-Master thesis proposal-

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

Supervision:

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Introduction

Theoretical background

Extra-pair paternity and density dependence.

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 single putative father. Recent molecular studies revealing new insights into avian mating systems show that sexually monogamous species are very rare with about 90% of all species showing extrapair paternity. Even among socially monogamous species over 11% of the offspring and 18.7% percent of broods contain extrapair offspring (Griffith et al. 2002).
Variation in breeding density is a traditional explanation for intraspecific variation in the rate of EPP because as density increases so do also social interactions, both cooperative and competitive, such as extrapair copulations. EPP is likely to increase with frequency of extrapair copulations. In addition the probability that extrapair males are neighbours is also great.
As logical as this relationship between frequency of EPP and density may seem, there are only few studies which clearly established this relationship. This fact doesn't mean though, that there is no such relationship but merely shows the need for further studies investigating the intraspecific relationship between it. Griffith et al. (2002) criticize the design of many studies as a reason why this relationship between EPP and density has not been clearly established so far: 1) most studies have been observational rather than experimental. 2) low statistical power due to the small number of populations involved. 3) usually very little variation between populations in both density and EPP. 4) the tests fail to acknowledge the large standard error around the estimates of EPP for any one population. We chose an alternative, intraspecific approach to prevent some of the problems described before. We will compare degree of EPP within pockets of high density versus pockets of low density of the same population. In the Hoopoe population in Valais there are big density differences due to the clumping of the broods, so that the premises to detect correlations between EPP and density are given.
Griffith et al. also investigate the role of phylogenetic relationships in the degree of EPP. Big evolutionary lineages explain a lot of the interspecific EPP-level variations, mainly between passerines and non-passerines. As a non-passerine, the Hoopoe is therefore expected to have a low degree of EPP. Non-passerines have an average EPP frequency of 3% ± 5 % compared to 18% ± 17% in passerines. The highest level of EPP observed in non-passerines so far was 18% (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=36) and 7.7% of offspring (5/65).

Risks inherent to extra-pair paternity and conspecific brood parasitism

To my knowledge there are no conservation risks known to be linked with level in 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. Our study will have to show if this actually happens.
Yet, if there is a strong positive correlation between frequency of EPP and density it may indicate alteration of a population social behaviour through artificially increased density, with perhaps unknown population dynamics consequences in the long term.

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.
There are several reasons why females may lay parasitically: 1) Females gain reproductive benefits without incurring the physiological costs or risks associated with incubation and parental care. 2) 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.
3) Parasitism among conspecifics might be facilitated if parasites and hosts are closely related. 4) 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. In our case we are interested in this last possibility, searching for a correlation between frequency of CBP and density.
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 2 years density was artificially reduced and hatching success increased again to 60%. These results show clearly a connection between frequency of CBP and population density and 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. An inverse relationship which is not only specific to wood ducks between total number of eggs laid and hatching success has been documented (Belrose & Holm 1990; Semel et al. 1988, 1990; Semel & Sherman 1995). Thus, increase in the number of laid eggs actually leads to a decline in individual reproductive success so that population growth rates become very small.
The relationships among 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 (Eadie & Fryxell 1992; May et al. 1991; Nee & May 1993). It has been shown with different assumptions (one with females being either parasitic or non-parasitic, the other allowed conditional parasites) that CBP can significantly impact population dynamics. CBP can lead to stable populations, populations that oscillate cyclically, or populations that fluctuate chaotically even leading to extinction of whole populations. Local extinction becomes possible when the frequency of parasitism is very high and the “inertia” (the 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 1). This simulated population crash occurred without any external, stochastic factors such as predators, bad weather, human impact, etc. Thus density does influence relative reproductive success of a population and, in turn, parasitism can regulate density of local populations.
There were simulations run with varying frequency of parasitism and population size. It was 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 2). Astonishingly initial population size (range from 10 to 127 individuals, which is the range of our Hoopoe population in Valais) did only have a minimal effect.

Artificial high density with unlimiting breeding sites availability may 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 the secondary cavity nester to breed again on the plain of the Rhone. The reason of the decline of that population was a lack of breeding sites on the intensively cultivated plain and the parents were therefore forced

to fly long distances between nest sites on the slopes 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 et al. 1998; Arlettaz et al. 2000; Schaad et al. 2001; Sierro et al. 2003; Sierro et al. 2002).
As a consequence of this action the population underwent a dramatic augmentation. Between 1998 and 2003 it increased from about 20 to 63 broods per year. This increase was accompanied by higher and higher local densities, probably matching the clumped distribution of the mole-crickets, the main prey with over 90% of biomass supplied to chicks (Arlettaz et al. 2000). In densely populated areas there have been several cases of polygamous males observed in the recent years which is unusual in this normally monogamous bird. Also there is several evidence for clutches destroyed by "enemy" females, for infanticide (chicks found murdered on the ground under the nests) and even for cases of adult females found dead, probably killed by competitors (Arlettaz et al. 2000; Schaad et al. 2001; Sierro et al. 2003; Sierro et al. 2002). 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 due to the artificially high breeding density caused by the unlimiting nestbox offer. The overall density in Valais is now around 1.4 broods/km^2 (63broods/46km^2 in 2003). Locally the densities found reach >3 territories/4ha which is about 75 bp/km^2. Densities in Europe are by far the highest in the Iberian Peninsula, averaging some breeding pairs per km^2. High densities are also found in SE Europe with average densities ranging between 0.04 to 0.12 bp/km^2 (France, Greece, Slovenia, Croatia, Bulgaria, Moldova, Ukraine and Belarus). Exceptions are Italy (0.02) and Hungary (0.25). All other West, Central and Eastern European Countries have fewer than 0.004 bp/km^2 (Hustings 1997), compare with Table 3, Appendix). Thus, also in comparison with other densities in Europe this is indeed a very high density found in Valais (Arlettaz et al. 2000).
Does the availability of unlimiting number of breeding sites affect population dynamics through altered and intraspecific social interactions such as extrapair paternity, polygamy and perhaps even conspecific brood parasitism? These alterations may lead to counterintuitive effects through reduced individual reproductive success, increased population instability and finally lead to a decline of the local population through the implementation of a nestbox program which was initially designed to help an endangered population. Do we have eventually to optimise the density of supplied nestboxes in specific areas to correct for this possible detrimental effects?

Important is to stress that, even though we are also addressing fundamental questions for which an experimental approach would be best (Griffith et al. 2002), we are working with an endangered population for which we have chosen a correlational approach. I don't know how nestbox density could be manipulated without possible detrimental effect for this important Swiss population.
But as the density of the provided nestboxes has great variation in our study area, we can work with an intraspecific approach and look at the density as almost experimentally varied.

Research model

Global status of the Hoopoe

Upupa epops epops has been widespread and common over all central Europe with regular breeding in Denmark and southern Sweden. Due to climatic changes to colder and wetter weather there has been a severe decline in northern peripheral populations and everywhere in central and western Europe since the end of the 19th century until the middle of the 20th century when some temporarily recovery took place, probably thanks to a warmer and therefore more favourable climatic period. Since 1950-55 a new retraction took place throughout all of Europe but mainly in the industrialized countries of central Europe. Causes are thought to be habitat

change through intensification of agriculture and thereby alteration of the favoured habitat of low cultivated landscapes, 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).

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 remaining breeding events (63 broods in 2003; (Sierro et al. 2003)) are found in the Upper Rhône valley (Valais). Other remaining breeding pairs are 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 ("stark gefährdet") in Switzerland (Keller et al. 2001).
Apparently the Valais population is one of the only ones which has increased steadily in the recent past. Yet, it suffers from variations in annual breeding success which are most likely caused by weather fluctuations affecting the availability of molecrickets (Table 1 in Appendix) (Schaad 2002).

Behaviour

In the Hoopoe the length of the male song strophe is a sexually selected trait demonstrating male quality and thus determining female mating choice. The more strophes (range between 2 to 6) a male includes in its song the more attractive it seems to be for a female (Martín-Vivaldi et al. 1998, 1999a, 2000; Martín-Vivaldi et al. 1999b). Hoopoes are regarded as brood reduction strategists, thus laying optimistic clutches for the best case (good weather, lots of prey for the young) and letting starve the youngest chicks depending on disposable food abundance (Martín-Vivaldi et al. 2002; Martín-Vivaldi et al. 1999c).

Questions

Primary questions

-Is EPP density-dependent? → Relationship between EPP and local density

EPP in Spain occurred in 10% (36) of the broods and 7.7% (5/65) of the fledglings. This level is in the middle of the range described for bird species but pretty high for non-passerines (Martín-Vivaldi et al. 2002).
I expect a degree of EPP higher than in Spain because of the locally higher density and the easily detectable nestboxes in Valais. Furthermore I expect to detect a positive correlation between frequency of EPP and local density index which would further support the hypothesis that density is responsible for intraspecific variation in EPP.

-Does EPP level affect reproductive success?→ Relationship between brood failures and EPP

I will try to analyse genetically as many fertilized, unhatched eggs and dead chicks as possible to see if there is a correlation between brood failure occurrence and EPP.
I will also look for a correlation between hatching/fledgling success and EPP.
Martin-Vivaldi has already shown that only low-quality males suffer losses in paternity through EPP. Therefore there is perhaps a higher mortality to be expected in broods containing EPP.

-Does CBP occur? Is it density-dependent? → Relationship between CBP and local density

In a Spanish Hoopoe population in Grenada (Martín-Vivaldi et al. 2002) there was no case of CBP found. It will be interesting to see if CBP occurs in Valais, due to a higher local density. I will also look at correlation between CBP and a density- index if enough cases of CBP.
Expectation: hatching success is still very high and clutch size constant in Valais. So no obvious signs of CBP detectable (compare Table 1, Appendix). In the Goldeneye's the percentage of CBP is smaller than in wood ducks, probably due to their aggressive defence of territory. As hoopoes don't defend their territory but only their nest surroundings against intruders they are perhaps more susceptible to CBP than Goldeneye's.

Secondary questions (only investigated if time allows, arranged according to priority)

-Is polygamy occurring more frequently in high density areas?

Comparing the occurrence of polygamy with the density index.

-Are there costs of polygamy?

Because both strategies (monogamy and polygamy) coexist in the same population there must be evolutionary costs for both strategies for the system to be stable. Calculation of reproductive success (number of fledglings and fledglings/no of eggs) of females and males depending on the mating system (monogamous vs. Polygamous and number of other mates) to see probable costs of polygamy.
For example there was a female observed that attacked a neighbouring female with already old chicks while their common polygamous male was feeding a third female further away. This means high costs for the male through losses of successful fledglings in all clutches due to insufficient feeding and disturbance of breeding females.

-Relationship between breeding success and density?

But as breeding density is also an indicator of habitat quality this may also lead to higher breeding success through better breeding habitat. But if the breeding success is lower even in high density areas which are probably better habitat this really indicates negative effects of breeding density.

Are the extrapair males breeding neighbours?

Check if the fathers of the extrapair chicks stem from neighbouring sites. If yes this supports also the hypothesis that EPP and density correlate: more close neighbours lead to a higher frequency of EPP.

Are the females rearing CBP-chicks related with their parasites?

As one explanation for CBP is close relatedness it would be interesting to investigate kinship as we already have the genetic data. If time allows and loci are informative enough this is technically possible to examine.

-Are the males engaged in EPP low or high quality males?.

If the EPP males are low quality and thus low dominance males there should be a lower survival rate of its EPP offspring and unhatched but fertilized eggs observable. If high quality and dominance male there would higher survival rate than true monogamous offspring observable.

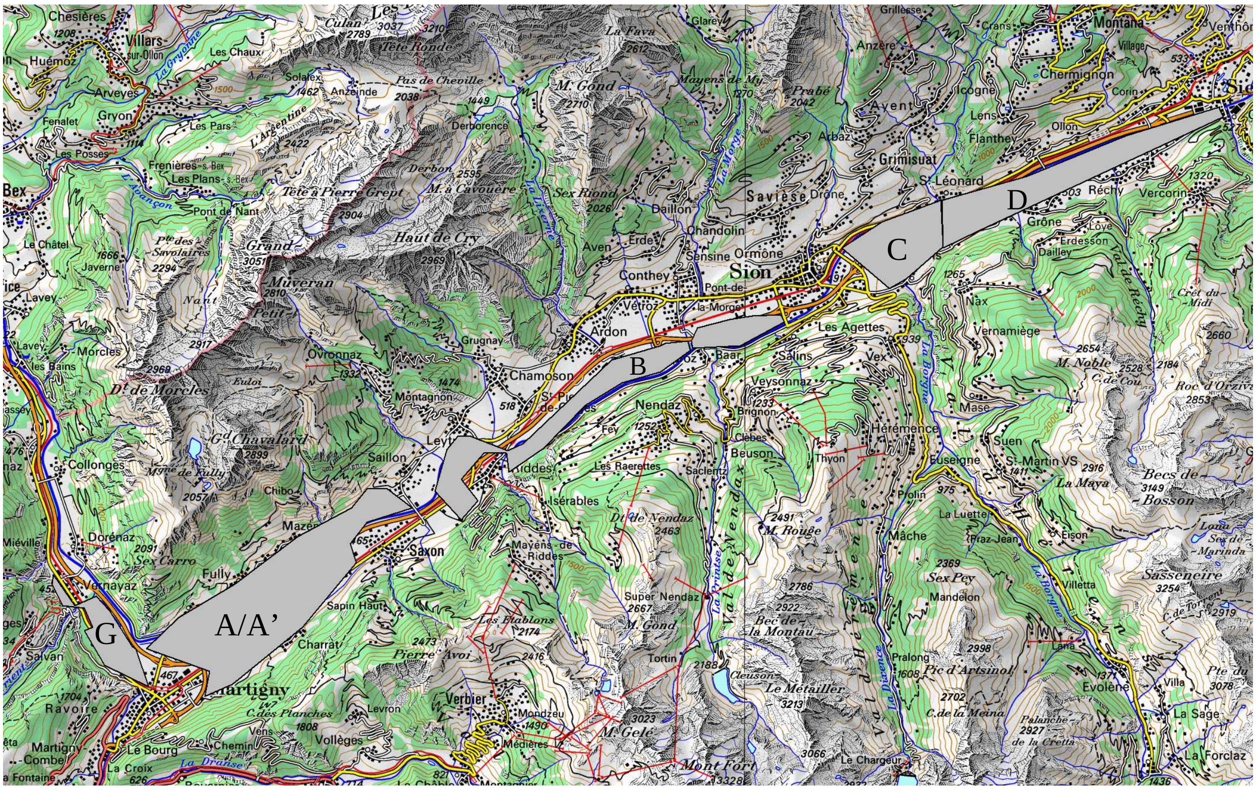
Additionally there are 31 dead chicks of complete families 2000-2002 and 7 dead chicks of 2003 (questionable if their DNA is still stable and intact) available to see if among them is an over proportionate high number of EPP offspring.
As the dominance status (strophe length) of the male is likely to correlate with Body-Condition Index (BCI), with males with high BCI males providing more molecricket biomass to nestlings, we could take the BCI as an indication for quality and dominance. (Schaad 2002).

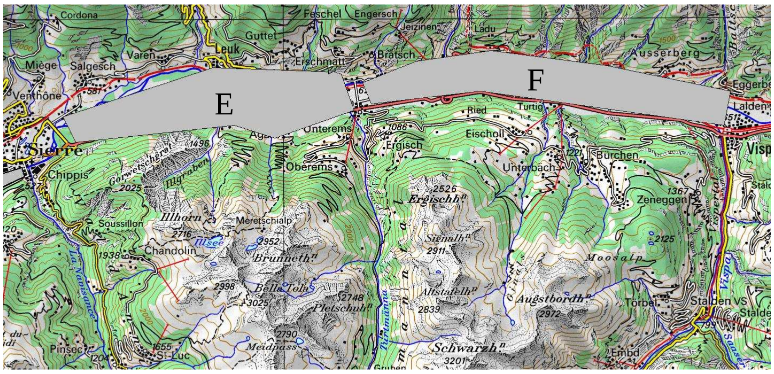
Are the females engaging in EPP low or high quality ones?

Female quality can be estimated directly through yearly fledgling success (except if CBP is common) and be compared to degree of EPP.

Material and Methods

Study site





The study site is the plain formed by the Rhône river between Vernayaz and Sierre in the canton Valais, Switzerland. It is divided into five zones from A to G with 46 km of length and an elevation from 452 m (Vernayaz) to 520 m (Sierre) and a total of 708 nestboxes (diameter of the entrance hole 55 mm). Its area is about 45.2 km^2 (Zone G 3.2 km^2; Zone A,A' 18 km^2; Zone B 7.5 km^2; Zone C 7.7 km^2; Zone D 8.8 km^2 - own calculation according to polygons on the map above). The five zones were equipped with nest boxes at different times. Nest boxes were installed first in zone A (winter 1997/98), then in zone B (March 2000), in zone C (March 2001), in zone D (December 2001) and finally in zone G (spring 2003). There is an additional Zone E (76 nestboxes but none used till 2003) and F (not equipped with nestboxes, still natural cavities available) both in the upper Valais with its extensive agriculture.
Apart from this historical nestbox equipment of the study site, the zones also differ in their habitat and probably resource availability. Farming is most intensive in the zones A and B. Only few traditional orchards and tall trees are left there. In the zones G, C and D there are still more traditional orchards, and a larger part of the zone is covered with gardens (zone D in particular). This means that the availability of natural holes is probably much more limited in zones A and B than in zones C, D and G.

Sampling design

Fieldwork

We will control the nestboxes every two weeks (to make sure that no brood is overseen as the incubation period is about 16 days long (Martín-Vivaldi et al. 1999c)). By using a mirror and a torch the nest boxes can be inspected through the entrance hole without opening the nest box and thereby disturbing the birds too much. Nestbox contact will be noted, but the number of eggs will not be evaluated because the breeding female must be disturbed as little as possible.
The newly detected broods will be inspected often to be able to collect as many dead eggs and chicks as possible for subsequent parentage analysis before they can be removed or eaten. Storage in the deepfreezer in Sion.
According to the knowledge of the age of the clutches feather-sampling of the hatchlings when they are 15 days old. The whole nestling period lasts between 24-30 days. At the end of the nestling period controls every few days to detect the number of successful fledglings. The whole cycle of a brood is around 44 days (Martín-Vivaldi et al. 1999c).

To catch adults we have to install mist nets in front of nestboxes. It is better to do this in the morning to avoid the wind which normally starts later in the day. A mean time-value to catch a bird is around three hours. At dusk or dawn chances of a successful catch are probably higher because the bird can see the net less well.

Measurements taken (adults):

-Blood sample (only if not yet ringed and probed with sufficient amount of blood in the past, classified as "enough" or "medium" in the filemaker database)
-Phenotypic sex determination
-Ringing or recording the already existing ring on the recaptures sheet.
-Measurements to determine BCI (mean tarsus length and weight)
-Pictures of "huppe", beak, wing open and closed and tail.

Measurements (young):

-Feather sample
-Ringing
-Tarsus length and weight

Storage of the blood samples will be in Eppendorf tubes containing queen lysis buffer (Seutin et al. 1990) (recipe see Appendix) in the deepfreezer in Sion prior to transportation to Bern all together in a cooled box. Feathers will be stored in paper envelopes.
Every capture event will be registered in a filemaker database. Observations of any kind will be noted in the field journal. Especially observations of other birds than those of the breeding pair visiting or staying close to the nest box will be interesting. Also females leaving the nestbox while having young chicks or eggs.
The more detailed planning of the week and each single day will be done from week to week depending on present knowledge about age of the clutches, weather and other data.

A big advantage of this study is that we have access to most of the breeding pairs (estimation: 80-90% of the broods) as it was found in the previous years that all Hoopoes switched voluntarily from the few remaining natural sites on the foothill slopes to our nestboxes in the plain. I will only take into account the breeding pairs where both parents were caught and sampled to have an absolute method where we have the data of all the family members. In these conditions it should also be possible to scan the whole population to find the genetic father of the extrapair chicks.

Method to determine density

The most common way density is measured and cited in literature is breeding pairs per area unit. But this is a pretty arbitrary method as the definition of area is completely arbitrary. To have a much more accurate information about density we will use the mean neighbour distance which is the mean distance between a breeding site and all other breeding sites. With this method we will have a density index for each nestbox which enables us to calculate correlations between density and EPP/CBP for each brood.

Method to determine adult fitness, eventually dominance status

The Body-Condition-Index (BCI) which we will use as a probable surrogate for the strophe length method to determine dominance status is calculated as follows:

$BCI = {}^{m}/_{l}$ (eventually m/l^3)

m: mass (g)
l: mean tarsus length (mm)

Timetable

Month	Week	Work	Place	Details	Persons
Mrz 04	9	Proposal	Bern		
	10	Proposal	Bern		
	11	Proposal	Bern		
	12	Proposal	Bern	hand in 1st version of proposal	R.Arlettaz
Mrz/Apr	13	Proposal	Bern		
Apr 04	14	Proposal	Bern		
	15	Proposal	Bern	hand in 2nd version of proposal	R.Arlettaz
	16	Proposal	Bern		
			Sion (Valais)	implementation field work; de-	
	17	Proposal/Field		pending on weather	R.Arlettaz
				blood samples,handling birds,	
Mai 04	18	Field	Sion (Valais)	etc	R.Arlettaz
	19	Field	Sion (Valais)		ev. helpers [1)]

	20	Field	Sion (Valais)		ev. helpers
	21	Field	Sion (Valais)		ev. helpers
Jun 04	22	Field	Sion (Valais)		ev. helpers
	23	Field	Sion (Valais)		ev. helpers
	24	Field	Sion (Valais)		ev. helpers
	25	Field	Sion (Valais)		ev. helpers
Jun/Jul	26	Field	Sion (Valais)		ev. helpers
Jul 04	27	Field	Sion (Valais)		ev. helpers
	28	Field	Sion (Valais)		ev. helpers
	29	Field	Sion (Valais)		ev. helpers
	30	Field	Sion (Valais)		ev. helpers
Aug 04	31	Field/Lab	Sion (Valais)		ev. helpers
	32	Holidays	?		
	33	Holidays	?		
	34	Lab work	Lab Bern	adjust microsats	L. Fumagalli
Sep 04	35	Lab work	Lab Bern	adjust microsats	
	36	Lab work	Lab Bern	adjust microsats	
	37	Lab work	Lab Bern	adjust microsats /analysing microsats	
	38	Lab work	Lab Bern	analysing microsats	
	39	Lab work	Lab Bern	analysing microsats	
Okt 04	40	Lab work	Lab Bern	analysing microsats	
	41	Lab work	Lab Bern	analysing microsats	
	42	Lab work	Lab Bern	analysing microsats	
	43	Lab work	Lab Bern	analysing microsats	
Nov 04	44	Lab work	Lab Bern	analysing microsats	
	45	Lab work	Lab Bern	analysing microsats	
	46	Lab work	Bern	statistics	L. Fumagalli
	47	Analysis	Bern	statistics	
Nov/Dez	48	Analysis	Bern	statistics	
Dez 04	49	Analysis	Bern	statistics	
	50	Analysis	Bern	statistics	
	51	Analysis	Bern	statistics	
	52	xmas	?		
Jan 05	1	writing Thesis	Bern		
	2	writing Thesis	Bern		
	3	writing Thesis	Bern	Hand in of 1st version of diploma work	
	4	writing Thesis	Bern		
Feb 05	5	writing Thesis	Bern	Hand in of 2nd version diploma	R. Arlettaz
	6	writing Thesis	Bern		
	7	writing Thesis	Bern	Hand in of 3rd version of diploma work	R. Arlettaz
	8	writing Thesis	Bern		

[1)] During peak period I may need additional help to be able to control and sample most of the birds. Possible helpers are: Raphaël Arlettaz, Paul Mosimann, Antoine Sierro (controlling the sectors C and D).

Labwork

We expect about 270 samples (200 chick feathers and 70 adult blood samples) in 2004. If there are less samples this year we can take an additional number of samples from previous years.

Microsatellite analysis

First the DNA will be extracted from blood and feather samples. Then the different microsatellite loci will be amplified with PCR, using 3 different PCR conditions for the various primers.
After amplification the microsatellites will be ordered in 3 Multiplexes and analysed on the capillary sequencer of the CMPG in Bern. The unequally long microsatellite fragments will run with different speed through the capillary. These time differences in the migration makes the microsatellites reach the laser (which detects the fluorescent signal of the fluorescent primers) in different time lags. The results of the Laser, with peaks showing the amount of microsatellites passing, will be read on Gene Scan 3.1. and interpreted by me.

Time planning:

Technical labwork:

-Extraction:

with the Quiagen-extraction tubes ca. 6h for 48 individuals. 300 Individuals will take 36 hours, **3 days.**

-PCR:

3 different PCR's per Individual to be done as we have 8 primers with 3 different PCR conditions. One PCR takes about 75 minutes. With one PCR machine only amplification of all 8/9 primers takes 4 hours for 12 individuals, makes 24 Individuals in 8 hours and thus per day as 2 hours before the first PCR is needed for preparation. In total **12 days** for PCR needed.

-Agarosegel:

Eventually testing the extractions and amplifications on an agarosegel. 2h for 9 individuals à 8/9 primers make 10h for 48 individuals, 300 individuals take **6 days**.

-Sequencer Preparation

3 Multitplexes for 48 Individuals, 1 day
300 Individuals will take **6 days**.
I don't know how long I will have to wait to get the results of the sequencer of the CMPG lab.

-Genetical sexing of offspring

Extractions will aready be done. Thus PCR of 200 Individuals with CHD-Primers has never done by me or in this lab, inconsistencies , say 1 week. Doing the Agarosegels 48 Individuals per Gel, 2h per Gel, 8 hours in total.

I expect that the sexing of the offspring of 2004 and 2003 (N=325) should be possible to do in a little more than one week.

TOTAL: **27 days (6 weeks)** for technical lab work. 1week for sexing. Additionaly at least 4 weeks at the beginning for adjusting the microsatellites and probably some weeks until the newly installed lab works proberly. For this reason I put **13 weeks** for labwork into my planning, if this is too cautiously calculated sample size can be augmented with samples of previous years.

Analysis of the gels

-Reading the microsatellite analysis. N x 16 individuals per Run. all 300 take probably around **6 days**

TOTAL: I planned **5 weeks** for this analysis. This includes enough time for solving problems and dealing with unexpected things. Furthermore I have to see with Luca Fumagalli how to do this which means I will probably have to travel to Lausanne which takes also time.

Genetical sexing

If still enough time, additionally to the chicks used for my analysis I will do genetical sexing of all the juveniles ringed and sampled in 2004 and 2003 for subsequent population demographic questions not included in this diploma work. We will use the CHD-Gene-Method described by Griffiths et al. (1998) already used for Hoopoe sex determination by Schaad (2002). PCR is done according to the method described by Griffiths et al. (1998). The resulting PCR products will be analysed with electrophoresis (2h at 60V) on a 3.5% MetaPhor-Agarose gel.

Sample size

The sample size will depend on weather conditions. I assume that this year there will be around 200 fledglings and about 100 adult birds (50 breeding pairs) in the Valais. Given that not all adult individuals will be captured, we are probably going to have a sample size of ca. 70 adults and 200 juveniles. If necessary we are going to analyse additional clutches of previous years. There would be 71 families with 574 Individuals available of previous years (Tab 4, Appendix).
According to (Griffith et al. 2002), for a comparative, interspecific study about EPP there should be at least 150 offspring analysed to get an acceptable range inside which an estimate of EPP would be found 99% of the times. This error around the estimate would be ca 18% with 150 individuals for an average EPP frequency of 15%. They suggest to analyse about 200 offspring which gives a possible error range of 10% between the two estimates of EPP. Thus a N of 200 offspring is considered a good compromise between the costs of further sampling and the potential reduction in error to be gained. Therefore I also aim to analyse at least 200 offspring or all completely sampled broods of 2004 (see Appendix 3). If time and budget allow I can always analyse more broods to increase the possibility of detecting CBP.

Already existing samples

20 to 40 microliter (0.02-0.04ml) of blood are necessary to perform a successful DNA-Extraction with the Qiagen extraction tubes. We stored the blood with 10 fold dilution in Queen Lysis buffer which makes 0.22 to 0.44 ml of buffer-blood mix necessary for a successful extraction. Our blood samples are stored in 1.2 ml Eppendorfer tubes, which means that the tube has to be a little less than half full (with the assumption of a ration blood-buffer 1:10) to contain enough DNA. I distinguished in the filemaker database three amounts of blood: “enough” (more than half full; >0.6 ml), “medium” (around 0.5 ml) and “little” (less than 0.5 ml, very bright red which shows a high dilution), assuming that samples considered as “little” don’t have enough blood for a successful extraction whereas. Therefore, completely sampled broods (social father and mother inclusive all fledglings) containing samples with too little blood where excluded from our reference sample for the previous years (Table 4).

Statistical analysis

If I have time to investigate additional previous years samples I will do a comparison between the years. If there is no significant difference (for example an increase in CPB or EPP over the years going alongside with increase in density) I can pool all years together for the subsequent analyses.
The adults will be assigned to their offspring comparing the length of the different microsatellites. This will be done on a specific computer program eventually also with an assignment test.

Sampling unit

We will have each brood as a statistically independent unit. To avoid pseudoreplication we exclude broods with adults which have been sampled on different years so that we never include the same individuals twice.

Possible constraints

Field:

-Weather is very unpredictable if hot summer big amount of work, if bad summer less work, if very hot and dry weather too many breeding adults to catch in time.
-Difficult, sometimes impossible, to catch both parents of a breeding pair. We have to get as many complete families as possible.
-The dead chicks are very difficult to collect as they often get eaten by the other chicks or parents or are easily lost in other ways if not collected quick enough.

Laboratory:

-I will be the first working in our newly installed DNA-Lab at the Erlachstrasse. Due to this problems of any kind may occur in a new, untested laboratory; Machines have to be installed and calibrated of an experienced person.
-Time necessary for adjusting the microsatellites until they work perfectly may be longer than expected. Calculation was done according to opinions of people who work with microsatellites: another 2-3 weeks might be necessary.
-Even if the PCR-Machine is the same, there can occur differences in the PCR due to maintenance differences and other unknown factors. If this happens this can cause big delays.

Budget and Material

Amount	**Material**	**Who** [1)]	**Costs**
	Field		
1	Car (Fiat)	U	670.- [1)]
2-3	Mist nets (2x3m, 1x9m)	R. Arlettaz	?
4-6	Poles for nets	R. Arlettaz	?
	Tent pegs to fix the nets	R. Arlettaz	
	Line / rope to fix poles	R. Arlettaz	
	Bird rings	R. Arlettaz	
	Ringlists	R. Arlettaz	
1	„Beringunszange“	R. Arlettaz	
1	Compass	U	
	Maps of study area	FL	
1	Mobile phone	FL	
	Protocols	FL	
500	Eppendorf tubes	U	
1	Freezer (in House in Sion)	U	
1	Laptop	FL	
1	Binocular	FL	
1	Subscription for 1 Month Lausanne-Bern for “Forschungspraktikum für Fortgeschritten“ (18.01.2004)	U	223.-
1	Gleis 7 and Halbtax	FL	
1?	Caliper rule	R.Arlettaz	

3	Bags to store birds	R. Arlettaz	
1	Light bulb with mirror	R. Arlettaz	Ca. 40.-
Ca. 200	Envelopes for feather storing	FL	5.-
Ca.70	Mikrohämatokryten	FL	
	"Spritzennadeln" 0.5mm durchmesser	FL	
	Fieldjournal	FL	
	Lab (consumable material)		
1	Queen lysis buffer	FL	140.-
2	QIAamp DNA Blood Mini Kit –for 50 extractions	FL	434.- [3)]
2	QIAamp DNA Mini Kit –for 250 extractions	FL	1371.-[4)]
1	Primer 925 ned	FL	300.- [5)]
20	Fluorescent Primers	L	[6)]
1	Taq DNA Polymerase (250 U)	FL	860.- [7)]
TOTAL			**4063.-**

0) Person/Instiution that owns or orders or is responsible for the material:
U = University of Bern
FL = Fabio Leippert
L = University of Lausanne

1) (1.33 SFr per litre gas, 8l /100km, 3,5 months field season (7 days per week, 5 working days per week. Total of ca. 14 weeks x 5 days = 70 working days. Approximately 90 km per day, as the study site is 45 km long.)
Calculation: 70*90=6300km; 63 x 8 x 1.33 ≈ 670 SFr.

3) 2 Mini Kits à 217.- = 434.- (QIAamp DNA Blood Mini Kit –for 50 extractions 217.- ; expected 100 extractions → 434.-)

4) 1 Mini Kit à 1121.- for 250 extractions (QIAamp DNA Mini Kit –for 250 extractions)
1 Mini Kits à 250.- for 50 extractions
If I am going to do the sexing of the remaining chicks of 2004 and 2003 we would need an additional number of about 350 extractions which would cost additional about 1121.- (250 extractions) + 250 (50 extractions) = 1376.-

5) To be ordered at the beginning of the labwork

6) Already bought in Lausanne, volume should last for my analyses.

7) Taq-polymerase: 0.1 µl per PCR reaction needed. 50µl in one tube (250 units). 500 PCR's possible with one Tube. 8 Primers per Individual, 300 Individuals =2400 reactions.
1 Taq DNA Polymerase (1000 U; 4 tubes à 50 µl) costs 665.-
1 Taq DNA Polymerase (250 units) costs 195.-

Expected results

Even though the European Hoopoe *(Upupa e. epops)* is still numerous in southern locations, mainly Spain, it makes much sense to protect it in Switzerland because populations at the edge of their distribution area bear a great value due to their potential of local adaptations which drastically increase overall genetic diversity. Conserving marginal populations is therefore a goal of Conservation Biology. For this reason a program was launched in order to offer artificial breeding cavities in Valais. After this has been introduced with remarkable success, manifested in a dramatic population increase, it is important to know if an unlimiting offer of nestboxes may have detrimental effects. Is the social system of the Hoopoe altered by this offer of nestboxes? Do we have to optimize the number of nestboxes provided? Does this possibly apply to all nestbox programmes?

If we can detect CBP, this may point to future problems since this Hoopoe population is expected to increase further. Given that no CBP was found in a natural Hoopoe population in Grenada, Spain, these may be the first signs of an alteration of the social system.

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Appendix

Table 1: Breeding success Valais between 1998 and 2003

YEAR	ZONE	NO. CLUTCHES		NO. EGGS		PROPORTION OF HATCHED EGGS	NO. FLEDGLINGS	PROPORTION FLEDLINGS/ HATCHED	PROPORTION FLEDGLINGS/ TOTAL EGGS	NO. HATCHED PER NEST	NO. FLEDGLINGS/ CLUTCH	REMARKS
		SURE	TOTAL	TOTAL	HATCHED							
1998	A	16	16	104	77	0.740384615	68	0.883116883	0.653846154	4.8125	4.25	good weather
1999	A	14	16	93	23	0.247311828	13	0.565217391	0.139784946	1.642857143	0.928571429	
	B	2	3	15	3	0.2	3	1	0.2	1.5	1.5	
	TOTAL	16	19	101	26	0.257425743	16	0.615384615	0.158415842	1.625	1	bad weather
2000	A	20	20	142	104	0.732394366	81	0.778846154	0.570422535	5.2	4.05	
	B	11	12	70	64	0.914285714	55	0.859375	0.785714286	5.818181818	5	
	C	7	7	22	22	1	17	0.772727273	0.772727273	3.142857143	2.428571429	
	TOTAL	39	39	239	195	0.815899582	158	0.81025641	0.661087866	5	4.051282051	good weather
2001	A	21	21	131	93	0.709923664	61	0.655913978	0.465648855	4.428571429	2.904761905	
	B	11	12	73	59	0.808219178	47	0.796610169	0.643835616	5.363636364	4.272727273	
	C	6	6	35	24	0.685714286	19	0.791666667	0.542857143	4	3.166666667	
	D	5	9	23	15	0.652173913	13	0.866666667	0.565217391	3	2.6	
	TOTAL	43	48	262	191	0.729007634	139	0.727748691	0.530534351	4.441860465	3.23255814	bad weather
2002	A	15	15	104	72	0.692307692	56	0.777777778	0.538461538	4.8	3.733333333	
	B	12	12	74	61	0.824324324	56	0.918032787	0.756756757	5.083333333	4.666666667	
	C	7	7	49	37	0.755102041	33	0.891891892	0.673469388	5.285714286	4.714285714	
	D	17	17	120	92	0.766666667	66	0.717391304	0.55	5.411764706	3.882352941	
	TOTAL	51	51	347	262	0.755043228	211	0.805343511	0.608069164	5.137254902	4.137254902	
2003	A	17	17	127	114	0.897637795	102	0.894736842	0.803149606	6.705882353	6	
	A'	1	1	4	4	1	4	1	1	4	4	
	B	22	22	155	134	0.864516129	106	0.791044776	0.683870968	6.090909091	4.818181818	
	C	3	3	23	15	0.652173913	14	0.933333333	0.608695652	5	4.666666667	
	D	20	20	161	146	0.906832298	105	0.719178082	0.652173913	7.3	5.25	
	TOTAL	63	63	470	413	0.878723404	331	0.801452785	0.704255319	6.555555556	5.253968254	Very hot summer
MEAN						**0.73947202**		**0.82176458**	**0.61087537**	**4.66243198**	**3.833304518**	

Table 2: Comparison breeding succes from the literature:

NO. CLUTCHES	NO. EGGS	PROPORTION OF HATCHED EGGS	NO. FLEDGLINGS	PROPORTION FLEDLINGS/ HATCHED	PROPORTION FLEDGLINGS/ TOTAL EGGS	NO. HATCHED PER NEST	NO. FLEDGLINGS/CLUTCH	REMARKS
		0.73947202		**0.82**	**0.61**	**4.66**	**3.83**	**Valais 1998-03**
		0.71		0.47			2.97	Granada Spanien (Martín-Vivaldi et al. 1999c)1996
21		0.8	71	0.592	0.473		3.4	Mähren (Glutz von Bolzheim 1980)
			39				4.3	TÜP Jüteborg West (Oehlschlager)
							3.5	TÜP Jüteborg Ost(Oehlschlager)
			21				5.3	Spreewald (Oehlschlaeger & Ryslavy 2002)
3	6.2	0.76						Kaiserstuhl(Stange & Havelka 2003) 1993
14	8.4	0.86					5.2	Kaiserstuhl (Stange (Stange & Havelka 2003) 2002
	7.3	0.655		0.794	0.5252		4.5	Markgräferland (Baldi & Sorace 1996)
Ø	**7.3**	**0.75407867**		**0.669**	**0.5360667**	**4.66**	**4.125**	

Table 3: Density

0.3 bp/km2	"alle anderen Abundanzwerte in Mitteleuropa bei 0.3 "		In mittelmeerländern Nestabstände von nur wenigen 100 m möglich Mitteleuropa 1-2km "höchste Dichte in Mitteleuropa im Wallis"	(Glutz von Bolzheim 1980)
0.15-0.16bp/km2	TÜP Jüteborg Ost	Deutschland		(Oehlschlaeger & Ryslavy 2002)
0.22-0.26 bp/km2	Jüteborg West	Deutschland		oehlschlager
1.1-1.4 bp/km2	Wriezen	Deutschland		oehlschlager
0.24-0.34 bp/km2	Müllrose	Deutschland		oehlschlager
0.75-1.4bp/km2	Oberspreewald	Deutschland		oehlschlager
0.8-2.3bp/km2	Polana Gebirge	Slovakei		oehlschlager (aus kristin)
0.34 bp /km2	Neusiedlergebiet	Deutschland		(Dvorak et al 1993)
0.04-0.6 bp/km2	Truppenübungsplätze	Deutschland		(Robel & Ryslavy 1996)
1-2.69 bp /km2	2 sehr kleine Tüp	Deutschland		(Robel & Ryslavy 1996)
2.1-2.5 territories /km2	**Extremadura, Spanien 1)**		**Fläche 34km2, vergleichbar mit Wallis**	**(Rehsteiner 1996)**
1.1-1.6 bp /km2	Wallis	Schweiz		(Arlettaz 1983)
1.4 territories /km2 up to 3 territo-ries/km2	**Wallis**	**Schweiz**		**Comparison of suivis200-2003**
5.5-5.7 bp/km2	Grandada	Spanien		(Rehsteiner, briefl. Von Martin-Vivaldi)

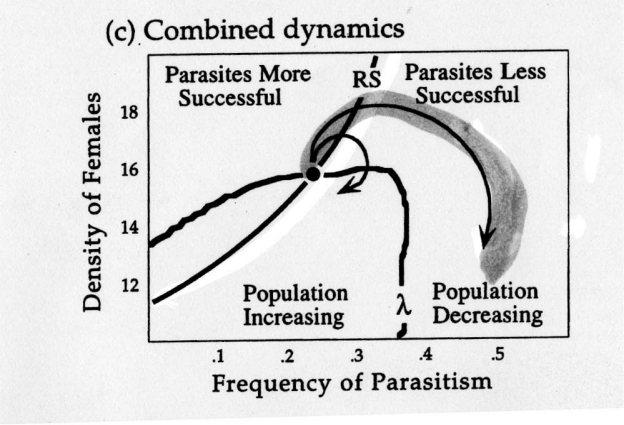
1) very variable, up to 12-14 bp /km2 („ in aufgelockerter Siedlungsfläche und in Landwirtschaftsflächen mit Schafställen und Bauernhöfen.“)

Table 4: Completely sampled families

Completely sampled families (social father and mother inclusive all fledglings) with bloodsamples assumed to contain enough blood (classified as “enough” and “medium”) for an extraction of the previous years are:

2003	25 families	220 individuals	220 extractions to do (55 blood; 165 feather)
2002	17 families	141 individuals	141 extractions to do (39 blood; 102 feather)
2001	18 families	116 individuals	0 extractions to do
2000	10 families	85 individuals	0 extractions to do
Total:	**71 families**	**574 individuals**	**373 extractions to do (94 blood, 167 feather**)

Appendix 1: Modelling population dynamics



Appendix 2: Simulation extinction risk

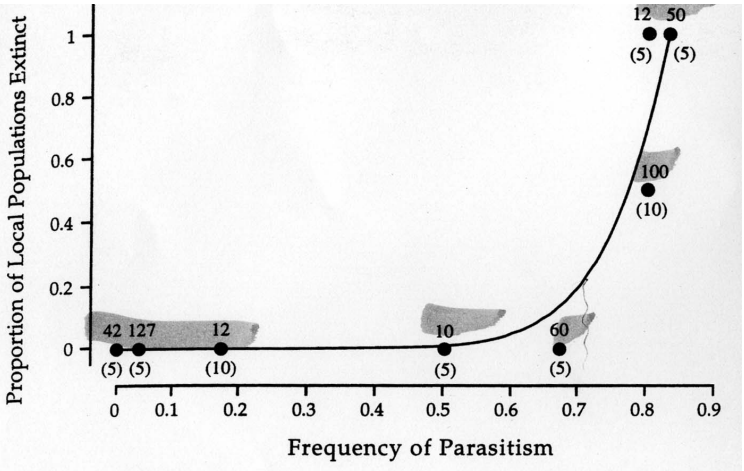


Figure 12-9 Results of a simulation model on the likelihood of local population extinction as a function of the frequency of conspecific brood parasitism. Values above each point indicate the population size for that set of simulations, and the values in parentheses below each point are the number of simulations conducted for those parameters. The logistic regression fit to the points is given by the equation $Y = 5.778 \times {}^{9.414}$.

Appendix 3

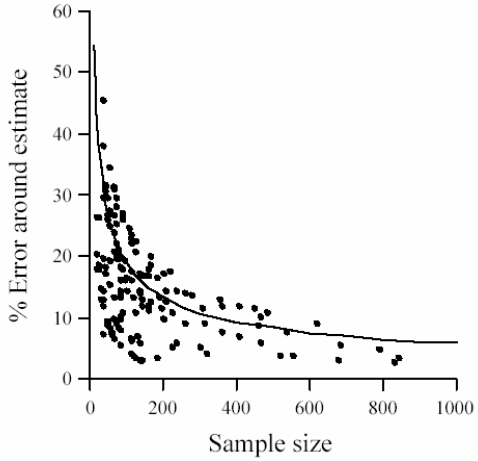


Fig. 2 The magnitude of error around actual estimates of EPP levels against the sample size of those studies. '% error' on the uertical axis refers to the magnitude of the difference between the upper and lower 95% confidence intervals around an estimate. The line plotted is this '% error' for a hypothetical population with a rate of 15% EPP across different sample sizes.

Recipe Queen lysis buffer

For a stock 10 times concentrated you need (Seutin et al. 1990):

0.1M Tris pH 8.0
0.1M NaCl
0.1 M EDTA
10% N-lauroylsarcosine (SLS) pH 7.5