DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS

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THE GREATER SPOTTED EAGLE *AQUILA CLANGA* AND THE LESSER SPOTTED EAGLE *A. POMARINA*: TAXONOMY, PHYLOGEOGRAPHY AND ECOLOGY

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TARTU 2004

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The dissertation is accepted for the commencement of the degree of Doctor of Philosophy in zoology on March 11, 2004 by the Doctoral Committee of the Faculty of Biology and Geography, University of Tartu

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Commencement: room 301, Vanemuise St. 46, Tartu,
on April 30, 2004 at 10.15 a.m.

The publication of this dissertation is granted by the University of Tartu

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Tartu Ülikooli Kirjastus
www.tyk.ut.ee
Tellimus nr. 109

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is as summary of the following papers, which are referred to in the text by the Roman numerals:

I Väli, Ü. 2002. Mitochondrial pseudo-control region in old world eagles (genus *Aquila*). *Molecular Ecology* 11: 2189–2194.

II Väli, Ü., Treinys, R. & Poirazidis, K. 2004. Genetic structure of Greater *Aquila clanga* and Lesser Spotted Eagle *A. pomarina* populations: implications for phylogeography and conservation. In: Chancellor, R.D. & Meyburg, B.-U. (eds.) *Raptors Worldwide*. WWGBP, in press.

III Väli, Ü. & Lõhmus, A. 2002. Parental care, nestling growth and diet in a Spotted Eagle *Aquila clanga* nest. *Bird Study* 49: 93–95.

IV Väli, Ü. & Lõhmus, A. 2004. Nestling characteristics and identification of the Lesser Spotted Eagle *Aquila pomarina*, Greater Spotted Eagle *A. clanga* and their hybrids. *Journal of Ornithology*, in press.

V Lõhmus, A. & Väli, Ü. Breeding habitat of a threatened Greater Spotted Eagle *Aquila clanga* population interbreeding with Lesser Spotted Eagles *A. pomarina*. (submitted manuscript)

VI Väli, Ü., Treinys, R. & Lõhmus, A. 2004. Geographic variation in macrohabitat use and preferences of the Lesser Spotted Eagle *Aquila pomarina*. *Ibis*, in press.

VII Lõhmus, A. & Väli, Ü. 2004. The effects of habitat quality and female size on the productivity of the Lesser Spotted Eagle *Aquila pomarina* in the light of the alternative prey hypothesis. *Journal of Avian Biology*, in press.

VIII Väli, Ü. 2004. Sex ratio of Lesser Spotted Eagle *Aquila pomarina* nestlings in good and poor breeding years. *Bird Study*, in press.

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The dissertant's contribution to the papers was following: I, VIII – 100%, II – 90%, III...VII – 50%.

1. INTRODUCTION

Twelve percent of the world's bird species pose a risk to become extinct in the next one hundred years (Hilton-Taylor 2000). However, two thirds of avian conservation research is conducted on sensitive but non-threatened species, and the studies on endangered species have been mostly restricted to a few examples (e.g. Spotted Owl *Strix occidentalis* and Bald Eagle *Haliaeetus leucocephalus*; Marzluff & Sallabanks 1998, Lõhmus 2003b). Usually, the knowledge of threatened species relies on anecdotes and guesswork rather than methodologically reliable and accurate surveys. Initial ideas about the reasons for the rarity or decline of the species tend to be biased to the obvious or simple, and frequently turn out to be erroneous when examined in detail (Green & Hirons 1991, Derrickson *et al.* 1998). Thus, adequate research to guide conservation actions is urgently needed for most endangered birds.

In the current thesis, I explore two closely related bird species of conservation concern that breed semisympatrically in Eurasia. The **Greater Spotted Eagle** *Aquila clanga* Pallas 1811 is a rare, globally threatened species, while the more abundant **Lesser Spotted Eagle** *A. pomarina* Brehm 1831 has an unfavourable conservation status in Europe (Tucker & Heath 1994). The spotted eagles will be shortly introduced in Methods, except the Indian Spotted Eagle *A.* (*pomarina*) *hastata*, probably a separate species (Parry *et al.* 2002) that will not be treated henceforth.

Research of population limitation factors belongs to the highest priorities of the conservation of spotted eagles (Meyburg *et al.* 2001). However, these birds are not easy to study, the problems emerging already from field identification and taxonomic status. The two eagles diverged about one million years ago (Seibold *et al.* 1996) and have usually been considered as separate species (Cramp & Simmons 1980, Meyburg 1996, Bergmanis 1996). However, identification problems and the variability of proposed 'species-specific' characters have induced suggestions to unite the two species into one as well (Dementjev & Gladkov 1951; Zhezherin 1969). An increasing number of hybridisation records (see references in **IV**) and changing views about the validity of various species concepts (e.g. Mayr & Ashlock 1991, Helbig *et al.* 2002) give occasion to revise the taxonomic status of spotted eagles.

Like in most bird species, distribution and numbers have been the first and most frequently studied issues about spotted eagles as well. General distribution maps have been obtained long ago (e.g. Dementjev & Gladkov 1951), but there are still several gaps of knowledge, such as the eastern range border and distribution in Asia (Meyburg *et al.* 2001, Mischenko *et al.* 2001) of even the better-studied Lesser Spotted Eagle. In the Greater Spotted Eagle, only small local populations have been monitored. Population sizes of spotted eagles are still questionable (Meyburg *et al.* 2001). The phylogeography of both species has remained unexplored, though the history of species distributions, as established through genetic analyses of populations, has relevance to present genetic diversity and, hence, to conservation and management (Avise 2000). Hitherto performed molecular analyses of spotted eagles mean only a phylogenetic study (Seibold *et al.* 1996) as a part of a wider survey of raptor evolution (Wink *et al.* 1998).

Most ecological studies on spotted eagles have described their basic natural history. In addition to general articles (e.g. Glotov 1959, Bergmanis *et al.* 1990, Drobelis 1994, Pugacewicz 1995, Abuladze 1996, Vlachos & Papageorgiou 1996, Ivanovsky *et al.* 1999), there are reports on breeding habitat (Skuja & Budrys 1999, Langgemach *et al.* 2001, Scheller *et al.* 2001, Treinys 2001), foraging and diet (Galushin 1962, Palášthy & Meyburg 1973, Gedeon & Stubbe 1991), behaviour (Meyburg 1974, Scheller & Meyburg 1996, Komischke *et al.* 2001), reproductive success (Švehlik & Meyburg 1979, Matthes & Neubauer 1991, Rodziewicz 1996, Bergmanis *et al.* 2001), siblicide (Meyburg 1971, 2001, Meyburg & Pielowski 1991, Haraszthy *et al.* 1996) and migration (Meyburg *et al.* 1995a,b, 2001, Danko *et al.* 1996, Hedenström 1997, Abuladze 2001). Most of the studies have explored the Lesser Spotted Eagle, and material about the Greater Spotted Eagle is usually based on only one or a few pairs. Indeed, the latter species is the least studied eagle in Europe (Meyburg *et al.* 2001), and not only due to its rarity — a number of papers and several books have been devoted to the twenty times less numerous Spanish Imperial Eagle *Aquila adalberti* (Ferrer 2001). Hopefully, the shortage of knowledge about the Greater Spotted Eagle can be partly overcome thanks to its close similarity with the Lesser Spotted Eagle, whose data could possibly be extrapolated to the rare Greater Spotted Eagle. Also in this thesis, I could not always compare the two species, and more complicated ecological studies were performed only in the Lesser Spotted Eagle.

Though natural history of species is essential information for conservationists, an understanding of ecological factors influencing biological characters is necessary for efficient conservation as well (Derrickson *et al.* 1998). Yet in spotted eagles these deeper questions have been seldom asked. Obviously, it is difficult to perform experimental ecological manipulations in rare and hardly accessible species (Gaston 1994: 159), but among spotted eagle studies, correlative approaches (e.g Bergmanis *et al.* 2001) and, in fact, any statistical hypothesis-testing have been scarce, too. In conclusion, advanced scientific approach is needed for an effective conservation of spotted eagles.

The aims of this thesis are to fill some gaps of knowledge in the biology of spotted eagles, and to help the conservation research of the species to pass on from the descriptive stage. My studies concentrated on three issues. (1) I revised the taxonomy of spotted eagles; (2) explored the genetic structure and diversity of spotted eagle populations to obtain information about their history and current status; (3) analysed ecological factors limiting the eagles, primarily habitat. For these purposes, I conducted genetic studies among and within species, analysed their morphological characters, and tested correlations between the incidence of nests or reproductive success of the eagles and their habitat variables.

Paper **I** revises phylogenetic relationships between *Aquila* eagles and explores the genetic divergence between spotted eagles by analysing a large sample of birds within the sympatric area where the clinal transition from one species to another has been suggested (Zhezherin 1969). This study also provides the first data on genetic diversity in the two species, a subject that is analysed in more detail in paper **II**. I ask whether low genetic variability — often characteristic to rare species and associated with population bottlenecks and inbreeding (Haig & Avise 1996) — could be a threat to the Greater Spotted Eagle. Since the present genetic diversity is an outcome of population history, phylogeography of the species is a subject of study in paper **II** as well. Paper **III** deals with breeding biology of the poorly studied Greater Spotted Eagle, specifically its parental care and daily activity, and the accuracy of a method used in diet studies. Additionally, I followed nestling growth, to develop a method for analysing morphological variation of spotted eagle nestlings of different age. This method is used in paper **IV**, where the main question beside the species identification is whether the reported overlap in the appearances of the two species could be due to hybrids. Paper **V** compares the breeding habitats of two spotted eagles (with an emphasis on the poorly studied Greater Spotted Eagle) and looks for variables distinguishing species at the landscape and nest site scales. The location of interbreeding pairs on the habitat gradient between species is of particular interest. The three last papers are devoted to habitat relationships of the Lesser Spotted Eagle, for which relatively large samples were available. Paper **VI** explores geographically distinct landscape use and responses to habitat availability. With respect to temporal changes, paper **VII** explores the relative contributions of habitat and individual quality to reproductive success in years of different prey availability, and checks whether the Lesser Spotted Eagle switches to alternative prey in the years of food shortage. Since spotted eagles can rear only one nestling per year, they cannot vary the number of offspring in response to inter-annual variation of habitat quality. However, they may allocate resources by growing up a nestling of different sex. This hypothesis is tested in paper **VIII**.

2. MATERIAL AND METHODS

2.1. The species

Spotted eagles are medium-sized migratory raptors breeding in the Eurasian temperate zone. These forest-dwelling birds build their nest into tree crown and use it for several years. Usually two eggs are laid, but after five to six weeks of incubation and eight weeks of nestling period only one young fledges. Spotted eagles forage in open landscapes, preferably grasslands. Their diet consists mostly of small mammals, but other small- to medium-sized animals (amphibians, birds etc.) are taken as well. For detailed data on the natural history of the species in Estonia, see Väli & Lõhmus (2000) and Väli (2003).

The world population of the Greater Spotted Eagle consists of about 3000 pairs distributed sparsely in a huge area from the Baltic Sea to the Pacific Ocean; the twenty thousand pairs of the Lesser Spotted Eagle breed mainly in Central and Eastern Europe (Meyburg *et al.* 2001). The distribution and breeding numbers in Estonia were for the first time well-surveyed only in the 1990s, resulting estimates of 20–30 pairs for *A. clanga* and about 500–600 pairs for *A. pomarina* (including 10–15 interbreeding pairs; Lõhmus 1998). During 1988–2003, spotted eagles have been registered in 423 breeding territories in Estonia, and in 234 territories at least one individual has been determined to species (Ü. Väli, unpublished data).

2.2. The samples

Most data on spotted eagles were gathered during fieldwork in Estonia, 1995–2003. Depending on analysis, I explored up to 50% and 30% of the national Greater and Lesser Spotted Eagle populations, respectively.

For studying phylogenetic relationships between eagle species (**I**), additional blood samples from two Golden Eagles *Aquila chrysaetos*, Eastern Imperial Eagles *A. heliaca*, and Steppe Eagles *A. nipalensis*, as well as samples of a Greater Spotted Eagle and a Lesser Spotted Eagle were collected from captive individuals held at Tallinn Zoo. For analysing the phylogeography of the Lesser Spotted Eagle (**II**), 29 DNA samples from Lithuania and four from Greece were obtained. The macrohabitat data of 55 nest sites in Lithuania were used to study geographic variation of Lesser Spotted Eagle's habitats (**VI**).

2.3. Field methods and habitat descriptions

The nesting territories (an area occupied by a pair of mated birds over successive years; Steenhof 1987) of spotted eagles were mapped and nests were searched for throughout the breeding season. Productivity (annual number of offspring per pair; Postupalsky 1974, Steenhof 1987) was recorded, eaglets were described and blood samples were taken in late July (i.e. shortly before fledging). Pellet analysis (taking into account also other bones from the nest) appeared to be a suitable method for analysing the eagles' diet as verified by direct visual observations while studying parental care of the Greater Spotted Eagle from the hide near a nest (**III**). Therefore, pellets and prey remains were collected and analysed in the diet studies (**III, VII, VIII**) using standard methods (Marti 1987) and reference collections. To measure female size — an indicator of individual quality (**VII**) — moulted flight feathers were collected during nest visits.

In habitat studies (**V–VII**), landscape composition and heterogeneity within 2 km around nests, distances from nests to certain landscape elements and number of conspecific nests within 5 km were measured from digital maps, whereas stand and nest tree characteristics as well as shorter distances from nests to landscape elements were measured in the field. To compare nest sites with available habitat, random plots on forest land were described in a similar way.

2.4. Genetic analyses

I used blood samples of nestlings and feathers of adult birds as sources of DNA. Blood was taken from the brachial vein of a nestling and stored in a vacuntainer with EDTA buffer at room temperature during fieldwork days. After this, blood cells were separated from serum and stored at –20°C until further analysis. Total DNA was isolated from blood cells using proteinase K lysis and salt-extraction of proteins, and ethanol precipitation of DNA. For DNA extraction from feathers, I used DNeasy Tissue kit (Qiagen) and manufacturer's directions.

Mitochondrial DNA (mtDNA) — a maternally inherited, non-recombining, and relatively rapidly evolving part of the genome — is one of the most effective markers for detecting genealogical structure within and among avian populations, and for reconstructing phylogenetic relationships among taxa (Quinn 1997). For identification of species-specific mitochondrial lineage (**I, IV–VII**) and analysis of the genetic structure of populations (**I, II**), the recently discovered pseudo-control region (Mindell *et al.* 1998, Haring *et al.* 1999) was amplified by polymerase chain reaction and sequenced. All mutations were checked by re-amplification and re-sequencing.

In papers **III, IV, VII** and **VIII**, spotted eagles were sexed using the sex-specific chromo-helicase-DNA-binding (CHD) gene. A part of the gene was amplified according to Griffiths *et al.* (1998) and the product was analysed by VspI restriction and electrophoresis. Female-specific (CHD-W) product of the gene contains two restriction sites, whereas the product of CHD-Z, amplified from both sexes, has only one site. This leads to three DNA bands for females and two bands for males — a clearly visible difference on 2% agarose gel.

2.5. Data processing

According to the aims and type of data, a wide array of statistical methods was used and performed mainly by Statistica 6.0 software. In morphological analysis (**IV**), a character index (cf. Mayr & Ashlock 1991: 95) was calculated for summarizing the plumage descriptions. The size variables of nestlings had to be corrected for their growth, and residuals of regressions between each variable and wing length (according to **III**, Figure 1 in **IV**) were used to get indices of relative size. Original sets of strongly intercorrelated variables (measuring size and habitat; **V–VII**) were reduced to a few principle components. For principal components analyses and parametric tests, the variables were checked for normality and transformed where appropriate (arcsine-square-root transformation used for proportions, logarithmic or square-root transformation for other variables). Logistic regression was used for building multivariate explanatory models for binary dependent variables (**V, VII**).

DNA sequences were aligned using Clustal W program (Thompson *et al*. 1994) and were improved manually. Phylogeny and population structure (**I, II**) were analysed using FastDNAml 1.0 (Olsen *et al*. 1994), DNASP 3.53 (Rozas & Rozas 1999), ARLEQUIN 2.000 (Schneider *et al*. 2000) and MEGA 2.1 (Kumar *et al*. 2001) software. Coalescence time for a population (**II**) was calculated according to Saillard *et al*. (2000) using minimum spanning network based on the variable sites (Bandelt *et al*. 1995).

3. RESULTS

3.1. Phylogenetic relationships in *Aquila* eagles

The pseudo-control region of mtDNA was described in five species of eagles. Despite difficulties caused by large species-specific indels, I reconstructed a network of phylogenetic relationships based on a total of 413 bp of the non-repetitive part of pseudo-control region. Three methods (neighbour joining, maximum parsimony, maximum likelihood) yielded trees of identical topology: Greater and Lesser Spotted Eagles were most closely related with each other, as were Imperial and Steppe Eagles, and the Golden Eagle had similar genetic distances to both of these species-pairs (Figure 1 in **I**). The most important result was, however, that the relatively large samples of spotted eagles confirmed the existence of two clearly separated groups. Comparison of genetic distances between species showed that the pseudo-control region is a suitable marker for population analysis, since its evolutionary rate is about three times greater than has been estimated for cytochrome b gene.

3.2. Genetic structure of spotted eagle populations

Analysis of the pseudo-control region indicated a higher genetic diversity in the Lithuanian than in the Estonian Lesser Spotted Eagle population (Table 1 in **II**).

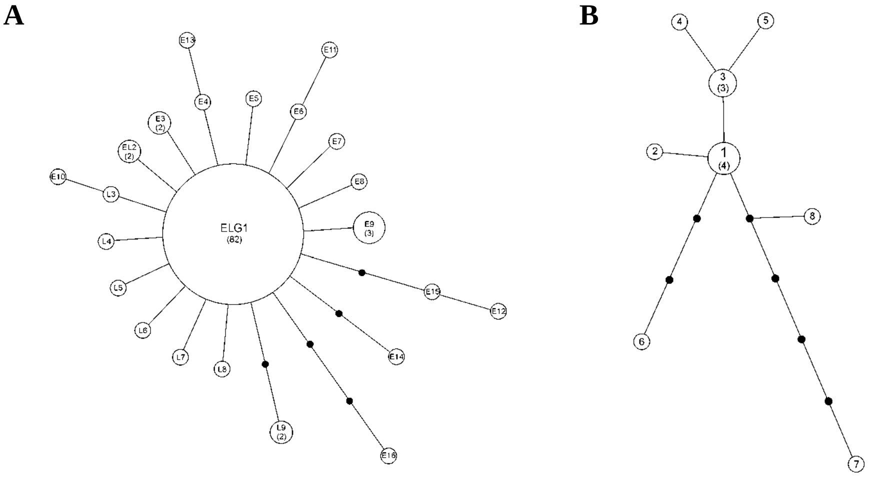

Figure 1. Minimum spanning networks based on the 867-bp sequence of the pseudo-control region describing population genetic structure in the Lesser Spotted Eagle (A), and the Greater Spotted Eagle (B; **II**). Circles represent haplotypes (sample sizes larger than one are given in brackets) and black nodes refer to substitutions or indels

Otherwise, there was a low differentiation between these two local populations. A star-like genetic structure (Figure 1A), significantly negative Tajima's *D* value and the concordance between observed and expected distribution of pairwise genetic differences showed that the Baltic population of the Lesser Spotted Eagle has expanded rapidly after a recent bottleneck. The calculated coalescence time (8000±1500 years) indicated an expansion after the last ice-age, and occurrence of the most common northern haplotype in Greece suggested that the Balkan refugium could be the source of the postglacial Baltic population. Compared with the Lesser Spotted Eagle, genetic diversity was found to be three times higher in the Estonian Greater Spotted Eagle population (Table 1 in **II**; Figure 1B).

3.3. Morphology of spotted eagle nestlings

Although the analyses of mtDNA showed the existence of two species-specific lineages (**I**), some nestlings had characters of or intermediate to both species. After pre-identification of all nestlings according to mtDNA, nape patch (the most reliable species-specific character; Meyburg 1974) and the appearance of their parents, the complex analysis of morphological characters distinguished two species and showed that the hybrid nestlings have intermediate scores of plumage and relative size indices (Tables 1–3 in **IV**; Figure 2). Since it is impossible to perform genetic analyses and combine size variables in the field, a three-step identification cue for separation of the two species and their hybrids was developed. Using this, we were able to identify correctly 164 of 168 birds (Table 5 in **IV**).

3.4. Ecology of spotted eagles

3.4.1. Species-specific differences of habitats

Distance to waterbodies clearly separated Greater Spotted Eagle nest sites from random forest plots; a similar tendency in the Lesser Spotted Eagle was much less pronounced (Figure 1A in **V**). The two species were well segregated in their use of open landscapes, such that the Greater Spotted Eagle tended to prefer natural areas, while the Lesser Spotted Eagle rather avoided these (Table 1 and Figure 1B in **V**). A large area of grasslands (whether anthropogenic or natural origin) was characteristic in the territories of the Lesser Spotted Eagle compared with the available habitat (Table 2 and Figure 2 in **VI**; Table 1).

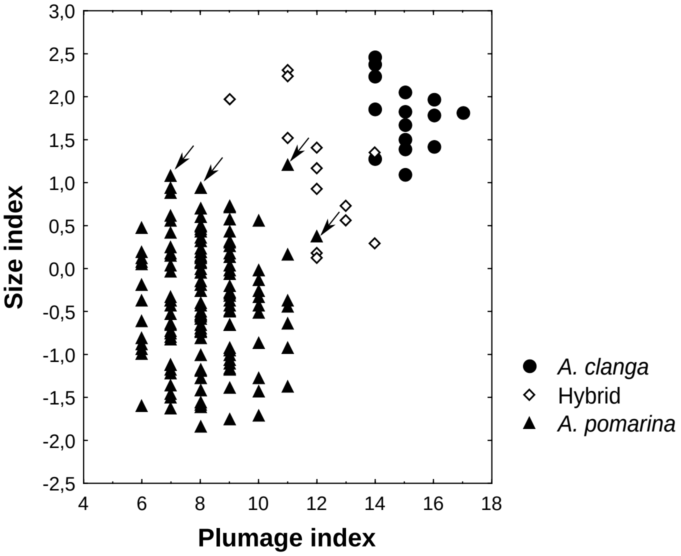

Figure 2. Size and plumage indices of the nestlings of the two spotted eagle species and the hybrids. Nestlings, which were not possible to separate from hybrids using step-by-step identification, are indicated by arrows (**IV**).

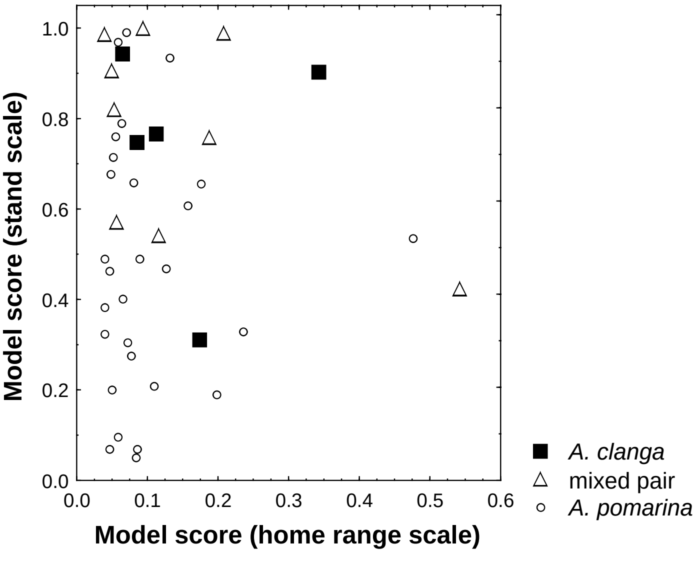

Figure 3. Distribution of the nest sites of Greater and Lesser Spotted Eagles and their mixed pairs according to the values predicted by species-specific habitat models at home-range and stand scales (**V**).

Within the same landscape, Greater Spotted Eagle nests were situated in old wet stands containing black alder, birch and pine, while the nearest Lesser Spotted Eagle rather used old spruce stands. There was also a tendency of a more pronounced old-growth structure in the Greater Spotted Eagle nest sites. All these species-specific differences in nest stands became significant only in the multivariate model (Table 1 and Table 2 in **V**), indicating some interaction between them. The species-specific models did not reveal a distinct position of mixed pairs within the Greater Spotted Eagle sample (Figure 3).

3.4.2. Geographic variation in habitat use and preferences

The landscape use and geographically distinct responses of the Lesser Spotted Eagle to habitat availability were studied on the northern edge (Estonia) and near the centre (Lithuania) of its European distribution range (**VI**). Nearly all possible types of habitat relationships with respect to geographic area were found. (1) Two characteristics (landscape heterogeneity, area of optimal foraging habitat) did not differ geographically, although, the optimal foraging habitats were preferred in both countries (Figure 2B in **VI**; Table 1). (2) Spatial variation in the use of two land cover types (area of suboptimal foraging habitats and forests) can be mostly attributed to differences in their availability. The eagles tended to avoid forests (Table 2 and Figure 2B in **VI**; Table 1), although this trend was not significant in *post-hoc* country-comparisons and within Estonia (Table 3 in **VI**). (3) Geographically distinct preferences occurred in the distances between nests and landscape elements. The species avoided anthropogenic edges in Lithuania but tended even to prefer their proximity in Estonia, and while the eagles selected nest sites near remote waterbodies throughout Estonia they had no such preference in Lithuania (see paper **VI** for details of these combined variables). There was no significant variation in these two distances from the eagle nests within Estonia. There was a tendency towards higher selectivity of the eagles in Estonia than in Lithuania but no differences between the four zones within Estonia.

3.4.3. Consequences of the annual variation in food supply

Annual changes in the abundance of main prey (microtine rodents) determined the breeding success of the Lesser Spotted Eagle in 1990–2002 in Estonia. The productivity peaked after every three years (Figure 1 in **VII**). However, there was no evidence of switching to alternative prey — no correlation between the annual productivity and proportion of voles in the diet. Productivity was also not related to the area of any land cover type in the breeding territory, but was positively correlated with heterogeneity of landscape (Table 1) and negatively with the number of other spotted eagles breeding nearby (though not signifycantly in vole-poor years). Individual quality (female size) was positively related to productivity in years with scarce food supply, and there was a significant negative interaction between body size and the number of conspecifics. In contrast to habitat quality effects, the eagles did not select heterogeneous landscapes (**VI**) and the female size was not correlated with habitat characteristics (**VII**).

Lesser Spotted Eagles responded to decreased food supply also by reducing the cost of rearing offspring — more male (smaller sex) nestlings were grown up in vole-poor years (Table 1 in **VIII**). In vole-rich years, more female nestlings were produced, and over all the years, the sex ratio did not differ from parity.

Table 1. Landscape variables determining the habitat preferences (**VI**) and productivity in vole-poor and vole-rich years (**VII**) of the Lesser Spotted Eagle. The numbers are the significance levels for differences of nest sites and random sites according to ANOVA (preference), and probability of a higher-than-average productivity according to univariate logistic regression.

Variable	Habitat preference	Productivity	
		Poor years	Rich years
Within 2 km around nest:			
Landscape heterogeneity	0.33	0.106	0.006
Forest area [a]	0.009	0.979	0.473
Area of optimal foraging habitat	<0.0001	0.421	0.286
Area of suboptimal foraging habitat	0.16	0.354	0.904
Proximity of landscape elements:			
Anthropogenic edges	0.03		
Remote waterbody	0.005		

[a] for productivity, a principal component ('anthropogenic landscape'), which was strongly correlated with forest area (r = –0.86).

3.4.4. Parental care in the Greater Spotted Eagle

As the nestling matured, the duration of nest attendance by adults and feedings by the female decreased almost linearly (Figure 2 in **III**). Visits of the male were mostly prey deliveries and took less than a minute. Most of daily activity of the adults was concentrated to late morning and midday and formed a smaller peak in the evening (Figure 3 in **III**). However, no significant impacts of weather on activity were detected. Neither significant general trends in the number and biomass of prey in relation to age of the chick nor a decline in prey-delivery rate before fledging were detected.

4. DISCUSSION

4.1. Taxonomy of spotted eagles

Taxonomic status has been one of the most intriguing questions about the spotted eagles. Their morphological similarity and frequent interbreeding in a wide hybrid zone suggests the subspecific status (Mayr & Ashlock 1991: 101). However, the mtDNA difference of 2%, showing divergence about one million years ago (Seibold *et al.* 1996), supports rather the full species status, although there is no precise limit between subspecies and species (Helbig 2000). The current study analysed relatively large samples of eagles from the area of sympatry and the sequences of pseudo-control region supported the occurrence of two lineages (**I**). The same has been shown by a complex analysis of morphological characters (Bergmanis 1996, **IV**), and by the differences in habitat use that were for the first time quantified and modelled in this study (**V**). After adding also the highly concordant data of species determination by morphology and mtDNA (**IV**), which suggest very limited gene flow between the two forms, there are few evidence left for assigning subspecies rank to spotted eagles.

The thesis showed that the morphological overlap of the two ‘species’ is caused by their hybrids (**IV**). This explains the frequent occurrence of intermediate phenotypes in the area of sympatry (Zhezherin 1969), because — uniquely among raptors — hybridisation occurs regularly in spotted eagles (Panov 1989, Väli & Lõhmus 2000, Lõhmus & Väli 2001a, Dombrovski 2002).

Though several species concepts have been proposed, each of these faces difficulties in hybrid zones (Helbig *et al.* 2002). I suggest that in sympatrically coexisting sexually reproducing animals, the biological species concept (based on reproductive barrier) should remain the ground of decision. Although roughly 10% of bird species are able to hybridise in nature (Grant & Grant 1992), reproductive incompatibility at the taxon level (i.e., non-random mating and restricted gene flow) is more important than the lack of interbreeding *per se* (Short 1969, Mayr & Ashlock 1991). Low numbers of the Greater Spotted Eagle make the examination of assortative mating difficult. However, exchange of genetic material between the two spotted eagles seems to be limited since, despite the regular hybridisation in a broad zone, only few probable cases of backcrosses (**IV**) have been recorded. Hence, following Panov (1989: 9–11) and Helbig *et al.* (2002), the semispecies status seems to be most appropriate for sympatric spotted eagles (such as in Pied and Collared Flycatchers *Ficedula hypoleuca et albicollis*). Similarly, closely related pairs of allopatric *Aquila* eagles are defined as allospecies (Seibold *et al.* 1996, Helbig 2000). Both of these intermediate situations between full species and subspecies should be taxonomically considered as species (Short 1969, Panov 1989: 9, Helbig *et al.* 2002).

4.2. Genetic diversity and population history

Two lineages of mitochondrial pseudo-control region showed about threefold difference in genetic diversity between spotted eagles (**I, II**). Possible explanations for the high variation among the Estonian Greater Spotted Eagles include a lack of severe bottlenecks, origin from several glacial refugia, intensive gene flow between populations and recent decrease in population numbers. Whatever the reason, it is evident that genetic impoverishment is not affecting the Greater Spotted Eagle.

The Baltic population of the Lesser Spotted Eagle showed a typical genetic structure of a population that has expanded rapidly in numbers and range after a recent bottleneck (Figure 1A). Similar population structures have been registered, for example, in the Greenfinch *Carduelis chloris* (Merilä *et al.* 1997) and the Great Tit *Parus major* (Kvist *et al.* 1999). During last glacial maximum in Pleistocene, when large areas of Northern and Central Europe were covered by ice and permafrost, animals and plants were concentrated to refugia on southern peninsulas of the continent (Hewitt 2000). Among many other species of Eastern Europe (Hewitt 1999), the Balkan Peninsula was probably the glacial refugium for the European population of the Lesser Spotted Eagle. After the retreating of ice, vegetation — spreading rapidly over Europe — enabled the northward expansion of animals. Broad-leaved forests, that afford suitable nest-sites for the Lesser Spotted Eagle, started their spread in early Holocene and reached rapidly to Estonia (Saarse & Veski 2001, Brewer *et al.* 2002, Petit *et al.* 2002). This fits well with the calculated expansion time for the eagle. The broad-leaved forests reached their maximum area in Estonia during the Atlantic period and their gradual retreat — along with the colonisation by Norway spruce from the east — started in the Sub-boreal period (Valk 1974, Lagercrantz & Ryman 1990, Saarse & Veski 2001). The changes in forest composition probably caused a change of breeding habitats of the Lesser Spotted Eagle. Nowadays, it breeds in spruce-dominated forests in Estonia (Väli 2003, **V**) while in Central Europe, the species still prefers broad-leaved forests (Drobelis 1994, Langgemach *et al.* 2001). It is not possible to determine the timing of such a probable response to habitat availability, because this could have been very rapid. For example, the shift of main foraging habitats — from seminatural meadows to anthropogenic grasslands in Estonia — required only a few decades of the previous century (Lõhmus & Väli 2001b).

4.3. Variation in habitat relationships and food supply

Geographic differences in breeding macrohabitats of the Lesser Spotted Eagle (**VI**) can be explained by different availability of habitats as well as by different selection by eagles. The results did not support the existence of latitudinal or range centre-to-periphery gradients in habitat relationships of the species; the main factor behind the geographic variation was probably land use history. Due to such a variability, extrapolating of local habitat relationships to other areas may give erroneous results, and large-scale conservation planning regarding species' habitat may be ineffective.

Temporal changes of food abundance and availability determine the numbers and/or reproductive success of many raptors, some of which may switch to alternative prey during the shortage of staple food (Newton 1979). Vivid examples of such responses are recorded in vole-eating species, such as the Common Buzzard *Buteo buteo* (Reif *et al.* 2001, Lõhmus 2003a). Diet of the Estonian spotted eagles consists also mainly of voles (Väli & Lõhmus 2000, Väli 2003) and vole cycles are followed by fluctuating reproductive success. However, the Lesser Spotted Eagle did not switch to alternative prey during vole-poor years (**VII**).

Productivity of the Lesser Spotted Eagle depended on the heterogeneity of the landscape around its nests (**VII)**, which may guarantee the presence of prey during different seasons and years. The negative effect of other spotted eagle territories in the surroundings, causing intraspecific competition on food resources, was expectable as it has been shown in many raptors (Lõhmus 2003a and references therein) and indicates a density-dependent mechanism of population limitation. In vole-poor years also the individual quality of eagles became important. Positive correlation between the female size and the productivity could be mostly related to the possibility of a large body to store more body reserves and/or to use them more efficiently during continuous nest attendance (**III, VII** and references therein). However, the negative effect of large body in the presence of competitors indicates the cost of being large for female raptors suffering under limitation of food resource.

4.4. Causes and consequences of hybridisation

Avian speciation is allopatric and no cases of speciation without spatial isolation are known (Grant & Grant 1997a, Helbig *et al.* 2002). Hence, hybridisation occurs as a secondary contact between differentiated species that are not yet reproductively isolated. Several concentrations of hybrid zones (suture zones) of plants and animals derived from different glacial refugia are known (Taberlet *et al.* 1998, Hewitt 2000) and a zone joining several western and eastern populations has been determined recently in Eastern Europe (Jaarola & Searle 2002). This is also the sympatry area of the Lesser Spotted Eagle, a member of the European (western) faunistic complex, and the Greater Spotted Eagle, distributed typically to the Siberian (eastern) complex (Kumari 1954). The secondary contact between spotted eagles has been probably established after Pleistocene glaciations, but earlier hybridisation cannot be excluded too.

Hybridising species must be close in respect to their ecology and ethology. Despite probable eco-morphological differentiation (Kirmse 1998), spotted eagles are still close species as revealed also by the current thesis. Although their habitats generally differ, there is a wide overlap (Figure 3), and parental care of the Greater Spotted Eagle (**III**) does not differ from that of the Lesser Spotted Eagle (Meyburg 1974 and references therein). Also the courtship display, the most important ecological isolation factor (Randler 2000) is very similar in spotted eagles (personal observations) and cannot therefore avoid the formation of mixed-species pairs.

Spotted eagles represent a typical situation of interbreeding, where one species is rare and another is much more abundant (Hubbs' principle; Hubbs 1955, Short 1969, Grant & Grant 1997b, Wirtz 1999, Randler 2002). Rarity (low breeding density) is one of the main reasons of hybridisation since the probability to find a conspecific mate is low. The main suggested factors for the decline of the Greater Spotted Eagle population are the loss of habitats, massive poisoning (in Russia; Belik 1999) and the shooting on migration or wintering (Meyburg *et al.* 2001). The opinion that the mixed pairs are likely to occupy former territories of the Greater Spotted Eagle is supported by the lack of obvious differences between the nest sites of pure and mixed pairs (Figure 3). Hence the Greater Spotted Eagle habitats seem to be not saturated in Estonia, which deserves urgent study. Obviously, the nest sites of interbreeders should be protected as those inhabited by Greater Spotted Eagle pairs to enable the population to recover in the future.

Why do the abundant Lesser Spotted Eagles hybridise? A typical raptor population contains non-breeding birds waiting for a vacant territory and possibility to breed (Newton 1979). Intraspecific competition should be more intense among the abundant Lesser Spotted Eagles, and the surplus birds may decide to mate with single Greater Spotted Eagles instead of not breeding at all. Indeed, other authors have hypothesized that interbreeders of the more abundant species are subordinate individuals — inexperienced (young) or late breeders (Grant & Grant 1997b, Veen *et al.* 2001). However, the results of current thesis suggest that the interbreeding of spotted eagles may also be adaptive and related to sexual selection. The male is Lesser and the female Greater Spotted Eagle in eight out of nine known interbreeding pairs in Estonia, which makes the size difference maximal within pair. Since female's size was positively related to productivity (**VII**), large females could be preferred as mates. Hence, in the presence of larger Greater Spotted Eagle females, Lesser Spotted Eagle males are probably influenced by supernormal stimulus (Grant & Grant 1997b) and may not accept the mates of their own species. A common view to females as selective partners (Wirtz 1999) suggests that smaller males should be also preferred in spotted eagles. Future studies should answer whether the small size of males is advantageous (like in some other raptors; see references in **VII**), or the scarcity of conspecific mates is the only reason for hybridisation for the Greater Spotted Eagle.

What are the consequences of hybridisation? Usually, the proportion of interbreeding pairs or hybrids in bird population is not high (Grant & Grant 1997b, Randler 2000, Kabus 2002). The fitness of hybrids is often lower (Arnold & Hodges 1995; Veen *et al.* 2001, Price & Bouvier 2002) and their fertility is nearly always restricted to one sex (according to Haldane's rule; Haldane 1922, Orr 1997, Turelli 1998, Helbig *et al.* 2001, Veen *et al.* 2001; Price & Bouvier 2002). However, hybridising individuals seem to form a large proportion of Greater Spotted Eagles in Europe. Interbreeding wastes reproductive effort and has negative impact to species maintenance even in the case of sterility of hybrids (Rhymer & Simberloff 1996). The situation worsens when the hybrids are fertile. Especially introgression — a gene flow between species — puts a serious threat to rare species, and may even lead to extinction (Haig & Avise 1996, Rhymer & Simberloff 1996, Soltis & Giltzendanner 1999). Breeding spotted eagle hybrids are difficult to identify because of similarity and variability of morphological characters, and until now only a few suspected cases are known (**V**). Studies of assortative mating, hybrid fertility, the proportion of first- and later-generation hybrid individuals in breeding populations and the extent of introgression should belong to the most important issues in future conservation research of spotted eagles.

SUMMARY

The Greater Spotted Eagle *Aquila clanga* Pallas and the Lesser Spotted Eagle *A. pomarina* Brehm are threatened but insufficiently studied species, although the research of factors limiting their population has high priority for conservation. In the current thesis, I used molecular, morphological and ecological methods to explore relationships between the two spotted eagle species, their population history and ecology, particularly habitat relationships.

The analysis of mitochondrial DNA showed the divergence of two lineages. Complex analyses of morphological characters and habitats confirmed the existence of two distinct groups. The occurrence of hybrids caused an overlap in morphological characters, but gene flow at the taxonomic level is probably restricted. Hence, despite the similar behaviour and regular hybridisation, spotted eagles should be treated as separate species. According to the super-species concept, the semispecies status seems to be most appropriate.

The spotted eagle species differed by their genetic diversity and population structure. The Baltic population of the Lesser Spotted Eagle is genetically homogeneous and is probably originated from a single glacial refugium on the Balkan Peninsula. The population expanded rapidly in numbers and range 8000±1500 years ago. Genetic variation in the Estonian Greater Spotted Eagle population is about three times higher than in the Lesser Spotted Eagle, probably due to different glacial history (higher numbers, larger range, several refugia) or recent factors (intensive gene flow between populations, crash in numbers).

Relationships between eagles and their habitats were studied mostly in the Lesser Spotted Eagle. Landscape use and preferences differed geographically. In vole-poor years the species did not switch to alternative prey, the productivity was lower, and reproductive cost was further reduced by rearing smaller-sized (male) nestlings. Breeding success was positively related to landscape heterogeneity and negatively to the number of other spotted eagles breeding nearby. In years with scarce food supply, larger females were more productive. Only in the presence of competitors, the large size became disadvantageous.

At least half of the Greater Spotted Eagle territories in Estonia are probably occupied by mixed-species pairs. The possible reasons for this regular hybridisation include rarity of the Greater Spotted Eagle and competition for mates and territories in the Lesser Spotted Eagle. Since larger females are more successful breeders, the Lesser Spotted Eagle males could prefer to mate with the Greater Spotted Eagle females. Though only future studies can ascertain the number of hybrids in the breeding populations, the hybridisation — due to its high frequency — has obviously negative impact to the Greater Spotted Eagle even in the case of limited fertility of hybrids and the lack of introgression.

SUMMARY IN ESTONIAN

Suur- ja väike-konnakotka taksonoomia, fülogeograafia ja ökoloogia

Suur-konnakotkas *Aquila clanga* Pallas ja väike-konnakotkas *A. pomarina* Brehm on ohustatud, kuid ebapiisavalt uuritud liigid. Seega kuulub nende liikide arvukust ja levikut limiteerivate faktorite selgitamine looduskaitseliste prioriteetide hulka. Käesolevas töös vaadati üle konnakotkaste taksonoomiline kuuluvus, analüüsiti populatsioonide seisundit ja ajalugu ning uuriti nende lindude bioloogiat levilate kattuvusalal, kasutades geneetilisi, morfoloogilisi ja ökoloogilisi meetodeid.

Konnakotkaste mitokondriaalse DNA uuringud andsid tunnistust kahest selgesti eristuvast liinist. Morfoloogiliste tunnuste kompleksne analüüs ning elupaiga-uuring kinnitasid diferentseerunud gruppide olemasolu. Välistunnuste kattuvus tulenes hübriidide esinemisest, kuid geneetilise materjali vahetus taksonite vahel on ilmselt piiratud. Seetõttu võib konnakotkaid, hoolimata sarnasest käitumisest ja regulaarsest ristumisest, pidada eri liikideks. Ülemliigi (*superspecies*) kontseptsiooni alusel peaks konnakotkaid määratlema poolliikidena (*semispecies*).

Geneetiline mitmekesisus ja populatsioonide struktuur on liikidel erinev. Väike-konnakotka Balti populatsioon ei ole geneetiliselt diferentseerunud ning pärineb ühisest jääaegsest refuugiumist Balkani poolsaarel. 8000±1500 aastat tagasi hakkas liigi arvukus kiiresti kasvama ning levila laienes põhja poole. Suur-konnakotka Eesti populatsiooni geneetiline mitmekesisus on ligi kolm korda suurem kui väike-konnakotkal, see võib tuleneda populatsiooni erinevast ajaloost viimasel jääajal (kõrgem arvukus, suurem levila, erinevad refuugiumid) või tänapäevastest faktoritest (aktiivne geenisiire, arvukuse hiljutine langus).

Seoseid elupaigaga uuriti põhjalikumalt väike-konnakotkal. Areaali eri piirkondades olid elupaigakasutus ja -eelistused maastikuparameetrite suhtes erinevad. Eestis ei lülitunud väike-konnakotkas toiduvaestel aastatel alternatiivsele saagile, sigimisedukus oli siis madalam ning sigimiskulutust vähendati väiksemakasvulisi isaseid järglasi üles kasvatades. Produktiivsus sõltus ka territooriumi maastikulisest mitmekesisusest ja lähedal pesitsevate konnakotkaste hulgast. Toiduvaestel aastatel määras sigimisedukust ka emaslinnu suurus, kusjuures konkurentsi kasvades suuruse positiivne efekt kadus.

Ilmselt on enam kui pooltele Eesti suur-konnakotka pesitsusterritooriumidest moodustunud segapaarid väike-konnakotkastega. Hübridiseerumise tõenäolisteks põhjusteks on ühelt poolt suur-konnakotka haruldus ja teisalt konkurents territooriumide ning paariliste pärast väike-konnakotkaste seas. Kuna suured emaslinnud on edukamad pesitsejad, võivad väike-konnakotka isaslinnud partnerivalikul isegi eelistada suur-konnakotkaid. Edasised uuringud peavad selgitama hübriidide osa pesitsevates populatsioonides, kuid kõrge sageduse tõttu on ristumine suur-konnakotkale kahjulik isegi hübriidide piiratud viljakuse ning tagasiristumise puudumise korral.

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ACKNOWLEDGEMENTS

First of all, I want to express my respect and gratitude to the eagles who still have survived in the Man's world. While following them, I have reached many wonderful refugia of Nature. I also appreciate the people working to save the eagles and other values surrounding us. Not only direct conservation, but also the scientific research of spotted eagles has been based on a huge amount of fieldwork carried out by the members of the Eagle Club. Among numerous people who have climbed to the trees and held a hand on the eagle's wing, I thank especially Asko Lõhmus, a friend, a co-worker and a supervisor at the same time for many years, and Rimgaudas Treinys, a good colleague who widened my view to spotted eagles in several respects.

I am very thankful to my supervisor professor Jüri Kärner for his support throughout my *studium*. Without him, I probably would have not started the spotted eagle studies at the Tartu University. I am also grateful to professor Richard Villems who always kept the lab-door open for me to reach the secrets hidden in the genes of eagles. People at the department of evolutionary biology created a nice atmosphere for work during the dark half of the year and provided all kind of help I needed in the lab. I also thank all other friendly people at the Institute of Molecular and Cell Biology, and the Institute of Zoology and Hydrobiology.

I thank my parents and sisters for their support, as well as all my friends.

And finally I thank Elo, for everything.