BREEDING ECOLOGY AND BEHAVIOUR OF A COLONIAL HIRUNDINE: A STUDY OF THE SAND MARTIN (*Riparia riparia*) USING DNA FINGERPRINTING.

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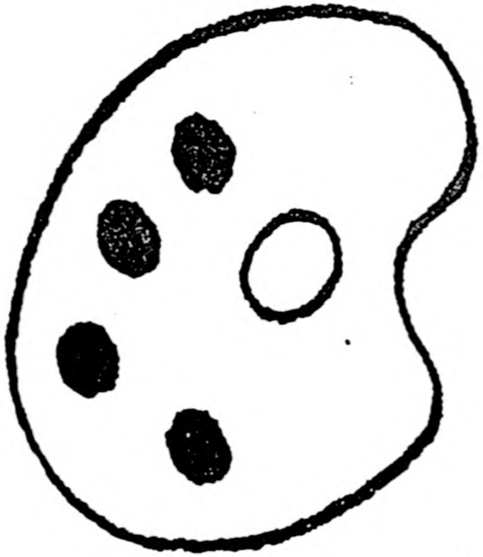
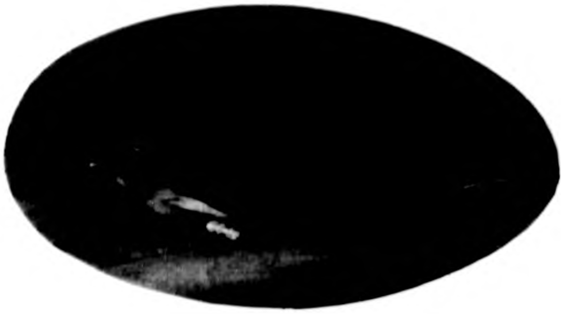
NUMEROUS ORIGINALS
IN COLOUR



To my parents and to Fernando



'Am not I consanguineous? am I not of her blood?'
W. Shakespeare

ABSTRACT

Some potential benefits and costs of coloniality in birds were investigated, including ectoparasitism, anti-predator behaviour, and social competition with particular emphasis on social-sexual behaviour associated with genetic parentage. The species studied was the sand martin (*Riparia riparia*), which was abundant and highly colonial in the study area in central Scotland.

Population decreases from 1990-1992, including a particularly notable decrease in the number of pairs in 1991 (46% lower than 1990), were associated with a decrease in mean adult body-size, apparently due to natural selection for smaller sizes related to adverse conditions in the wintering areas. During breeding, most losses occurred before hatching mainly due to desertion or predation of eggs.

Anti-predator behaviour, specifically the time until a predator was detected and mobbing started, tended to decrease with subcolony size, and was interpreted as a benefit of coloniality. Nests treated with a pesticide to kill arthropod ectoparasites had heavier chicks than control nests, suggesting that ectoparasitism may be a cost for sand martins. The cost was possibly dependent on colony size since the effects of ectoparasites on nestling growth tended to be more marked in a larger colony.

Artificial eggs placed in nests at the pre-laying and laying stages were more often rejected prior laying (46%) than after the start of laying. Amongst 46 broods (excluding 6 used for male removal experiments) and 170 offspring analyzed by multilocus DNA fingerprinting, 41.3% of the broods, and 20.6% of the offspring were derived from extra-pair fertilizations (EPFs), quasi-parasitism (QP) and intra-specific brood parasitism (IBP). Amongst these, EPFs were most frequent, with 34.8% of broods containing at least one offspring fathered by an extra-pair male, accounting for

14.7% of the offspring. The proportion of nestlings resulting from QP was 4.1%, while IBP accounted for 1.8% of chicks.

The occurrence of extra-pair paternity in broods was unrelated to the body size of the attendant male parent, clutch or brood size, or age of the male (estimated from ringing date). Late broods tended to have a greater incidence of EPFs, however, and males were cuckolded more frequently in second broods than in first broods.

Male removal experiments and observations of mate-guarding behaviour gave some indication that the risk of cuckoldry for males increased as the intensity of mate-guarding decreased, but other factors (such as female choice) might also have been involved in regulating the frequency of EPFs. Cuckolded males (as determined by DNA fingerprinting) did not reduce their level of parental effort, measured as rate of brood provisioning, suggesting that EPFs represented a reproductive cost for the cuckolded individuals, while benefiting the individuals which achieved EPFs.

The potential costs and benefits of coloniality, and the implications of mixed-reproductive strategies (MRS) in avian parental care and mating systems are discussed.

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CHAPTER 1 - INTRODUCTION

The costs and benefits of social living have often been discussed in relation to resource distribution and abundance on the one hand, and predation pressure on the other (Vine 1971, Hamilton 1971, Pulliam 1973, Ward and Zahavi 1973, Treisman 1975, Emlen and Wrege 1986). The major factors considered to favour group living or the formation of colonies are, a) increased foraging efficiency and b) increased protection from predators (Krebs and Davies 1987). Shields *et al.* (1988) also proposed the idea of 'ideal-free coloniality', where individuals aggregate because suitable nest sites are patchily distributed and in short supply. Some food supplies, such as marine resources or aerial insects, have a very variable abundance or availability; coloniality has been shown to be advantageous in exploiting unpredictability of this type, since colonies can serve as information centres for the location of food patches (Ward and Zahavi 1973, Emlen and Demong 1975, Brown 1986). A colony is here defined as a place where a number of individuals or pairs are nesting at a more or less centralized place from which they recurrently depart in search of food (Wittenberger and Hunt 1985).

Large groups of individuals may be more conspicuous to predators, but on the other hand living in groups can increase overall levels of vigilance, and therefore offer protection against predation at an individual level (Krebs and Davies 1987). Defense also occurs due to 'swamping' (Clark and Robertson 1979) and 'selfish herd' effects (Hamilton 1971); or to alarm calls and mobbing (Wittenberger and Hunt 1985).

The costs associated with living in groups or colonies, include: a) competition for critical resources located at or close to the colony, for example mates (sexual harassment) and nests, and also food (Birkhead and Furness 1985, Bryant 1978); b) increased risk of disease and parasite transmission (Alexander 1974, Hoogland and Sherman 1976). Intraspecific interactions, like competition for critical resources at a colony may lead to increased chances of offspring death by infanticide and/or siblicide (Wittenberger and Hunt 1985), egg destruction (Brown and Brown 1988a) or an increased probability of rearing genetically unrelated offspring due to cuckoldry, intraspecific brood parasitism or mix-up of young (Hoogland and Sherman 1976). Intracolonial destruction of eggs and young has been observed in many colonial birds (Hoogland and Sherman 1976, Brown and Brown 1988a). Colonies containing a large number of nests provide many opportunities for intraspecific brood parasitism (Emlen and Wrege 1986), which occurs widely in certain populations of colonial cliff swallows (Brown and Brown 1988b, Brown and Brown 1989).

Costs and benefits of coloniality may be in conflict, according to colony size. In seabirds for example, decreased risks of predation may favour individuals breeding in large colonies (Wittenberger and Hunt 1985), whereas intraspecific competition for food (increasing risk of starvation) may favour individuals breeding in smaller colonies, implying the existence of an optimal colony size (Birkhead and Furness 1985).

Sand martins can breed in very large colonies, with up to a thousand nests in close proximity (Jones 1985, Turner and Rose 1989), offering a good context for the study of social interactions during breeding. A study of costs and benefits of coloniality was done by Hoogland and Sherman (1976) on bank swallows, in which a reduction of predation was concluded to be a major benefit of coloniality in this species. This same study by Hoogland and Sherman pointed to the likelihood of misdirected parental care as a cost of coloniality for bank swallows, since sexual chases could end in extra-pair copulations (EPCs). Beecher and Beecher (1979) presented some evidence that bank swallows actively pursue a mixed-reproductive strategy (MRS), although they lacked genetic evidence that EPCs end in extra-pair fertilizations

(EPFs). At the start of this study no long term research on the breeding cycle of sand martins, particularly associated with aspects of social behaviour such as predation, ectoparasitism and particularly socio-sexual behaviour, including genetic parentage, had been done. The present study aimed to fill this gap for the sand martin, as well as establish more general patterns characteristic of highly colonial species. Preliminary results using DNA fingerprinting had shown EPFs (18% of the offspring) in 5 sand martin broods (Riley 1992), indicating that sexual chases and extra-pair copulations can indeed end in EPFs. This small sample size suggested that MRSs were likely to be frequent in this species.

The implied trade-off between anti-predator benefits and costs of ectoparasitism increasing with subcolony size was considered in the present study. Other potential costs for some individuals (that can be considered as benefits to other individuals), such as intra-specific brood parasitism and MRSs, were also a main interest during this study. Futhermore, consanguinity between parents and offspring, and the implications of a MRS for mating systems and parental care were investigated. For example, whether the presence of cuckoldry results in reduced parental effort.

The thesis is divided into seven chapters, including this general introduction (Chapter 1). These are:

Chapter 2. Describes the study site and general methodology. More specific details of methods are given within each chapter.

Chapter 3. Contains information on reproductive biology and ecology of sand martins, including population changes during the study period, and an investigation of space use and behaviour by radio-tracking.

Chapter 4. An experimental investigation of the effects of ectoparasites on the growth of nestlings, and the effectiveness of anti-predator behaviour of individuals in subcolonies of different sizes.

Chapter 5. Contains behaviourial and genetic evidence

(DNA fingerprinting) of intraspecific brood parasitism, including experimental introduction of eggs into pre-laying and laying nests.

Chapter 6. Behaviourial and genetic evidence (DNA fingerprinting) for extra-pair paternity, including experimental manipulations of the intensity of male mate-guarding to investigate the influence of genetic paternity on male paternal care.

Chapter 7. A discussion of a) coloniality in sand martins and other hirundine species and b) the cost of coloniality in sand martins due to cuckoldry (for at least some males), and its effects on parental care and mating systems in wild birds.

CHAPTER 2 - GENERAL METHODS AND STUDY SITE

2.1. Study site

Field work was carried out mainly at Barbush sand quarry, Dunblane, central Scotland (56° 12′ N, 3° 59′ W: OS 57 NN 785025 of British National Grid), from March to September in 1990, 1991 and 1992. Two other smaller sand quarries were used for supplementary experiments. One of those, Argaty quarry (NN 742020), was located in Doune, approximately 4km to the west of Barbush. The other area (NN 796049) was at Kinbuck about 2km to the north (Figure 2.1).

2.2. Catching, marking and measuring

Adult sand martins were captured with mist nets, hand nets (Figure 2.2) and 'cones' (made with card and mist net)(Figure 2.3). Hand nets were preferred for catching specific individuals. Juveniles were captured mainly with mist nets and nestlings extracted from burrows with a thin bamboo stick with a wire hook in the end. The wire was covered with soft plastic tubing to avoid injury to nestlings. A torch was used to light the burrow while nestlings were pulled out.

Adult birds were marked with metal rings (provided by British Trust for Ornithology, BTO), and with colour markers. A design requirement of the colour marks was that they could be easily detected while the bird was in flight. In 1990 pieces of coloured wool (5cm long) were glued with superglue to the tail feathers. In subsequent years the wool was replaced with pieces of coloured 'Sellotape' on the tail feathers, after Best (1990). Coloured 'Tippex' was also used on the tips of the outer feathers of the tail, particularly for birds which required minimal handling (e.g. birds in the pre-laying or laying stage). Sometimes the coloured tape

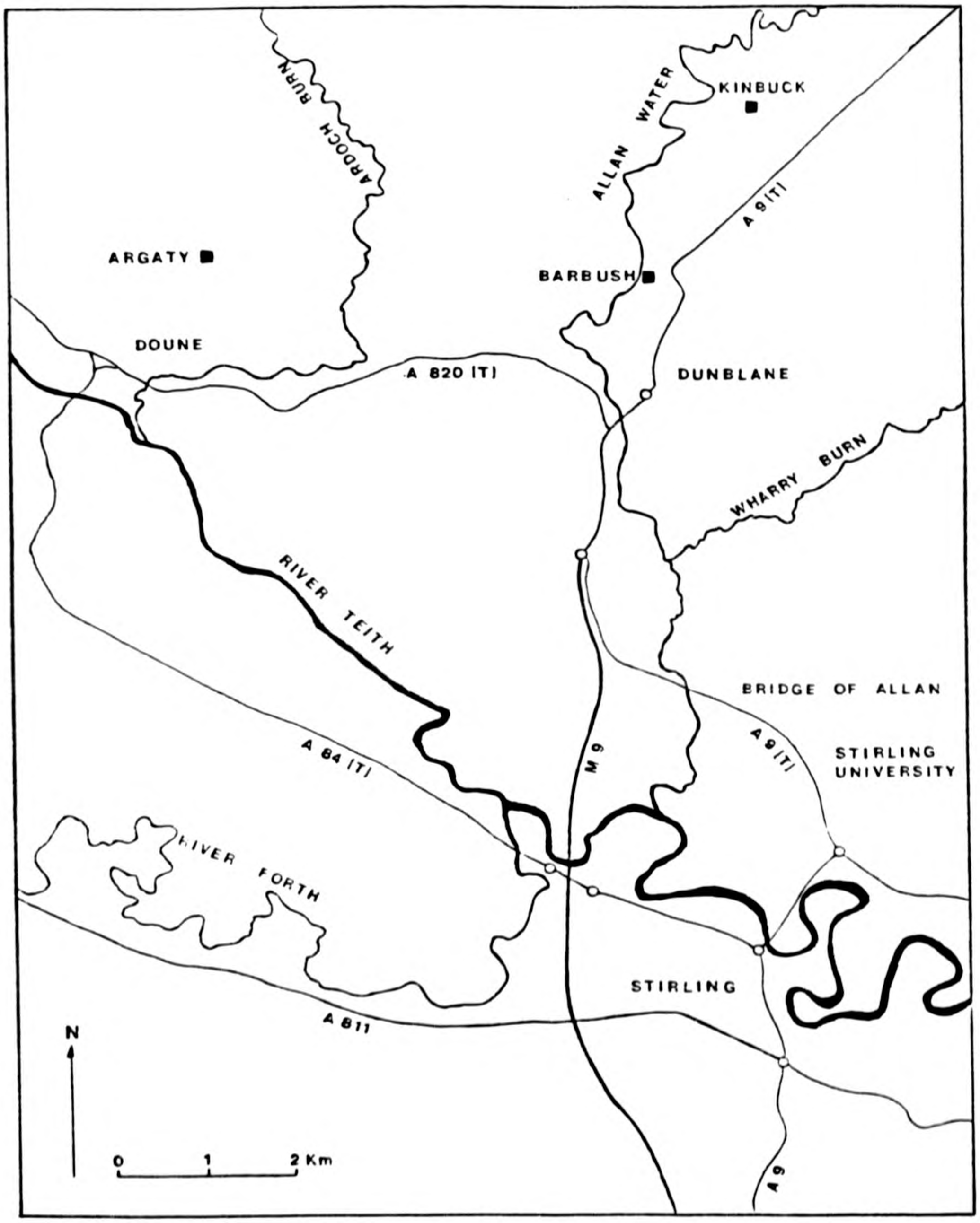


Figure 2.1 - Map of the study area in central Scotland, showing the sand martin study colonies (square symbols).

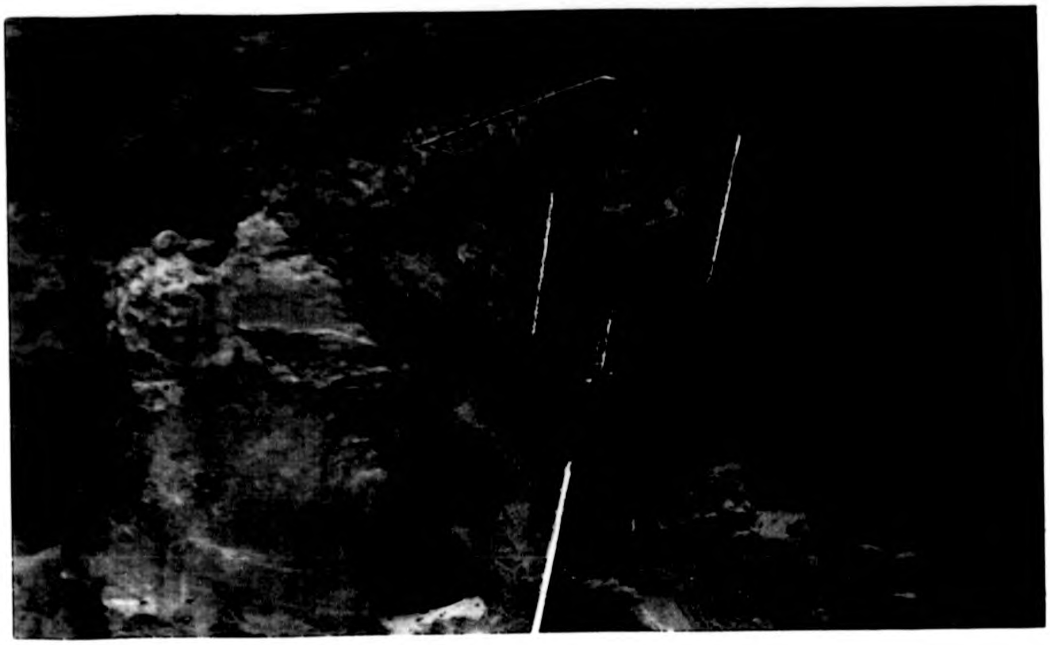


Figure 2.2 - Hand net used for catching sand martins.

Figure 2.3 - Cone used for trapping sand martins.

method was used along with 'Tippex'. These marks were found to be more easily seen and longer lasting than pieces of wool. In addition, the throats of most individuals were coloured using dye markers. These dyes lasted for two or three weeks and the birds were re-marked when recaptured.

Adults were also sexed (breeding females had conspicuous brood patches compared with males - Asbirk 1976, Svennson 1969), weighed (with a 50g balance - Pesola to the nearest 0.25g) and measured (by dial callipers to the nearest 0.1mm). The following measures were taken: wing length (maximum chord), keel length (length of sternum from tracheal pit to hind margin), head and bill length (the maximum distance from the tip of the bill to the back of the skull), tarsus length (the length of the tarso-metatarsal bone from the notch of the inter-tarsal joint to the centre of the underside of the foot where the toes diverge). Nestlings were ringed and weighed on the 13th day after hatching (from the day of the last egg to hatch).

2.3. Aging

Free and Belanger (1981) used the extent of wear on the inner primaries combined with the degree of skull pneumatization to identify second year or older bank swallows. However, it was not used in this study due to the time it took to process the birds, and age was estimated through ringing date instead. Sand martins were therefore categorized as pullus or juvenile/fledgling during their first summer, and thereafter as '1 year', and '2 years and older'.

2.4. Censuses

Subcolonies at Barbush Sand Quarry were surveyed throughout each breeding season and censused twice (1990 and

1991) or once a week (1992) to estimate the number of occupied burrows. Fresh claw marks at the entrance of the burrows, and observations of birds going in and out, were used to register burrows as occupied. Burrows with no fresh marks, or with spider's webs blocking the entrance, were not counted as occupied.

2.5. Artificial cliff

An artificial cliff, similar to one used by Asbirk (1976), was built at Barbush, firstly beside a natural subcolony (1990) and later transferred to the bank of a pond (1991). The aim was to observe breeding behaviour in nests directly, and to simplify manipulations of natural eggs.

The artificial cliff consisted of a wooden observation hut with 40 holes (diameter 5 cm, and 15cm between each horizontally) on the front facing the pond. 'PVC' tubes 40cm long ran from the holes into wooden nest boxes (60x100x10cm), each divided into 5 chambers. These nest boxes (and the pipes) were filled with sand from the ground of local natural cliffs, topped with transparent hard plastic 0.3cm thick (individually adjusted for each chamber in a nest box, with a stone as a weight on top). Each chamber had a sliding metal door at the rear. The front of the hut was covered with a mixture of plaster and sand as camouflage. Grass, soil and green waterproof canvas, and wood on the back, were also used to camouflage the hut (Figure 2.4). The sand of the nest boxes was kept moist by spraying it with water once a week.

The hut was 6x2m wide and 2.5m high, with an entrance door at the back. The first line of holes was placed at a height of 90cm from the base, and the second 1m higher than the first line (each line contained 4 nest boxes, with 5 holes each, hence 20 potential nests). The hut was made in sections (to make it easy to move) and was placed on a gravel platform 1.5m high in 1990. In 1991 the water level

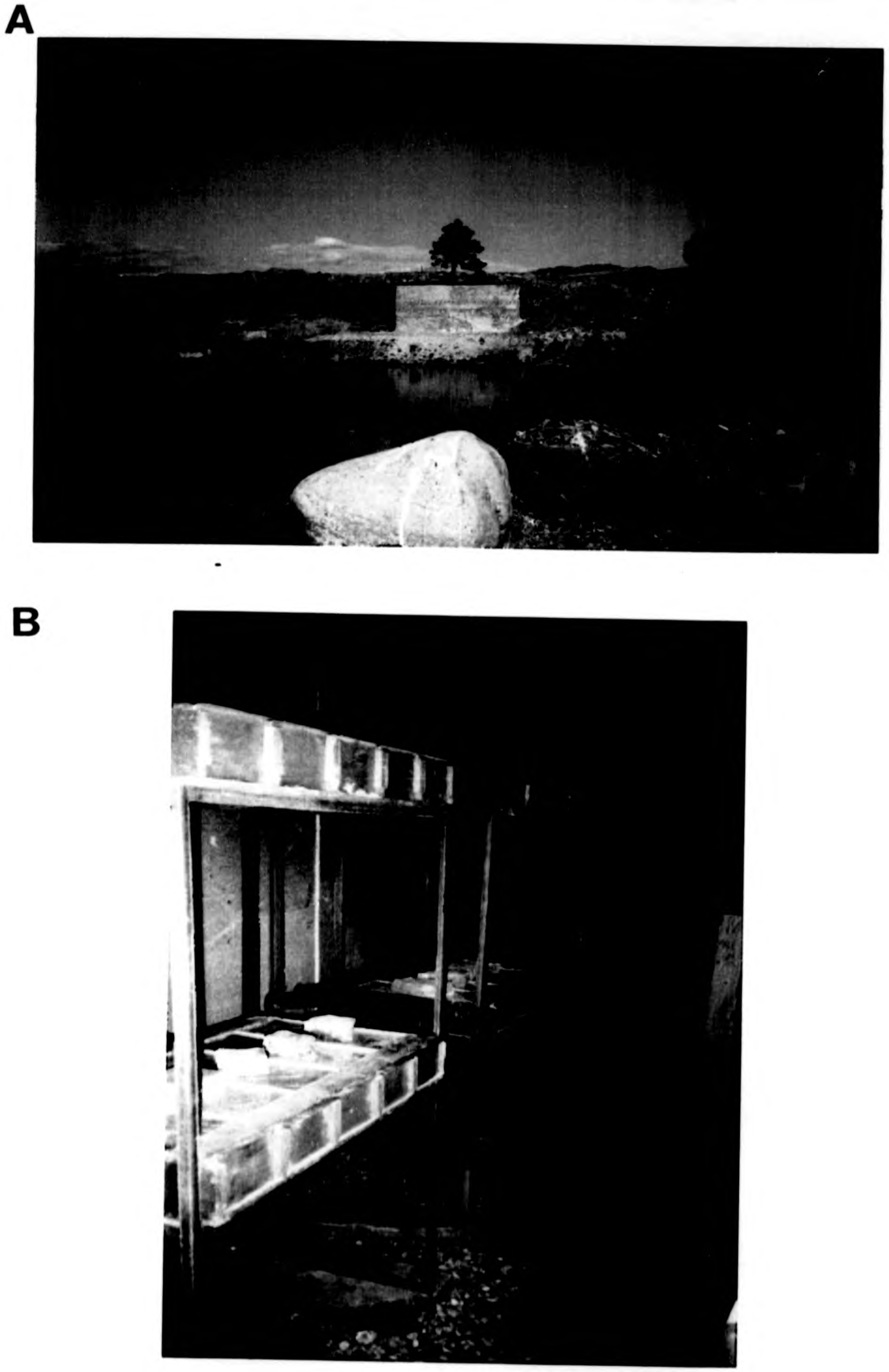


Figure 2.4 - Artificial cliff provided for sand martins at Barbush, 1991. A) External view; B) internal view.

was normally 1.5-2.0m from the bottom of the hut.

Play-back of sand martin calls (once a week, early in the morning for 2h) and two stuffed sand martins (left occasionally for a day on the entrance of the pipes, by the top row) were also used to attract the birds.

Sand martins, however, did not nest in the nest boxes, despite some attempts (Appendix 1).

2.6. Checking nests

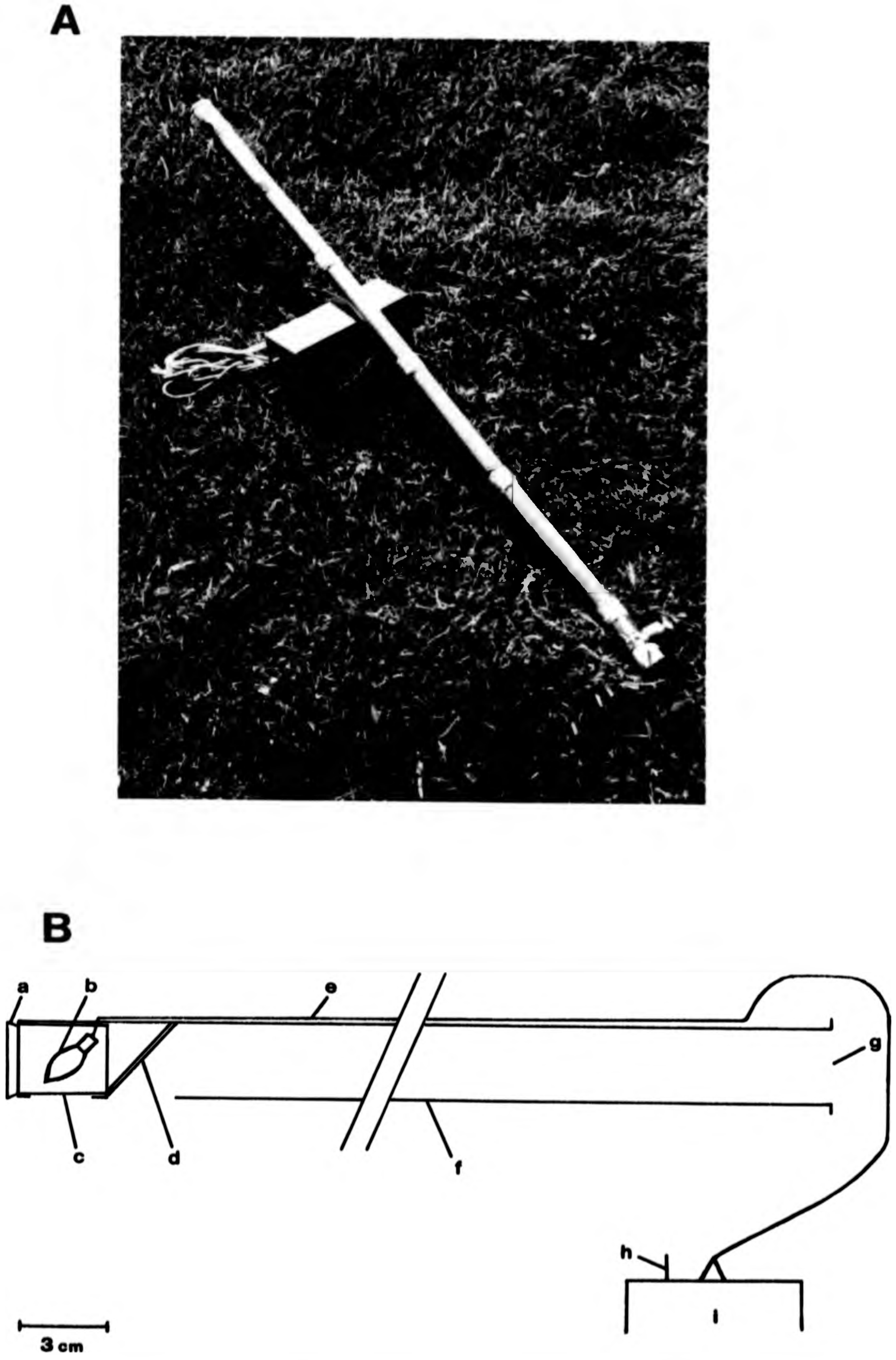
Nests were marked by driving pieces of wire with attached plastic labels into the sand cliff. Photographs of the cliffs were also taken so that nests could be identified from a distance. A light metal ladder was used to gain access to nests. A metal platform was also used to reach the upper nests in two subcolonies at Barbush. The nests were checked using a 'ripariascope' (Figure 2.5), a periscope designed for observing bank swallows (Petersen 1955). The end with the mirror was covered by 'Clingfilm' to avoid loose sand blocking the scope.

2.7. Breeding observations

Nests were checked every day (early afternoon), or every other day, until the clutch was complete, and regularly (usually twice a week) thereafter. From the 10th day of incubation (considered to start from the last egg laid) the nests were again checked daily or every other day until the clutch hatched; and after that, they were checked twice a week until the chicks fledged.

Breeding information was taken on laying, incubation, and rearing periods. Time budget observations were made with binoculars, using a car as a hide (or a green cotton hide when a car could not be driven sufficiently close to the colony). These observations were made during the following stages:

Figure 2.5 - A) 'Ripariascope' used to check sand martin nests. B) Diagram of the 'ripariascope' (1m long) used to check sand martin nests. a=removable cover to provide access to light bulb, b=light bulb, c=transparent plastic window, d=mirror, e=power supply to light bulb, f=metal tube, g=eyepiece, h=switch, i=battery (6V).



1) laying and pre-laying - 6h/d/nest between: 7-9h, 10-12h, and 16-18h;

2) incubation - 8h/nest: 2h a day for 4 days (7-9h and 14-16h) between the 4th and 12th days of incubation;

3) nests with chicks - 8h per nest spread over 2 days (10-12h and 14-16h), between the 6th and 12th days after hatching.

The time spent in the nest and the number of visits made by the male and female were also registered.

2.8. Mate-guarding

Seasonal variation in mate-guarding was investigated in 1991, within one hour of midday to avoid diurnal changes in the intensity of this behaviour. Burrow exits from different nests (range 23-81) from 5 subcolonies were observed each week (most of them in the main subcolony). Records were made as to whether a bird left a burrow alone or if members of a pair left within 5 seconds of each other. In this case mate guarding was inferred (Jones 1985).

Mate-guarding was also registered by time budgeting pairs in their nests (as a measure of the time members of a pair stayed together in the nest) at the pre-laying (-5th to +4th day, with 0 being the first egg day) and laying stages. The nests were time budgeted 6 hours a day split in three sections (early morning: 7-9h, late morning: 10-12h and late afternoon: 16-18h). For time budgeting, an average time for each activity (min/h) was calculated for each nest.

2.9. Food supply and weather data

The food supply was measured using a 12.2m high suction trap (Taylor and Palmer 1972, Taylor 1973a, Bryant 1973). It was located on the University of Stirling campus, 8km from Barbush site. Taylor (1973b) found a strong correlation between the volume of insects daily caught at suction traps located over 100km apart. Therefore, the data collected was assumed to be representative of the study area. The trap was run continuously and samples removed daily at 10h, giving a daily measure of aerial food abundance by volume of insects caught (there were settled volumes in a measuring cylinder). Large insects, of >1.5cm winglength, not usually eaten by hirundines, were excluded from the measured volumes of insect catches.

Maximum and minimum temperature, rainfall and wind were recorded daily at the Parkhead station on the University of Stirling campus, adjacent to the suction trap. Mean temperature was calculated by dividing the sum of maximum and minimum daily temperature by 2.

2.10. Radio-tracking

2.10.1. Radio-tags and radio-tracking.

Birds were caught using a hand net as they left their burrows. They were weighed, measured, radio-tagged, marked with coloured dye as required and released within 5min. A trial was carried out in which a radio-tag was glued (cyanoacrylate) between the shoulders of a Sand martin directly above the centre of gravity, following O'Connor *et al.* (1987) and Brigham (1989). The radio-tag fell off, or was removed by the bird within 24h, so this technique was abandoned in favour of tail-clips.

Radio-tags were attached using miniature tail-clips (East and Hofer 1986, Greig-Smith 1985, Johnstone 1992) so that they were hidden by the undertail coverts. Tail-clips weighed 0.3g and consisted of two perspex plates clamped together using a pair of nylon bolts. The single stage SS-2 radio-tags (Biotrack, Dorset, UK) weighed 1.0g, resulting in a total package mass of 1.3g. The 15cm long external whip antenna projected from the tip of the tail. Only the central four tail feathers were contained within the clip so that the eight outer feathers could be spread as required. In

Sand martins, the post-nuptial moult includes the tail (Turner and Rose 1989) so tags would soon be lost. However such an additional load on the tail may change the centre of gravity of the bird, potentially increasing flight costs or compromising manouverability (Evans and Thomas 1992).

Information on nest attendance was collected for two hours during late morning while they were being radio-tracked. A neighbour's nest at the same stage was used as a control. The birds were marked with dyes so that they could be easily distinguished with binoculars when entering and leaving their nest burrows.

Three sand martins were caught in the same subcolony and radio-tagged using tail-clips. Only the pair provisioning nestlings was radio-tracked during daytime. Birds were also searched for at night throughout the life-time of the radio-tags to locate roost sites.

All radio-tracking was done using hand-held three element Yagi antennal and RX-81 receivers. The $\theta_{.95}$ error arc (Springer 1979) of the antenna was $\pm 1.5°$. During daylight, birds were monitored by two observers from two fixed vantage points near the nesting subcolony. The compass bearings of radio-signals were simultaneously recorded from the vantage points every five minutes during each bout of tracking. These were then converted to locations on a 1:10,000 scale map of the study area. Four bouts of tracking each lasting 2h, were distributed over the day (early morning, late morning, afternoon and late evening). The radio-tracking was spread over a 48 hour period during which the weather was warm, calm and sunny. Analysis of radio-tracking data is described in Chapter 3.

2.11. DNA fingerprinting

2.11.1. Collection and storage of blood samples

Blood samples were taken from adults and nestlings to determine parentage by DNA-fingerprinting analysis. The blood was usually taken from the tibio-tarsal vein (above the intertarsal joint) of adults and from the brachial vein of chicks, using a sterile syringe needle (after cleaning the area to be bled with ethanol). The blood was collected in glass heparinized capillary tubes of 50µl, pipetted into 1.5ml Eppendorf tubes and immediately transferred to an ice box. The wound was firmly pressed with a finger to stop bleeding and a small amount of antiseptic cream applied. As soon as possible (no more than 4h later) the blood was transferred to a freezer at -70° (1990 and 1992 samples) or - 20°C (1991 samples).

The adults were usually bled during late incubation or when the nests already had chicks (usually on the second capture, to avoid risk of desertion). Nestlings were blood sampled on the 13th day after hatch. If a chick died, naturally or by accident, blood was taken from the jugular vein when possible; samples of the liver were also taken, and all the carcasses were stored in the freezer at -20°C. The blood samples of members of the same family were kept together in boxes (Nunc boxes for Eppendorf tubes).

2.11.2. DNA extraction

A small amount of blood (15-20µl) was pipetted into an Eppendorf tube containing 465µl of 1xSET buffer (0.05M Tris, 0.15M NaCl, 1mM EDTA pH 8.0) and mixed to a 'pink' solution. The cells were lysed by adding 7.5µl of SDS (sodium dodecyl sulphate) at 25% (weight/volume). The tube was then shaken and 15µl of proteinase K (10mg/ml) was added. After gentle mixing the tubes were incubated overnight at 55°C (usually 12 samples at one time). The proteinase K was used to inactivate the nuclease enzymes and disrupt the DNA-protein complex.

To separate and remove the DNA from the proteins a series of extractions was made with immiscible organic solvents. To each Eppendorf tube was added 150µl of TE buffer (10mM Tris, 1mM EDTA pH 8.0) and 500µl of phenol. The tubes were stirred by gentle rotation (15rpm) for 30min and the phases separated by centrifuge (11,600g) for 10min. The upper layer, where the DNA remained as an aqueous solution, was transferred to a fresh Eppendorf tube, trying to avoid material from the interface. The proteins and other material from the blood were dissolved in the phenol or the interface. Phenol extraction was repeated once again, adding more TE if the volume of the upper layer fell below 300µl. The extraction was done twice using a 24:23:1 (v/v) mixture of phenol/chloroform/isoamyl alcohol until the upper layer was clear and colourless and no protein precipitated at the interface. To remove all the traces of phenol from the DNA solution a final extraction with chloroform was carried out.

The volume of the solution resulting from the extractions was estimated and 2 volumes of pure ethanol at -20°C added to precipitate the DNA. The samples were then mixed by gentle rotation for 10-15min and kept at -20°C overnight to allow the DNA to precipitate out. To pellet the DNA the tubes were centrifuged at 11,600g for 10min. The ethanol was poured off leaving the pellet of DNA in the tubes. A wash with 1ml of 70% ethanol was carried out, samples were centrifuged for a further 10-15min, and the ethanol poured off. The pellets were then dried by leaving the tubes on an open bench or in a 37°C incubator with the lids open for 30-40min. To each sample 100-150µl of TE buffer was added (less if the pellet was small); and the samples were left in a 55°C waterbath overnight to allow resuspension of the DNA. Samples of DNA were stored at 4°C until required, with each sand martin family in a separate box.

The DNA from the liver tissue was extracted by removing about 0.5mg of the frozen tissue with a spatula and grinding it to a fine powder with a pestle and mortar, using liquid nitrogen to keep the tissue frozen. The powdered tissue was immediately transferred to a Eppendorf tube containing SET buffer. Extraction was then carried out as described for blood samples.

2.11.3. DNA restriction

From the isolated DNA 30μl was pipetted into an Eppendorf tube and to it was added 4μl of spermidine trichloride, 4μl of the reaction buffer and finally 2μl of the restriction enzyme (Hae III). The samples were left overnight at 37°C, and then assayed using a Fluorimeter taking 2μl of each for a reading. The standard used was 2μl of 1mg ml^{-1} calf thymus DNA in 2ml of working dye solution (TNE buffer, 1μl of Hoechst 33258 10mg/10ml per 100ml of the buffer). The reading of DNA in the standard (1μ μl^{-1}) was 1000u (units). To check if the DNA was cut a minigel assay was carried out. From each sample 4μl was removed and loaded onto a 0.8% agarose mini-gel with 5μl of ethidium bromide (10mg/ml per 100ml of solution). The minigel was run for an hour at 100V and the resulting smear was examined.

The restricted samples were diluted with a solution of 2xBromophenol Blue (BPB): Ficoll R 400, EDTA, xylene cyanide FF. The BPB and xylene cyanol allow the DNA to be visualised, acting as tracking dyes during the electrophoresis. The following formula was used to dilute DNA samples with BPB to the same concentration (6μg in 40μl):

$$\left[\frac{X\ (\text{units})}{150} \times Y\ (\text{volume})\right] - Y$$

X = Fluorimeter reading in units
Y = sample volume used (in the case of this study, 40μl was used).

2.11.4. Electrophoresis

Fragments of DNA were separated according to size in an agarose gel under the influence of an electrical field. Large fragments move more slowly than small ones. Since the DNA fragments are negatively charged, samples were loaded onto gels near to the cathode.

A 1% agarose gel (Seakem LE) was prepared with 375ml of TAE buffer solution (1x TAE, 0.004M Tris acetate, 1mM EDTA, pH 8.0), dissolved by microwave and cooled to 55°C and poured in a gel-mould (22x20cm) to set. The gel running kits obtained were Gibco BRL and were used with 15, 8x2mm loading wells. After it had set, the gel was placed in the electrophoresis tank containing 2.5l of 1xTAE buffer.

Samples of 40µl of restricted DNA and markers (bacteriophage lambda DNA digested with the restriction enzyme Hind III, 1µg/10µl) were loaded into the gel. The samples were left for 10min to equilibrate with the electrophoresis buffer prior to switching on the powerpack at 20V. After 1h the voltage was increased to 40V and electrophoresis carried out for 48h.

2.11.5. Southern blotting

The DNA fragments were transferred from the gel to a solid support by Southern blotting onto a nylon membrane (Zeta-probe), so maintaining their relative positions. This technique provided a permanent copy of the gel, which was stored at room temperature.

To prepare the Southern Blot, the following procedure was used following electrophoresis. The gel was carefully removed from the loading tray, inverted and soaked in a shallow tray containing 0.2M HCl for 10min. The acid breaks up some of the larger fragments allowing them to be more easily transferred from the gel during blotting. The gel was then soaked in 1.5M NaCl, 0.5M NaOH for 35min. This alkaline solution separates the double stranded DNA, which also eases transference of the DNA. The gel was finally soaked in neutralizing solution of 3M NaCl, 0.5M Tris pH 8.0 for 45min.

The 'blot' was then set up. A strip of 3mm of 'Whatman' chromatography paper, wider than the gel, was wetted with 20x SSC (3M NaCl 0.3M sodium citrate) solution in a tray. The chromatography paper was then placed on a piece of glass laid across the tray, allowing the ends of the paper to dip into the solution and act as wicks. The air bubbles were removed by rolling the damp paper gently with a pipette. The gel was then placed on the top of the paper. A piece of Zetaprobe GT membrane 20x20cm was wetted in double distilled water and placed carefully over the gel to cover the areas containing DNA. The rest of the gel was trimmed away with a scalpel, and surrounded with strips of parafilm to ensure no 'short circuits' (transfer solution passing straight into the paper towels). Two pieces of 3mm of 'Whatman' chromatography paper (slightly larger then the gel) were then wetted in double distilled water and placed on top of the membrane, again removing air bubbles by gently rolling them flat with a clean pipette. Another two dry pieces of this paper were placed on top, followed by layers of paper towels and a glass plate. Finally, a weight was put on top (not exceeding 500g) and the whole left overnight. The solution (SSC) drawn up from the gel towards the paper towels carries fragments of DNA with it to the membrane, keeping them in the same positions as on the gel. The transference of the DNA from the gel to the membrane is likely to take place during the first 4 hours. After that the gel becomes crushed and DNA movement is inhibited.

The next day, the membrane was removed, washed in 2xSSC for 30s, air dried for about 1h and baked at 80°C for 30min. The membrane was then kept in a sealed plastic bag at room temperature.

2.11.6. Probing the membranes

A hybridization probe is a piece of nucleic acid which binds to specific target sequences of the 'minisatellite' DNA immobilized on the membrane surface. The RNA probe used was prepared at Nottingham University, and grown by Carole Campbell (technician of Stirling University - Avian Group Laboratory), who also helped with probing the membranes. RNA probes were produced by inserting human probes 33.6 and 33.15 into EcoRI and HindIII sites of the transcription vectors pSPT 18 and pSPT19 to yield four recombinants - pSPT 18.6, 19.6, 18.15 and 19.15 (Carter 1989; Carter *et al.* 1989). The one used in this study was pSPT 19.6, derived from the human probe 33.6 (Jeffreys 1985). RNA probes have a greater ability to incorporate radioactivity than DNA probes allowing autoradiographs to become exposed more rapidly.

The probing process was done in three steps. First, the pre-hybridization, which prevented non-specific hybridization of the labelled probe to positive charged sites on the membrane. The membrane was washed with proteinaceous 'blocking' agents, e.g. 500ml of 1xSSC, 2% SDS and 1% BLOTTO (1% non fat powdered milk and 0.02% sodium azide, sterile distilled water and 1µl of diethylpyrocarbonate). The labelled membranes were soaked (one by one) in the pre-hybridization solution kept in a plastic box (up to 10 membranes were processed each time) and left in a shaking water bath at 65°C for 4-6h. Second, the hybridization, in which one 1µg of plasmid containing the minisatellite region (that had already been linearized) is labelled in a reaction containing unlabelled nucleotides (UTP, GTP, and ATP), transcription buffer DTT, T_7 RNA polymerase and a radioactive nucleotide, α^{32}P CTP. RNA copies were produced by transcription, using T7 RNA polymerase enzyme and nucleotides containing radioactive phosphorous. The transcription reaction producing multiple RNA copies of the minisatellite, and incorporating into them α^{32}P CTP, was done using a commercial kit (Riboprobe Gemini System II, Promega). The labelled probe was then added to the pre-hybridization solution, and the membranes left to hybridize overnight in a shaking water bath at 65°C. Finally, the membranes were washed four times (every 30 min) in a solution of 1xSSC, 0.1%SDS at 65°C.

2.11.7. Autoradiographs

The radioactive probe, attached to areas of DNA on the membranes which have a complementary sequence of nucleotides, can be detected by autoradiography. After the post-hybridization washing, the damp membranes were wrapped in 'Saranwrap' and exposed for 2-6 days at -70°C or for 1-3d at room temperature, using pre-flashed Fuji RX X-Ray film and two intensifying screens.

If re-probing was necessary, the membranes were stripped twice (for 15 minutes) in 0.4M NaOH at 45°C, and then twice in 0.2M Tris/HCl pH 7.5, 0.1% SDS, 0.1% SSC at 45°C.

2.12. Statistical analysis

Statistical procedures followed Zar (1984), and were performed with the assistance of Minitab and SPSSX. Parametric statistics were used for normal data distributions (or for $\log_{10}$ transformed data, such as insect availability measured by suction trap), but in cases where data violated the necessary assumptions (or in cases of small sample size) non-parametric tests were used instead. The tests were two-tailed, unless specified, and the 5% level was accepted as the level of statistic significance. Mean±sd is given throughout, unless otherwise stated.

Latin names of all species mentioned in the text are given in Appendix 2.

CHAPTER 3 - REPRODUCTIVE ECOLOGY AND BEHAVIOUR

3.1. INTRODUCTION

3.1.1. The study species

Sand martins, or bank swallows as they are known in America, have one of the most extensive ranges of any swallow; breeding in North America, Europe and Asia, to northern India, southeastern China and the northern islands of the Pacific cost (Cramp 1988). They are migratory, wintering in South America, Africa, India and southern Asia. The main wintering sites for western Europe and Siberian populations are the African Sahel zone and in eastern Africa south to Mozambique (Turner and Rose 1989).

The Sand martin is the smallest hirundine species breeding in Britain, where they arrive in mid-April (Cramp 1988). Nests are built at the end of burrows dug into sand and earth banks along rivers, sand and gravel pits (Morgan 1979). Sand quarries may have increased the number of available breeding sites, affecting the distribution or abundance of the species (Jones 1986a). Colonies in sand quarries are considerably larger than most natural colonies, reaching up to several hundred pairs (Hjertaas *et al.* 1988, Jones 1986a).

Sand martins are aerial insectivores and serve as an example of social 'central place foragers' (Bryant and Turner 1982). They can be highly colonial, with burrows often only 30cm apart (Sieber 1980). An obvious feature of nuptial behaviour is sexual chases (Tobby 1947) by males seeking extra-pair copulations (Jones 1986b).

3.1.2. Space use by breeding sand martins

Space use by breeding birds can be divided into two broad categories. Some birds exploit and defend relatively small and exclusive territories, containing the nest site and sufficient food supplies. Others forage over much larger areas, tracking ephemeral food patches, which are not defended. The latter often benefit from nesting and feeding in groups. Examples of the second category include seabirds, swifts and hirundines (Lack 1968).

Despite extensive work on breeding birds at colonies, relatively little is known about where individuals roost or go when away from the colony. Radio-tacking was used in the present study to investigate where sand martins go or roost, if members of a pair use the same space when away from colony, and how far away from their colony they travel. Radio-tracking facilitates studies where direct observation is made difficult by cover, distance or darkness, but it has seldom been applied to small birds (East and Hofer 1986, Greig-Smith 1985, Hanski and Haila 1988, Johnstone 1992, Nygard and Einavik 1992, O'Connor *et al.* 1987). Although radio-tags have been used on hirundines (Brigham 1989), and on bats weighing less than 10g (Lunney *et al.* 1985), the sand martin is the smallest bird to be radio-tagged to date.

The aims of the space-use investigation (done in collaboration with I.G. Johnstone) were 1) to assess the feasibility of radio-tagging sand martins and 2) to illustrate the type of novel information radio-tracking can provide on behaviour away from the colony, foraging ranges and roost site selection in aerial feeding birds. Particular reasons for using radio-tracking in this study were: a) to determine if individuals during the pre-laying stage (when males guard their mates) stay together when away from their nests (where possibly extra-pair copulations can take place); b) and if individuals rearing a brood use similar space when away from the nest site.

This Chapter also presents information on aspects of breeding biology and ecology of sand martins, such as colony size, nest burrow success, clutch and brood-sizes and frequency of second broods, as a baseline to understand the social organization of these birds.

3.2. METHODS

Methods for recording population variation through the breeding season (censuses), clutch-size, mate-guarding and time budgets of adults at nests, and also radio-tracking, are described in the general methods (Chapter 2).

3.2.1. Body-size measurements

Keel-length was considered a reliable measurement of body size and correlated with body mass in both males and females (Jones 1985). It is likely to be heritable, and does not increase in length once a bird has reached independence; contrary to wing-length, which is influenced by age, and to body mass which depends on food abundance and stage of the reproductive cycle (Bryant and Jones unpubl., Jones 1985). Therefore, in the present study, keel-length was used as a measure of body size. For the analysis, each individual was considered only once in the three years (i.e. retrapped individuals were not considered).

3.2.2. Analysis of radio-tracking data

Estimates of home range sizes require behavioural data which are statistically independent (White and Garrot 1990). The degree of statistical independence between radio locations was evaluated by comparing colony attendance estimated from radio-tracking data with nest attendance measured by direct observation over the same period. Both individuals attended the nest burrow between consecutive locations. Therefore, using a sample interval of 5min, locations were considered independent following the 'rule of thumb' that the time taken to cross the home-range is equal to the time to independence (White and Garrot 1990). Space use was quantified by using the grid-cell technique since this is a non-statistical range estimator which makes the fewest assumptions about the utilization distribution (White and Garrot 1990).

Two variables are required to describe the use of space by an animal relative to a central place: direction and distance. The direction of the location estimates from the nest burrow were measured to the nearest 10° on the map of the study area. Locations that were less than 100m from the burrow were considered to be at the burrow and had no bearing from it. Distances of locations from colony and river were measured to the centre of grid cells and rounded down to the nearest 100m.

Since both members of the pair were tracked concurrently and the successive locations were independent, it was possible to measure the degree of interaction between the birds. A measure of static interaction can provide information on the degree to which two utilization distributions overlap, and also whether the shared area contains the least or most utilized parts of each range. The degree of static interaction was calculated using the 'Static' computer program of Doncaster (1990).

Simultaneous pairs of locations can also provide an estimate of dynamic interaction between a pair of animals. Positive dynamic interaction indicates two individuals occur close together more often than would be expected at random (mutual attraction), negative dynamic interaction indicates mutual avoidance (Doncaster 1990). A critical separation distance between pairs of locations of 150m was used to include the eight cells surrounding the occupied cell. Dynamic interaction was calculated using the 'Dynamic' program (Doncaster 1990).

3.3. RESULTS

3.3.1. Population variation at Barbush site

The birds usually arrived late March, early April. The earliest recorded arrival at Barbush was 29th March, in 1990, and the latest on the 9th April. They were qualitatively observed to settle first in the higher and central places in a subcolony. The earliest date of the first egg registered was 1st May, but they probably had laid before that date in nests inaccessible to the observer.

Barbush colony was composed of several subcolonies (separated by more than 25m), which varied slightly in number and distribution between years, according to sand extraction locations. The number of occupied subcolonies was 9 in 1990, 7 in 1991, and 5 in 1992. The main study subcolony (subcolony 3) remained unquarried for two years (1990 and 1991). It was located near the top of a large and relatively stable cliff (Figure 3.1).

The settlement pattern of sand martins in each year at Barbush for all subcolonies combined is presented in Figure 3.2. The maximum number of occupied burrows in the breeding season was greater in 1990 than in 1991 and 1992. The total number of occupied burrows in 1991 (n=413) was 46% less than 1990 (n=770); and in 1992 (n=516) it was 33% less than 1990. The colonization pattern of the 3 largest subcolonies in 1990 is presented in Figure 3.3.

The total number of birds ringed in the three years of the study was 956. Of these were 341 adults and the rest nestlings or juveniles (hatched in the same season they were first captured). The adults included 149 males, 164 females and 28 of unknown sex. The number of adults recorded breeding in subsequent seasons over the three years was 57 (34 males and 23 females), accounting for 22.8% of the males and 14% of the females marked (including birds ringed from earlier studies in Britain or elsewhere).

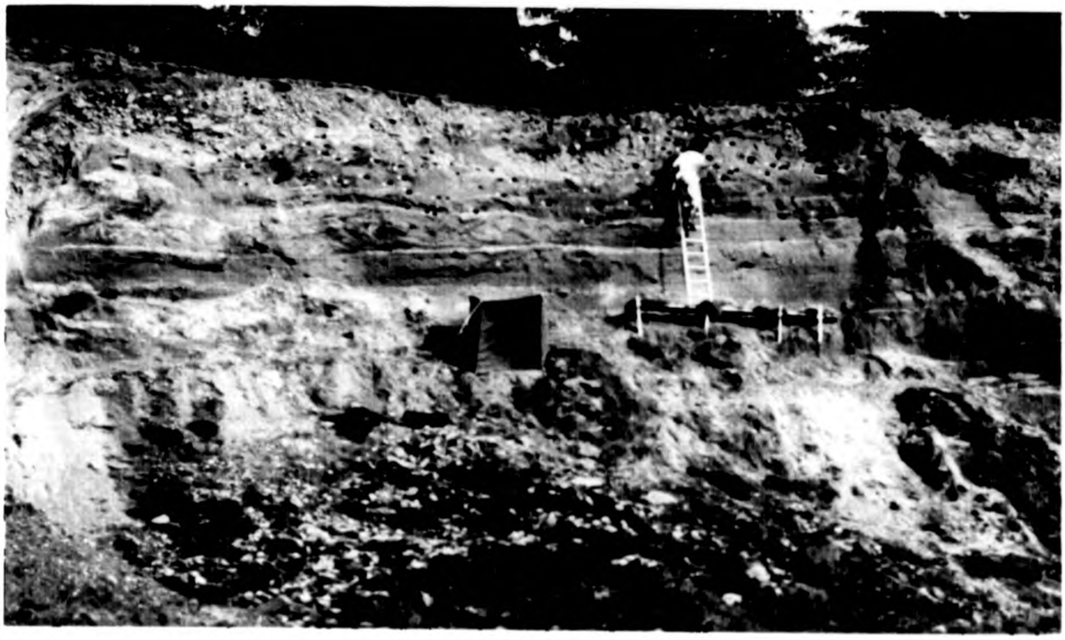


Figure 3.1 - Sand martin subcolony 3 at Barbush, 1991.

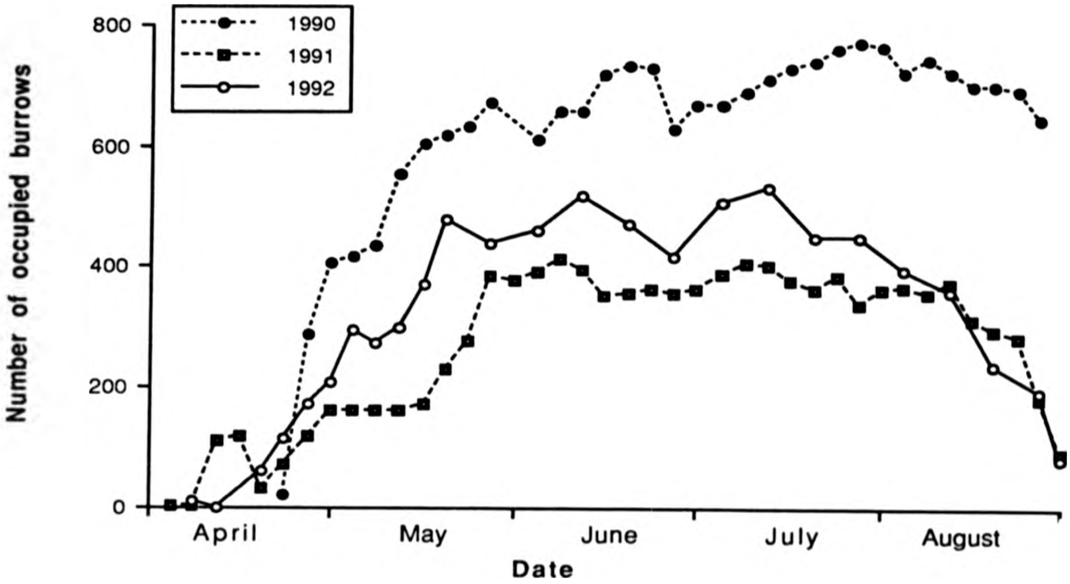


Figure 3.2 - Seasonal changes in the estimated number of occupied sand martin burrows at Barbush in 1990, 1991 and 1992.

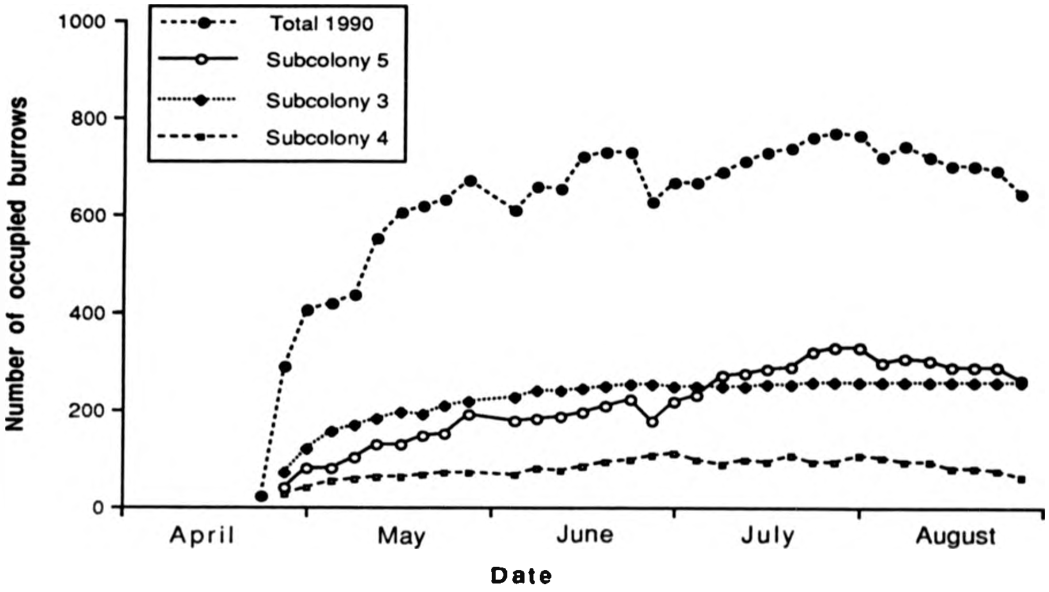


Figure 3.3 - Seasonal changes in the estimated number of occupied burrows at the three largest sand martin subcolonies at Barbush in 1990.

3.3.2. Body-size variation

Keel-length was the chosen measure of body-size. An analysis of variance (ANOVA two-way) of the effects of sex and year on keel-length of adult sand martins (Table 3.1) showed that a) measured keel-length did not vary consistently with sex; b) keel-length varied significantly between years, and c) there was an interaction between year and sex affecting keel-length, suggesting that keel-length variation between years was different for males and females. Separating males and females, there was a significant difference ($p<0.001$) in keel-length between years for males and females (Table 3.2). The Tukey range test indicated that female keel-lengths in 1990 were significantly higher than in 1991 ($q=11.83$, $p<0.001$) and 1992 ($q=5.82$, $p<0.001$). The keel-length of males was also higher in 1990 compared to 1991 (Tukey test, $q=4.00$, $p<0.05$), and 1992 (Tukey, $q=6.11$, $p<0.001$). Keel-length did not differ between 1991 and 1992 for females, but was higher in 1991 than in 1992 for males (Tukey, $q=35.0$, $p<0.001$). The frequency distribution of keel-lengths for males and females in the three years is illustrated in Figures 3.4 and 3.5. In 1991, males with keel-lengths in the range of 19+mm were found in a similar proportion as in 1990 (6%). Females, however, in the same category almost disappeared in 1991, being reduced from 21% to 2% of measured individuals. In 1991 63% of females were found in classes <18mm compared to 26.3% in 1990; while 31% of males were found in that class compared to 45.6% in 1990. In general, smaller individuals became more frequent during the course of the study, with a particular marked change between the 1990 and 1991 breeding seasons.

3.3.3. Nest burrow success

The number of unmanipulated nests with eggs and/or chicks at Barbush subcolonies and their fate through the

Table 3.1 - Comparison by ANOVA (two-way) of adult sand martin keel-length(mm) between years (1990, 1991 and 1992) and sexes (n=198).

Factor	F	df	p
Sex	0.00	1	0.99
Year	13.95	2	<0.001
Interaction	3.98	2	<0.02

Table 3.2 - Average keel-length measures (± sd) of female and male adult sand martins in 1990, 1991 and 1992.

	Year				
	1990	1991	1992	F	p
Females	18.48±0.63 (38)	17.77±0.59 (47)	17.84±0.75 (9)	13.37	<0.001
Males	18.23±0.59 (47)	18.03±0.65 (46)	17.68±0.45 (11)	3.91	<0.05

F= variance ratio for significance testing of one-way ANOVA

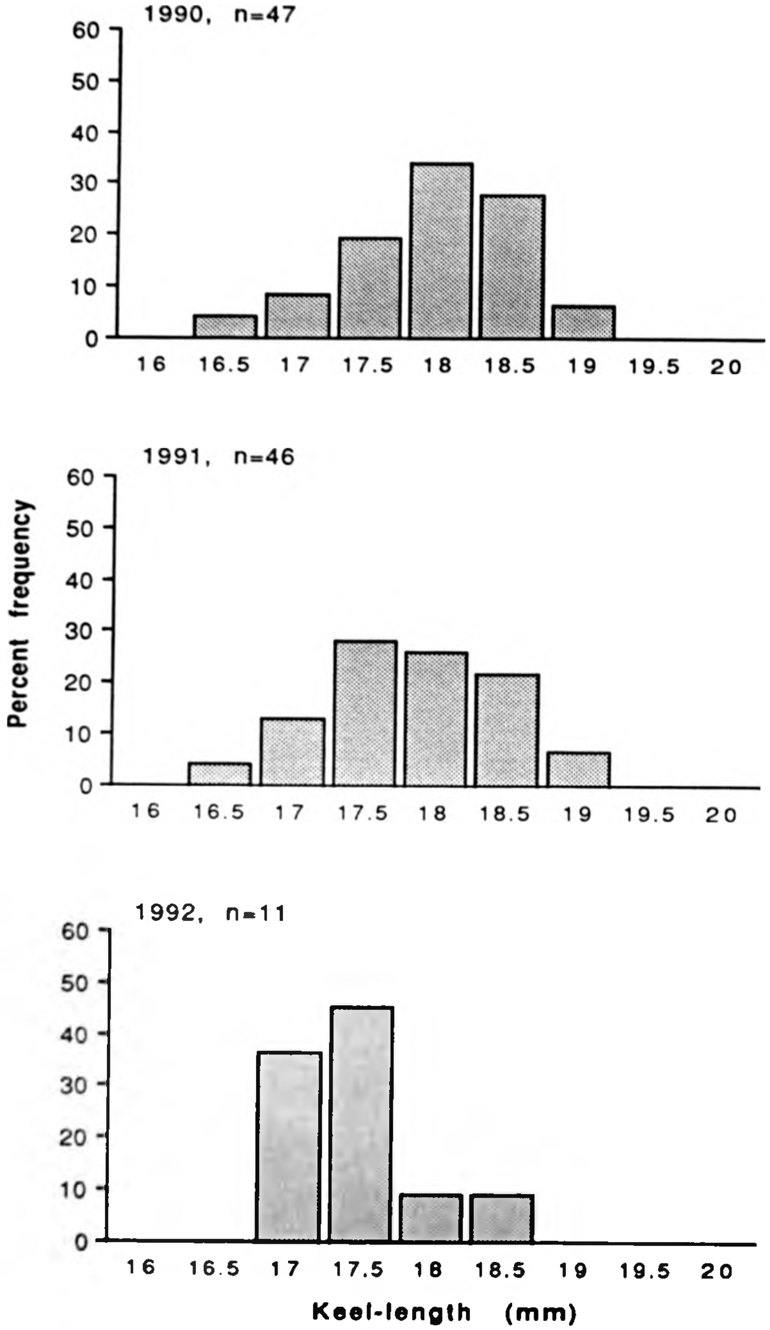


Figure 3.4 - Frequency distributions of keel-lengths in male sand martins at Barbush between 1990 and 1992. Intervals labelled (i.e.) 16, include values in range 16.01-16.50, those labelled 16.5, include values 16.51-17.00, and similarly for the other values shown.

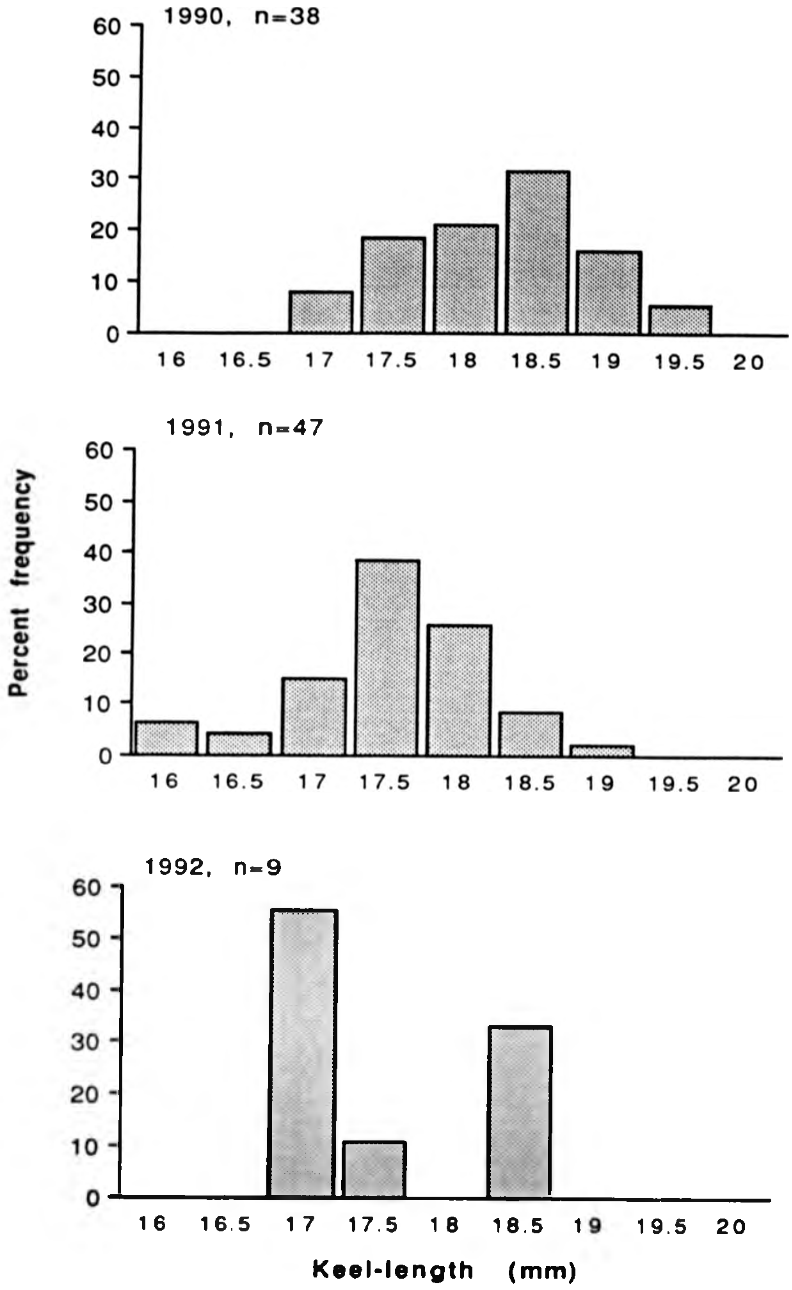


Figure 3.5 - Frequency distributions of keel-lengths in female sand martins at Barbush between 1990 and 1992. Intervals labelled (i.e.) 16, include values in range 16.01-16.50, those labelled 16.5, include values 16.51-17.00, and similarly for the other values shown.

breeding seasons of 1990, 1991 and 1992 are shown in Table 3.3. 'Second clutch' nests were those that were reused in the same season, but may include a few nests where birds relaid and some exceptionally late first clutches, or even birds which moved in after failing elsewhere, as well as 'true' second clutches.

Hatching and fledging success of nests was recorded when nests had at least one egg hatched or one chick fledged. The total number of fledglings was not counted, but the number of chicks coming to the burrow entrance to be fed before fledging was generally similar to the number counted at the 13th day after hatch, taking approximately 24 days to fledging.

The overall hatching success (excluding manipulated nests, and nests with observer disturbance from catching) in 1990, 1991 and 1992 was 61% for 'first clutches', and 85% for 'second clutches' (Table 3.4). Overall fledging success (again excluding manipulated nests, and nests with observer disturbance caused by catching) in the three years was 82% for 'first clutch' nests, and 78% for 'second clutch' nests (Table 3.5).

Most of the nests monitored through the breeding season in 1990 and 1991 came from subcolony 3 at Barbush. The overall hatching success of the nests in these two years in subcolony 3 was 79% for 'first clutch' nests, and 87% for 'second clutch' nests; while the fledging success was 92% for the 'first clutch' nests and 86% for the 'second clutch' nests. The overall success of nests with eggs or chicks that produced at least one fledgling was 54% (including 'first' and 'second clutch' nests).

Sand martins seemed to desert their nests more easily when caught at the pre-laying and laying stages, particularly females, as was also observed by Riley (pers. comm.).

Table 3.3 - Fate of non-manipulated sand martin nests through the seasons of 1990, 1991 and 1992. F= nests with fledglings, P=mammal predation, OD=deserted possibly due to observer disturbance (catch), HD=deserted by human disturbance (vandalism), UR=deserted for unknown reason. 'First clutches' = first nests found in burrows in the breeding season; 'second clutches' = nests found in burrows that had already been used for nesting in the same breeding season. Spaces = zero.

		'First clutches'						'Second clutches'					
Year	Subcolony	Nests with eggs or chicks	F	P	OD	UR	HD	Nests with eggs or chicks	F	P	OD	UR	HD
1990	1	14		8	1	5		5	1	1		3	
	2	5		1	2	2		1	1				
	3	22	15		2	5		1				1	
	8	6	2		2	2							
	9	2	1			1							
	Total	49	18	9	7	15		7	2	1		4	
1991	1	5		3		2		3	3				
	6	6	3		1	2		2	2				
	10	4				3	1						
	3	28	19	2	1	6		9	6		1	2	
	Total	43	22	5	2	13	1	14	11		1	2	
1992	10	20	12			8		7	5			2	
	<u>Total</u>	112	52	14	9	36	1	28	18	1	1	8	

Table 3.4 - Hatching success of unmanipulated sand martin nests excluding those disturbed by catching in the 1990, 1991 and 1992 breeding seasons. 'First clutches' = first nests found in burrows in the breeding season; 'second clutches' = nests found in burrows that had already been used in the same breeding season. H = nests with at least one hatched egg. Nests from subcolony 3 are given in brackets.

	First clutches			Second clutches		
	Nests with eggs or chicks	H	%	Nests with eggs or chicks	H	%
1990	42 (20)	27 (17)	64 (85)	7	5	71
1991	41 (27)	24 (20)	58 (74)	13 (8)	12 (7)	92 (87)
1992	20	12	60	7	6	86
TOTAL	103	63	61 (79)	27	23	85 (87)

Table 3.5 - Fledging success of unmanipulated sand martin nests (excluding those disturbed by catching) in the 1990, 1991 and 1992 breeding seasons. 'First clutches' = first nests found in burrows in the breeding season; 'second clutches' = nests found in burrows that had already been used in the same breeding season. F = nests with at least one fledgling. Nests from subcolony 3 are given in brackets.

	First clutches			Second clutches		
	Nests with eggs hatched	F	%	Nests with eggs hatched	F	%
1990	27	18	67	5	2	40
	(17)	(15)	(88)			
1991	24	22	92	12	11	92
	(20)	(19)	(95)	(7)	(6)	(86)
1992	12	12	100	6	5	83
TOTAL	63	52	82	23	18	78
			(92)			(86)

3.3.4. Clutch size and brood size

The overall mean clutch size was 4.78±0.69 (n=129). The mean brood size at hatching was 3.90±0.01 (n=72) on the last day of hatching, and 3.79±1.02 (n=83) on the 13th after hatch. It was sometimes difficult to count newly hatched chicks with the 'ripariascope', due to their small size and presence of large number of lining feathers. Brood size on the 13th day was determined by counting chicks removed from the nest at that stage (see general methods, Chapter 2).

The mean incubation period (from last egg laid) was 14.93±1.60 days (n=69), and the average fledging period (from last hatched until the last chick fledged) was 23.85±3.08 days (n=52).

Overall seasonal changes in clutch size and brood size at Barbush between 1990-1992 are shown in Figures 3.6 and 3.7. Both clutch size and brood size decreased significantly through the season. Sand martins sometimes attempted second broods in a given breeding season. Since they often changed mates and subcolonies few records of 'genuine' second broods were obtained. During the three years, of 93 adults only 9 (9.7%), including 4 males and 5 females were proven to have second broods. In all these cases the birds changed partners after the first brood. One female moved to a different subcolony, and 4 females and 2 males moved to different nests in the same subcolony. The other 2 males did not change subcolony or nest for the second brood.

An estimated proportion of first broods which were followed by second broods was calculated from the bimodal distribution of first egg dates from 1991 and 1992 (1990 was excluded due to a small sample size). The first egg date distributions were not so clearly separated in 1991 as in 1992 (Figure 3.8). Therefore separation between the distributions of 1991 was done using the percentage cumulative frequency diagram method, making use of probability graph paper (Lewis and Taylor 1967)(Figure 3.8A). The mean estimated values for clutch size and brood

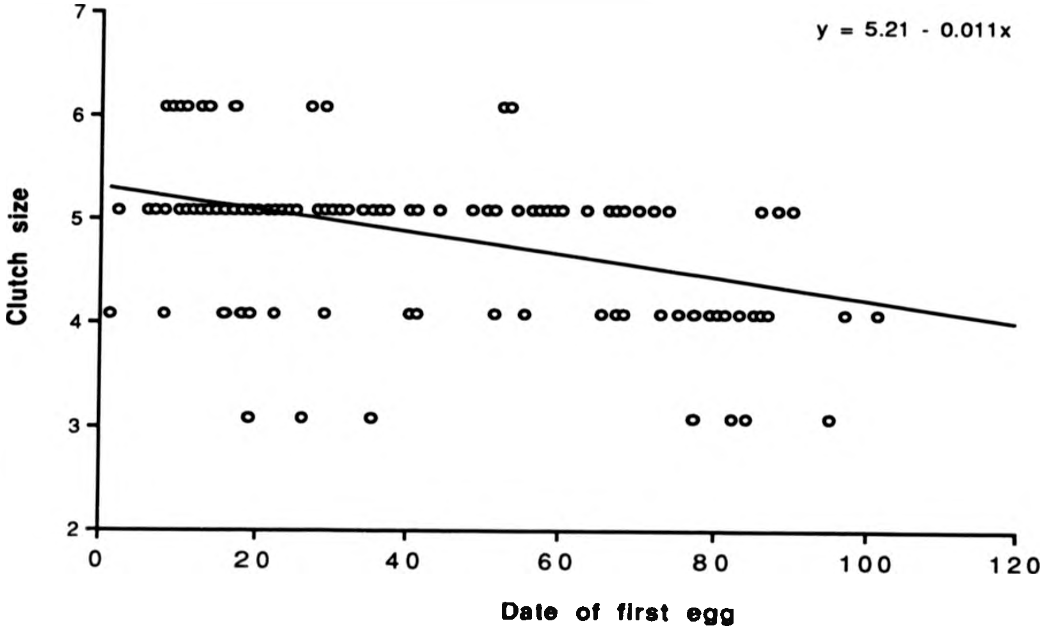


Figure 3.6 - Seasonal trend in clutch size for sand martins at Barbush, 1990-1992 (n=129, F=26.23, $p<0.001$, $r^2=0.16$). Date of the first egg given in days (day 1 = 1st of May).

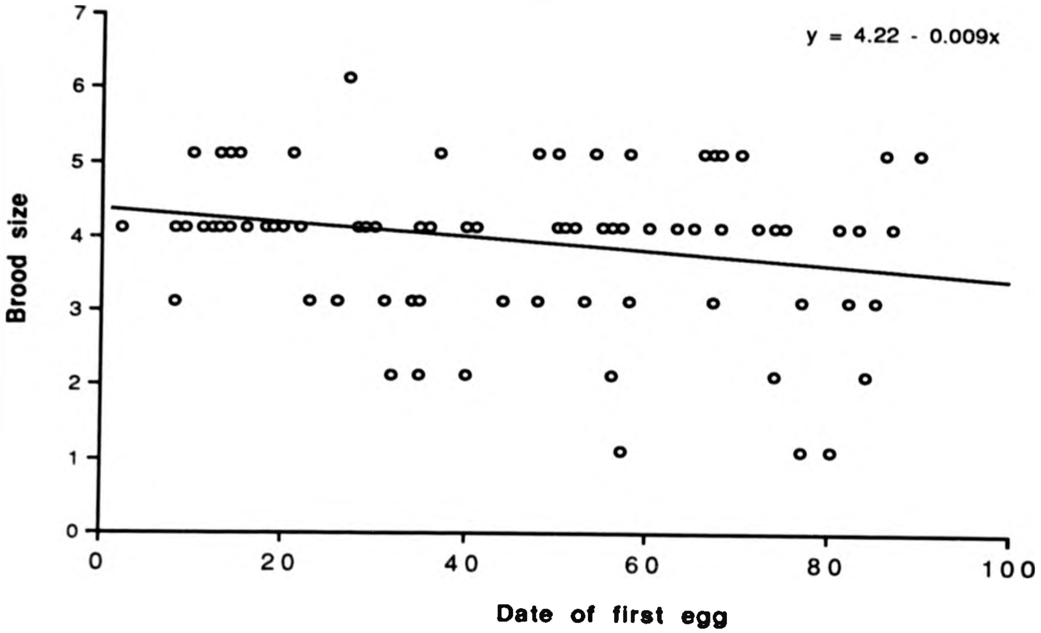


Figure 3.7 - Seasonal trend in brood size (number of nestlings on the 13th day after hatch) at Barbush, 1990-1992 (n=83, F=4.42, p=0.039, r^2=0.04). Date of the first egg given in days (day 1 = 1st of May).

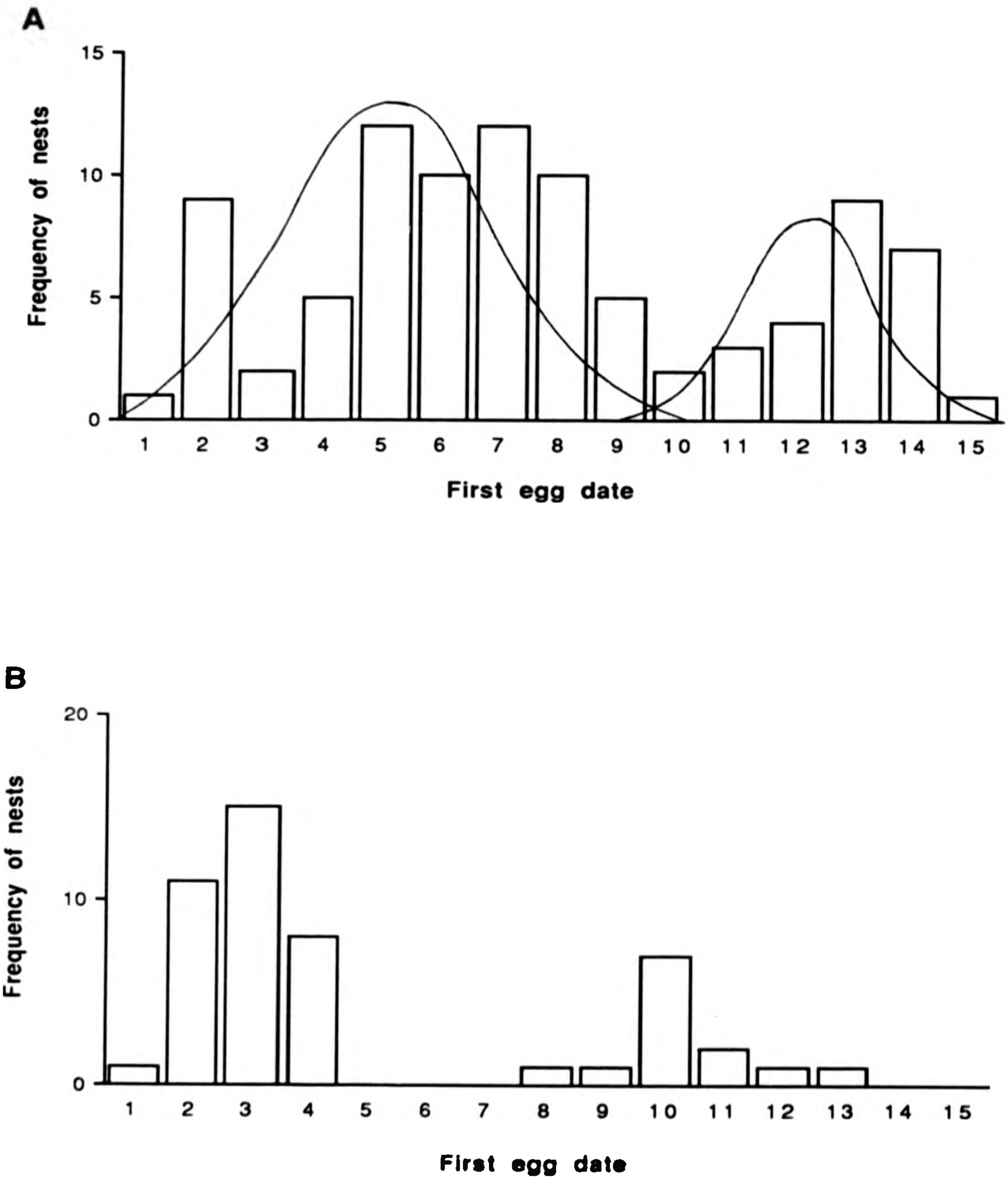


Figure 3.8 - First egg date distribution of sand martins at Barbush in A) 1991 and B) 1992. Distributions from A were separated using the percentage cumulative frequency diagram method. Date of the first egg given in weeks (week 1 starts on 1st of May).

size, derived from these distributions, for both first and second broods are presented in Tables 3.6 and 3.7. First and second clutch sizes did not differ significantly between the two years (Table 3.6). Second brood size also did not differ significantly between 1991 and 1992, although first brood size was significantly higher in 1992 (Table 3.7). This result could be explained by broods in 1992 being counted earlier in the season than broods from 1991.

The estimated frequency of birds attempting second broods was 27.1% (n= 48) in 1991, and also in 1992 (n=48). The frequency of second broods calculated by the 'ideal' distribution of first and second clutches presented in Figure 3.8A was 27.5%.

The seasonal decline in clutch size, presenting first and second clutches estimated by the first egg date distributions, is shown for 1991 (Figure 3.9A) and for 1992 (Figure 3.9B). Those patterns suggest a slight decline from earlier to late clutches in the first clutches, while second broods tend to have a steeper decline but not significantly so. Also, birds attempting earlier second broods seem to lay slightly larger clutches than the late first clutch layers. No significant difference was found, however, for the regression of separated first and second clutch sizes with date in 1991 and in 1992. Data from first clutches and second clutches in 1991 and 1992 were therefore combined and plotted against date, which was standardized for mean and standard deviation (zscore). Again, the regression lines were not significant at 5% level (r^2=10.3%, F=1.72, p=0.19, n=70 and r^2=8.37%, F=3.28, p=0.08, n=26, for first and second clutches respectively), although there was a trend for second clutches to decline (b=-0.25) more rapidly than first clutches (b=-0.10) (Figure 3.10). Clutch size was indeed significantly larger in first clutches than in second clutches (Table 3.6), but no significant difference in brood size was found between them (Table 3.7).

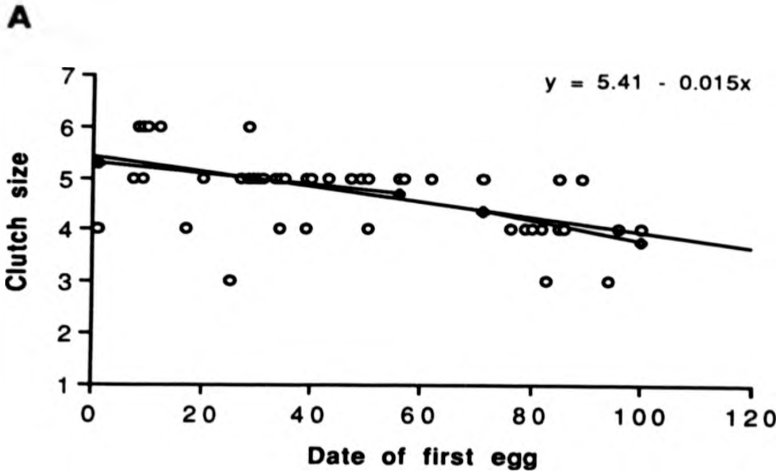
Table 3.6 - Means and standard deviations of clutch size for presumed first and second sand martin clutches at Barbush, in 1991 and 1992.

		Year			
		1991	1992	t	p
Clutch size	First	4.9±0.66 (35)	4.9±0.64 (35)	0.18	0.85
	Second	4.1±0.64 (13)	4.4±0.77 (13)	-1.11	0.28
t		4.26	2.34		
p		<0.001	<0.01		

Table 3.7 - Means and standard deviations of brood size for presumed first and second sand martin clutches at Barbush, in 1991 and 1992.

		Year			
		1991	1992	t	p
Brood size	First	3.6±1.03 (23)	4.4±0.72 (16)	-2.73	<0.01
	Second	3.6±1.33 (9)	3.9±1.22 (11)	-0.61	0.55
t		0.11	1.14		
p		0.92	0.27		

t = t-test



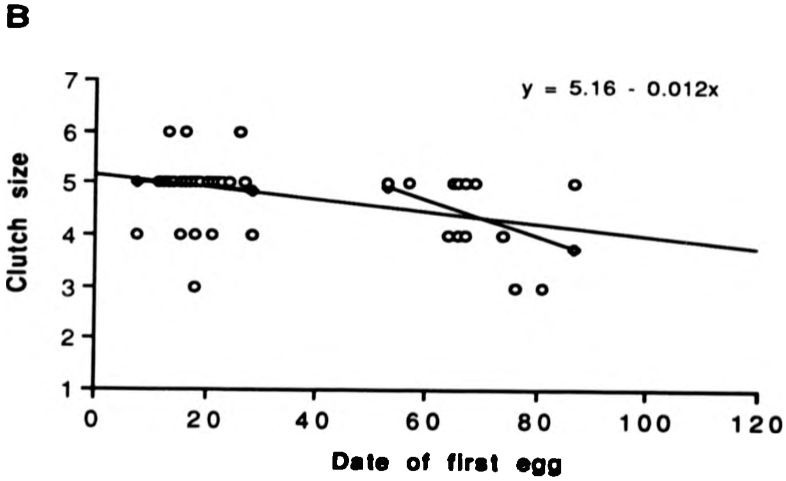


Figure 3.9 - Seasonal change in clutch size of sand martins, with first and second clutches separated by first egg distribution, at Barbush in A) 1991 ($F=20.73$, $p<0.001$, $r^2=0.29$, $n=50$) and B) 1992 ($F=8.36$, $p<0.01$, $r^2=0.13$, $n=48$). Date of the first egg given in days (day 1 = 1st of May).

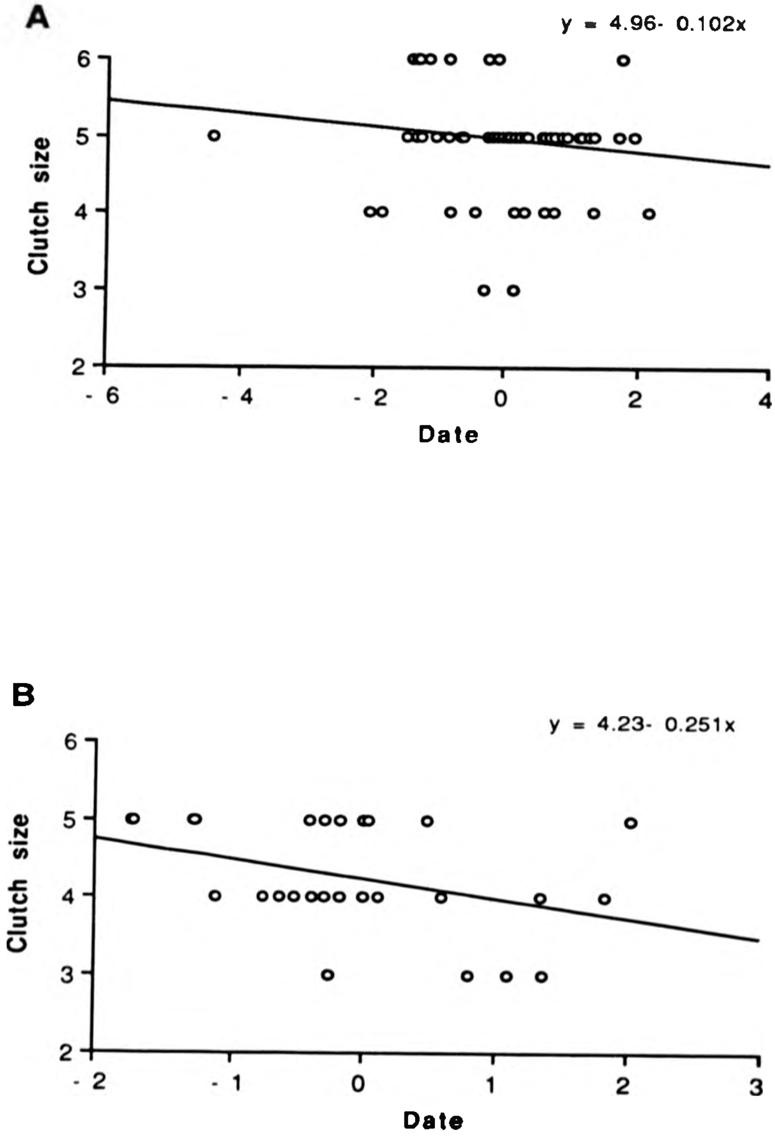


Figure 3.10 - Seasonal changes in clutch size of sand martins for combined first (A) and second (B) clutches from 1991 and 1992. Dates were standardized for mean and standard deviation (zscore). A) F=1.72, p=0.19, r^2=0.10, n=70 ; B) F=3.28, p=0.08, r^2=0.84, n=26.

3.3.5. Time budgeting at different breeding stages

The proportion of time (min.h^{-1}) during daytime spent in the nest at different stages of the breeding cycle by males and females in 1991 is shown in Figure 3.11 (means and their variation are given in Appendix 3.1). The median number of visits.h^{-1} to nests is presented in Figure 3.12 (means and their variation are given in Appendix 3.2). Data come from nests where each was observed for 6h in the pre-laying (n=10) and laying (n=12) stages, and 8h during incubation (n=14) and chick-rearing (n=15). Observation time was equally distributed for all observed nests in the pre-laying, laying and incubation stages, as described in Chapter 2; and the total nest-hours of observation was 364. There was no significant difference in time spent in the nest per hour, nor in the number of visits to nests, by males and females in the pre-laying stage (Wilcoxon matched pairs signed-ranks test, respectively $z=-1.34$, $p=0.180$ and $z=-1.83$, $p=0.07$). During laying females spent more time in nests than males (Wilcoxon matched pairs signed-ranks test, $z=-2.20$, $p=0.028$), while males made more nest visits (Wilcoxon matched pairs signed ranks test, $z=2.37$, $p=0.018$). During laying, males were often observed flying around the nest entrance, frequently entering for short periods.

Females spent more time incubating than males (Wilcoxon matched pairs signed-ranks test, $z=-2.79$, $p=0.005$), while males visited the nests more often (Wilcoxon matched-pairs signed-ranks test, $z=-2.10$, $p<0.036$). During chick rearing the time spent in the nest and the number of visits did not differ between males and females (Wilcoxon matched-pairs signed-rank test, respectively $z=-0.87$, $p=0.382$, and $z=-0.65$, $p=0.351$).

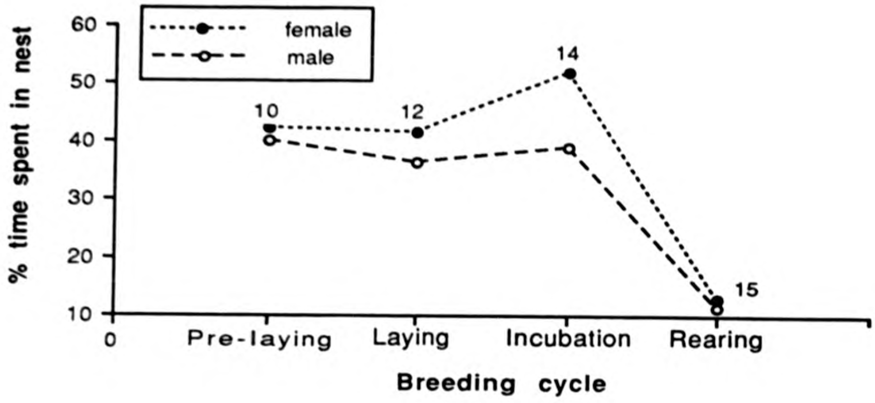


Figure 3.11 - Proportion of time spent in nests by adult sand martins during daylight in the pre-laying and laying stages (6h/nest); and incubating and chick-rearing stages (8h/nest) at Barbush colony in 1991. Ranges are given in Appendix 3.

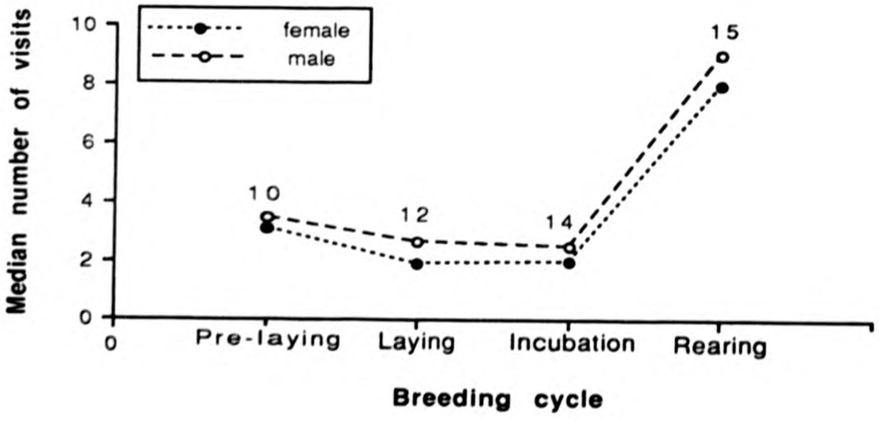


Figure 3.12 - Median number of visits h^{-1} by adult sand martins during the pre-laying and laying stages (6h/nest); and incubation and chick-rearing stages (8h/nest). Ranges are given in Appendix 3.

3.3.6. Space use using radio-tracking

3.3.6.1. Radio-tag performance and accuracy of locations

The radio transmitters had a maximum range of 1.2km and a cell life of approximately 7 days.

During daytime tracking, bearings could not be obtained on 5% of occasions for the male and 1% of occasions for the female. Sudden changes in signal strength suggested that this was due to birds being close to the ground where local topography blocked the signals, rather than to the birds being out of range.

All locations were assigned to one hectare grid-cells. Measurement of error polygons indicated that 95% of the locations conformed to this level of accuracy, following the criteria of Kenward (1987). The remaining 5% percent were less accurate, but as outlying locations, contributed little to the utilization distribution. Sand martins are highly mobile, so movement error may be an inevitable source of inaccuracy in the radio-locations. However, since pairs of bearings from each individual were always recorded within a few seconds of each other, this was thought to be of little importance.

3.3.6.2. Effects of radio tags

Radio-tags represented 10% of the mean body mass of the sand martin (13g), twice the widely recommended limit of 5% (Cochran 1980). Accordingly, the minimum power required for level flight with and without radio-tags was derived using the programs of Pennycuick (1989). This indicated that a sand martin of mean mass carrying a 1.3g tail-mounted radio-tag will incur a 15% increase in the power required for level flight. Laying sand martins (15g) routinely incur a 27% increase in minimum power, therefore, the additional load represented by the radio-tags fell well within that which female sand martins normally experience. It is likely, however, that laying birds anticipate increases in flight cost associated with mass change and may adjust their physiology accordingly.

Tagged birds behaved apparently normally. They flew with a mixed flapping and gliding gait typical of the species and could not be distinguished from other birds in flocks. When perching at the entrance of the burrows they were never seen to peck at the tags. Only small changes in body mass were shown between radio-tagging and recapture (Table 3.8), and no apparent damage to rectrices was observed.

The pair rearing chicks exhibited normal attendance behaviour just after tagging. They fed chicks at a broadly similar rate to a pair in a neighbouring nest used as a control (Table 3.8). The subsequent behaviour of the birds that were tagged is shown in Table 3.8.

3.3.6.3. Space use and habitat selection

The radio-locations obtained from the pair provisioning chicks, along with the locations of the subcolonies and the nearby river, are shown in Figure 3.13. The male and the female visited 69 and 39 one hectare grid-cells respectively.

On average the male showed little directional preference, while the female showed a strong preference for locations to the west of the subcolony (Figure 3.14).

For both male and female, over 70% of the locations occurred within 300m of the burrow. There was no significant difference in the distance from the subcolony between the two birds (Mann-Whitney W=22164, p=0.11, male n=154 and female n=148). Distances from the subcolony at different times of the day (early morning, late morning, afternoon and evening) differed for the male (Kruskal-Wallis, H=8.63, p=0.04), but not for the female (Kruskal-Wallis, H=5.74,

Table 3.8 - Capture and recapture details of sand martins that were equipped with radio-tags.

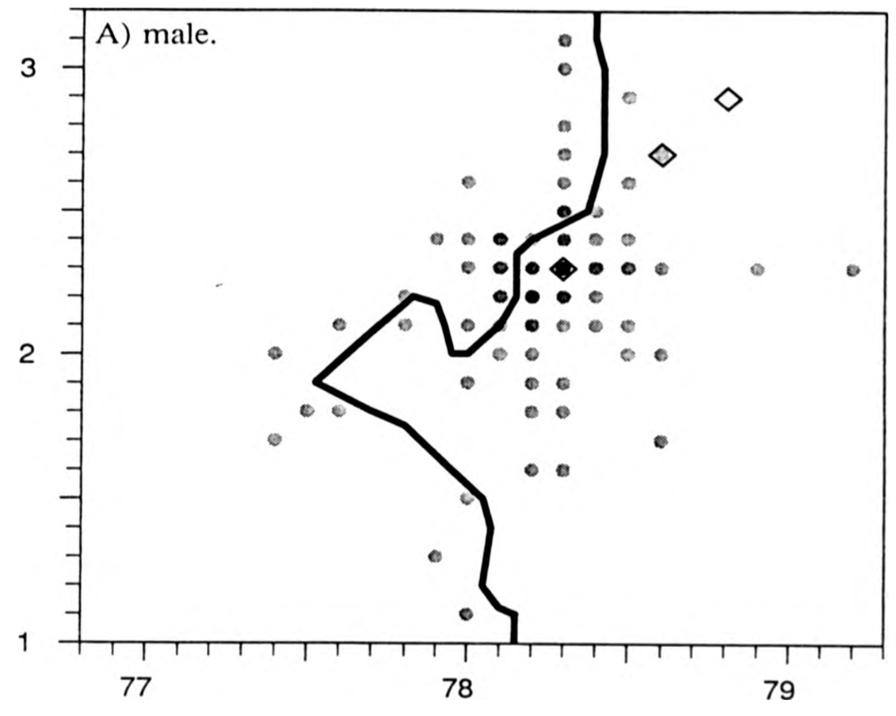
Sex	Date tagged	Breeding stage	Initial mass (g)	Duration (days)	Mass change on recapture (g)	Nº burrow visits[$]	Subsequent behaviour
Female	8th June	Provisioning nestlings (1st brood)	12.5	3[*]	0	34 (21)	1st brood fledged 2nd brood attempted 39 days after being radio-tagged.
Male	9th June	Provisioning nestlings (1st brood)	13.0	14[#]	+0.25	28 (23)	1st and 2nd broods fledged
Male	24th June	Mate guarding (2nd brood)	12.5	5[#]	-0.5		Mate deserted while attempting 2nd brood
Mean (±sd)			12.7 (0.29)	7.3 (5.9)	-0.1 (0.4)		

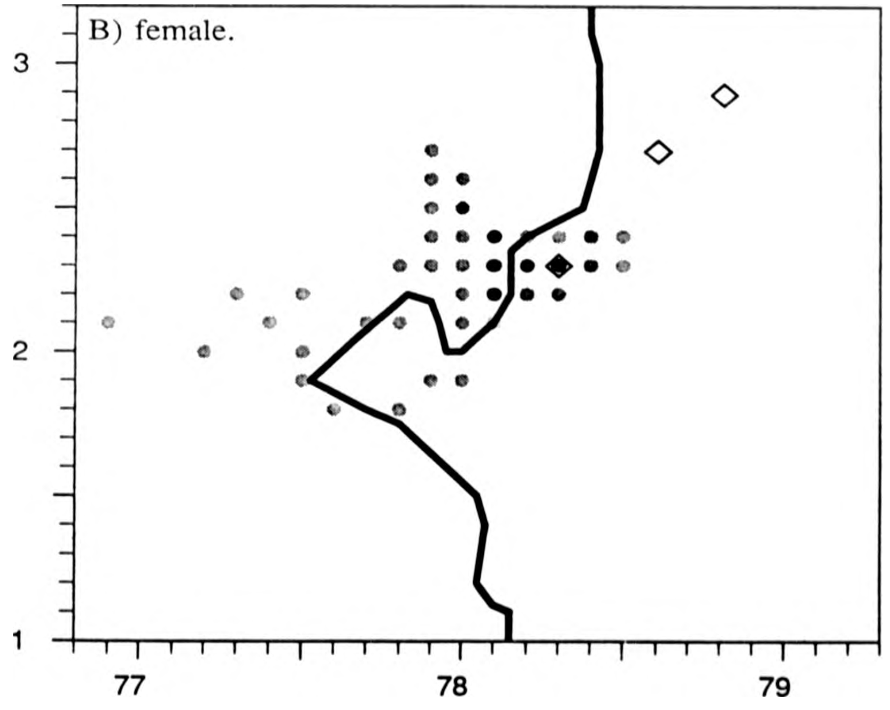
[*] Radio-tag not subsequently recovered

[#] Radio-tag removed on recapture

[$] Number of burrow visits recorded in two hours of observation during late morning (control birds in brackets)

Figure 3.13 - Locations at which a pair of radio-tagged sand martins were recorded, A) male n=148, B) female n=154. Black symbols represent >5% of the total number of locations. The diamond symbols represent subcolonies and the solid line the River Allan. Axes refer to the 100km grid square NN of the British national grid, north is the top of the figure. In both cases, the locations were concentrated around the subcolony containing the pair's nest burrow.





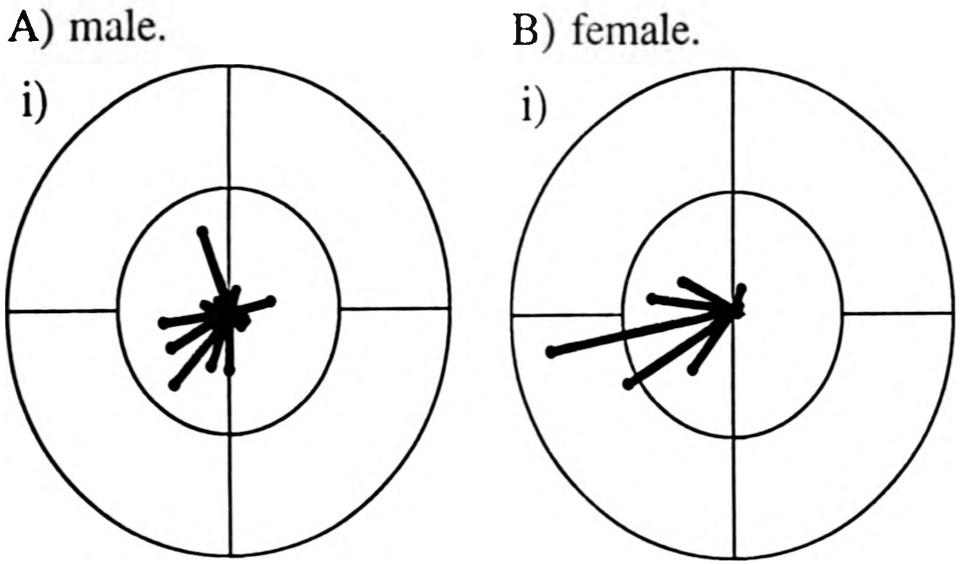


Figure 3.14 - Percentage of occasions on which birds were located in different directions from the nest burrow, bearings rounded to the nearest 10°. A) male (n=118), B) female (n=114). The radius of the inner circles represents 20% of the total number of locations. North is towards the top of the figure. Locations placing the birds at the nest burrow were excluded.

p=0.13). The male was located further from the subcolony in late morning than in early morning (non-parametric Tukey comparisons, Q = 2.912, $p<0.05$).

The birds were located near the River Allan and over grassland and agricultural crops, when away from the colony. The birds were assumed to be foraging most of the time when away from their nest burrow during daytime, since they were rearing chicks. Locations which placed the birds at their own subcolony were excluded from the sample, since such space use could be the result of the need to feed young rather than selection of habitat for foraging. Over the whole period that the birds were tracked, the male occurred significantly further from the river than the female (Mann-Whitney W=11676.0, $p<0.001$, male n=118, female n=114). Considering locations with respect to the river, both birds showed a significant tendency to be further from the river when they were at larger distances from their nest burrow (male $r_s=0.27$, $p<0.01$, n=118; female $r_s=0.52$, $p<0.01$, n=114). Locations which placed the birds at their burrow were again excluded.

3.3.6.4. Interaction between the individuals

Of the 77 grid cells used by at least one member of the pair, only 29% were visited by both. Spearman's coefficient was then used to test for a correlation between the utilization distributions of the pair, following Doncaster (1990). For the pair of sand martins ranges, r_s was +0.43 ($p<0.05$). For ranges that overlap by the observed value of about 30%, a positive value for r_s will indicate that the shared area contains the most utilized parts of the range, a negative value will indicate the reverse (Doncaster 1990). Although male and female shared the most utilized parts of their home ranges, an analysis of dynamic interaction showed no tendency for the male and female to be separated by less than 150m more often than would be expected at random

(X^2=0.008, df=1, p>0.20).

3.3.6.5. Mixed-reproductive behaviour

In contrast to its mate, the male visited other subcolonies; a behaviour which could be interpreted as seeking extra-pair copulations (EPCs). On the occasions that the male was recorded closer to other subcolonies than to its own subcolony, 90% occurred in the afternoon and evening.

3.3.6.6. Roost selection during the rearing period

The locations of roost sites chosen by the radio-tagged sand martin pair with chicks are shown in Figure 3.15. On the nights of the 9th and 10th June, immediately after tagging, the female was located roosting in its burrow, while the male was not found within a 1km radius. Both birds were located on the 12th June away from the colony, the first night that a search of the area surrounding colony was carried out. The pair was found roosting together in a field of oilseed rape, 4.7km from their nest burrow. On the following night they were located together in the same field, 50m from the previous roost site. At dusk a few swallows foraged in the area, but there were no sand martins visible and there was no contact calling or social interaction to suggest the presence of a large communal roost. Both birds were again located roosting together on the 14th and 15th June. On these occasions the sites were in a field of wheat 3km from the subcolony, and in slightly different locations on each night. Again there was no suggestion as to the existence of a large communal roost. On the 16th June the birds were located together just before dusk. Fluctuating signals indicated that the birds were still active 2.7km from the subcolony and heading in the

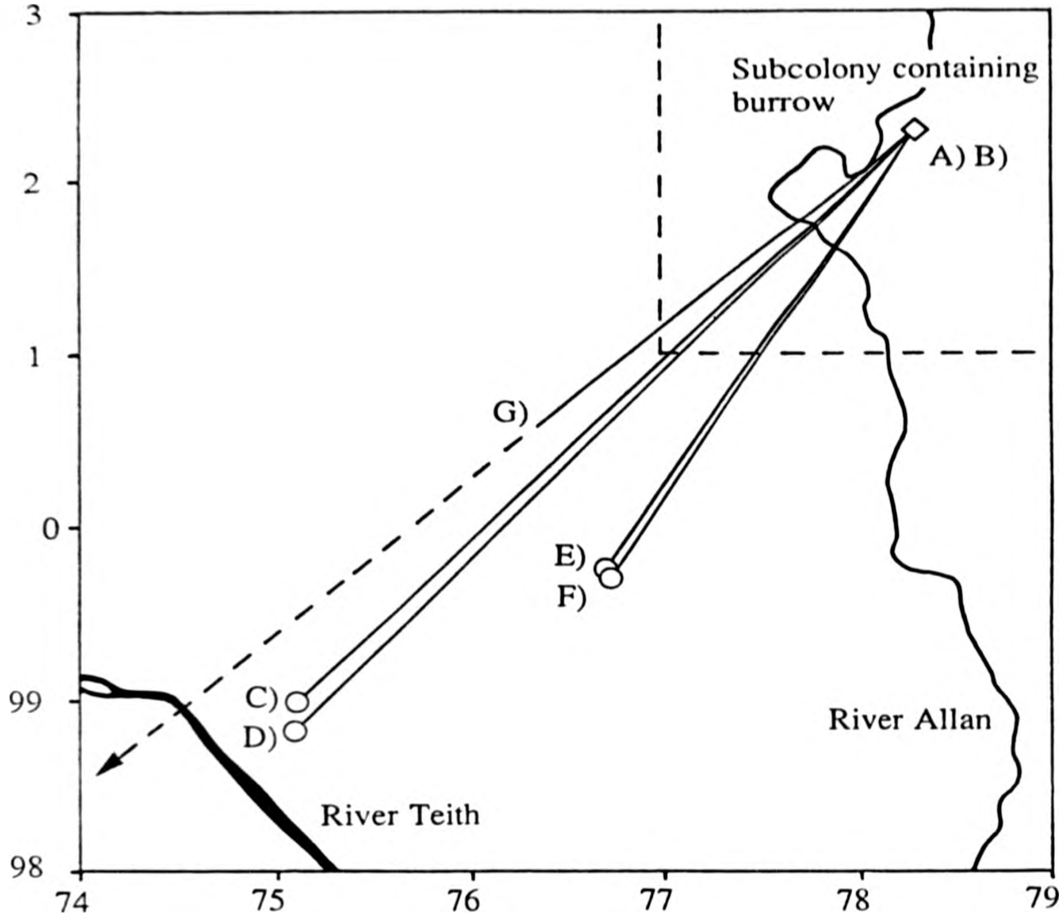


Figure 3.15 - Selection of roost sites by a pair of sand martins during brood provisioning. A) and B) 9th and 10th June, nest burrow, female only, male not found. C) 12th June, oilseed rape, male and female together. D) 13th June, oilseed rape, male and female together. E) 14th June, wheat, male and female together. F) 15th June, wheat, male and female together. G) 16th June, still active just before dusk, male and female together, not found after dusk and probably crossed River Teith. The square bounded by the hatched line represents the area covered by Figure 2. Axes refer to 100km grid squares NS and NN of the British national grid, north is towards the top of the figure.

same direction as the roost sites of previous nights. However they were not located after dusk despite an extensive search covering approximately 30km^2, suggesting that the birds could have crossed the River Teith and the roost site was more than 6km from the colony. On the night of 17th June, the last time the birds were searched for, neither was located. By then the chicks were 17 days old and only the male fed the brood.

The second male was radio-tagged during the pre-laying period, and was recorded roosting at night in the same burrow that it defended during the daytime.

3.4. DISCUSSION

3.4.1. Colony size

Comparatively to other colonies of sand martins/bank swallows, the colony at Barbush was very large. Although 100 breeding pairs is not unusual and sometimes colonies can reach up or more than 1000 pairs, the average colony size is less than 50 pairs (Table 3.9). Most large colonies are at artificial sites (i.e. sand quarries), but some occur at river banks (Table 3.9, also see Turner and Rose 1989). In 1982 Barbush reached up to 920 pairs, and has been probably one of the largest in Scotland in recent years (Jones 1986a). Even after a population crash in 1984, the numbers at Barbush were around 150 breeding pairs (Jones 1985). The large number of breeding colonies in central Scotland in the last few decades is suggested to be associated with an increased available nesting habitat due to increased levels of sand quarrying (Jones 1986a).

No regular censuses were carried out at the Argaty or Kinbuck colonies, but both quarries were not active and smaller than Barbush, with a maximum estimate of 200 and 50 occupied burrows respectively.

Table 3.9 - Sand martins and bank swallows colony sizes, estimated by number of pairs breeding. Sample sizes in brackets if available.

Location	Average number or range	Reference
Britain	37.6 (57)	Morgan 1979
Belgium	31.0 (31)	Billen and Tricot 1977*
Northwest Germany	25.0 (10)	Kuhnen 1978
Central southern Swenden	46.0 (136)	Anderson 1982*
Denmark	19-42 (134)	Moller 1979*
Stirling area (Scotland)	1-920 (27)	Jones 1986a
Barbush (Scotland)	413-720 up to 920	present study Jones 1986a
Westfalen (West Germany)	up to 450	Loske, 1983*
Lower Saxony and Bremen (Germany)	up to 400	Oelke, 1964
Southern Swenden	up to 140	Person 1987
St. Kern (Switzerland)	1-350	Sieber, 1980
Switerzland	50% <25, 3% >100 (123)	Sieber, 1982*
Qu'Apelle River (Canada)	1-48	Hjertaas *et al.*,1988
Oneida Lake (New York)	12-300	Stoner, 1936
Michigan (USA)	2-451	Hoogland and Sherman, 1976
Sacramento River (California, USA)	20-1784	Humphrey and Garrison, 1986

* from Cramp, 1988

3.4.2. Population fluctuations at the Barbush study site

The number of occupied burrows at Barbush was used as estimate of number of pairs breeding, and therefore as an indication of population trends over the study period (Jones 1985, Jones 1987a). As found by Jones, there was a more or less linear increase between the date of first arrival (usually April) until early June, levelling out thereafter because most of the birds had arrived. The pattern of subcolonies settlement in 1990 was not, however, the same as found by Jones (1985) for sand martins; which involved birds settling first in a subcolony, and once this filled up establishing another subcolony. In this study the birds settled in the three major subcolonies more or less syncronously, although the main subcolony reached a plateau earlier than the others. A possible explanation for this may be that all three subcolonies were equally favoured nesting sites (i.e. large cliff, suitable sand consistency at top of cliff, and inaccessible to mammalian predators).

The reduced population numbers in 1991 and 1992 compared to 1990, was accompanied by a reduction in keel-length (i.e. body-size). The same trend had earlier been described for sand martins between 1980 and 1991 (Bryant and Jones unpubl., Jones 1985). A severe drought in the Sahel in 1983 and 1984 was related to a dramatic crash in sand martin numbers in Britain, when the numbers breeding in central Scotland in 1984 were 27% of the birds in 1983 (Bryant and Jones unpubl.). Selection for small size (keel-length reached a minimum) was demonstrated at this time (Jones 1987b). By 1990 the breeding population of central Scotland had recovered the density of the early 1980s, followed by a rise in body-size at Barbush up to 1989 breeding season (Bryant and Jones unpubl.). This was possibly associated with better conditions in the winter grounds, with the rainfall in Sahel increasing from 1987 to 1988, when it showed a normal pattern (Nicholson and Palao 1993). In 1990 the rainfall dropped markedly again, although the drought was not as extreme as in 1982-1984, but was followed by a decrease in sand martin numbers and associated with a decrease in body-size (Bryant and Jones unpubl., present study).

Bryant and Jones (unpubl.) considered three hypotheses to explain the correlation between body-size and colony size: 1) large individuals could be attracted to large colonies while small individuals have to settle in small colonies; 2) body mass may be on average large following a year of high food supply; 3) average body-size may be maintained by stabilizing selection, and variation from long-term size averages are most likely to be caused by intense directional selection, apparently acting on the winter grounds. The first of these hypothesis has implications for mate competition in a species with a mixed-reproductive strategy, since a large colony (such as Barbush) would be a site for large and therefore more competitive individuals, in terms of their reproductive success.

The first hypothesis seemed unlikely to apply, however, since the data were collected only in a large colony, Barbush, and the body-sized decreased in that colony from one year to the next. There was also no evidence to support the second hypothesis, because the aerial insect catch was actually higher in 1990 than in any other year in 1980s decade, except 1982 (Bryant and Jones unpubl.). The third hypothesis is supported by data from the present study, and was proposed by Bryant and Jones to explain why sand martins co-varied in body-size and population over 10 years (since 1980), and also in 1991 at the same site (Barbush). Severe weather conditions in the winter grounds leads to a decrease in food availability and a subsequent high mortality, affecting the number of migrants coming back to the breeding sites.

Jones (1985) did not find a difference in sand martin keel-length between the sexes in 1984, possibly due to small sample sizes. In the present study, however, the intensity of over-winter selection acting on body-size differed between the sexes in 1991 after a severe reduction in numbers. Females had a greater keel-length reduction than males suggesting that severe climatic conditions in the wintering grounds had a greater effect on females, for reasons unknown. Reproductive costs in hirundines involving winter mortality have been found to affect mainly females, probably to due to their higher breeding contributions (i.e. double broods)(Bryant 1979, Persson 1987). Among other species, moreover, the costs of reproduction have been reported to usually affect females more than males (Partridge 1989).

A similar directional selection on body-size was reported for Darwin's finches. In this species, contrary to sand martins, large body-size was favoured in recurring drought conditions, with decreased food supply and high adult mortality (Price *et al.* 1984). Selection on females was less intense than on males, with males being larger than females (Price 1984b).

Natural selection often affects the sexes in different ways (Price 1984b). Heritability estimates of body size measures are usually similar for both sexes if they are monomorphic, or differ slightly morphologically (Price 1984a). However, additional directional selection for large size in males may occur through sexual selection (Bryant 1979, Price 1984a). Females may mate selectively with large males, which may be high quality parents (Price 1984b), while small females may breed when younger (Price 1984a).

A possible benefit of being large for females is an increased reproductive output per season, probably due to an improved flight efficiency compared to small birds, as shown for house martins (Bryant and Westerterp 1982). On the other hand large females can to some extent be subjected to counter selection due to high mortality rates of females rearing more offspring per season. Benefits of small body size may be associated with their reduced total energy expenditure compared to large birds (Jones 1985).

It seems that there is a trade off in some hirundines between being large during the breeding season and small, when the climatic conditions may be severe, outside the breeding season.

Drought in South Africa was suggested to affect swallows, causing heavy mortality and perhaps poor breeding condition, with a subsequent reduced reproductive output. This had the potential to lead to a decrease in population levels (Møller 1989c). A decrease in the swallow breeding population in central Scotland was reported for swallows in 1991 (Ward 1992), following an increase in 1988 (Thompson 1992). However, no significant change in structural size of female swallows was found between 1987-1991, suggesting no comparable selection pressure upon body size in that species (Ward 1992). The reason may be the small sample size taken in 1991 did not permit an effect to be detected (only 15 birds). Alternatively, swallows may not experience the severity of conditions affecting sand martins.

3.4.3. Breeding biology

Breeding parameters are presented in Table 3.10 for sand martins and bank swallows. Sand martins are among the earliest summer migrants to return to Britain in the spring, laying regularly in early May (Morgan 1979). Mean clutch size and mean brood size, as determined in this study, were similar to values quoted from others studies. Values for mean clutch size and brood size were also similar to the mean values for northern Britain (5.0, n=23 and 3.6, n=57 respectively), which are slightly greater than the values for southern Britain (Morgan 1979). Incubation time, and fledging time were close to values quoted by Turner and Bryant (1979) for the same colony.

3.4.3.1. Second broods

Some sand martins raised two broods in a season, as found by Morgan (1979) and Cowley (1983). Information about first and second clutches/broods is rare, possibly because sand martins often change partners and or nests/subcolonies after first broods. Nevertheless, second clutches (or late broods) are apparently smaller than first clutches (Table 3.10). The estimated proportion of second brood attempts found in the present study was higher than the estimated value found by Stoner (1936)(Table 3.10), but similar to values quoted by Cowley (1983). Cowley found 26.6% of those birds which raised a first brood successfully, attempted another brood; while 5.7% of the birds were proven to have had second broods, similar to values found in this study. The occurrence of second broods may be related to the timing of arrivals and the start of laying (Table 3.10). The highest estimate for second broods in sand martins was registered in Switzerland where the birds also arrived in early April, and it was also at the lowest latitude (Table 3.10); suggesting that the proportion of second broods increases with a decrease in latitude (Table 3.10). In the far north one brood is usual for sand martins (Turner and Rose 1989). Many of the factors which influence clutch size also affect the number of broods produced in a season, such as food supply and latitude (Cody 1971). The number of broods raised by an individual per year is considered to depend mainly on the length of the time for which conditions are suitable for feeding young (Lack 1954). A latitudinal cline in number of broods is apparent in species of swifts (which have similar ecology), with a decrease from lower to higher latitudes, reaching one brood in northern Europe (Lack 1954, Cody 1971).

Despite Stoner (1936) quoting the occurrence of second broods for bank swallows (Table 3.10), Petersen (1955) found no cases of genuine second broods in his study, attributing all late clutches to re-nestings following nest destruction.

Table 3.10 - Breeding parameters of sand martins/bank swallows.

	Sand martins				Bank swallows		
	Present study	Asbirk (1976)	Svensson (1969)	Sieber (1980)	Hjertaas *et al.*(1988)	Petersen (1955)	Stoner (1936)
Date of arrival	Early April	End April	-	4th April	-	22th April	Late April
Date of earliest egg	1th May	24th May	11th June	-	Middle May	-	19th May
Estimate mean date clutch:							
first	24th May	30th June	-	-	-	-	8th June
second	15th July	23th July	-	-	-	-	9th July
Estimate mean of clutch size:							
first	5.0 (70)	4.8 (38)	-	5.0 (145)	-	5.0[$] (104)	5-4[e] (200)
second	4.2 (26)	-	-	4.6 (17)	- -	4.0[*] (21)	4-3[l]
total	4.8	4.8	4.8	-	5.0	4.8	-

$ = before 15th June, * = after 15th June
e = early broods, l = late broods

Continuation Table 3.10.

	Sand martins				Bank swallows		
	Present study	Asbirk (1976)	Svensson (1969)	Sieber (1980)	Hjertaas *et al.* (1988)	Petersen (1955)	Stoner (1936)
Mean brood size first	3.9 (39)	-	-	-	-	-	-
second	3.7 (20)	-	-	-	-	-	-
total	3.8 (83)	-	-	-	4.4^{F}	-	3-6
Occurrence of second broods	Yes	Yes	-	Yes	-	No	Yes
Estimated % of second broods	27	-	-	50-66	-	-	4
Colony size	413-720	40-50	60	1-350	1-48	-	12-300
Study site	Barbush Scotland	Roskilde in Zeland Denmark	Ammarnäs Sweden	Kt.Bern Switzerland	Qu'Apelle Canada	Madison Wisconsin	Oneida New York
Latitude	56°12'N	55°39'N	65°58'N	46°57'N	50°40'N	43°04'N	43°04'N

F = fledglings
O = occasionally

In America, bank swallows are usually quoted as being single brooded (Hoogland and Sherman 1976) presumably related to the period for which conditions remain suitable for breeding.

3.4.3.2. Why clutch size declined through the season

Seasonal decrease in clutch size has been found for several species of birds (Lack 1954, Perrins 1970, Crick *et al.* 1993), including sand martins (Svensson 1986). Sand martins showed a tendency to decrease clutch sizes within both first and second clutches similar to the trend in swallows (McGinn and Clark 1978), although with a greater decrease in second compared to first clutches.

Seasonal decreases in clutch size often show no correlation with decrease in food supply (Bryant 1975, Perrins 1970). The general decrease in clutch size as the season advances may be linked with daylength, decreasing after mid-June, leading to a decreasing time available for feeding (see McGinn and Clark 1978). This hypothesis, however, does not explain the clutch size pattern at the start of second clutches (second half of the season), which show a slight elevation compared to the late first. Daylength did not explain the seasonal decrease in clutch size of house martins either, which instead was suggested to be due to an adaptive response associated with lower feeding efficiency of late breeding birds (Bryant 1975). The decrease in first clutches, followed by an increase at the beginning of second clutches found in sand martins can be explained by experienced adults arriving back to the breeding sites before less experienced (i.e. young birds) and laying earlier (in first and also second broods) and larger clutches than younger birds arriving later, as suggested to occur in swallows (McGinn and Clark 1978) and house martins (Bryant 1975, Bryant and Weterterp 1983).

The proportion of nests with young that fledged from nests with eggs that hatched, was greater than hatching success, suggesting that most losses occurred before hatching, mainly by desertion and sometimes predation.

The reproductive aspects covered in this Chapter are relevant in several aspects to the main part of the present study, the socio-sexual behaviour of sand martins. For instance, the most successful individuals within a breeding season are double brooded; early arrival might therefore be important to increase the chances of having a second brood, in competition amongst males for favoured nesting places and for early laying ('good') females. Early arrivals can also potentially increase their reproductive success by settling in presumed 'best' subcolonies, as found for sand martins by Jones (1985, 1987b)(but with no clear indication of the same pattern in this study); and by laying larger clutches (although this trend was weak in this study). Early arrivals could further increase their reproductive success through the increased opportunities for mixed-reproductive strategies and re-mating in the course of the breeding season (see Chapter 6).

3.4.4. Space use by radio-tracking

3.4.4.1. Effects of tags

Only a few studies of small birds present quantitative results about the effects of radio-tags on condition and behaviour (Brigham 1989, East and Hofer 1985, Johnstone 1992). Studies on birds with high aspect ratio wings such as sand martins are also rare (Brigham 1989, Nygard and Einavik 1992). The effect of packages on the flight of such birds is of great importance since they are aerodynamically efficient, leaving relatively little scope for carrying additional loads, and also spend much of their time flying to collect food.

The present study suggests that the tail clips and associated change in centre of gravity caused no obvious problems to sand martins. However, Wright and Cuthill (1990) have shown that subtle changes in provisioning rate may occur as a result of adding tail weights. Larger samples of birds and longer periods of observation would be necessary to test for any such changes in provisioning between tagged birds and controls.

Over the period that the birds carried radio-tags they showed on average only a slight decrease in body mass; consistent with the general decline in mass of the population during the breeding season (Jones 1986b). The pair provisioning chicks, however, showed a slightly higher rate of nest attendance, in comparison with a neighbouring control nest. If the increase in wing loading due to the radio-tags caused a reduction in the mass of the prey bolus that birds could economically carry, burrow visits could became more frequent to maintain a constant rate of delivery to the chicks. The observed rates were, however, both within normal limits (as quoted by Turner 1980) and the difference could be due to different demands of the broods or individual variations. Furthermore, the female began a second clutch while tagged, indicating that radio-tags did not impair egg laying. The results are consistent with tail mounted radio-tags having no major effects on condition or behaviour of sand martins.

3.4.4.2. Space use and habitat selection

The use of radio-tracking techniques to determine foraging range and feeding locations by chick rearing shags, also a similar highly mobile social 'central place forager', showed no significant sex difference in feeding locations (Wanless *et al.* 1991). But the radio-tagged male and female sand martins commonly took different directions during the chick rearing period. They visited only 11% and 6% respectively of the area accessible within the maximum flight distance they used, which suggests a high degree of site selectivity. The bearings of the locations from the burrow suggested that the female concentrated foraging in the direction of the river, and the male had a similar preference although with much more variation. Although the male showed a significant tendency to be further from the river than the female, the results from the present study showed over 70% of the locations to be within 100m of the river. A possible reason was that the local topography caused concentrations of insects along the river valley at the height favoured by foraging sand martins (Waugh 1978). The birds being located further from the river at greater distances from the burrow, was almost certainly due to them taking beeline flight paths to and from prey concentrations at different places along the meandering course of the river. This increased their probability of being located while overflying the intervening farmland.

3.4.4.3. Information centres

Nestling feeding by both parents provides an opportunity to investigate ideas generated by the information-centre hypothesis. That is, individuals in roosting and other assemblies (including breeding colonies) could serve as information-centres, where individuals benefit by following successful ones to profitable feeding areas, particularly for unevenly distributed resources (Bayer 1982, Brown 1986, Ward and Zahavi 1973). Radio tracking can provide a way to answer the question: do pairs co-operating to rear nestlings exchange information about the location of profitable prey patches?

As would be expected for a 'central place forager', the cells that were most utilized by each bird, were used by both of them and were concentrated at and close to their burrow. Both birds must repeatedly have flown through these cells to deliver food to the chicks. The absence of any degree of dynamic interaction suggests that the birds were not using cues from each other to locate food.

3.4.4.4. Mixed-reproductive strategy

The tendency for the male to visit more grid cells, and to be located on average further from the river than its mate, was possibly due to the male looking for potential extra-pair copulations (see Chapter 6). Sand martin chasing behaviour shows its highest peak early in the morning, decreasing in the middle of the day and increasing again in the afternoon to reach another peak by evening (Jones 1986b). Fertile sand martins could be located by males in a flock of foraging birds by a distinctive gait during flight (Jones 1986b). Radio-tracking sand martins during the pre-laying and laying stages (when the males guard their mates) would give us useful information about mate-guarding and opportunities for extra-pair copulations when the birds are away from their nest burrows. While this information is difficult to obtain, the limited results of this study indicate that an approach using radio-tracking would be likely to be successful.

3.4.4.5. Roost selection

Published information about roosting when away from the nest burrow during breeding is scarce in hirundines and swifts. In sand martins, the females and sometimes the males roost in the nest burrow until the nestlings are 10 days old (Petersen 1955), after which there is no information. There is potential at this time for either males and females to investigate extra-pair opportunities overnight, without impinging on their daytime routines at the nest. Huxley (1949) records house martins, barn swallows and chimney swifts returning to ground level from a great height at dawn, suggesting the possibility of aerial roosting. However, Smart (1990) recorded house martins roosting in oak trees in June before a night of wet and windy weather. Common swifts (Holmgren 1993) have also been observed going into trees at dusk, and white-throated needletails were radio-tracked, and shown to be roosting in trees (Tarburton 1993) .

Records of massive communal roosts of hirundines in autumn during migration are widespread (Cramp 1988), sites usually being located in reed beds. Ford and Elphick (1993) observed the progressive formation of such a roost by swallows in maize during August and September.

The use of roost sites in crops presented in this study suggests an explanation for the absence of previous records of roosts away from the burrow during breeding. It is possible that single birds or pairs scatter into countryside surrounding a colony at dusk, and select separate locations in the highly abundant suitable sites represented by agricultural crops. Birds roosting in these situations would seem to be impossible to detect without radio-tracking. These sites become increasingly rare during the late summer and autumn due to the harvesting of crops. This might account for the progressive formation of communal roosts after breeding when the birds became concentrated into the remaining sites such as reed beds.

The ability to locate roost sites many kilometres from the colony demonstrated by this study could open the possibility of monitoring the post breeding movements of individuals. This pilot study suggests that there were no measurable effects on the behaviour, body mass and short term survival of sand martins equipped with tail-mounted radio-tags. Thus the application of radio-tracking to such a small aerial foraging bird can provide information on foraging range, habitat selection and social behaviour. It might be particularly helpful in studies of mating behaviour and therefore mixed-reproductive strategies in birds, where observation of socio-sexual behaviour (such as copulations) is made difficult by cover (for instance, nest burrows) or distance, such as in sand martins.

CHAPTER 4 - INTERSPECIFIC INTERACTIONS

4.1. INTRODUCTION

4.1.1. Ectoparasitism

Nest predation has long been recognized as a major determinant of reproductive success in passerine birds (Ricklefs 1969). Recently, attention has also been given to the effects of ectoparasites as a selective agent affecting reproductive fitness of birds (Brown and Brown 1986, Møller 1990, Møller *et al.* 1990, Møller 1991b, Møller 1993, Johnson and Albrecht 1993).

The fitness effects of ectoparasites on reproductive parameters of passerines include: a) delay of egg laying (Møller 1990), b) decrease of clutch size (Moss and Camin 1970), c) fewer offspring fledged (nestling survivorship can be reduced by up to 50% in cliff swallows parasitised by bugs, Brown and Brown 1986) and d) a decrease of body mass and condition of the offspring (Brown and Brown 1986, Møller 1990, Moss and Camin 1970), which can affect future survival (see reviews by Magrath 1991 and Møller *et al.* 1990).

Ectoparasitism and predation may interact; for instance nest predation may increase if parents have to visit nests more frequently, or chicks beg more or stay longer in the nests than nestlings with no ectoparasites (Møller 1989a). Even heavy loads of ectoparasites, however, may sometimes have little apparent effect on nestlings, suggesting that parents increase their own energy expenditure to compensate for the effects of ectoparasites on nestlings (Johnson and Albrecht 1993).

Colonially breeding and hole-nesting species are often subject to intense competition for nests, and may re-use nests making them susceptible to high rates of ectoparasitism (Møller *et al.* 1990). Alexander (1974) suggested that ectoparasitism may be one of the major costs of social behaviour since parasites can spread rapidly due to close proximity and frequent interactions or contact between members of a group. Individuals of group-living passerine species incur a greater risk of acquiring contact transmitted ectoparasites than individuals of solitary species (Poulin 1991).

Sand martins nest in burrows in close proximity in large colonies, therefore rates of disease transmission are predicted to be high, possibly increasing with colony size. Fleas (sand martin fleas) have been the most commonly observed parasite in sand martins and bank swallows (Hoogland and Sherman 1976, Kaczmarek 1988, Walter and Streichert 1984).

The questions addressed in this chapter are:

1. Do ectoparasites have any effect on the body mass of sand martin nestlings? To answer this question, a pesticide was used to kill parasites in some nests. Provided that a) the pesticide was effective in killing the ectoparasites, with no harm to the hosts; b) control nests were matched in time and place with the experimental nests; and c) nestlings from experimental and control nests were weighed on the same day of the nesting cycle (when the body mass reaches a peak), it would be expected that the nestlings of experimental nests would be heavier than the controls.

2. Is there any effect on nestling body mass in larger subcolonies? Given that breeding conditions are broadly similar in the vicinity of different subcolonies, the predictions include (Figure 4.1): 1) subcolony size and parasites affect body-mass of nestlings in both experimental and control nests; 2) only subcolony size affects nestling body-mass; 3) neither parasites nor subcolony size affects nestling body-mass; 4) parasites, but not subcolony size affects nestling body-mass; 5) the peak body mass of nestlings from treated nests in different subcolony sizes will not differ significantly since the absence of ectoparasites should allow chicks to grow to an optimum peak weight. Because the levels of ectoparasite infection can

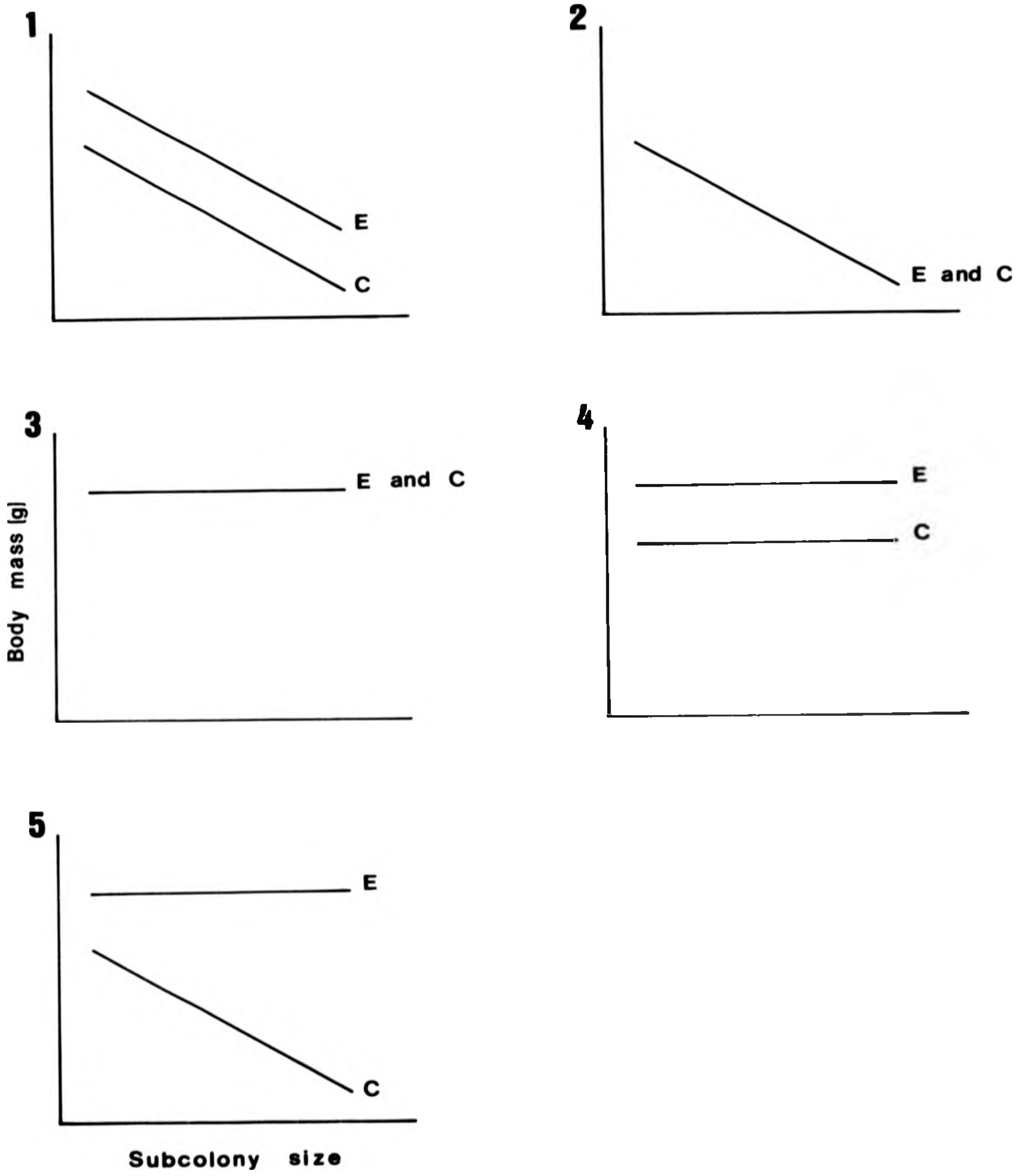


Figure 4.1 - Predicted average peak body mass for sand martin nestlings, in nests treated with pesticide to kill parasites (experimental) and control nests. 1) If subcolony size and ectoparasites affect body mass on both experimental and control nests; 2) if only subcolony size affects body mass; 3) if neither ectoparasites nor subcolony size affects body mass; 4) if ectoparasites but not subcolony size affect body mass; 5) if ectoparasites and subcolony size affect body mass of control nests only. E=experimental, C=control.

increase with colony size (Hoogland and Sherman 1976), nestlings from untreated control nests in bigger subcolonies can be predicted to be lighter than in small colonies.

4.1.2. Predation

One of the advantages of living in groups or in colonies is a decreased risk of predation. Grouped individuals can afford to be less vigilant for predators than solitary conspecifics (Brown and Hoogland 1986, Pulliam 1973). Despite a decreased vigilance level per individual, a group can have the same or greater probability of detecting a predator as a highly vigilant solitary individual (Pulliam 1973). In addition, grouped individuals have a smaller chance of being attacked due to dilution and 'selfish herd' effects (Dehn 1990, Hamilton 1971, Vine 1971). The last one refers to individuals positioning themselves behind potential conspecific prey, in order to reduce their chance of being predated. Brown and Brown (1987) suggested that colonial nesting may confer some 'selfish herd' benefit on cliff swallows, given that they do not appear to mob predators very effectively.

Among colonial birds, mobbing is the most common form of group defense against predators (Hoogland and Sherman 1976). The risk of mobbing is not negligible, for example where large falcons that prey on adult birds are involved (Sordahl 1990). Adult hirundines are unlikely to be injured while mobbing kestrels and other falcons or hawks, however, because of their greater flying agility (Lima 1993, Pettifor 1990).

Sparrow-hawks are recorded as predators of sand martins in Britain (Mead and Pepler 1975), and were considered the predominant aerial predators of sand martins in Scotland (Bryant and Turner 1982). Bank swallows have been reported as an important prey of the american kestrel in North America (Freer 1973). Adult sand martins are unlikely to be at high risk of predation while foraging, because they feed in flocks which are able to detect and avoid predators (Bryant and Turner 1982). Nestlings begging at the nest burrow entrance are the main target of predators. Adult sand martins give alarm calls and mob predators, and have also been seen to be taken by sparrow-hawks close by the nest (Jones 1985). Another risk to be considered is human predation of eggs (by vandalism of nest burrows) and attempts by vandals to catch adults at the burrows entrance, if the nest burrows are easily accessible (pers. obs.).

Although suggestions that ectoparasitism depresses fitness of bank-swallows/sand martins (Stoner 1936), and colony size affects positively the number of ectoparasites in active nest burrows (Hoogland and Sherman 1976), there is no study quantifying the effect of ectoparasites on fitness of this species. The present study investigates the effect of ectoparasites on nestlings body mass, concurrent with the effects of colony size. Apart from the study by Hoogland and Sherman (1976) on bank swallows, testing the anti-predatory behaviour in different colony sizes, and demonstrating the effect of colony size on the reaction to predators (which were detected more quickly in lager colonies), no other study on this aspect has been done for this species. In the present study, the effect of subcolony size on the time of reaction to predators was tested.

Do sand martins react faster to predators in larger subcolonies? Because there are more individuals in larger subcolonies, it was predicted they would do so. This prediction was tested by exposing subcolonies of different sizes to a model aerial predator.

4.2. METHODS

4.2.1. Ectoparasite experiment

The topography of the quarry at Barbush changed from year to year as sand extraction progressed, and so the locations of subcolonies also changed annually. Moreover, several experiments and observations were carried out at the same time, so nests used for the ectoparasite experiment came from three different subcolonies in two years, comprising one subcolony at Barbush in 1991 (subcolony 3), a second subcolony at Barbush (subcolony 4), and another at Argaty in 1992.

Experimental nests were treated with a pesticide to kill ectoparasites. The product used was 'Alugan', a dusting powder, insecticide and acaricide, normally used for domestic animals, including pet birds. About 0.5g of pesticide per week was spread into experimental nests using the apparatus shown in Figure 4.2; beginning as soon as the nest was complete, or the birds were laying, and continuing until the eggs hatched. A graduated syringe was placed 5cm from one of the ends of the stick (1m long) to allow the pesticide to be dispersed in the nest chamber as a whole. After hatching, the pesticide was added once more when the chicks were 4-10 days old, using a plastic spoon attached to the tip of a stick. The ideal situation would be to kill the ectoparasites in the nests without introducing another variable (such as a pesticide, for which the effect on the birds was not known for certain). For instance, ectoparasites have been killed using liquid nitrogen in starling nests (H. Smith, pers. comm.), or using a microwave, as has been done for great tits (Richner *et al.* 1993). These methods are not suitable for cavity breeders, such as sand martins, however, because their nests are hard to gain access to without damage.

Body mass was used as a measure of the extent to which nestlings were affected by ectoparasites. Chicks were extracted from their nests (as described in general methods, Chapter 2), ringed and weighed on the 13th day after hatch, the average day of peak body mass (Turner and Bryant 1979).

Average body mass for all the nestlings in a given nest was used for the analysis, since measurements from nestlings

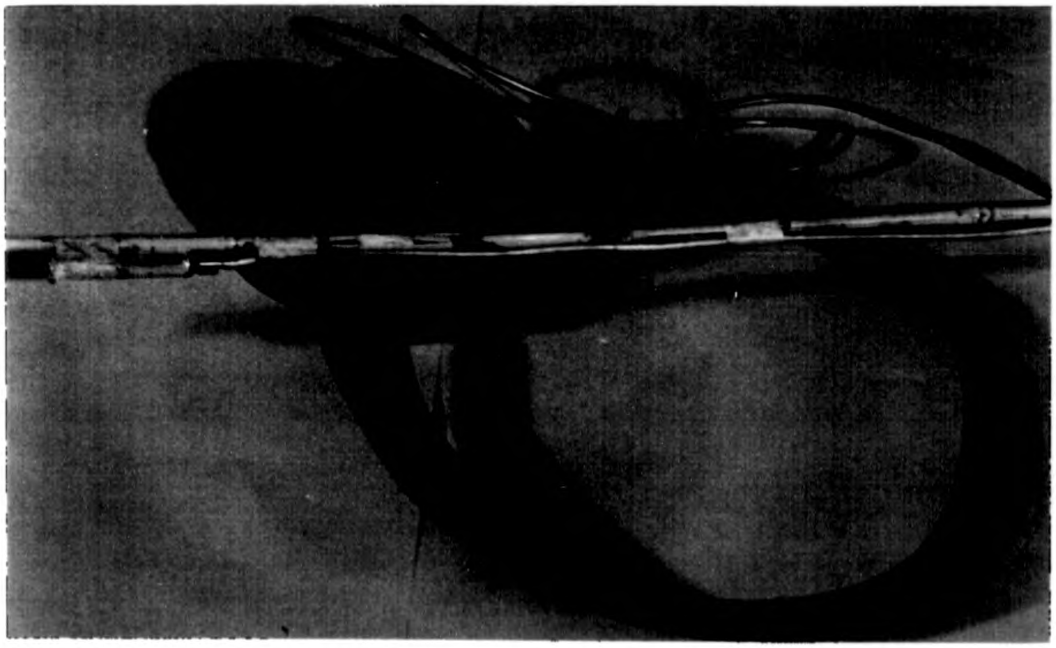


Figure 4.2 - Apparatus used to dispense a powdered pesticide in sand martin nests to kill ectoparasites.

raised in the same nest cannot be considered as independent for the purpose of statistical analysis. Peak nestling body mass from neighbouring nests, matched in time and location in the subcolony with the experimental nests, were used as controls.

4.2.2. Predator experiment

A stuffed sparrow-hawk (Figure 4.3) was ''flown'' across subcolonies of three different sizes, categorized as small, medium and large (according to number of occupied nests in each subcolony). The model was released from above the subcolony and run down a fine nylon line attached to posts at the top and bottom of the sand face. A fishing reel operated from inside a hide positioned above the subcolony was used to wind the model up and down; while an observer in a car recorded the reaction of the birds. The model was concealed in the hide between runs. The mean (±sd) time taken to 'fly' the model down and wind it up again was 40.5±12.0 sec. During each predator-run, the time from the start of the run to the first sand martin alarm call, and the start of mobbing, was recorded using a digital stopwatch.

Sand martins habituated quickly to the presence of the hide and car and ceased responding to the presence of humans once they had entered the blinds. A 'trial' consisted of predator-runs at several subcolonies on the same day, so each trial could be carried out at the same breeding stage and under similar weather conditions. Predator runs were carried out on days of relatively ''good'' weather (i.e. little wind and clear sky).

To avoid habituation to the model, no more than 5 runs per day were done at the same subcolony, and trials were never carried out at the same subcolony on consecutive days. A total of 3 trials (12 runs) in each subcolony was carried out, visiting each subcolony on 3 separate days, at

Figure 4.3 - Sparrow-hawk model presented to sand martins.

intervals of 3-9 days. A rest period of at least 10 minutes was given between predator runs, to allow sand martins to recover from their state of alarm. Trials were carried out during the time most of the nests at a given subcolony had nestlings.

4.3. RESULTS

4.3.1. Effects of ectoparasites on body mass of nestlings

Ectoparasites found in sand martin nests included sand martin fleas, sand martin ticks and hippoboscid flies, which were not identified to species (the likely species, based on Jones' (1985) study at the same colony, are given in Appendix 2). Fleas and hippobicid flies could often be seen at the entrance of the nest burrows, and sometimes on the body of adults when handled. Ticks were usually found on chicks.

4.3.2. Ectoparasite experiment

The pesticide was effective in killing parasites, and caused no obvious harm to the birds. Few or no ectoparasites (mainly fleas) were detected at the burrow entrances of the experimental nests, after being treated with pesticide, whereas they were usually observed in the nest burrow entrances of the control nests. The ectoparasites in the control nests were noticed mainly when the nestlings were removed, such as on occasions when the whole nest had to be removed to bring the chicks out; very few or no parasites were seen on experimental nests so removed.

Clutch size frequencies were exactly the same for treated and control nests (i.e. clutch size (cs) 6=4, cs 5=31, cs 4=8, cs 3=1, for both experimental and control groups). Brood size frequencies also did not differ significantly between treated (cs 6-5=8, cs 4=25, cs 3=5, cs

2-1=6) and control nests (cs 6-5=11, cs 4=19, cs 3=8, cs 2-1=6)(X^2=1.98, df=3, n=88, $p<0.50$).

There was no significant difference between average body mass of nestlings on the 13th day from nests with clutch sizes of 4-6 in experimental or control nests (Table 4.1). Body mass of chicks from nests with different brood sizes also did not differ between treated and control nests (Table 4.2).

There was no significant difference in incubation period between control nests at 3 different subcolonies. However, incubation periods differed significantly between treated nests at the 3 different subcolonies. Birds breeding at Barbush in 1991 incubated for significantly longer than birds from both Barbush and Argaty 1992 (Tukey comparisons test q= 4.48, $p<0.05$); while incubation periods did not differ between subcolonies at Barbush and Argaty in 1992 (Table 4.3). There was, however, no significant difference in incubation period between paired treatment and control nests at the three subcolonies (one-way ANOVA F=0.52, p=0.598), suggesting that incubation was unaffected by ectoparasite effects.

The average peak body mass of chicks in experimental and control nests in the three subcolonies is shown in Table 4.4. Two-way ANOVA on nestlings body mass between treatment and subcolonies, showed that: a) nestlings body mass varied significantly with treatment (meaning that there was an effect of the pesticide on body mass, with nestlings being heavier in treated nests); b) nestling body mass also varied significantly between subcolonies; and c) there was no interaction between treatment and subcolony, indicating that the variation in body mass between subcolonies was not caused by the treatment (Table 4.4).

There was a significant difference between the three subcolonies in the average body mass of nestlings from experimental nests (one-way ANOVA, F=4.15, p=0.02); chicks reared at Barbush 3 were lighter than chicks reared at both Barbush 4 and Argaty (respective Tukey comparisons q=3.46

Table 4.1 - Variation in average body mass (g) at 13th nestling day of sand martin nestlings with clutch sizes, in nests treated with pesticide (experimental) to kill ectoparasites and control nests. (± standard error).

Nests	mean±se body mass of nestlings in clutches of:		
	6	5	4
Experimental	18.56±0.12	18.86±0.19	18.68±0.46
n	4	31	8
ANOVA one-way	F=0.20, n=43, p=0.819		
Control	17.42±0.62	18.25±0.28	17.01±0.38
n	4	31	8
ANOVA one-way	F=2.60, n=43, p=0.087		

Table 4.2 - Variation in average body mass (g) at 13th nestling day of sand martin nestlings with brood sizes, in nests treated with pesticide (experimental) to kill ectoparasites and control nests. (se±standard error).

Nests	mean±se body mass of nestlings in broods of:			
	5	4	3	2
Experimental	18.67±0.43	18.81±0.23	18.50±0.45	18.50±0.39
n	7	25	5	4
ANOVA one-way	F=0.19, n=41, p=0.905			
Control	17.79±0.57	18.37±0.26	17.81±0.56	18.27±0.64
n	10	19	8	5
ANOVA one-way	F=0.51, n=42, p=0.677			

Table 4.3 - Average incubation period (mean±se) of sand martins in nests treated with pesticide to kill ectoparasites and control nests, in three subcolonies. B3 and B4 = Barbush subcolonies, A = Argaty subcolony.

	Subcolonies				
Nests	B3 1991	B4 1992	A 1992	F	p
Treated	15.4±0.20	14.1±1.27	14.1±0.38	6.74	0.003
n	16	14	14		
Control	14.8±0.41	14.1±0.45	14.1±0.33	1.08	0.350
n	16	14	14		

F= variance ratio for significance testing of one-way ANOVA

Table 4.4 - Average body mass at 13th nestling day (mean±se) (g) nestlings in experimental and control sand martin nests. Experimental nests were treated with a pesticide to kill ectoparasites.

Year	Subcolony size	Subcolonies	n
1991		Barbush 3	
Experimental		18.20±0.25	
Control	77	17.45±0.34	16
1992		Barbush 4	
Experimental		19.09±0.32	
Control	100	17.68±0.41	14
1992		Argaty	
Experimental		19.10±0.20	
Control	50	18.90±0.36	14

Two-way ANOVA of average body mass of sand martin nestlings (g) in relation to treatments and subcolonies.

Factor	F	df	p
Treatment	9.22	1	0.003
Subcolonies	6.97	2	0.002
Interaction	2.57	2	0.184

and q=3.42, showed no difference between Barbush 4 and Argaty). There was also a significant difference between average body mass of chicks from control nests (one-way ANOVA, F=4.45, p=0.02); with Barbush 3 having lighter chicks than Argaty, Tukey comparison q=4.03). A regression with nestling body mass against subcolony size (n=3), however, showed no difference between experimental or control nests, although a trend was found for body mass amongst control broods to decrease in larger subcolonies (Figure 4.4).

Conditions of weather and food supply at the three subcolonies on the day the nestlings were weighed, and means over 5 and 10 days before that are compared in Table 4.5. Neither maximum and mean daily temperature, nor wind, varied significantly between the three subcolonies. Food supply was significantly higher at Barbush 1991, but only on the days the chicks were weighed, which could possibly affect body mass. This result, however, does not explain the reduced nestling body mass in that subcolony. Minimum temperature was also significantly higher at Barbush in 1991, both on the day the chicks were weighed and over the previous 10 days (being almost significantly higher at the 5% level during the 5 days before). Rain was however significantly more intense at Barbush in 1991 than at the other subcolonies, on the day the chicks were weighed and also during the preceding 5 and 10 days.

4.3.3. Predators

Observed predation attempts on sand martins included both birds and mammals. Birds were mainly kestrels, carrion crows and jackdaws. When they are close to fledging, sand martin chicks were often seen waiting at the entrances of burrows to be fed, where they were vulnerable to attack. Crows and jackdaws were seen to take nestlings twice, when they fell from their nest burrow entrances. In the first case, the predator picked up the chick from the ground and

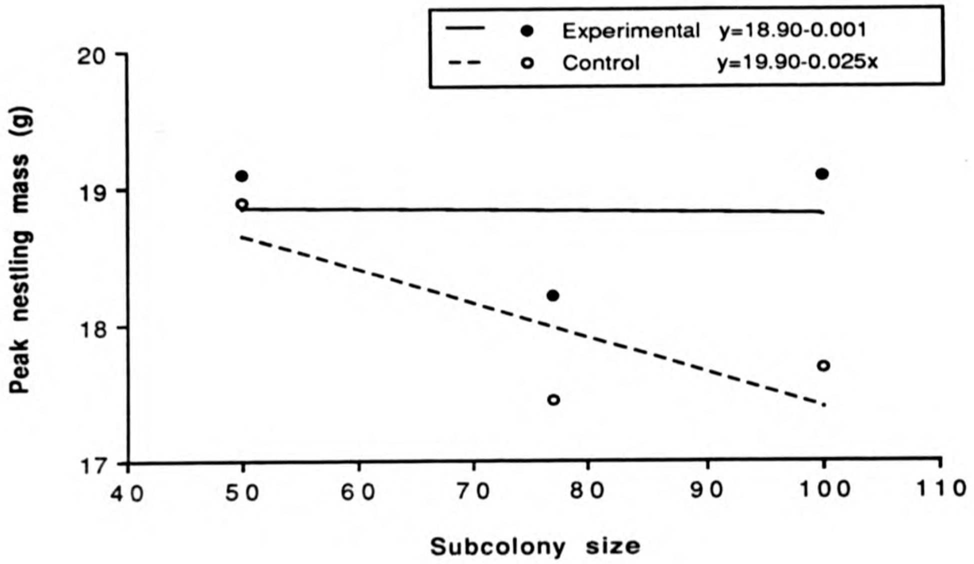


Figure 4.4 - Average body mass at the 13th nestling day of nestlings in nests treated with pesticide to kill ectoparasites (experimental) and control nests, and subcolony size. Experimental, F=0.00, p=0.964, r^2=0.00; control, F=1.92, p=0.398, r^2=0.31.

Table 4.5 - Weather and insect availability at 3 sand martin subcolonies treated with pesticide. Values have been compared for the day the nestlings were weighed (13th day), and the means calculated over 5 and 10 days before. MaxT = maximum temperature, minT = minimum temperature, meanT = mean temperature, trap (insect availability). Values are means±standard deviations (maxT, minT, meanT and trap) and medians (wind and rain). B3 and B4 = subcolonies of Barbush, A = Argaty subcolony. Trap values (daily insect volumes) were $\log_{10}$ transformed.

	Subcolonies				
	B3 1991 (n=16)	B4 1992 (n=14)	A 1992 (n=14)	F	p
MaxT	18.84±2.59	19.42±2.89	19.95±2.73	0.62	0.543
5	18.69±2.00	19.54±1.64	19.70±1.24	1.63	0.209
10	18.89±1.60	19.08±1.12	19.08±1.12	0.88	0.421
MinT	10.75±2.84	8.58±2.35	7.67±2.55	5.61	<0.01
5	10.69±2.99	9.41±1.07	9.04±0.69	3.01	0.060
10	10.92±2.13	9.27±0.59	9.16±0.49	8.09	<0.001
MeanT	14.79±2.54	14.00±1.82	13.81±1.51	1.00	0.376
5	14.69±2.44	14.48±0.65	14.37±0.67	0.16	0.854
10	14.90±1.83	14.17±0.44	14.33±0.38	2.99	0.060
Trap	1.01±0.26	0.71±0.34	0.82±0.20	4.68	<0.05
5	0.96±0.25	0.93±0.22	0.84±0.15	1.21	0.310
10	0.95±0.22	0.93±0.16	0.85±0.09	1.57	0.220

				H	p
-Wind	2.00	2.00	3.50	0.49	0.784
5	3.60	5.40	4.00	4.05	0.132
10	3.25	5.00	4.10	3.66	0.160
Rain	0.50	0.00	0.00	13.17	<0.001
5	1.00	0.50	0.25	16.73	<0.001
10	1.00	0.25	0.25	20.87	<0.001

F = variance ratio for significance testing of one-way ANOVA
H = Kruskal-wallis test

carried it away, while being mobbed by the male parent and other sand martins from the same subcolony. In the second case, the crow ate the chick on the ground in front of the subcolony, where it had fallen.

A flying sand martin in a mobbing group was seen to be caught only once, by a crow. Adult sand martins were often pursued by kestrels, and these birds of prey were often seen perching at burrow entrances. Other potential predators not seen to take sand martins in the present study included, sparrow-hawks and black-headed gulls.

Mammal predators included mink, seen only once running along the ground below a subcolony (also reported from the same place by workers at Barbush colony), weasel and stoat, seen once and twice respectively.

All the bird and mammal predators mentioned above were usually mobbed by sand martins, when they came near the breeding subcolonies. Rabbits that had burrows in the vicinity of a breeding subcolonies were also mobbed occasionally.

With so many predators, there is likely to be intense selection on sand martins to choose subcolonies where predator accessibility is minimized, as was noticed by Jones (1985); or the greatest advantage can be gained from the presence of conspecifics. The first birds arriving to breed choose high and inaccessible subcolonies (pers. obs.). These were possibly older birds, which therefore have a wider choice of subcolony and are likely to settle in the best quality colony (Jones 1987a).

Nests in the most accessible parts of subcolonies were occasionally found to be predated. In 1990, a subcolony at Barbush (subcolony 1, Table 3.3, Chapter 3), only 2.5m high, suffered from predator attacks in approximately 50% of the nests. The predators were probably mammals, but no clear indication of their identity was found from marks on the cliff face. Disarranged nests were found with no eggs (once, one egg was found in the tunnel, and another on the ground below the nest burrow) or chicks. This colony had a maximum of 48 occupied nests in the beginning of season, but it was found to be almost devastated in the second week of June (after that, it had a maximum of 9 occupied nests).

In 1992, a subcolony at Argaty with 50 occupied burrows experienced a massive predator attack late in the season. This subcolony was very accessible and many nests could be checked without using ladders. The predator was probably a weasel, judging by marks left on the sand face (a weasel was also seen during daytime, close by the time of the attack, crossing the road near the entrance of the sand quarry, approximately 500m from the subcolony). Many chicks were found dead in their nests, with marks of sharp teeth on their corpses. Some were half eaten while others in the same nest were dead but uneaten. The first recorded attack was on 9th June when a nest in which 3 chicks had been counted a few days previously was found empty. Subsequent attacks were detected mainly between 15th and 18th June, involving 8 nests. Before the attacks these nests contained a total of approximately 30 chicks. Of these, 23 (77%) were found dead after the attacks, and a further chick was found alive in a burrow where the rest of the brood had been killed. These attacks probably involved only one or two predators, because of a great proportion of dead chicks were left uneaten over the 3 day period.

The anti-predator response of sand martin nestlings observed by Asbirk (1976) was to crouch in the nest. When well grown, young struggle and utter distress calls (Stoner 1936). Observed parental anti-predator strategies of adults included warning calls and mobbing, but they were usually reluctant to enter nest holes in the presence of a predator, as quoted by Cramp (1988). Mobbing was apparently effective in deterring predators (Table 4.6).

4.3.4. Predator experiment

Sand martins reacted to the model sparrow-hawk with

Table 4.6 - Observed bird predator attempts on young sand martins at the entrance of the nests listed are: predators deterred by mobbing, and successful predation attempts. 'Deterred' means the predator flew away once mobbing had started by adults.

	Attempts	
Predators	Deterred	Succeeded
Black-headed gull	1	0
Carrion crow	4	1
Jackdaw	5	1
Kestrel	5	0
Sparrow-hawk	2	0
Total	17	2

alarm calls and mobbing. The median initiation time of these anti-predator behaviours after the appearance of the model varied significantly between the three subcolonies of different sizes (Table 4.7). Sand martins initiated alarm calls and started mobbing soonest in the largest subcolony, and latest in the smallest subcolony. The three subcolonies were all found to be significantly different as far as time to first alarm call and the start of mobbing were concerned, with the exception of mobbing time between the small and medium subcolonies)(Table 4.7).

4.4. DISCUSSION

4.4.1. Effects of ectoparasites

As predicted, sand martin nestlings reached higher peaks of body mass in nests treated with pesticide compared with those in untreated nests, suggesting that ectoparasites significantly depressed nestling growth.

It would be expected that, in the absence of ectoparasites (in nests treated with pesticide), nestlings in different subcolonies would reach a similar peak body mass; provided that breeding conditions were similar. Nestlings of treated nests from Barbush 3 subcolony (1991), however, reached a lower average body mass on the 13th day than nestlings in the two subcolonies sampled in 1992. This suggests that in 1991, nestlings could not reach the same, presumably optimum, peak body mass in the absence of ectoparasites as in 1992. It was perhaps due to differences in weather, food supply, parental effort, or big loads of parasites in the nests. The 1991 subcolony was used for a second year in succession, and is likely that some burrows were reused. The body mass of the control chicks in 1991 was also a bit lower than in that in the other two subcolonies, which suggests differences in weather and food supply.

There was a tendency for peak nestling body mass to

Table 4.7 - Time interval between the appearance of a bird predator model and i) the first alarm call and ii) initiation of mobbing in three sand martin subcolonies of different size. N=estimated number of breeding individual adults present at the subcolonies.

		Median time (sec)			
Subcolony size	N	Alarm call	n	Mobbing time	n
Large	50	1.07	12	4.32	12
Medium	20	3.11	12	9.50	12
Small	6	8.66	11	19.48	10
H		26.58		19.87	
df		2		2	
p		<0.001		<0.001	
Comparisons (Q)					
Small x large		5.06		4.41	
Small x medium		2.57		1.83	
Medium x large		2.54		2.71	

H = Kruskal-Wallis test among groups
Q = Kruskal-Wallis comparisons between groups ($Q_{0.05,3}$=2.394)

decrease as subcolony size increased, although no significant difference was found (Figure 4.4). It is possible that the relationship between nestling body mass and subcolony size was obscured by different conditions in the intermediate size subcolony (Barbush 1991), producing a decrease in body mass of nestlings from both experimental and control nests. Relatively higher temperatures could possibly have favoured the growth of ectoparasites at Barbush in 1991, thus reducing nestlings growth. It is also possible that higher rain fall reduced parental feeding rates, leading to lower chick masses in subcolony Barbush 3. No data on body condition of parents were collected. The conditions at Argaty during the chick rearing period seemed to be better than at the other two subcolonies, since chicks had a higher body mass at Argaty. It was a new and relatively small subcolony, and the level of ectoparasite infection may therefore have been lower than in the other longer-established subcolonies; since the weather conditions and food supply were similar to Barbush 1992. Nevertheless local variation in food abundance, cannot be discounted.

Flea infestations were reported to increase with colony size in bank swallows (Hoogland and Sherman 1976) and cliff swallows (Brown and Brown 1986). Ectoparasitism by fleas was probably not an important cost of coloniality in cliff swallows; but bugs caused a significant reduction in body mass and survival of nestlings (Brown and Brown 1986). It is possible that the parasites which affected nestling sand martins' body-mass the most were fleas, although the relative importance of the effects of fleas and other ectoparasites is not known. Post-fledging survivorship of chicks was not compared between treated and control sand martin nests, but it may have differed, given the mass differences between large and small subcolonies.

Nest ectoparasites did not seem to influence clutch size and/or brood size in sand martins. However, in a related species, the purple martin, nest ectoparasites were apparently important in the determination of clutch sizes

(Moss and Camin 1970). The similarity of average body mass between nestlings subject to different levels of ectoparasites reported by Moss and Camin may have been due to parasite-induced brood reduction in parasitized nests Møller (1993).

When arriving at breeding sites sand martins seem to avoid reusing old nests, and usually dig new burrows, perhaps because of the risk of parasite infection (Turner and Rose 1989). Cliff swallows are more likely to build new nests rather than reuse old ones in large colonies compared with small colonies, probably in response to heavier infestations in nests from large colonies. Barn swallows regularly use old nests, but avoid old nests with mites, as demonstrated by experimental manipulations of mite loads (Møller 1990).

Ectoparasitism represented a cost of coloniality in sand martins, causing a decrease in chick growth, a trend apparently more severe in larger colonies, supporting model number 5 in Figure 4.1 (while the others were rejected).

4.4.2. Predator experiment

As predicted, sand martins detected and started mobbing the predator model faster in bigger subcolonies. Predators were also detected more quickly by bank swallows, and mobbed by a greater number of adults in larger colonies of bank swallows (Hoogland and Sherman 1976).

Large colonies should detect predators more quickly than smaller colonies, because they contain more pairs of eyes (Pulliam 1973). Approaching predators that are quickly detected, and subsequently mobbed, are more likely to be deterred; thus conferring an advantage on individuals in larger colonies (Hoogland and Sherman 1976). The effectiveness of mobbing also increased with group size (and consequently colony size) in the yellow-rumped cacique (Robinson 1985).

It is predicted (Pulliam 1973) that: a) the speed with which a predator is detected increases with colony size; b) individual vigilance should decrease with colony size, because there are more pair of eyes ; and c) the vigilance level stays the same, independent of the group size (lines A, B and C respectively on Figure 4.5).

The probability of detection and level of vigilance per individual would not be predicted to decrease with colony size if predation is not a major selective force for the evolution of coloniality. Cliff swallows, for example, did not effectively mob predators, and thus were unable to deter them, regardless of group size (Brown and Brown 1987). Although these authors suggested that avoidance of predation was a selective force for group leaving, their results shown that predation is probably not the main selective force in the evolution of coloniality in that species.

Hoogland and Sherman (1976) have demonstrated experimentally that mobbing in bank swallows can in some instances deter predators. They tested the assumption that mobbing decreases the risk of predation to the mobber or its offspring; and suggested that reduced predation on eggs and young, resulting from group defense and 'selfish herd' effects, is an important advantage of coloniality for bank swallows. Bank swallows (and possibly sand martins) could hypothetically have A and B distributions, while cliff swallows D and E (Figure 4.5).

In the present study, there was no difference in the time taken to initiate mobbing between the intermediate and smaller subcolonies. This may have been caused by too much variation in the amount of time to the start of mobbing in the small subcolony (sometimes with no mobbing time recorded during a run of the predator model). It may also be possible, although it was not tested, that there is a minimum colony size above which variation in the number of breeding pairs does not influence the time taken to detect a predator.

The results of the present study give support to

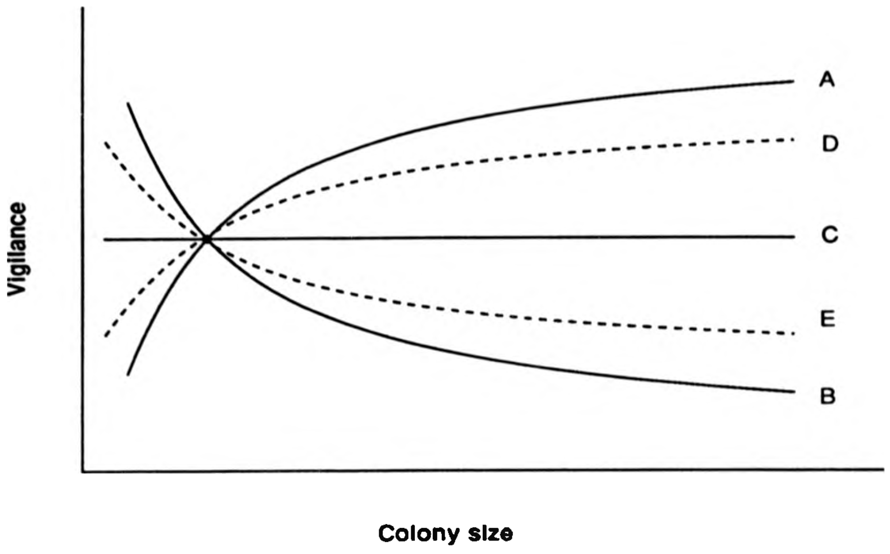


Figure 4.5 - Probability of detecting a predator (A), level of vigilance per individual (B), and total vigilance level (C), related to colony size. Hypothetical values of vigilance for colonial species which do not mob predators, and/or do not have predation as a main force of group leaving, are indicated by D and E.

Hoogland and Sherman's (1976) suggestions, although the ectoparasitism experiment carried out was simpler and did not make use of many different subcolony sizes. Sand martins seemed to effectively detect and mob predators, and were apparently more successful in large subcolonies. The results of the ectoparasitism and the predator experiments imply a trade-off when breeding in large colonies. Ectoparasites were shown to have a negative effect, in terms of nestling growth, while predators had a positive effect in bigger subcolony sizes. Other positive and negative effects of coloniality, however, such as intra-specific interactions, including food competition or mating competition, for example, are likely to be involved in regulating the size of sand martin colonies. Some of these factors are considered in the following chapters.

CHAPTER 5 - INTRASPECIFIC BROOD PARASITISM

5.1. INTRODUCTION

Intraspecific brood parasitism (IBP), involves laying eggs in the nests of conspecifics without taking part in incubation or caring for the nestlings (Yom-Tov 1980). It is considered to be a strategy whereby females increase their reproductive success either by producing more young or breeding again, or reducing their 'costs of breeding' hence increasing their survival (Brown and Brown 1988b).

IBP has been assumed to be rare among altricial species (Rohwer and Freeman 1989, Yom-Tov 1980) because species which feed their young should experience intense selection pressure for effective defense against being parasitized, since their reproductive success is limited by parental care. Selection against IBP should be stronger than that against extra-pair fertilization (EPF) because IBP represents a genetic cost to both males and females (Petrie and Moller 1991). Also IBP does not require cooperation of one individual or pair, whereas both EPFs and quasi-parasitism (QP) do. However, increasing numbers of altricial species are being found to exhibit IBP. It suggests that the presumed rarity of IBP, particularly among altricial birds, may be partially a consequence of methodological constraints (McWhirter 1989, Payne 1977).

Colonial species are suggested to be more prone to IBP (Rohwer and Freeman 1989) than non-colonial species because laying females have more chances to locate nests at the same stage of the nesting cycle (Hamilton and Orians 1965) and are therefore less conspicuous as they approach conspecific nests because of all the other birds around.

Females which lay eggs in the nests of conspecifics, may be unmated or mated; and may or may not raise young in their own nest (Jackson 1993). In other words, IBP can be an alternative for females which cannot raise young in another way (including females which lose their nests or mates) or serve as a way of increasing reproductive success for mated females. It may be particularly frequent where nest sites are limited (Yom-Tov 1980) as has been found in the tree swallow (Lombardo 1988). Egg-dumping occurred widely in the colonial cliff swallows of Nebraska (Brown 1984), where transference of eggs between nests increased the chances of fledging at least some offspring in a risky environment (Brown and Brown 1988b, Brown and Brown 1989). Electrophoretic analysis of body tissue samples indicated that 22-43% of the nests had IBP (Brown and Brown 1988c). However, a much lower rate of IBP was found in a population of this species in Sierra Nevada (only 3.7% of nests), as determined by analysis of egg white proteins (Smith *et al.* 1993). In this species IBP may be dependent on local environmental, demographic or geographical considerations (Smith *et al.* 1993).

No case of IBP was detected in tree swallow populations in Canada, using DNA fingerprinting (Lifjeld *et al.* 1993), although evidence of irregular sequences of laying was found in an American population (Lombardo 1988). This could possibly be another example of variation between populations, which could be confirmed by future genetic studies of the American population.

To avoid IBP, birds could eject parasitic eggs laid in their nests, begin incubating midway through laying, lay synchronously with pairs of the same species, guard the nest, and desert. Many bird species eject eggs placed in their nests before laying had begun. After the clutch is initiated, in contrast, parasitic eggs are usually accepted (Brown 1984, Davies and Brooke 1988, Møller 1987b, Petrie and Møller 1991, Stouffer 1987).

There are several ways for a human observer to detect whether a nest has been parasitized (McWhirter 1989, Yom-Tov 1980), including: a) direct observations, b) the presence of abnormally large clutch sizes, c) individual recognition of the eggs, requiring that egg patterns, including dimensions, marks and/or colour, vary among individuals, but are constant for each female, d) an irregular sequence of laying, such as the appearance of more than one egg per day, e) the appearance of new eggs after clutch completion, f) irregularities in hatching, e) genetic evidence.

The chance of observing a parasitic individual laying or removing an egg in a host nest is very small, demanding many hours of observation in field. Removal of eggs has been observed, in cliff swallows (that were seen carrying eggs in their beaks between nests)(Brown and Brown 1988b). The detection of IBP is usually best achieved using a combination of observational and genetic methods (McWhirter 1989, Romagnano *et al.* 1990), since only genetic analysis can detect cryptic forms of this behaviour. DNA fingerprinting in conjunction with detailed long-term behaviour observations of individually marked birds is a very good way to get information about IBP (McWhirter 1989).

This Chapter presents information on the occurrence of IBP in sand martins through observations, experimental additions of artificial eggs to nests during the pre-laying and laying stages, and DNA fingerprinting. The experiments of adding artificial eggs to nests were used to test the tendency of birds to reject added eggs before laying, and to accept them after clutch initiation. DNA fingerprinting also provided an opportunity to investigate if observation gives an estimate of IBP, which compares closely with the true frequency of this behaviour in a highly colonial species.

5.2. METHODS

During the pre-laying and laying periods, sand martin nests were inspected at 1-2 days intervals with the 'ripariascope' (Chapter 2) until the clutch was apparently complete. Nests were checked in the afternoon (1200-1800h).

Artificial eggs of similar shape, size, mass and colour as natural sand martin eggs were experimentally introduced into the nests, using a tool made from bamboo pole with a plastic teaspoon attached at one end (Figure 5.1). Each artificial egg was marked with a number in pencil, and directed into the nest chamber with the aid of a head torch illuminating the burrow from its entrance. Eggs were introduced in the morning (0900-1200h) and nests quickly checked with the 'ripariascope', to make sure the egg was placed in the nest cavity. Only one artificial egg was introduced to each nest.

Artificial eggs were added to nests during the pre-laying stage (when the nest was complete with a chamber already completed to receive eggs) and laying stages (Figure 5.2). Neighbouring nests at similar stages were used as controls, in which the tool was introduced empty (immediately after or before treating the experimental nests) to simulate disturbance.

Measures of weather conditions and food supply were collected as described in Chapter 2.

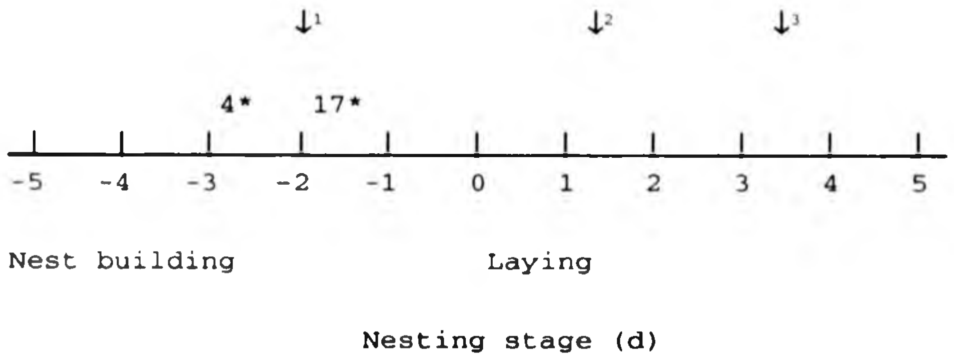


Figure 5.2 - Diagram of pre-laying and laying period showing when artificial eggs were added to sand martin nests, as indicated by arrows (0 = first egg day). The number of eggs added at each stage in 1991 and 1992 were: stage 1, n=39; stage 2=19; stage 3, n=13. 4*=number of eggs known to have

been introduced between -3 and -2 days of the pre-laying stage; 17*=number of eggs known to have been introduced between -2 and -1 days of the pre-laying stage.

In 1991, artificial eggs were added to a total of 32 nests during the pre-laying (24) and laying stages (10), but controls were not subjected to the 'empty tool' treatment. In 1992 a total of 39 nests was treated experimentally (17 in pre-laying and 22 at laying stages), with the same number of controls. There was no difference in clutch size between experimental and control nests (Table 5.1).

Experimental and control nests were checked daily or every other day until the clutch was complete and a week later to check if the birds had persisted with breeding. In some cases the nests that had artificial eggs added were occasionally checked through the nestling period (to see if the added eggs were still in the nests). Acceptance of artificial eggs was considered if there were signs of activity in the nest within the week following.

DNA fingerprinting (Chapter 6) was also used to determine the frequency of IBP.

5.3. RESULTS

5.3.1 - Evidence of IBP from observations

Of a total of 76 nests checked during the laying period (excluding experimental egg-dumping and ectoparasitism nests), 56 clutches (73.7%) increased by one egg per day, laying was interrupted (i.e. no eggs laid on one or more days) in 18 clutches (23.7%); and in a further 2 nests (2.6%) the clutch size increased by 1 egg in a day except on the last day, when 2 new eggs were detected.

Details of laying anomalies are shown in Table 5.2. In

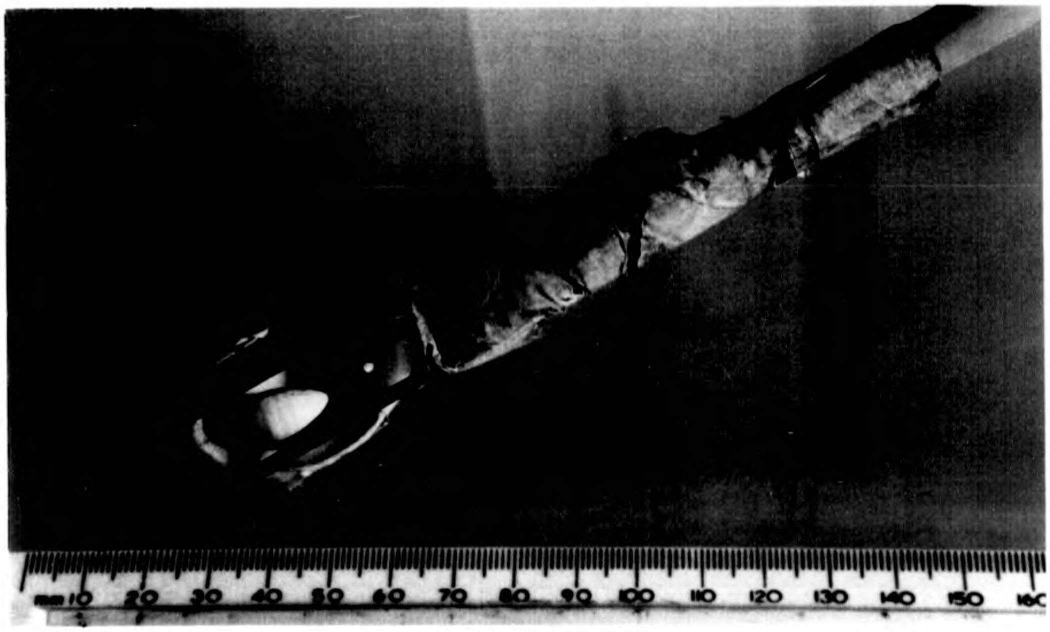


Figure 5.1 - Tool used to introduce artificial eggs into sand martin nests.

Table 5.1 - Comparison of clutch sizes between sand martin nests where artificial eggs were introduced and control nests (no eggs added) at pre-laying and laying stages (Barbush, 1991).

	Clutch size				
	mean	sd	n	t	p
Pre-laying					
experimental	4.90	0.738	10		
control	4.31	0.793	16	1.62	0.14
Laying					
experimental	5.14	0.710	22		
control	4.86	0.941	22	1.24	0.23

t = paired t-test

Table 5.2 - Clutch size, number of offspring and occurrence of extra-pair offspring from extra-pair fertilizations (EPFs), quasi-parasitism (QP) and intra-specific brood parasitism (IBP) in sand martin nests with abnormal laying sequences (n=20). Clutch size was termed apparently complete when no eggs were added in a 24h interval. Since extra eggs appeared in some nests that were not checked regularly after apparent clutch completion, these were assigned a minimal estimate of the number of days before an extra egg could have been laid.

	1990			1991												1992				
Clutch size after laying was apparently complete	5	3	3	4	4	3	3	4	3	3	3	5	5	4	4	5	4	5	3	3
Final clutch size	6	5	5	5	5	4	5	5	5	4	5	6	6	5	5	6	6	6	5	4
Number of days after apparent clutch completion that extra eggs were detected	4*	1	0	1	1	1	1	1	0	3*	2	1*	1*	1*	1	1*	1	2	1	1
Number of eggs hatched	4	4	-	5	4	4	4	4	3	3	3	4	5	-	5	4	-	-	4	-
Number of chicks on the 13th day after hatch	4	4	-	5	4	3	4	4	3	3	3	4	5	-	5	-	-	-	4	-
DNA fingerprinting data	+				+			+	+			+						+		
EPFs					+ (2)				+ (2)											
QP																				
IBP																				

* minimum estimate of the number of days.

most of the cases (70%), eggs appeared 1 day after apparent clutch completion. Overall, the clutch size after the first apparent completion was significantly smaller than that after extra eggs had appeared (paired t-test, $t=-12.37$, $p<0.001$, $n=20$)(Table 5.2). In 1991, however, when the greatest number of nests with abnormal laying sequences were observed, clutch sizes after the appearance of new eggs did not differ from those with a normal sequence of laying (Table 5.3). This result indicates laying was interrupted and the clutch size was not completed until new eggs appeared in the nests. Laying was occasionally interrupted for periods of at least 4 days; but in most clutches only for one day (Figure 5.3). At nests with normal and abnormal laying, the eggs usually hatched over 1 or 2 days, although in most cases 1 or more eggs failed to hatch (Figure 5.4).

Interruption of laying can be due to unfavourable weather conditions (Bryant 1975, 1979, Hoogland and Sherman 1976) or may be an artifact caused by variations in timing of nest examination (Hoogland and Sherman 1976). For example, one of the sand martin nests, where 2 eggs were apparently laid in one day, was checked early on the previous day before any new egg was found in the clutch (so early the female might not yet have laid). The other nest with 2 eggs per day, however, was examined at a similar time (middle of the day) on the previous day; even so, laying probably does not occur at exactly 24h interval. Laying anomalies observed might also in some cases be due to the difficulty of observing sand martin nests using a 'ripariascope'.

To test if the weather or food supply was related to the abnormal laying sequences, values of these variables for the day of the first egg, up to five days before and four days later were compared between nests with normal and abnormal laying sequences (Tables 5.4 and 5.5). The analysis was confined to 1991 because of the high incidence of laying abnormalities in that year compared with 1990 and 1992.

Nests with abnormal laying sequences had experienced

Table 5.3 - Clutch size of sand martin pairs with normal and abnormal laying sequences. Before = apparently complete, after = after apparently complete.

	Mean	Standard deviation	n	t	p
Normal	4.59	0.73	22		
Abnormal (before)	3.75	0.75	12	3.14	<0.01
Normal	4.59	0.73	22		
Abnormal (after)	5.00	0.60	12	-1.75	0.09
Abnormal (before)	3.75	0.75	12		
Abnormal (after)	5.00	0.60	12	-4.49	<0.001

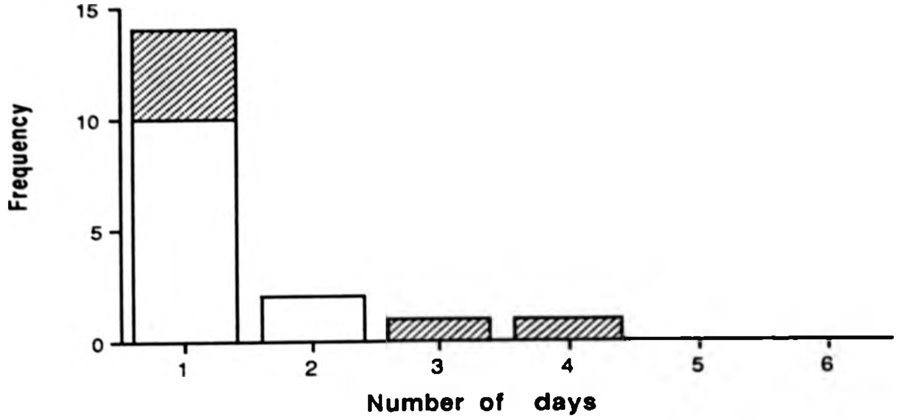


Figure 5.3 - Frequency of interrupted laying in sand martins. Shaded areas represent clutches where a minimum interruption period was estimated.

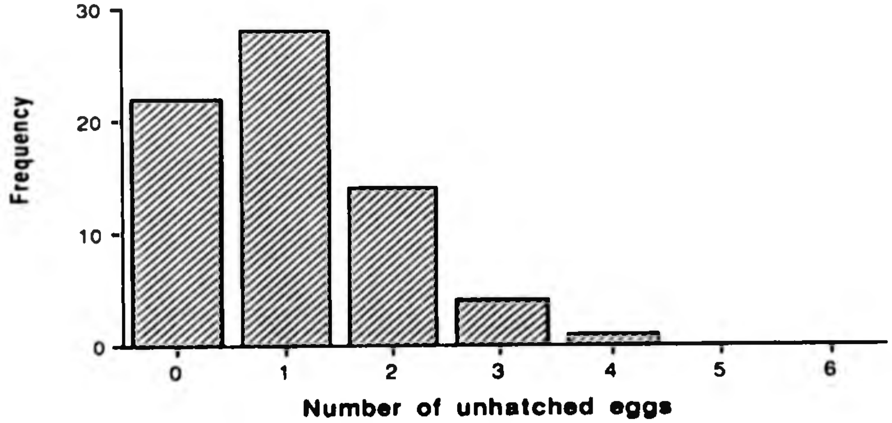


Figure 5.4 - Number of unhatched eggs in 69 sand martin clutches.

Table 5.4 - Weather and food availability (insects) variables associated with normal and abnormal laying sequences in sand martins,1991. Mean values have been compared for the day of the first egg, and the five days before laying began (-1 to -5). Max T = maximum temperature, Min T = minimum temperature, Mean T = mean temperature, Trap (insect availability). Values in the table are means ± standard deviation for MaxT, MinT, MeanT Trap, and mean ranks for Rain and Wind. Normal, n=22; abnormal, n=12. Trap variables were $\log_{10}$ transformed.

	Normal laying	Abnormal laying	t	p
Max T	18.20 ± 3.56	14.70 ± 1.92	3.71	<0.001
Max -1	18.62 ± 3.31	15.02 ± 1.87	4.04	<0.001
Max -2	18.21 ± 3.17	15.10 ± 1.33	4.00	<0.001
Max -3	18.49 ± 2.54	14.60 ± 1.93	4.61	<0.001
Max -4	18.35 ± 2.91	15.95 ± 1.87	2.57	<0.05
Max -5	18.04 ± 2.56	15.29 ± 1.96	3.23	<0.01
Min T	10.55 ± 3.43	5.66 ± 3.46	3.96	<0.001
Min -1	9.86 ± 3.05	5.12 ± 3.41	4.16	<0.001
Min -2	10.01 ± 2.64	5.38 ± 2.77	4.80	<0.001
Min -3	10.36 ± 2.60	6.35 ± 2.95	4.10	<0.001
Min -4	9.69 ± 2.56	5.63 ± 2.96	4.18	<0.001
Min -5	9.94 ± 1.82	5.82 ± 3.78	4.31	<0.001
Mean T	14.37 ± 3.14	10.18 ± 2.45	4.00	<0.001
Mean -1	14.24 ± 2.95	10.07 ± 2.32	4.22	<0.001
Mean -2	14.11 ± 2.54	10.24 ± 1.65	4.75	<0.001
Mean -3	14.43 ± 2.23	10.47 ± 2.04	5.06	<0.001
Mean -4	14.02 ± 2.34	10.79 ± 2.06	4.00	<0.001
Mean -5	13.99 ± 2.07	10.55 ± 2.54	4.26	<0.001
Trap	0.10 ± 0.36	0.91 ± 0.47	0.62	0.537
Trap -1	0.97 ± 0.31	0.85 ± 0.45	0.85	0.401
Trap -2	0.99 ± 0.34	0.81 ± 0.36	1.43	0.161
Trap -3	1.04 ± 0.29	0.92 ± 0.42	0.99	0.330
Trap -4	1.07 ± 0.30	0.99 ± 0.23	0.80	0.428
Trap -5	1.07 ± 0.32	0.87 ± 0.32	1.85	0.074
Rain	17.14	18.14	W = 218	p = 0.742
Rain -1	17.36	17.75	W = 213	p = 0.907
Rain -2	18.86	15.00	W = 180	p = 0.226
Rain -3	18.86	15.00	W = 180	p = 0.226
Rain -4	18.59	15.50	W = 186	p = 0.332
Rain -5	19.95	13.00	W = 156	p < 0.05
Wind	15.16	21.79	W = 261.5	p < 0.05
Mean -1	13.82	24.25	W = 291.0	p < 0.001
Mean -2	15.30	21.54	W = 258.5	p = 0.066
Mean -3	15.11	21.88	W = 262.5	p < 0.05
Mean -4	17.41	17.67	W = 212.0	p = 0.938
Mean -5	16.43	19.46	W = 233.5	p = 0.361

t = t-test
W = Mann-Whitney test

Table 5.5 - Weather and food availability (insects) variables associated with normal and abnormal laying sequences in sand martins. Mean values have been compared for the day of the first egg, and the four days after laying began (1 to 4). Max T = maximum temperature, Min T = minimum temperature, Mean T = mean temperature, Trap (insect availability). Values in the table are means ± standard deviation for MaxT, MinT, MeanT Trap, and mean ranks for Rain and Wind. Normal, n=22; abnormal, n=12. Trap variables were log$_{10}$ transformed.

	Normal laying	Abnormal laying	t	p
Max T	18.20 ± 3.56	14.71 ± 1.91	3.71	<0.001
Max 1	18.66 ± 3.73	16.02 ± 1.45	2.93	<0.01
Max 2	18.90 ± 4.23	15.70 ± 1.56	2.18	<0.05
Max 3	18.21 ± 4.16	15.79 ± 2.14	2.24	<0.05
Max 4	18.34 ± 4.15	15.90 ± 1.54	2.46	<0.05
Min T	10.55 ± 3.43	5.66 ± 3.46	3.96	<0.001
Min 1	9.85 ± 3.13	5.97 ± 3.44	3.33	<0.01
Min 2	9.34 ± 3.52	6.17 ± 2.90	2.65	<0.05
Min 3	9.94 ± 4.56	7.69 ± 2.26	1.59	0.121
Min 4	9.29 ± 4.20	6.86 ± 2.65	1.81	0.080
Mean T	14.37 ± 3.14	10.18 ± 2.45	4.00	<0.001
Mean 1	14.26 ± 3.09	11.00 ± 2.13	3.24	<0.01
Mean 2	13.62 ± 3.70	10.94 ± 2.08	2.31	<0.05
Mean 3	14.07 ± 4.16	11.74 ± 2.05	2.19	<0.05
Mean 4	13.81 ± 3.84	11.38 ± 1.83	2.49	<0.05
Trap	0.10 ± 0.36	0.91 ± 0.47	0.62	0.537
Trap 1	0.98 ± 0.29	0.97 ± 0.35	0.11	0.912
Trap 2	0.86 ± 0.39	1.07 ± 0.32	-1.55	0.130
Trap 3	0.89 ± 0.46	1.03 ± 0.35	-0.95	0.349
Trap 4	0.98 ± 0.44	1.05 ± 0.34	-0.53	0.603
Rain	17.14	18.17	W = 218	p = 0.742
Rain 1	20.09	12.75	W = 153	p < 0.05
Rain 2	18.18	16.25	W = 195	p = 0.515
Rain 3	16.36	19.58	W = 235	p = 0.321
Rain 4	17.48	17.54	W = 210.5	p = 0.984
Wind	15.16	21.79	W = 261.5	p < 0.05
Mean 1	17.41	17.67	W = 212.0	p = 0.936
Mean 2	17.34	17.79	W = 213.5	p = 0.892
Mean 3	17.52	17.46	W = 209.5	p = 0.984
Mean 4	16.77	18.83	W = 226.0	p = 0.539

t = t-test
W = Mann-Whitney test

significantly lower maximum, minimum and mean temperatures on the day of the first egg, and up to five days before that (Table 5.4). Nests with abnormal sequences of laying also had experienced significantly lower maximum and mean temperatures four days after the first egg; while they were subjected to significantly lower minimum temperatures only up to two days after the first egg (Table 5.5). These nests had also experienced significantly higher wind speeds on the day of the first egg, one day and three days earlier. Rain was significantly greater for nests with normal laying sequence only five days before the first egg. No significant difference was found for measures of food supply on the day or up to five days before, or four days after, the first egg was laid (Tables 5.4 and 5.5).

5.3.2 - Evidence of IBP from DNA fingerprinting

DNA fingerprints were available for complete families from 6 nests with abnormal laying sequences (Table 5.2). No cases of IBP were detected, although there was a case of QP in one nest where an extra egg was detected after clutch completion. Two nests contained offspring fathered by an extra-pair male (Table 5.2). Considering all sand martin nests for which DNA fingerprints were available (Chapter 6), IBP was detected in 4.3% of nests (n=46), and 1.8% of offspring (n=170) hatched from dumped eggs. The sequence of laying was apparently normal in all nests with IBP, and also in most of the cases of quasi-parasitism (QP). Quasi-parasitism occurred at a higher rate than IBP, with at least one offspring as an outcome of this behaviour in 17.4% of broods, and accounted for 6.5% of offspring (Chapter 6).

Average weekly maximum and minimum temperatures, during the breeding season, the frequency of nests with normal and abnormal sequences of laying (categorized by date of the first egg), and nests with IBP and QP detected by DNA fingerprinting are shown in Figure 5.5 for 1990 and in significantly lower maximum, minimum and mean temperatures on the day of the first egg, and up to five days before that (Table 5.4). Nests with abnormal sequences of laying also had experienced significantly lower maximum and mean temperatures four days after the first egg; while they were subjected to significantly lower minimum temperatures only up to two days after the first egg (Table 5.5). These nests had also experienced significantly higher wind speeds on the day of the first egg, one day and three days earlier. Rain was significantly greater for nests with normal laying sequence only five days before the first egg. No significant difference was found for measures of food supply on the day or up to five days before, or four days after, the first egg was laid (Tables 5.4 and 5.5).

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Average weekly maximum and minimum temperatures, during the breeding season, the frequency of nests with normal and abnormal sequences of laying (categorized by date of the first egg), and nests with IBP and QP detected by DNA fingerprinting are shown in Figure 5.5 for 1990 and in

Figure 5.5 - Temperature and frequency of normal and abnormal laying sequences in sand martins in 1990. A) Average maximum and minimum temperatures per week. B) Frequency of nests with normal and abnormal laying sequences (by date of the first egg). C) DNA fingerprinting evidence of quasi-parasitism (QP) in nests with normal and abnormal laying sequences (by date of the first egg laid).
Week 1 starts on 1st of May.

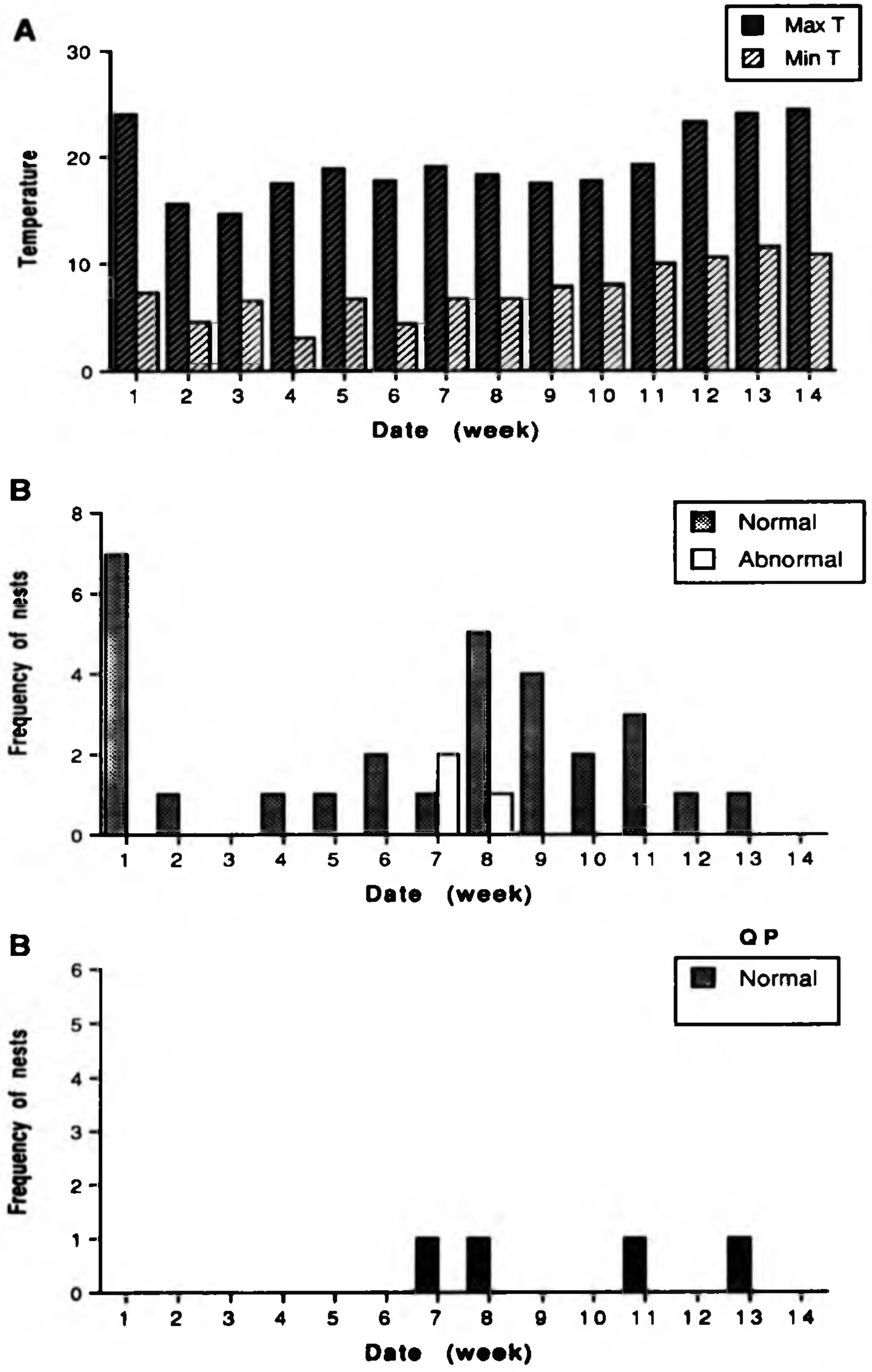

Figure 5.6 for 1991. Abnormal sequences of laying seemed to be crudely related to low temperatures, although two nests with an abnormal sequence of laying were recorded when temperatures increased (Figure 5.6). All the nests with QP in 1990 (5) had an apparently normal sequence of laying, except one. In 1991, the one clutch with IBP and QP had apparently normal sequence of laying. As mentioned before, laying anomalies were found to be related to weather conditions. Hence observations of the sequence of laying in sand martins did not yield conclusive evidence about the occurrence or frequency of IBP or QP; in only one case was an apparent laying abnormality attributable to a genuine dumped egg.

5.3.3 - Experimental egg dumping

The artificial eggs matched well visually with the natural eggs. Sand martins reacted to artificial eggs introduced into their nests in different ways: accepting or ejecting the eggs, deserting the nest, or building over the eggs (Table 5.6). In one case the whole nest disappeared, apparently due to predation by a mammal, followed by nest material removal by unknown birds. A total of 19 of 41 eggs (46.3%) was rejected at the pre-laying stage in 1991 and 1992 (Table 5.6), while only 4 of 32 eggs (12.5%) were rejected at laying nests. Comparing experimental and control nests in 1992, sand martins accepted artificial eggs significantly less in experimental nests, compared to control nests of the pre-laying stage (i.e. control nests could reject their own eggs as a result of disturbance) (Table 5.7). No difference was found between experimental and control nests during the laying stage.

A total of 15 in 41 nests was deserted (36.6%) after artificial eggs were introduced into them in pre-laying stage in 1991 and 1992, while only 3 in 32 nests were deserted (9.4%) during the laying stage (Table 5.6).

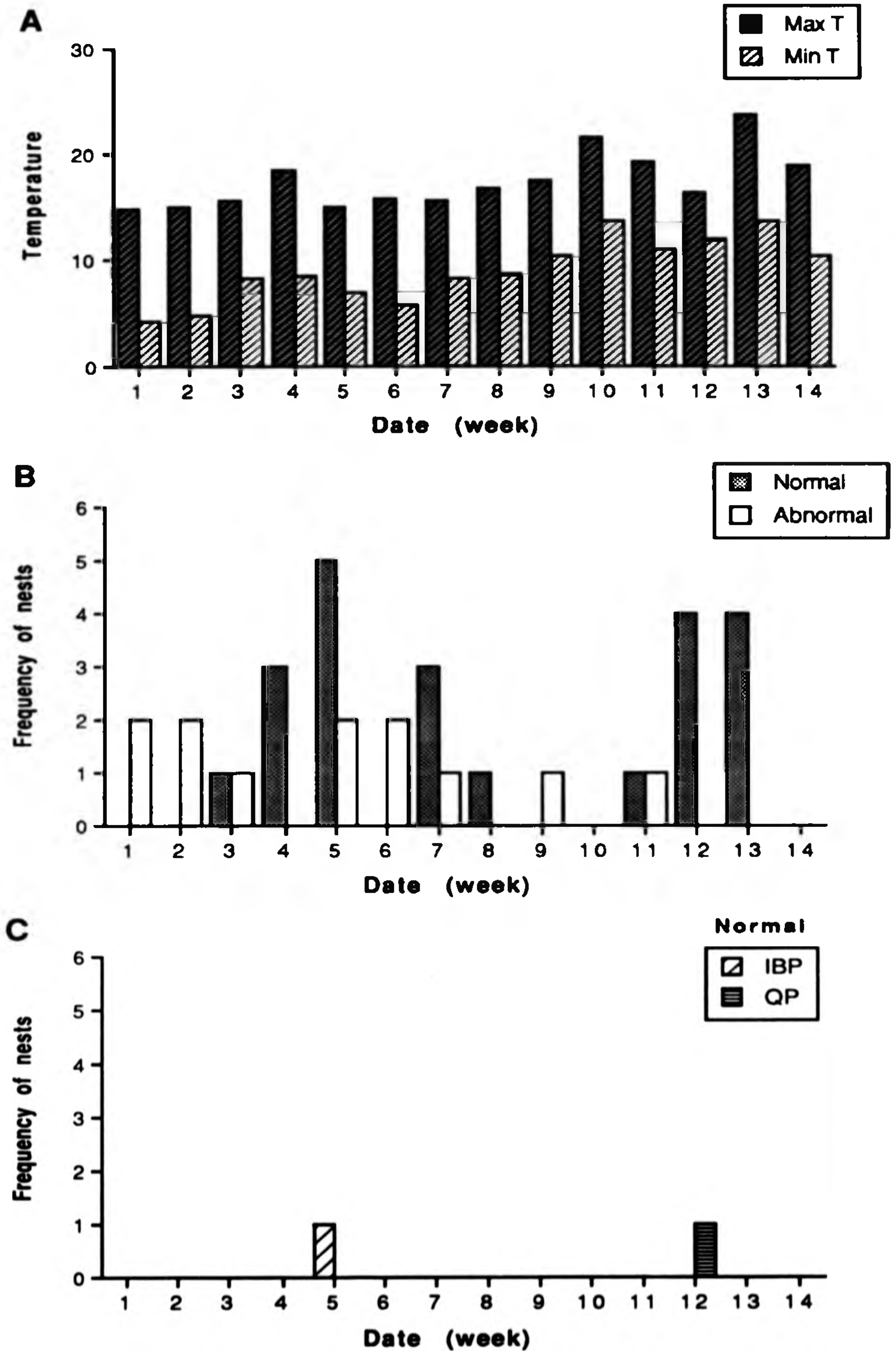

Figure 5.6 - Temperature and frequency of normal and abnormal laying sequences in sand martins in 1991. A) Average maximum and minimum temperature per week. B) Frequency of nests with normal and abnormal laying sequences (by date of the first egg); excluding all experimental nests (ectoparasitism experiment). C) DNA fingerprinting evidence of intra-specific brood parasitism (IBP) and quasi-parasitism (QP) in nests with normal laying sequences (by date of the first egg laid).
Week 1 starts on 1st of May.

Table 5.6 - Responses to introduction of artificial eggs into sand martin nests at the pre-laying and laying stages. Artificial eggs rejected include eggs that were ejected from the nests (found on the ground by the subcolonies), deserted (the nest was deserted by the pair) and built over (birds built a nest over the previous nest containing the artificial egg).

	Number of nests						
	Artificial eggs rejected						
Year/stage	ejected	deserted	built over	Predated	Artificial eggs accepted	Rejections/ total	%
1991							
Pre-laying	2	10	-	1	11	12/24	50.0
Laying	-	2	-	-	8	2/10	20.0
1992							
Pre-laying	-	5	2	-	10	7/17	41.2
Laying	-	1	1	-	20	2/22	9.1

1991 and 1992	deserted/total	X^2	p	rejections/total	X^2	p
Pre-laying	15/41			19/41		
Laying	3/32	3.43	0.064	4/32	4.15	0.042

Table 5.7 - Frequency of rejection of artificial eggs at experimental and control sand martin nests. Control nests were disturbed but had no eggs added.

1992	Accepted	Rejected	G	p
Pre-laying				
experimental	10	7		
control	16	1	4.34	<0.05
Laying				
experimental	20	2		
control	21	1	0.07	0.75

G = G test

5.4 - DISCUSSION

5.4.1 - Observations and DNA fingerprinting evidence

The interval between the laying of successive eggs by most female birds is usually 24h (Sturkie 1976). Bank swallows normally lay one egg per day until the clutch is complete (Petersen 1955, Stoner 1936). The absence of widespread variation from the one egg per day pattern suggested little or no intra-colonial brood parasitism in bank swallows (Hoogland and Sherman 1976). Interruptions of laying might also occur if females dump eggs in nests other than their own. Interruption of the laying sequence in bank swallows, however, was interpreted as a response to unfavourable weather rather than IBP (Hoogland and Sherman 1976).

The present study demonstrated interruption of laying in sand martins, and a low rate (2.6%) of more than two eggs laid per day in a single nest. A similar frequency of clutches with 2 eggs per day was found in bank swallows (Hoogland and Sherman 1976), and European bee-eaters (C.M. Lessells pers. comm.).

Interruption of laying in sand martins was apparently related to unfavourable weather conditions, particularly low temperatures just before and on the day that laying started. Interruption of egg laying was found to be dependent on food availability in blue tits; when females were experimentally provided with extra-food, the frequency of laying gaps decreased (Nilsson and Svensson 1993). Food supply was not found to be related to abnormal sequences of laying in sand martins, although it is possible that the suction trap collections on the University campus did not reflect the real variations in insect abundance at the study site. This is likely, given the tendency for sand martins to feed by water (Waugh 1978); more so since the suction trap was remote from the running water frequented by radio tagged sand martins (Chapter 3).

Interruption of egg laying occurs in a variety of different species. Weather conditions were found to cause laying interruptions in house martins (Bryant 1975, Bryant 1979), swallows (Thompson 1992, Ward 1992), and bank swallows (Hoogland and Sherman 1976), and the present study also gave evidence for this in sand martins.

Laying anomalies were least common in swallows (10% Thompson 1992 and 14% Ward 1992), followed by sand martins (24% present study), house martins (35% Bryant 1979) and swifts (range 25-60%, O'Connor 1979). All these species had a higher frequency of laying interruption than non-aerial feeding birds, as for example tits (6.7% Dondht *et al.* 1983). This is consistent with the prediction that aerial foraging birds are more prone to laying interruptions because they have a less predictable food supply (Ward 1992). In conclusion, interruptions of laying occur in response to adverse environmental conditions, or poor female condition; and are therefore not conclusive evidence that IBP has occurred (McWhirter 1989).

DNA fingerprinting provided evidence for both IBP and QP in sand martins, with IBP occurring at a lower rate. There was also evidence of EPFs (Chapter 6). Laying abnormalities were not detected in any the nests that had IBP or QP. Thus, observations of laying patterns apparently cannot be reliably used to detect cases of egg-dumping (IBP or QP) in this species.

The parasitic female sand martins could have dumped eggs when the host female was laying, and/or after the clutch was completed. If they added eggs during the laying sequence of the host female, they probably removed one egg of the host since a very low rate of 'two eggs per day' was observed.

Removal of eggs by brood parasites is a tactic that may occur to reduce the chance of detection by the host (Petrie and Møller 1991), and it has been recorded in several species including white-fronted bee-eaters (Emlen and Wrege 1986) and cliff swallows (Brown and Brown 1989). Only a few sand martin eggs were found freshly broken on the ground in the vicinity of the colony, suggesting that the parasitic females did not actually remove eggs very often, or if they did, they carried eggs well away from the colony (or the eggs were rapidly eaten by predators). Neither of these possibilities was judged likely, however, although proof of their importance was not available. The appearance of 2 eggs in one day was detected in two nests, in both cases on the day the last egg was laid. At last one of these was probably a true case of parasitism because the nest was definitely checked after the host female had laid on the previous day. However, this nest was deserted and so DNA fingerprinting evidence was not available. The other case where 2 eggs were apparently added on the same day could have been explained by the timing of nest examinations. In this case only 3 of 5 eggs hatched, and DNA fingerprinting of the 3 chicks did not reveal IBP or QP.

If intraspecific brood parasites dump eggs while the host female is laying then this gives their eggs a greater chance of hatching. Presumably parasites might monitor potential hosts and their nests (Petrie and Møller 1991), waiting for a chance to dump eggs. Since neighbouring nests are more easily monitored, this could explain the tendency of parasites to dump eggs in nests of close neighbours, as found in swallows (Møller 1987b). Most of the parasitic sand martin eggs were actually fertilized by the host males. In this case the parasite females may monitor the nests of the males with which they mate, and target these nests when dumping eggs. Alternatively, males could 'lead' females to their nests to attempt extra-pair copulations.

5.4.2 - Experimental dumping of artificial eggs

Sand martins seemed able to recognize and not accept parasitic or artificial eggs before clutch initiation. However, they seldom rejected eggs added after clutch initiation, suggesting that hosts do not recognize that they have been parasitized (i.e. cannot 'count' eggs) or cannot distinguish their own eggs from those of parasites (sand martin eggs are white, lacking marks).

The tendency to remove eggs before, but not after laying has started, has been demonstrated in a number of species, including white-fronted bee-eaters (Emlen and Wrege 1986), European bee-eaters (C.M. Lessells pers. comm.) acorn woodpeckers (Mumme *et al.* 1983), european starlings (Stouffer *et al.* 1987), and swallows (Møller 1987b). The usual response is to reject the majority of introduced eggs (around 100%) before laying, but not after clutch initiation.

Sand martins, however, accepted approximately 50% of the artificial eggs added during the pre-laying stage (time between the nest completion of the nest and the appearance of the first egg). Bichoff and Murphy (1993) found a similar relative high frequency of pre-laying acceptance of eggs in eastern kingbirds, and suggested that this species was not very efficient at rejecting eggs (although egg ejection is an important defence against IBP in this species during pre-laying). In sand martins the high acceptance rate could be due to the eggs being mainly introduced very close to the start of laying (41.5% of the eggs introduced in the pre-laying stage). All the accepted eggs were introduced between -3 and -1 day of the nest cycle. Eggs introduced during pre-laying in lined but empty nests were all accepted by yellow warblers, while eggs introduced to unlined nests were buried due to continued nest building behaviour (Sealy *et al.* 1989). Cliff swallows rejected some eggs added more than 4 days before they began laying (Brown and Brown 1989).

The fact that sand martins reject some artificial eggs before laying begins indicates that they can recognize that the nest has been parasitized at that stage and are able to avoid some of the costs of brood parasitism. The cost of ejecting one of their own eggs once laying has started is obviously high if the birds are not able to distinguish their own eggs from parasitic eggs. However, the question of who rejects the eggs, the male or the female, remains. The female is perhaps more likely to be aware if she has begun to lay and therefore to reject eggs, at least until she lays her first egg. Females have been shown to be the sex which ejects experimentally added eggs during the pre-laying stage in European bee-eaters (C.M. Lessells pers. comm.) and swallows (Møller 1987b).

5.4.3 - Why accept a dumped egg

Several hypothesis have been proposed to explain why some hosts accept being parasitized (Gibbons 1986). Some hypothesis are: a) the dumper may be related to one of the host pair; b) there may be a benefit to the host; c) the host pair may be unaware of being parasitized, and d) the host male may have copulated with the dumping female.

The three first hypotheses (a, b and c) were not tested in this study. DNA fingerprinting, however, provided proof that sand martin host males had copulated with the dumping females (QP, Chapter 6). It is also possible that a pair could be unaware of being parasitized, although it is more likely that they accept parasitic eggs because they cannot discriminate these eggs from their own white eggs (Hoogland and Sherman 1976). Recognition of conspecific eggs has not been reported for any bird species (Smith *et al.* 1993).

Egg dumping was found to be a cost of living in colonies for at least some individual sand martins which raised parasitic chicks, while it may be a benefit for individuals which increased their reproductive success. It was not possible to determine which individuals increased their reproductive success through IBP. Rejection of eggs before laying is selectively advantageous to sand martins, enabling them to avoid the cost of being parasitized at this stage of laying at least.

Parasitism seems to be particularly costly for this species, not only because of the risk of raising brood parasites but because desertion was a common response to artificially introduced eggs, accounting for 36.6% in the pre-laying and 9.4% of nests in the laying stage. Desertion is apparently a costly strategy adopted by hosts against parasitism due to the time and energy costs of relaying, and is expected to occur only if the cost of rearing parasitic eggs outweighs the cost of rebuilding a nest and relaying (Petrie and Møller 1991). A further fitness cost of relaying may be a reduced opportunity to raise a second brood (Bryant 1979).

CHAPTER 6 - DNA FINGERPRINTING AND BEHAVIOUR

6.1. INTRODUCTION

6.1.1. DNA fingerprinting technique

Investigations into the human myoglobin gene revealed a number of hypervariable regions of DNA consisting of repeats of short core sequences or minisatellites of about 12 nucleotides (Jeffreys *et al.* 1985a). Two human probes (33.6 and 33.15) were isolated (Jeffreys 1985), and when radioactively labelled, and hybridized to DNA, which had been digested by restriction endonuclease enzymes (after using electrophoresis through an agarose gel), were able to detect hypervariable minisatellites at many loci. Because of this, they are usually called multilocus probes. The pattern of bands, or DNA fingerprint, can be visualized through autoradiographs, using a technique called Southern Blotting (see General Methods). Using a given probe, the pattern of bands of a given individual was found to be stable (Jeffreys 1985); that is, for a given individual the same pattern of bands was produced independently of the tissue used as the source of DNA.

These minisatellite fragments vary considerably between individuals and are transmitted from parents to offspring in a Mendelian fashion. Hence, on average half of the bands in the DNA fingerprint of a given individual come from the father, and half from the mother. One fragment in 300 could not be traced to the father or the mother, and was thought to be the result of a mutation (Jeffreys 1985). Each individual has a unique band pattern, which can be used to identify that individual. This led to the popular name for the technique of DNA fingerprinting.

The applications of the DNA fingerprinting technique are various, including forensic analysis and parentage studies in man (Dodd 1985, Gill *et al.* 1985, Helminen *et al.*

1988, Jeffreys *et al.* 1985b, Logtenbergh and Barker 1988, Thompson and Ford 1990), gene mapping and medical research (Jeffreys *et al.* 1990, Nakamura *et al.* 1987), verification of pedigrees and segregation analysis, registration of breeding stock and characterization of genetic relationships between strains of the same species (Jeffreys *et al.* 1986, Jeffreys and Morton 1987, Parkin 1987). It can also be applied to investigations of genetic diversity of wild populations with applications in ecological studies (Burke 1989a, Hill 1987, Parkin 1989, Parkin *et al.* 1988).

DNA fingerprinting is more precise as far as paternity exclusion (parent-offspring) is concerned than, for example, classic protein polymorphisms (Dodd 1985, Hill 1987, Jeffreys 1985, Logtenberg and Bakker 1988). It is also much more sensitive for detection of extra-pair fertilizations (EPFs) than some other techniques, such as those using heritability analysis of body size parameters like tarsus or wing length (Dhondt 1991, Lessells and Ovenden 1989) and plumage polymorphisms (Westneat *et al.* 1987). DNA fingerprinting can detect variation at many loci simultaneously. Most minisatellites appear to be inherited independently, allowing the direct assignment of parentage. Attempts to ascertain relatedness between randomly chosen individuals by means of DNA fingerprinting should proceed with caution, since unrelated individuals of a given species tend to share a variable number of bands and may have F values close to the theoretical value for second degree relatives (0.25), giving only an approximate estimate of relatedness (Lynch 1988).

Assumptions for DNA fingerprinting to be valid in determining true genetic relationships include, (i) that every individual has a unique fingerprint (except identical twins) and (ii) that the minisatellites are inherited in a Mendelian fashion. This means that each scorable fingerprint fragment (or band) should segregate independently at meiosis with a probability of 50% that a parental band will be inherited by an offspring. Segregation analysis of bands in a large family (10 or more offspring) (Burke 1989a) is usually used to check if the bands are inherited independently. The use of large families avoids overestimation of linkage and allelism. Human minisatellite probes (isolated by Jeffreys, 1985) revealed similar hypervariability in dogs, cats (Jeffreys and Morton 1987) and other animals (Hill 1987, Parkin 1989). Burke and Bruford (1987) and Wetton *et al.* (1987) showed that these probes also detected highly variable regions in DNA of birds. Since then, DNA fingerprinting has been extensively used in studies of the behaviourial ecology of wild birds.

6.1.2. Mixed-reproductive strategies (MRS)

The term mating system refers to the way mates are acquired, the number of mates, characteristics of pair bonds and division of parental care by each sex (Emlen and Oring 1977).

Mating systems can be classified as: monogamous, polygynous, polyandrous and promiscuous (Perrins and Birkhead 1983). Monogamy occurs when one male forms a pair bond with one female, and both provide parental care. In polygynous systems, one male forms a pair bond with several females simultaneously or successively, with parental care provided mainly by the females. Polyandry is a system where a female forms pair bonds with several males simultaneously or successively, with the males usually providing parental care. A promiscuous system involves both male and female mating with several individuals of the opposite sex, simultaneously or successively; either/or both sexes may provide parental care.

In a species in which monogamy is the dominant mating system, it would seem adaptive for males to pursue a mixed-reproductive strategy (MRS), since it is a way to increase their reproductive output without the costs of raising young. For example, a male might help raise offspring with one female, while at the same time attempting to copulate with other females whose young they will not raise (Trivers 1972, Fitch and Shugart 1984). In monogamous systems individuals of both sexes can try to maximize their reproductive success by pursuing a MRS (Fitch and Shugart 1984). A MRS might include: a) extra-pair copulations (EPCs), that is copulations by paired birds with partners other than their mates, and b) intraspecific brood parasitism (IBP), where females dump eggs in nests of conspecifics. Males could maximize their reproductive success by fertilizing eggs laid by their own mates and copulating with as many extra females as possible. Females can increase their reproductive success by mating with high quality males (choosing males or selectively accepting EPCs), or maximizing the number of eggs laid (which includes IBP). Birds conventionally described as monogamous have been shown with evidence from genetic studies to be promiscuous (Birkhead and Møller 1992). The term monogamy may therefore often describe the parental care shared by one male and female but not their genetic contributions (Davies 1992a).

Sperm competition refers to competition between spermatozoa of different males to fertilize ova within a reproductive cycle of a single female (Birkhead 1988, Birkhead and Møller 1992), and can be expected to occur in monogamous species that pursue a MRS. In a narrow sense, sperm competition could refer to the physiological processes occurring within a female's reproductive tract after multiple mating. In a broader sense, it includes features such as number, size and structure of sperm, morphology and physiology of male and female reproductive tracts, sperm storage, courtship, copulation and social systems (Birkhead and Møller 1992, Birkhead and Møller 1993b).

EPCs have been recorded for many bird species (Oring, 1982) and provide a means for males to increase the number of offspring they father; while females might increase the quality of the offspring produced, through copulating with males with 'good genes' (Birkhead and Møller 1992). EPCs can be forced (despite female resistance), sometimes causing risk or injury to the female, like in waterfowl (Cheng *et al.* 1983, Mckinney *et al.* 1984, Mineau and Cooke 1979), bee-eaters (Emlen and Wrege 1986) and cliff swallows (Brown and Brown 1988c). Female resistance to EPCs may be genuine (certain female behaviour has been described as a way to avoid 'rape' attempts, Butler 1982), or a ploy to test male quality (Westneat *et al.* 1990). Females may also solicit EPCs (Arvidson 1992, Kempenaers *et al.* 1992).

Evidence of observed EPCs in monogamous birds is increasing (Birkhead *et al.* 1987, Gladstone 1979, McKinney 1984), and many recent studies on birds have shown that EPCs can result in extra-pair fertilizations (EPFs) in monogamous or other mating systems (Birkhead *et al.* 1987, Birkhead and Møller 1992, Westneat *et al.* 1990).

The potential for individuals of one sex to accumulate multiple mates increases with increasing degree of asyncrony in timing of breeding among members of the other sex (Emlen and Oring 1977). The frequency of sexual chases and EPCs involving female mates can be reduced by males mate-guarding or by females breeding synchronously (Oring 1982).

6.1.3. Mate-guarding

Mate-guarding is a reproductive tactic which serves to minimize the risks of the male losing paternity through EPC during the fertile period of the female (Birkhead and Møller 1992). It can be defined as the close following of the female partner by the male prior to and during egg-laying (Beecher and Beecher 1979). Mate-guarding, which also serves to protect females from harassment (Mckinney 1973), is probably the most widespread paternity defence and has been described in many species of birds (Aguilera and Alvarez 1989, Beecher and Beecher 1979, Birkhead *et al.* 1987, Bjorklund and Westman 1983, Hatch 1987, Møller 1985, Møller 1987cd, Morton 1987, Seiler and Prys-Jones 1989, Wagner

1992). Not all birds species show mate-guarding (Birkhead *et al.* 1987). The frequency of EPC was found to be inversely related to the intensity of mate-guarding in swallows (Møller 1987d), and the lack of mate-guarding in tree swallows was thought to be related to a low probability of EPC (Leffelaar and Robertson 1984). However, Morrill and Robertson (1990), using coloured glass microspheres inserted into the cloacae of males, found that EPCs were more frequent than was expected. This conclusion was later confirmed by DNA fingerprinting techniques (Dunn and Robertson 1993).

Birkhead and Møller (1992) reviewed the frequency of extra-pair paternity (offspring not related to male attending nest) in different species of birds and found that the percentage ranged from 0 to 65%. The use of genetic techniques to determine paternity, particularly DNA fingerprinting, has increased and strengthened the evidence of EPFs in many species of birds.

6.1.4. Why sand martins were chosen for a DNA fingerprinting study

The sand martin is monomorphic, monogamous, and both sexes participate in nest building, incubating and provisioning of nestlings (Cramp 1988). However, this species can be highly colonial which might be expected to exacerbate tendencies toward cuckoldry in males. EPCs were predicted to be more frequent in colonial birds simply because of the proximity of conspecifcs (Alexander 1974). Accordingly, colonially nesting species have been found to have a higher rate of EPC than solitary ones (Birkhead *et al.* 1987, Birkhead and Møller 1992), and are predicted to have a greater incidence of extra-pair paternity; but this idea was not tested due to lack of suitable data (Birkhead and Møller 1992).

Sand martin/bank swallow males guard their mates during the pre-laying and laying periods, when females are chased by other males (Emlen and Wrege 1986). Females at these stages can be detected by their laboured flight (Jones 1986b). These sexual chases can end in EPCs (Hoogland and Sherman 1976, Beecher and Beecher 1979). Copulations in sand martins are rarely seen, however, making observations of EPCs impractical and sampling subject to bias.

Parental care describes any form of parental behaviour which appears likely to increase parental fitness (Clutton-Brock and Godfray 1991). The provisioning of offspring can be regarded as a key component of parental care. One possible cost of females engaging in EPCs could be reduced parental investment from mates aware of being cuckolded (Trivers 1972). Males of monogamous bird species might therefore be expected to invest more in broods with higher paternity than broods with lower paternity.

This Chapter describes the variability of sand martin DNA fingerprints and the validity of this technique for determining parent-offspring relationships in this species. The questions that arise are: a) How frequent are extra-pair fertilizations (EPFs) and intra-specific brood parasitism (IBP) as determined by DNA fingerprinting? b) Is mate-guarding effective as a tactic for paternity defence? The importance of mate-guarding by males in preventing EPC was tested experimentally. Males were removed temporarily when the females were likely to be fertile.

The DNA fingerprinting results on paternity were combined with behaviourial observations to answer questions which include: a) Is the rate of food delivery to the brood by males influenced by their paternity? In other words, do male sand martins adjust their level of parental effort according to their level of paternity? b) Do male sand martins temporarily removed when the females are probably fertile reduce their parental effort compared to control males not removed? This assumes that males would perceive their mates were more liable to EPFs in their absence. The alternative is that males may be unaware of this possibility when this arises from captivity.

6.2. METHODS

6.2.1. DNA fingerprinting technique

Collection and storage of blood samples and DNA fingerprinting techniques are described in Chapter 2. Blood samples were taken from 93 adult individuals and 194 chicks belonging to 52 complete families. No difference in quality of DNA was found between blood samples stored at -20°C and -70°C by Seutin *et al.* (1990). Thus, due to lack of space in the -70°C freezer sand martin blood samples collected in 1991 were stored at -20°C and the rest at -70°C.

To validate the technique for sand martins the general level of variability of DNA fingerprints of unrelated individuals was established, and the percentage of DNA fragments which segregated independently was determined. A group of 12 randomly selected adults (6 males and 6 females presumed unrelated) were run together in a single gel, to assess the variability of DNA fingerprints. The probe used was the same used for all the other fingerprints (see General methods).

For a segregation analysis, two broods with the same male or female parent were run in a single gel. The segregation analysis was done separately for a male and a female with two broods, because no pair of sand martins was observed to stay together to raise a second brood in a given breeding season. The respective mates of the male and the female for the first and second broods were also run in the same gel. To facilitate scoring, the DNA samples of the parents were run on both sides of the offspring samples. The bands were scored in the region of 23.1 - 6.5kb, as was done for all other fingerprints.

The total number of hypervariable loci detected by the human probe 33.6, assuming that the number of distinct loci

(L) is a random sample of N heterozygous loci and 2N bands in a DNA fingerprint, was estimated as (Jeffreys *et al.* 1986):

$$N = 1/2 \left| \frac{(L + a)\ (L + a - 1)}{2a} \right| + 1 \qquad (1)$$

Where a is the number of allelic pairs.

6.2.2. Scoring the DNA fingerprints

The fingerprints were assessed visually. Bands at similar positions on the gel were assumed to represent the same minisatellite fragment. Some very faint bands were not scored. The band patterns in two individuals were compared by calculating the band sharing coefficient (Meng *et al.* 1990):

$$F = \frac{2\ N_{AB}}{(N_A+N_B)} \qquad (2)$$

Where N_{AB} is the number of bands shared by two individuals with respectively N_A and N_B bands. Theoretically, F values should be approximately 0.5 for first degree relatives and 0.25 for second degree relatives. F values only approach theoretical values if large number of bands are scorable in each individual, each individual has the same number of bands and average band sharing between unrelated individuals is essentially zero. In practice these conditions cannot be fully reached, caution is therefore needed, particularly for estimating F values of second degree relatives (Lynch 1988). In the present study, F values have been used as an indication of parent-offspring relationships between individuals (in other words, to exclude paternity).

The probability that a band present in a given individual A will be present in the individual B, is:

$$x = \frac{N_{AB}}{N_A} \tag{3}$$

The band sharing probability (x) is used to calculate the probability, pf, that two randomly chosen individuals have identical fingerprints (Jeffreys and Morton 1987):

$$pf = (1- 2x + 2x^2)^{n/x} \tag{4}$$

The mean population frequency of alleles at minisatellite loci, q, is calculated as (Jeffreys *et al.* 1985b):

$$q = 1 - (1 - x)^{1/2} \tag{5}$$

The probability that two siblings have identical fingerprints (ps) can be calculated by (Jeffreys and Morton 1987):

$$ps = [1 - 1/2q(1 - q)^2 \ (4 - q)]^{n/x} \tag{6}$$

The primary criterion used to distinguish between chicks that were fully related or unrelated to at least one of their putative parents was the number of mismatching bands, after comparing the frequency distribution of the number of mismatching bands with a Poisson distribution, as described in the results section. Two or fewer mismatching bands were considered to represent mutations.

The term extra-pair offspring (EPO) included extra-pair fertilization (EPF), quasi-parasitism (QP) and intraspecific brood parasitism (IBP). The term 'no EPO' excludes all cases of EPF, QP and IBP.

6.2.3. Male removal experiment

To catch members of pre-laying or laying pairs, the entrance of the nest burrow was blocked the night before using a cardboard cone. This was replaced early the following morning (0530-0700h) by a hand net (Figure 2.2) or a cone with a mist net (Figure 2.3) to catch the birds. The female was marked if not caught previously (usually with 'Tippex' dots on the tail and dyes on the throat), and released quickly. The male was kept in a cotton bird bag for 2-5 hours, and fed every 2 hours with minced meat. Controls were provided by neighbouring nests, matched in time as far as possible with the experimental ones (i.e., at a similar nesting stage). Cones were also put on the entrance of the control nests at night and removed the next morning. No attempt was made to catch the control pair for practical reasons (since the birds do not usually came out of the nest burrow at the same time, and may delay coming out when disturbed by catching operations, compromising the start of observation at the experimental nest). Behaviourial observations at experimental and control nests were made for 2 hours after male removal, and for another hour after releasing the males.

6.3. RESULTS

6.3.1. Variability of DNA fingerprints

The DNA fingerprints of 12 randomly chosen adults (6 males and 6 females) are shown in Figure 6.1. A total of 80 bands was distinguished in the size range 23.1-6.5kb. An average of 17.1 (± 2.43) bands per individual were scorable within this range. No species specific bands (i.e. bands

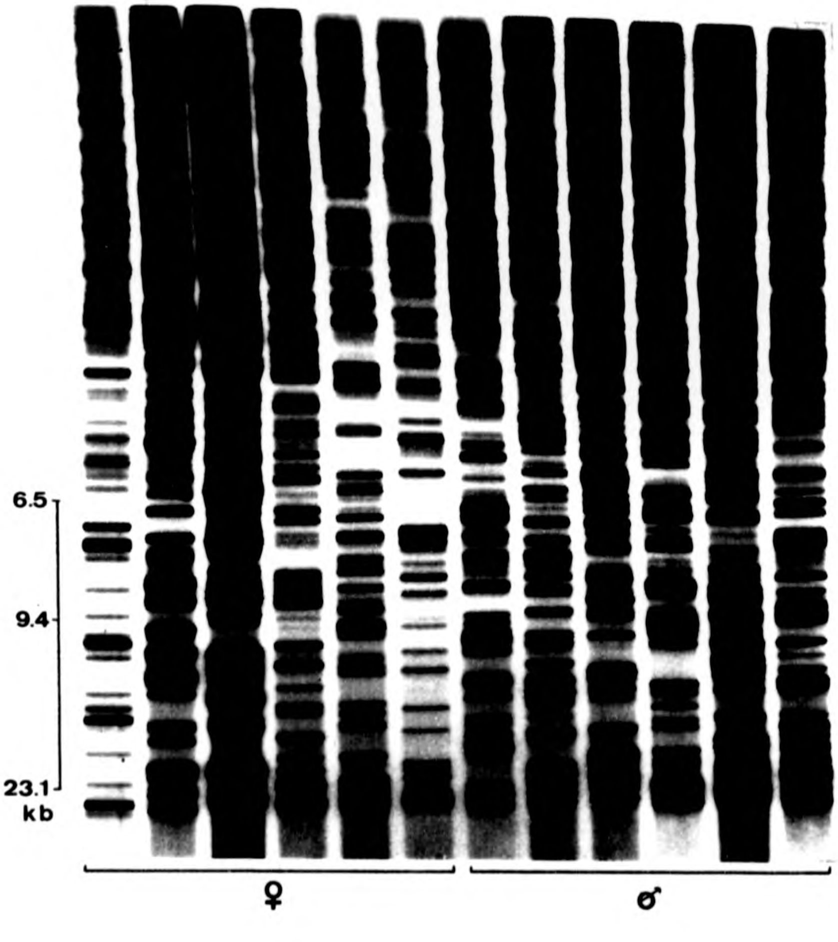

Figure 6.1 - DNA fingerprints of twelve randomly chosen adult sand martins (females n=6, males n=6). Size of fragments (in kilobases, kb) are indicated to the left, determined using the molecular weight marker lambda Hind III.

appearing in all individuals), nor sex-linked bands were identified. The males (n=6) had an average of 17.7 (± 2.58) and the females (n=6), 16.5 (± 2.34) bands.

The average band sharing coefficient (F) for 66 pair-wise comparisons of these 12 randomly chosen adults (Table 6.1) was 0.196 ± 0.090. Comparisons between males showed a statistically non-significant tendency for males to have a higher average F than females (Mann-Whitney, W=190.0, p=0.08).

The probability that two randomly chosen individuals had identical fingerprints was 5.0×10^{-15} (equation 4, section 6.2.2), which means that the probability of two unrelated sand martins having identical fingerprints is negligible. The mean population frequency of alleles at minisatellite loci, q (equation 5, section 6.2.2), was 0.111. As q may serve as a measure of homozygosity, this low value indicates that most of the minisatellites fragments scored are heterozygous. The probability of two siblings having identical fingerprints (equation 6, section 7.2.2) was 2.5×10^{-7}, which means that it is very unlikely that even closely related sand martins have identical fingerprints.

One case of 2 siblings having identical fingerprints was found. In theory, these 2 birds could have been identical twins, or a mistake may have been made in the field (one chick bled twice) or in the laboratory. Evidence for having twins would be: clutch size would be smaller than the brood size, because identical twins would come from the same egg (Romanoff and Romanoff 1949) and should have a lower mass than their siblings. However, the clutch size was 4 and the brood size was 4, and the mass of all nestlings was similar at least on the 13th day after hatching. To check if a mistake had been made in the laboratory another gel was run to establish whether one sample had been loaded twice. The same pattern occurred and so, DNA was re-extracted from the original blood samples, and again the same result was produced. Therefore, it is likely that two blood samples were taken from the same nestling in error.

Table 6.1 - Band sharing coefficients (F) for pair-wise comparisons of 12 randomly chosen adult sand martins (F values calculated by equation 2, section 7.2.2). Number 1 to 6 = females, number 7 to 12 = males.

	2	3	4	5	6	7	8	9	10	11	12
1	0.069	0.200	0.250	0.187	0.143	0.345	0.143	0.258	0.138	0.250	0.229
2		0	0.114	0.229	0.129	0.250	0.129	0.235	0.187	0.114	0.316
3			0	0.056	0.062	0.182	0.187	0.114	0.061	0.222	0.103
4				0.263	0.294	0.114	0.235	0.270	0.286	0.316	0.195
5					0.059	0.343	0.118	0.216	0.286	0.158	0.286
6						0.129	0.333	0.242	0.323	0.176	0.270
7							0.193	0.059	0.250	0.229	0.316
8								0.121	0.129	0.235	0.162
9									0.118	0.108	0.400
10										0.229	0.263
11											0.293

The DNA fingerprint patterns of sand martins and house martins are compared in Table 6.2. The values found for sand martins in the present study were similar to those found by Riley (1992).

6.3.2. Segregation analysis

Segregation analysis is usually done with large families, since in these families instances of linkage and allelism observed are likely to be real. Analysis of small families (six or fewer offspring) are likely to produce inflated estimates of linkage and allelism due to chance factors (Jeffreys and Morton 1987).

For a male raising two broods in a given season (Figure 6.2) a total of 26 bands was scored. Of these, 20 were specific to the male, 4 were shared with the female of the first brood and 3 with the female of the second. The respective band sharing coefficients (F) between the male and the first and second female were 0.216 and 0.162. Both values are close to the average for unrelated individuals (section 7.3.1). Female 2 had an usually low number of bands (11) compared to the average number for females, which was 17.7 (see section 6.3.3.1).

All offspring bands could be traced to one or other of the parents, except for one chick of the second brood (chick number 3), which was related to the female but not to the male. Thus band segregation between a male parent and 9 offspring was analyzed.

Linkage and allelism between bands were detected visually, derived from 91 pairs of bands comparisons. Two pairs of male bands segregated as linked (that is, all were absent, or all were present, in an offspring)(bands number 1 with 20, 5 with 12). This suggests that the male is heterozygous at almost all (93.7%) minisatellite loci. Only one apparent allelism was found (each offspring inherited either one or other of the bands, never both); it consisted

Table 6.2 - Comparison of DNA fingerprint patterns of unrelated adult sand martins (SM) and house martins (HM). F = band sharing coefficient, x = band sharing probability, q = allele frequency, pf = probability of identical unrelated adults, ps = probability of identical unrelated siblings. The probe used was 33.6, isolated by Jeffreys (1985).

Species	DNA fragments size (kb)	Number of fragments per individual (mean ± se)	F (mean ± se)	x (mean ± se)	q	pf	ps
SM (present study)	23.1 - 6.5	17.1 ± 0.76	0.196 ± 0.090	0.208 ± 0.100	0.110	$5.0\text{x}10^{-15}$	$2.5\text{x}10^{-7}$
SM (Riley, 1992)	25.0 - 4.3	18.4 ± 1.51	0.153 ± 0.044	0.154 ± 0.030	0.080	$2.2\text{x}10^{-16}$	$6.6\text{x}10^{-7}$
HM (Riley, 1992)	30.0 - 6.0	14.5 ± 1.63	0.157 ± 0.023	0.152 ± 0.016	0.079	$4.3\text{x}10^{-13}$	$1.5\text{x}10^{-6}$

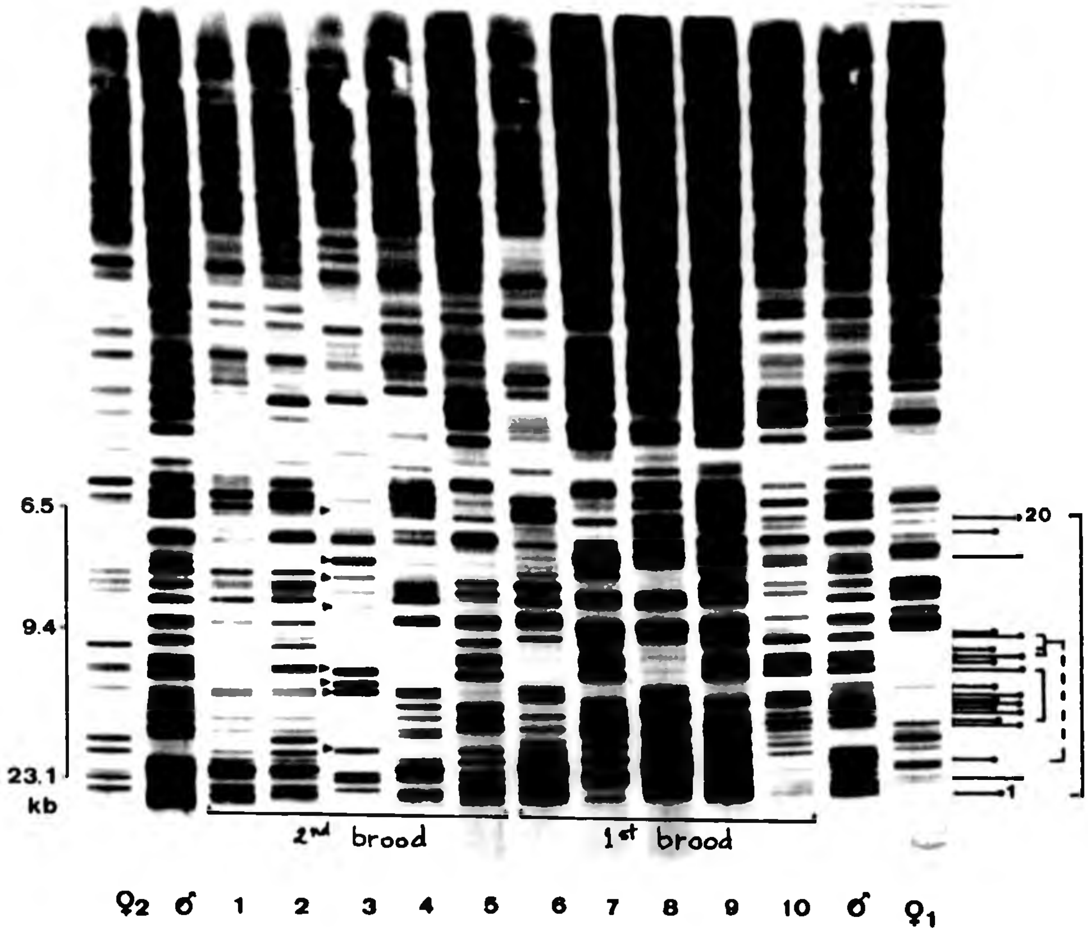

Figure 6.2 - DNA fingerprints for a male sand martin and his first and second broods; ——• male band, ---joins allelic bands, —— joins linkage bands, ▶ mismatching bands.

of a fragment (band number 3) apparently allelic to a group of 3 fragments linked in sequence (bands number 14, 15 and 16)(Figure 6.2).

For a female with two broods (4 and 5 offspring in the first and second brood respectively) a total of 18 bands was scored (DNA fingerprints not shown), of which 12 were specific to the female (not shared with the first and/or the second male). The band sharing coefficients (F) between the female and the first and second males were respectively 0.129 and 0.296.

All the bands in each offspring could be traced to one or other of the parents. Bands present in both parents of the two broods were excluded from the analysis. No female bands were present in all offspring, suggesting that the female was heterozygous at all minisatellite loci involved. Two bands segregated as linked and one pair of apparently allelic bands was detected (DNA fingerprint not shown).

The segregation of minisatellite fragments from the DNA fingerprints of the male and female with second broods is shown in Table 6.3, indicating that 75% (14 of 20 bands) of the male and 83.3% (10 of 12 bands) of the female scorable fragments represented distinct loci, and were therefore inherited independently.

The transmission frequency of hypervariable fragments passed to the offspring was compared with the expected number given by a binomial distribution. Fragments present in all offspring (just one case for the male), were not considered in this analysis, since they may derive from homozygous loci (Jeffreys *et al.* 1986). The pattern of transmission of male and female fragments was consistent with a binomial distribution and a probability of band inheritance of 0.5 (Table 6.4). Fourteen specific paternal bands were transmitted to an average of 51.6% of offspring, and 10 maternal fragments had a transmission frequency of 40%. These results suggest that sand martin minisatellite bands are inherited in a Mendelian fashion, and the parentage of offspring can be established from DNA

Table 6.3 - Segregation of minisatellite fragments from DNA fingerprints of a male and a female sand martin with double broods (mates changed for the second broods in both cases).

	Male	Female
Number of parent-specific bands scored	20	12
Number of homozygous loci	1	0
Number of allelic pairs (a)	1	1
Number of linked bands minus linkage groups (b)	4	1
Number of loci scored	15	10
Estimated total loci (N)	53	28

Table 6.4 - Transmission of DNA minisatellite fragments from a male and a female sand martin to double broods of nine offspring.

Number of offspring with band	Male bands		Female bands	
	observed	expected	observed	expected
0	0	0.003	0	0.002
1	0	0.246	1	0.176
2	3	0.984	2	0.703
3	0	2.296	2	1.640
4	3	3.445	1	2.461
5	3	3.445	3	2.461
6	4	2.296	1	1.640
7	0	0.984	0	0.703
8	1	0.246	0	0.176
9	0	0.003	0	0.002
	X^2	11.35		8.45
	df	9		9
	p	>0.05		>0.05

Grouping categories to give expected values greater than 5 failed to yield values of X^2 with significance $p<0.05$.

fingerprints.

The number of distinct loci scored (L) was estimated by subtracting the number of allelic pairs (a) and the number of linked bands minus linkage groups (b) from the total number of parent-specific bands scored. The estimated total number of hypervariable loci (equation 1, section 6.2.2) was 53 heterozygous loci or 106 fragments for the male, and 28 heterozygous loci or 56 fragments for the female (Table 6.3).

6.3.3. Parent-offspring relationships

6.3.3.1. General characteristics of the fingerprints.

The principal criterion for separating legitimate from illegitimate offspring was the number of mismatching bands. The distribution of mismatching bands is shown in Figure 6.3. This distribution was compared to a Poisson distribution, using a chi-square goodness-of-fit test to establish a cut-off point above which chicks should be considered illegitimate. The frequency of mismatching bands departed significantly from that expected under a Poisson distribution when 4 or more mismatching bands were included (Table 6.5). More than three mismatching bands was therefore the critical number to confirm illegitimate offspring, although three mismatching bands was found to lie on the boundary of significance ($p=0.053$).

A second criterion to categorize offspring as legitimate was the band sharing coefficient. The band sharing coefficients (F) for parent-offspring relationships of 46 sand martin families were analyzed, excluding 6 families that were experimentally manipulated (male removal experiments). The band sharing coefficient (F) between first degree relatives (parents and chicks), was significantly higher for males than for females (medians: male-offspring 0.591 and female-offspring 0.516, $n=135$, Mann-Whitney, W=

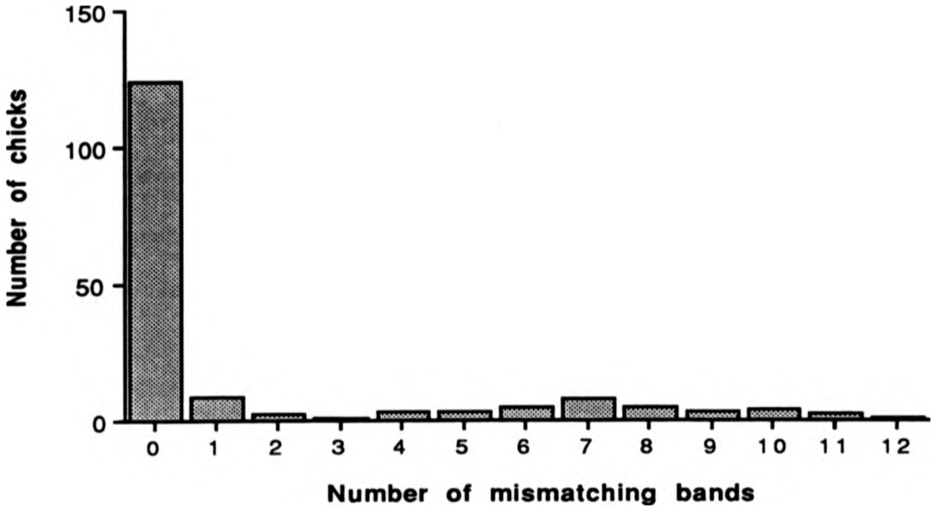

Figure 6.3 - Frequency of mismatching bands in sand martin chicks (including those with zero mismatches), n=170.

Table 6.5 - Observed frequency of mismatching bands in sand martin chicks, compared to that expected (E_2 to E_5) under a Poisson distribution. A chi-square goodness-of-fit test identifies significant differences between the numbers of observed and expected mismatching bands as a progressivelly greater number of mismatches are included (E_2, 0-2 mismatches; E_3, 0-3 mismatches; E_4, 0-4 mismatches; E_5, 0-5+ mismatches).

Number of mismatching bands	Obs.	E_2	E_3	E_4	E_5	X^2	df	p
0	124	123	120	114	35	-	-	-
1	9	12	14	23	55	-	-	-
2	2	1	1	2	44	1.76	2	0.414
3	1		0	0	23	5.87	2^{*1}	0.053
4	3			0	9	17.40	2^{*2}	<0.001
≥5	31				4	512.17	5	<0.001

Categories were combined to encourage expected values to approach or exceed 5: hence, $*^1$ categories 2 and 3 were combined and $*^2$ categories 2,3 and 4 were combined. Rounded values are given for E_2 to E_5, and were used for goodness of fit calculations.

20218, $p<0.001$). Thus (F) was plotted separately for each sex (Figures 6.4 and 6.5). The band-sharing distribution excluded birds with more than 3 mismatching bands. The confidence limits of the distributions are shown in Figure 6.6. Legitimate and illegitimate offspring, based on the criterion of mismatching bands are shown in association with the band sharing coefficient to the putative father and putative mother.

It is expected that 5% of the chicks would fall outside the 95% confidence limits by chance (8.5, $n=170$). In practice nine chicks were found outside the limits for unrelated and related individuals, more than expected for a single tail of the frequency distribution (Figure 6.6). The chicks positioned beyond the 95% confidence intervals for unrelated and related individuals were principally categorized as legitimate or illegitimate based on presenting zero, 1, 2 or 3 mismatching bands, or more than 3 mismatching bands respectively. Chicks with 3 mismatches, however, presented attribution problems since they differed significantly, at $p=0.053$, from that expected to occur at random (Table 6.5). Since expected frequencies were in one case less than 5 for the goodness of fit test, the derived significance level was considered unreliable. In such a case, the additional evidence of band sharing coefficients was considered. In practice only one chick had three mismatching bands; this chick was considered to be derived from an EPF, based on the low band sharing coefficient with the putative father ($F=0.18$), and high band sharing coefficient to the putative mother ($F=0.59$)(see Figure 6.6). This chick belonged to a brood which had another illegitimate chick, also derived from an EPF.

One illegitimate chick (with 6 mismatching bands) fell within the 95% confidence interval for band sharing coefficients for both relatives and non-relatives. It was thus unclear whether it was derived from an EPF or from IBP. This chick was categorized as being derived from an EPF for the following reason:

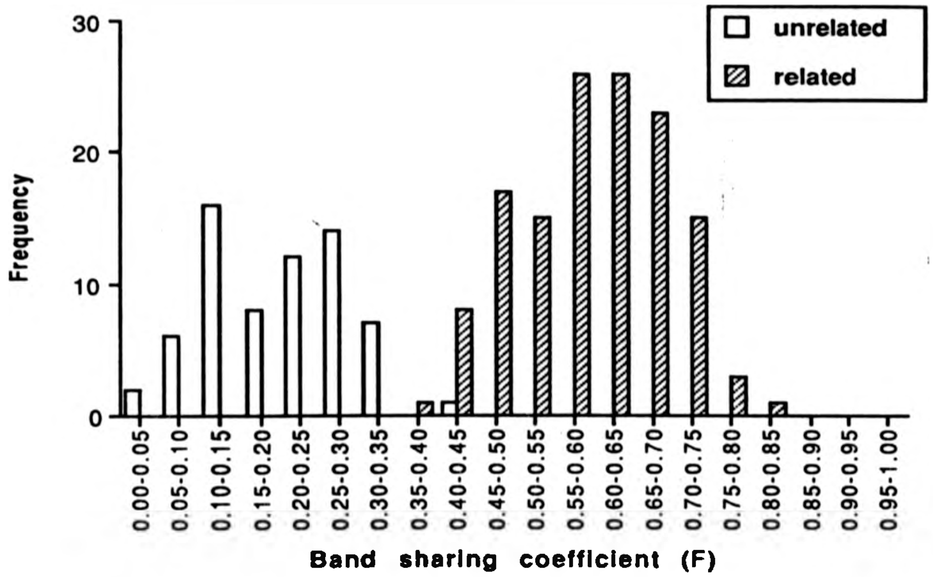

Figure 6.4 - Band sharing coefficients (F) for a group of 12 randomly chosen unrelated sand martin (6 males and 6 females) and for fathers with their offspring.

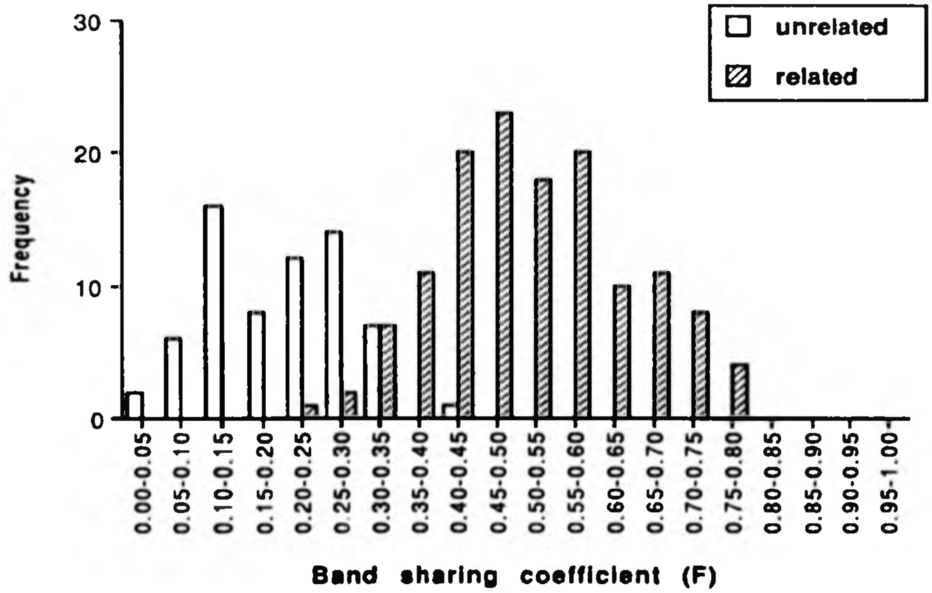

Figure 6.5 - Band sharing coefficients (F) for a group of 12 randomly chosen unrelated sand martin (6 males and 6 females) and for mothers with their offspring.

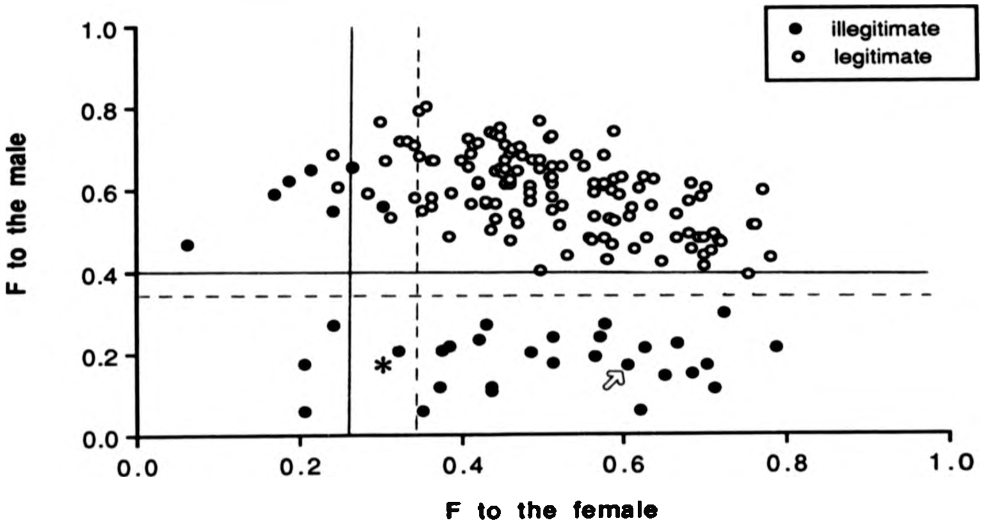

Figure 6.6 - Band sharing coefficients (F) of chicks with their putative father and putative mother. Chicks with 4 or more mismatching bands were categorized as illegitimate, while chicks with 0 to 3 mismatches were considered as related to their putative parents (except one case with 3 mismatches, see arrow). Dashed lines represent the upper 95% confidence interval for unrelated birds, while solid lines are the lower 95% confidence intervals for related birds. The attribution of the chick marked with * is discussed in the text (p 93).

The chick belonged to a brood of 3 (see ✱ on Figure 6.6), which also had a chick derived from an EPF, as well as a legitimate chick. The 2 illegitimate chicks had 6 and 7 mismatching bands respectively; many of which they shared (n=5, ≥70%). The band sharing coefficient between the 2 illegitimate chicks was F=0.61, indicating that they were full siblings with the same mother and father. Since one was certainly derived from an EPF, it follows that the individual difficult to attribute had the same parentage and was therefore categorized as an EPF. Further, but not in itself conclusive, the band sharing coefficients between the 2 illegitimate chicks and the legitimate chick were F=0.34 and F=0.21, values around those expected amongst half siblings.

The number of scorable bands was found to be significantly higher for males than for females (mean ± sd, males 22.1 ± 3.17 (n=46) and females 17.7 ± 2.65, t=7.13 (n=46), $p < 0.001$). One reason for males to have more bands than females might be due by the fact that the females are the heterogametic sex in birds. The sex chromosomes are termed W and Z, with the male being ZZ and the female WZ. The W chromosome carries fewer genes and is largely inert, giving rise to the possibility that the small number of minisatellite fragments observed on some sand martin fingerprints were carried on the sex chromosomes, as previously suggested to occur in house martins (Riley 1992).

The average number of scorable bands for chicks was 20.1 ± 3.79 (n=170). The mean number of bands (± standard error) shared between parents and first degree related chicks (in each case using a mean for one nest) was again significantly higher for males than for females with unsexed offspring (males 10.2±0.36, and females 7.4±0.37, t=5.49, $p<0.001$). Thus, compared to the distribution of F values between males and related chicks (Figure 6.4), the distribution of F values between females and related chicks was displaced to the left (Figure 6.5). A similar pattern has been found for house sparrows (J. Wetton pers. comm.).

Thirty four chicks had 4 or more mismatching bands and were considered to be unrelated to one or both parents (mean of 7.5±2.11 mismatches, range 4-12).

Single (n=9) or 2 (n=2) mismatching bands were found in DNA fingerprints of 11 offspring, possibly representing mutations. This observed rate of mutation in sand martins (13 in a total of 2761 offspring scored bands, that is 0.47%) was similar to the rate of 1 fragment in 300 (0.33%) found by Jeffreys (1985) for humans.

6.3.3.2. Frequency of extra-pair offspring.

The frequency and percentages of extra-pair offspring in nests and amongst chicks of sand matins are shown in Table 6.7. Extra-pair offspring derived from different parents were sometimes found in a same nest. Thus some single broods were found to have multiple paternity and maternity. The occurrence of EPF was most frequent, with QP and IBP much less common.

6.3.3.3. Attributes of males and females in relation to paternity.

The mean values of five body size variables of males and females in broods with at least one EPF are compared with broods with no EPO in Table 6.8. No significant difference was found for any of the parameters included. Therefore, there is no apparent relationship between body size parameters and the likelihood of the brood having EPFs.

No statistically significant difference was found between the age (estimated by ringing date) of the male or the female parent and the likelihood that the brood contained EPFs (Table 6.9).

Table 6.7 - Extra-pair offspring in sand martins determined by DNA fingerprinting. EPF = extra-pair fertilizations, QP = quasi-parasitism, IBP = intra-specific brood parasitism and Nil = no EPF, QP or IBP.

	Broods Total = 46	%	Offspring Total = 170	%
Nil	27	(58.7)	135	(79.4)
EPF only	12	(26.1)	19	(11.2)
EPF + QP	3	(6.5)	6	(3.5)
EPF + IBP	1	(2.2)	5	(2.9)
QP only	2	(4.3)	4	(2.3)
IBP only	1	(2.2)	1	(0.6)
Totals				
At least one EPF	16	(34.7)	25	(14.7)
At least one QP	5	(10.9)	7	(4.1)
At least one IBP	2	(4.3)	3	(1.8)

Table 6.8 - Average body size ± sd of adult sand martins in relation to the occurrence of at least one extra-pair fertilization (EPF) in their broods. All the measurements are in mm, except mass (g).

Sex	Body size measure	EPF	n	NO EPO	n	t	p
Male	keel	18.05 ± 0.682	16	18.18 ± 0.625	22	-0.61	0.54
	wing	105.75 ± 4.450	16	107.86 ± 2.620	22	-0.89	0.38
	tarsus	11.22 ± 0.695	13	10.90 ± 0.401	22	1.50	0.15
	head and bill	25.28 ± 0.443	16	25.18 ± 0.435	22	0.70	0.49
	mass	12.75 ± 0.835	15	12.67 ± 0.814	22	0.29	0.78
Female	keel	18.14 ± 0.741	15	18.00 ± 0.707	19	0.57	0.57
	wing	104.73 ± 2.630	15	105.78 ± 3.920	18	-0.91	0.37
	tarsus	11.07 ± 0.474	14	10.92 ± 0.265	19	1.07	0.30
	head and bill	25.10 ± 0.364	14	25.14 ± 0.438	19	-0.32	0.75
	mass	13.19 ± 1.070	16	13.47 ± 0.777	19	-0.89	0.38

Table 6.9 - Age of adult male and female sand martins in relation to the occurrence of at least one extra-pair fertilization (EPF) in their broods.

Sex	Age	EPF	NO EPO	X^2	p
Male	1	13	19		
	≥2	3	3	0.001	0.981
Female	1	14	16		
	≥2	2	3	0.043	0.835

Age estimated by ringing date. Birds categorized under age '1' are likely to include a few individuals >1 year old, whereas those under '≥2' are certainly 2 or more years old.

6.3.3.4. Factors related to paternity

a) Colony size. The estimated number of occupied burrows for three years at Barbush colony is shown in Table 6.10. No significant difference was found in the frequency of fingerprinted nests which had EPFs (Table 6.11). The difference in the frequency of EPFs was greatest when comparing 1991 and 1992. This may have been due the fact that all the birds bled in 1992 came from a relatively small subcolony (25 occupied nests in the season), whereas most of the birds in 1991 came from a large subcolony (approximately 500 occupied nests during the season). EPFs could be expected to be more frequent in larger than in small subcolonies, due to a large number of birds breeding together in close proximity, increasing the chances of interactions with other conspecifics.

b) Occurrence of second broods. Because birds often changed mates and/or subcolonies when relaying after a failed first brood attempt, or when attempting second broods, it was not certain if late season broods were genuine second broods, first breeding attempts or late arriving birds. None of the 4 males or 5 females that was known to attempt a second brood stayed with their first brood partner. Accordingly, two categories were defined: 1) 'genuine' second broods - cases which the birds had been caught rearing a first brood, and 2) 'probable' second broods, i.e. those that were laid during July and August (see Chapter 3). All the cases of genuine second broods also fell in the category of 'probable' second broods. The frequency of nests with EPFs was higher for males with 'genuine' second broods (Table 6.12). However, no significant difference ($p>0.05$) was found for females in first compared with 'genuine' second broods. No significant differences in nests with EPFs and no EPO were found in the 'probable' category between first and second broods (Table 6.13).

Table 6.10 - Estimated number of occupied burrows and adult sand martins in the seasons of 1990, 1991 and 1992 at Barbush colony.

Year	Occupied burrows	Adult birds
1990	770	1540
1991	413	826
1992	516	1032

Table 6.11 - Frequency of nests with at least one extra-pair fertilization (EPF) in different years at Barbush colony.

	1990	1991	1992
EPF	6	8	2
NO EPF	9	14	13

Comparisons	X^2	p
1990 x 1991	0.015	0.903
1990 x 1992	1.534	0.215
1991 x 1992	1.373	0.241

Table 6.12 - Frequency of extra-pair fertilizations (EPFs) for males and females in broods with 'genuine' second broods, compared to broods without extra-pair offspring (EPO).

Sex	Brood	EPFs	NO EPO	Fisher exact test
Male	first	0	5	
	second	3	1	0.048
Female	first	0	5	
	second	2	2	0.167

Table 6.13 - Frequency of extra-pair fertilizations (EPFs) in first and second sand martin broods categorized by date (first egg laid in May-June = first, July and August = second).

Date	Brood	EPF	NO EPO	X^2	P
May/ June	first	7	17		
July/ August	second	9	8	1.47	0.225

c) Clutch size and brood size. No significant difference was found in clutch size or brood size between nests which had at least one EPF compared with broods with no EPO (Table 6.14). The same was found for broods with QP and IBP compared with no EPO (Table 6.14). A regression of brood size (on the 13th day after hatching) on laying date (date of the first egg laid) was negative and significant ($F=4.42$, $p<0.05$, $n=83$). The proportion of the variation in brood size explained by date of laying was only 4.0% (as measured by r^2)(Figure 6.7). Considering the 16 broods with at least one EPF (closed circles in Figure 6.7), there was no significant difference in the proportion above and below the line (8 points above and 8 below). Therefore brood sizes for nests with at least one EPF did not differ significantly from those expected from their dates of laying. This result suggests that the smaller broods of nests with EPF were explained by the tendency of EPF to occur later in the breeding season.

d) Time of breeding. It might be expected that nests of pairs laying early in the season were less likely to have an EPF, since they have fewer extra-pair males available to interfere with the laying females. The observed pattern was consistent with this expectation since the percentage of nests with EPFs (using a nest as a unit) was positively correlated with date (Spearman correlation $r_s=0.93$, $p<0.01$) (Figure 6.8).

6.3.4. Copulations

Sand martin copulation attempts were observed 5 times in 1990, once in the air and 4 times on the ground. The individuals involved were unmarked (except one). Each attempt lasted approximately 30s, although they were not seen clearly enough to be sure cloacal contact had been made.

Table 6.14 - Average clutch size and brood size (± standard deviation) of sand martins in nests with extra-pair fertilizations (EPF), and quasi-parasitism (QP) plus intraspecific brood parasitism (IBP), compared to nests without EPO.

	EPF	NO EPO	t	p
Clutch size	4.6 (±0.83)	4.7 (±0.78)		
n	15	21	-0.42	0.68
Brood size	3.4 (±1.20)	3.9 (±1.08)		
n	16	22	-1.29	0.21

	QP + IBP	NO EPO	t	p
Clutch size	4.4 (±0.79)	4.7 (±0.78)		
n	7	21	0.83	0.42
Brood size	3.4 (±0.98)	3.9 (±1.08)	1.00	0.34
n	7	22		

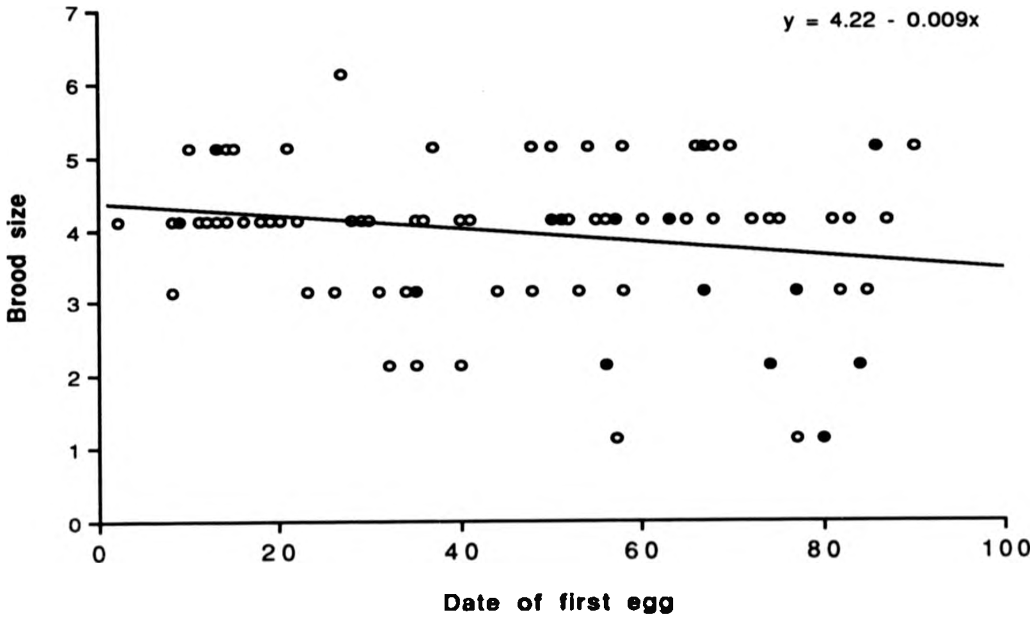

Figure 6.7 - Brood size of sand martins in relation to the date of the first egg ($F=4.42$, $p<0.05$, $r^2=0.04$, $n=83$). Black points represent broods with at least one extra-pair fertilization (EPF). Date of the first egg given in days (day 1 = 1st May).

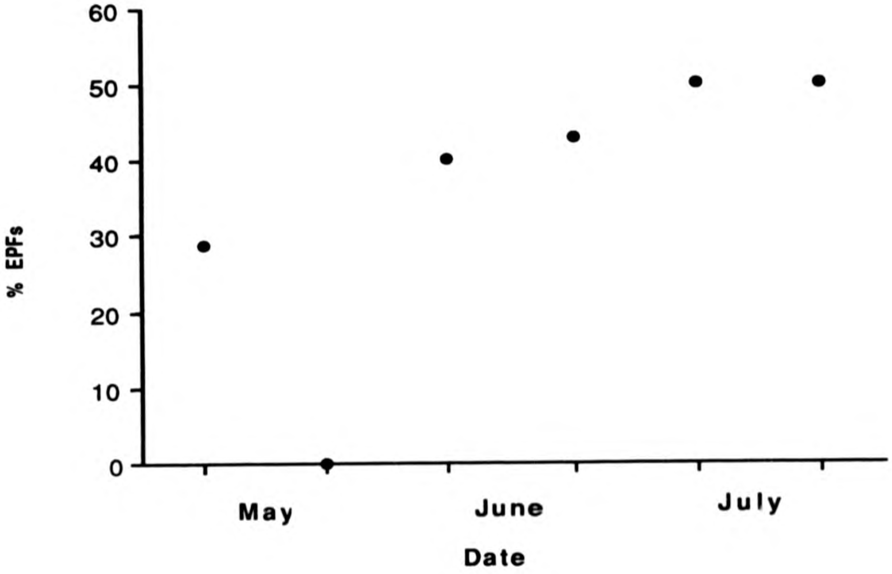

Figure 6.8 - Proportion of extra-pair fertilizations (EPF) in sand martin broods in relation to the date of the first egg laid. The time interval used to derive the proportion of EPFs was two weeks (Spearman correlation $r_s=0.93$, $p<0.01$, $n=6$).

On 21st June (09.30h), a marked male came out of its nest burrow apparently with its unmarked mate. Both came to the ground and the male mounted the unmarked bird. Immediately following, another unmarked adult bird joined the pair. The marked male fought the second unmarked bird on the ground for approximately 30s, and then returned to its nest burrow (it was not noted whether the mounted bird returned to the nest burrow).

On 5th May (17.00h) a copulation attempt was seen to take place on the ground, approximately 30m from a subcolony; both birds were unmarked, and took off together after mounting. On 11th July (10:00h) another attempt was observed, also taking place on the ground, about 20m from a subcolony where one of the birds (unmarked) had landed previously. Another unmarked bird came from behind and attempted to mount. The first bird took off avoiding the second.

On the 12th July (11.30h), an attempted mating took place in the air near a nest burrow entrance. One unmarked bird mounted another, pecking at its nape. The participants subsequently flew away together, followed by two other unmarked birds.

On the 6th July, a nestling close to fledging fell from its burrow entrance onto the ground in front of the subcolony. Two unmarked birds were subsequently seen approaching the nestling and mounting it for less than 30s.

6.3.5. Mate-guarding

Mate-guarding usually started a few days before the first egg was laid in a given nest. For Barbush as a whole the percentage of nests with birds mate-guarding tracked the percentage of nests where laying had started (Figure 6.9).

The percentage of time that females were guarded at nests (n=10) which were watched for 6h on one day during the pre-laying and laying period, reached a peak (100%) 3 days

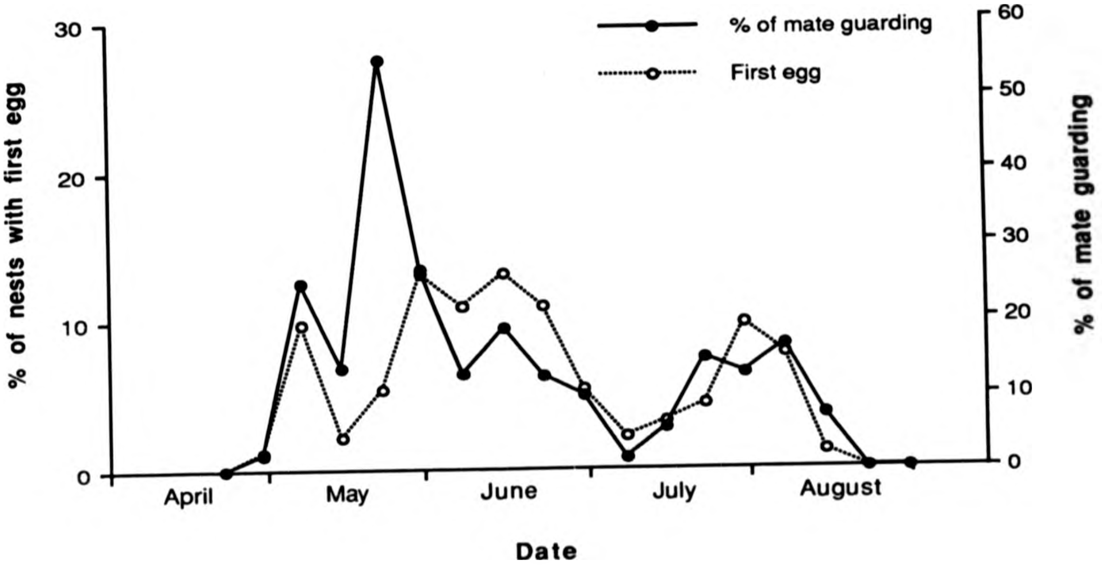

Figure 6.9 - Brood size of sand martins in relation to the date of the first egg ($F=4.42$, $p<0.05$, $r^2=0.04$, $n=83$). Black points represent broods with at least one extra-pair fertilization (EPF). Date of the first egg given in days (day 1 = 1st May).

before the first egg, and decreased after the first egg was laid (Figure 6.10). The percentage of time the male was together with the female became very low on the 1st day of incubation (the day after the last egg), and also in the incubation period (a mean value for day 4-12 of incubation is shown), when members of a pair used to alternate time spent in the nest.

There was no significant difference in time spent in the nest per hour at different times of the day in the pre-laying stage for males (Kruskal-Wallis, H=3.70, df=2, p=0.158, n=10), or females (H=4.13, df=2, p=0.127, n=10). Also the number of nest visits did not differ significantly for different times of the day amongst males (H=0.27, df=2, p=0.874, n=10) or females (H=1.20, df=2, p=0.548, n=10). The time spent in the nest by males and females differed significantly with time of day during the laying stage (Kruskal-Wallis range test, males H=11.06, df=2, p<0.01, n=12; females H=17.23, df=2, p<0.001, n=12). A non parametric range test for males showed that they spent significantly more time in the nest early in the morning (7-9h) than in both the late afternoon (4-6h) (Q=12.67, df=2, p<0.001), and late morning (10-12h) (Q=5.12, df=2, p<0.001). Males also spent more time in the nest late in the morning than late in the afternoon (Q=7.56, df=2, p<0.001). Females spent more time in their nests early in the morning compared with the late afternoon (Q=4.15, df=2, p<0.001) and the late morning(Q=2.42, df=2, p<0.02), but there was no significant difference between late morning and late afternoon (Q=1.72, df=2, ns)($Q_{0.05,3}$=2.39).

6.3.6. Male removal experiments

Male removal experiments were carried out on 16 nests during the pre-laying (3 days before the first egg was laid, n=1) and laying periods (day of the second egg, n=15). Only 6 nests produced chicks that survived long enough to be

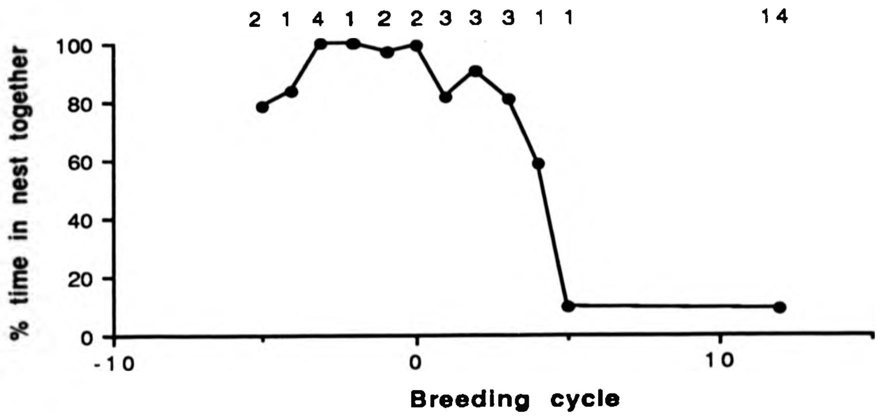

Figure 6.10 - Percentage of time female sand martins were accompanied by the pair male in the nest, at Barbush in 1991. Day of the first egg=0. Nests in pre-laying, laying (-4 to +4 day), and first day of incubation (+5 day) stages, were observed for 6h each (6h/day). Nests in incubation stage (+12 day) were observed for 8h each (2h/day). The numbers above the curve shows number of nests included in each sample. The total hours of observation was 250 (pre-laying=60h, laying=78h and incubation=112h).

blood sampled; and chicks fledged from 5 of these nests. Of the others, 6 were deserted on the day of male removal, 3 on the next day, and one after the chicks had hatched.

a) Behaviour of females during and after removal of their mates

Excluding females that did not come out of the nests when the males were caught, seven females took 3-103min to come back to their nests after release. For the control nests where males were not removed, the females returned in 5-21min (n=5). After the males were released, their mates took a range of 1-27min (n=6) to come back to their nests, while the range for the female controls was 2-14min (n=4). Twelve of the laying females at nests where the male was removed laid an egg on the day of removal; one did not lay the second egg in her nest, and the other 3 nests were not checked on the day of male removal and were deserted on the same day or by the next day.

The time spent in the nest per hour, while the males were held, did not differ significantly between females which had their mates removed and control females; nor did the number of visits to the nests per hour differ between experimental and control nests (Table 6.15).

b) Male behaviour after release

A total of 10 males was seen returning to the nest within one hour of release, in an average of 15.8±11.74min (range of 1-39min). Two other males were seen around the nest 46 and 54min, respectively, after release, but did not enter.

c) Intrusions

Although females were often chased back to their nest by extra-pair birds while their mates were removed, the

Table 6.15 - Behaviour of sand martin females at nests where males were removed in comparison to nests with males present (based on 2h observations at each nest).

	Male removed	Male Not removed	W	p
Median time (min) in nest per hour	20.75	31.75	124	0.141
Median number of visits per hour	1.50	2.00	130	0.254
Number of nests	12	12		

W = Mann-Whitney test

chasers usually stayed at the entrance of the nest burrow. Intruders were seen entering male removal nests in only four cases, when the pair-males were removed for 5 hours. No intruders were seen to enter control nests. The difference between male removal nests and control nests in terms of number of intruders seen entering was not significant at the 5% level (Fisher's exact test, p=0.07)(Table 6.16). There was, however, a significant difference in intruders coming to nests where the males were removed for 5 hours and nests where the males were removed for 2-3 hours (Fisher's exact test, p=0.02)(Table 6.17). This suggests that only prolonged absence by males led to an increased rate of intrusions.

Chasers could sometimes be identified; they were always males, some of which had their own nests, while some were failed breeders. In two cases, marked intruders, which had lost their mates were found in the nests with the females by the pair-male after release. In both cases fights occurred; in one case the owner of the nest displaced the intruder; but in the other nest the owner was displaced by the intruder. In the first case the nest was deserted first by the female and then later by the male; in the second case both birds deserted apparently on the same day, while the intruder stayed some days in the nest, and was later seen singing at the entrance of the burrow.

d) Effects of male removal on paternity

The frequency of extra-pair fertilizations in male removal nests and controls is given in Table 6.18. The frequency of EPFs was higher in male removal nests than controls, but the difference was not significant (Fisher's exact test, p=0.175) (Table 6.19). The comparisons were made using the number of nests with at least one EPF, rather than the total number of chicks fathered by an extra-pair male, since one EPF could in theory result in several chicks. A similar result arose, however, when the percentages of chicks in broods due to EPFs were compared, instead of the

Table 6.16 - Frequency of intruders (I) at nests with the male experimentally removed and control nests.

	I	No I	p
Experimental	4	12	
Control	0	14	0.07

Fisher's exact test, one tailed.

Table 6.17 - Frequency of intruders (I) at nests with the male removed for 2-3 hours and 5 hours.

	I	No I	p
5 hours	4	3	
2-3 hours	0	9	0.02

Fisher's exact test, one tailed.

Table 6.18 - Extra-pair paternity determined by DNA fingerprinting for offspring and nests at which the male was removed experimentally and control nests. EPF = nests with at least one extra-pair fertilization.

	Experimental				Control			
	Nests	(%)	Offspring	(%)	Nests	(%)	Offspring	(%)
EPF	4	(66.7)	9	(39.1)	1	(20.0)	1	(5.0)
Faithful	2	(33.3)	12	(52.2)	4	(80.0)	19	(95.0)
Totals	6		21		5		20	

Table 6.19 - The occurrence of extra-pair paternity (EPFs) in nests where the male was experimentally removed compared with nests where the male was not removed (control).

	EPFs	Control	p
Experimental	4	2	
Control	1	4	0.175

Fisher's exact test, one tailed.

number of nests, between experimental and control groups (Mann-Whitney, W=44.5, p=0.144).

6.3.7. Behavioural observations and paternity

Behavioural observations during incubation and chick rearing periods were compared between nests which had at least one EPF and nests with no EPO; in terms of time spent incubating (and number of visits made) and rate of feeding chicks (and time spent in nest). The provisioning rate to offspring was regarded as a measure of parental effort. It could be expected that males which were cuckolded would invest less in the offspring. The question addressed here was: is the rate of food delivery to the brood by the male influenced by his paternity, as determined by DNA fingerprinting?

No significant difference in total time spent incubating (observations made not less than a week after the experiment) per hour by individuals with EPF and with no EPO was found for either sex (Table 6.20). However, females at nests with EPFs spent significantly more time incubating than their respective mates. Comparing male-removal nests with controls, no difference in total incubating time was found for either sex, but females of the experimental nests spent significantly more time incubating than their mates (Table 6.21).

The number of visits made to nests per hour during the incubation stage was higher for males at nests with EPFs compared with males at nests without EPO, but did not differ for females (Table 6.22). Males at nests with EPFs made significantly more nest visits per hour than their mates, although there was no difference between the sexes at nests without EPO (Table 6.22). There was no difference in incubation nest-visit rates of either sex between male-removal and control nests, nor between males and females of male removal or control nests (Table 6.23).

Table 6.20 - Time spent incubating by male and female sand martins at nests with at least one extra-pair fertilization (EPF) and no extra-pair offspring (EPO). Ranges given in brackets.

Median time(min/h)

	Males	Females	W	p	n
EPF	20.50	32.62	119.00	0.004	9
	(17.37-35.50)	(28.62-41.00)			
No EPO	23.87	29.12	161.00	0.025	11
	(13.25-33.62)	(23.75-46.75)			
W	90.00	103.50			
p	0.761	0.518			

W = Mann-Whitney test

Table 6.21 - Time spent incubating per hour by male and female sand martins at nests with male removed (experimental) and not removed (control). Ranges given in brackets.

Median of time (min/h)

	Males	Females	W	p	n
Experimental	22.50	33.12	40.00	0.012	5
	(11.75-25.25)	(27.75-35.25)			
Control	23.87	29.12	34.00	0.210	5
	(16.62-33.62)	(23.75-41.00)			
W	23.50	29.00			
p	0.465	0.837			

W = Mann-Whitney test

Table 6.22 - Number of visits per hour made by incubating male and female sand martins at nests with at least one extra-pair fertilization (EPF) and nests without extra-pair offspring (EPO). Ranges given in brackets.

Median visits (min/h)

	Males	Females	W	p	n
EPF	3.12 (1.87-4.37)	2.37 (1.50-3.12)	58.50	0.019	9
No EPO	2.25 (0.75-3.37)	1.75 (0.50-3.00)	106.00	0.187	11
W	123.00	110.50			
p	0.033	0.237			

W = Mann-Whitney test

Table 6.23 - Number of nest visits per hour made by incubating male and female sand martins at nests with male removed and controls. Ranges given in brackets.

Median feeding visits (min/h)

	Males	Females	W	p	n
Experimental	2.87 (1.25-7.00)	2.75 (1.25-5.50)	26.00	0.833	5
Control	2.25 (1.62-3.12)	1.87 (1.62-3.00)	24.50	0.600	5
W	33.00	32.00			
p	0.296	0.403			

W = Mann-Whitney test

There was no difference in the median time per hour spent in the nest during the chick-rearing period by males and females at nests with at least one EPF, and nests with no EPO (Table 6.24). Both males and females at nests with at least one EPF tended to spend more time in the nest per hour, but the difference was only significant for females. No difference was found in the time spent in the nest at the rearing stage between experimental and control nests for either sex (Table 6.25).

As far as feeding visits were concerned, no significant difference was found in the number per hour made by individuals from nests with EPF and no EPO for either sex (Table 6.26). The number of visits per hour made by males and females at nests with male removal and controls also did not differ significantly (Table 6.27). These results suggest that males either cannot detect cuckoldry, or if they do, they do not adjust their level of parental care.

6.4. DISCUSSION

6.4.1. Extra-pair paternity and maternity in sand martins

DNA fingerprinting indicted that 20.6% of offspring in the present study were not related to one or both of their putative parents. A total of 14.7% of nestlings was unrelated to the attendant male, a similar rate to that found for house martins (15%, Riley 1992) and house sparrows (13.6%, Wetton and Parkin 1991). There was evidence of quasi-parasitism (4.1% of offspring) and IBP (1.8% of offspring). The rates of QP and IBP were not consistent with observations at sand martin nests during the egg laying period, when apparently no more than one egg per day was observed in a clutch. However, abnormal laying sequences were observed in some nests during laying or when the clutch was apparently complete. These laying patterns could not be completely disentangled from effects of variable weather

Table 6.24 - Time spent in the nest during the chick rearing stage by male and female sand martins at nests with at least one extra-pair fertilization (EPF) and no extra-pair offspring (EPO). Ranges given in brackets.

	Median time (min/h)				
	Males	Females	W	p	n
EPF	8.13 **(1.50-20.37)**	9.06 **(1.87-19.87)**	158.50	0.665	12
No EPO	3.06 **(0.75-19.12)**	4.56 **(0.37-9.75)**	265.00	0.985	16
W	209.50	218.50			
p	0.104	0.041			

W = Mann-Whitney test

Table 6.25 - Time spent in the nest during the chick rearing stage by male and female sand martins at nests with male experimentally removed and at control nests. Ranges given in brackets.

	Median time (min/h)				
	Males	Females	W	p	n
Experimental	2.49 **(1.00-16.00)**	8.49 **(3.00-13.75)**	21.00	0.470	4
Control	3.18 **(2.25-9.75)**	4.62 **(1.00-7.62)**	19.00	0.885	4
W	16.00	20.00			
p	0.665	0.665			

W = Mann-Whitney test

Table 2.26 - Feeding visits per hour by male and female sand martins in nests with at least one extra-pair fertilization (EPF) and no extra-pair offspring (EPO). Ranges given in brackets.

Median feeding visits (min/h)

	Males	Females	W	p	n
EPF	8.94 **(4.50-13.87)**	8.37 **(3.75-16.00)**	150.00	1.000	12
No EPO	9.00 **(3.83-13.25)**	7.94 **(0.25-14.37)**	239.50	0.365	16
W	160.00	191.00			
p	0.530	0.443			

W = Mann-Whitney test

Table 6.27 - Feeding visits per hour by males and females at nests with male removed and at control nests. Ranges given in brackets.

Median feeding visits (min/h)

	Males	Females	W	p	n
Experimental	10.93 **(6.25-16.50)**	6.56 **(5.12-15.87)**	15.00	0.468	4
Control	8.68 **(6.62-9.00)**	7.87 **(3.75-12.37)**	16.00	0.665	4
W	18.00	17.00			
p	1.000	0.885			

W = Mann-Whitney test

conditions, although the latter was considered the more important factor (see Chapter 5).

Hoogland and Sherman (1976) found no evidence of IBP in bank swallows through observations of nests. Riley (1992) also did not find evidence of IBP in house martins or sand martins, as determined by DNA fingerprinting. In the last case, this may have been due to a small sample size for sand martins (5 nests). Beecher and Beecher (1979) found that male bank swallows appeared to pursue a MRS routinely and actively, a pattern which seems consistent with the sand martins in the present study. It is possible, however, that extra-pair males could have been non-paired males, which pursued an itinerant or promiscuous reproductive behaviour. For example, some unpaired marked males (n=5) were seen chasing mated females. Behavioural observations were also consistent with the occurrence of a MRS in sand martins, however, since some marked males (n=6) were seen chasing other females when they were fertile, at the same time they were attending their own mates. At the present time, therefore, combined genetic and behavioural evidence for a MRS in sand martins is lacking, although its occurrence appears to be likely.

Although there was no evidence that more than one egg per day was added to a sand martin nest, two broods had an increased number of eggs (1-2) 2 days after the clutch was apparently completed. As shown in Chapter 5, sand martins accepted only 41.5% of artificial eggs added to nests at the pre-laying stage, but accepted almost all of them during laying. Thus it is possible that parasitic females had laid eggs in 'laying' nests, or nests where the clutch had just been completed. If they dump eggs during laying, they probably removed one egg of the host. A few eggs were indeed found fresh on the ground beneath the colonies, but it was not possible to detect from which nest they came (except in one case, where the nest was apparently predated). These eggs may alternatively have been ejected, possibly because they were damaged by parents or interlopers. Parasitic females could be those which had lost their nests or partner, or which had been fertilized by the male owner of the parasitic nest (quasi-parasitism).

Quasi-parasitism is quoted as rare (Petrie and Møller 1991). Evidence of QP was found for zebra finches (Birkhead *et al.* 1990), white-fronted bee-eaters (Emlen and Wrege 1986), blue-fronted bee eaters (Stader, pers. comm.) and swallows (Bryant pers. comm.). There is no obvious reason why female sand martins should show a relatively high incidence of quasi-parasitism. It may be possible that males seek extra-pair copulations mainly with females from accessible and hence neighbouring nests and that this association, or simply chance, leads the female to choose their nests as the most appropriate in which to dump her eggs.

Both EPFs and IBP resulted in multiple parentage, but the first was much more prevalent. The EPF rate was very similar to that found in house martins (Riley, 1992), although no IBP or QP was detected in house martins. The same author found higher rates of EPF for 5 sand martin nests (18% of offspring) possibly due the fact that larger colonies were sampled. Although EPF rates were similar in both species, the greater variety of behaviours (EPF, IBP, and quasi-parasitism) found in sand martins compared with house martins may be explained by the fact that sand martins generally live in larger colonies with nests in closer proximity, at least in the UK. In zebra finches, DNA fingerprinting also revealed EPFs, IBP and QP (one case), but IBP was more frequent than EPF (Birkhead, *et al.* 1990).

6.4.2. Variability and validity of DNA fingerprinting in sand martins.

The band sharing coefficient between supposedly unrelated birds was comparable to the values found for house martins, and within the range usual for other species (Riley

1992). Unrelated individuals had very variable fingerprints, and the probability of two individual sand martins (even siblings of a same brood) having two identical fingerprints was very small.

Some of the problems that might arise in the interpretation of parentage studies include: insufficiently large sample sizes, incorrect storage and handling of blood samples and misinterpretation of electrophoretic results (Romagnano *et al.* 1989). Another source of error can be misidentification of the putative parent (Birkhead and Møller, 1992). Attempts were made to minimize these possible errors in the present study. A large number of families was sampled (n=52), samples were kept in individually marked Eppendorf tubes, using a different box for each family, and kept in ice in the field before storing at -20° or -70°C. Putative parents were usually individually marked early in nest cycle and their behaviour recorded to establish attendance at a particular nest. In cases where the behaviour was not observed earlier in the nesting cycle, all birds were watched for some days in advance (≥2d) and their blood samples collected during the time when they were feeding nestlings regularly. Of the 52 families bled, 40 (77%) had both attendant parents marked, 6 (11.5%) had one member of the pair marked and 6 (11.5%) had neither member of the pair marked. Birds were invariably bled when they could be accurately sexed in sand martins; that means during incubation and rearing, using brood patch development as the criterion (see Chapter 2). This is a simple criterion for sexing sand martins, and can be considered a negligible source of error in this study. This view is supported by the fact that a concurrent study of sand martin body size at the same colony (Bryant and Jones, unpubl.), involving capture, ringing and sexing of birds (>100/annun) found no discrepancies with this present study amongst recaptured individuals.

Sand martin DNA fingerprints were found to be sufficiently variable for the technique to be used with confidence to investigate parent-offspring relationships. Riley (1992) found that 75% of scorable DNA fragments segregated independently in house martins. In the present study, 75% of the fragments for a single male and 83.3% for a female were found to segregate independently. Since no sand martin pairs were observed to stay together and raise two broods, the segregation analysis was done separately for each sex, so double broods with one common parent were used as for red deer (Amos *et al.* 1992).

6.4.3. Correlates of extra-pair paternity

No relationship was detected between paternity and body size or age of both sexes, colony size, clutch and brood size. Males tended to be cuckolded more frequently in second broods than in first broods. Weatherhead and McRae (1990) suggested that EPC/EPFs in american robins are more likely in second broods, because males cannot guard their mates closely when rearing chicks. This might apply to sand martins, since the female starts to lay a second clutch just before the previous brood has fledged. In some cases in this study (at least 3), the female sand martin left the first brood a few days before the chicks fledged, leaving the male to finish rearing the brood. Similarly, Cowley (1983) found that female sand martins re-mated while their first brood young were still dependent, leaving them in the care of the males, and occasionally visiting and feeding the first brood young. This suggests a tendency for sequential polyandry in sand martins. Sequential polyandry was also found in the common redpoll, with a female observed feeding almost fully grown nestlings at one nest while building a second nest 200m away while being followed closely by another male (Seutin *et al.* 1991). However, in that case it was unknown whether or not the second male fathered the second clutch in part or in whole.

By re-mating before the first brood fledges, the female can start a second brood earlier than with her original mate and perhaps increase her annual reproductive success. On the other hand, males can increase their annual reproductive success by attempting EPCs while not guarding their own mates (i.e. during incubation and nest rearing).

Annual reproductive success can increase with age, seeming to peak in the second year of life for house martins (Bryant 1989) and in purple martins where older males achieved 96% paternity in their nests at the expense of younger males (Morton *et al.* 1990). Experienced adult sand martins began nesting earlier in the season than first time breeders, were more likely to attempt a second brood (Cowley 1983), and consequently had a higher reproductive success (Jones 1987b). In the present study the effect of age on breeding success of sand martins could not be detected due to the small sample size, and the fact that most of the parent birds were marked for the first time as breeding adults and were therefore of unknown age.

Since it is rare to observe copulations in sand martins and the period for which females can store sperm after copulation has not been determined, some observed EPF may therefore be a result of rapid mate-switching rather than EPC (Birkhead and Møller 1992); possibly involving fertilization with sperm stored from matings with previous mates. Males of the sequentially polyandrous spotted sandpiper, for instance, pairing early in the season can cuckold their females' subsequent mates through stored sperm (Oring, *et al.* 1992). The EPF rate was higher for males in second broods so this is also a possibility for sand martins.

6.4.4. Male removal experiments

The frequency of EPF was not significantly higher (at 5%) in nests which had the male removed compared to control nests. While the sample size was too small to be conclusive, the results suggested that although mate-guarding seems to help to prevent EPFs it is not fully effective. The number of intruders tended to be higher in nests where the male had been removed compared to the controls; and was significantly higher in experimental nests where the males were held for longer periods (5h compared to 2-3h). This is also evidence that mate-guarding may function as a paternity defence. Considering nests from which the male was removed, in 4 out of 5 cases EPFs were found (male removed on the second day of laying). In only one case, where the male was removed 3 days before the first egg was laid, was no EPF detected.

Non-pair males chase female sand martins when they are forming/laying eggs, apparently using the females laboured flight as a proximate cue (Jones 1986b). As shown in this study, mate-guarding by male sand martins matched the first egg distribution through the breeding season at a whole colony. Individually, mate-guarding became very intensive from 3 days before the first egg until the second egg was laid. During this period the female is invariably followed by her mate when entering and leaving of the nest. Since intruders were rarely seen entering nests when the pair male was present, it is possible that EPCs may take place when the pair is away from the nest. For practical reasons the birds could not be followed away from their nests. Attempts were made to radio-track a pre-laying pair to check if they stayed together all the time when away from the nest. The attempts failed, however (see Chapter 3).

The behaviour of fertile females during male removal experiments varies between different species of bird. In pied flycatchers, after removing males 1-3 days before laying, the rate at which other males visited the territory increased as did the frequency of EPCs compared to controls (Bjorklund and Westman, 1983). Amongst tree swallows, male removal on the day of the first egg showed that females actively selected or rejected copulations with extra-pair males, suggesting that females have control over sperm competition in this species (Lifjeld and Robertson 1992). In swallows, colonially breeding females experienced more extra-pair copulations while their mates where removed, but solitary breeding females did not (Møller 1991a). Male removal in dunnocks three days after the first egg showed no decrease in paternity of removed males in monogamous or polyandrous systems (Davies *et al.* 1992). Also there was an increased probability that an intruder would enter a nest when the pair male was absent in house martins (Riley 1992).

The sample size of nests with male removal was small for sand martins, and no significant difference was found in the frequency of EPFs between experimental and control nests. Nevertheless, the results suggest that mate-guarding may be important in reducing the risk of EPFs, although other factors may be involved, such as female choice.

Female choice may be widespread in birds, often occurring in a subtle manner, through a variety of behaviourial and physiological mechanisms, including sperm competition in the female reproductive tract (Gomendio and Roldan 1993, Birkhead and Møller 1993b).

6.4.5. Mate-guarding and MRS

Males probably face a trade-off between the need to guard the nest against conspecific brood parasites and nest material robbers, and the need to guard their mates when fertile. House martins, for instance, unlike sand martins, do not attempt to guard their mates continuously during the fertile period (Riley 1992); suggesting that the risk of EPCs to females is higher in sand martins than in house martins. The present study, however, showed a very similar rate of EPFs in both species. The fact that sand martins do not guard the nest as much as house martins could explain why no IBP or QP was detected in house martins.

In house martins the slacking of mate-guarding by some males earlier in the laying period of their female mates may have resulted in them being more likely to be cuckolded

(Riley, 1992). The percentage of time that male sand martins spent following the female between days -5 and +4 of the breeding cycle (0 = day of the first egg) ranged from 59-100%, remaining at around 100% between days -3 and 0 (Figure 6.11). Thus male sand martins seem to slacken mate-guarding only slightly after the day of the first egg, but are still present in the nest with the female for a high proportion of the total time she spends in there. The time together in nest decreased to approximately 60% on the last egg day, reaching a very low value (10%) on the first day of incubation (Figure 6.11). After the day of the first egg, sand martin males were observed entering and leaving the nest many times while the female was still inside. This behaviour may be related to inspection of the colony seeking opportunities to copulate with extra-pair females.

Male sand martins were found to follow their mates very intensively over a similar period as bank swallows (Beecher and Beecher 1979). Male and female sand martins were found to spend more time in the nest early in the morning in the pre-laying and laying stages, which was also the time of the day that most of the sexual chases were observed by Jones (1985).

In the bank swallow, which is usually considered to raise just one brood per season (Beyer 1938), mate-guarding begins 3-5 days before the first egg is laid and ends by the day the fourth egg is laid (although it is more intense 3 days before the first egg)(Beecher and Beecher 1979). Also, there is no apparent synchrony in the nesting cycle of the whole colony, so fertile females are available to males throughout an extended period of the breeding season. That is, within the colony as a whole there is little synchrony, although small groups in a big colony show intra-group synchronization (Emlen and Demong 1975, Beecher and Beecher 1979). A similar pattern was observed for sand martins in the present study. Thus, it is theoretically possible for a mated male of this species to seek promiscuous copulations with females who have just paired or are about to lay

(Beecher and Beecher 1979).

6.4.6. Sperm competition

In birds copulation frequency normally peaks a few days before the first egg is laid and then drops markedly. Despite this, females remain fertile until the penultimate egg is laid (Birkhead and Møller 1992, Sturkie 1976). The differences in temporal patterns of copulation with respect to the first day of laying among different birds are consistent with the hypotheses that copulations are less efficient once egg laying starts, and with the sperm competition hypothesis (Birkhead and Møller 1993a).

The sperm competition hypothesis proposes that temporal patterns of copulation differ between mating systems, according to the intensity of sperm competition. It means that if sperm competition is minimal (i.e. lekking species) copulations cease soon after the onset of laying; when it is intensive (i.e. in species with polyandry), males continue copulation with their mates through the egg-laying period until the last egg is fertilized. In monogamous birds, however, factors other than, or in addition to, sperm competition must be influencing the variation in temporal copulation patterns (Birkhead and Møller 1992). In sand martins it is unknown when males stop copulating with their partners.

Indications of sperm competition were found in sand martins: EPF accounted for 14.7% of the offspring. EPF in wild bird species has been found to be in a range of 0-54.8%, including Hirundinidae (2-34.6%)(Birkhead and Møller 1992). Birkhead *et al.* (1987) found that EPCs tended to be more frequent among non-guarding species (including many colonial birds) than in guarding birds. Sand martins show a relatively high frequency of EPF compared to other species, despite male mate-guarding by close following.

When copulations are temporally separated, the last male to inseminate a female before ovulation (usually 24 h before the egg is laid) fertilizes the majority of the eggs (Birkhead and Møller, 1992). Females may therefore choose the paternity of offspring using last male sperm precedence (Birkhead and Møller 1992). The benefits of choosing paternity for females could be to seek 'good' genes or increase genetic diversity, although there may be costs involved such as transmission of diseases. Two different predictions apply for female choice (Westneat *et al.* 1990). Females could refuse to copulate with their partners and copulate with a preferred male; in this case the extra-pair male could fertilize the majority of the eggs or all the eggs. A prediction for genetic diversity is that females should copulate with different males. By selectively copulating throughout the laying period, a female could in theory have each egg fertilized by a different male. But even in this case, an alternative explanation could be that females seek 'good' genes from different males (Birkhead and Møller, 1992). It is likely that in some sand martin nests EPFs could have come from more than one extra-pair male, as revealed by DNA fingerprinting. It was not possible using multilocus DNA fingerprinting to know which sand martin individuals increased their reproductive success by EPFs (males), or QP or IBP (females). Thus success of males and females in parasitizing parental care of conspecifics could not be compared. The identification of the parents of extra-pair offspring (to compare reproductive success with apparent reproductive success) has been shown to be most successfully achieved by using locus-specific minisatellite probes for DNA fingerprinting (Bruford *et al.* 1992, Burke 1989, Burke *et al.* 1991).

Sand martin males did not apparently adjust their parental effort according to their level of paternity. Even in nests where males were temporarily removed (i.e. mate-guarding was interrupted). Thus, male sand martins cannot detect cuckoldry or if they do, they do not adjust their level of parental care. Probably because males cannot discriminate between their own and extra-pair offspring, and reduced feeding would incapacitate both their own as well as cuckolded young.

The next Chapter discusses the effect of cuckoldry on parental care and mating systems, thereby placing sand martins in a general context.

CHAPTER 7 - GENERAL DISCUSSION

7.1. Coloniality: costs and benefits

Colonial breeding occurs in only 13% of bird species, being most common among marine birds and relatively rare among passerines (Lack 1968), with strict coloniality occurring only within the Hirundinidae and Corvidae (Shields *et al*. 1988). Factors proposed to be the most important in the formation of colonial breeding are: a) abundant food outside the limits of the nesting territory; b) enhanced defence against predators; c) shortage of suitable nesting sites. Factors that might play a secondary role, maintaining coloniality once it is established, include intraspecific relationships between individuals (Siegel-Causey and Kharitonov 1990).

Shields (1988) concluded that coloniality in the swallow family evolved as a response to nest site shortages. This conclusion was based on the traditional aggregation hypothesis, that hirundines re-use nest cavities or nest sites within and across seasons, particularly when they have bred there successfully before. According to Shields' (1988) hypothesis, the size of breeding groups, and therefore the level of coloniality in hirundines, is determined by the availability of breeding sites and the type of nests. So, species like sand martins, which dig their own nest burrows, are less limited by types of breeding sites than, for example, hole nesters (i.e. tree swallows and purple martins) or open-cup mud nesters (i.e. swalows). The size of sand martin breeding groups can be larger at traditional sites, even when suitable but non-traditional sites remain unused nearby. Thus, hirundines seem to be reluctant to use new sites when traditional sites are available.

Siegel-Causey and Kharitov (1990) concluded that the chief causal factor in the development of colonial breeding in seabirds is the presence of unpredictable or abundant food resources located outside the limits of the nesting habitat. Other factors maintaining coloniality include features such as breeding synchronization and social facilitation.

Coloniality in birds is, however, a multi-faceted phenomenon, with some hypotheses more likely to provide an explanation in some groups than in others, and no single hypothesis likely to provide a general explanation (Wittenberger and Hunt 1985). It is also possible that the factors primarily responsible for the establishment of coloniality are now subordinated to those involved in the maintainance of coloniality. Many studies have identified common features of coloniality, and proposed explanations for its occurrence, although very few have tested hypotheses to explain the evolution of coloniality in birds, such as those done by Siegel-Causey and Kharitonov (1990). Therefore, information for determining which hypotheses are most likely to apply for each species is essential to understanding avian coloniality, such as basic studies on costs and benefits of coloniality.

A detailed consideration of the costs and benefits of coloniality, as was done for bank swallows by Hoogland and Sherman (1976), was outside of the scope of the present study. Instead, some potential costs and benefits of coloniality for sand martins were considered.

7.1.1. Sand martin colonies as information centres

Ward and Zahavi (1973) suggested that roosting and breeding assemblies of birds serve as information centres, and that these assemblies (or colonies) have evolved primarily for the efficient exploitation of unevenly distributed resources. That is, individuals would benefit by following successful foragers to profitable feeding areas (Bayer 1982, Brown 1986).

Enhanced foraging efficiency by means of information centres (Ward and Zahavi 1973) has been shown to be a benefit of coloniality for cliff swallows (Brown 1988). Emlen and Demong (1975) proposed that social foraging increased the feeding efficiency of bank swallows, although this was not supported by Stutchbury's (1988) observations on colonies of this species. Hoogland and Sherman (1976) found that coloniality had seemingly not evolved in the context of social facilitation in bank swallows. In the present study, while a preliminary investigation using radio-tracking produced no evidence to support the idea that sand martin colonies act as information centres (Chapter 2), it did indicate that radio-tracking of sand martins could be used to investigate this hypothesis in more detail.

7.1.2. Decreased risk of predation

Colonies can attract predators, since for them they represent localized concentrations of food, but the question of whether predation pressure per individual member of a colony is increased or decreased by coloniality remains (Wittenberger and Hunt 1985). Collective defense against predation can be conferred by 'swamping' (Clark and Robertson 1979), by 'selfish herd' effects (Hamilton 1971), alarm calls, mobbing, and vigilance. 'Swamping', however, only confers protection above some critical colony size (Wittenberger and Hunt 1985).

Protection against predation as a fundamental factor of coloniality is supported by the observation that predator mobbing and vigilance effects appear important for colonial species which show no apparent transfer of information about food (Clark an Robertson 1979). For example, yellow warblers nesting synchronously with their own and neighbouring species resulted in a decrease in the rate of nest predation, suggesting a 'swamping effect' on the local predators (i.e., a vast production of young at the same time results that predators can eat a few of). The fact that this species breeds in synchrony with conspecifics, as well as the proximity of red-winged blackbirds, resulted in a lower incidence of brood parasitism by brown-headed cowbirds; suggesting that selection for defense against predators and nest parasites may lead to multi-species aggregations (Clark and Robertson 1979).

Recruitment of neighbours for mobbing predators is more successful at high local densities, so that the effectiveness of predator deterrence increases with colony size. Results of the present study suggested that decreased predation risk played a important role in the maintenance of coloniality in sand martins, supported by an apparent increase in the effectiveness of mobbing and alarm calls with increased subcolony size (Chapter 4). Mobbing has been demonstrated to be effective against predators in bank swallows, and decreased risk of predation was found to play a major role in the maintenance of coloniality in this species (Hoogland and Sherman 1976). It is likely, however, that mobbing benefits have a non-linear pattern, and that studies with larger groups would be worthwhile to determine the colony size above which no further benefit accrue.

7.1.3. Increased transmission of ectoparasites

Alexander (1974) suggested that increased levels of infestation by ectoparasites could be a disadvantage of colonial living. This study suggests that colony size may have an effect on the degree ectoparasites negatively affect breeding success (Chapter 4). All swallow species might suffer costs when breeding at high densities, either as a result of nest ectoparasites, or through increased social competition (Shields *et al.* 1988).

Barn swallow pairs with infected first nests built a new nest for the second clutch more often than pairs with uninfected first nests (Møller 1990). Sand martins tended to change mates and nest sites for second broods. It is possible that avoidance of ectoparasites may be partly responsible for this behaviour; as well as, or instead of mate choice (see Chapter 6).

Hamilton and Zuk (1982) suggested that animals choose mates with genetically determined resistance to disease by examining characters whose full expression is dependent on health and vigour. This hypothesis could not be tested in the present study, but it would be interesting for future studies. For example, DNA fingerprinting could be used to determine whether males from nests treated with a pesticide to kill ectoparasites, were able to increase their reproductive success through extra-pair fertilizations to a greater extent than males with ectoparasites (untreated nests).

If ectoparasites tend to negatively affect larger colonies more than smaller colonies, and predation rate is lower in bigger than smaller colonies, it implies a possible trade-off resulting from breeding in small and large colonies. It seemed to be the case for sand martins (Chapter 4). Another negative factor of breeding in big colonies is competition for food, thus affecting nestling growth, such as shown for house martins (Bryant 1979). However, advantages and disadvantages of breeding in different colony sizes, such as mixed-reproductive strategies, should also be considered, and may contribute to determination of the optimum colony size.

7.1.4. Coloniality and extra-pair offspring

Are colonial species more prone to EPFs and therefore to misdirecting their parental care? Although EPCs are more frequent in colonial than in solitary species, no difference in the percentage of extra-pair offspring was found between these two categories (Birkhead and Møller 1992). It may be that cross species comparisons are confounded by factors such as variation in intensity of mate-guarding or female responses to EPCs attempts, obscuring the trends between coloniality and EPFs (Riley 1992). Some colonial species show high rates of EPFs, such as hirundine species (Riley 1992, present study) and shags (Graves *et al.* 1992); while others present no EPFs or IBP, such as the northern fulmar (Hunter *et al.* 1992). In the last case, frequent copulations by the pair male is apparently a way to assure his paternity.

Within a individual species, however, greater density of breeding individuals may lead to a higher incidence of extra-pair paternity, as found for red-winged blackbirds (Gibbs *et al.* 1990). In sand martins, no difference in the rate of EPFs was found between 1991 and 1992 in this study, although the number of breeding pairs at Barbush colony differed during that period. This colony was, however, still very large and there was no comparison between distinct colonies.

7.1.5. Likelihood of misdirected parental care

The main focus of the present study was the potentially increased likelihood of individuals misdirecting their parental care due to EPFs, QP and IBP, suggested as a reproductive cost in large colonies of bank swallows by Hoogland and Sherman (1976). In fact these facets of a so called mixed-reproductive strategy (Trivers 1972) can be viewed as both a benefit for individuals which gain offspring in extra-pair nests; as well as a cost for individuals which are cuckolded and/or parasitized. That means that some individuals can increase their reproductive output at the expense of others.

Morton *et al.* (1990) suggested that colonial breeding in purple martins evolved as a means of increasing the opportunities for EPFs by older males. Increased chance for EPFs, however, is more likely to be a consequence of coloniality; if individuals live to adulthood, the costs and benefits for individual males should balance out with age, with individuals being cuckolded when young and gaining EPFs when older.

The sand martin population studied here exhibited a considerable proportion of extra-pair offspring, mainly due to EPFs. Rates of EPFs in sand martins are comparable to other hirundine species (Table 7.1). Individuals which pursue a MRS can increase reproductive success without the cost of increased parental effort. Since it is mainly males that suffer genetic costs of MRS in sand martins, and males help to incubate and feed the brood, the question of the effects of paternity on male parental care arises.

7.2. Paternity and parental care

In most animal species a male's reproductive success is limited by the number of matings with different females achieved, while females are limited by the number of eggs produced and young to be fed (Trivers 1972). Therefore, female reproductive success is not limited by access to males but only to resources, such as food or nest sites (Davies 1992b).

This asymmetry between sexes may force a trade-off between providing parental care and trying to find additional mates (Trivers 1972). If male assistance is needed for rearing offspring, males themselves may become an important resource to enable females to raise any young at all, or to increase their reproductive success (Emlen and Oring 1977, see Davies 1992b). Monogamous males may invest heavily in parental care (Breitwisk 1989), and most also adopt a MRS (Chapter 6). Polygynous males have also been shown to pursue a MRS, as for example in red-winged blackbirds, which increased their reproductive success by gaining EPFs in other nests, reaching a rate of 24-28% (Gibbs *et al.* 1990, Westneat 1993).

In american robins males provided most of the parental care for fledglings from previous nests, after their mates had initiated second clutches. Despite reduced opportunities to mate-guard females during the pre-laying period of the second clutch, which might increase the risk of cuckoldry, males did not appear to feed second brood nestlings at a lower rate (Weatherhead and McRae 1990).

It would be expected that parents would invest more in their own offspring than unrelated offspring (Trivers 1972). However, investigation of the variation of parental care with paternity shows that males of monogamous pairs, such as indigo buntings (Westneat 1988), house martins (Riley 1992), tree swallows (Lifjeld *et al.* 1993, Whittingham *et al.* 1993), sand martins (present study), do not necessarily decrease their parental effort in response to reduced paternity in their own nests.

Morton *et al.* 1990 working on purple martins found that young males had a higher rate of cuckoldry than older males, and they suggested that younger males decrease their feeding rates in response to a low level of paternity.
Alternatively, younger males may be less efficient at both ensuring paternity and rearing nestlings than older males (Riley, 1992).

Male swallows, apparently, reduced their parental care in response to an induced increase in the rate of EPCs by experimental male removals (Møller 1988). The interpretations of this experiment were, however, confounded by increased nestling mortality leading to brood reductions in experimental nests (Wright 1992); and the fact that detailed genetic relationships between individuals were not confirmed.

Temporary removal of male sand martins for 2-5h during the pre-laying or early laying period did not seem to affect male parental effort, as measured by rate of brood provisioning (Chapter 6). In monogamous pairs of dunnocks, reduction in paternity due to removal of pair males during the fertile period did not decrease their rate of feeding nestlings (Davies 1992b, Davies and Hatchwell 1992). In polyandrous mating arrangements, however, male dunnocks delivered food to offspring in proportion to their mating access to a laying female, which also corresponded to paternity as determined by DNA fingerprinting (i.e., males with less mating access delivered less food to the offspring and also had a lower share of paternity)(Davies 1992b). Dunnocks are apparently able to monitor their likely paternity, and they can afford to reduce parental effort in polyandrous systems, where another male can take up the slack. The same is not possible for monogamous pairs, because parent male birds of any species are apparently unable to discriminate between their own and extra-pair offspring before fledgling, and a reduction in parental care will affect the brood as a whole. In species where parents feed young at the nest, it is advantageous for chicks to 'conceal' relatedness until fledgling (Beecher 1988).

So far there is no direct evidence that monogamous species reduce their level of parental care in response to their confidence of paternity. Whittingham *et al.* (1992, 1993) suggested, that in species studied so far, paternity has not been low enough to reach the threshold at which the costs of male parental care exceeds the benefits gained from recruiting a few related young, as was found for tree swallows. Captive monogamous tree swallow males allowed to 'see' their mates engaging in EPCs in pre-laying and laying stages did not reduce their parental effort over a wide range of paternity, although the male's access to his fertile mate was reduced by up to 50%. However, if their paternity was zero male tree swallows were reported to commit infanticide (Whitingham *et al.* 1993). Males that had no access to a female during the fertile period sometimes did not feed offspring at all (Burke *et al.* 1989, Davies *et al.* 1992). Indigo buntings, occasionally polygynous, and known to have been cuckolded through genetic analysis, tended to feed young less often than uncuckolded males (Westneat 1988). It is not clear, however, whether reduced parental effort is an evolutionary result of selection caused by increased rates of cuckoldry or the frequent opportunities for extra-pair matings or both (Westneat and Sherman 1993).

7.3. Extra-pair paternity (EPF) in six species of hirundines

Occurrence of EPF, QP and IBP and intraspecific comparisons of levels of extra-pair paternity of 6 hirundines are shown in Table 7.1. These species are similar in morphology and foraging ecology, and nest colonially or semi-colonially (although swallows sometimes breed singly), apart from the tree swallow which is described as territorial (Robertson and Gibbs 1982). They are predominantly monogamous breeders, although male tree swallows, and to some extent purple martins and swallows, may breed polygynously (see Turner and Rose 1989). EPFs are expected to occur at a higher rate in colonial species breeding in close proximity (Birkhead and Møller 1992). Sand martins, however, which form some of the largest colonies, apparently have lower rates of EPFs than purple martins which breed in smaller colonies and monogamous tree swallows which are territorial. Riley (1992) also found no indication that EPFs increased with colony size between different hirundine species. No apparent intraspecific variation in the incidence of EPFs in sand martins was related to subcolony size (Chapter 6). Cliff swallows, which form the largest colonies had the lowest rate of EPFs, but a high incidence of IBP compared to the other species. Brown and Brown (1989) suggested that IBP in cliff swallows was a risk spreading strategy. However, a different population of the same species presented a much lower level of intraspecific-brood parasitism, suggesting that IBP may be dependent on local environmental, demographic or geographical variations (Smith *et al.* 1993).

Mate-guarding may be effective in paternity defence, as has been suggested for house martins and sand martins

(Chapter 6). Unlike the other hirundines in Table 7.1, male cliff swallows and male tree swallows do not guard their mates before or during the laying period (Leffelaar and Robertson 1984). The lack of mate-guarding in tree swallows was supposed to be due to a low risk of cuckoldry and a need for territorial defence by both members of a pair, since there is intense competition for nest sites (Leffelaar and Robertson 1984). However, EPCs were found to occur at a high rate in tree swallows (Table 7.1). In cliff swallows, males may be unable to mate-guard because the nest has to be defended continuously by at least one member of a pair against conspecific brood parasitism (Brown and Brown 1989). Purple martin males mate-guard with varying intensity (Morton *et al.* 1990) and also show a high incidence of EPFs. Therefore, although mate-guarding may help to protect paternity in some species, such as sand martins and house martins (Chapter 6), it is not the only factor influencing the occurrence of EPFs.

In sand martins, females attempting second broods were found to show a higher rate of EPFs (Chapter 6). The divorce rate (from first to second broods) was 100% for known individuals. This contrasts with house martins, where members of a pair usually stayed together for the second broods, laying in the same nest with only 10% of pairs splitting after first broods (Bryant 1988b). Faithful double-brooded house martin pairs seemed less likely to be cuckolded (in first or second broods) than single brooded pairs, or pairs that switched partners for a second brood (Riley 1992). Swallow pairs also normally stay together for second broods, but no published information on the incidence of EPFs in second broods is available. In sand martins the rate of EPFs increased through the season, suggesting that males who arrived and presumably began breeding earlier might have had more opportunities to gain EPFs compared to late arriving males. Female sand martins which arrived earlier may also have benefited in terms of reproductive success by having the opportunity to mate with better

Table 7.1 - Percentage occurrence of extra-pair fertilization (EPF) and intraspecific brood parasitism (IBP), presence/absence of extra-pair fertilization (EPF), quasi-parasitism (QP), intraspecific brood parasitism (IBP) based on genetic parentage and details of the breeding ecology of six hirundine species.

Species	% EPF Broods	% EPF Offspring	N	EPF	QP	IBP	Method	Mate guarding	Second Broods	% Divorce	% Male feeding	Reference
Sand martin C, M	35(4)	15(2)	46, 170	+	+	+	D	Yes	Yes	100	47.2	This study
	40	18	5, 17	+	0	0	D	"	"	-	-	Riley (1992)
House martin C, M	38	15	21, 72	+	0	0	D	Yes	Yes	10	53.9	Riley (1992)
Barn swallows SC,M	7	26	464, 2320	-	-	-	H	Yes	Yes	-	46.7$^{\$}$	Møller (1989d)
	-	22(-)		+	0	+	D	-	-	-	-	Wellbourn unpubl.
	17		128, -	+	+	+	D	-	-	-	-	D.Bryant (pers. comm.)
	45	22	11,45	+	-	-	D	Yes	-	-	-	Smith *et al.* (1991)

Percentage IBP given in brackets in columns 2 and 3. A dash (-) means no data available, + and 0 means presence and absence, N = number of broods and number of offspring respectively. C = colonial, SC = semicolonial (facultative colonial), T = territorial. Method: D = DNA fingerprinting, A = allozyme, E= electrophoretic analysis of egg albumin, H = heritability. Divorce refers to % of observed pairs which did not stay together from first to second broods.

$ = from Møller and Birkhead, 1993.

Continuation Table 7.1

Species	% EPF Broods	% EPF Offspring	N	EPF	QP	IBP	Method	Mate guarding	Second Broods	% Divorce	% male feeding	Reference
Cliff swallow	6(43)	2(-)	105, 349	+	0	+	A	No	No	-	-	Brown and Brown (1988c)
	-(4)	-	54, -	-	-	+	E	-	-	-	-	Smith *et al.* (1993)
Tree swallow T, M	50	38	18, 86	+	0	D	D	No	No	-	47.6$^{\$}$	Lifjeld *et al.*(1993)
P	100	77	7, 44	+	0	0	D	"	"	-	-	Dunn and Robertson (1993)
Purple martin C, M	54(-)	35(-)	13, 52	+	0	+	D	Yes	No	-	45.6$^{\$}$	Morton *et* al. (1990)

quality males (Jones 1987a) and more time to attempt a second brood. In house martins and purple martins older birds are the first to arrive at the colony and also lay earliest (Bryant 1979, Morton *et al.* 1990). In sand martins older birds were also found to return to their nest sites before the first-years and choose burrows high on the face of the sand bank (Sieber 1980). It seems likely that early sand martin arrivals are older birds, and are more successful at fathering young in their own nests and other nests, but future investigation of age and realized reproductive success will be necessary to confirm this.

Females could also have increased their reproductive output through IBP and QP. It is possible that females having poor quality nests or poor quality mates had accepted EPCs from extra-pair males benefiting from that.

Thus, a MRS depends on various factors such as competition and access for mates, mate-guarding, nest guarding, female choice and sperm competition, and therefore has implications for mating systems. It also can depend on local, demographical or geographical variation, even within species.

Although there is variation on the proportion of extra-pair paternity within the six species of hirundines presented in Table 7.1, there was not much variation in the proportion of parental effort due to males (i.e. male feeding rate), which was generally around 50%. There are, however, no available data for the cliff swallow, which remains to be reviewed. These comparative data for hirundine species, therefore, are not consistent with the trend for parental care to decrease with an increase of the frequency of extra-pair paternity in cross-species comparisons that was found by Møller and Birkhead (1993).

7.4. Mating systems in wild birds

Bird mating systems are thought to be related to the spatial distribution of individuals, which in turn are assumed to be determined by the spatial and temporal distribution of resources (Emlen and Oring 1977, Davies 1992b). Where males provide parental care, they represent a resource for the females and influence female dispersion. Comparative studies and male removal experiments show that mating systems also vary in relation to costs and benefits of desertion by either sex (Davies 1992b). It is not necessarily true that the sex investing more would be less likely to desert. It may depend on the opportunities for males and females to gain further mates (Trivers 1985).

Monogamy was suggested to be the predominant avian mating system, comprising 93% of the bird species (Lack 1968). The reason was thought to be that if both parents raised a brood together they would leave more descendants on average. However, under conditions of decreased demand for parental care, males can allocate time and energy towards gaining additional mates or EPCs as well as raising their own young (Carey 1990). The predominance of monogamy in many birds may therefore arise not because each sex has greatest success with monogamy but because of limited opportunities for polygamy (Davies 1992b).

In cases where both sexes share the duties of rearing chicks, females can increase their provisioning rate in the absence of their mates. Male removal experiments indicate that in some cases females cannot compensate males' absence in raising chicks, and male help can increase reproductive success, although it is not always essential (Wright and Cuthill 1989, Hatchwell and Davies 1992ab, Davies 1992b). The influence of male parental care may depend on the period for which the male was removed. If males are removed in the period immediately after hatching, it may significantly affect female breeding success in species that hatch heterothermic young, as for instance was suggested for house wrens by Johnson *et al.* (1993).

Male removal experiments in tree swallows (Dunn and Hannon 1992) indicated that male parental care had little effect on reproductive success or survival of females or their offspring. Cases of polygyny (due to greater food abundance during laying), allowed females mated to polygynous males to lay more eggs and produce more fledglings than females mated to monogamous males. DNA fingerprinting results indicated a higher incidence of cuckoldry in polygynous tree swallows.

The sex most prone to desert a current breeding attempt to start a new one depends on the opportunity to gain further mates (Trivers 1972). If young can be raised by one of the partners, one of the pair can attempt desertion. Usually it is the male which deserts, since they can potentially fertilize eggs at a faster rate than the female can lay (Oring 1982 in Davies 1992b). Female sand martins, however, tended to desert before laying when disturbed in the nest; or sometimes when chicks of the first broods were nearly fully grown, attempting second broods with different males (Chapter 6).

In sand martins desertion of first broods by partners with fully grown chicks was observed 4 times in the present study, of which females deserted three times. The nest which the male deserted had 3 chicks subsequently fed only by the female, from which 2 fledged. In the nests that females deserted, their mates carried on feeding the chicks; in one of those nests the chicks (4) fledged successfully (and the male went on to raise a successful second brood in the same nest with another female). In another case of female desertion, the chicks were probably predated; and in the last case of female desertion all the chicks died, despite the fact that the male continued feeding, possibly due to food scarcity at the end of the season. No quantitative measures of feeding rate were made at these nests, so it is not known whether the individual which stayed provisioned the brood at a greater rate to compensate for the lack of its partner.

Mating systems can change according to the environment. In rich environments, a female's seasonal reproductive success is potentially greatest with polyandry, less in monogamy and least in polygyny; whereas male's reproductive success usually follows the reverse order of females success, i.e. polygyny>monogamy> polyandry. Such sexual conflict occurs in dunnocks (Davies 1992b). In house sparrows monogamy seems to be the optimum mating situation as far as female reproductive success is concerned, whereas polygynous males raised more young per year than monogamous males (Veiga 1990).

Polygyny is not always the most successful reproductive tactic for males. In tree swallows, polygynous males were found to produce fewer genetically related fledglings from two nests combined, than monogamous males from a single nest (both polygynous and monogamous males had similar proportion of unrelated young per nest)(Dunn and Robertson 1993). This was because secondary females of polygynous males had very low fledging success. In other populations of the same species secondary females enjoyed higher fledgling success, and in this cases polygyny, even with the costs of EPP, may be advantageous to females (Dunn and Hannon 1992).

Although polyandry seems to be the best option for females, it is rare in birds because females require substantial resources to reproduce and have limited ability to monopolize males (Oring 1986). For example, polyandry among female red-necked phalaropes seems to be limited by the availability of unpaired males (Whitfield 1990).

As Davies (1992b) noted, mating systems can be viewed as outcomes of the behaviour of individuals competing to maximize their reproductive success. Therefore, mating systems are not species specific nor static and could change according to the chances each sex has to maximize its reproductive success. Mating systems may vary within some species nesting in different habitats, as for example the pied flycatcher, which is opportunistically polygynous (Atalalo *et al.* 1984). Mating systems in a population has been considered as a result of many factors which can vary ecologically and geographically (Dunn and Robertson 1992).

According to Birkhead and Møller (1992), mating systems determine only the type of social bond, but not the sexual relationship between individuals. Thus, sperm competition is a means for monogamous species to escape the constraints of mate choice apparently imposed by their mating system.

7.5. Mating systems in sand martins

If the habitat has an important influence on female reproductive success then females should compete for habitat while males attempt to monopolize females, and the outcome of male-male competition would determine the mating system, as suggested for dunnocks and possibly for other species (Davies 1992b).

The view that females choose suitable habitats and the outcome of male-male competition for mates determines the mating system (Davies 1992b) is in contrast to the current emphasis on female preference for high quality males, even in territorial systems, including that females actively seek EPCs with high quality males (Birkhead and Møller 1992, Kempenaers *et al.* 1992).

It is possible, however, that females can use habitat quality to access the quality of the male defending it (Davies 1992b). The present study showed evidence that males breeding earlier tended to suffer fewer EPFs, and could have a better chance of later increasing their reproductive success through EPCs, and also attempting second broods. It is possible that male age, that is longevity, is linked to male quality (Bryant 1989) but since the present study had no precise information on sand martin age, further studies are need to confirm this point.

The social bond of sand martins is essentially monogamous, possibly because bi-paternal care appears to be necessary for chicks to survive to fledging. However, the mating system seems to vary according to the opportunity to gain access to mates. Males increase the number of females they mate with through EPCs, i.e. a tendency to polygyny. The females desert full grown chicks and switch partners for second broods because this allows them to lay earlier. Therefore, at least some, and perhaps the majority of females are sequential polyandrous. The sand martin mating system could therefore be viewed as a continuun, from monogamy, to monogamy plus EPCs, to polygamy (both polygyny and polyandry). Both sexes are opportunistic breeders, and the mating system is determined by the behaviour of both sexes to maximize their reproductive success. Sperm competition enables males and females to escape the constraints of monogamy. Therefore, males can father more young through EPFs, whereas females can mate with higher quality males and also dump eggs in other conspecifics nests.

Future studies using single locus DNA probes, to identify which individuals increase their reproductive success at the expense of others, are essential to investigate in more detail the mechanisms involved in MRS in sand martins and in other wild birds.

SUMMARY AND CONCLUSIONS

1. Barbush is one of the largest sand martin colonies that has been studied to date. A population decline over the study period, with a considerable drop in numbers in 1991 (46% lower breeding pairs) was associated with a decrease in body-size, apparently due to natural selection for smaller sizes due to adverse environmental conditions on the winter grounds. Since extra-pair paternity was not related to body size, its implications for the present study were small, and allowed data from all years to be considered together.

2. Clutch size declined through the season. Estimated values for first and second clutches indicated that first clutches were smaller than second clutches. There was also a tendency for clutch sizes to decline within first and mainly second broods. The apparent increase in clutch size at the start of second clutches could been explained by experienced adults arriving earlier to breed than less experienced (i.e. younger) birds arriving later. Birds breeding earlier could also start second broods (estimated as 27.1%) as a strategy to increase their seasonal reproductive success. The main losses of nests occurred before hatching, mainly due to desertion and predation.

3. The utility of radio-tracking for investigating space use by sand martins was shown to be potentially valuable for behavioural, i.e. mate-guarding, studies; and indicated no evidence for the 'information centre hypothesis'. It also allowed the first known determination of roosting sites of sand martins rearing chicks, when away from their nest burrows.

4. Chicks in nests which had their ectoparasites eliminated with a pesticide were heavier than chicks in control nests, while nestling body-mass from control nests tended to

decrease with an increase in colony size.

5. Anti-predator behaviour, in response to an aerial predator model presented to different subcolony sizes, indicated that sand martins effectively detect and mob predators, and are apparently more successful in large subcolonies.

6. Eggs experimentally dumped in nests were more often rejected prior to laying than once birds had started laying. The low incidence of intra-specific brood parasitism was probably due to rejection of eggs added at the pre-laying stage. It was shown that brood parasitism could not be detected reliably from observations of laying sequences alone, being difficult to distinguish from laying abnormalities caused by unfavourable weather conditions.

7. Extra-pair fertilizations (EPFs), quasi-parasitism (QP) and intraspecific brood parasitism (IBP) were detected by multilocus DNA fingerprinting, which was found to be a useful method of determining genetic relationships in sand martin families. The total number of broods fingerprinted was 52, including six experimental broods which had the males temporarily removed. Of 46 broods (excluding the experimentals) and 170 offspring, EPFs, QP and intraspecific brood parasitism IBP accounted for 41.3% of broods and 20.6% of offspring. Some broods contained offspring sired by an extra-pair male and also offspring not related to the putative mother (QP); cases of EPFs and IBP were also found in the same brood. EPFs occurred at a greater rate than QP and IBP (4.1% and 1.8% of all offspring respectively), accounting for 34.8% of broods containing at least one offspring sired by an extra-pair male, and 14.7% of all nestlings. Therefore, EPFs and QP represent a cost for at least some males and females, respectively, although are also a potential benefit to individuals which perform those behaviours.

8. Broods with at least one EPF tended to occur later in the breeding season, with males apparently being cuckolded more frequently in second broods than in first broods. Individuals starting to breed earlier could possibly have a reproductive advantage, with males being more likely to have a second brood and less likely to be cuckolded and sometimes fathering at least some offspring of their first brood mates' second brood (as demonstrated by DNA fingerprinting in one case). Females could also gain from starting early, since they could leave a nearly-fledged first brood to the care of their first brood mates, and attempt an earlier second brood.

9. Behaviourial observations indicated that mate-guarding by males was very intensive at the time their mates were likely to be fertile, although it decreased after the first egg. Temporary male removal experiments (at 6 nests) on the day the second egg was laid (except in one case, 3 days before the first egg) suggested that mate-guarding may help to assure paternity (as determined by DNA fingerprinting), since EPFs tended to occur more commonly in male-removal nests. Two broods did not contain EPFs, however, despite males being removed, suggesting that other factors might have been involved, such as female choice.

10. Sand martin males that had been cuckolded did not reduce their levels of parental effort, measured as a rate of food delivery to the brood, a result that fits with other similar studies of monogamous birds.

11. Future investigations should concentrate on identifying the individuals which increase their reproductive output through a MRS, and on analyzing the differences between observed and realised reproductive success at the individual level, through the use of the single locus probe DNA fingerprinting technique. This would help to clarify the mechanism involved in MRSs in sand martins. Comparative

studies to investigate the effects of colony size (and also the interaction between coloniality and social organization), on the parameters of survival and reproductive success, will be useful to understand the basis of potential costs and benefits of coloniality, and therefore its adaptive significance.

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The view that females choose suitable habitats and the outcome of male-male competition for mates determines the mating system (Davies 1992b) is in contrast to the current emphasis on female preference for high quality males, even in territorial systems, including that females actively seek EPCs with high quality males (Birkhead and Møller 1992, Kempenaers *et al.* 1992).

It is possible, however, that females can use habitat quality to access the quality of the male defending it (Davies 1992b). The present study showed evidence that males breeding earlier tended to suffer fewer EPFs, and could have a better chance of later increasing their reproductive success through EPCs, and also attempting second broods. It is possible that male age, that is longevity, is linked to male quality (Bryant 1989) but since the present study had no precise information on sand martin age, further studies are need to confirm this point.

The social bond of sand martins is essentially monogamous, possibly because bi-paternal care appears to be necessary for chicks to survive to fledging. However, the mating system seems to vary according to the opportunity to gain access to mates. Males increase the number of females they mate with through EPCs, i.e. a tendency to polygyny. The females desert full grown chicks and switch partners for second broods because this allows them to lay earlier. Therefore, at least some, and perhaps the majority of females are sequential polyandrous. The sand martin mating system could therefore be viewed as a continuun, from monogamy, to monogamy plus EPCs, to polygamy (both polygyny and polyandry). Both sexes are opportunistic breeders, and the mating system is determined by the behaviour of both sexes to maximize their reproductive success. Sperm competition enables males and females to escape the constraints of monogamy. Therefore, males can father more young through EPFs, whereas females can mate with higher quality males and also dump eggs in other conspecifics nests.

Future studies using single locus DNA probes, to identify which individuals increase their reproductive success at the expense of others, are essential to investigate in more detail the mechanisms involved in MRS in sand martins and in other wild birds.

SUMMARY AND CONCLUSIONS

1. Barbush is one of the largest sand martin colonies that has been studied to date. A population decline over the study period, with a considerable drop in numbers in 1991 (46% lower breeding pairs) was associated with a decrease in body-size, apparently due to natural selection for smaller sizes due to adverse environmental conditions on the winter grounds. Since extra-pair paternity was not related to body size, its implications for the present study were small, and allowed data from all years to be considered together.

2. Clutch size declined through the season. Estimated values for first and second clutches indicated that first clutches were smaller than second clutches. There was also a tendency for clutch sizes to decline within first and mainly second broods. The apparent increase in clutch size at the start of second clutches could been explained by experienced adults arriving earlier to breed than less experienced (i.e. younger) birds arriving later. Birds breeding earlier could also start second broods (estimated as 27.1%) as a strategy to increase their seasonal reproductive success. The main losses of nests occurred before hatching, mainly due to desertion and predation.

3. The utility of radio-tracking for investigating space use by sand martins was shown to be potentially valuable for behavioural, i.e. mate-guarding, studies; and indicated no evidence for the 'information centre hypothesis'. It also allowed the first known determination of roosting sites of sand martins rearing chicks, when away from their nest burrows.

4. Chicks in nests which had their ectoparasites eliminated with a pesticide were heavier than chicks in control nests, while nestling body-mass from control nests tended to

decrease with an increase in colony size.

5. Anti-predator behaviour, in response to an aerial predator model presented to different subcolony sizes, indicated that sand martins effectively detect and mob predators, and are apparently more successful in large subcolonies.

6. Eggs experimentally dumped in nests were more often rejected prior to laying than once birds had started laying. The low incidence of intra-specific brood parasitism was probably due to rejection of eggs added at the pre-laying stage. It was shown that brood parasitism could not be detected reliably from observations of laying sequences alone, being difficult to distinguish from laying abnormalities caused by unfavourable weather conditions.

7. Extra-pair fertilizations (EPFs), quasi-parasitism (QP) and intraspecific brood parasitism (IBP) were detected by multilocus DNA fingerprinting, which was found to be a useful method of determining genetic relationships in sand martin families. The total number of broods fingerprinted was 52, including six experimental broods which had the males temporarily removed. Of 46 broods (excluding the experimentals) and 170 offspring, EPFs, QP and intraspecific brood parasitism IBP accounted for 41.3% of broods and 20.6% of offspring. Some broods contained offspring sired by an extra-pair male and also offspring not related to the putative mother (QP); cases of EPFs and IBP were also found in the same brood. EPFs occurred at a greater rate than QP and IBP (4.1% and 1.8% of all offspring respectively), accounting for 34.8% of broods containing at least one offspring sired by an extra-pair male, and 14.7% of all nestlings. Therefore, EPFs and QP represent a cost for at least some males and females, respectively, although are also a potential benefit to individuals which perform those behaviours.

8. Broods with at least one EPF tended to occur later in the breeding season, with males apparently being cuckolded more frequently in second broods than in first broods. Individuals starting to breed earlier could possibly have a reproductive advantage, with males being more likely to have a second brood and less likely to be cuckolded and sometimes fathering at least some offspring of their first brood mates' second brood (as demonstrated by DNA fingerprinting in one case). Females could also gain from starting early, since they could leave a nearly-fledged first brood to the care of their first brood mates, and attempt an earlier second brood.

9. Behaviourial observations indicated that mate-guarding by males was very intensive at the time their mates were likely to be fertile, although it decreased after the first egg. Temporary male removal experiments (at 6 nests) on the day the second egg was laid (except in one case, 3 days before the first egg) suggested that mate-guarding may help to assure paternity (as determined by DNA fingerprinting), since EPFs tended to occur more commonly in male-removal nests. Two broods did not contain EPFs, however, despite males being removed, suggesting that other factors might have been involved, such as female choice.

10. Sand martin males that had been cuckolded did not reduce their levels of parental effort, measured as a rate of food delivery to the brood, a result that fits with other similar studies of monogamous birds.

11. Future investigations should concentrate on identifying the individuals which increase their reproductive output through a MRS, and on analyzing the differences between observed and realised reproductive success at the individual level, through the use of the single locus probe DNA fingerprinting technique. This would help to clarify the mechanism involved in MRSs in sand martins. Comparative

studies to investigate the effects of colony size (and also the interaction between coloniality and social organization), on the parameters of survival and reproductive success, will be useful to understand the basis of potential costs and benefits of coloniality, and therefore its adaptive significance.

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APPENDIX 1

Artificial cliff

Sand martins were apparently attracted to the play-back of their song and calls and to the decoys at the entrance of the nest pipes.

In 1991 adult birds were sometimes seen perching at the entrance of the holes, but they did not dig more than 10cm into the pipes. Sand was therefore removed from 3 pipes to check if the birds would try to enter and dig more sand from the nest boxes, but no sign of subsequent digging was found.

In 1992, on 24th April, one adult sand martin was seen to perch at the entrance of a burrow which had a decoy. Following this, other birds were attracted; 5 birds were seen perching around the decoys, and some started excavating the sand at the entrance of pipes filled by sand. Later on that day, claw marks of sand martins were detected at the entrance to 4 pipes. Next day, one of the pipes was found with fresh marks, 5cm deep. This and previous attempts to excavate occurred on the top row holes of the artificial cliff. Three days after the first attempt, one of the decoys was found on the ground with a wing pulled off. On April 28th, when the decoys were placed on the usual top row and the tape recorder was playing, one adult sand martin perched close to one of the decoys and another on the bottom row of pipes. On May 5th one of the decoys was found missing 1.5h after being placed on artificial cliff. Four days later, the other decoy was found missing 11h after it was placed on the cliff. By that time, a kestrel was seen flying around. After that day, sand martins were not seen trying to use the cliff for a while.

Some of the pipes had some sand removed to check if the birds would find it easier to settle. On the 14th of June, three or four sand martins were reported by local workers to be seen coming in and out of the pipes early in the morning.

On that same day, from inside the shed three potential nests could be seen through the transparent plastic cover of the nest boxes (Figure A.1). The lengths of the three burrows were 47, 47.5 and 58cm, located on the top row of the cliff. Three days later the artificial cliff was found to have been vandalized, with a large hole on the roof made by stones (one big stone was found on the top of a middle nest box). After that incident, no birds were observed attending the artificial cliff.

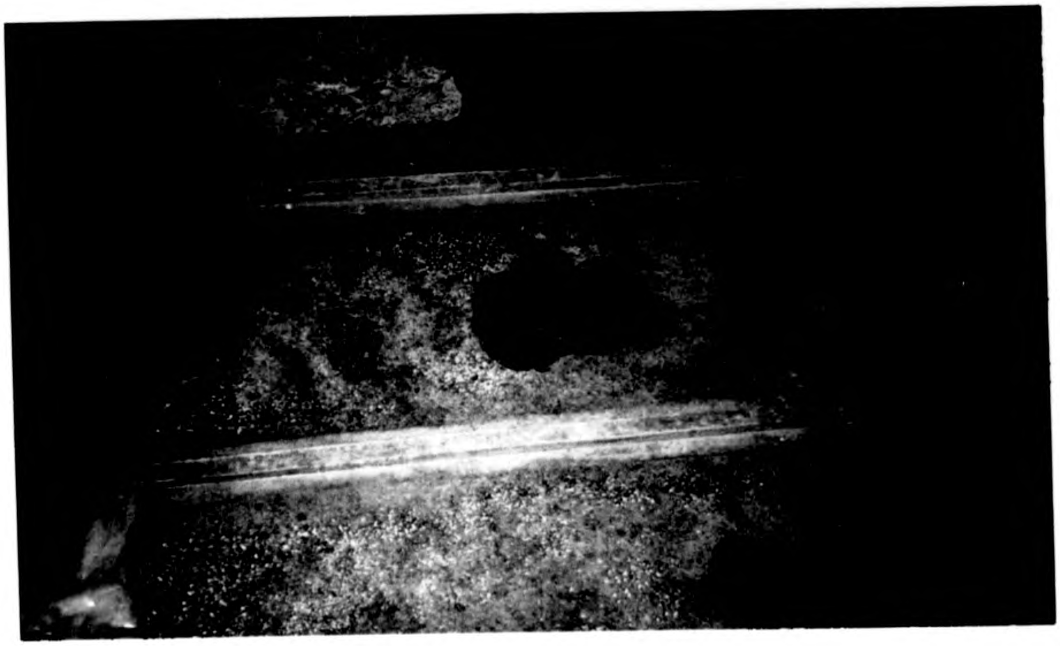

Figure A.1 - Sand martin nesting attempts on the artificial cliff at Barbush, 1991.

APPENDIX 2

List of common and latin names of species quoted in the text.

Acorn woodpecker, *Melanerpes formicivorus*
American kestrel, *Falco sparverius*
Black-headed gull, *Larus ridibundus*
Blue-throated bee-eater, *Merops viridis*
Blue tit, *Parus caeruleus*
Brown-headed cowbird, *Molothrus ater*
Chimney swift, *Chaetura pelagica*
Cliff swallow, *Hirundo pyrrhonota*
Carrion crow, *Corvus corone*
Common redpoll, *Carduellis flammea*
Common swift, *Apus apus*
Darwin's finch, *Geospiza fortis*
Dunnock, *Prunella modularis*
Eastern kingbird, *Tyrannus tyrannus*
European bee-eater, *Merops apiaster*
European starling, *Sturnus vulgaris*
Great tit, *Parus major*
House wren, *Troglodytes aedon*
House martin, *Delichon urbica*
House sparrow, *Passer domesticus*
Indigo bunting, *Passerina cyanea*
Jackdaw, *Corvus monedula*
Kestrel, *Falco tinnunculus*
Maize, *Zea mays*
Mink, *Mustela vison*
Northern fulmar, *Fulmarus glacialis*
Oak, *Quecus sp*
Oilseed rape, *Brassica napus*
Pied flycatcher, *Ficedula hypoleuca*
Purple martin, *Progne subis*
Rabbit, *Oryctolagus cuniculus*

Red dear, *Cervus elaphus*
Red-necked phalarope, *Phalaropus lobatus*
Red-winged blackbird, *Agelaius phoenicus*
Sand martin/bank swallow, *Riparia riparia*
Sand martin flea, *Ceratophyllus styx*
Sand martin tick, *Ixodus lividus*
Sand martin hippoposcid flie, *Sterepterix hirundinis*
Shag, *Phalacrocorax aritotelis*
Sparrow-hawk, *Accipiter nisus*
Spotted sandpiper, *Actitis macularia*
Stoat, *Mustela erminea*
Swallow/barn swallow, *Hirundo rustica*
Tree swallow, *Tachycineta bicolor*
Weasel, *Mustela nivalis*
Wheat, *Triticum aestivum*
White-fronted bee-eater, *Merops bullockoides*
White-throated needletail, *Hirundapus caudacutus*
Yellow-ramped cacique, *Cacicus cela*
Yellow warbler, *Dendroica petechia*
Zebra finch, *Taeniopygia guttata*

APPENDIX 3

Mean time spent in nest and number of visits to nests by sand martins during different stages of the breeding cycle.

3.1 - Mean time spent in nest±standard error by male and female sand martins during different stages of the breeding cycle. The observation time per each nest was 6h for pre-laying and laying, and 8h for incubation and rearing stages.

Stages	Male	Female	n
Pre-laying	24.2±2.78	25.3±2.66	10
Laying	21.9±2.76	24.9±2.35	12
Incubation	23.3±1.08	31.2±1.26	14
Rearing	6.9±1.51	7.8±1.58	15

3.1 - Mean number of visits in nest±standard error by male and female sand martins during different stages of the breeding cycle. The observation time per each nest was 6h for pre-laying and laying, and 8h for incubation and rearing stages.

Stages	Male	Female	n
Pre-laying	4.5±1.16	3.7±1.06	10
Laying	2.9±0.47	2.0±0.36	12
Incubation	2.6±0.25	2.1±0.14	14
Rearing	9.2±0.73	8.2±0.99	15