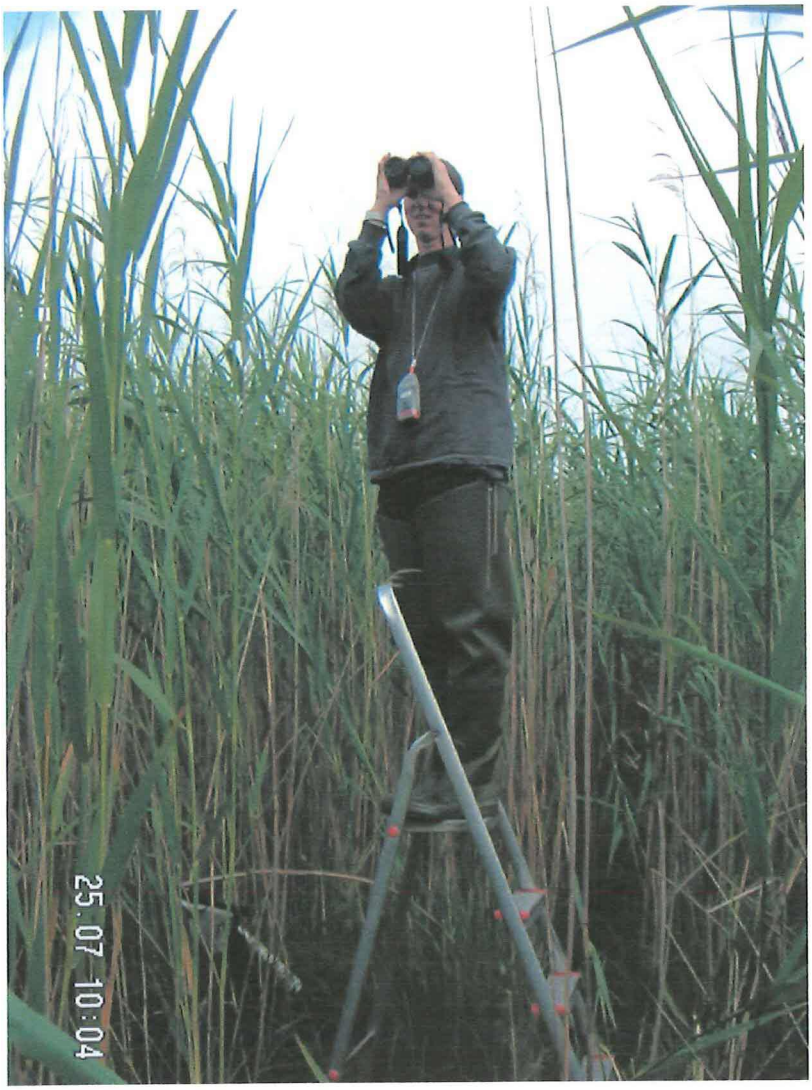
Paternal care for non-related offspring in the Reed bunting



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Summary

In this study I investigated paternal care in reference to the proportion of extra pair young in the nests. The study organism was the Reed bunting *Emberiza schoeniclus,* a passerine bird, with a high rate of extra pair paternity. The study site is located on the Southeast shore of lake Neuchâtel in Switzerland. The data were collected during the field season 2004. To our knowledge we are the first group videotaping nests for the whole nestling period and during the whole day. Paternity was assigned using microsatellite techniques. The results of this study showed first that females invest significantly more in offspring than males do. Second, there is a negative correlation between parental care and the proportion of extra pair young in double-brooded pairs. Third, there is no correlation between paternal investment and the proportion of extra pair young on day two and day six in single-brooded pairs.

Zusammenfassung

In dieser Studie untersuchte ich, welchen Beitrag der Vater zur Aufzucht der Jungen im Verhältnis zur Anzahl der fremden Jungtieren im eigenen Nest leistet. Als Organismus diente die Rohrammer *Emberiza schoeniclus,* ein Vogel aus der Familie der Singvögel mit einer hohen Rate von fremden Jungtieren. Das Forschungsgebiet liegt am Südostufer des Neuenburgersees in der Schweiz. Die Daten wurden während der Feldsaison 2004 gesammelt. Unseres Wissens sind wir die erste Gruppe, die Nester während der ganzen Nestlingsperiode gefilmt hat. Die Vaterschaft wurde mittels Mikrosatellitentechnik bestimmt. Die Resultate der Studie zeigen: 1. Weibchen investieren signifikant mehr in die Aufzucht der Nachkommen als Männchen. 2. Es gibt eine negative Korrelation zwischen der Investition des Paares in die Jungen und der Anzahl von fremden Jungtieren bei Paaren, die zwei Bruten in einer Saison aufzogen. 3. Am zweiten und sechsten Tag gibt es keine Korrelation zwischen der Investition der Männchen und der Anzahl fremden Jungtieren bei Paaren, die nur eine Brut aufzogen.

Résumé

Dans cette étude, j'ai examiné la contribution que le mâle souscrit par rapport au nombre de jeunes produits hors couple. Comme organisme j'ai choisi le bruant des roseaux *Emberiza schoeniclus*, un oiseau de la famille des passerines qui a beaucoup de jeunes produits hors couple. Le site de recherches se situe sur la rive sud-est du Lac de Neuchâtel en Suisse. Les données ont été récoltées durant la saison 2004. A notre connaissance, nous sommes le premier groupe qui a filmé les nids pendant toute la période d'élevage. La paternité a été déterminée par la technique de microsatellites. Les résultats indiquent en premier que les femelles s'investissent de façon plus significative dans l'élevage des jeunes que les mâles. Deuxièmement, il y a une corrélation négative entre l'investissement des couples et le pourcentage de jeunes produits hors couple chez des couples qui ont nourri deux nichées dans une saison. Troisièmement, il n' y a pas de corrélation entre l'investissement des mâles et le pourcentage de jeunes produits hors couple au deuxième et au sixième jour chez les couples qui ont nourri seulement une nichée dans une saison.

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1 Introduction

Social monogamy is the most common breading system in birds. Usually male and female contribute to brood care (Lack, 1968; Silver *et al.*, 1985). Since the early nineties reliable molecular paternity analysis is possible (Westneat *et al.*, 1990; Birkhead *et al.*, 1992; Griffith *et al.*, 2002). There is evidence that extra pair paternity (EPP) is wider spread than assumed. Trivers (1972) proposed that monogamous males should pursue a "mixed" reproductive strategy in which they seek for extra pair copulation (EPCs) but invest only in the offspring of their bonded mates. Parker *et al.* (2005) took up Trivers idea. They investigated three different models. In one they assumed a socially monogamous bird population in which each male has a social mate to whom it is bonded, but where EPCs can occur; EPCs and hence EPFs (extra pair fertilisation) are assumed to be at their ESS (evolutionarily stable strategy) levels. Only deviations in male parental care were taken into consideration. Parker *et al.* (2005) came to the conclusion that only if the function of the fitness of each offspring follows diminishing returns it will be an ESS for a male to invest exclusively in his home nest, provided that this is where he has sired the greatest expected number of offspring.

The Reed bunting is a socially monogamous passerine bird species with a high rate of extra pair paternity (EPP) (Dixon *et al.*, 1994: 55% of young and 86% of nests contained at least one extra pair young; Bouwman *et al.*, 2005: 49.7% of young and 80% of nests contained at least one extra young). Extra pair paternity is defined as offspring of a male other than the male attending the nest whereas intra pair paternity (IPP) is defined as offspring of the male attending the nest (Bouwman *et al.*, 2005). Almost all females have extra pair young. It seems that females accept or even seek for extra pair copulation in spite of risking the loss of their mate's solidarity. This breeding system results in different clutch patterns, clutches only with intra pair young (IPY) or only with extra pair young (EPY) and mixed broods. Dixon *et al.* (1994) showed that Reed bunting males adjust their feeding effort to their amount of paternity whereas Buchanan (2001) and Bouwman *et al.* (2005) couldn't find such a relationship. How males could estimate their paternity is not known but there could be several cues: males could estimate the number of fertile females in the same period nearby (Margarth *et al.*, 1997); males could assess the likelihood of being cuckolded (Kempenaers *et al.*, 1996); olfaction after the eggs hatched is a cue in bluegill sunfish (*Lepomis macrochirus*) (Neff *et al.*, 2003).
Parental care consists not only of feeding the young although it is the greatest part. As females build the nest alone other parental investments of males are breeding, covering the young and defending the nest site. Breeding and searching for food might constrain males from advertising and going for extra pair copulation (EPC).

For my thesis I investigated the question: Does the male investement in parental care correlate with paternity? The hypothesis to test is: The more extra pair young there are in a nest the less the social male should invest in parental care.

2 Material and Methods

2.1 Biology of Reed bunting

Reed buntings (*Emberiza schoeniclus*) are distributed in the palaearctic fauna in wetlands (lakes, moor) or along watercourses. Reed buntings are social monogamous passerine birds. Males weigh in average 20.0 g and females 18.4 g. There is a strong dimorphism between males and females: males have a black head and breast patch and a white neck band, females are brownish. (**Figure 1**)

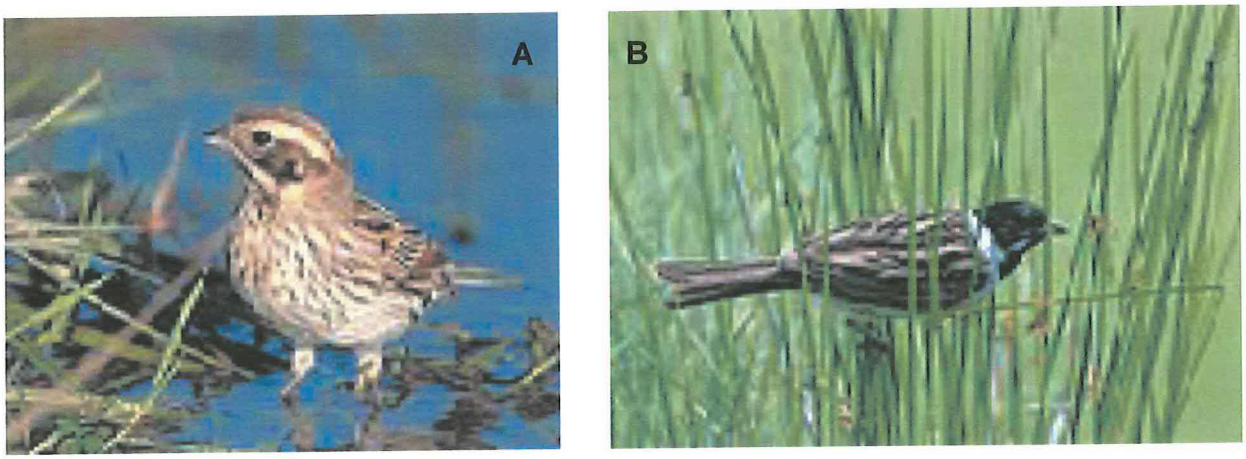

Figure 1 Reed bunting (www.fotonatur.de); **A** female; **B** male in breeding plumage

Reed buntings breeding in Northern Switzerland spend the winter in Spain or southern France. They arrive at the breeding site between February and May. Males start to sing to defend their territory and to attract females (Blümel, 1995). Food is mostly collected in nearby forests or on the lakeside, rarely in the territories. They form social pairs and usually raise two broods during one breeding season. Females choose a site to build a nest 10 to 30 cm above water level hidden in the vegetation. The construction period lasts between 2 and 7 days (Blümel, 1995). Males do not help to build the nest. Females lay 4 to 6 eggs. The incubation period is about 12 to 14 days and the nestling time lasts from 8 to 12 days.

2.2 Study site

Our study site is located at the Southeast shore of lake Neuchâtel in Switzerland in the "Grand Cariçaie" at Gletterens (46°54' N / 6°56' E) (**Appendix 1**). The Southeast shore of lake Neuchâtel is girded with a reed belt and marshland of about 800 ha. The typical vegetation consists of reed (*Phragmites australis*), great fen-sedge (*Cladium mariscus*) and tufted sedge (*Carex elata*). The study site in Gletterens has an area of 32.25 ha. A mowing regime has been established in 2000. Therefore the study site contains plots of reed with age between one and five years after mowing in winter (**Figure 2**).

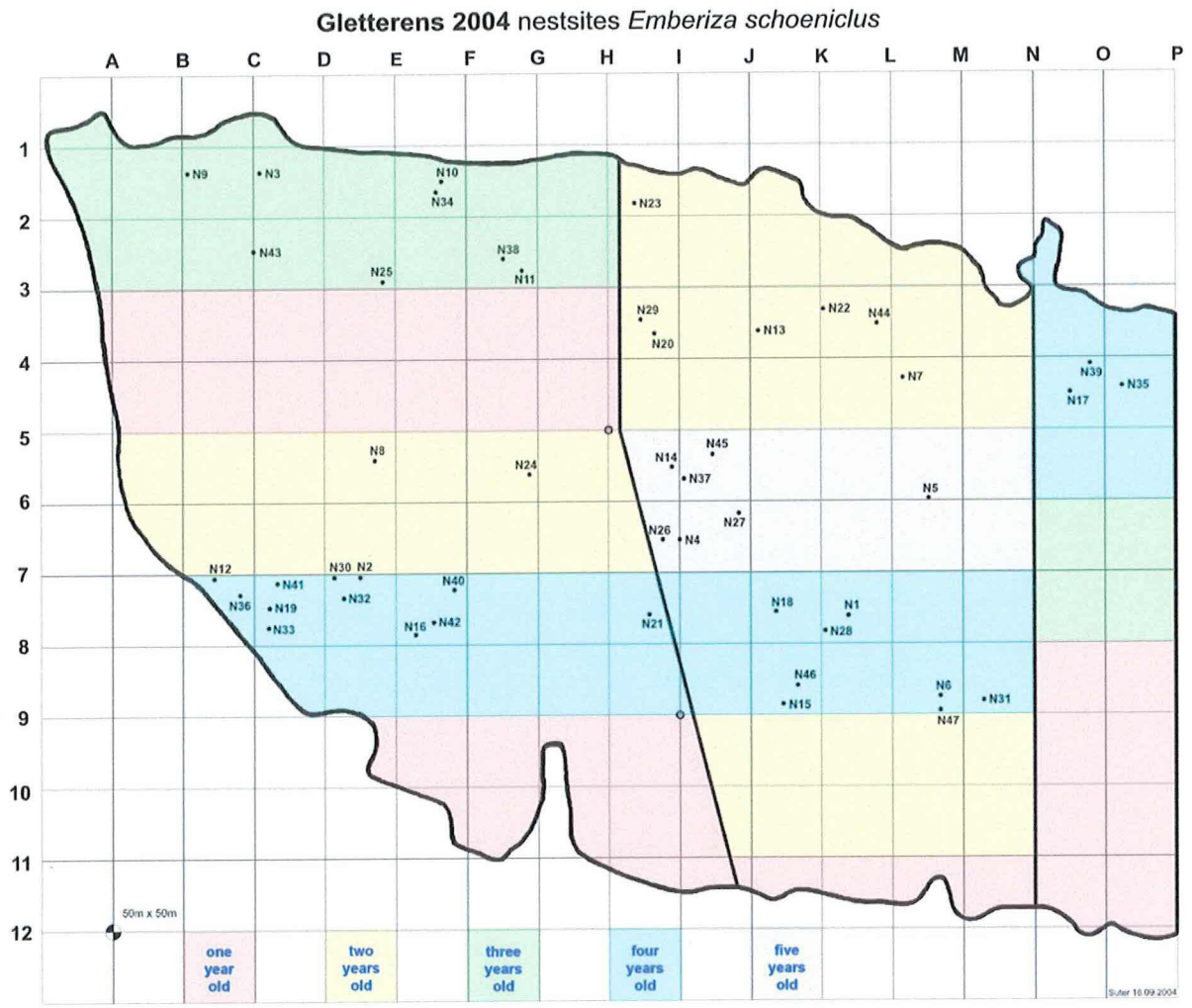

Figure 2 Study site with mowing regime in 2004. The N# indicate the nests in the order we found them.

2.3 Field work

First we laid a virtual grid of 50 x 50 m^2 over the study site. The corners were marked with high bamboo sticks. We mainly moved along the lines of this grid to avoid disturbing the vegetation. Distances where determined with a laser measuring device ("Geovide", Leica).

Observation

Through observing males and writing down every singing position and movements on maps we could determine the territories. Nests were found through observations of the females during the construction or incubation period when females returned to their nests. Nests were controlled regularly to determine the exact hatching date.
25.5 % of the nests where lost, ten nests through predation, two nests through high water level. Whereas from one of the two nests we have information about IPY/EPY (12/47 nests).

Ringing and blood sample

Adults where caught with mist nets placed around their nests on day 4 or 5 after hatching of their young. The nestlings were ringed on day 8 after hatching. All birds were ringed with 3 coloured plastic rings and a numbered aluminium ring from the Swiss ornithological station "Vogelwarte Sempach" for individual identification.
The following size parameters were measured by adults and nestlings:Tarsus length (mm); wing length (mm); 8th primary length (mm); tail length (mm); bill culmen length (mm); bill nostril-tip length (mm); bill width (mm); bill height (mm); weight (g) and fat/muscles (categories from 1 to 3). We also looked for ectoparasites.
About 50 µl blood was taken from the brachial vein, collected in a 70 µl capillary tube and stored on ice in the field and then at -18° C for paternity analyses.
Dead young and eggs which didn't hatch were collected.
Photographs from the patch of each male were taken in a standardised way with a Konica/Minolta Di MAGE x20 digital camera with the help of a constraining transparent tube (**Figure 3**).

Figure 3 Standardised photograph from a male's patch

During the field work 2004 we ringed and collected blood samples from 58 adults (28 male; 30 female) and 161 offspring (4 eggs, 12 dead young, 15 after leaving the nest, 130 eight days old fledglings) out of 47 nests.

Video

CMOS Video cameras CLVMPC2 were placed about 10 to 15 cm away from the nest. The ARCHOS AV140 Video Recorders were placed about 5 m away from the nest in a box to protect them from rain and intense sunshine. The video recorders and two full Sonnenschein A506/4.5 ks (Akku 6.0 V 5 Ah, Pb) batteries were placed in the box before 6 a.m. The video recorders were programmed to record from 6 a.m. until 12 a.m., 12 a.m. until 6 p.m. and 6 p.m. until 10 p.m. After 8 p.m. we started to collect the video recorders and the batteries. The video films were then transferred through a G5 MAC computer to a LaCie 400 GB hard disk. Recordings were made from the hatching day until the last nestling left the nest. In two cases we installed a camera nine and eight days before hatching.

2.4 Laboratory work

DNA Isolation

DNA was isolated from blood samples, dead young and from embryos in the eggs. For the DNA isolation we used E.Z.N.A.® Blood DNA Mini Kits (Classic-Line) following the protocol on page 5 of the manual. The main steps are lyse; charge and binding; wash I; wash II; elution (contains DNA). The two elution of every bird are stored in 2 ml tubes in different freezers at about -20° C.

Sexing of the young

In birds males are homogametic (ZZ) and females are heterogametic (ZW). To determine the sex of the young we used the CHD-W gene (chromobox-helicase-DNA-binding gene) that appears on the W-chromosome and the CHD-Z gene that appears on the Z-chromosome. A single set of PCR primers (P2 / P8) can be used to sex birds. CHD-Z occurs in both sexes. It should also be amplified to serve as a control (**Figure 4**). The PCR products vary in size. Gel electrophoresis reveals one band in males (about 345 Kb) and two in females (second band at about 300 Kb) (Griffiths, 1998).

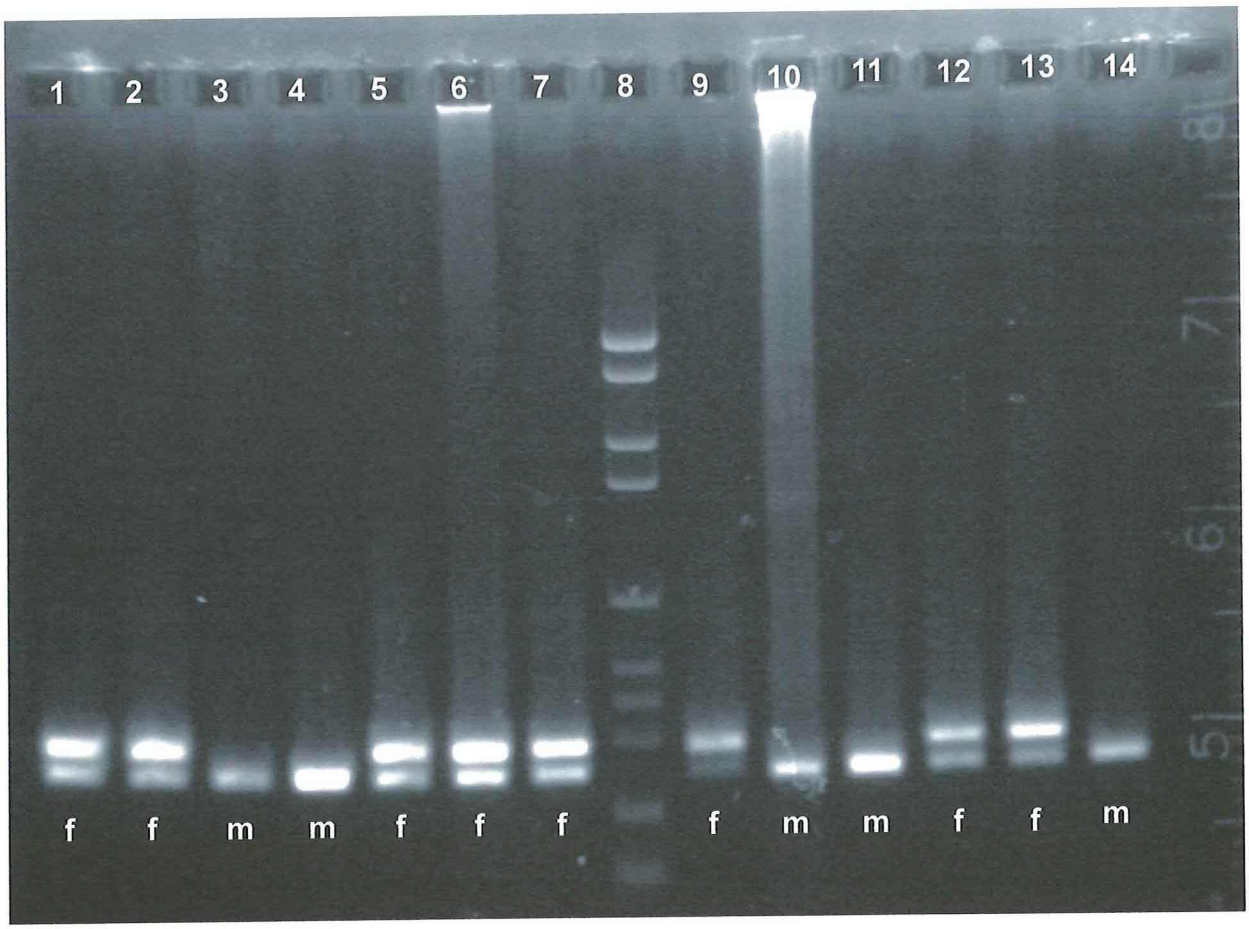

Figure 4 example of a gel electrophoresis for sexing of the young. Hole 1-7: young; hole 8: standard; hole 9-12: young; hole 13 and 14: adults for control.

Paternity

To determine paternity 5 microsatellites were used. Microsatellites loci consist of tandem repeats of short DNA sequences (≤ 5 bp), usually of less than 100 bp total length. These loci can be polymorphic due to variation between alleles in the number or repeat units. We used four Reed bunting primers (Escμ1, Escμ3, Escμ4 and Escμ6) (Hanotte, 1994) and one House sparrow (*Passer domesticus*) primer (Pdo5) (Griffith, 1999) (**Table 1**).

Locus	Repeat motif	Number of alleles	Primer sequences (5'-3')	colour of fluorescent dye
Escμ1	(CA)	12	F: TTCTCTTGGTCTATGGAAGGTG R: GCTTGAAAGACAGTCACCAGG	blue
Escμ3	(TC)	17	F: CTCCTGACAGAGTTTTTCTGGT R: TGCTTGTGGTTGTCAAGAT	green
Escμ4	(TG)	14	F: TTCCCTCACAATTTTCCGAC R: TATGTGCTGAAGTGAACCATCC	green
Escμ6	(CA)CG(CA)GTA(CA)	12	F: CATAGTGATGCCCTGCTAGG R: GCAAGTGCTCCTTAATATTTGG	blue
Pdo5	(CA)	20	F: GATGTTGCAGTGACCTCTCTTG R: GCTGTGTTAATGCTATGAAAATGG	yellow

Table 1 The five microsatellites loci used for the determination of the paternity

By PCR technics microsatellites were amplified. The forward primers were 5' endlabelled with a specific fluorescent dye in different colours for multiplex amplification length analysis in an ABI 310 Genetic Analyzer (Applied Biosystems Instrument). In one run we mixed Escμ3 and Escμ6 or Escμ1, Escμ4 and Pdo5 together.
Each mother and each father transmit one allele of these repeated sequences of specific length to their offspring. First we compared the alleles from the social mother to the offspring in one nest. Afterwards we compared the alleles from the social father or neighbouring males if the social father's alleles did not match to the offspring's allele in the nest. Fatherhood was assigned when at least 4 of 5 loci matched (**Table 2**).
The 15 offspring we caught after leaving the nest came to our territory out of the surrounding sites. They could not be assigned to neither a female nor to a male. There was no allusion to cuckoldry, so the social female of a nest was always the genetic mother of all the offspring in a nest. 4.8 % of the eggs or offspring could not be assigned to a father (7/146 eggs or offspring). This was due to the fact that no or not enough DNA could be isolated, especially out of eggs. In one nest (N25) we could not catch the female because of heavy rain and drown of the nest. For 5.8 % of the offspring where we could assign a father we could not say whether they are intra pair young or extra pair young (8/139 offspring). These 8 offspring came out of two nests (N13 and N15) where we only observed the female feeding. We assume that these two females did not have a social partner. 32.1 % of the offspring are extra pair young (42/131 offspring) and 58.8 % of the nests contained at least one extra pair young (20/34 nests).

	Escμ1		Escμ4		Pdo5		Escμ3		Escμ6	
status	1st allele	2nd allele	1st allele	2nd allele	1st allele	2nd allele	1st allele	2nd allele	1st allele	2nd allele
extra pair male	135,95	144,25	150,32	174,41	249,01	253,22	156,19	156,19	126,88	134,50
social male	136,41	150,76	147,71	154,54	232,00	269,17	152,18	154,15	129,91	129,91
social female	136,66	136,66	150,00	171,83	227,74	256,59	152,56	152,56	127,71	141,77
extra pair young	136,24	144,25	150,00	174,00	227,95	252,70	156,18	156,19	135,35	141,62
extra pair young	136,24	144,25	150,00	174,04	227,86	248,43	152,14	156,19	135,22	141,68
intra pair young	136,32	150,75	154,60	171,86	256,74	269,18	152,26	154,21	127,64	129,57
intra pair young	136,42	136,41	154,49	171,78	227,91	269,30	152,01	154,01	129,98	141,88

Table 2 Example of nest no. 26. One allele is inherited from the mother, the other from the father. The extra pair male ist homozygous in Escμ3, the social male in Escμ6 and the social female in Escμ1 and Escμ3. The alleles of Escμ3 from the first extra pair young couldn't be assigned to the social female, male or to the extra pair male.

2.5 Video analysis

Cameras were placed at 22 nests in the eastern part of the study site (**Appendix 4**). From mid May until mid July we collected 1990 hours of video material. For video scanning I used the program Quicktime Pro for MAC OSX. First the films were cut in one hour pieces to display a picture every 4 seconds instead of every 40 seconds in 6 hour films. Thus I made sure not to miss a visit from the parents. Some visits late in the nestling period lasted not more than two seconds but thanks to the behavioural change of the young shortly before the parents arrive a picture every 4 seconds was sufficient. For every visit, the visiting parent, the duration of the visit, the activity and if possible, the kind of food, was written down in a table for each nest. The following activity categories were established: feeding, covering, cooling, toilet (removing the excrement from the young), order (diving head first into the nest) and control (watching on the nest rim). Cooling behaviour was only manifested at high temperatures. Sometimes I could not distinguish where order started and covering ended. Therefore I put order, covering and cooling in the same category (covering).
In order to devise a system of spot-checks I decided to screen first the complete set of recordings from four pairs including first and second brood. Feeding, covering and total activity (all categories together) corrected with the total amount of recording time per nest were calculated (**Formula 1**)

$$\text{category rate corrected} = \frac{\text{sum or count of activity}}{\text{total duration of video in seconds}} \qquad \textbf{(1)}$$

The analysis revealed that until day two after hatching covering was the greatest part in brood care (**Figure 5**). From day six after hatching feeding was the greatest part in brood care (**Figure 6**). The analysis also showed that the busiest time of the day is the morning (**Figure 7**). Due to equipment failures we were left with many incomplete recordings. To obtain a representative data set I decided to screen recordings on day two (9 nests) and day six (12 nests) after hatching from 7 a.m. until 11 a.m.
For the analysis on day two from 7 a.m. until 11 a.m. I measured covering and total activity (all categories together) in seconds.
For the analysis on day six from 7 a.m. until 11 a.m. I measured feeding and total activity (all categories together) in counts.
All analyses were performed with JMP 5.0.1.

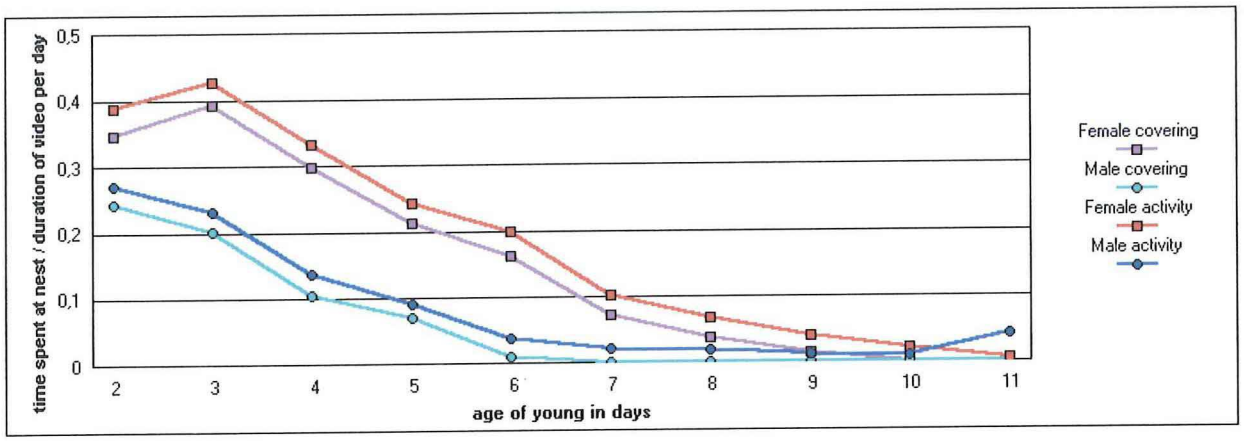

Figure 5 Distribution of male and female total activity and covering rates, from the data set of pairs breeding twice during nestling period (n=8).

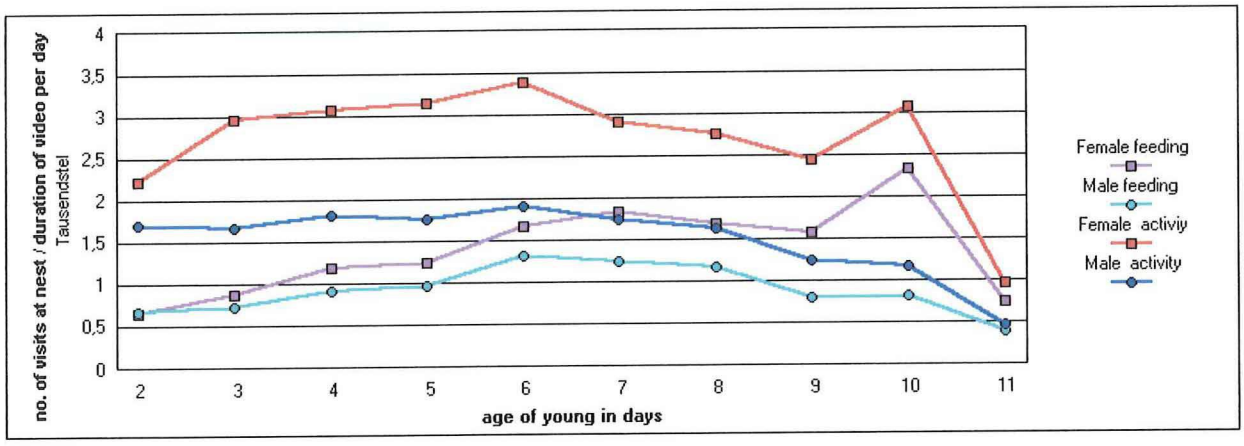

Figure 6 Distribution of male and female total activity and feeding rates, from the data set of pairs breeding twice during nestling period (n=8). Between day 10 and 11 the first young already left the nest.

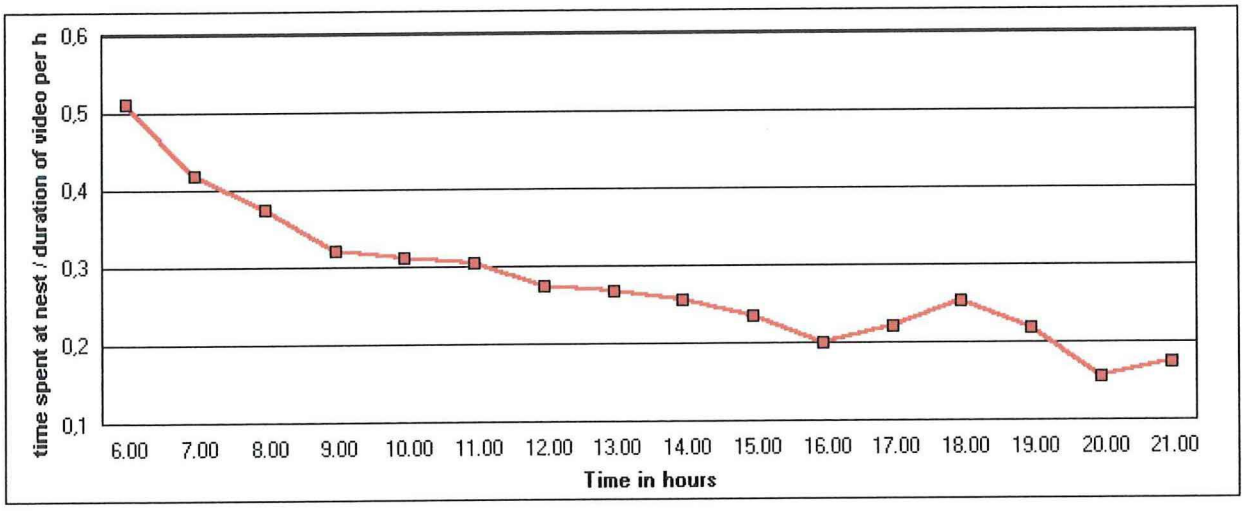

Figure 7 Distribution of male and female activity rate, from the data set of pairs breeding twice during a day (n=8).

2.6 Temperature as a factor of weather conditions

Weather condition is a factor that can influence the behaviour of the birds in parental investment. Weather conditions changed from the beginning until the end of the season. The day 2 or day 6 in different nests was not on the same date. I first controlled whether temperature, as a factor of weather conditions, has an influence in parental investment. It could be that a couple covers the offspring longer because of low temperature at that time or a couple feeds less because at that point there is not much food available because of lower temperature. Temperature data were provided by MeteoSchweiz.

To test whether temperature influences parental investment in offspring an analysis of variance (ANOVA) was made (**Figure 8**). Mean temperature is defined as the mean from 5 measurements (one measurement per hour) between 6:40 a.m. and 11:40 a.m. There were no significant correlations between mean temperature and all activities that were tested. For that reason temperature was not included in further analysis.

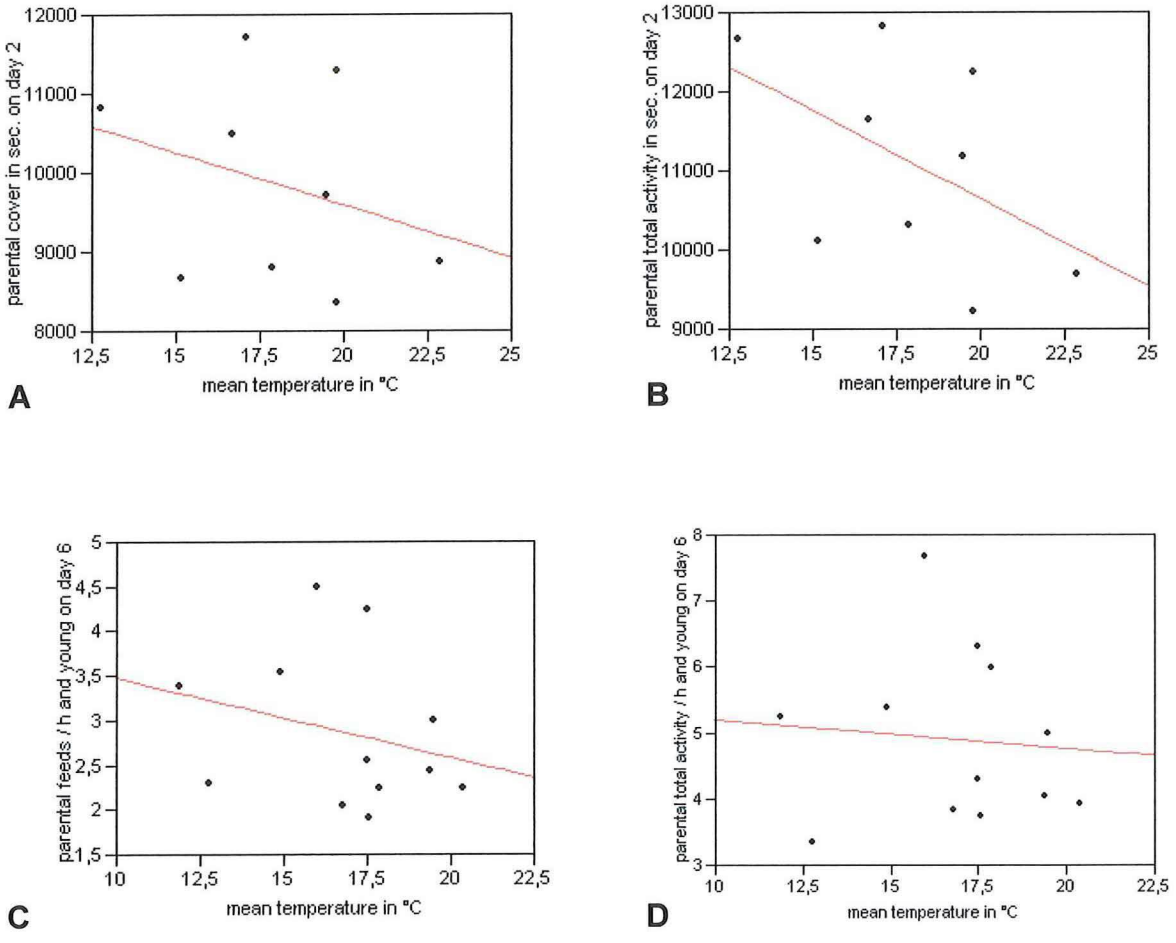

Figure 8 Parental care in reference to the temperature out of the data set from videos from 7a.m. until 11a.m. Mean temperature is defined as the mean from 5 measurements (one measurement per hour) between 6:40 a.m. and 11:40 a.m.; **A** covering in seconds on day 2 in reference to the mean temperature (n=9, F Ratio=0.7710, p=0.4090); **B** total activity in seconds on day 2 in reference to the mean temperature (n=9, F Ratio=2.2952, p=0.1735); **C** feeding per hour and young on day 6 in reference to the mean temperature (n=12, F Ratio=0.7682, p=0.4013); **D** total activity per hour and young on day 6 in reference to the mean temperature (n=12, F Ratio=0.0761, p=0.7883)

3 Results

3.1 Female effort compared to male effort in parental care

For this analysis the data set from the films from 7 a.m. until 11 a.m. were taken. Female covering and total activity on day 2 and female feeding and total activity on day 6 were compared to male investment with a paired t Test. Females invest significantly more than males in covering on day 2 (t-Ratio=-7.62107, df=8, p<0.0001), in total activity on day 2 (t-Ratio=-8.21902, p<0.0001) and in total activity on day 6 (t-Ratio=-3,4423, df=11, p=0.0055) but not in feeding on day 6 (t-Ratio=1.70074, df=11, p=0.1171) (**Figure 9**).

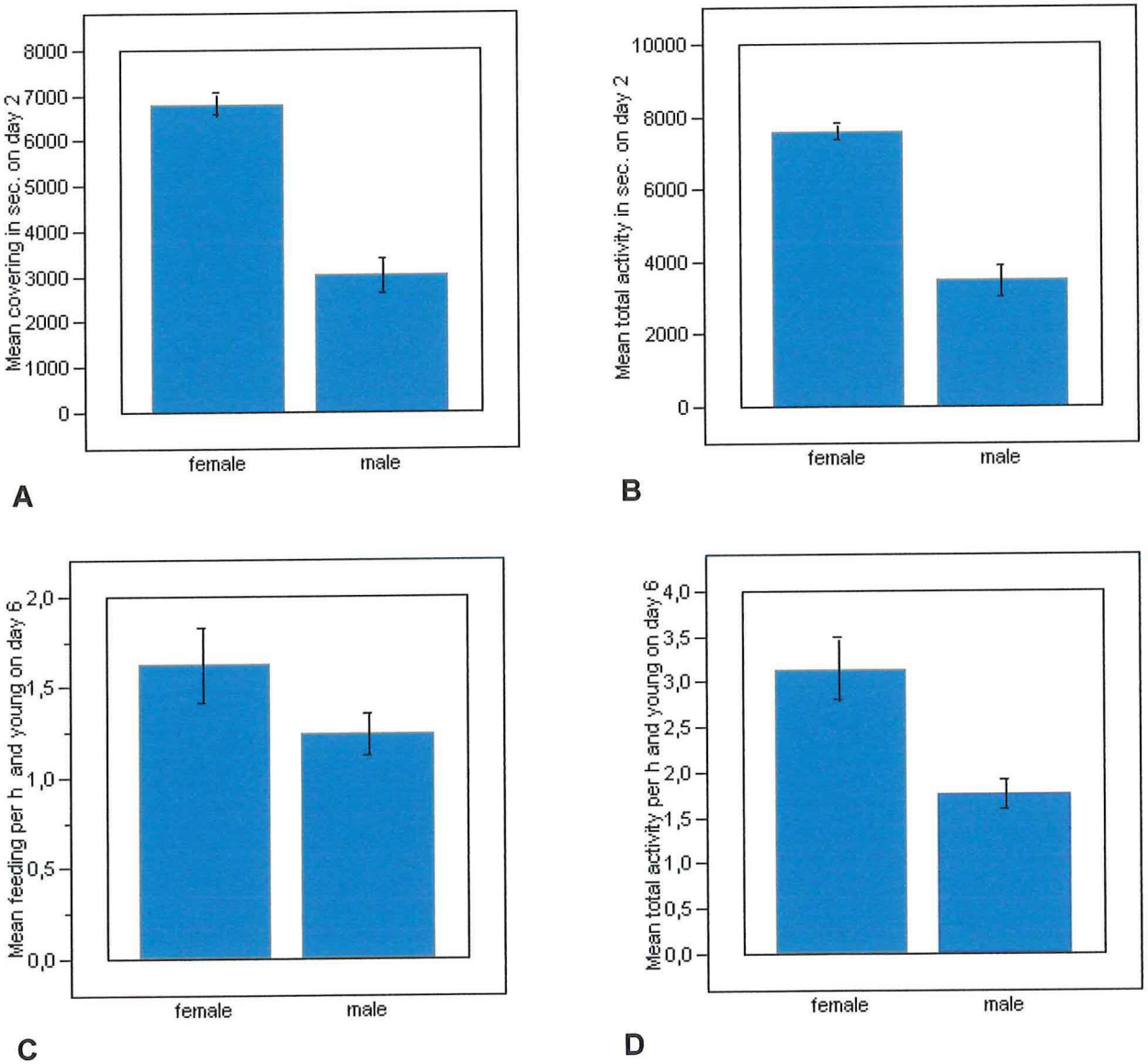

Figure 9 Female effort compared to male effort in parental care out of the the data set from videos from 7a.m. until 11a.m.; **A** Covering in seconds on day 2 (t-Ratio=-7.62107, df=8, p<0.0001); **B** Total activity in seconds on day 2 (t-Ratio=-8.21902, df=8, p<0.0001); **C** Number of feeding per hour and young on day 6 (t-Ratio=-1.70074, df=11, p=0.1171); **D** Number of total activity per hour and young on day 6 (t-Ratio=-3.4423, df=11, p=0.0055)

3.2 Paternal care in reference to extra pair paternity

For this analysis the data set from the films from 7 a.m. until 11 a.m. were taken. In this analysis I looked for correlation between paternal care and the proportion of extra pair young in the nest with an analysis of variance (ANOVA). Male effort plus female effort equals 100 %. There is no correlation between the proportion of extra pair young in % and male covering in % on day 2 (n=9, F Ratio=0.2631, p=0.6238), male total activity in % on day 2 (n=9, F Ratio=0.2126, p=0.6587), male feeding in % on day 6 (n=12, F Ratio=0.1857, p=0.6757) and male total activity in % on day 6 (n=12, F Ratio=0.0002, p=0.9888) (**Figure 10**).

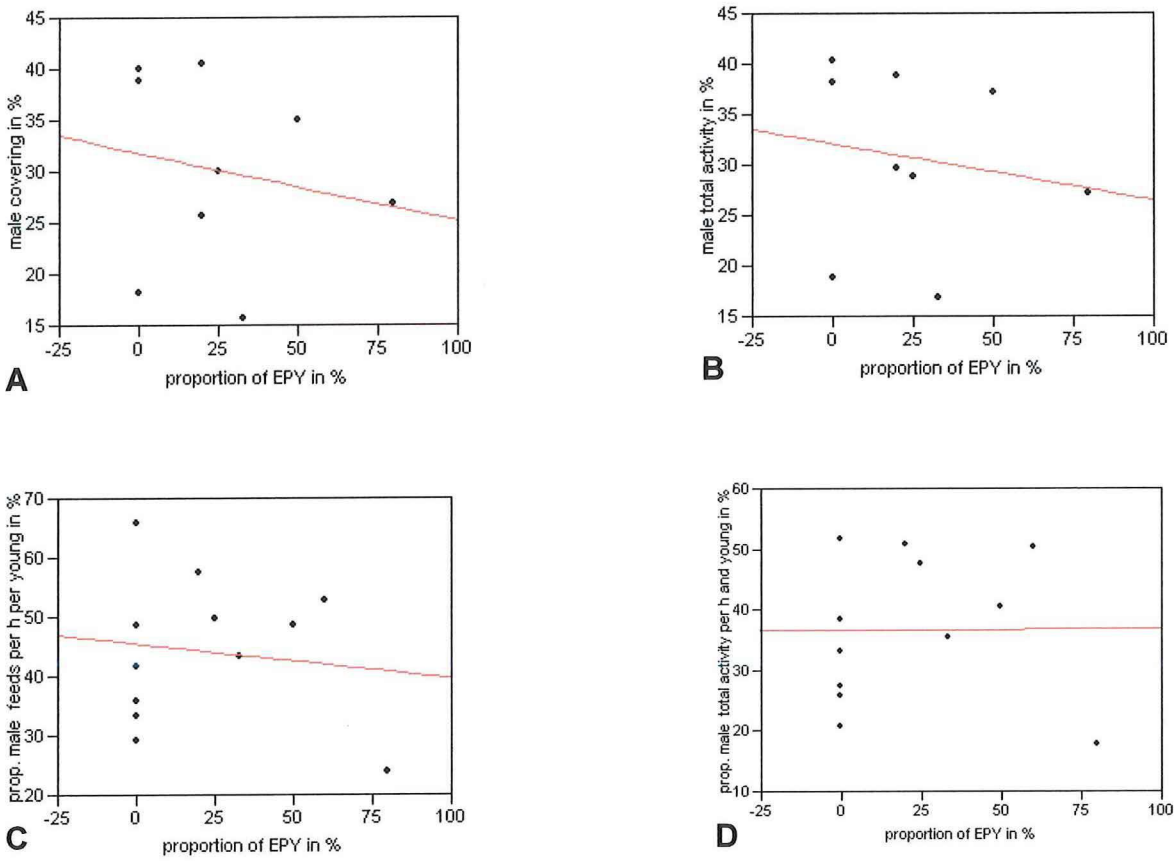

Figure 10 Paternal care in reference to extra pair paternity (EPP) out of the data set from videos from 7a.m. until 11a.m. Male effort plus female effort equals 100%; **A** male covering in % on day 2 in reference to the proportion of extra pair young (EPY) in % (n=9, F Ratio=0.2631, p=0.6238); **B** male total activity in % on day 2 in reference to the proportion of extra pair young (EPY) in % (n=9, F Ratio=0.2126, p=0.6587); **C** male feeds per hour and young in % on day 6 in reference to the proportion of extra pair young (EPY) in % (n=12, F Ratio=0.1857, p=0.6757); **D** male total activity per hour and young in % on day 6 in reference to the proportion of extra pair young (EPY) in % (n=12, F Ratio=0.0002, p=0.9888)

If paternal investment is found to be directly related to the level of extra pair paternity, then it might be the result of a facultative assessment by a male of the level of paternity in a brood. Alternatively, the level of paternity achieved may partly be determined by paternal phenotype, which may also determine the level of paternal care. In this case a relationship between parental care and paternity might be a non-facultative consequence of phenotype. To discriminate between these two hypotheses, paternity and provisioning data from each brood of double-brooded pairs shall be compared, thus allowing for any effect of consistent differences among males to be excluded from the analysis (Dixon, 1993).

In this analysis I looked for correlation between differences in paternal care from first to second brood in reference to the differences in extra pair young from first to second brood with an analysis of variance (ANOVA). There is a negative correlation between the difference in male feeding rate and the difference in the proportion of extra pair young in the first and second brood (n=4, F Ratio=4.4337, p=0.1699; attention, very small n) (**Figure 11A**). There is a negative correlation between the difference in male total activity rate and the difference in the proportion of extra pair young in the first and second broods (n=4, F Ratio=6.3545, p=0.1279; attention, very small n) (**Figure 11B**). There is a negative correlation between the difference in female feeding rate and the difference in the proportion of extra pair young in the first and second brood (n=4, F Ratio=8.2207, p=0.1032; attention, very small n) (**Figure 12A**). There is a negative correlation between the difference in female total activity rate and the difference in the proportion of extra pair young in the first and second brood (n=4, F Ratio=4.1348, p=0.1790; attention, very small n) (**Figure 12B**). There is a negative correlation between the difference in male and female total feeding rate together and the difference in the proportion of extra pair young in the first and second brood (n=4, F Ratio=6.4882, p=0.1257; attention, very small n) (**Figure 13A**). There is a negative correlation between the difference in male and female total activity rate together and the difference in the proportion of extra pair young in the first and second brood (n=4, F Ratio=5.1973, p=0.1502; attention, very small n), (**Figure 13B**).

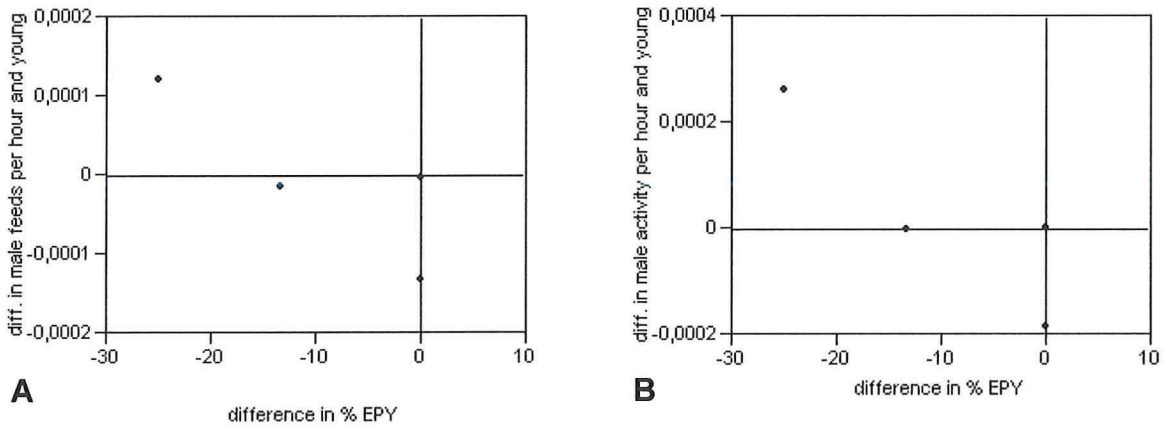

Figure 11 Paternal care in reference to extra pair paternity (EPP) out of the data set from double-brooded pairs; **A** relationship between the difference in male feeding rate and the difference in proportion of extra pair young (EPY) in first and second broods of double-brooded pairs (n=4, F Ratio=4.4337, p=0.1699); **B** relationship between the difference in male total activity at the nest and the difference in proportion of extra pair young (EPY) in first and second broods of double-brooded pairs (n=4, F Ratio=6.3545, p=0.1279).

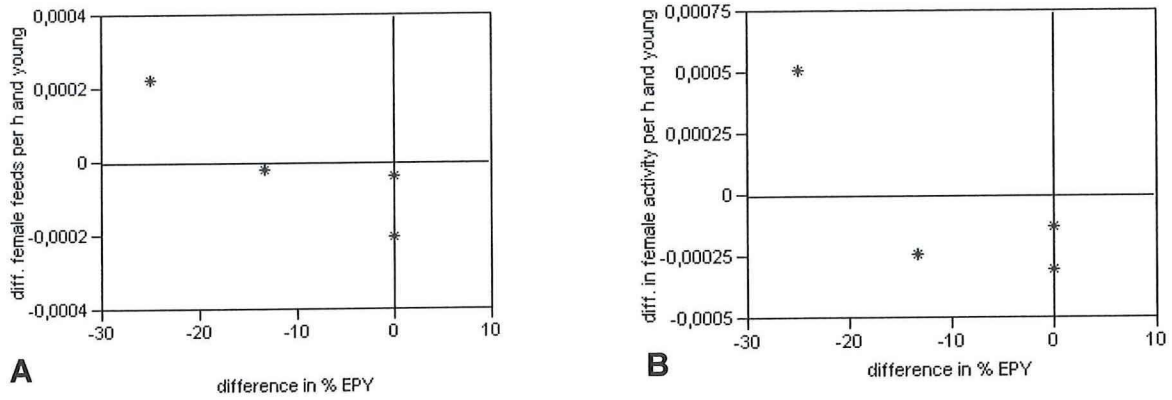

Figure 12 Maternal care in reference to extra pair paternity (EPP) out of the data set from double-brooded pairs; **A** relationship between the difference in female feeding rate and the difference in proportion of extra pair young (EPY) in first and second broods of double-brooded pairs (n=4, F Ratio=8.2207, p=0.1032); **B** relationship between the difference in female total activity at the nest and the difference in proportion of extra pair young (EPY) in first and second broods of double-brooded pairs (n=4, F Ratio=4.1348, p=0.1790).

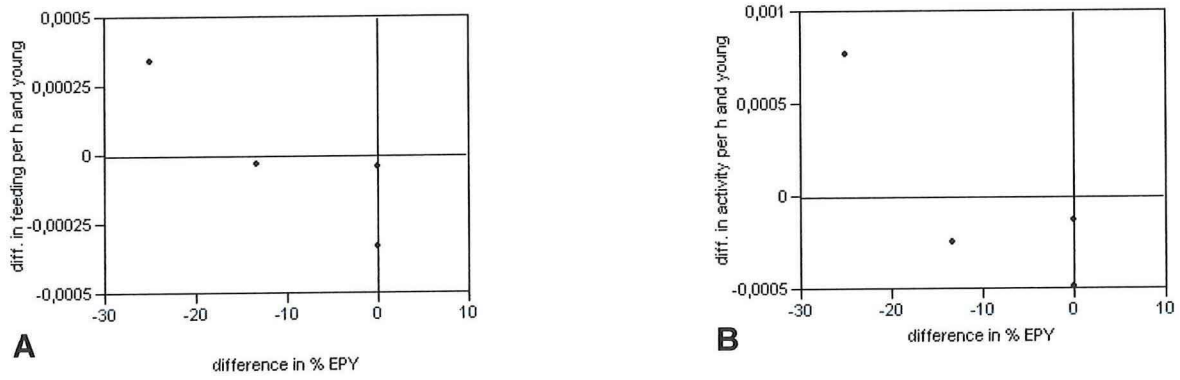

Figure 13 Parental care in reference to extra pair paternity (EPP) out of the data set from double-brooded pairs; **A** relationship between the difference in feeding rate and the difference in proportion of extra pair young (EPY) in first and second broods of double-brooded pairs (n=4, F Ratio=6.4882, p=0.1257); **B** relationship between the difference in total activity at the nest and the difference in proportion of extra pair young (EPY) in first and second broods of double-brooded pairs (n=4, F Ratio=5.1973, p=0.1502).

4 Discussion

For my thesis I investigated the question: Does the male investment in parental care correlate with paternity? The hypothesis to test was: The more extra pair young there are in a nest the less the social male should invest in parental care.

Males help significantly less to rise the offspring than females except for feeding on day six. At that day males and females fulfil about the same amount of work. Males contribute little to breeding (result of recordings from two nests). Feeding rate increases from day two until day five of the age of the nestling (**see Figure 6**). In the beginning of the nestling period the males are often singing, defending their territories and probably seeking for extra pair copulations. There is probably a trade off for the males between paternal care and singing, defending its territories and seeking for extra pair copulation.

The analysis revealed that there is a correlation between paternal, maternal and parental care in reference to extra pair paternity (EPP) from the data set of pairs breeding twice (**see Figure 11-13**). This result confirms Dixon's (1994) but in our study the sample size is very small. There was no correlation between paternal care and extra pair paternity in the data set from 7 a.m. until 11 a.m. of pairs breeding only once (**see Figure 10**).
In theoretical models three assumptions are necessary for males to adjust their paternal care toward the entire brood in relation to their paternity (Westneat *et al.*, 1993; Whittingham *et al.*, 1992). First, extra pair paternity levels have to be different from one to the next breeding attempt of the same male. This allows males with low paternity to achieve higher reproductive success in another brood. In my data set I had two pairs with a higher amount of extra pair paternity in the second brood than in the first and two pairs with the same amount of extra pair paternity in the two broods but no pair where the amount of extra pair paternity was lower in the second brood than in the first one. This was due to the very small sample size. Dixon (1994) and Bowmann (2005) had the same amount of nests with more or fewer extra pair young in first and second broods.
Second, males should be able to assess their share of paternity. It is not known whether males can estimate their paternity and if they are able to, how they do it. There are different studies where certainty of paternity was experimentally manipulated. Some had positive results (Sheldon *et al.*, 1998) and some had negative results (Kempenaers *et al.*, 1998). In some species it has been shown that males judge their share of paternity using access to the female during her fertile period (Davies *et al.*, 1992), frequency of extra pair copulation (Ewen *et al.*, 2000; Møller, 1988) or absence of female during the egg laying period (Sheldon *et al.*, 1997). There is no study about how Reed bunting males estimate their paternity. Possible cues could be: the absence of the female during her fertile period, the number of intruding males into the territory or how the female reacts to these males (Bouwman *et al.*, 2005). In our population in 2004 there were two nests containing 100% extra pair young. In both nests males contributed to brood care (direct observations). This is an indicator that Reed buntings can not exactly estimate the amount of extra pair young in their nest. Dixon (1994) had nests in his study with 100% extra pair young where the social males contributed to brood care.

Third, benefits of reducing paternal care should outweigh the costs. Costs of reduced paternal care are obviously that offspring survival rate will decrease, affecting the male's own offspring (Bart *et al.*, 1989; Wolf *et al.*, 1988; Davies *et al.*, 1992). Benefits are not as obvious as costs. One could be lower male mortality (Nur, 1984; Yezerinac *et al.*, 1996), another benefit could be more opportunities for additional mating (Magrath *et al.*, 1997; Smith, 1995, reviewed in Magrath *et al.*, 2003). I also found a weak negative correlation between female parental care and the proportion of extra pair young. An explanation is not at hand. None of the females in our study site was cuckolded due to egg dumping.
It turned out that feeding and covering are important parameters to measure parental care but feeding is quite difficult to quantify. It depends not only on how many times females or males arrive at the nest with food and feed the offspring, but also on the amount and the quality of the food they carry to the nest. Body length of caterpillars, dragonflies or other insects could be measured and then converted to biomass using length-mass regressions (Rodenhouse, 1986; Nagy *et al.*, 2005; Omland *et al.*, 1994; O'Neill Goodbred *et al.*, 1996). In our case it was impossible to measure biomass of the food. In most of the cases it was not detectable on the recordings what kind of food or how many insects the birds were carrying.
Until now it was not possible to explain the male behaviour in brood care in a breeding system with high rates of extra pair young in the nests. Here a proposition for a new hypotheses: a strategy in such a breeding system could be that males fulfil a fix but reduced amount of brood care because they can not estimate the proportion of extra pair young in their nests. In our study males fulfilled a reduced amount of brood care. This behaviour could also lead to an ESS (evolutionary stable strategy).

Finally some difficulties that turned up during our field work: First, the power supply. We connected the two batteries for the power supply wrong together so the batteries discharged to fast and the recordings stopped sometimes in the middle of the day. We fixed this problem after the first brood.
Second, position of the camera. We often videotaped the adults from the back so that it was not detectable what kind of food they were carrying or what activity they exactly were doing. The cameras should have been positioned diagonal above the nest for a better view.
Third, protection of the sun. When there was broad sunshine the contrast in the films was very week. Furthermore, if it was a very windy day there were only black and white spots on the film. The nests and the cameras should be protected from direct sunlight so that the light condition stays the same during the whole day.
Fourth, number of camera sets. Ten camera sets were provided. One to three video recorders always were out of order due to technical problems.
Fifth, which will be double-brooding pairs in this season? It is impossible to know which pair establishes a second brood and which not. It is very important to have enough camera sets to videotape every nest from the first brood.
All these reasons made the sample size decrease.

5 Conclusion

In this study I investigated paternal care in reference to the proportion of extra pair young in the nests. The study organism was the Reed bunting *Emberiza schoeniclus,* a passerine bird, with a high rate of extra pair paternity. The data were collected during the field season 2004. 22 out of 47 nests were videotaped from 6 a.m. until about 8 p.m. from one day after hatching until leaving the nest. To our knowledge, we are the first group that placed cameras permanently by the nest for the whole nestling period and videotaped the nests during the whole day.
After analysing the different data sets it came out first, that females invest significantly more in offspring than males do except in feeding on day six. Second, there is no relationship between paternal care and proportion of extra pair young in the data set from 7 a.m. until 11 a.m. suggesting that males can not discriminate intra pair young from extra pair young. Third, there was a negative correlation between parental care and proportion of extra pair young in the data set of double-brooded pairs and a negative correlation between male investment and proportion of extra pair young in the data set of double-brooded pairs.
All these analysis should be repeated with bigger sample sizes, especially the analysis of double-brooded pairs. This data set can only be increased with further field seasons. There is still a large amount of videos that were not looked through. For activity the data set from 7 a.m. until 11 a.m. could be enlarged to all nestling days.

6 Acknowledgements

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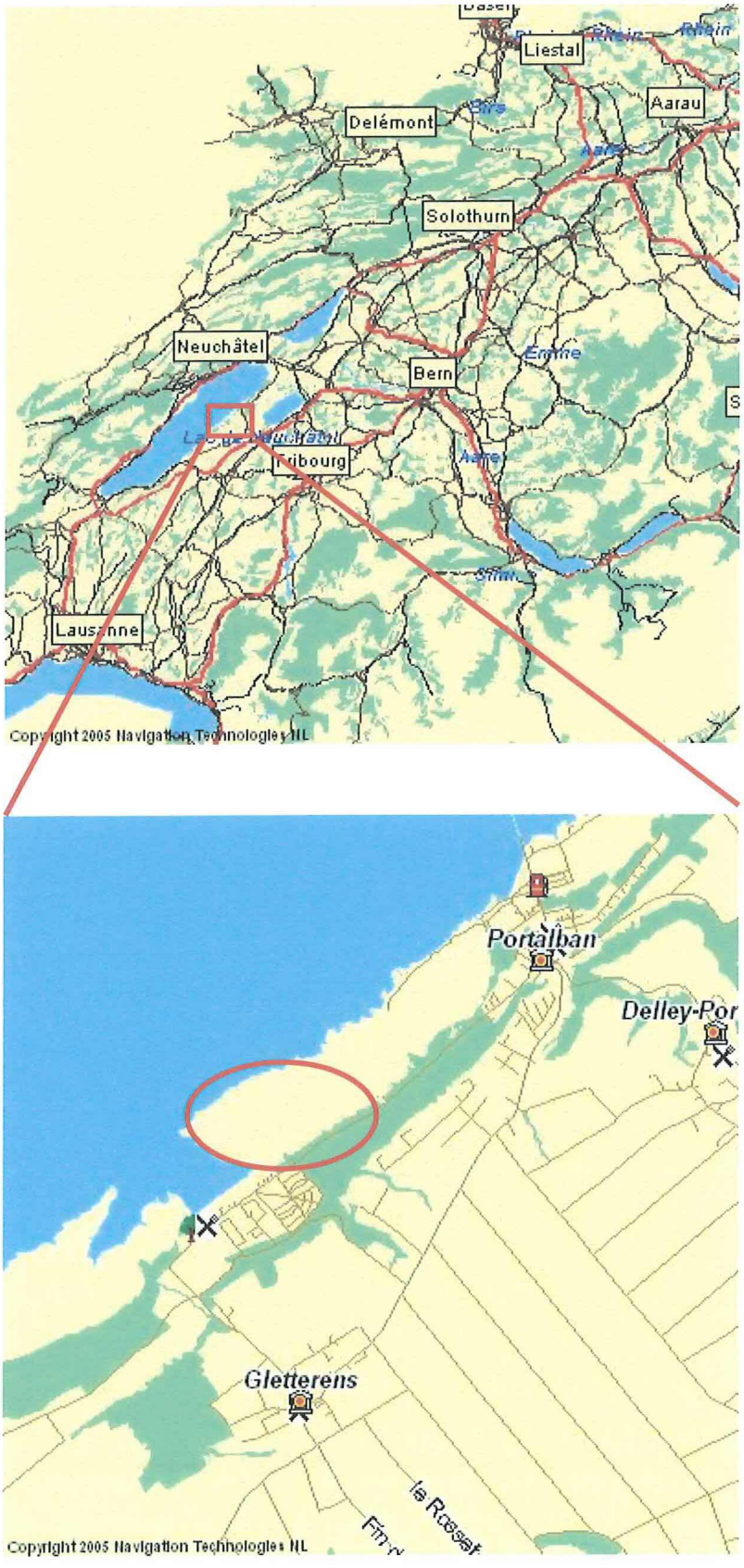
8 Appendixes

Appendix 1: Study site: Gletterens



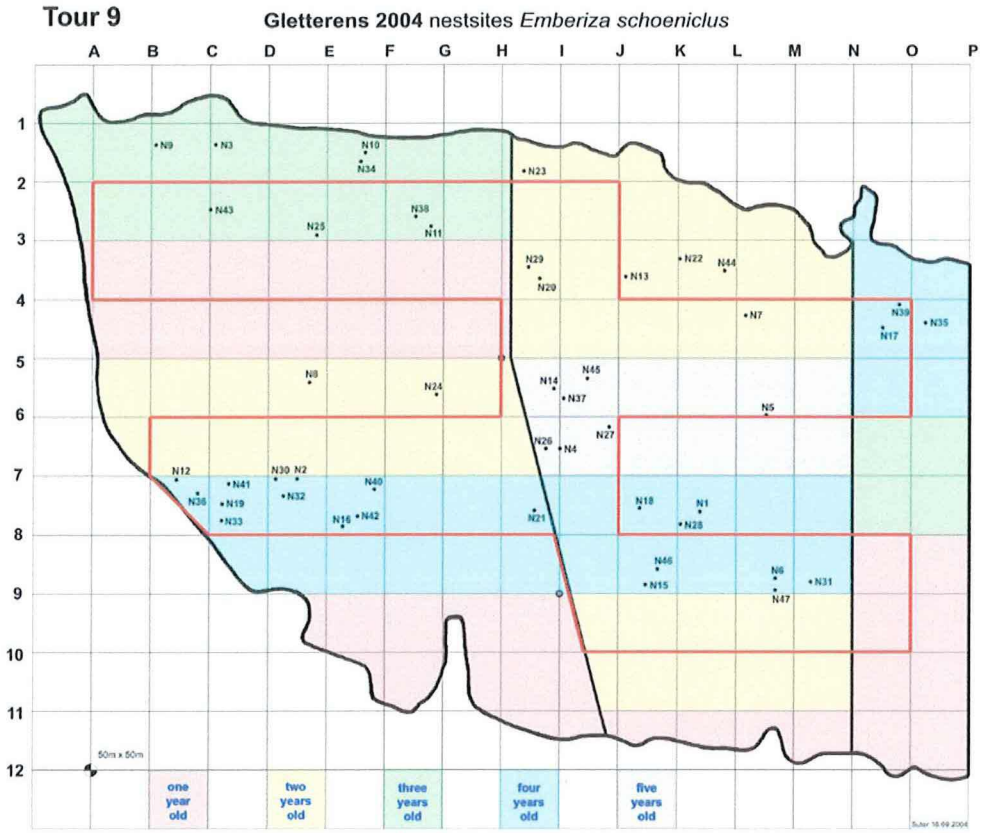
Appendix 2: Tours

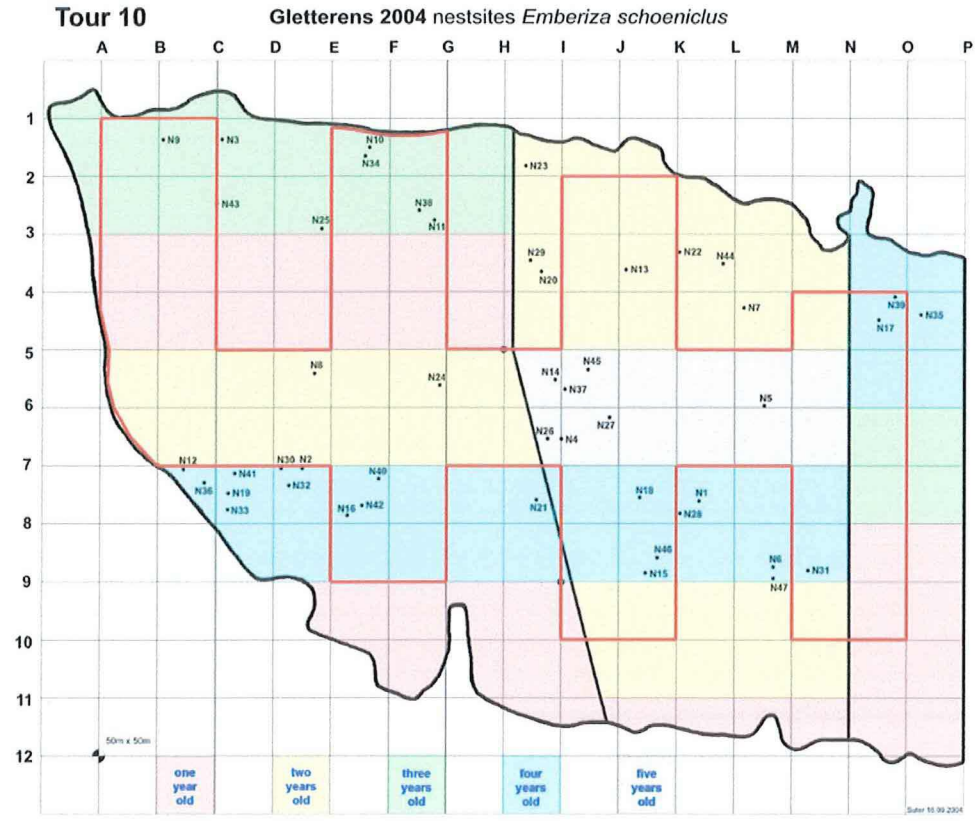
Every morning (start between 5:30 a.m. and 7 a.m.) and evening (start between 5:30 p.m. and 7 p.m.) a tour through the study site was made and the activities (male singing, female/male flying away, two male fighting, ec.) were written down. Also recorded was the start and end time of the tour and the weather pattern at the start and end time. During trials in the beginning of the field season we defined four different tours where we were not further away than 50 m from every marked point and which did not take more than one and a half hour to complete (**Appendix 3**). The tours starting/ending point and the direction were picked out by draw. The four tours alternated always the same way (**Table 3**).

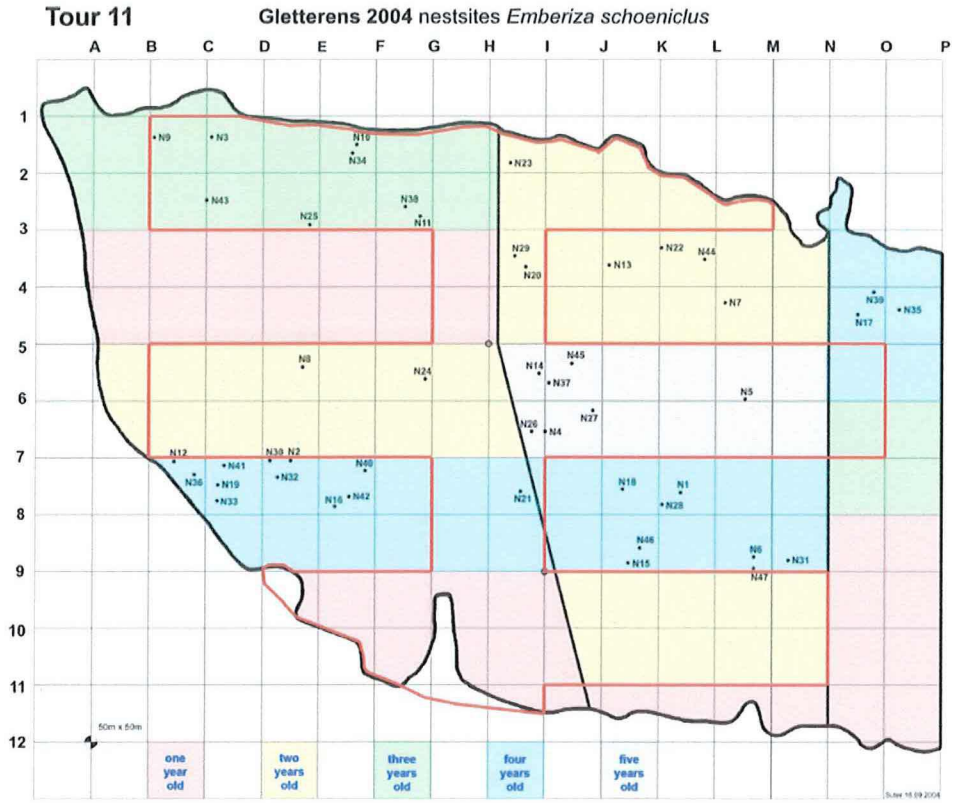
day	time	tour	distance
1	morning	10	3650m
1	evening	9	3200m
2	morning	11	3700m
2	evening	12	3200m
3	morning	9	3650m
3	evening	10	3650m
4	morning	12	3200m
4	evening	11	3700m
5	morning	10	3650m
5	evening	9	3650m
ec.	ec.	ec.	ec.

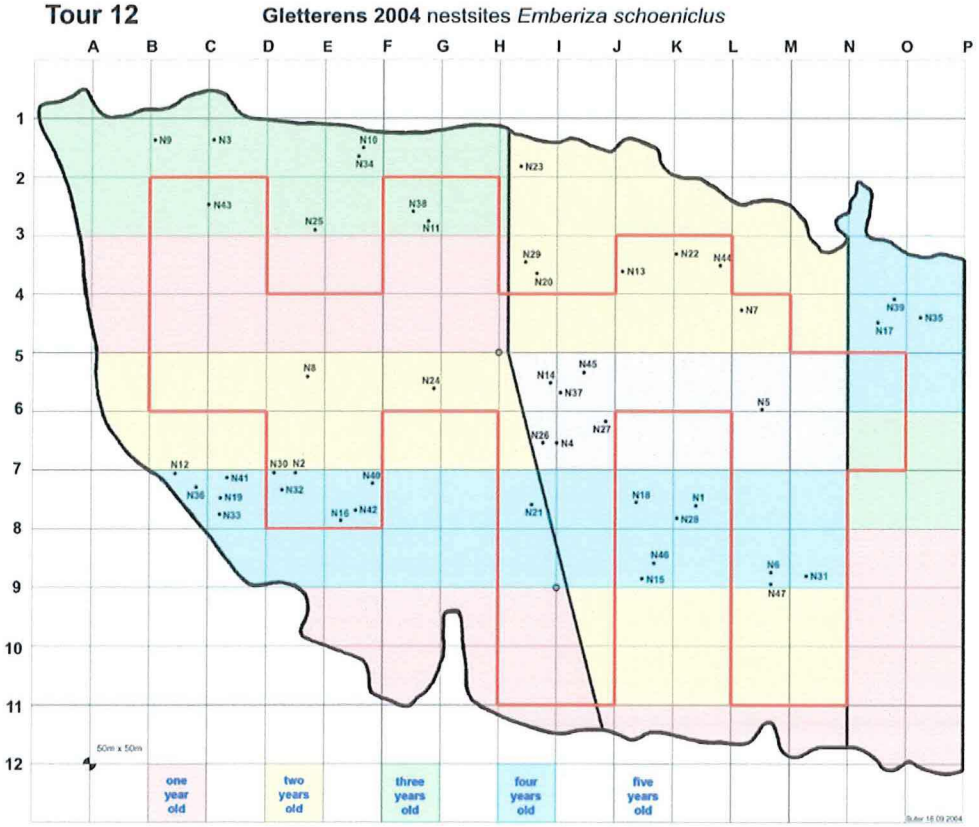
Table 3 alternated tour order. Day 5 corresponds with day 1, day 6 corresponds with day 2, ec.

Appendix 3: Study site: the four tours we walked over in an alternated tour order (**Table 3**) twice a day.

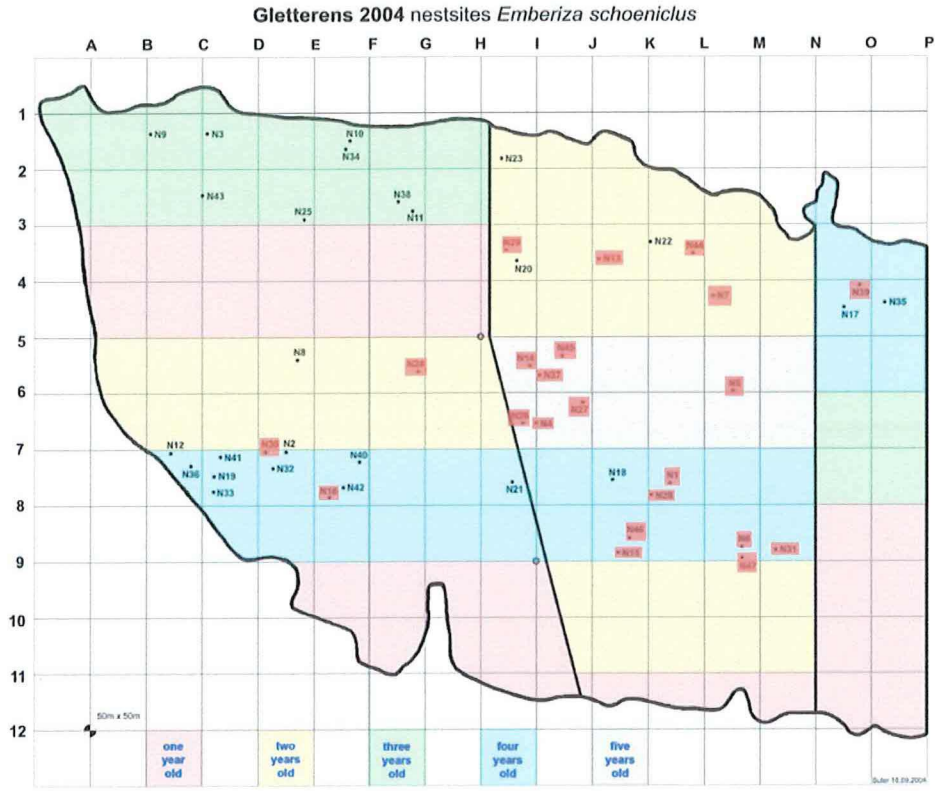




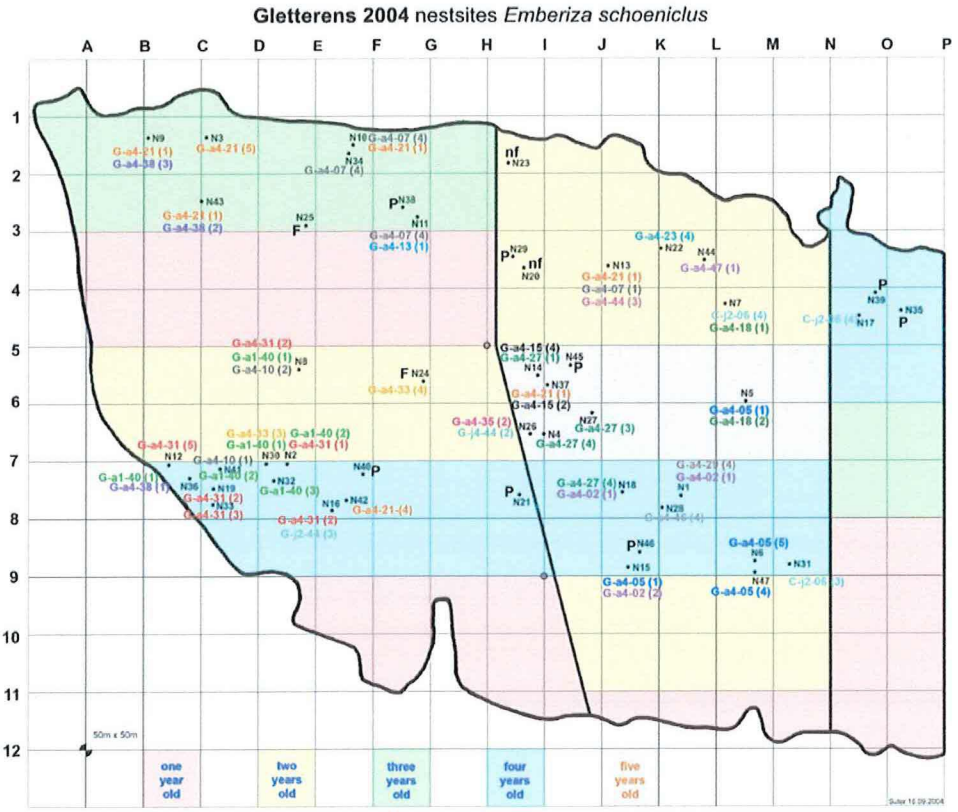

Tour 11
Gletterens 2004 nestsites Emberiza schoeniclus
A B C D E F G H I J K L M N O P
1 2 3 4 5 6 7 8 9 10 11 12
N9 N3 N10 N34 N23 N43 N25 N38 N11 N29 N20 N22 N44 N13 N7 N39 N17 N35 N8 N24 N14 N45 N37 N5 N26 N4 N27 N12 N36 N41 N19 N33 N30 N2 N32 N40 N16 N42 N21 N18 N1 N28 N46 N15 N6 N47 N31
50m x 50m
one year old
two years old
three years old
four years old
five years old


Tour 12
Gletterens 2004 nestsites Emberiza schoeniclus
A B C D E F G H I J K L M N O P
1 2 3 4 5 6 7 8 9 10 11 12
N9 N3 N10 N34 N23 N43 N25 N38 N11 N29 N20 N22 N44 N13 N7 N39 N17 N35 N8 N24 N14 N45 N37 N5 N26 N4 N27 N12 N36 N41 N19 N33 N30 N2 N32 N40 N16 N42 N21 N18 N1 N28 N46 N15 N6 N47 N31
50m x 50m
one year old
two years old
three years old
four years old
five years old

Appendix 4: Study site: the red defeated N# are the nest that were videotaped



Appendix 5: Study site: Distribution of offspring from every male, for example: the male with the no. G-a4-07 had 13 offspring in 4 different nests. The numbers in brackets indicate how many offspring a male had in this particular nest.



Appendix 6: further results

In this analysis I compared different size parameters with the patch size (in pixel), the patch colour (3 categories: 1=dotted, 3=black, 2=in between) and the patch shape (3 categories: 1=patchy, 3=one piece, 2=in between) (**Figure 14**). Definition of song parameters see Rieille (2005). I couldn't find any correlation between the different parameters (**Table 4**).

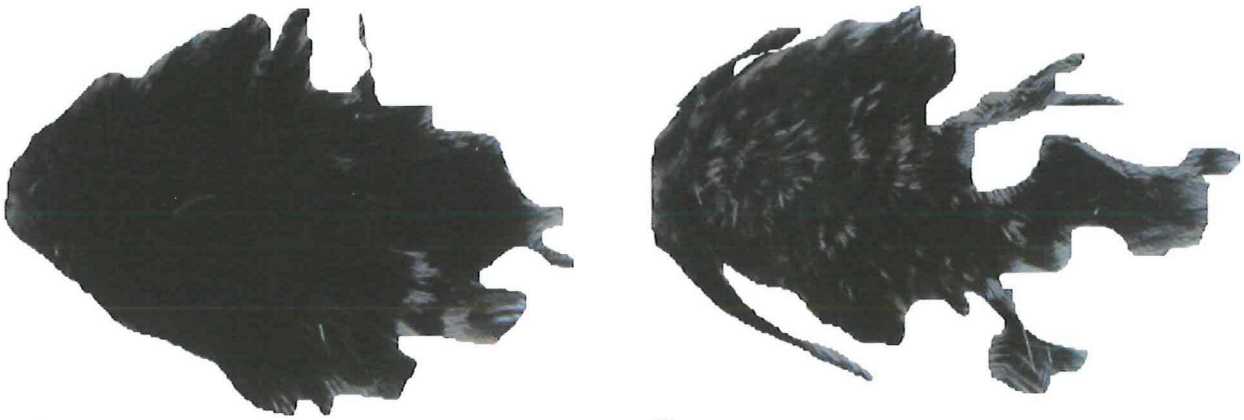


Figure 14 Example of two different patches; **A**: male G-j2-44; patch size: 178'857 pixel; patch colour: 3; patch shape: 3; **B**: male G-a4-23; patch size: 142'955 pixel; patch colour: 1; patch shape: 1

Analysis of variance	n	patch size P-value	patch colour P-value	patch shape P-value
tarsus	27	0,8837	0,2425	0,7813
wing	27	0,7908	0,2236	0,9448
bill nostril-tip	27	0,4957	0,1842	0,3665
weight	27	0,1675	0,8263	0,8931
tail	27	0,1918	0,8209	0,7913
song rate	23	0,7163	0,2264	0,2547
song rep. size	23	0,3291	0,8209	0,2548
male covering in sec.	9	0,2342	0,7283	0,8799
male activity in sec.	9	0,2638	0,7774	0,8389
male feeds per h per young	10	0,9873	0,3560	0,9832
male activity per h per young	10	0,9879	0,3571	0,9675

Table 4 Summary of the analysis of variance of different size and song parameters compaired with patch size, patch colour and patch shape.

In this analysis I compared different size parameters with the total number of young per male, the intra pair young (IPY) per male and the extra pair young (EPY) per male. There is a tendency that male with a big tarsus have more young (n=27, F Ratio=3.4093, p=0.0767) and more extra pair young (n=27, F Ratio=3.8008, p=0.0625). There is a tendency that male with a big patch have more extra pair young (n=27, F Ratio=3.8298, p=0.0616). Male with a black patch have more intra pair young (n=27, F Ratio=3.7562, p=0.0381) (**Table 5**). All these correlation disappear when corrected with Bonferroni.

Analysis of variance		total no. of young	IPY	EPY
	n	P-value	P-value	P-value
tarsus	27	*0,0767*	0,2126	*0,0625*
wing	27	0,0669	0,1600	0,1758
bill nostril-tip	27	0,4063	0,7226	0,3405
weight	27	0,4447	0,4699	0,6490
tail	27	0,2636	0,3300	0,5090
patch size	27	0,4681	0,4326	*0,0616*
patch colour	27	0,1085	**0,0381**	0,3640
patch shape	27	0,5228	0,2005	0,4444

Table 5 Summary of the analysis of variance of different size parameters compaired with the total number of young, number of intra pair young (IPY) and number of extra pair young (EPY) per male.