on intensive field surveys since 1980 (Bagyura *et al.* 2002). The population size has been increasing since the early 1990's and maximal population size has not yet been reached (Horváth *et al.* 2011).

Due to sampling reasons only turnover of female eagles was determined. The large bias in sex ratio of the feathers collected under the nests was most probably caused by the different behaviour of the sexes. Females spend more time on the nests and in their immediate vicinity than males during the breeding season (Margalida *et al.* 2007, MH's own observations).

The population in the Carpathian Basin inhabits two different habitat types: lowland areas and mountainous areas. The latter area can be considered as traditional, because it served as refuge for the species until 1990, when the first nest in the lowland was observed. Even though turnover rates differed in the two habitat types the difference, most likely because of the relatively small sample size was statistically not significant.

According to recent studies (Horváth 2009) feeding opportunities are better in the lowlands, but there is also bigger human disturbance, which could mean more risks for the birds. However, no trend was found in breeding success according to habitat type but non-adult pairs, where at least one of the birds in a pair has non-adult plumage were significantly less successful. The changes in productivity were also explored as the expansion progressed and the newly established lowland territories had better productivity than traditional mountain territories. Unlike in the case of the Spanish Imperial Eagle (Ferrer & Donázar 1996, Ferrer & Bisson 2003) in the lowland area of the Carpathian Basin new nests are established both in the peripheral zone and in between existing territories and despite the increasing density of territories there was no change in the breeding success in the lowland area (Horváth 2009). Our data implies a much higher turnover rate among breeding females than was estimated by field methods or expected from a long-lived raptor species. Such high turnover rate significantly exceeds the estimated yearly loss of other populations of both Eastern and Spanish Imperial Eagles (Ferrer & Calderon 1990, Rudnick *et al.* 2005, Gonzalez *et al.* 2007, Ortega *et al.* 2009), or other large sized eagles (Real & Mañosa 1997, Whitfield *et al.* 2004).

The extraordinary high frequency in exchange of resident birds can be explained either by (1) the instability of territories, (2) emigration or (3) high mortality of residents.

Considering that in our study only two cases of territory swapping were found, and even though breeding pairs occasionally changed the location of the used nest they mostly stayed within the average nearest nest distance, thus we can assume the stability of territories. This is also supported by the strong mate and nesting site fidelity of the species (del Hoyo *et al.* 1994, Rudnick *et al.* 2005). Moreover, replaced females were mostly adults therefore the high turnover rate could not be explained by the inexperience of breeders. Although we cannot exclude the possibility of emigration outside of the studied population, this scenario is highly unlikely in a continuously growing, geographically isolated population. The nearest breeding populations to the Central European one are in Macedonia (500 km), Bulgaria (600 km) and Southeast Ukraine (1000 km), which distances are significantly higher than the recorded natal dispersal distances of Imperial Eagles (Ferrer 1993, Horváth & Kovács 2009).

We rather suggest that this surprisingly high turnover rate is caused by high mortality in this breeding population. In recent years the two main causes of mortality of Imperial Eagle in Hungary are electrocution and illegal poisoning (Horváth *et al.* 2011). While the effect of electrocution remained more or less stable during the last decades, the prevalence of poisoning incidents has increased dramatically. In the last seven years 69 carcasses of poisoned

Imperial Eagles were found (Horváth *et al.* 2011). The most commonly used poisons are withdrawn pesticides that kill so quickly that most of the bait remains intact and can attract predators and scavengers. Therefore even if eagles are not the primary targets of poisoning they are greatly affected.

Despite the unexpectedly high turnover (mortality) rates the population of our study area has been increasing meaning that missing individuals are replaced and new territories are established every year. Replacement by immigrants from genetically non surveyed populations cannot be the main process as our study area covers approximately 60% of the eagle population of Central Europe.

High natality and therefore a large number of floater individuals that are able to replace missing individuals and hence fill up the vacant territories are realistic explanations of the growth of the population (Kenward 2000). Unfortunately, as in most field surveys, we cannot estimate the size of the floater population. As we are genetically identifying most of the newly-hatched chicks in the population, we will be able to find out in the following years if they are the main source of the new breeders.

Our findings on the high turnover rate and thus on the possibly extraordinary high mortality rate of Imperial Eagles underline the needs for urgent conservation measures in order to decrease the prevalence of illegal poisoning incidents and to modify the electric power lines into a bird friendly design at the key habitats of the species.

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Acknowledgement – We are grateful to the members of the Hungarian Imperial Eagle Working Group for collecting the samples for this study. The comments of Dr. Todd Katzner and an anonymous reviewer improved greatly the quality of the manuscript. We also would like to thank Dr. Louise Deering and Katalin Demeter for their valuable comments on the manuscript. The studies were partly financed by the LIFE-Nature Fund of the European Union (LIFE02NAT/H/8627, LIFE10NAT/HU/019), National Research and Technical Development Program (NKFP3-3B023-04) and the Spanish Ministry of Education and Science and Doñana Biological Station (ICTS-RBD 2007).

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Revised version received January 18, 2013, accepted August 1, 2013, published September 30, 2013