-Master thesis-

May the availability of an unlimiting offer of artificial breeding sites induce detrimental density-dependent effects on the reproductive behaviour of an endangered, recovering Hoopoe *(Upupa e. epops)* population?

Supervision:

Prof. Raphaël Arlettaz, Conservation Biology, Zoological Institute University of Bern

- by Fabio Leippert, 2005 -

UNIVERSITÄT BERN

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1 Abstract

1. The Hoopoe *(Upupa epops)* was once widespread and common over all central Europe. A severe decline has taken place recently. As a consequence, the Hoopoe is today considered as one of the most endangered bird species in western and central Europe. In Switzerland about 70-80% of the broods of this red listed bird ("highly endangered") are now found in the Upper Rhône valley. The valais population is recovering thanks to an extensive nestbox concept.

2. In Valais, along with the number of broods, local density has steadily increased during the last five years, leading to one of the highest densities in Europe. 2004 was again a new record year with an overall increase of broods of about 79% compared to 2003.

3. As a consequence of high density there may occur alterations of social behaviour such as increase of conspecific brood parasitism (CBP, females that lay eggs in foreign nests) and extrapair paternity (EPP, proportion of fertilizations resulting from copulations outside the traditional social bonds). Both may cause detrimental effects on population dynamics which may be simply avoided by optimising nestbox supply.

4. In a microsatellite maternity analysis of 273 offspring stemming from 44 broods we found no case of CBP. Level of EPP was extremely low, with only one among 273 chicks (0.4%) and one of 44 broods (2.5%) being EPP.

5. Consequently we do not have to worry about detrimental alterations of social behaviour in the Valais hoopoes, at least at the present density. Further monitoring and paternity assessments may be carried out in the future to regularly check for nestbox supply suitability.

1 Zusammenfassung

1. Der Wiedehopf *(upupa epops)* war in ganz Zentraleuropa weitverbreitet. Nach einer markanten Bestandesabnahme gehört er heute zu den meistgefährdeten Vogelarten in West- und Zentraleuropa. In der Schweiz finden sich 70-80% der Bruten dieser seltenen Art (Rote Liste: „stark gefährdet") im Unterwallis. Dank einem Nistkastenprogramm hat sich diese Population gut erholt und wächst weiter.
2. Durch das Populationswachstum nahmen die lokalen Dichten in den letzten fünf Jahren im Wallis ebenfalls konstant zu und gehören heute zu den höchsten in ganz Europa. 2004 war ein neues Rekordjahr mit einer Zunahme der Bruten um 79% im Vergleich zu 2004.

3. Die hohen Brutdichten könnten einen Anstieg an CBP (konspezifischer Brutparasitismus = Weibchen, welche ihre Eier parasitisch in Nester ihrer Artgenossen legen) und EPP (extrapair paternity = prozentualer Anteil der Jungen, welche von einem anderen Vater, als dem traditionell vom Sozialsystem anerkannten, abstammen) zur Folge haben und dadurch das Sozialverhalten verändern, was negative Auswirkungen auf die Populationsdynamik hätte. Die Frage ist nun, ob man die Anzahl an Nistkästen optimieren muss, um solche schädlichen Auswirkungen zu verhindern.

4. In einer Elternschaftsanalyse von 273 Jungen aus 44 Bruten mittels Mikrosatelliten, fanden wir keinen Fall von CBP. Mit nur Einem von 273 Jungen (0.4%) und Einer von 44 Bruten (2.5%) war der EPP Anteil ebenfalls sehr tief.

5. Aufgrund dieser Ergebnisse müssen wir uns für die Walliser Population momentan keine Sorgen über eventuelle Veränderungen des Sozialverhaltens machen, wenigstens bei der heutigen Dichte. Weiteres Monitoring und Vaterschaftsanalysen könnten in Zukunft durchgeführt werden um regelmässig zu überprüfen ob die Nistkastenzahl optimal ist.

2 Introduction

2.1 Status

2.1.1 Global status of the Hoopoe

The hoopoe *(Upupa epops epops)* was once widespread and common over all central Europe, with regular breeding as far north as Denmark and Sweden. Probably due to climatic changes to colder and wetter weather there has been a severe contraction of northern, central and western Europe populations from the end of the 19th century until the middle of the 20th century when some temporary recovery took place, probably thanks to a climate improvement. Since 1950-55, a new retraction took place throughout Europe, but mainly in the industrialized countries of central Europe. Causes are thought to be habitat change through intensification of agriculture such as removal of old trees (loss of nest sites) and large scale application of insecticides (reducing number of prey) (Bauer & Berthold 1997). As a consequence, the Hoopoe is today considered as one of the most endangered bird species in western and central Europe (Hustings 1997).

2.1.2 Status in Switzerland

In the 1950's the Hoopoe was relatively widely distributed. Since then the lowlands of the "Mittelland" and the Northwest have been continuously abandoned. Nowadays about 70-80% of the Swiss breeding events (63 broods in 2003, Sierro et al. 2003) occur in the Upper Rhône valley (Valais). Other remaining breeding pairs are commonly reported in Ticino and Graubünden. In total there was a decline of 60 percent in the number of occupied atlas squares between 1972-76 and 1993-1996 (Arlettaz & Fournier 1998). As a consequence the Hoopoe is red-listed as an endangered species ("highly endangered") in Switzerland (Keller et al. 2001).

Apparently the Valais population is one of the few ones which has increased steadily in the recent past in central Europe. Yet, it suffers from variations in annual breeding success (Appendix 1), which are most likely caused by weather fluctuations affecting the availability of molecrickets (Schaad 2002).

2.2 Extra-pair paternity and density dependence.

According to Griffith et al. (2002), the frequency of extrapair paternity (EPP) is defined as the proportion of fertilizations resulting from copulations outside the social bonds recognized by the traditional mating system classification. Hence, in socially monogamous

species such as the Hoopoe, extra-pair young are those sired by males other than the social father. Recent molecular studies revealing new insights into avian mating systems showed that sexually monogamous species are very rare, with about 90% of all species showing extrapair paternity. Even among socially monogamous species, on average, over 11% of the offspring and 18.7% of the broods contain extrapair offspring (Griffith, Owens & Thuman 2002).

Variation in breeding density is a traditional explanation for intraspecific variation in the rate of EPP (Westneat & Sherman 1997; Møller & Ninni 1998). In a review, Westneat & Sherman (1997) report that there is a general trend for high density populations to have a higher rate of EPP than con-specific populations at lower density because, as density increases, so do also social interactions, both cooperative and competitive, such as extrapair copulations. EPP is likely to increase with the frequency of extrapair copulations. In addition, the probability that extrapair males are neighbours is also greater. As appealing and logical as this relationship between frequency of EPP and density may seem, there are only few studies which clearly established this relationship (summarized in Griffith et al. 2002).

In the Hoopoe population in Valais there are big density differences due to the spatial clumping of the broods, so that the premises to detect correlations between EPP (if it occurs) and density are given. We aimed to compare the degree of EPP with the local density, measured as the mean distance to all other occupied nests during the whole breeding season.

Griffith et al. (2002) also investigated the role of phylogenetic relationships in the degree of EPP. Major evolutionary lineages explain over 50% of the interspecific EPP-level variations, mainly between passerines and non-passerines. As a non-passerine, the Hoopoe is expected to have a low degree of EPP. Non-passerines have an average EPP frequency of 3% ± 5% (SD) of the offspring, compared to 18% ± 17% (SD) in passerines. The highest level of EPP observed in non-passerines so far was 18% of the offspring (Westneat & Sherman 1997). Interestingly the only study investigating genetic relationships in the Hoopoe (Martín-Vivaldi et al. 2002) found a pretty high EPP frequency for a non-passerine: 10% of broods (n = 2/20) and 7.7% of offspring (n = 5/65).

2.3 Risks inherent to extra-pair paternity

So far there are no conservation risks known to be linked directly with level of extrapair paternity per se. Perhaps if males could recognize "wrong" kin and abandon it, or refuse to

feed extrapair chick, this could lead to a decrease in breeding success. Yet, if there is a strong positive correlation between frequency of EPP and density, this may indicate an alteration of a population social behaviour through artificially increased density, with perhaps unknown population dynamics consequences in the long term.

2.4 Conspecific brood parasitism and density

Concerning conspecific brood parasitism (CBP) the situation is different. Recent studies on cavity nesting Barrow's goldeneyes and Wood ducks have shown that populations living in artificial nestboxes supporting very high population densities may suffer detrimental effects on population dynamics through intraspecific social interactions, particularly due to an increase of CBP which reduces demographic output (Eadie, Sherman & Semel 1998). There are several reasons why females may lay parasitically. First, females gain reproductive benefits without incurring the physiological costs or risks associated with incubation and parental care. Second, females are unable to locate a suitable nest site of their own or are unable to lay an entire clutch on their own and might still achieve some reproduction through laying parasitically. Third, parasitism among conspecifics might be facilitated if parasites and hosts are closely related. Fourth, parasitism might represent nest-site competition between females as each of them attempts to lay in the same high quality cavity or in a cavity defended by a dominant sexy male (Eadie, Sherman & Semel 1998). The first explanation is unlikely, as CBP is not known as an alternative strategy used by Hoopoe females under natural conditions, the second hypothesis is not plausible as nestsites are not limiting in our population. Third, individuals in our population do not seem to be too closely related as we observe every year a relatively high proportion of unringed immigrants and neighbours are rarely relatives. We were thus interested in the fourth possibility and searced for a correlation between frequency of CBP and density if there is at all CBP occurring.

Haramis & Thompson (1985) showed in a 7 year study of box nesting wood ducks that the frequency of CBP increased with duck density. By year 5 of their study, reproductive success had crashed, with only 22% of all eggs hatching (compared to 79% at the start of the study) due to CBP. In the following two years density was artificially reduced and hatching success increased again to 60%. These results show a clear connection between frequency of CBP and population density. It also shows the possibly detrimental effects of CBP at high frequencies for local populations. These damaging effects are due to dramatically decreased hatchling success because of inefficient incubation of supernormal

clutches, broken eggs and subsequent fungal infections, disturbance of laying females by parasitic females and, eventually, nest abandonment (Semel, Sherman & Byers 1988, 1990; Semel & Sherman 1995). An negative relationship has been documented for wood ducks and barrow's goldeneyes (Eadie, Sherman & Semel 1998; Semel, Sherman & Byers 1988; Belrose & Holm 1990; Semel, Sherman & Byers 1990; Semel & Sherman 1995). Thus, an increase in the number of laid eggs actually leads to a decline in both hatching success and individual reproductive success so that population growth rates decrease (Eadie, Sherman & Semel 1998).

The relationships between population density, CBP and reproductive success suggest that social behaviour can play an important role in demography. There are three studies that modelled these interactions (May, Nee & Watts 1991; Eadie & Fryxell 1992; Nee & May 1993). Although these models made different assumptions and focused on different aspects of parasitic behaviour, remarkably similar conclusions were obtained. CBP can lead to stable populations, populations that oscillate cyclically, or populations that fluctuate chaotically, even leading to extinction of whole populations (Eadie & Fryxell 1992; Nee & May 1993). In short, CBP can significantly impact population dynamics. Local extinction becomes possible when the frequency of parasitism is very high and the "inertia" (females adapt their reproductive strategy too slow to regain a positive population growth rate before the population goes extinct) of the population may prevent a return to the equilibrium, thus leading to an irresistible population crash (vortex theory, outer arrow, Appendix 3). It is important to notice that this simulated population crash occurred without any external, stochastic factors such as predation, bad weather, human impact, which could in reality further increase extinction risk (Eadie, Sherman & Semel 1998). Thus density does influence relative reproductive success of a population and, in turn, parasitism can regulate density of local populations.

Based on the model by Eadie and Fryxell (1992), Eadie et al. (1998) ran simulations with varying frequency of parasitism and population size. They found that the risk of extinction exponentially increases, starting at about 60% CBP and increasing rapidly to a local population extinction risk of 55% at a CBP frequency of 80% (Apppendix 4). Astonishingly, initial population size (range from 10 to 127 individuals) did only have a minimal effect (Eadie, Sherman & Semel 1998).

2.5 Could an artificial high density of breeding sites potentially lead to increase in EPP and CBP in the Valais Hoopoe population ?

In Valais, a nestbox program was launched in 1998 with the aim to help this secondary cavity nester to breed again on the plain of the Rhône. The reason for the decline of that population was a lack of breeding sites on the intensively cultivated plain, which forced the parents to fly long distances between nest sites on the slope and best feeding grounds on the plain. This was energetically costly and caused a low breeding success (Fournier & Arlettaz 2001). Between 1998 and 2003, more than 700 nestboxes were installed in agricultural buildings on the plain as a remedy (Arlettaz, Fournier & Zbinden 1998; Arlettaz et al. 2000; Schaad et al. 2001; Sierro et al. 2002; Sierro et al. 2003).

As a result, the population underwent a dramatic augmentation. Between 1998 and 2003, it increased from about 20 to 63 broods per year, with a new record of 113 broods in our study year 2004. Production of juveniles increased by 62% from 2003 to 2004 (531 fledglings in 2004, 331 in 2003, Appendix 1), which may induce a further increase in the next years. This increase had progressively lead to higher and higher local densities, with a clumped distribution matching that of the main prey molecrickets which represented over 93% of the biomass supplied to chicks in 2001 (Schaad 2002). In densely populated zones, there have been several cases of polygamous males observed in recent years, which is unusual in this normally monogamous bird and let envision social problems emerging. Also there has been evidence for clutches destroyed by "enemy" females, for infanticide (chicks found murdered on the ground under the nests [see also (Martín-Vivaldi et al. 2002)] and even for cases of adult females found dead, probably killed by competitors (Arlettaz et al. 2000; Schaad et al. 2001; Sierro et al. 2002; Sierro et al. 2003). All these observations may be indications of conflicts between females competing for the same breeding cavity, or between different males competing for the same female, possibly as an indirect consequence of the artificially high breeding density caused by the unlimiting nestbox offer.

In Europe, Hoopoe densities are by far the highest in the Iberian Peninsula, averaging several breeding pairs per km^2. All other European populations lower density, ranging from 0.25 bp/km^2 to 0.004 bp/km^2 (Hustings 1997) (Appendix 2).

In this study we try to evaluate the amount of EPP and CBP occurring in the Valais Hoopoe population to test the hypothesis that a high breeding density may cause alterations of hoopoe social interactions such as increased polygamy, EPP and CBP. Consequences could be reduced individual reproductive success, fortified population instability and, finally,

a decline of the local population through the implementation of a nestbox program which was initially designed to help an endangered population. Finally, we should be able to answer the question whether we have to optimise the number of supplied nestboxes in specific areas to avoid or correct for this possible detrimental effects, if any.
Important is to stress that, even though we are addressing fundamental questions for which an experimental approach would be best (Griffith, Owens & Thuman 2002), we are working with an endangered population of a rare bird which precludes to rely on experimental manipulation.

3 Methods

3.1 Study area

The study was conducted on the plain of the Rhône in Central Valais (46.2°N, 7.4°E, 482 m elevation). This study area stretches along the valley axis from 452 m (Vernayaz) to 520 m (Sierre) elevation. In total, there were 708 nestboxes available in 2004 on an area of 45km^2. Nest boxes were installed from 1998 to 2003 from west to east (Appendix 5).

3.2 Sampling design

Fieldwork was carried out between 26.4. and 15.8.2004. Nestboxes were controlled every two weeks (n = 7 controls in total) with a non disturbing method (mirror and torch), between 26.4. and 20.7.2004. Newly detected broods were inspected as often as possible to time hatching date and collect unhatched eggs and dead chicks. From the 15th day after hatching onwards we ringed the chicks and collected a feather sample from each chick (second tail feather from the right, which was stored in a paper envelope).
Provisioning adults were mist–netted at the nest sites from the 8th day after hatching of offspring onwards, i.e. when chicks start thermoregulation and females start to help with feeding. On average, it took about 3h to catch an adult. Adults feeding at the nestbox were considered as social parents. Blood samples were collected from captured adults using an haematocrit collector (20 µl), after brachial vein puncture. Adults could be sexed morphologically. Blood samples were stored in a deep freezer.

3.3 Estimation of breeding density

Local density was calculated as the mean neighbour distance (MND), which is the distance of a certain breeding site to all other active breeding sites (n = 91) in 2004. MND distances of all broods were not normally distributed (Table 1).

3.4 Paternity analysis

Blood samples (40 µl) were stored in Queen's Lysis Buffer (10x: 0.1M Tris ph 8.0, 0.1M NaCl, 0.1 M EDTA, 10% N-lauroylsarcosine (SLS) pH 7.5; (Seutin, Bradley & Boag 1990), and frozen together with feather samples. Feather tissue and blood samples were digested with 20µl-40µl Proteinase K and 500µl of TNES-UREA buffer (10mM Trizmabaze, 0.3M NaCl, 1% SDS, 10mM EDTA, 4M UREA, H2O, pH8) and incubated / shaked at 55°C between 3 to 6 hours. Genomic DNA was extracted using 400µl of the digestion with 20µl of MagneSil® Solution (Promega, Wallisellen, Switzerland), being shaked for 5 minutes and then placed on the MagnaBot® (Promega, Wallisellen, Switzerland). After removal of the digestion the MagneSil® pellet was washed twice with 99% and 70% alcohol, respectively and finally dissolved in water. Primers were designed with microsatellite enriched-libraries for hoopoe ordered at GIS (Genetic Identification Services, Chatsworth, US). DNA fragments containing double and triple repetitions were transferred in One Shot® Top10 cells (Invitrogen AG, Basel, Switzerland). Positive colonies were kept and the "ingested" fragment were amplified using primers M13f and M13r (Invitrogen). PCR products were checked on an agarose gel, then they were purified using QIAquick PCR Purification Kit (Qiagen) and sequenced. Primers were designed for sequences containing microsatellites using Oligo V 4.0 (Molecular Biology Insights, Inc., Cascade, US). Microsatellites amplifications were checked on agarose gel and PCR conditions were optimised.
In total, 31 microsatellite loci were identified, 17 showed good amplification in PCR and where ordered in fluorescent primers. Of these, 7 loci where chosen which seemed to be sufficiently polymorphic and could be discriminated either in dye colour or different size range of the alleles, and thus be placed in one single multiplex set (Table 1, Appendix 7). Polymerase Chain Reaction (PCR) was performed in a reaction volume of 10 µl on GeneAmp® PCR System 9700 (Applied Biosystems, Rotkreuz, Switzerland) using Qiagen (Basel, Switzerland) Multiplex DNA kit according to the manufacturers protocol with denaturation at 95°C for 15 min followed by 30 cycles of denaturation at 94°C for 30 s annealing at 57°C for 90 s and extension at 72°C for 90 s. There was always a negative control performed. Total volume was 10µl whereof 4µl template DNA. Forward primers were labelled with 6-FAM™ ,VIC™, HEX™ and NED™ fluorescent dyes (Applied Biosystems, Rotkreuz, Switzerland). PCR products were diluted 1:3, whereof 1µl was added to 19.75 µl of Hi-Di Formamide and 0.25 µl of LIZ 500 size standard (ABI, Rotkreuz, Switzerland). PCR fragments were visualized by capillary electrophoresis on an ABI 3100 Genetic Analyser (ABI, Rotkreuz, Switzerland) and scored on Genemapper® v 3.0 (ABI,

Rotkreuz, Switzerland). To guarantee comparability of different runs on the Sequencer, there were always 2 Individuals with known genotype included in each new run. The genotype of these Individuals had always been scored identically.

Allele frequency was calculated on CERVUS Version 2.0 (Marshall et al. 1998). Using a χ^2 test, six microsatellite were not significantly different from Hardy-Weinberg equilibrium with four loci in almost perfect Hardy-Weinberg equilibrium (computed using an iterative algorithm based on the difference between observed and expected frequency of homozygotes). The seventh loci (UpuD3) showed a severe excess of homozygotes with an estimated frequency of null alleles of 0.56 and was therefore removed from analysis (Table 2). Two loci (Upu935, a2) showed minor deviations from HWE, with estimated frequency of null alleles of 0.074 and 0.015 respectively (Table 2). These loci where used for parentage analysis, taking into consideration that they may contain null alleles which arise from the nonamplification of one allele due to a mutation or polymorphism in the flanking sequence and thus looking like a homozygote, creating homozygote excess and deviation from HWE and causing mismatches with the genotype of their putative parents. With both putative parents known null alleles are very easy to detect because they result in incompatibilities between the parents and offspring that invariably involve homozygous genotypes (Jones & Ardren 2003). Sex linkage as alternative explanation for deviation from HWE was tested by comparing allele frequencies of each sex separately. As there was no such difference this explanation could be ruled out. Combined exclusion probability of these six loci was 0.98709 for the first parent, and 0.998908 for the second parent when the first is known or already positively assigned. Exclusionary probabilities represent the average probability of excluding a single randomly-chosen unrelated individual from parentage at one or more loci (Chakraborty, Shaw & Schull 1974; Marshall et al. 1998). Exclusion probabilities (P_{excl}) were calculated on CERVUS with the genotypes of all scored adult birds (Tables 2 and 3).

Genotypes of all nestlings were checked for allelic mismatches with their presumed genetic mothers (= social mothers) by exclusion, performed on Excel. As mentioned in the review by Jones & Arden (2003) of parentage analysis methods, we tolerated one mismatch for an exclusion to be considered valid, making our method more robust for scoring errors, mutations or as in our study most of the time being the case, null alleles.

We compared the genotype of the offspring to the genotype of their social mother assuming correct paternity if the maternal allele was matching at least at 5 different loci ensuring an exclusion probability of at smallest 0.96, which seems to be far enough considering that the

mothers caught feeding at the nestbox are very likely to be also the real genetic mother. For all samples showing mismatches, PCR and genotype scoring were replicated.

As a second step the putative males which were caught feeding at the nestbox were assigned using the same technique. If there wasn't more than one mismatch at locus Upu935 or UpuA2 it was considered as genetic father with an exclusion probability of 0.994 or higher (Table 3.).

As control of the male-offspring relationships identified by this exclusion method I did also a CERVUS 2.0 parentage analysis. CERVUS uses a likelihood-based approach to infer parentage. The natural logarithm of the likelihood ratios is termed the LOD (logarithm of the odds) score (Marshall et al. 1998). A LOD score of zero implies that the putative father is as likely to be the father as a randomly chosen male. A positive LOD score implies that the male under scope is more likely to be the father than a randomly chosen male (Marshall et al. 1998; Hatchwell et al. 2002). I used CERVUS just to see if the observed father-offspring relationship confirmed by exclusion was also confirmed if all genotyped males (n = 38) were tested as possible fathers of each genotyped nestling (n = 273). If the social father was one of the two with the highest LOD score I assumed it to be also the true genetic father.

Note that six broods analysed in our sample contained adults which have been used twice. This is due to the fact that we analysed them already for interesting cases with suspected polygamy and other special behavioural observations.

According to Griffith, Owens & Thuman (2002), for a comparative, interspecific study about EPP there should be at least 150 offspring analysed to get an acceptable estimate of EPP, assuming that EPP can be found 99% of the times. These authors thus suggest to analyse about 200 offspring, which gives a possible error range of 10% between the upper and the lower 95% confidence interval around an estimate of EPP (Appendix 8). With our sample of 273 offspring we are on the safe side to detect EPP if it exists.

4 Results

4.1 Demographic aspects

2004 was a new record year for the recovering Hoopoe population in Valais, this in every demographic aspect, except for the reproductive success which decreased from 6.38 chicks/brood (2003) to 5.33 chicks/brood in 2004 (range between 1 - 9 chicks per brood). The number of broods increased by 79% (63 broods in 2003, 113 in 2004) and number of fledglings increased by 62% (331 juveniles in 2003, 531 in 2004, Sierro et al. 2004). Consequently density had again increased, giving ideal premises for detecting density dependent EPP and CBP.

For the first time since the nestbox program started there had been at least four breeding attempts on the less favourable slopes again, which may indicate that the population is getting saturated on the plain, at least in the very occupied western part of the study area (Appendix 5 and 6, zone D). 69% (n = 156) of all breeding adults (n = 226) have been caught. At least 33 birds performed a 2nd clutch (20 females, 13 males) and 3 (2 females, 1 male) even a 3rd clutch.

4.2 Density

In 2004, overall breeding density was around 2.45 broods/km^2 (113 broods on 46km^2). In a very attractive region in the east (St-Léonard) there were 15 broods on 1 km^2. On a very small scale, densities reached sometimes more than 3 territories/4 ha, which is about 75 bp/km^2 (Appendix 6).

Overall MND ranged between 10.81 and 20.47 km (mean = 13.83, median = 13.506, not normally distributed) (Fig. 1). In our sample, mean was 13.47, median 13.28. Density distribution of our sample was therefore reflecting densities of the whole population. Compared to other European regions breeding densities in the Valais population are only surpassed in some Mediterranean countries (Appendix 2).

4.3 Paternity analysis

In 2004 we genotyped 44 broods with both putative parents sampled (70 adults: 35 males, 35 females; 273 juveniles). In the sampled broods, the average number of hatchlings and fledglings were 6.06 and 5.33, respectively, which was slightly higher than in the target population in 2004 (5.03 and 4.67, respectively). This is an advantage for detecting EPP and CBP.

Among 43 broods, all 258 juveniles could be assigned to their respective social mother with no mismatch (n = 206) or one mismatch (n = 52) in one of the null allele containing loci, either in Upu935 or UpuA2. In one brood we had three of six chicks which showed null allelic mismatches in both null allele containing loci. But as the four non-null allele containing loci show no mismatches it is still highly possible that the putative mother is also the genetic one ($P_{excl.}$ = 0.92, if the two mismatches at the null allele loci are ignored, Table 3). In spite of a replication of the extraction and also PCR, four juveniles could only be typed at four loci and one only at three, probably due to bad sample quality. All four, respectively three loci, matched the genotypes of their putative mothers. Therefore no case of CBP was detected among these 43 broods.
One single brood (7 offspring) showed many mismatches with maternal genotype until we found out that the potential mother was in fact a visiting, possibly helping male. In all 44 broods no case of intraspecific brood parasitism was detected.

Fatherhood could be clearly assigned to 242 juveniles in 38 broods with no more than one mismatch at either Upu935 or UpuA2, which could be similarly explained by null alleles since there were otherwise at least 5 matching loci. Except three individuals which were only scored at four loci with a P_{excl} of 0.98 , all other males were assigned with a P_{excl} of at least 0.99 (Table 3). In one brood (G) there were three offspring showing two mismatches at Upu935 and UpuA2, whereas only the mismatches at UpuA2 can be explained by the presence of null alleles. The mismatches at Upu935 could be explained by wrong scoring at Upu935 but this hypothesis could not be evaluated because amplification of this locus in the replicate of the sample did not work. This favours the idea that the male may have been misscored at Upu935 due to a very small peak height, and therefore artefacts instead of the real allele peak may have been scored. All other four loci still matched and it would be very unlikely that these three offspring were extra pair chicks. But this possibility cannot be excluded for sure.
At a second brood (A), a possible polygamous neighbouring male (A'; 100m apart), could be genetically confirmed to be the actual genetic father of three of four offspring. We found one chick to be extra pair with mismatches at five of six scored loci.
In total we found between 0.4-1.6% (n = 1-4) of the offspring (n = 253) and 2.5-5% (n = 1-2) of the broods (n = 40) to contain extrapair offspring. It is notable that the one assured case of EPP occurred in a polygamous brood.

CERVUS paternity analysis with the same six loci confirmed our first analysis. For 220 juveniles the social father was also the one with the highest LOD score and assigned as the most probable genetic father. There were 28 juveniles where paternity was assigned to another father than the social one, but there the social father had the second highest LOD score and the ones assigned in first place had only a nonsignificant higher LOD score. Moreover the first-assigned fathers mostly stemmed from broods much further away, and the attribution to several different fathers within one brood questioned the validity of these assignments. In four cases the social father came in third place and once only in fourth place, but always with positive LOD scores (0.827-1.887), which means that the social father is still more likely to be the true genetic father than a randomly chosen male. These cases might also represent extrapair paternity offspring but it seems quite unlikely as all the males with higher LOD scores stemmed from different zones and relocation between zones and successive broods during one year is seldom among males. Also the fact that these more likely males never fitted perfectly to the offspring genotype and always showed some mismatches, indicates that they are not more likely to be the real genetic fathers. They might be relatives with similar genotypes.

For the extrapair chick from A the social father did not get a positive LOD score at all. The most probable father of this chick stemmed from a neighbouring nestbox (A"; 600m away) where the breeding male had been caught 20 days earlier.

In G where we could not rule out three cases of EPP, CERVUS assigned for all three doubtful cases the social father also as the genetic one with the highest LOD score, making it even more probable that these three chicks are not extrapair. This means that we have only one single case of EPP in our whole sample.

The 7 pulli where by mistake the "male mother" was caught, did not get a consistent picture for identifying a genetic father because it was probably absent from our adult male sample.

Among our 44 broods there were also six broods with incomplete sampling which were analysed all the same after special observations made in the field: four as possible cases of polygyny, one as adoption and one as a simultaneous breeding event. They will be discussed in the subsequent chapter. In one of these broods there were possibly two more cases of EPP occurring. As we did not capture the corresponding male in this nestbox, the possible occurrence of two further EPP cases here is based solely on likelihood calculation with CERVUS, and only with 5 typed loci, with two and one mismatch, respectively.

4.4 Relationship between brood failures and EPP

Of all 46 chicks that died older than 15 days of age in 2004, 27 (58%) were analysed in our sample. None of them was extra-pair offspring. Thus there is no indication of a relationship between brood failures and EPP.

4.5 Polygyny and reproductive success

There were four cases where males were caught while breeding within 14 days of initial capture, but at a second breeding site. These were cases of highly suspected polygamy. Three were situated in a most dense inhabited zone (MND = 10.8-11.8), the other one in a very densely populated area with 15 broods / km^2.

In a first brood (A) the male of a 80 m neighbouring site (A') could be genetically determined as being the true father of three among four offspring. Interestingly six of nine pulli at its official nest (A') starved even tough they were almost fully grown up (in total 7/13 survived). In another nestbox (B) 4 of 5 almost grown up chicks starved while their father was feeding at a new brood (B'), 400 m apart (in total 6/10 survived). Another male at two sites (C and C') 1 km apart fledged only 2 and 3 pulli, respectively (total 5 pulli). A last male at two sites located 400m apart (C and C') was pretty successful with 9 and 5 pulli raised (14 chicks raised).

4.6 Special observations

4.6.1 Simultaneous breeding in the same building

Despite the fact that most artificial sites consist of two nestboxes installed in the same building, only one case with two females breeding in the same building (E), 4 m apart (MND = 15.163) was recorded. For these two broods parentage could be determined genetically. Both females were the true genetic mothers of their respective offspring, as mentioned previously, whereas the only male caught, clearly had conceived only the offspring of one brood. CERVUS assigned a male from a site (E') 400 m apart as the most probable genetic father of the second brood. It showed no mismatches for all four offspring and a pretty high LOD score of 1.89 - 3.17, which makes it very plausible. The fifth chick couldn't be analysed in full as only three loci had been successfully genotyped, but all three matched the fatherly genotype. Note that the afterborn brood was abandoned, with all four chicks starving shortly before fledging age, probably as a consequence of conflict between these two males. It is

remarkable that the male caught had missing feathers around the neck, looking as if attacked by a competitor.

4.6.2 Adoption

Another striking observation was made at F. A first breeding pair raised five chicks there. As the brood was abandoned, three of them starved and the two remaining, weighing not more than 30 g, were about to die when a different female started a new clutch in this same nestbox, with the two remaining chicks starting to be fed by the male. We obtained blood samples of the first breeding pair and of the second different breeding female. Paternity analysis showed that at least six of the seven offspring of the new clutch were not from the same father as the five of the previous clutch. This was clearly a case of adoption, where a new couple accepts two unrelated offspring in their nestbox and feed them.

5 Discussion

5.1 Design

As mentioned in the methods we constrained our analysis prioritarily to those broods with both parents captured in order to do the simplest and most accurate kind of paternity analysis, an exclusion analysis. Our sampling collection design is considered as "best-case scenario" in Jones & Arden (2003). We have to consider though that biases may be linked to this approach. If only loyal partners were caught whereas unloyal birds (those engaging in EPP) could not be sampled, there would emerge a bias towards an EPP estimate which is too low. For the females and CBP estimate this is not a problem as the social female caught is not the parasitic one and therefore females on CBP containing broods are loyal ones. Also social males feeding their putative chicks which are in fact EPP offspring would be caught. According to Martin-Vivaldi et al. (2002) these are most likely subdominant males which are themselves not engaging in EPP in other broods and therefore loyal. Furthermore, the fact that we were able to capture the observed polygynous males with the same time investment indicates that our method is not biased towards loyal birds. Last it is noticeable that Martín-Vivaldi et al. (2002) chose the same approach, so EPP and CBP data are comparable with the Spanish study. If we had chosen a different approach, for example, if we also analysed broods where only a female or a male were caught, we would have needed more loci to obtain the same exclusion probabilities. Also, parentage was assigned by a purely probabilistic approach (e.g. CERVUS), which, as seen in our analysis, gives only likelihoods which does not yield to such a clear picture as we obtained. Another

possible bias is due to the fact that naive birds with probably different behaviour concerning EPP and CBP, are caught more easily. 57% (40 / 70) of our analysed birds were controls, whereof 70% (28 / 40; 40% of all 28 / 70) were ringed as nestlings which shows that naive immigrants seem to be well balanced against locally recruited breeders.
As our subsample contains large clutches, we think that our error range around our estimate is significantly smaller that the 10% suggested by Griffith, Owens & Thuman (2002), quoted in the method chapter. The same is true for estimated amount of CBP as we screened many large clutches where CBP would have been more likely.
Microsatellite paternity analysis is the method now widely used for assessing paternity in birds as well as in many other species. With our six loci we have exclusion probabilities for the second parent, as in many studies assessing paternity with microsatellites (Griffith et al. 1999; Webster, Chuang-Dobbs & Holmes 2001; Hatchwell et al. 2002; Hughes et al. 2003; Jones & Ardren 2003) and slightly lower exclusion probabilities for the first parent (female). It would have been possible to ameliorate our multiplex set, involving more loci with no null alleles and augmenting the exclusion probability. But as our P_{excl} is more than satisfactory for an exclusion analysis were the knowledge of the social bonds is also included, we resigned from further multiplex adjustments.

5.2 Conspecific brood parasitism

Of 185 bird species with documented occurrence of conspecific brood parasitism 49 are cavity nesting bird species (Eadie, Sherman & Semel 1998). Hoopoes are not among them. We found no evidence for CBP in our Hoopoe population and have therefore reason to doubt that this breeding strategy exists in the hoopoe at all. This shows that this recovering population is not threatened by the feared detrimental effects which may potentially be induced by an unlimiting offer of nestboxes (May, Nee & Watts 1991; Eadie & Fryxell 1992; Nee & May 1993; Eadie, Sherman & Semel 1998). The fact that average reproductive success remained stable over the years, despite a dramatic population increase, confirms this view. Also, there was no evidence for steadily increasing clutch size in our population which is a prerequisite for CBP to negatively affect population dynamics (Semel, Sherman & Byers 1988, 1990; Eadie, Sherman & Semel 1998). In CBP, classically hatchling success decreases through inefficient incubation of supernormal clutches, breaking of eggs and subsequent fungal infections, disturbance of laying females by parasitic ones, and, eventually, nest abandonment (Semel, Sherman & Byers 1988, 1990; Semel & Sherman 1995). Nothing like this occurred on a regular basis in our population in 2004. Therefore as

long as hatching success and other key demographic factors such as fledging success remain constantly high, while mean clutch size is not increasing, there is no reason to worry. If the population increases further and clutches start to become larger, then a new analysis of CBP should be envisioned.

An explanation for the absence of CBP may be that the females stay almost constantly on the eggs during incubation, therefore avoiding lack of attendance which may promote alien females to engage in CBP. Eadie, Sherman & Semel (1998) have explained the big discrepancy in CBP between two similar nesting duck species, the Barrow's Goldeneyes and the Wood Ducks, by a comparable behavioural pattern. According to them, Barrow's Goldeneyes have a smaller occurrence of CBP probably due to their aggressive defence of territory, preventing foreign females to enter their territory which keeps breeding densities under a certain threshold. Even though hoopoes do not defend a territorial homerange and density is therefore not regulated, they chase off intruders that come too close to their nest, rendering them probably less susceptible to CBP, like the Barrow's Goldeneyes.

5.3 Extrapair paternity

We found a very low degree of extrapair paternity concerning proportion of offspring (1/253, 0.4%, n = 253), as well as proportion of broods (1/40, 2,5%, n = 253). It is notable that the only case of EPP occurred in a polygamous brood. Compared to the range described for bird species (Westneat & Sherman 1997) this is remarkably low (Griffith, Owens & Thuman 2002). Non-passerines are known for lower EPP levels of 3% ± 5% (mean ± SD) than passerines (18% ± 17%) (Westneat & Sherman 1997). As Martin-Vivaldi et al. (2002) found relatively high EPP levels in their Spanish population (7.7% among 65 offspring, 10% among 20 broods) we thought that hoopoes might be an exception. At this stage the question is why is there such a discrepancy between the to studies? The prevailing explanations for intraspecific variance of EPP between populations is a mixture of differences in density, breeding synchrony (initiation of the broods synchronous within a population) and the extent of genetic variation; in short, differences in the possibility to indulge in alternative reproductive strategies (Westneat & Sherman 1997; Griffith, Owens & Thuman 2002).

We do not think that there is a difference in breeding synchrony between the two populations as Hoopoes are asynchronous breeders (Martín-Vivaldi et al. 1999b). Genetic diversity is most likely not the cause of the difference in EPP because a genetic exchange with other populations is taking place in the Valais population shown by the capture of many

unringed, immigrated birds every year. The same is possibly true for the Spanish population as it is not isolated from other populations at all. But if our Valais population was less diverse than the Spanish population this could probably explain some difference, as EPP level is assumed to be higher in genetically more diverse populations (Griffith, Owens & Thuman 2002). Remains the hypothesis of differences in breeding density. Yet, breeding density is even higher in the Valais population than in Grenada (Griffith, Owens & Thuman 2002), giving no explanation for the difference in EPP levels.
Alternatively it is possible that our population, contrary to Spain, may be far below local carrying capacity, as it is still steadily increasing, recovering from a bottleneck. EPP and maybe even CBP might simply be postponed until saturation takes place and breeding opportunities become scarce, making it then more profitable to switch to alternative reproductive strategies.
Another explanation for the discrepancy between our and the Spanish study lies in the different molecular methods employed. Whilst we used microsatellites, they used multilocus DNA fingerprinting. But considering their methods and conservative way of determining extrapair offspring, this is most likely not the reason for the discrepancy. Perhaps their small sample size (n = 65 offspring) may explain the discrepancy as 150 offspring is the minimal requirement (Griffith, Owens & Thuman 2002).

As level of EPP was low, we cannot infer related costs such as reduced reproductive success. As we analysed only late dead chicks we know nothing about the genetic identity of the chicks that died at a younger age, but considering that our detected degree of EPP was very low it is sensible to assume that there was also a quite low degree of EPP among the dead young chicks, except if all EPP young were the ones that hatched last and starved at early age. If so, however, we should have detected EPP in large clutches with many successful fledglings, as Hoopoes, which adopt a brood reduction strategy, let starve the youngest chicks if the breeding conditions are suboptimal (Martín-Vivaldi et al. 1999b). The excellent breeding conditions in 2004 enabled us to analyse nests with complete broods, with up to 8 fledglings (n = 2 broods) and even 9 fledglings (n = 1) per brood.

5.4 Polygamy

Interesting in this study was the discovery of several cases of polygyny, which has not been mentioned in the literature (Hirschfeld & Hirschfeld 1973; Glutz von Bolzheim 1980; Kern 1984; Baldi & Sorace 1996; Rehsteiner 1996; Bauer & Berthold 1997; Hustings 1997;

Martín-Vivaldi et al. 1999a; Martín-Vivaldi et al. 1999b; Keller et al. 2001; Oehlschlaeger 2001; Martín-Vivaldi et al. 2002). Polygyny could already be assessed through ringing in our population, with on average a minimum of 2.5% of the broods (n = 8/309) being raised by ascertained polygynous males (Arlettaz et al. 2000; Schaad et al. 2001; Sierro et al. 2002; Sierro et al. 2003; Sierro et al. 2004). Even though the proportion of confirmed polygynous broods in 2004 remained low (3.5%), there are indices that polygyny bears high costs for the males engaging into multiple broods, with only one male among four polygynous males exhibiting a breeding success equivalent to monogamous males (mean = 4, median = 3.25 vs. mean = 4.68 chicks / brood in all broods of 2004) . Provisioning investment is so demanding for Hoopoe males (Fournier & Arlettaz 2001; Schaad 2002) that they obviously fail to rear two broods at the same time in an efficient manner. Three broods were abandoned early because the male stopped feeding it, having switched to a new brood. In all cases young starved which would have otherwise most probably survived as they were shortly before fledging. In all cases the males came from closeby territories (< 1 km distance) indicating a density dependent effect. Polygyny and similar phenomenon could cause real problems if they would develop further, reducing overall reproductive success. As both polygyny and monogamy presently coexist in this population, there must be evolutionary costs for either, rendering the system evolutionary stable and making it unlikely that this will become a real threat for our population.

The case of adoption under natural conditions is very interesting. Up to now it was only reported in captivity (Arlettaz, unpublished).

5.5 Conclusions and implications for conservation

Our hypothesis that CBP and EPP correlate positively with local Hoopoe density, and that an unlimiting offer of nestboxes might negatively influence social interactions, reproductive success and eventually population dynamics, was not supported. Bearing in mind that the Spanish study did actually report no case of CBP either, this phenomenon is probably not of concern for the Hoopoe as a species, especially as the only two studies so far, the Spanish (Martín-Vivaldi et al. 2002) and ours, examined dense populations where an occurrence of CBP would be more likely. In the future, it will be interesting to see how monogamous versus polygamous strategies dynamically equilibrate in the rapidly expanding Swiss population. This study already evidenced the negative costs of polygamy in terms of breeding success for both partners engaging in this strategy.

For the future we should keep an eye on the occurence of chick starvation or even infanticide, which are symptoms of polygamy. If these events become more frequent, the interplay between pair and nestbox density may start to play a role in population dynamics. But at present we can be happy and confident about the fate of this recovering Valais population!

6 Acknowledgements

I would like to thank Prof. Dr. Raphaël Arlettaz for enabling and supervising this diploma study and helping me out many times in the field and with the writing of this diploma. Many thanks also to Antoine Sierro for helping with the nestbox controls and digitalizing field data. Great thanks to Paul Mosimann for helping with the captures and giving many precious advice on capture and handling of the birds and support in the field. Thanks also to Stephanie Geiser for collaboration with the nestbox controls and to all people living at the Valais field station in summer 2004.

Great thanks also to the people from the University of Lausanne who introduced me into the field of paternity analysis, especially Dr. Luca Fumagalli, Sylvain Ursenbacher and Nadège Duvoisin who showed me the practical part of labwork.

I am very grateful for the help I was given by the members of the CMPG in Bern, especially by Dr. Carlo Largiadèr and Ghislaine Rigoli. Thank you so much David 'Speedy' Bittner for helping to build up the DNA lab and taking care of me when I didn't know anymore where to search for help. Special thanks also to Mathias Ritschard who showed me his work on paternity analysis and gave me helpful insights. I am most thankful for the help and support provided by my office partner Martin Fischer and my co lab worker Judith Zbinden for the fruitful discussions.

Without the back up and support of my dear family and my friends throughout hard times I wouldn't have made it - thank you so much! Special thanks to Lukas Burkhard for being so kind to provide me with his binoculars during the whole field season.

I would also like to acknowledge the financial support of the Division of Conservation Biology, University of Bern and the Schweizerischen Vogelwarte Sempach.

Last but no least I am very thankful for the friendly and great support of all the farmers and private people in the Valais who support this fascinating and increasing Hoopoe population by allowing them to live in their buildings.

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8 Tables, Figures and Appendix

8.1 Tables

Table 1. Hoopoe primer sequences, ordered from Applied Biosystems and Microsynth, with annealing temperatures (Ta) for each of the microsatellite loci analysed

Locus	Forward primer sequence	T_a (°C)
UpuD3	5' FAM-TGCACTGCTGAGCAGCTCTC	60
Upu935	5' NED-ACCCTGTACCCACACAAGTC	60
UpuA3	5' FAM-CTGTTGTTACCACAGTGTC	60
UpuA7	5' VIC-GCCTTTCCACTTAGGCACCCG	60
UpuA2	5' NED-TGTATCTGAGTCCATGGGGA	64
Upu907	5' FAM-TGAATGAGCCTCCACTCTCC	60
Upu921	5' HEX-AGCTCTTGGTGAGGGCACTG [1)]	60

[1)] Upu 921 would better be labelled with VIC

Table 2. Characterization of genetic parameters of the seven microsatellite loci used in this study. Heterozygosity frequencies and allele numbers were calculated using all adult breeding birds.

Locus	k	N	Range	H(O)	H(E)	PIC	Excl(1)	Excl(2)	HWE	Null freq
Upu907	13	76	116-142	0.882	0.855	0.833	0.54	0.704	NS	-0.0172
Upu921	13	76	107-119	0.934	0.832	0.808	0.497	0.668	NS	-0.0669
Upu935	16	68	199-255	0.779	0.903	0.888	0.657	0.793	NS	0.0704
UpuA2	18	71	137-177	0.62	0.848	0.83	0.545	0.708	NS	0.1514
UpuA3	9	76	187-217	0.763	0.697	0.657	0.294	0.475	NS	-0.0543
UpuA7	15	76	160-192	0.855	0.854	0.834	0.545	0.708	NS	-0.0057
UpuD3	26	57	217-387	0.263	0.948	0.937	0.784	0.879	NA	0.5633

k = number of alleles, N = number of individuals typed, Range = size range of all alleles, H(O) = observed heterozygosity; H(E) = expected heterozygosity, PIC = polymorphic information content, Excl (1) = exclusion probability for the first parent; Excl (2) = exclusion probability for the second parent, when first parent is assigned, HWE = Hardy-Weinberg equilibrium, Null freq = estimated frequency of null alleles

Table 3. Overall exclusion probabilities according to number of loci analysed.

Missing loci	Mean no of alleles/locus	Mean proportion of individuals typed	Mean heterozygosity expected	P_{excl} first parent	P_{excl} second parent
none	15.71	0.940	0.848	0.997494	0.999889
UpuD3	13.83	0.976	0.826	0.986609	0.998908
UpuA2, UpuD3	13.00	0.984	0.826	0.972978	0.996634
Upu935, UpuD3	13.40	0.986	0.810	0.960009	0.994564
UpuA2 ,Upu935, UpuD3	12.25	1.000	0.806	0.919298	0.983243

8.2.1 Figure captions

Fig. 1. Distribution of distances among sites (mean neighbour distances, MND) for all broods in 2004

Fig. 2. Distribution of LOD scores of the social fathers of 248 offspring. Distribution is normal (Shapiro Wilkinson W test, Prob < W = 0.13, mean = 3.17, SD 0.96, upper 95% confidence interval = 3.3, lower 95% confidence interval = 3.06)

8.2.2 Figures

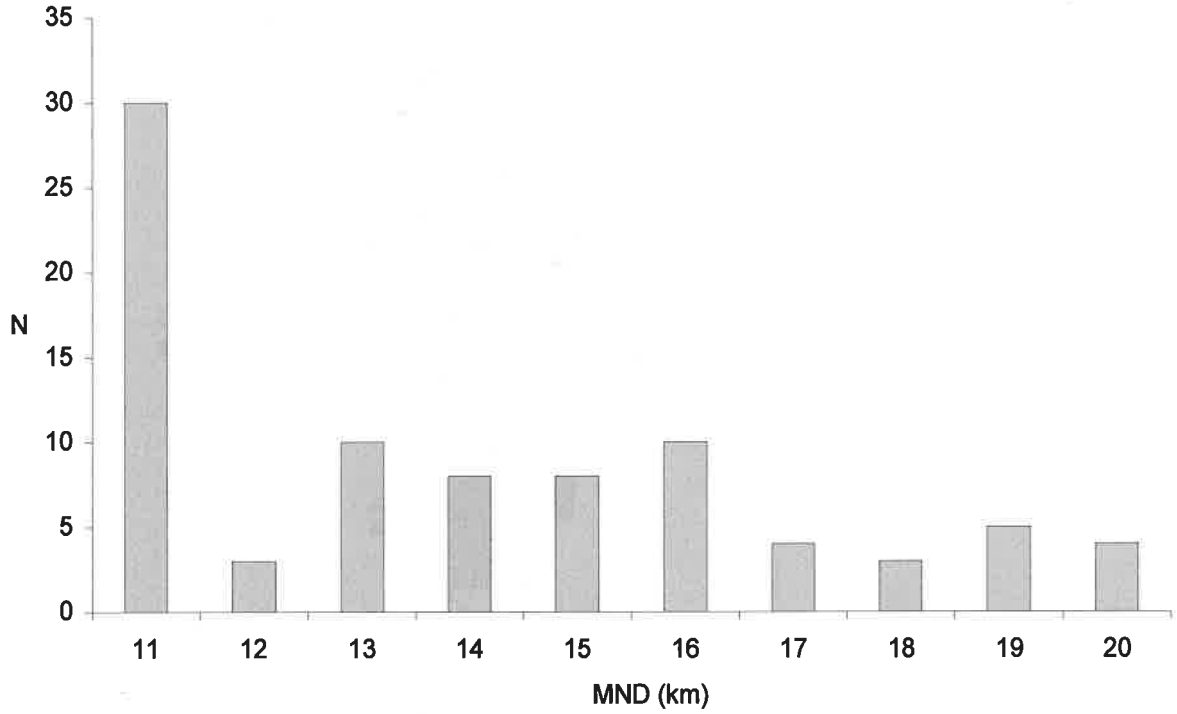


Fig. 1

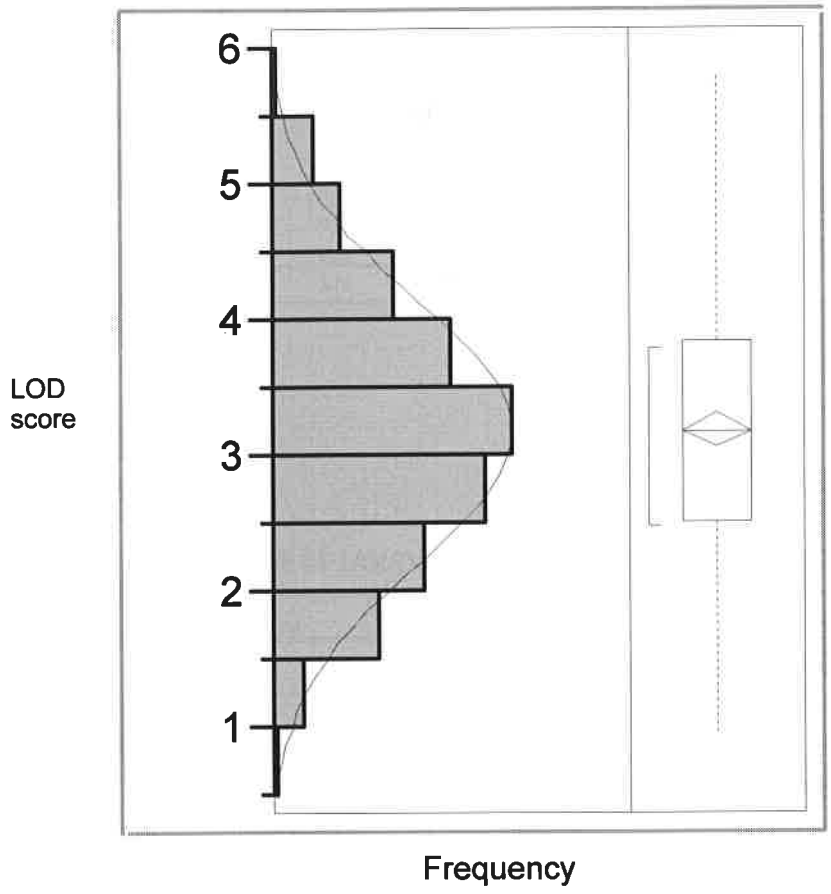


Fig. 2

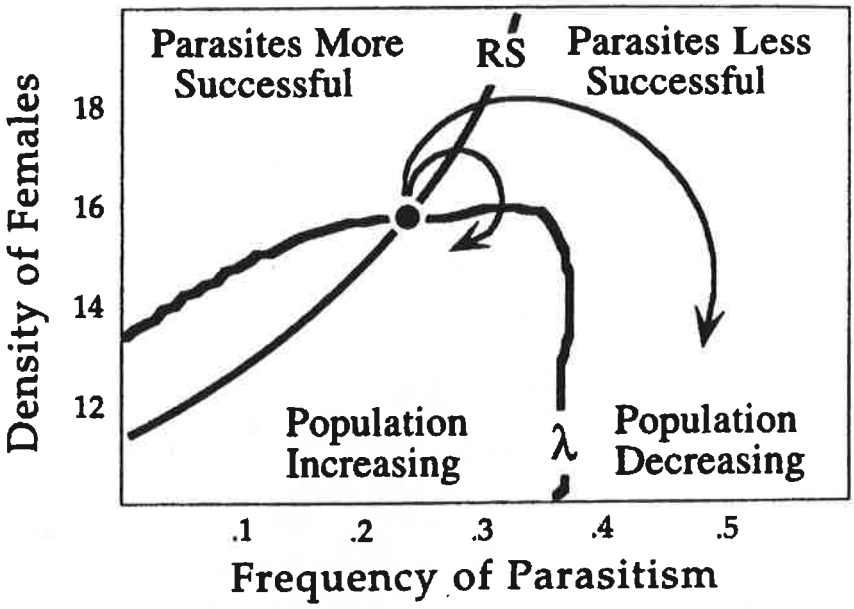
8.3 Appendices

Appendix 1. Breeding success between 1998 to 2004

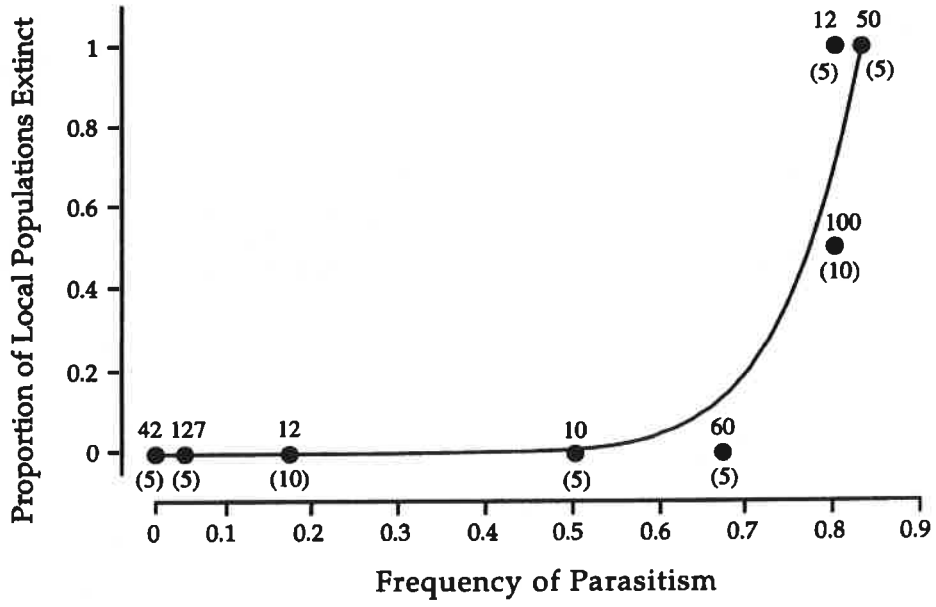
Year	no. clutches	no. eggs total	no. eggs hatched	mean clutch size	proportion of hatched eggs	no fledlgings	proportion fledglings / hatched	proportion fledglings / total eggs	no. hatched / nest	no. fledglings / clutch	remarks
1998	16	104	77	6.50	0.74	68	0.88	0.65	4.81	4.25	good weather
1999	16	101	26	6.31	0.26	16	0.62	0.16	1.63	1.00	bad weather
2000	39	239	195	6.13	0.82	158	0.81	0.66	5.00	4.05	good weather
2001	43	262	191	6.09	0.73	139	0.73	0.53	4.44	3.23	bad weather
2002	51	347	262	6.80	0.76	211	0.81	0.61	5.14	4.14	
2003	63	470	413	7.46	0.88	331	0.80	0.70	6.56	5.25	very hot summer
2004	112	753	594	6.72	0.79	524	0.88	0.70	5.30	4.68	
Mean	48.57			6.35	0.75		0.83	0.62	4.70	3.90	

Appendix 2. published densities of *Upupa epops* populations in Europe

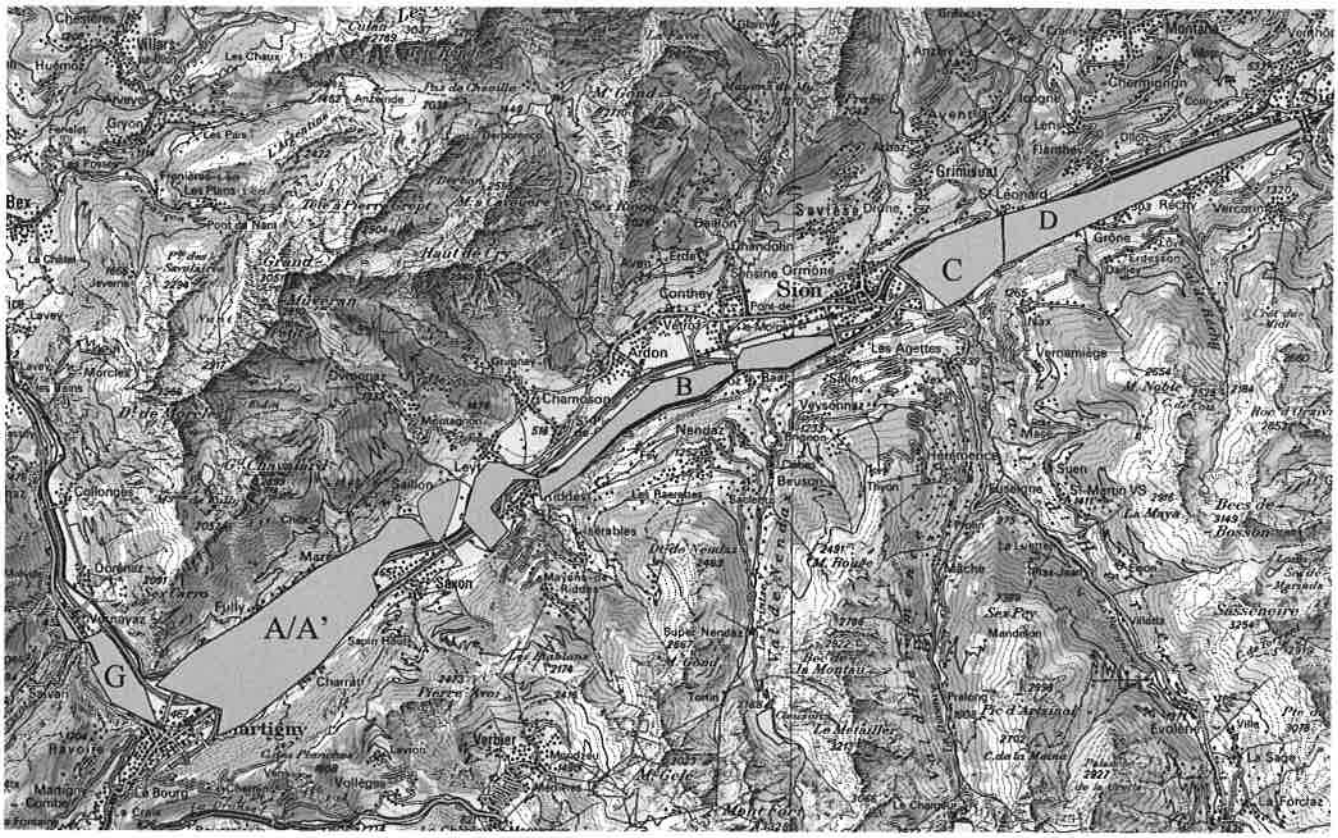
Density	Place	Country	Reference
breeding distance a few 100 m		mediterranean countries	(Glutz von Bolzheim 1980)
breeding distance 1-2 km		middle Europe	(Glutz von Bolzheim 1980)
highest density of middleurope in Valais .	Valais	Switzerland	(Glutz von Bolzheim 1980)
0.15 - 0.16bp/km^2	TÜP Jüteborg Ost	Germany	(Oehlschlaeger & Ryslavy 2002)
0.75 - 1.4bp/km^2	Oberspreewald	Germany	(Oehlschlaeger & Ryslavy 2002)
1.1 - 1.4 bp/km^2	Wriezen	Germany	(Oehlschlaeger & Ryslavy 2002)
0.8 - 2.3bp/km^2	Polana Gebirge	Slovakia	Dvorak et al, 1993
2.1-2.5 territories /km2, up to 12-14 pb / km^2	Extremadura	Spain	(Rehsteiner 1996)
5.5-5.7 bp/km^2	Grandada	Spain	Rehsteiner, by letter of Martin-Vivaldi
1.1-1.6 bp /km^2	Valais	Switzerland	(Arlettaz 1983)



Appendix 3. Combined dynamics of the simulation of Eadie and Fryxell (1992), vortex theory. λ = growth rate of 1, on the right λ>1, on the left λ<1, RS = equlibrium where parasites and nonparasites are equally successful



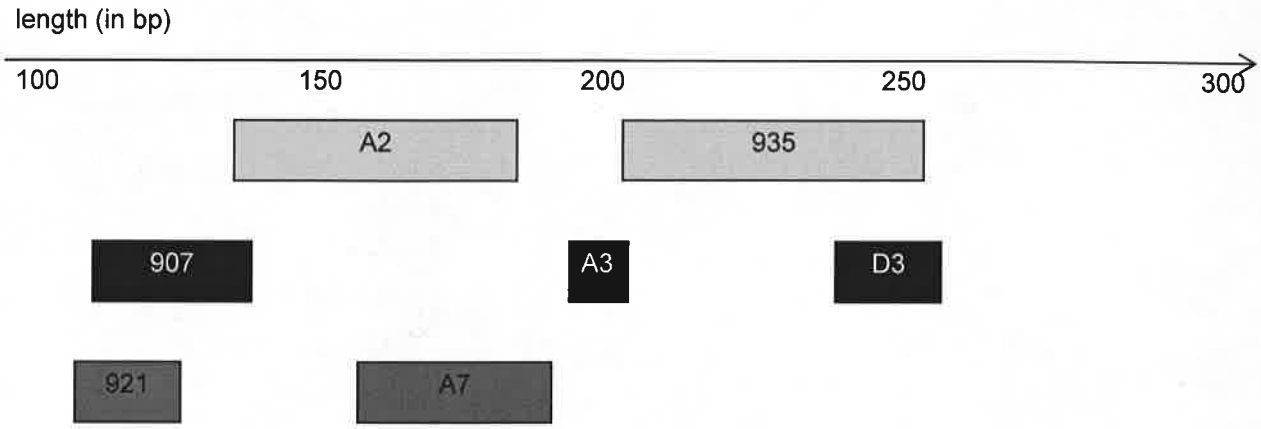
Appendix 4. Results of a simulation model on the likelihood of local population extinction as a function of the frequency of conspecific brood parasitism. Values above each point indicate the population size for that set of simulations, and the values in parentheses below each point are the number of simulations conducted for those parameters. The logistic regression fit to the points is given by the equation $Y = 5.778 \times ^{9.414}$ (Eadie et al. 1998).



Appendix 5. Study area with the different nestbox zones

Appendix 6. Distribution of breeding sites 2004

Appendix 7. Size range and arrangment of the different loci in the multiplex set



Appendix 8. The magnitude of error around actual estimates of EPP levels against the sample size of those studies. % error on the vertical axis refers to the magnitude of the difference between the upper and lower 95% confidence intervals around an estimate. The line plotted is this % error for a hypothetical population with a rate of 15% EPP across different sample sizes. (Griffith et al. , 2002)