Reproductive success in martins (Hirundinidae) Studies of the behaviour and ecology of individuals using DNA fingerprinting

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NUMEROUS ORIGINALS IN COLOUR

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Plate 1. The study species: A pair of house martins in the early stages of nest building (above), and an adult and fledgling sand martin near the entrance to a nest burrow (below). Note the dusky throat and scalloped wing feathers of the fledgling.

Abstract

The aim of the study was to isolate some of the sources of individual variation in reproductive success in wild bird populations, with particular emphasis on the number of clutches produced per year, and the consanguinuity between parents and offspring. The main study species was the house martin, with some comparative work on the sand martin (Plate 1).

Both at an individual and a population level double-broodedness was implicated as the annual breeding strategy with the highest fitness benefits for house martins, in terms of total annual output of fledged young. Older females laid earlier than first year females, and, in contrast with earlier work on the same species (Bryant 1979, 1988a), there was a hint that small size in males might be associated with increased annual reproductive success.

Experimental manipulations of first brood size in house martins indicated that the interval between breeding attempts increased with first clutch size, and that nestlings in enlarged broods grew more slowly and suffered an increased rate of mortality. Enlarging or reducing the size of the first brood also had an effect on the probability of a second clutch in the same breeding season. Pairs with enlarged broods seemed less likely to produce a second clutch, whereas there was an increased chance that pairs with reduced first broods would lay again.

Over the time period 1972-1989, house martins breeding at study colonies in Central Scotland have apparently undergone a decline in annual reproductive success. Variation in food abundance apparently had some effects on the timing and success of breeding, but could not fully account for the observed changes. It is debatable whether this decline is real, or a result of changes in food availability, nest site preference, and possibly population age structure of house martins in Central Scotland over the same period.

There was no evidence of egg dumping or intraspecific brood parasitism (IBP) in either of the study species. DNA fingerprinting indicated that 38% of house martin broods contained at least one offspring that had been fathered by an extra-pair male, with 15% of all young being unrelated to their putative father. Preliminary results indicated a slightly higher incidence of extra-pair paternity in sand martins.

Observations of behaviour indicated that male sand martins and house martins guard their

mates during the prelaying and laying periods, although the degree of guarding differed between the two species, probably due to differing risks of extra-pair copulations (EPCs). There was some indication that the risk of cuckoldry for male house martins was linked to the intensity of mate guarding. In the absence of the pair male, female house martins apparently chose whether or not to accept EPCs from males intruding into their nests. House martin males that have been cuckolded do not reduce their parental effort in terms of rate of food delivery to the brood.

Extra-pair fertilisations (EPFs) therefore represent fitness or fecundity costs of reproduction for at least some males in both of the study species, and former estimates of apparent male reproductive success in house martins (Bryant 1988a, 1989) must now be revised. Unfortunately the fathers of extra-pair offspring were not identified, but there were indications that male house martins which achieved full paternity in their own families might also be likely to increase their reproductive success through extra-pair fertilisations. This finding is in accordance with the basic assumption of Trivers (1972), that individuals should pursue a mixed reproductive strategy in order to increase their lifetime reproductive success, although if it is older males that increase their fecundity at the expense of younger males then realised lifetime reproductive success in house martins is unlikely to be very different from apparent success. In addition,the pursuit of EPCs may represent an alternative reproductive tactic for unmated house martin males.

A preliminary review of consanguinuity in wild birds failed to isolate any consistent themes. For each species, even closely related species within the hirundine family, the observed rates of EPF and IBP seem to be a result of unique interactions between behavioural and ecological factors. Mate guarding apparently varies in intensity and effectiveness within and between species, as does the response of females to attempted EPCs. A cross species comparison indicated that the occurence of EPFs and IBP was apparently unrelated to breeding dispersion and breeding system, although within species an increased density of breeding individuals may lead to a higher frequency of non-kin offspring. What is clear is that EPCs probably occur in the majority of bird species, even though in some they rarely if ever lead to EPFs. It is no longer possible to ignore the effects of sperm competition and IBP, and no study of individual reproductive success in wild bird populations can now be considered complete unless it incorporates DNA fingerprinting.

Summary of contents

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1. Introduction

Biological fitness can be defined as the contribution of an individual of a distinct genotype to the next generation relative to that of other concurrent individuals (Newton 1989). For populations that are neither growing nor shrinking in size, as is thought to be the case for most bird species, lifetime reproductive success (LRS), measured as the number of offspring produced by an individual in its lifetime that survive to breed, is generally considered a good measure of fitness (Schaffer 1983, Nur 1988, Newton 1989). The average LRS of an individual from a population of a bird species is given by the following equation (modified from Tinbergen *et al* 1987):

$$LRS = BL\ (CY \times CS \times F \times R) \qquad 1.1$$

LRS is the product of breeding lifespan (BL) and annual reproductive success, the latter represented by the variables within parentheses. CY is the average number of clutches produced per year (simultaneously or in succession); CS the average clutch size; F the proportion of young that fledge; and R the proportion of fledged young that recruit into the breeding population.

It has now been established that both male and female birds may attempt to increase their reproductive success by exploitation of other individuals of the same species. Males by copulating with non-mate females in an attempt to secure extra-pair fertilisations (EPFs; Trivers 1972, Birkhead & Møller 1992), females by 'dumping' eggs in the nests of other pairs of the same species (Yom-Tov 1980, Petrie & Møller 1991). Thus it cannot be assumed that all the young in the nest of a given pair of birds are genetically related to the male and female attending that nest, and the calculation of average LRS must be re-written as follows:

$$LRS = BL\ [\ (CY \times CS \times CO \times F \times R) + (E \times EF \times ER)\ ] \qquad 1.2$$

Where CO is the cosanguinuity coefficient, the proportion of eggs in the clutch that are the true genetic offspring of the parent individual; E the average number of EPFs achieved by a male bird for eggs laid in another nest, or the number of eggs laid or 'dumped' by a female parent in another nest; EF the proportion of extra-pair offspring that fledge; and ER the proportion of extra-pair offspring that recruit into the breeding

population. This time the square brackets enclose components of annual reproductive success, and the parentheses within them separate events in the nest(s) of an individual from events in the nest(s) of extra-pair birds. Since both CO and E are likely to vary between males and females, the equation would be best used to calculate average values for LRS for the two sexes.

LRS can also be measured directly, by long-term studies of known individuals throughout their breeding lifespan, and the results of a number of such studies of birds have recently been published in books edited by Clutton-Brock (1988) and Newton (1989). In some species, especially long distance migrants or those with sex differences in dispersal, LRS was measured as total lifetime production of fledglings rather than as the number of local recruits to the breeding population. In studies where both these measures of LRS were available they were normally correlated (chapters 2, 4, 8, 9, 11, 15, 17, 18, 19, 20, and 23 in Newton 1989), indicating that both lifetime fledging production and lifetime production of breeding recruits can be used as measures of fitness.

The indication of studies of LRS in birds is that breeding lifespan is the major determinant of lifetime fledging production in both long-lived and short-lived species. Where LRS was measured as recruitment to local breeding populations the survival of offspring between fledging and recruitment became relatively more important than lifespan (Newton 1989). Offspring survival and recruitment may be influenced by environmental factors outside the control of the individual, as in great tits where the size of the beech crop in the year of hatching had a major effect on juvenile recruitment (van Noordwijk & van Balen 1988, McCleery & Perrins 1989). In co-operatively breeding species such as the Arabian babbler (Zahavi 1989) factors in the social environment such as breeding group size or social rank can have profound effects on LRS. Phenotypic characters such as body size also appear to affect components of LRS in some species, where it is often the case that larger individuals are more successful. In male house martins for example, larger body size was positively correlated with lifespan, the total number of eggs incubated, and the total number of young reared (Bryant 1988a, 1989).

There is often considerable variation in LRS between the individuals of a population, both within and between the sexes. In many cases, a small proportion of the breeding adults produce a large proportion of the recruits to the next generation. Evolutionary theory predicts that individuals in a population should attempt to maximise their LRS, so as to maximise their genetic contribution to succeeding generations. The causes of individual variation in LRS are therefore of value for understanding the way that

selection pressures act on populations, and in the evolution of life history strategies. For instance, individuals with low LRS may either be genotypes that are being eliminated from the population by directional selection, or a result of chance factors such as unpredictable environmental variation. Hence caution is needed when interpreting the results of studies of LRS, since like any other phenotypic character it is subject to variation that is beyond genetic control (Grafen 1988, Newton 1989).

The aim of this study was to isolate some of the sources of individual variation in reproductive success within bird populations, with particular focus on two components with potentially large influences on LRS, the number of clutches produced per year (CY), and the consanguinuity between parents and offspring (CO). The main study species was the house martin *Delichon urbica*, with some comparative work on the sand martin *Riparia riparia*. Because the study was carried out over only a three year period, and it was not possible to collect data for many individual birds for more than one breeding season, I have looked at variation in annual rather than lifetime reproductive success. In species such as the house martin where few individuals have a reproductive lifespan of more than two years variations in the former parameter are likely to have a profound effect on the latter since it is the second most important determinant of LRS after breeding lifespan (Bryant 1988a, 1989).

I deal first with the variation in annual reproductive success of house martins breeding in Central Scotland (Chapter 2), using data collected during the present study (1987-89) combined with data collected by D M Bryant between 1972 and 1983. A variable number of pairs attempt two broods each year whereas others raise only one. Since a double-brooded individual can nearly double its annual reproductive success over an individual that attempts only one brood, it is surprising that all house martins do not attempt two broods. Probably intra-seasonal reproductive costs associated with the size or timing of the first brood discourage some pairs from attempting a second. To investigate this, the progress of breeding was monitored, and experimental manipulations of brood size were carried out to assess the effect of enlarged or reduced brood size on nestling growth and mortality, and the probability and timing of a second clutch. Experimental manipulations are important because they can help to identify causal relationships between reproductive effort and success. These cannot normally be identified from natural variation because differences in individual quality tend to obscure relationships between effort and success (Reznick 1985, 1992; Partridge 1989).

DNA fingerprinting, which allows genetic relationships between individuals to be

characterised (Jeffreys *et al* 1985a & b, Burke & Bruford 1987, Wetton *et al* 1987), was used to look at the difference between apparent reproductive success (assuming that all the offspring within a family are the true offspring of the male and female attending the nest) and realized reproductive success in house martins, and a closely related species, the sand martin (Chapter 3). Sand martins were included to extend the range of colony sizes examined and hence the consequences of variation in local population sizes. House martins usually occupy small colonies, a range of 3 to 18 breeding females in the present study, whereas sand martins establish very large colonies, with 100 or more pairs not uncommon in a single sand bank (pers obs). Thus the opportunity for EPFs is likely to be higher in sand martin colonies, because of the large number of individuals nesting in close proximity.

Behavioural aspects of a mixed reproductive strategy in house martins and sand martins were also examined (Chapter 4) by making observations of marked individuals at the nest during the prelaying and laying periods, and the late nesting period when both parents are feeding the offspring (house martins only). Attention was focused on the situations where pair copulations and extra-pair copulations might occur; the response of females to attempted extra-pair copulations; and the form, effectiveness and variation between individual males in paternity defence behaviours such as mate-guarding. Paternity has been shown to influence nestling provisioning in the Dunnock (Burke *et al* 1989, Davies *et al* 1992), and rates of nestling provisioning by house martin males were examined for individual differences that might be related to paternity. In addition to the observations, males were temporarily removed from their nests in an attempt to experimentally reduce the effectiveness of mate guarding, and perhaps the level of male parental care if removed males responded to an increased risk of cuckoldry by reducing their parental effort.

Finally, I discuss the implications of a mixed reproductive strategy for measurements of lifetime reproductive success in birds, and compare the findings of the present study with those of other published studies of wild bird populations that have used DNA fingerprinting, paying particular attention to studies on other hirundine species.

2. Variation in annual reproductive success in house martins

2.1 Introduction

Lack (1947, 1954, 1968) suggested that the average clutch size of individuals within a population of birds maximises the number of surviving offspring, and that in nidicolous birds it is the ability of the parent to feed the young that limits clutch size. However, studies have shown that birds of many species can successfully rear enlarged broods (for example, Haymes & Morris 1977, Cronmillar & Thompson 1980, De Steven 1980, Roskaft 1985, Dijkstra *et al* 1988, Linden 1988), suggesting that natural brood size may often be smaller than the most productive brood size.

One explanation for this is that reproduction carries with it a cost in terms of future survival and/or fecundity to breeding adults and/or their young. Thus natural clutch sizes may be adjusted to maximise lifetime reproductive success, rather than output from the current breeding attempt (Williams 1966, Charnov & Krebs 1974, Stearns 1976).

Costs of reproduction are trade-offs among different components of an organism's life history, and are believed to be fundamental to the evolution of breeding strategies, including the frequency of reproduction and the number of offspring produced (Reznick 1992). Costs may be physiological or ecological. The former involves competition for resources between different body functions such as growth, maintenance and reproduction. Ecological costs are imposed by the external environment, for example reproductive activity may expose an individual to risks such as predation, disease, or injury (Magnhagen 1991, Reznick 1992).

The most convincing evidence for reproductive costs comes from studies where reproductive effort is experimentally manipulated, which in birds is usually achieved by enlarging or reducing the size of the brood. Results of such studies indicate that the cost of reproduction may be expressed as reduced adult survival to the next breeding season (Askenmo 1979, Nur 1984a, 1988a), or as a reduction in future reproductive success (Gustafsson & Sutherland 1988, Nur 1988 a & b). It is not necessarily the case that every species manifests the same cost, or that the cost is detectable in every breeding season (Nur 1988a).

The existence of a reproductive cost is more difficult to assess from non-experimental studies which correlate subsequent adult survival or fecundity with natural clutch or brood sizes. A correlative approach using natural variation to study reproductive trade-offs may be inappropriate, as such studies measure the combined effect of genotypic, phenotypic and environmental factors (Reznick 1985, 1992), and it is possible that some individuals may adjust their reproductive behaviour according to phenotype or environment in order to reduce reproductive costs. A positive relationship between clutch size and adult survival has been demonstrated for some species (for example Smith 1981, Hogstedt 1981) which seems to disprove the cost hypothesis, but could be explained by individual adjustment of clutch size by females based either on their own condition, or enhancement of both reproductive success and survival by factors in the environment such as territory quality or food availability (Drent & Daan 1980, Daan *et al* 1988, Nur 1988a, Pettifor *et al* 1988).

In birds that commonly attempt more than one brood within a breeding season, it is possible to look for intra-seasonal reproductive costs; that is, the effect that raising a first brood might have on the success of subsequent broods in the same season. In the extreme case, effort expended on a first brood might prevent a pair from attempting a second. A variable proportion of pairs of house martins breeding in Central Scotland attempt two broods each year. If a pair that successfully raises two broods could nearly double its annual reproductive success (measured as number of fledged young) compared with a pair that raises only one brood, why don't all pairs attempt two broods? There are a number of factors that might influence this decision. Birds that lay their first clutch early in the season will obviously have more time to attempt a second brood than those which lay later. The larger the size of the first brood the more effort is likely to be required to raise the nestlings (Hails & Bryant 1979, Bryant & Westerterp 1983b) and this may influence the decision to attempt a second brood. In addition, other factors that have been shown to affect the timing or success of breeding in birds, including environmental influences such as food abundance (Perrins 1970, Bryant 1975a, Daan *et al* 1988), and characteristics of individuals such as condition and age (Drent & Daan 1980, Clutton-Brock 1988, Bryant 1988a) might also influence the decision to attempt a second brood. A second brood might reduce the chances of the adults surviving to the following year, or lower their subsequent reproductive output.

Experimental manipulation of first brood size has been shown to affect the probability that a second clutch will be attempted in the same season in great tits (Smith *et al* 1987, Tinbergen 1987, Linden 1988) and swallows (Thompson 1992), but not in house wrens

(Finke *et al* 1987, Robinson & Rotenberry 1991).

Brood manipulation experiments may also affect the growth and survival of the nestlings. A number of studies have demonstrated that young from enlarged broods fledge at a lower mass, show increased mortality in the nest, or reduced survival to the next breeding season, compared with young in natural or reduced broods (Cronmillar & Thompson 1980, Linden 1988, Smith *et al* 1989, Nur 1984b), although this is not always the case (for example, Haymes & Morris 1977). In some species at least, it is possible that there is a trade off between the number of offspring produced and the quality of offspring (Smith & Fretwell 1974, Smith *et al* 1989). The brood size that produces the most recruits to the next breeding population may therefore be smaller than the maximum number of young a given pair of birds is capable of raising to fledging.

This chapter deals with the variation in annual reproductive success of house martins breeding in Central Scotland, building on work already done on this species (Bryant 1979, 1988a, 1989). Particular emphasis was placed on the reasons why some pairs attempt two broods per season, whereas others attempt only one. Brood manipulation experiments were carried out in an attempt to assess the affect of enlarged and reduced brood size on the probability that a given pair would attempt a second clutch, and on the growth and mortality of nestlings.

2.1.2 A model for the timing of breeding

A qualitative model for the timing of breeding in a double-brooded, seasonally breeding bird species such as the house martin is given in Figure 2.1, based on models given in Tinbergen & Van Balen 1988.

The start of season is the date on which the earliest first clutch is initiated. For house martins breeding in south east England, Bryant (1975a) found that the start of season coincided with the appearance of flying aphids in suction trap catches, and suggested that the aphids might act as a cue to the birds to begin breeding. It is not known if this applies in Central Scotland.

The slope of the line representing the cumulative proportion of pairs laying first clutches depends on the synchrony of laying. First clutches at house martin colonies are typically started over a period of at least one month, which raises the question of why some pairs begin to lay earlier than others. For the purposes of simplification the model has assumed

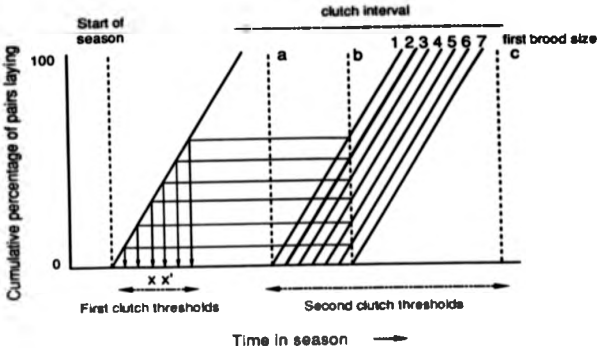


Figure 2.1 A model for the timing of breeding in a double-brooded, seasonally breeding bird species such as the house martin. Plotted lines represent the cumulative percentage of pairs laying a first clutch (single line from start of season), and a second clutch after raising first broods of 1-7 nestlings (parallel lines from a). The start of season is the date that the first. first clutch is begun. The clutch interval is the number of days between the laying of the first and second clutches. The first and second clutch thresholds, the dates x and x', and the lines a, b and c are explained in section 2.1.2 of the text.

a constant rate of laying, although in reality there is a peak around the mean date of first egg.

The clutch interval as depicted in the model is the period of time between the laying of the first egg of the first and second clutches. However, in the analysis I have used the interval between the laying of the last egg of the first clutch and the first egg of the second clutch, in an attempt to eliminate the effects of variation in clutch size between pairs.

A number of studies have shown that clutch interval increases with first brood size (McGillivray 1983, Slagsvold 1984, Hegner & Wingfield 1987, Smith *et al* 1987, Tinbergen 1987; although this is not always the case, see Finke *et al* 1987) and this assumption has been built into the model. The parallel, sloping lines indicate the date of second clutch initiation for first broods of one to seven young, assuming that all pairs will attempt a second clutch if they have time. I have used the term "brood" rather than "clutch" so as to cover experimentally enlarged broods. Some house martin pairs were given broods of seven young to rear, but the maximum first clutch size of this species in Central Scotland is five eggs.

The second clutch threshold (SCT) is defined as the date after which no more second clutches are started, the assumption being that after this date there is not sufficient time for a pair to raise a second brood successfully. The first clutch threshold (FCT) is then the latest date that a first clutch can be started by a pair that also attempt to lay a second clutch. Assuming that clutch interval increases with first brood size, then first clutch threshold will also vary with this parameter.

The lines labelled a, b and c illustrate the effect of moving the SCT while holding the start of season constant. With the SCT at position a, no pairs will attempt a second clutch; whereas at position c all pairs will lay again. If the SCT lies at position b, some pairs will lay a second clutch, depending on the date the first clutch was started, and the number of young reared. For example, pairs that begin laying first clutches before date x, and raise first broods of four nestlings or less, would be expected to lay again. Similar effects on the proportion of pairs laying two clutches could be obtained by moving the start of season while keeping the SCT constant.

The model can be used to consider the likely effects of brood manipulation experiments. The parallel arrows indicate the latest date that a first brood of a given size would be

expected to be followed by a second clutch assuming that line b represents the SCT. Thus a first brood of 4 laid on day x will produce a second clutch. However, if this brood is enlarged to six, the increased clutch interval will mean that the start date for laying the second clutch will fall after the threshold date for second clutches. Thus pairs with enlarged first broods are expected to attempt second clutches less often than unmanipulated pairs. The only pairs with enlarged broods that would be expected to attempt a second clutch would be those laid early in the season.

A female laying a first brood of 4 on date x' would not be expected to lay a second clutch. However, if this brood is reduced to 2, then the female will have time to lay a second clutch. Pairs with reduced first broods are thus expected to produce second clutches more often than pairs with unmanipulated, or enlarged broods.

The predictions of this model are therefore that the proportion of pairs that attempt a second clutch in a given season will depend on the date at which breeding starts and finishes, the synchrony of laying, and the interval between clutches. Brood manipulation experiments should affect the proportion of second clutches by altering the length of the clutch interval. Increasing the size of a first brood should reduce the probability that it is followed by a second clutch, whereas reducing first brood size should increase the likelihood that a second clutch will be attempted.

2.2 Methods

I studied variation in annual reproductive success at a total of nine house martin colonies in the Central Region of Scotland between 1987 and 1989. The location of the colonies, which were between 15 and 23 km from Stirling, is shown in Figure 2.2, and map references and colony sizes are given in appendix A. In addition, the analysis in this chapter includes data collected by David Bryant at the Naemoor colony (Figure 2.2) between 1972 and 1983.

The analysis includes pairs breeding in nest boxes and in natural nests (Figure 2.3). Between 1987 and 1989, 39 of 87 first broods were in nest boxes, and between 1972 and 1983, 162 of 183 first broods. Nest boxes were found more convenient to work with since it was easier to count eggs and extract nestlings without damaging the nest. The contents of natural nests were checked using a torch and a dental mirror. Young were extracted for ringing and measurement by enlarging the entrance hole and scooping them out with a metal spoon.

It is not known if the provision of nest boxes was likely to have artificially increased the number of available nest sites within the house martin colonies studied, and therefore had an effect on apparent reproductive costs, as has been implied for other studies (Linden & Møller 1989, Møller 1989a, 1992). Since nest boxes rarely if ever attract house martins to a new site (D M Bryant, pers comm), this is perhaps unlikely. Nest boxes were routinely cleared of nesting material at the end of each breeding season to help control the build-up of parasites, and old mud nests were knocked down to encourage birds to occupy nest boxes in the next year. Thus very few pairs included in the analysis had occupied natural nests built in previous years, and pairs breeding in natural nests are therefore unlikely to have suffered increased parasite load compared to birds breeding in nest boxes (Møller 1989a, 1992). The results from different nest types are therefore suitable for comparison, although they could over-estimate absolute success rates if heavy parasite loads routinely depress reproductive success in more natural situations.

Checking for nest occupation at colonies was begun in mid-April. Thereafter I visited each colony at least twice a week throughout the breeding season, making daily visits to the most intensively studied colonies.

Breeding adults were captured at the nest using the nest traps shown in Figure 4.1. If they were not already ringed, birds were given individually numbered BTO aluminium

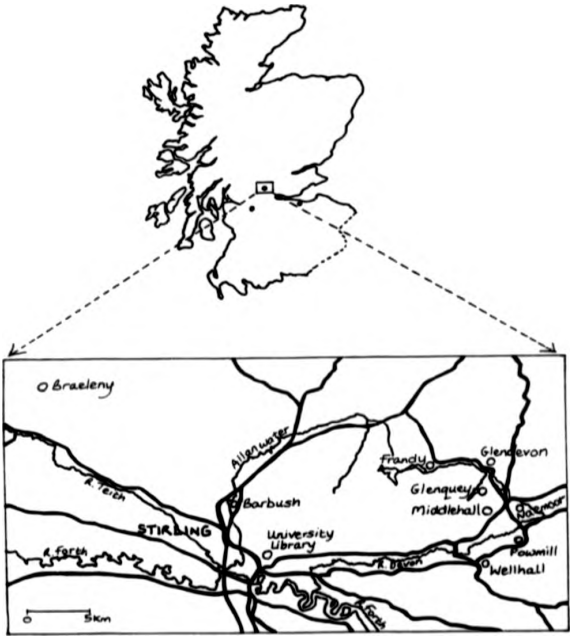
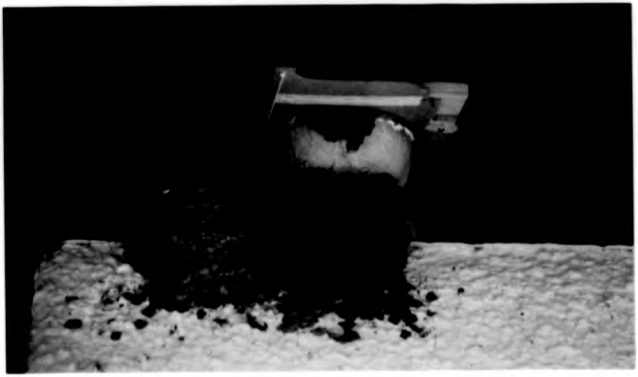


Figure 2.2 The location of house martin colonies used in the present study. Map references and colony sizes are given in Appendix A. The location of the single sand martin colony at Barbush is also shown (see Chapter 4). The three dots on the upper map represent, from east to west, Edinburgh, Stirling and Glasgow.



(a)

(b)

Figure 2.3a House martin nest box and natural mud nests, b, lid of nestbox opened to reveal nestlings

rings and colour marked as described in section 4.2.1. The following measures of body size were recorded: mass (measured to the nearest 0.1g using a Pesola spring balance); wing length (maximum chord, to the nearest mm); keel length (length of sternum from tracheal pit to hind margin, to the nearest 0.1mm); head and bill length (the maximum distance from the tip of the bill to the back of the skull, to the nearest 0.1mm); and tarsus length (the length of the tarso-metatarsal bone from the angle of the inter-tarsal joint to the base of the last complete scale before the toes diverge, to the nearest 0.1mm). Normally, adults were captured at least twice during a given breeding attempt to verify their association with a given nest. Body size measurements used in the analysis represent the mean for a given bird in a given year. Measurements of female mass during the pre-laying and laying periods were excluded from calculations of mean mass, since at this time female mass increases as eggs are formed. The five univariate measures of body size described above were also entered into a principal component analysis and principal component 1 (PC1) was used as a multivariate measure of body size (see for example Rising & Somers 1989).

Breeding adults were sexed according to the development of the brood patch (Bryant 1975b). If a colony had not been studied in the previous year then no attempt was made to estimate the age of breeding birds. House martins captured at colonies that had been intensively studied for at least one year previously were divided into two age classes, first year and older. Birds caught for the first time as breeding adults at these colonies were assumed to be in their first year and assigned a 'minimum known age' of one (Bryant 1979). A small number of birds had been ringed in previous years either as nestlings, or as breeding adults, and could thus be confidently assigned to one of the two age classes.

For each nest the following were recorded: date of first egg, clutch size, date of hatch (the day that the first nestling hatched for clutches that hatched over a period of more than 24 hours), brood size at hatch and after any manipulation, the number of nestlings fledged and the date of fledging. The latter is difficult to measure accurately since young were observed to return to the nest after they had fledged, and the adults would continue to feed them. Fledging date was generally taken as the first day that the nest was empty when checked during daytime (0900-2000hrs). If more than two days had elapsed between nest checks then the fledging date was estimated as the median of the two dates between which the young left the nest.

Nestlings were weighed and the length of the wing measured on the first occasion the nest was checked after hatching. This allowed the date of hatch of the first nestling to

be estimated at nests that were not inspected daily. House martin nestlings reach peak mass at 14-16 days after hatch, and thereafter decline in weight until fledging (Figure 2.4; Bryant & Gardiner 1979). To compare growth between broods of different sizes at fixed periods after hatch, nestlings were controlled at 15-16 days after the first chick had hatched (peak), and again at 25-26 days (fledging). The following measures were taken: mass, wing, keel, head and bill, and tarsus.

2.2.1 Brood manipulation experiments

Between 1987 and 1989 the size of first broods was manipulated by swapping nestlings between nests within five days of hatch. In 1987 and 1988 roughly a third of available nests were assigned to each of three manipulation categories: unmanipulated, reduced and enlarged. A smaller proportion of first broods was manipulated in 1989 because of the need to preserve a large number of unmanipulated broods for observations of nestling feeding.

The assignment of nests to manipulation categories was haphazard (in the terminology of Martin & Bateson 1986). Because first clutches at house martin colonies are laid over a period of more than one month, the opportunity to carry out manipulations depended on the availability of at least two broods hatching within 2-3 days. In the first year of the study, 1987, 1-3 nestlings were added or taken away from each manipulated brood. In 1988 and 1989 I used a fixed degree of manipulation, either plus two or minus two nestlings. Broods were thus adjusted relative to their original size, rather than to achieve a fixed size for enlarged and reduced broods. This method was adopted because it was the most practical, and assumes that the original clutch size was optimal for the laying female, time of the season or environmental conditions. The overall range of brood sizes produced varied between one and seven nestlings. The natural maximum first brood size for house martins in Central Scotland is five, but a pair is capable of raising up to seven young if the weather and food supply remain reasonable (D M Bryant, pers comm). By keeping the range of experimental brood sizes within the limit that house martins are known to be able to raise, I hoped to detect more subtle reproductive costs than an increased level of nestling mortality. In reality, parent birds are more likely to be faced with the choice of increasing their clutch size by one or two eggs, than an extra four or five (Linden & Møller 1989).

Small young that were fostered into another nest were marked with colour rings so they

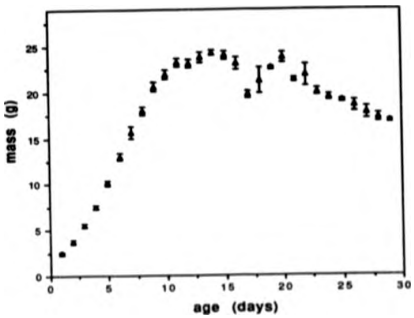


Figure 2.4 Growth of nestling house martins. Includes data from nestlings of known age from all manipulation classes (reduced, unmanipulated and enlarged) 1987-89, ± standard errors. Day 0 = day of hatching.

could be identified when they had grown sufficiently to be given BTO aluminium rings.

Between 1972 and 1983 brood manipulations were also carried out, for a different purpose (Hails & Bryant 1979, Bryant & Westerterp 1980, 1982, 1983a & b). Where nestlings were removed or added within 10 days of hatching I have considered the brood to be manipulated, and included it in the analysis discussed in section 2.3.5. Up until this time there is no significant difference in age-specific weights of nestlings in broods of different sizes, but thereafter the largest broods tend to hold lighter nestlings (Bryant & Gardiner 1979). Broods that were manipulated more than ten days after hatch have been excluded from consideration.

2.2.2 Measurement of food abundance

Food abundance was monitored using an insect suction trap (Taylor & Palmer 1972, Bryant 1973) sampling at 12.2m above ground level. The trap was permanently sited at the University of Stirling, at the centre of the study area but 15-23km away from the most distant study colonies. The measure of food abundance used was the settled volume in alcohol of insects caught in each 24hr period, the trap being emptied at 1000hrs daily.

Taylor (1973) reported a high positive correlation between the volume of insect catches at suction traps sited 80km apart. Thus it seems reasonable to assume that the suction trap at Stirling University gives an adequate measure of food abundance at the study colonies, all located less than 25km away. The possibility remains that local environmental conditions might influence food availability to a different extent at different house martin colonies.

Variables used in the analyses in this chapter are listed in appendix B.

2.3 Results

2.3.1 Variation in annual reproductive success

Between 1972 and 1989, house martins breeding in Central Scotland fledged between 0 and 9 young per pair per year. The mean annual reproductive success was 4.7 fledged young (206 pairings, standard error=0.15). This analysis includes only pairs raising unmanipulated first broods, and double brooded pairs where no change of male or female was known to have taken place for the second brood. Pairs that abandoned first clutches before hatching have been excluded.

Pairs attempting two broods per season fledged significantly more young and also laid the first egg significantly earlier than single brooded pairs (Table 2.1). In addition, there was a positive correlation between first egg date and annual reproductive success (1972-89, (Pearson correlation coefficient) -0.53, $p<0.001$, n=200; 87-9, (Spearman) -0.38, $p<0.01$, n=51). Thus both the date at which breeding starts, and the number of broods attempted per season have a significant effect on annual reproductive success.

The start of laying at house martin colonies is typically spread over a period of several weeks. Between 1972 and 1989, first clutches at the study colonies were started between 15th May and 15th August, although late first clutches may occasionally have been laid by birds that have moved from other colonies for a second brood. The mean date of first egg was the 10th June (330 pairings, standard error 0.98 days).

A one way analysis of variance indicated that the mean first egg date varied significantly between years ($F_{15,314}=1.99$, $p<0.05$). Use of the Tukey range test indicated that this was due to a significant difference at the 0.05 level between mean lay date in 1989 (19th June) and 1973 (2nd June). Data from 1981 were excluded because the date of the first egg was known for only one pair.

2.3.2 Individual characteristics and annual reproductive success (1987-89)

The following analysis was confined to pairs raising unmanipulated first broods. Most birds were captured for the first time as breeding adults, and assigned a 'minimum known age' of one. Of 32 males for which age could be estimated (see section 2.2), only six were known not to be yearlings. In the case of females, six of 34 aged adults were

Table 2.1 Mean annual reproductive success (total fledged young per year) and first egg date by the number of broods attempted per season for house martins breeding in Central Scotland 72-89 (unmanipulated broods only)

	No second brood	Second brood attempted	t	2-tailed p
Annual reproductive success (se)	3.1 (0.1)	5.8 (0.2)	13.37	0***
First egg date (se, days)	June 30th (2.0)	May 31st (1.0)	9.12	0***
n	82	124		

se = standard error; *** $p<0.001$

definitely older birds. Thus for both sexes, less than 20% of the birds used in the analysis were known to have survived beyond their first breeding year. Since previous studies found a higher incidence of adults in the '2+' age class (Bryant 1979, 1988a, 1989), this implies either that a number of older birds have been wrongly assigned to the yearling age class, or that there has been a change in the age structure of the house martin population breeding in Central Scotland, with less birds surviving beyond their first breeding season. The small numbers of older birds found in the present study also means that age effects can be identified, but not excluded.

(i) Body size

(a) Annual Reproductive Success

After all age classes were lumped, no relationships were found between the body size of males and females and their annual reproductive success (Table 2.2). For older males, a negative correlation between PC1 and annual reproductive success suggests that smaller individuals in this category may be more successful, but the sample size was small. In addition, this analysis involved a large number of correlations, and it is possible that some significant results occur by chance alone.

(b) Number of reproductive attempts

No body size measure of males or females was found to be related to the number of broods attemped per season when all age classes were considered together (Table 2.3). Yearling males attempting two broods had significantly shorter tarsi, whereas double-brooded females in this age class had significantly larger head and bill measurements.

(c) First egg date

When all age classes were lumped, mass and wing length of males were found to be negatively associated with first egg date (Table 2.4), indicating that larger individuals paired with females that laid earlier. Among yearling males the relationship between body size and first egg date was more ambiguous. Wing length was negatively related to first egg date, but keel length showed a positive association with this parameter.

For females, when all age classes were lumped, and when older birds were considered alone, no body size measure was related to first egg date (Table 2.4). In the yearling class, keel length and tarsus length were positively related to first egg date, implying that smaller birds laid earlier.

Table 2.2 Pearson correlation coefficients for the relationships between body size and annual reproductive success (total fledged young per year) in house martins

Sex	Age	Size measure*	Correlation with annual reproductive success (r)	2-tailed p
Male	all	mass (47)	0.16	0.29
		wing (47)	-0.08	0.60
		keel (47)	0.12	0.44
		head & bill (47)	0.06	0.71
		tarsus (42)	-0.11	0.49
		PC1 (42)	-0.08	0.64
	1	mass (26)	0.29	0.15
		wing (26)	0.10	0.62
		keel (26)	0.00	0.99
		head & bill (26)	0.20	0.32
		tarsus (23)	-0.27	0.21
		PC1 (23)	-0.04	0.88
	2+	mass (6)	-0.55	0.25
		wing (6)	-0.42	0.41
		keel (6)	-0.36	0.49
		head & bill (6)	-0.58	0.23
		tarsus (6)	-0.78	0.06
		PC1 (6)	-0.90	0.01*

Table 2.2 continued

Sex	Age	Size measure+	Correlation with annual reproductive success (r)	2-tailed p
Female	All	mass (47)	0.14	0.37
		wing (48)	0.19	0.20
		keel (47)	0.10	0.50
		head & bill (48)	0.16	0.29
		tarsus (45)	0.00	0.98
		PC1 (44)	0.21	0.17
	1	mass (28)	0.09	0.63
		wing (28)	0.21	0.28
		keel (27)	0.09	0.67
		head & bill (28)	0.32	0.10
		tarsus (27)	-0.10	0.64
		PC1 (26)	0.16	0.42
	2+	mass (5)	-0.03	0.96
		wing (6)	-0.34	0.51
		keel (6)	0.14	0.79
		head & bill (6)	-0.08	0.88
		tarsus (5)	-0.11	0.86
		PC1 (5)	0.33	0.59

Units of body size measures as follows: mass (g); wing, keel, head & bill, tarsus (mm); PC1 = principal component 1 is a multivariate measure of body size which has no meaningful units; + sample size given in brackets; *, $p<0.05$.

Table 2.3 Variation in body size of male and female house martins with the number of broods attempted per season

Sex	Age	Size measure	Mean size: No 2nd brood	Mean size: 2nd brood attempted	t	2-tailed p
Male	all	mass	18.6	18.3	0.97	0.34
		wing	112	112	0.10	0.92
		keel	19.5	19.6	0.75	0.46
		head & bill	26.4	26.7	1.41	0.17
		tarsus	11.8	11.7	0.59	0.56
		PC1			0.64	0.53
			(n=34)	(n=13)		
	1	mass	18.6	18.6	0.04	0.97
		wing	112	113	0.45	0.66
		keel	19.7	19.7	0.09	0.93
		head & bill	26.6	26.6	0.23	0.82
		tarsus	12.0	11.7	2.08	0.05*
		PC1			0.91	0.37
			(20)	(6)		
	2+	mass	19.1	18.7	0.46	0.67
		wing	114	114	0.14	0.90
		keel	19.4	19.5	0.09	0.94
		head & bill	26.4	26.2	0.76	0.49
		tarsus	11.6	11.6	0.12	0.91
		PC1			0.13	0.92
			(4)	(2)		

Table 2.3 (continued)

Sex	Age	Size measure	Mean size: No 2nd brood	Mean size: 2nd brood attempted	t	2-tailed p
Female	all	mass	19.1	19.1	0.05	0.96
		wing	112	112	0.04	0.97
		keel	19.4	19.5	0.53	0.60
		head & bill	26.2	26.4	0.92	0.36
		tarsus	11.8	11.6	1.14	0.26
		PC1			0.30	0.77
			(35)	(13)		
	1	mass	18.9	18.9	0.08	0.94
		wing	112	113	1.16	0.26
		keel	19.4	19.7	0.80	0.43
		head & bill	26.3	26.8	2.27	0.03*
		tarsus	11.8	11.7	0.71	0.49
		PC1			0.56	0.58
			(22)	(6)		
	2+	mass	20.8	19.0	2.83	0.07
		wing	111	109	0.64	0.56
		keel	19.3	19.7	0.49	0.65
		head & bill	26.2	26.5	0.49	0.65
		tarsus	12.0	11.3	2.71	0.07
		PC1			1.47	0.24
			(3)	(3)		

Units of body size measures as follows: mass (g); wing, keel, head & bill, tarsus (mm); PC1 = principal component 1 is a multivariate measure of body size which has no meaningful units. Symbols: *, $p<0.05$.

Table 2.4 Spearman non-parametric correlation coefficients for the relationships between body size and first egg date for male and female house martins (1987-89)

Sex	Age	Mass	Wing	Keel	Head & bill	Tarsus	PC1
Male	all	-0.29	-0.21	-0.01	0.01	0.01	-0.09
p		0.004**	0.049*	0.893	0.935	0.941	0.409
n		91	91	91	90	82	81
	1	-0.26	-0.30	0.31	0.02	0.08	0.14
		0.065	0.035*	0.029*	0.912	0.580	0.369
		50	50	50	49	45	44
	2+	-0.20	0.29	-0.14	0.25	0.06	0.08
		0.520	0.337	0.649	0.406	0.840	0.713
		13	13	13	13	13	13
Female	all	-0.11	-0.14	0.19	0.10	0.14	0.05
p		0.281	0.188	0.072	0.346	0.191	0.636
n		93	96	95	96	92	89
	1	-0.18	-0.11	0.33	0.18	0.28	0.18
		0.202	0.450	0.017*	0.202	0.046*	0.209
		52	52	51	52	51	50
	2+	-0.01	0.11	0.01	-0.12	0.21	-0.02
		0.979	0.714	0.968	0.687	0.519	0.938
		11	13	13	13	12	11

Notes: * $p<0.05$; ** $p<0.01$

(ii) Age

(a) Annual Reproductive Success
No relationship was found between the age of males or females and their annual reproductive success (Table 2.5).

(b) Number of reproductive attempts
No significant relationship was found between the age of males and females and the number of broods attempted per season (Table 2.6), although there was some suggestion that older females were more likely to attempt two broods. Three of six older females (50%) laid a second clutch, compared with only 6 of 28 (21%) yearling females.

(c) First egg date
Older females were found to begin laying significantly earlier than younger females (Table 2.7), but there was no corresponding difference for males.

2.3.3 Interval between breeding attempts

The following analysis includes only double brooded pairs where the female was not known to have changed between the first and second brood, although eight pairs where a female acquired a different mate for a second brood have been included. Two measures of the interval between breeding attempts are considered. The clutch interval is the number of days between the laying of the last egg of the first clutch and the first egg of the second clutch. Breeding interval is the number of days between the fledging of first brood young and the first egg of the second clutch; this measure was available only for the years 1987 to 1989.

Considering the entire data set (1972-1989), the clutch interval increases with the size of the first brood (for enlarged or reduced broods the brood size after manipulation was used in the analysis), and the number of nestlings fledged from the first brood, with the number of fledglings explaining slightly more of the variation in clutch interval (Table 2.8). When manipulated and unmanipulated broods are considered separately the same trends are apparent, but both brood size and number fledged explain more of the variation in clutch interval in manipulated broods. Mean clutch interval is shortest for pairs raising reduced first broods, and longest for pairs with enlarged first broods (Table 2.9).

Table 2.5 Mean annual reproductive success (total number of fledged young per season) of male and female house martins by age

Sex	Age	Annual reproductive success (±se)	n	t	2-tailed p
Male	1	3.5 (0.3)	26	0.49	0.64ns
	2+	3.0 (1.0)	6		
Female	1	3.6 (0.3)	28	0.09	0.93ns
	2+	3.5 (0.3)	6		

Table 2.6 Number of broods attempted per season by male and female house martins of different ages

Sex	Age	Number of pairs		X^2	2-tailed p
		No 2nd clutch	2nd clutch attempted		
Male	1	20	6	0.00	1.00ns
	2+	4	2		
Female	1	22	6	0.86	0.35ns
	2+	3	3		

notes: ns, not significant

Table 2.7 Median values of first egg date by age for male and female house martins (1987-89)

Sex	Median date of first egg		Mann-Whitney Z	2-tailed p
	Age=1	Age=2+		
Male	June 10th	June 5th	-0.86	0.39
n	50	13		
Female	June 13th	May 30th	-2.54	0.01*
n	52	13		

* $p<0.05$

Table 2.8 Correlation coefficients for the relationships between clutch interval, breeding interval, and three measures of reproductive success in house martins

Manipulation category	Size of 1st clutch (r)	Size of 1st brood (r) before manip	Size of 1st brood (r) after manip	Number fledged 1st brood (r)	n
Clutch interval[+]					
all broods (72-89)	-0.02		0.38***	0.42***	152
unmanip (72-89)	0.04	0.21*		0.23**	120
manip (72-89)	-0.11	-0.23	0.55***	0.66***	32
all (87-89)	-0.15		0.16	0.37*	31
unmanip (87-89)	-0.10	0.13		0.30	14
Manip (87-89)	-0.27	-0.36	0.18	0.41	17
Breeding interval[$]					
all (87-89)	-0.19		0.14	0.26	30
Unmanip (87-89)	-0.00	-0.04		0.07	14
Manip (87-89)	-0.35	-0.40	0.16	0.36	16

+ Pearson's parametric correlation coefficient; $ Spearman's non-parametric coefficient; * p<0.05; **p<0.01; ***p<0.001; 'all' refers to manipulated (manip) and unmanipulated (unmanip) broods combined, and for manipulated broods the size of the brood after manipulation has been entered in the analysis.

Table 2.9 Average duration of clutch and breeding interval in house martin first broods of different manipulation classes

Manipulation	Mean clutch interval (days ± se)		Median breeding interval (days)
	Years 72-89	87-89	87-89
Reduced	45.3 (1.3)	46.7 (1.7)	4
n	23	12	11
Unmanipulated	49.6 (0.5)	48.1 (1.3)	6
n	121	14	14
Enlarged	53.7 (1.1)	51.8 (1.3)	10
n	9	5	5
ANOVA:			
between years	$F_{13,129}=1.10$	$F_{2,24}=0.74$	K-W$^+$ $X^2=0.33$
between manipulation	$F_{2,129}=10.58$***	$F_{2,24}=1.83$	K-W $X^2=4.88$

+ Kruskal-Wallis one way non-parametric anova; *** $p<0.001$; se standard error

For data collected between 1987 and 1989, the only significant correlation is a positive relationship between the number fledged from the first brood and clutch interval, when pairs in all manipulation classes were lumped (Table 2.8). When unmanipulated and manipulated broods were considered separately no significant correlations emerged. However, pairs raising reduced broods showed, on average, the shortest clutch and breeding intervals, and pairs raising enlarged broods had the longest average clutch and breeding intervals (Table 2.9). The sample size for breeding interval is less than that for clutch interval because in one case I considered that my interference caused nestlings to fledge early.

A two way analysis of variance indicated no significant difference in clutch interval between the years 1972 and 1989, but significant differences in clutch interval between manipulation classes (Table 2.9). The Tukey range test showed that this was due to a significant difference in mean clutch interval ($p<0.05$) between pairs raising reduced broods and pairs raising unmanipulated broods, and also between pairs raising reduced and enlarged broods. There was no difference between pairs raising unmanipulated and enlarged broods. For the 1987-89 data there were no significant differences in clutch interval or breeding interval between pairs in the three manipulation classes, although the trends were the same (Table 2.9).

The implication is that reducing the size of the first brood shortens the clutch interval, and tends to reduce the breeding interval, although the effect on the latter parameter was not significant. The breeding interval was normally positive, that is, the second clutch was started after the first brood young had fledged. Four pairs however laid the first egg of the second clutch before first brood young had fledged. These included two broods where the number of young was experimentally reduced, from respectively three to one, and five to two young; and also two natural broods where the number of young fledged was less than the clutch size laid. In one case three eggs were laid, two young hatched but only one fledged; in the other, four eggs were laid and three young fledged.

2.3.4 Nestling period, growth and mortality

Data on nestling period and growth were available only for 1988 and 1989. Information on nestling mortality was available for 1972 to 1989.

The nestling period is the number of days between the hatching of the first nestling and the fledging of the last nestling. Only pairs that fledged at least one offspring from the

first brood have been considered in this analysis. A two way analysis of variance indicated no significant difference in the nestling period between manipulation classes or between years, although average nestling periods tended to be shortest in reduced broods, and longest in enlarged broods (Table 2.10). There were no apparent relationships between nestling period and the size of the first clutch, the size of the first brood, or the number fledged from the first brood (Table 2.11). Thus the length of time that the young were in the nest seemed to be independent of brood size.

Table 2.12 shows the relationships between the average mass and wing length of first brood nestlings and three measures of brood size: the size of the first clutch, the brood size (after any manipulation), and the number fledged. The sample sizes for fledging measurements are smaller because I discontinued these measurements for broods in natural nests. The removal of nestlings at an advanced stage of growth from natural nests tended to cause them to fledge early.

In unmanipulated first broods, there was no relationship between nestling mass at peak or fledging and any measure of brood size. However, wing length at peak was positively correlated with the number of nestlings fledged, suggesting that the wings of nestlings in larger natural broods grew more rapidly to peak.

In manipulated broods there was no relationship between any measure of brood size and wing length, but mean peak mass was negatively correlated with the size of the first brood and the number of nestlings fledged.

Average peak nestling mass tended to be highest in reduced broods and lowest in enlarged broods. However, a two way analysis of variance indicated no significant difference in any nestling growth parameter between manipulation classes. There were however significant differences in wing length between years (Table 2.13). Mean wing lengths at peak and fledging were significantly shorter in 1988 than in 1989 (respective means and standard errors: peak wing, 55.3 ± 0.97, 59.0 ± 1.0; fledging wing, 97.2 ± 1.1, 101.3 ± 0.65).

Nestling mortality was measured both as the number and the proportion of nestlings per brood that died before fledging. Both measures of nestling mortality differed significantly between manipulation classes when data from 1972 to 1989, and 1987 to 1989 were considered (Table 2.14). Pairwise Mann-Whitney U tests between categories indicated that nestling mortality was significantly higher in enlarged broods compared with both

Table 2.10 Average duration of first brood nestling periods in house martin broods of different manipulation classes (1988-89)

Manipulation class	nestling period, days (se)	n
Reduced	27.7 (0.36)	7
Unmanipulated	28.4 (0.38)	36
Enlarged	28.7 (1.29)	7
ANOVA: between manipulation classes between years	$F_{2,44}$=1.79 $F_{1,44}$=0.30	7

se, standard error

Table 2.11 Spearman correlation coefficients for the relationships between first brood nestling period and three measures of annual reproductive success in house martins

	All broods	Unmanipulated	Manipulated
First clutch size	-0.06	-0.01	-0.18
p	0.687	0.505	0.532
First brood size	0.10	-0.00	0.23
	0.967	0.908	0.597
Number fledged, first brood	0.09	0.09	0.12
	0.538	0.420	0.695
n	50	36	14

Table 2.12 Spearman correlation coefficients for the relationships between nestling growth and three measures of reproductive success of the first brood in house martins (1988-89)

Manipulation category	Mean peak mass	Mean fledging mass	Mean peak wing	Mean fledging wing
All				
clutch size	-0.22	-0.24	0.14	-0.02
brood size	-0.35*	-0.38	0.20	-0.08
number fledged	-0.24	-0.20	0.31*	0.01
n	48	35	48	35
Unmanipulated				
clutch size	-0.20	-0.20	0.24	0.09
brood size	-0.22	-0.16	0.29	0.23
number fledged	-0.06	0.24	0.48**	0.36
n	34	24	34	24
Manipulated				
clutch size	-0.30	-0.34	-0.08	-0.43
brood size	-0.61*	-0.50	0.22	-0.50
number fledged	-0.55*	-0.49	0.17	-0.57
n	14	11	14	11

'All' refers to manipulated and unmanipulated broods combined, and for manipulated broods the brood size after manipulation has been entered in the analyses; *p<0.05; **p<0.01

Table 2.13 Average values of four nestling growth parameters in house martins by manipulation category (1988-89)

Manipulation category	Mean peak mass (g)	Mean fledging mass (g)	Mean peak wing (mm)	Mean fledging wing (mm)
Reduced	25.2	20.0	55.1	99.6
se	0.57	0.58	1.6	1.5
n	7	5	7	5
Unmanipulated	24.2	19.2	57.7	99.6
	0.42	0.18	0.97	0.96
	34	24	34	24
Enlarged	23.1	18.7	57.3	97.1
	0.91	0.83	1.41	1.27
	7	6	7	6
2-way ANOVA				
between manipulation classes	$F_{2,42}$=1.5	$F_{2,29}$=2.2	$F_{2,42}$=0.4	$F_{2,29}$=0.8
between years	$F_{1,42}$=1.3	$F_{1,29}$=1.7	$F_{1,42}$=5.5*	$F_{1,29}$=9.5**

se standard error; *p,0.05; **p<0.01

Table 2.14 Variation in nestling mortality in house martins with manipulation category

Manipulation category	Statistic	Number of nestlings dead		Proportion of nestlings dead	
		Years 72-89	87-89	72-89	87-89
reduced broods	mean	0.20	0.16	0.08	0.07
	median	0	0	0	0
	range	0-2	0-2	0-1	0-1
	n	35	19	35	19
unmanipulated	mean	0.25	0.46	0.08	0.14
	median	0	0	0	0
	range	0-4	0-4	0-1	0-1
	n	212	52	212	52
enlarged	mean	1.05	1.47	0.19	0.27
	median	0	1	0	0.1
	range	0-6	0-6	0-1	0-1
	n	22	15	22	15
K-W+	X^2	12.51**	11.21**	9.53**	8.80*

+Kruskal-Wallis one way non-parametric anova between manipulation classes; *p<0.05; **p<0.01

unmanipulated and reduced broods. There was no significant difference in nestling mortality between reduced and unmanipulated broods (Table 2.15).

Thus nestlings in enlarged broods did not spend longer in the nest than those in unmanipulated or reduced broods. They tended to reach lower peak weights, but not fledging weights, and also suffered increased mortality in the nest.

2.3.5 Second breeding attempts

This section considers the effect of experimental manipulations of brood size on the production of second broods. Before carrying out the analysis the data were controlled for lay date. It has already been shown that pairs attempting two broods begin to lay significantly earlier in the season than single-brooded pairs (Table 2.6). I have therefore included in the analysis only those pairs that laid on or before the observed first clutch threshold. This is the latest date that a pair raising an unmanipulated first brood and attempting a second clutch began to lay their first clutch (Figure 2.1; Section 2.1.2). Between 1987 and 1989 the estimated first clutch threshold was June 23rd, and between 1972 and 1983, and 1972 and 1989, it was was June 27th.

Considering data from 1987 to 1989, there were significant differences between the three manipulation categories in the proportion of pairs that went on to lay a second clutch (Table 2.16). Separate pairwise comparisons of the three manipulation classes indicate that pairs raising reduced first broods are more likely to attempt a second clutch than pairs raising unmanipulated first broods (X^2=5.7, $p<0.05$) . No difference was found between pairs with enlarged and reduced first broods (X^2=3.03, $p>0.05$), or between pairs with unmanipulated and enlarged broods (X^2=0.00, $p>0.05$).

Between 1972 and 1983, no significant differences were found between the proportion of pairs attempting a second clutch in the three manipulation categories (Table 2.16).

Lumping the entire data set for 1972 to 1989 produces different results. There were no significant differences between manipulation categories in the proportion of pairs that attempted a second clutch (Table 2.16). However, separate pairwise comparisons of the three categories indicated that there were significant differences between pairs with enlarged first broods and both unmanipulated (X^2=4.6 $p<0.05$) and reduced (X^2=4.5, $p<0.05$) broods. There were no differences between reduced and unmanipulated broods (X^2=0.42, $p>0.05$).

Table 2.15 Mann-Whitney Z scores for the difference in the number and proportion of dead nestlings in house martin first broods from different manipulation classes

Comparison	Number of nestlings dead		Porportion of nestlings dead	
	Years 72-89	87-89	72-89	87-89
Enlarged-unmanipulated	-3.40***	-2.57**	-2.96**	-2.12*
Enlarged-reduced	-2.69**	-3.06**	-2.38*	-2.86**
Unmanipulated-reduced	-0.42	-1.45	-0.38	-1.41

Sample sizes as given in Table 2.14; * $p<0.05$; ** $p<0.01$; *** $p<0.001$

Table 2.16 The proportion of house martin pairs from different manipulation categories that attempted a second clutch

Timespan (breeding years)	Number of pairs			X^2	First clutch threshold
	Reduced	Unmanipulated	Enlarged		
1987-89					
no 2nd clutch	5	25	9		
2nd clutch attempted	12	14	6	6.0*	June 23
% 2nd clutches	71	36	40		
1972-83					
no 2nd clutch	2	34	3		
2nd clutch attempted	11	116	4	2.0	June 27
% 2nd clutches	85	77	57		
1972-89					
no 2nd clutch	8	60	12		
2nd clutch attempted	23	130	10	5.5	June 27
% 2nd clutches	74	68	45		

* $p<0.05$

Thus data from 1987-89 indicate that reducing the size of the first brood increased the chance that a given pair might attempt a second brood, but that enlarging the first brood had no effect on the probability of a second clutch. Despite the lack of significant differences between manipulation categories over the period 1972-83, considering the data set from 1972-89 as a whole, changes the results. Separate pairwise comparisons then indicated that enlarged first broods were less likely to be followed by second clutches than unmanipulated and reduced broods, but that reducing the size of the first brood did not alter the probability that a given pair would attempt a second clutch. Both data sets indicated that manipulation of first brood size had an effect on the chance of a given pair producing a second clutch, but the effect differed with the timespan of the data included.

To attempt to explain these results I looked at the variation in the overall proportion of pairs with unmanipulated first broods that went on to produce a second clutch across years. There is a significant negative correlation between these two variables (Figure 2.5, Table 2.17), indicating a decline in the proportion of pairs attempting a second clutch between 1972 and 1989. Between 1987 and 1989, less than 40% of pairs went on to attempt a second clutch, thus over this timespan it might be predicted that the effects of manipulation experiments are manifest most strongly in reduced broods, since most unmanipulated first broods will not be followed by a second clutch. Between 1972 and 1983, 50% or more unmanipulated first broods were followed by a second clutch, thus when data from these years are included it might be expected that the effects of brood manipulations would show up more strongly in enlarged broods.

2.3.6 Variation in average annual reproductive success of house martins in Central Scotland 1972-1989

If the proportion of house martin pairs attempting a second clutch has declined between 1972 and 1989, then what is the cause of this, and has it lead to a concurrent decline in annual reproductive success for house martins breeding in Central Scotland?

Correlation matrices showing the relationships between reproductive success, the percentage of pairs attempting second clutches, the timing of breeding and food abundance are given in Tables 2.17 and 2.18. The analysis is based on average yearly values of the various parameters calculated for unmanipulated pairs only. Table 2.17 includes data from 1972 to 1989, and Table 2.18 shows the same analysis for 1972-83.

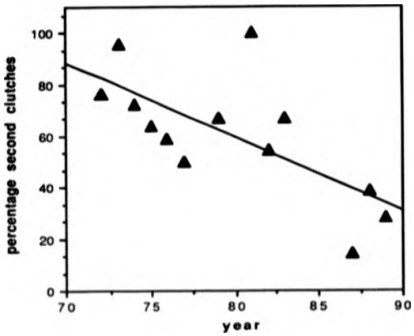


Figure 2.5 The percentage of house martin pairs attempting a second clutch in relation to breeding year. Includes only pairs raising unmanipulated first broods

Table 2.17 Pearson correlation coefficients for the relationships between measures of the timing and success of breeding and food supply for house martins in Central Scotland for the years 1972-89

YEAR	-0.69 0.009** 13						
LMJJL	0.60 0.040* 12	-0.42 0.132 14					
MDOE	-0.83 0.001** 12	0.65 0.021* 12	-0.52 0.099 11				
MCI	0.11 0.749 11	-0.40 0.222 11	0.44 0.200 10	0.07 0.830 11			
FCT	0.31 0.346 11	-0.63 0.039* 11	-0.10 0.777 10	0.06 0.855 11	0.26 0.436 11		
SCT	0.34 0.30 11	-0.31 0.356 11	0.39 0.270 10	0.01 0.986 11	0.66 0.028* 11	0.32 0.344 11	
ARS	0.94 0.000*** 13	-0.66 0.015* 13	0.62 0.032* 12	-0.82 0.001** 12	0.22 0.524 11	0.28 0.400 11	0.49 0.129 11
	PSC	YEAR	LMJJL	MDOE	MCI	FCT	SCT

Initials: ARS annual reproductive success; PSC the percentage of unmanipulated pairs attempting a second clutch; LMJJL the log of average daily suction trap catches in June and July; MDOE the mean date of first egg; MCI the average clutch interval; FCT the average first clutch threshold; SCT the average second clutch threshold. Values beneath coefficients are respectively probability and sample size; * $p<0.05$; ** $p<0.01$; *** $p<0.001$

Table 2.18 Pearson correlation coefficients for the relationships between measures of the timing and success of breeding and food supply for house martins in Central Scotland for the years 1972-83

YEAR	-0.18 0.621 10						
LMJJL	0.27 0.484 9	-0.11 0.742 11					
MDOE	-0.72 0.029* 9	0.02 0.954 9	-0.25 0.554 8				
MCI	0.03 0.941 9	-0.49 0.174 9	0.44 0.278 8	0.21 0.585 9			
FCT	0.04 0.920 9	-0.56 0.120 9	-0.48 0.228 8	0.60 0.090 9	0.16 0.676 9		
SCT	0.49 0.180 9	-0.37 0.330 9	0.38 0.348 8	-0.07 0.854 9	0.64 0.063 9	0.14 0.728 9	
ARS	0.97 0.000*** 10	-0.18 0.617 10	0.34 0.375 9	-0.73 0.026* 9	0.15 0.705 9	-0.05 0.887 9	0.58 0.104 9
	PSC	YEAR	LMJJL	MDOE	MCI	FCT	SCT

Initials: ARS annual reproductive success; PSC the percentage of unmanipulated pairs attempting a second clutch; LMJJL the log of average daily suction trap catches in June and July; MDOE the average date of first egg; MCI the average clutch interval; FCT the average first clutch threshold; SCT the average second clutch threshold. Values beneath coefficients are respectively probability and sample size; * $p<0.05$; ** $p<0.01$; *** $p<0.001$

Between 1972 and 1989 there were significant declines in average annual reproductive success and the observed first clutch threshold, as well as the percentage of second clutches. Over the same period the average first egg date became later. With the exception of an increasing mean first egg date, the same trends were apparent between 1972 and 1983, but they were not significant.

The average annual reproductive success was strongly correlated with the percentage of pairs that attempted a second clutch, both between 1972-89, and 1972-83. In addition, over both timespans, both average annual reproductive success and the percentage of second clutches were negatively related to the mean first egg date, suggesting that the time at which breeding starts is a major factor determining reproductive success in house martins.

The model discussed in section 2.1.2 predicts that changes in the first egg date and the first and second clutch thresholds should affect the percentage of pairs that attempt a second clutch. The observed negative relationship between first egg date and the percentage of second clutches supports this. Changes in the end of the season, measured by the first and second clutch thresholds, are less clear cut. Between 1972-83, and 1972-89 there was a non-significant tendency for the percentage of second clutches to increase as the first and second clutch thresholds fell later in the season, also in accordance with the predictions of the model. Both these thresholds also tended to fall earlier with year, with the negative relationship between first clutch threshold and year falling below the 0.05 significance level between 1972-89. It is also notable that the second clutch threshold tends to fall later in years when the mean clutch interval is longer.

What factors might account for the observed variation in annual reproductive success and the timing of breeding in house martins in Central Scotland between 1972-1989? Although there was no significant tendency for food abundance to change over this time period (Table 2.19), there were indications that food supply influenced components of breeding success.

Between 1972-89, average annual reproductive success and the percentage of second clutches increased with the total food abundance in June and July, the time when first brood young were in the nest. Neither of these variables were found to be influenced by food supply in any other month or combination of months (Table 2.19). There was also a negative relationship between food abundance in August and the first clutch threshold

Table 2.19 Pearson correlation coefficients for the relationship between the log of average daily food supply per month and components of timing and success of breeding in house martins in Central Scotland 1972-89

	May	June	July	August	May & June	June & July	July & August
PSC	-0.32 0.294 13	0.55 0.062 12	0.55 0.06 12	0.15 0.631 13	-0.19 0.548 12	0.60 0.04* 12	0.34 0.286 12
MDOE	0.08 0.798 12	-0.40 0.218 11	-0.49 0.126 11	-0.12 0.704 12	-0.04 0.915 11	-0.52 0.099 11	-0.28 0.396 11
FCT	-0.10 0.772 11	-0.15 0.677 10	-0.01 0.976 10	-0.66 0.027* 11	-0.15 0.677 10	-0.10 0.777 10	-0.49 0.151 10
SCT	0.07 0.846 11	0.40 0.254 10	0.36 0.306 10	-0.16 0.642 11	0.24 0.502 10	0.39 0.270 10	0.03 0.944 10
ARS	-0.11 0.724 13	0.55 0.065 12	0.60 0.041* 12	0.06 0.850 13	0.01 0.968 12	0.62 0.032* 12	0.28 0.376 12
YEAR	0.24 0.385 15	-0.42 0.134 14	-0.38 0.180 14	0.18 0.528 15	0.10 0.731 14	-0.42 0.132 14	-0.04 0.902 14

Intials: PSC percentage of unmanipulated pairs attempting a second clutch; MDOE average date of first egg; FCT average first clutch threshold; SCT average second clutch threshold; ARS average annual reproductive success; YEAR breeding year. Values beneath coefficients are respectively probability and sample size; *$p<0.05$

1972-1989 (Table 2.19), suggesting that low levels of food abundance late in the season may influence the decision of late layers to attempt a second brood.

2.4 Discussion

Both at an individual and a population level double-broodedness is implicated as the annual breeding strategy with the highest fitness benefits for house martins. Pairs that attempted two broods in a season fledged significantly more young on average than those that attempted only one brood, and in years when higher proportions of the study population attempted a second brood the average annual reproductive success per pair was also higher. Pairs that laid earlier also enjoyed higher annual reproductive success, and these are also pairs that had sufficient time to attempt a second brood.

Fitness has here been measured as the total number of fledged young per pair per season. Not enough returning young were recaptured in the present study to assess whether pairs that fledged more offspring also contributed more recruits to the breeding population. Bryant (1979) found that for young returning to a single study colony there was no significant difference in the proportions from small or large broods, or from broods reared by parents of different ages. Few second brood young returned, but he suggested that they were more likely to have settled elsewhere, rather than experiencing a mortality as heavy as that implied by the observed return rates. This the pattern found among second brood house martins in central Europe (Rheinwald & Gutscher 1969). Daan *et al* (1988) have shown for the kestrel that the reproductive value of eggs in terms of producing recruits to the next breeding season declines with increasing date in the season. It has also been shown for great tits that nestlings born later in the season have a lower chance of being recovered locally in the next breeding season (Perrins 1965, Kluyver *et al* 1977, Smith *et al* 1989). Thus the suggestion is that young born later in the season have a lower chance of surviving to breed. Even though second brood young in house martins probably do have a lower chance of surviving to breed than first brood young, it still seems reasonable to assume that the number of offspring recruited to the breeding population is broadly proportional to the number of fledged young since this has been shown for other species (Newton 1989).

2.4.2 Individual attributes and reproductive success

The results of an analysis of the relationships between individual attributes and annual reproductive success for house martins between 1987 and 1989 are summarised in Table 2.20. There was only one clear result; that older females laid earlier than first year

Table 2.20 Relationships between body size, age and annual reproductive success in house martins: the results of the present study (**bold type**) compared with those of previous work on the same species (Bryant 1988a, single type)

		Annual Reproductive Success	Number of breeding attempts	First egg date
Males	size	(-) PC1 0 + MA	(-) TS Y	- MA WL * - MA KL
	age	+	+	-
Females	size		(+) HB Y + KL	(+) KL TS Y
	age	+		- -

Notes: - or + signs indicate the direction of statistically significant relationships, the absence of a symbol means that no significant relationship was found. Signs in brackets indicate that the effect was significant in one age class only, as indicated by Y = first year birds, O = second year and older. Other letters indicate which size parameter was related to the measure of reproductive success concerned: PC1 = principal component 1 (section 2.2), MA = mass, WL = wing length, KL = keel length, TS = tarsus.
* relationship less clear when yearlings were considered alone, see section 2.3.2

females. Larger males also seemed to pair with females that laid earlier, although this relationship did not persist clearly when yearling males were considered alone. There were in fact some hints that smaller body size in males was associated with increased reproductive success. The suggestion from this study that older females were more likely to attempt two broods was supported by Bryant (1979), but not by the analysis in Bryant (1988a).

Table 2.20 also allows the results of the present study to be compared with those of earlier studies on house martins breeding at the Naemoor colony (Bryant 1979, 1988a). The main differences are the hint that small size may be associated with increased annual reproductive success in males, in contrast to the findings of Bryant (1988a); and the lack of any relationship between the age of males or females and any component of reproductive success. The suggestion from this study that smaller yearling females tended to lay earlier was also not supported by Bryant (1988a).

It would be unwise to draw any firm conclusions from the present study, because of the relatively small sample sizes, and the uncertainty of age measurements for most breeding adults. Since many birds were captured for the first time as breeding adults, I may have under-estimated the number of birds in the '2+' age category. This means that differences between breeding adults in the two age classes may not have been clearly identified and could explain why the results of this study sometimes conflict with those of earlier work on house martins (Bryant 1979, 1988a). Alternatively, there may have been a change in the age structure of the house martin population in Central Scotland, with few individuals surviving after their first breeding year. A third possibility is that changing environmental conditions, either on the breeding grounds or in the wintering areas, may have brought about changes in the observed relationships between individual attributes of house martins and annual reproductive success, especially since selection on body size has been shown to occur in a closely related migratory hirundine, the sand martin (Jones 1987a).

As far as lifetime reproductive success of house martins is concerned, Bryant (1989) found that for both males and females, the total number of eggs incubated and the lifespan (in years) explained the largest amount of variation. In addition, large size in males but not females was also associated with increased lifetime reproductive success.

2.4.3 The effects of brood manipulations

Experimental manipulations of brood size in house martins were found to have effects

on the interval between breeding attempts, the growth and mortality of nestlings, and the probability of a second clutch.

(i) Interval between breeding attempts

For house martins raising both natural and manipulated broods the clutch interval increased with both the number of nestlings, and the number of fledglings, from the first brood. Experimental manipulations seemed to exaggerate the effects of natural variation in clutch interval with clutch size; clutch intervals were found to be shortest for pairs raising reduced first broods and longest for pairs raising enlarged broods. An effect of first brood size on the interval between breeding attempts has been noted in both natural and manipulated broods of a number of multi-brooded bird species (natural broods: house sparrow, Mcgillivray 1983; song sparrow, Smith & Roff 1980; captive budgerigars Stamps *et al* 1985; eastern bluebird, Pinkowski 1977; manipulated broods: great tit, Tinbergen 1987, Smith *et al* 1989; house sparrow, Hegner & Wingfield 1987; swallow, Thompson 1992, house martin, this study), although this is not always the case (house wrens: Finke *et al* 1987; Robinson & Rotenberry 1991). Enlarged brood size may even cause delayed laying in the next year in single brooded species, as was found for rooks (Roskaft 1985).

The clutch interval spans the incubation and nestling periods and the breeding interval. Assuming that in house martins the incubation period is effectively independent of clutch size (Bryant 1975a) then the increase in clutch interval with the number of young in the first brood must be due either to an extended hatching and/or nestling period, or a longer breeding interval. The latter two parameters were examined in detail. Both nestling period and breeding interval tended to increase with first brood size and the number of young fledged, although neither relationship was significant. However, the average nestling periods of reduced and enlarged broods varied by only one day (Table 2.11), whereas the difference in breeding interval between reduced and enlarged broods was six days (Table 2.10). Thus the increased clutch interval in larger first broods seems to be due mainly to an increase in the breeding interval. This may imply that the increased effort required to raise an enlarged brood affects the body condition of the female so she requires more time to recover condition and lay again. No consistent measurements of adult mass at the end of the first brood nestling period were made, so there are no data available to test this hypothesis for house martins. However, in great tits and house sparrows, there is apparently no effect of female condition (measured as body weight) on the interbrood interval (Hegner & Wingfield 1987, Smith *et al* 1987, Tinbergen 1987), although in great

tits female weight may influence the decision whether or not to lay a second brood (De laet & Dhondt 1987). Adult house martins were also observed to feed their young after fledging, so the increased breeding interval associated with larger first broods might alternatively be a consequence of an extended period of post-fledging provisioning. These two possible explanations for the effect of first brood size on the clutch interval are not mutually exclusive, since an extended period of post-fledging provisioning may also extend the time taken for a female to recover condition sufficiently to lay again. A third possibility discussed by Smith *et al* (1987), that resources in the breeding territory may be depleted in relation to the size of the first brood and therefore increase the breeding interval, is unlikely to apply to an aerial insect feeder such as the house martin which does not defend a territory and exploits a food supply that is continually renewed.

(ii) Nestling growth and mortality

In house martins, nestling mass at peak but not at fledging decreased with brood size. Thus in enlarged broods nestlings tended to reach a lower peak mass, or perhaps reach peak mass later than 15-16 days after hatch, the time when measurements were made. These results are similar to those of Bryant & Westerterp (1983b), who noted a tendency for body mass of nestlings (measured between nine and 21 days) to be lower in broods of six and seven than in smaller broods. Growth of the wing was apparently unaffected by brood size in manipulated broods, although there was a tendency for the wing to grow more rapidly in larger natural broods.

Body size at fledging has been shown to affect subsequent survival in a number of bird species (Perrins 1965, Dhondt 1971, Garnett 1981, Coulson & Porter 1985, Smith *et al* 1989). It is not known if peak mass effects subsequent survival in species such as the house martin which show marked weight recession (Ricklefs 1968). The observed reduction in peak mass with brood size in house martins could be a consequence of slower growth in mass in larger broods, a difference that is not however, reflected in fledging weights. It is possible that the increased level of mortality in enlarged broods allowed nestlings to 'catch up' with young in smaller broods, instead of fledging at a lower mass.

Between 1972-89, despite the increased nestling mortality rate, pairs of house martin raising enlarged first broods fledged significantly more young on average than pairs with unmanipulated first broods (respective means and standard errors 5.0 $\pm$ 0.44, n=23; 3.4 $\pm$ 0.07, n=212; t=-3.6, $p<0.01$). However, between 1987 and 1989 there was no

significant difference between the number of fledglings from enlarged and unmanipulated broods, although pairs with enlarged broods did raise more young on average (respective means 4.4 ± 0.73, n=8; 2.8 ± 0.172, n=38; t=-2.08, p>0.05).

Bryant & Westerterp (1983b), predicted an upper limit of five young in the first brood for house martins in Central Scotland on the basis of the maximum foraging performance of the average bird. Since five is also the maximum natural first brood size in this area, this result supported Lack's (1947, 1954, 1968) prediction that it is the ability of the parents to feed young that limits brood size. However, analysis of house martin breeding data over an extended time period has indicated that artificially enlarged broods (including some with six or seven young), produce more fledged offspring on average than unmanipulated broods. The ability of individual pairs to sucessfully raise broods of six or seven has been noted, but also the susceptability of nestlings in large broods to reduced growth and increased mortality. Presumably the success of artificially enlarged broods is mediated by food supply. If food remains reasonably abundant (probably above the average level) then it is likely that the average house martin pair can raise six or seven young. However, if environmental conditions deteriorate, and food becomes scarce, as is not uncommon in mid-summer in Central Scotland, larger broods seem to suffer disproportionate mortality. In addition, parents raising enlarged broods are also likely to miss the opportunity of attempting a second clutch, as discussed below. Since some house martin pairs manage to raise up to four young in a second breeding attempt, it would not seem worth their while forgoing this opportunity by taking the risk that environmental conditions will occasionally and unpredictably enable them to raise more than five young in a first brood.

(iii) The probability of a second clutch

Manipulation of first brood size was found to have an effect on the number of house martin pairs attempting a second clutch, as predicted by the model in section 2.1.2. The effect of manipulations varied with the timespan of the data analysed. Between 1987 and 1989 pairs raising reduced first broods were found more likely to produce second clutches than pairs raising unmanipulated first broods. Over this time span, pairs with enlarged first broods were not less likely to produce a second clutch, possibly because of the increased level of nestling mortality. Considering the whole data set, between 1972 and 1989, pairs raising enlarged first broods were less likely to produce a second clutch than pairs raising both unmanipulated and reduced first broods. The fact that brood manipulations on the same species over different periods of time produced different

results may be explained by the decline in the average number of pairs producing a second clutch between 1972 and 1989 (Figure 2.5). These findings illustrate the values of long term studies in highlighting flexibility in the breeding strategy of a given species. Such flexibility can also be revealed by studies of populations of the same species inhabiting different areas. For example, experiments on different populations of great tits have produced evidence that enlarging the first brood may reduce the chance of a subsequent breeding attempt in the same season (Tinbergen 1987, Linden 1988), or that reducing the size of the first brood may increase the probability that it is followed by a second brood (Smith *et al* 1987).

The breeding interval increases with the size of the first brood, and reasons for this have been discussed in section 2.4.3 (i). Pairs with reduced first broods require less time to lay again, and, depending on the time in the season that they began laying the first clutch, may therefore be able to fit in a second brood. Pairs with increased first broods may find that by the time they are ready to relay, it is too late.

The model proposed in section 2.1.2 suggests that the laying of second clutches stops after a certain date - the 'second clutch threshold' or 'end of (laying) season'. This may ultimately be because the survival chance of eggs decreases precipitously with time after this date (Daan *et al* 1988, Smith *et al* 1989). In addition, in a migratory species such as the house martin, adults may face a trade-off between raising second brood offspring and building up reserves to begin the journey to the wintering grounds. What is the proximate cause for the end of the season, in other words, how do birds know that it is too late to lay again? One possibility is the shortening of daylength acting to suppress the reproductive system. It is difficult to support or discount this theory from the data available. If daylength changes are the only cue used by house martins then the end of season should fall on exactly the same date every year. The measured end of season does not (Tables 2.17 and 2.18), but this may be influenced by the number of pairs studied and the times at which they are ready to relay. It is also possibile that declining levels of food abundance cause birds to stop laying, a suggestion that is supported by the existence of a significant negative relationship between one measure of the end of season (the first clutch threshold) and food abundance in August (Table 2.19). Since August is the month that later second clutches are laid, low food abundance at this time may deter house martins from laying again. A possibility that has not yet been considered is that the end of season may vary from pair to pair. For instance, older birds that have less chance of surviving to breed in the next year may be prepared to to risk relaying later than first year birds (Pianka & Parker 1975, Pianka 1976). Individual differences in

foraging ability of adults, as noted by Bryant & Westerterp (1982, 1983a) may also allow some birds to continue breeding later than others.

Thus it seems likely that the end of season is related to more than one factor, perhaps being under the influence of daylength and food availability, the latter in turn influenced by characteristics of individual birds.

2.4.4 Changes in the timing of breeding and annual reproductive success of house martins in Central Scotland 1972-89

The average annual reproductive success and the percentage of second clutches attempted by house martins at the study colonies in Central Scotland has declined between 1972 and 1989. What are the underlying causes of these changes? The abundance of food is generally thought to have a profound effect on the timing and success of breeding in birds (Perrins 1970, Bryant 1975a, Daan *et al* 1988). There was no evidence of a decline in the abundance of insects measured by suction trap catches over the study period (Table 2.19), but there were indications that food abundance affected components of the timing and success of breeding in house martins.

The percentage of second clutches and annual reproductive success increased with food abundance in June and July, the time when first brood young are being reared. The implication is that when food is more abundant adults can feed nestlings and maintain higher body condition, enabling a second brood to be attempted. It is also possible that they may use food levels in June and July to predict food abundance later in the season, and assess the likelihood of success of a second breeding attempt.

The first clutch threshold (but less convincingly the second clutch threshold) declined with food abundance in August (Table 2.19). Since this is the month when later second clutches are laid, low food abundance at this time might deter second breeding attempts. In a migratory species such as the house martin, adults are likely to face a trade-off between attempting a second brood and allowing themselves enough time to aquire reserves for the start of the journey to the wintering grounds.

The average date of the first egg, identified as a major determinant of the percentage of second clutches and annual reproductive success, was not affected by any measure of food abundance between May and August. It is possible that it may be influenced by qualitative changes in food supply, as was found for house martins breeding in southern

England (Bryant 1975a). Changing conditions on the wintering grounds or migratory feeding areas might also effect laying dates.

Overall, the decline in the percentage of pairs laying second clutches, and the average annual reproductive success per pair, of house martins between 1972 and 1989 seemed to be a result of a contraction in the length of the breeding season, involving a decline in the average date on which first clutches were laid, and the first clutch threshold. Changes in food abundance were implicated as having some effects on the timing and success of breeding, but could not fully account for the observed decline. If there has been a change in population age structure during the same time period, as suggested in Section 2.4.2, then this might also contribute to the decline in annual reproductive success, since younger birds are generally less successful breeders (Table 2.20).

When trying to interpret these trends it should be remembered that data from 1972 to 1983 relate to a single large study colony (Naemoor), whereas data from 1987 to 1989 come from a total of nine colonies. It is difficult to judge whether a real decline in breeding success with time has been detected, or a difference in breeding success between the Naemoor colony, and the other colonies studied in 1987-89. It is however notable that over the time period considered here, the Naemoor colony declined from a peak of around 30 breeding pairs between 1972 and 1976, to a single breeding pair in 1988, and that in 1989 no house martins were recorded breeding there.

A decline in breeding success at the study colonies does not necessarily imply a general trend throughout the Central Scotland area. It seems rather that house martin colonies around farms have undergone a decline since the 1970s, as illustrated by the demise of the Naemoor farm colony between 1972 and 1988. At the same time, house martins have become commoner on new houses, often on new estates on the outskirts of towns or villages (D. M. Bryant, pers comm). This change in nest site preference seems to be a result of changes in farming practices such as the removal of hedges and belts of trees, the infilling of ponds, the replacement of ditches with drains, and the use of pesticides, all of which probably contribute to a decrease in the local abundance of insect food.

In the preceeding analysis annual reproductive success has been measured as the total number of fledged young produced by a given pair in a season. This assumes that the young in a nest are the genetic offspring of the adults that raise them. However, recent work has shown that birds may sometimes be duped into raising offspring that are not

genetically related to them, and that the *apparent* reproductive success of an individual bird (the number of young in the nest) does not always equal its *realised* reproductive success (the number of genetic offspring produced). The following chapter examines the extent of consanguinuity between parents and offspring in house martins and a closely related species, the sand martin. The implications of these so-called mixed reproductive strategies (Trivers 1972, Fitch & Shugart 1984) for measurements of reproductive success are considered in chapter 5.

3. DNA fingerprints of house martins and sand martins

3.1 Introduction

3.1.1 The DNA fingerprinting technique

DNA fingerprinting or genetic fingerprinting was developed by Alec Jeffreys and colleagues working at the University of Leicester (Jeffreys 1987, Jeffreys *et al* 1985a & b). Investigations into the human genome had revealed a number of hypervariable regions of DNA consisting of repeats of short core sequences or 'minisatellites' of DNA of about 10-30 base pairs (bp) in length. These hypervariable regions showed considerable polymorphism due to differences between individuals in the number of repeats of the core sequence at individual loci. Jeffreys prepared a number of 33-bp probes each containing a slightly different version of the core sequence. Of these probes, two, known as 33.6 and 33.15 (numbers after the point refer to the order in which they were prepared) proved especially useful. By radioactively labelling these probes and hybridising them to human DNA digested with restriction endonuclease enzymes and electrophoresed through an agarose gel, Jeffreys was able to detect a large number of DNA fragments (showing up as dark bands on an autoradiograph) and the pattern of these minisatellite bands varied considerably between individuals due to differences in size of the minisatellites detected. The two probes, 33.6 and 33.15, detected largely different sets of fragments, but with a given probe the banding pattern or DNA fingerprint of a given individual was somatically stable (it remained the same no matter which tissue was used as a source of DNA - with just a few exceptions, see Jeffreys 1987). Furthermore the minisatellite fragments detected were transmitted from parent to offspring in a Mendelian fashion. Each band present in a child was derived from one or other of its parents. About one offspring fragment in 300 could not be traced to either parent and was thought to be the result of a mutation.

The function of minisatellite DNA and the origin of the large number of alleles present at each locus have not yet been established. Because the core sequence is similar in length and base-pair composition to a known recombination 'hotspot' in the bacterium *Escherichia coli*, it has been suggested that the core sequence might serve as a recombination signal during cell division, and that the large number of alleles present at each locus might be generated by unequal crossing-over between sister chromatids during meiosis resulting in two mutant alleles containing unequal numbers of repeats of the core

sequence (Jeffreys *et al* 1985a, Jeffreys 1987). Because, as far as is known, minisatellite length is selectively neutral (alleles of different length confer no advantage or disadvantage to an individual) new mutant alleles are likely to be maintained and spread in a population in a process analagous to genetic drift. However, evidence for the recombination hypothesis is largely circumstantial (Jarman & Wells 1989) and recent work has shown that new mutant alleles at one human minisatellite locus at least, seldom if ever arise by unequal meiotic exchange (Jeffreys *et al* 1990). Other processes, such as slippage during DNA replication or sister chromatid exchange during mitosis, may be the main source of minisatellite mutation.

DNA fingerprinting has become very important in forensic and parentage studies in man. The probablility that two unrelated humans have identical DNA fingerprints is very small (in the order of $4x10^{-10}$, Jeffreys 1987) and it is therefore unlikely that two individuals will have the same fingerprint by chance. Only identical or monozygous twins will produce identical DNA fingerprint patterns. Even for first degree relatives such as siblings, the probability of identical fingerprints is about $3x10^{-14}$ (Jeffreys 1987). The technique can therefore be used with confidence to identify criminals from small samples of tissue such as blood, semen or skin (Gill *et al* 1985). DNA fingerprinting has also been used to resolve a number of paternity disputes, including immigration cases where entry to Great Britain depends on a proven relationship to a person already resident here (Jeffreys *et al* 1985). In addition, DNA fingerprinting has applications for gene mapping (Nakamura *et al* 1987) and medical research into inherited disease and cancer (Jeffreys 1987, Thein *et al* 1987).

The human DNA fingerprint probes can also be hybridised to the DNA of other animal groups including non-human mammals (for example Jeffreys & Morton 1987, Amos & Dover 1990, Faulkes & Abbot 1990, Paul *et al* 1992), birds (Wetton *et al* 1987, Burke & Bruford 1987, Meng *et al* 1990), reptiles, amphibians (Tegelstrom & Sjorgren 1990), fish, and a number of invertebrate groups; often revealing similar hypervariable fingerprint patterns to those given by human DNA. If the human core sequence does act as a recombination signal then it is perhaps not surprising to find that it is common to the DNA of many organisms, and that there is no obvious decrease in the intensity and complexity of hybridisation with phylogenetic distance from man (Jeffreys 1987). In addition, a number of DNA sequences isolated from other organisms have been found to hybridise to DNA to produce 'fingerprint' patterns (Georges *et al* 1987; Gyllensten *et al* 1989; Vassart *et al* 1987).

DNA fingerprinting has obvious potential for use by animal breeders, for example in the verification of pedigrees, the registration of breeding stock, the characterisation of genetic relationships between different strains of the same species (Kuhnlein *et al* 1989) and the identification of stolen animals. It also has applications for the design of captive breeding programmes for rare species, and could be used to register captive and perhaps wild stock of rare and endangered species such as birds of prey (Parkin 1987; Parkin *et al* 1988).

3.1.2 Parent-offspring relationships in wild populations

A further application of DNA fingerprinting lies in research into the population genetics of wild species, in particular the investigation of parent-offspring relationships. This is the concern of the present study.

The majority of bird species breed in monogamous pairs (Lack 1968); a male and a female co-operating to raise a brood of young. There is increasing evidence, however, that males and females of apparently monogamous species may pursue a mixed reproductive strategy (Trivers 1972, Fitch & Shugart 1984). Behavioural observations have detected extra-pair copulations (EPCs, where at least one participant is paired to a third individual) and intraspecific brood parasitism (IBP or egg dumping, where a female lays an egg in the nest of another pair of her own species). The occurrence of these so-called alternative reproductive tactics means that it cannot be assumed that all the nestlings in a brood are the true progeny of the adults that raise them. If a female has mated with an extra-pair male then some or all of the offspring may not be related to the male attending her nest. If an egg has been dumped into the nest then the resulting offspring may not be related to either of its putative parents. Quasi-parasitism, where a female participating in an EPC dumps the resulting egg into the nest of the male she has mated with (Wrege & Emlen 1987) might result in an offspring being unrelated to the female attending the nest but related to her partner. For research aiming to measure the seasonal or lifetime reproductive success of individuals it is obviously important to have estimates of the frequency of alternative reproductive tactics in the population under study.

3.1.3 Estimating parent-offspring relationships - the past and the future

A number of methods have been used to estimate the frequency of extra-pair fertilisations (EPFs) and IBP in wild populations, including inferences from behavioural observations, variations in the heritability of body size parameters, and the use of genetic markers. Behavioural observations are unlikely to provide accurate estimates of the number of young resulting from alternative reproductive tactics because it is normally impossible to obtain a complete record of behaviour, and different types of behaviour are likely to vary in their conspicuousness to an observer. In many species of bird, within-pair copulations are infrequently observed, and EPCs solicited by a female are likely to be even more furtive occurrences if she runs risk of desertion by her mate if she is discovered. On the other hand, forced EPCs, (Mckinney *et al* 1983) may be more obvious than pair copulations and lead to an over-estimation of EPFs. Even if copulations are observed, details of factors such as sperm competition or the timing of the fertile period of the female are not available for most species, so the chance that an insemination will lead to fertilisation is not known (Birkhead & Møller 1992). As far as IBP is concerned, most bird species lay eggs at constant intervals (one per day in passerines) until the clutch is complete, and the appearance of two eggs in a 24 hour period is often taken as evidence of an egg dump (Yom-Tov 1980). However, if parasitism is combined with the removal of an egg from the host nest then IBP may be very difficult to detect from observations alone.

Differences in the estimates of male-parent - offspring and female-parent - offspring heritability for various body size parameters have been used to estimate the frequency of cuckoldry in a few passerine species (Alatalo *et al* 1984, Møller 1987a, 1989, Payne & Payne 1989). Lifjeld & Slagsvold (1989) question the usefulness of this method because the standard errors of parent-offspring regressions are usually large, and consequently differences between male-parent and female-parent - offspring heritabilities are not normally significant. However, Lifjeld & Slagsvold's critique is not entirely convincing (Møller 1989b; Alatalo *et al* 1989) and in a number of studies heritability methods have produced estimates of the frequency of EPFs that were similar to those obtained from biochemical techniques (Payne & Payne 1989, Møller & Birkhead 1992). Heritability methods may therefore provide reasonably accurate estimates of the frequency of EPFs or IPB in a given population, but they are of limited value because they cannot be used for the direct assignment of parentage.

Genetic markers such as plumage polymorphisms, enzyme polymorphisms and restriction

fragment length polymorphisms (RFLPs) of DNA have also been used to assess parenthood in wild populations. Studies using plumage variations with known inheritence patterns have demonstrated the occurrence of multiple paternity and/or maternity in captive and wild populations (Payne & Kahrs 1961, Compton *et al* 1978, Burns *et al* 1980, Birkhead *et al* 1988, Lank *et al* 1989).

Starch gel electrophoresis is a technique for separating the enzyme products of different alleles (allozymes) at a single genetic locus (Evans, 1987, provides a review of the technique as applied to birds). Electrophoresis has been used to assess parentage in a number of bird species (Sherman 1981; Gowaty & Karlin 1984; Jeste *et al* 1985; Mumme *et al* 1985; Gavin & Bollinger 1985; Evarts & Williams 1987; Brown & Brown 1988; Price *et al* 1989) but the technique has usually been confounded by low levels of variability between individuals. The polymorphisms observed are usually the result of variation at a small number of genetic loci (2-9 in references quoted) and unrelated individuals will often share the same electrophoretically determined genotype by chance. Thus if a male responsible for an EPF has the same electrophoretic genotype as the putative father of a nestling the mismatch will go undetected. Electrophoresis is therefore likely to underestimate the true frequency of mismatched offspring and complex mathematical models are required to estimate the true frequencies of non-kin offspring from electrophoretic data (Westneat *et al* 1987, Wrege & Emlen 1987, Chakraborty *et al* 1988). Further, if an offspring has a genotype inconsistent with its parents, but the putative mother and father share the same genotype then exclusion is ambigious with respect to the male or female, and the likelihood of mismatched offspring resulting from EPFs or IPB must be inferred from behavioural observations (Gavin & Bollinger 1985; Westneat 1987a; Brown & Brown 1988; Sherman & Morton 1988).

Restriction fragment length polymorphisms (RFLPs) occur when a known sequence of DNA between two sites for a restriction enzyme differs between individuals. If this difference results in the incorporation of an extra restriction site then a large fragment in one individual will be matched by two smaller fragments in another. Alternatively, the length of the DNA sequence between the 2 restriction sites may vary between individuals. Mixed maternity and paternity was detected in broods of snow geese using 17 different RFLPs (Quinn & White 1987, Quinn *et al* 1987). This is a greater number of polymorphic loci than is available for most electrophoretic studies, but even so, some exclusions were still ambiguous with respect to the putative mother or father.

Because it detects variation at many highly variable loci simultaneously, and because

most minisatellite fragments appear to be inherited independently, DNA fingerprinting allows the direct assignment of parentage, provided DNA samples for all putative parents are available. Even if DNA from all putative parents is not available, the DNA fingerprint of a complete family will normally show clearly which parent, if any, a given offspring is unrelated to. In addition, the human probes isolated by Alec Jeffreys appear to hybridise to the DNA of a variety of different species to produce individual specific fingerprint patterns, obviating the need for development of species specific probes. DNA fingerprinting thus represents a major breakthrough for research aiming to understand the structure and function of animal breeding systems. In the future, DNA fingerprinting with multilocus probes, as used in this study, may eventually be replaced by locus specific probes. These detect minisatellite alleles at a single locus only and are simpler to use but more difficult to isolate (Burke 1989).

3.2 Methods

3.2.1 Collection and storage of blood samples

For the purposes of this study most DNA was extracted from blood samples. Adult house martins and sand martins were blood sampled on the second capture in a given season because sampling at first capture sometimes resulted in the desertion of a nesting attempt. Nestlings were blood sampled as soon as they had reached peak mass (about 13 days after hatch for sand martins and 15 days for house martins). In a few cases where nestlings died before reaching peak mass the carcass was collected and stored at -70°C, and DNA was extracted from muscle tissue.

Blood was taken from the tibio-tarsal vein (on the inside of the leg just above the intertarsal joint, Figure 3.1a) or the brachial vein (underneath the wing Figure 3.1b). The latter was routinely used for nestlings whereas the leg vein was preferred for adults since it was felt that flight movements of the wing might cause a wound to re-open. To take blood, the vein was pierced with a sterile syringe needle and 25-50 µl of blood collected in a glass capillary tube. The wound was closed by maintaining a firm pressure on the vein until blood had stopped flowing, and a small amount of antiseptic cream was applied to minimise the risk of infection. Birds normally showed no signs of discomfort or stress during blood sampling and so far as is known no bird was incapacitated or died as a result of sampling during the three years of the study. Stangel (1986) also concluded from controlled experiments that withdrawal of blood from small birds did not cause significant stress as indicated by changes in body weight or mortality rate, and noted that prolonged handling of wild birds is probably more stressful than taking blood.

Blood samples collected in the field were kept on ice until they could be transfered to a -70°C Freezer, in most cases within 5-6 hours of collection. In 1987 and 1988 frozen blood samples were stored in glass capillary tubes, but it was found that these tended to break during freezing and thawing and some samples were lost. Also, blood stored in micro-capillary tubes has a large surface to volume ratio and this may accelerate the rate of degredation of the DNA (Tegelstrom 1989). So in 1989 blood samples were pipetted out of capillary tubes into plastic 1.5ml eppendorf tubes before freezing.

(a)

(b)

Figure 3.1 Blood letting from a nestling house martin. a, the tibio-tarsal vein (on the inside of the leg), and b, the brachial vein (underneath the wing).

3.2.2 Extraction of DNA from blood samples

Between 10-25µl of whole blood was pipetted into 465µl of 1xSET (0.15M NaCl 0.05M Tris 1mM EDTA pH8.0) in a 1.5ml eppendorf tube. Initially 25µl of blood was used for extraction but this produced a viscous DNA solution that was difficult to handle. Using 10-15µl of blood normally yielded enough DNA for several fingerprints and the resulting DNA solution was more easily purified.

To the mixture of blood and SET was added 7.5µl of 25% (weight/volume) sodium dodecyl sulphate (SDS) and 15µl proteinase K (10mg/ml), the tube contents were mixed gently and incubated overnight at 55°C.

Nuclear DNA occurs in the cell in combination with proteins (chromatin or nuclear material), and proteinase K, which is active against a broad spectrum of cellular proteins, disrupts this DNA-protein complex. Proteinase K also inactivates native nuclease enzymes, present in the cells, which might otherwise degrade the DNA during the extraction process. SDS is present to lyse the blood cells (the SET buffer is isotonic to the cells) and to stimulate the action of proteinase K (Hilz *et al* 1975).

The next stage involved extraction with organic solvents, to separate the partially digested proteins from the nucleic acid. To each sample was added 150µl of TE (10mM Tris 1mM EDTA pH8.0) followed by 500µl of phenol. Phenol was prepared fresh for each set of extractions by ⅓ filling a 150ml glass bottle with solid phenol, adding about 75mls Tris pH8.0 and a very small amount (to a concentration of about 0.1%) of 8-hydroxyquinoline (a reducing agent), shaking the mixture and leaving it to separate into two layers. The phenol, which was coloured yellow by the hydroxyquinoline, formed the bottom layer.

After phenol addition, samples were stirred by gentle rotation (15rpm) for 30 minutes and then spun at 11,600g for seven minutes to separate the two phases. The DNA remained in aqueous solution (upper layer) whereas the proteins and other material from the blood dissolved in the phenol or precipitated at the interface of the two solutions. As much of the upper layer as possible was then transferred to a fresh eppendorf tube leaving behind the material present at the interface. Phenol extraction was repeated 1-2 times, adding more TE if the volume of the upper layer fell below about 300µl. The extraction process was then repeated 2-3 times using a 24:23:1 (v/v) mixture of phenol/chloroform/isoamyl alcohol until the DNA solution was clear and colourless and no protein precipated at the interface. Then a final chloroform extraction was carried out, to remove all traces of

phenol from the DNA solution.

To precipitate the DNA from aqueous solution, two volumes of absolute ethanol at -20°C were added to each sample. Tubes were mixed by rotation for 15-30 minutes and then left overnight at -20°C to allow the precipitate to form. At this stage a white, stringy precipitate of DNA was normally visible. Samples were centrifuged at 11,600g for 7-10 minutes to pellet the DNA. Excess ethanol was poured off, taking care not to dislodge the DNA pellet. Several different methods of pellet drying were used. For vacuum drying the ends of the eppendorfs were sealed with 'parafilm' and a few tiny holes pierced in the top of each tube. Samples were then placed in a vacuum drier for 30 minutes, or a centrifugal evaporator for 60 minutes. Alternatively, tubes were placed in a 37°C incubator with the lids open for about one hour to allow excess ethanol to evaporate. Occasionally pellets refused to dry properly, suggesting that traces of phenol or chloroform remained in the DNA. Such pellets were washed with 75% ethanol, spun down for about three minutes and re-dried.

DNA was resuspended overnight in a 55°C waterbath in 100-150µl TE, less if the pellet was small. Resuspended DNA samples were stored at 4°C until required.

The yield of DNA from this extraction process varied considerably between samples. It was difficult to estimate the concentration of large molecules of un-restricted DNA in solution because DNA often failed to resuspend evenly. Determination of DNA yield by weight was also likely to be inaccurate because the pellet might contain RNA and other contaminants. After cutting with restriction enzymes, DNA concentration was measured using either a spectrophotometer or a fluorometer, and average DNA yields determined by back-calculation (Table 3.1). Burke & Bruford (1987) commented that 50µl of avian blood typically yields over 100µg DNA, and the mean yields of DNA obtained in this study are roughly consistent with this. As Table 3.1 indicates, the yield of DNA from 10-15µl samples of sand martin blood was surprisingly low. This value was calculated from samples taken in 1989, and in addition to the low yields, the DNA was found to be rather degraded. Extractions from a small number of sand martin blood samples taken in 1988, and from house martin blood taken in 1988 and 1989 produced better yields of good quality DNA. Since there were no consistent differences in the methods of sample collection, storage or extraction for house martin and sand martin blood in 1989, it is hard to account for the differences in DNA yield.

Table 3.1 Average yields of DNA from whole blood of house martins and sand martins

Amount of whole blood, µl	Mean DNA yield, µg	
	House martin	Sand martin
25	117	168
10-15	80	21

3.2.3. Extraction of DNA from tissue

Carcasses for DNA extraction were removed from the -70°C freezer and sawn in half just above the upper end of the keel while still frozen solid. Using a metal spatula, about 0.5g of muscle tissue was scraped out and ground to a fine powder in a pestle and mortar in the presence of liquid nitrogen. Portions of powdered tissue of approximiately 50mg in weight were immediately transfered to eppendorf tubes containing SET, SDS and proteinase K in the same proportions as given for blood samples. Extraction was thereafter carried out as described for blood samples.

3.2.4 Restriction of DNA samples

For the preparation of fingerprints, 20µl of DNA solution was removed and incubated with 10 units of a restriction endonuclease overnight at 37°C in the presence of 4mM spermidine trichloride. Restriction endonucleases are enzymes which cleave DNA molecules at a specific site or sites, thus breaking up the large DNA molecules into a number of smaller fragments. Spermidine appears to speed up DNA restriction by binding to the DNA, removing its secondary structure, and allowing the enzyme easier access to the cleavage site (Roy Carter, pers comm). To check if the reaction was complete, 2µl of a restriction was removed and run in a 10cm agarose gel (minigel) containing 0.5µg/ml ethidium bromide at 80V for one hour. Ethidium bromide is a fluorescent dye which binds to DNA, allowing it to be seen whem illuminated with ultra-violet light. Visual assessment of DNA smears on a minigel was used to assess whether a DNA sample had been fully cut.

The time to completion for a restriction reaction varied considerably between samples. More dilute DNA solutions cut more quickly, whereas more concentrated solutions, and samples of DNA that had not been adequately cleaned, took longer to cut and occasionally did not cut at all. Extra aliquots (1µl, 10 units) of enzyme were added to samples which had not cut overnight. Addition of extra enzyme beyond 10% of the total reaction volume was avoided, because the glycerol in the enzyme buffer may then cause so-called 'star' activity (when the enzyme cuts the DNA at atypical sites).

Samples of restricted DNA were assayed using a Spectrophotometer at a wavelength of 260 nanometres (nm) with a solution of TE as a blank. An optical density of 1 at 260nm corresponds to a DNA concentration of 50µg/ml (Maniatis *et al* 1982). Alternatively DNA assay was carried out using a Fluorometer (Hoefer TKO 100) with 2µl of a 1mg/ml

solution of calf thymus DNA as a standard.

Completed restrictions were diluted with a solution of 2xBromophenol Blue (0.04M EDTA 4% ficoll 0.05% BPB 0.05% xylene cyanol w/v) so that 40µl contained 6µg. EDTA stops the activity of the restriction endonuclease and BPB and xylene cyanol act as tracking dyes during electrophoresis, allowing the progress of the DNA to be monitored visually. If a restriction was found to contain less than 6µg of DNA then more DNA from the same individual was restricted and the two restrictions were combined, adding 1/10 volume of 10xBPB as a loading buffer.

Fingerprint patterns obtained for both martin species using two enzymes, HaeIII and AluI, were compared. HaeIII was chosen for house martin DNA because it produced a relatively large number of scoreable bands and a higher level of individual variation than AluI. For the same reasons, AluI was selected for sand martins.

3.2.5 Agarose gel electrophoresis

For electrophoresis, 6µg samples of DNA were loaded into 22x20cm agarose (Seakem LE) gels using 1xTAE buffer (0.04M Tris-acetate 0.001M EDTA). Gel running kits were obtained from Gibco BRL and used with 16, 8x2mm loading wells. Agarose concentrations of 0.8-1.0% (w/v) were tried, but for both martin species a 0.8% gel was found to give the best results in terms of band definition and separation.

Loaded maxigels were left for 10 minutes to allow DNA to settle to the bottom of the wells and so that the salt concentrations of the samples could equilibriate with that of the buffer. Electrophoresis was then carried out for 48 hours at 40V.

Because DNA is negatively charged, samples are loaded into gels nearest the cathode. When the current is switched on the DNA fragments move through the gel towards the anode. The agarose gel acts as a selective filter, allowing small DNA fragments to move more quickly than large fragments. Thus the effect of electrophoresis is to sort the DNA fragments according to size.

3.2.6 Southern blotting

After electrophoresis, gels were Southern blotted onto nitrocellulose ('Schleicher & Shuell') or nylon membranes ('High-bond N'). Nylon membranes were preferred because

they were less brittle than nitrocellulose and therefore less likely to break as a result of handling.

Southern blotting is a technique whereby DNA is passed from an agarose gel to a sheet of filter paper or nylon membrane to which it becomes bound. The positions of DNA fragments in the gel are preserved as they are transfered onto the membrane, which thus provides a permant copy of an agarose gel. Once attached to the membrane, the DNA is stable and can be stored for some time before being probed with radioactive DNA. After probing, the radioactive DNA can be stripped from the membrane leaving the original DNA still in place and available for re-probing.

For both filter types the Southern Blot procedure was identical. Gels were placed in shallow melamine trays and soaked for 20 minutes in 0.2M hydrochloric acid (HCl). The acid partially depurinates the DNA, breaking up some of the larger fragments so they can move out of the gel more easily during blotting. Gels were then soaked for 35 minutes in 0.5M NaOH 1.5M NaCl. This denatures the DNA, causing the two strands to separate, again facilitating an easier transfer of DNA. Finally, gels were soaked for 45 minutes in a neutralising solution of 0.5M Tris 3M NaCl. The Southern blot was set up as shown in Figure 3.2, using 20xSSC (3M NaCl 0.3M sodium citrate) as the transfer solution and inverting the gel so that its original underside was against the filter. Gels were trimmed with a sharp knife so that only those portions that contained DNA were blotted and strips of 'saranwrap' or 'parafilm' were arranged on the wick, flush with each edge of the gel, to prevent transfer solution short-circuiting the gel and passing straight into the paper towels.

During blotting, the transfer solution is drawn upwards through the gel, carrying the DNA onto the surface of the filter. Blots were normally left to transfer overnight, although it is likely that most transfer takes place during the first four hours since after that time the gel becomes crushed and further DNA movement is inhibited. Filters were then rinsed for 2-3 minutes in 2xSSC, air dried for about 30 minutes and baked at 80°C under vacuum for 2 hrs sandwiched between 3MM paper. Baking fixes the DNA to the filter. A vacuum is only necessary for nitro-cellulose filters since these may explode if the temperature rises too far above 80°C.

3.2.7 Probing of Filters

Probing was carried out in the Department of Genetics at Nottingham University by Roy

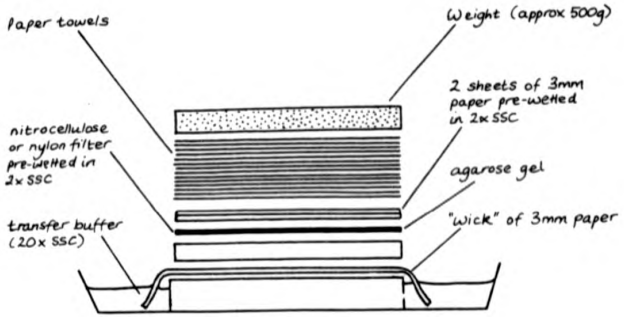


Figure 3.2 Southern blot (after Maniatis *et al* 1982)

Carter and Jon Wetton. Since I have little first hand experience of the procedure I will give only brief details here. A RNA probe was used, rather than a more conventional DNA probe. RNA probes were produced by inserting human probes 33.6 and 33.15 into the EcoRI and HindIII sites of vectors pSPT18 and pSPT19 (Carter 1989; Carter *et al* 1989). Many RNA copies of either strand of the human DNA probes were produced by the process of transcription, using T7 or SP6 RNA polymerase enzyme in the presence of nucleotides containing radioactive phosphorous. These RNA probes have a higher specific activity (that is, a better incorporation of radioactivity) than DNA probes, which means that autoradiographs (photographs taken with X-Ray film) of filters hybridised with RNA probes become exposed rapidly, and can be exposed without intensifying screens, giving better resolution of minisatellite bands.

Filters to be probed were pre-hybridised at 65°C for eight hours in 1xSSC 1%SDS 1%blotto (1% 'marvel' powdered milk and 0.02% sodium azide w/v) in a plastic container in a shaking waterbath or hybridisation oven. The probe was then added to the prehybridisation solution and the filters left to hybridise overnight at 65°C. During this time the radioactive probe RNA becomes attached to areas of DNA on the filters which have a complementary sequence of nucleotides. The filters were then washed in about five changes of 1xSSC and 0.1%SDS over three hours. The damp filters were wrapped in 'saranwrap' and exposed for four hours at -80°C using pre-flashed Fuji RX X-Ray film and two intensifying screens, followed by 6-8 days exposure at room temperature without screens. These autoradiographs are the DNA fingerprints.

RNA probes equivalent to both of Jeffreys original human probes, 33.6 and 33.15, were successively hybridised to a trial Southern blot of house martin and sand martin DNA. The pattern of minisatellite bands detected with each probe was found to be very similar for both martin species, but in each case 33.6 detected a greater number of bands and so this probe alone was used for the remaining filters.

3.3 Results

The use of DNA fingerprinting to investigate parent-offspring relationships is based on the assumptions that every individual has a unique fingerprint and that minisatellite fragments are inherited in a Mendelian fashion. This implies that each scorable minisatellite fragment should segregate independently at meiosis and that there is a probability of 0.5 that a given parental band will be inherited by an offspring. Thus before the technique is applied to the study of parent-offspring relationships of a particular species it is necessary to establish the general level of variability of DNA fingerprints of unrelated individuals, and to study the pattern of inheritance of minisatellite bands in a large family.

3.3.1 Variability of martin fingerprints

Figure 3.3 shows the DNA fingerprints of eight randomly chosen adult house martins, presumed to be unrelated, comprising four males and four females from four different colonies. The probe used (human minisatellite 33.6) has hybridized to a large number of fragments of house martin DNA, suggesting that the human probe has an identical or very similar nucleotide sequence to a minisatellite in house martin DNA. The variability of the fingerprints was assessed by comparing the positions of bands in the tracks of different individuals. Bands of similar mobility (position on gel) and intensity were judged to represent the same minisatellite fragment. A few faint bands, present in one or more tracks but likely to have been obscured in other tracks because of the presence of an intense band of similar mobility, were excluded from consideration.

A total of 74 bands was distinguished in the eight adults in the size range 30-6 kilobases (fragment size was determined by comparison with samples of DNA containing fragments of known size run at either end of the gel). Bands representing fragments smaller than 6 kb were considered too diffuse and closely spaced to score with any accuracy. Thus a large area of the house martin fingerprint was unscorable and in addition, the duration of electrophoresis was such that very small fragments of DNA were run off the end of the gel. The scorable region of the DNA fingerprint therefore represents a sample of the total genome.

An average of 14.5 (standard error 1.63) bands per individual were distinguishable in the size range 30-6 kilobases. There was no evidence of any species specific band, present in every individual, nor of any sex-linked bands present in every male or every female.

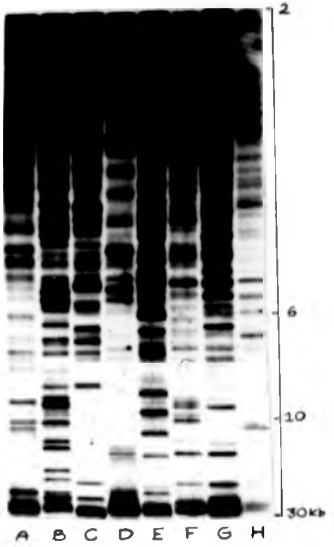


Figure 3.3 DNA fingerprints of eight randomly chosen adult house martins. The scale gives the approximate sizes of the DNA fragments (bands) in kilobases.

In this sample of eight adults, males had an average of 15.3 scorable bands and females 13.8 bands. In a larger sample of 19 males and 19 females the respective mean numbers of scorable fragments were 14.6 (standard error 0.76) and 12.5 (standard error 0.6), and a pairwise T-test between males and females run on the same gel indicated that this difference was significant (T=2.88, p=0.01). DNA fingerprints of male house martins therefore tend to possess an average of 2 extra bands in the size range 30-6kb. This might be accounted for by the fact that, in birds, the female is the heterogametic sex. The sex chromosomes in birds are termed W and Z and the female has genotype WZ while the male is ZZ. The W chromosome carries fewer genes and is largely inert. This would suggest that a small number of the minisatellite fragments observed in a house martin fingerprint are carried on the sex chromosomes, and that detailed investigation might isolate some sex-linked minisatellites. However, at the time of writing, no published reports of sex-differences in fingerprint patterns for other bird species had been seen.

A similar analysis of the variability of sand martin fingerprints was performed by comparing the fingerprint patterns of mated pairs of adult males and females from eight families (DNA fingerprints not shown). Although these adults were not randomly chosen, and the analysis involved only male-female comparisons, there is no evidence for assortative mating in sand martins and no reason to suppose that adults in mated pairs are any more similar or different than adults selected randomly from the population. In this sample of 16 adults a mean of 18.4 fragments was detected by human probe 33.6 in the size range 25-4.3 kb. As has been noted before, sand martin DNA samples were found to be rather degraded and this figure is probably an underestimate of the true number of bands that might be detectable in this size range given better quality sand martin DNA.

The fingerprint patterns in two individuals can be compared by calculating the band sharing coefficient F (Meng *et al* 1990):

$$F = 2N_{AB} / (N_A + N_B) \qquad 3.1$$

where N_{AB} is the number of bands shared by two individuals with respectively N_A and N_B bands. F values for all possible pairwise comparisons of the house martins in Figure 3.3 are given in Table 3.2. For each paired comparison, three F values are given: for fragments divided into two size ranges and for all fragments considered together. This illustrates that there is a tendency for house martin fingerprints to be more similar in the smaller size range (10-6 kb), as evidenced by the tendency for the F value between two

Table 3.2 Band sharing coefficients (F) for a group of eight randomly chosen adult house martins

	B	C	D	E	F	G	H
A	0.316	0.154	**0**	0	0	0.133	0.167
	0	0.143	**0**	0.125	0.143	**0**	0.200
	0.167	**0.148**	**0**	**0.065**	**0.074**	**0.069**	**0.182**
B		0.111	0.222	0.100	0	0.100	**0**
		0	0	0.095	0.105	0.105	0.133
		0.054	**0.125**	**0.098**	**0.054**	**0.138**	**0.063**
C			0	0.143	0.167	0.429	**0**
			0.182	0.222	0.250	0.500	**0**
			0.087	**0.188**	**0.214**	**0.467**	**0**
D				0	0.333	0.143	0
				0.154	0.545	0.182	0.286
				0.074	**0.435**	**0.160**	**0.111**
E					0	0.143	0.154
					0.333	0.333	0.286
					0.189	**0.235**	**0.222**
F						0.286	0
						0.375	0.167
						0.333	**0.087**
G							**0**
							0.167
							0.080

For calculation of F see equation 3.1 in the text. F values for each pairwise comparison are, from the top downwards, F:30-10 kilobases; F <10-6kb; F 30-6kb (bold type).

given individuals to be higher in this size range. This tendency, which is not statistically significant in house martins (Mann-Whitney U test, U=293.5 p>0.05) , is also found in humans (Jeffreys *et al* 1985b, Jeffreys 1987). It may indicate that smaller minisatellite fragments have a lower genetic variablity, or be an artefact caused by the tendency of smaller DNA bands to be more diffuse and closely spaced, thus making it more likely that two different fragments will be erroneously judged to be the same.

F values have been used as a crude indication of the relationships between individuals (Wetton *et al* 1987, F is equivalent to D; Morton *et al* 1990). In theory, F values should be approximately 0.5 for first degree relatives (parent-offspring or siblings in a brood), and 0.25 for second degree relatives. However, observed F values only approach their theoretical values if large numbers of bands are scorable in each individual, if each individual fingerprint contains roughly the same number of fragments and if the average band-sharing between unrelated individuals is essentially zero. In practice, unrelated individiuals 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. Use of DNA fingerprinting as a measure of relationships between randomly chosen individuals should therefore proceed with caution (Lynch 1988). Comparisons between individuals C-G and D-F (Figure 3.3) produced F values in the region of 0.5 (Table 3.2), the theoretical value for first degree relatives. Since these birds were first ringed as adults it is impossible to know if these degrees of band-sharing are due to chance alone. The mean F value for house martins, calculated as an average of all pairwise comparisons, is 0.157 (Table 3.3). This is much lower than 0.5, suggesting that birds C and G, and D and F may indeed be closely related.

Table 3.3 summarises the comparison of DNA fingerprints of unrelated individuals for house martins and sand martins. The probability, x, that a band present in a given individual A, will be present in another individual B, is given by:

$$x = N_{AB}/N_A \qquad 3.2$$

The band sharing probability can be used to calculate the probability, pf, that two randomly selected individuals have identical fingerprints as follows (Jeffreys & Morton 1987):

$$pf = (1-2x+2x^2)^{n/2} \qquad 3.3$$

Table 3.3 Comparison of DNA fingerprints of unrelated individuals in house martins and sand martins

Species	DNA fragment size (kb)	Fragments per individual (n ± se)	Band-sharing coefficient F (± se)	Band sharing probability x (± se)	Allele frequency
House martin	30-10	7.3 (0.77)	0.111 (0.023)	0.115 (0.017)	0.059
	<10-6	7.3 (0.98)	0.180 (0.028)	0.197 (0.024)	0.104
	total	14.5 (1.63)	0.157 (0.023)	0.152 (0.016)	0.079
Sand martin	25-4.3	18.4 (1.51)	0.153 (0.044)	0.154 (0.030)	0.080

Calculation methods for F, x and q are given respectively by equations 3.1, 3.2 & 3.4 in the text

For house martins this probability is 4.3×10^{-13}, and for sand martins the corresponding value is 2.2×10^{-10}. Thus the likelihood of a pair of unrelated house martins or sand martins having identical DNA fingerprints due to chance is extremely small, and the values for both martin species are of similar magnitude to those obtained for a number of other bird species (Table 3.4).

Assuming that shared fragments in different individuals always derive from the same alleles (Jeffreys *et al* 1985b), the mean population frequency of alleles at minisatellite loci, q, can be calculated as:

$$q = 1-(1-x)^{0.5} \qquad 3.4$$

Thus the mean allele frequency in house martins is 0.079 and for sand martins 0.080. It can be shown that q is equivalent to the homozygosity, and these low values indicate that most minisatellite loci in both martin species are heterozygous.

The probability that two siblings have identical fingerprints, ps, can be calculated from q, x and n as follows, assuming that minisatellite bands segregate independently (Jeffreys & Morton 1987):

$$ps = [1-\tfrac{1}{4}q(1-q)^2(4-q)]^{nx} \qquad 3.5$$

For house martins the probability of identity of siblings is 1.5×10^{-4}, and for sand martins 6.6×10^{-7}. Thus the variability of DNA fingerprints in these two species is such that it is very unlikely that even closely related individuals will have identical fingerprints. Moreover, because the minisatellite loci with which particular bands are associated are unknown, and because a large fraction of each gel track is occuppied by bands it is likely that a proportion of apparently co-migrating bands in different individuals will be derived from different loci. Thus estimates of F, x, q, pf and ps are maximal, and fingerprinting is likely to overestimate the genetic similarity of individuals by an unknown extent.

3.3.2 Segregation analysis of a house martin family

Figure 3.4 shows the fingerprint of a house martin family containing eight offspring from two broods. To make scoring easier, the parents have been run on both sides of the offspring so that parental bands can be traced across the gel. A total of 26 bands has been scored, of which 18 are present in the male and 13 in the female. The parental

Table 3.4 Comparison of DNA fingerprint patterns of birds and mammals

Species	DNA fragment size (kb)	Probe	Average fragments per individual (n)	Average band-sharing probability (x)	Average allele frequency (q)	Probability of identity		Source
						unrelated adults (pf)	siblings (ps)	
House martin	30-6	33.6	14.5	0.152	0.079	4.3×10^{-13}	1.5×10^{-6}	This study
Sand martin	25-4.3	33.6	18.4	0.154	0.080	2.2×10^{-16}	6.6×10^{-7}	This study
Purple martin		33.6	21.9	0.089	0.046	1.2×10^{-19}	5.9×10^{-10}	Morton *et al* 1990
House sparrow	20-2	33.15	24	0.24	0.128	2×10^{-20}	9×10^{-10}	Burke & Bruford 1987
		33.6	16	0.13	0.094	3×10^{-14}	5×10^{-7}	
		33.6	13	0.15	0.078	8.3×10^{-12}	5.7×10^{-6}	J Wetton pers comm
Dunnock	20-2.3	33.15	23.2	0.24	0.13	9×10^{-20}	1×10^{-9}	Burke *et al* 1989
Willow warbler		L17*	17.2	0.121	0.023	1.4×10^{-14}	1.8×10^{-5}	Gyllensten *et al* 1990
Wood warbler		L17	15.6	0.127	0.030	1.0×10^{-14}	8.3×10^{-6}	
Rothschild's Mynah		33.6	12.9	0.468	0.271	5.6×10^{-9}	1.8×10^{-4}	D Ashworth pers comm
		33.15	8.5	0.243	0.130	1.1×10^{-7}	6.2×10^{-4}	

Table 3.4 continued

Species	DNA fragment size (kb)	Probe	Average fragments per individual (n)	Average band-sharing probability (x)	Average allele frequency (q)	Probability of identity		Source
						unrelated adults (pf)	siblings (ps)	
Mute swan	30-3.5	33.6	23.7	0.284	0.154	1.2×10^{-19}	7.3×10^{-6}	Meng *et al* 1990
Whooper swan	30-3.5	33.6	17.7	0.284	0.154	7.4×10^{-15}	4×10^{-7}	
Bewick swan	30-3.5	33.6	18.6	0.206	0.109	2.9×10^{-16}	6×10^{-8}	
Human	20-4	33.6 & 33.15	36	0.25	0.134	4×10^{-30}	3×10^{-14}	Jeffreys 1987
Dog	20-3	33.6 & 33.15	32	0.46	0.265	2×10^{-21}	5×10^{-10}	Jeffreys & Morton 1987
Cat	20-3	33.6 & 33.15	21.1	0.47	0.272	3×10^{-14}	7×10^{-7}	

For published studies, values of n, x, q, pf and ps have been taken from the text. If a particular value was not quoted then it has been calculated using the appropriate formula (3.1-3.5) given in the text.
+ L17 is a DNA sequence from the willow warbler that also hybridises to the DNA of other bird species (Gyllensten *et al* 1990). Depending on hybridization conditions, this probe produces either a multiple locus (many bands) or a single locus (one band) pattern. The values of n, x, q, pf and ps for the warblers are for multiple locus fingerprints.

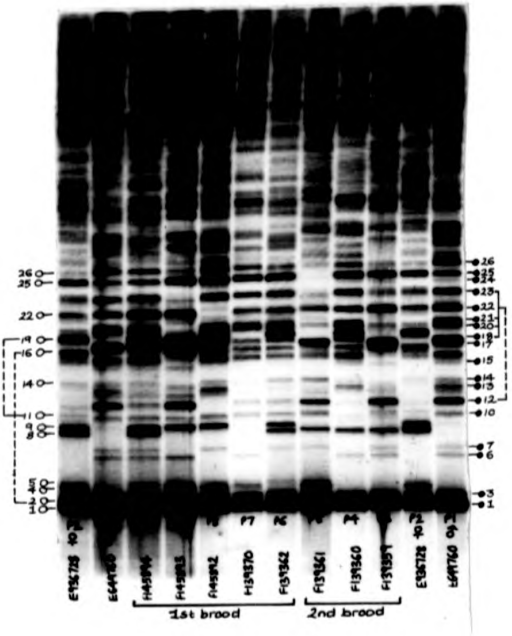


Figure 3.4 DNA fingerprints of a family of house martins (G3)
Key: o— female band; ●— male band; ------ joins allelic bands; —— joins linkage groups.

tracks contain five bands of similar intensity and mobility that have been assumed to represent the same minisatellite fragment (band numbers 1, 14, 22, 25 and 26) and their band-sharing coefficient F = 0.323 is rather higher than the average value for unrelated individuals (Table 3.3), which may imply some degree of relatedness.

In this family, every band in an offspring can be traced to one or other of the parents which indicates that the adults are the true parents of all the offspring. The track of offspring P5 does contain one band which does not seem to match any parental band, but evidence from another fingerprint of the same family (not shown) suggests that this band is equivalent to band 8 in the female. Alternatively, this band might represent a mutation that has generated a novel minisatellite fragment. Since all the other bands in the track of P5 are readily traceable to either parent there is no reason to suspect that this offspring mis-matches with either of the parents. Sex-linkage cannot be investigated since the sexes of the offspring are unknown.

The pattern of inheritance of the 21 bands that are derived from only one parent has been investigated to determine whether or not minisatellite fragments are inherited independently. Table 3.5 summarises the findings of this analysis. Bands that are present in both parents have been excluded from consideration because the parental origin of such a band in a given offspring is uncertain. No band that is derived from only one parent is present in all offspring, which suggests that both parents are heterozygous at all the minisatellite loci involved. The 13 paternal-specific bands are transmitted to an average of 55.8% of offspring, and eight maternal fragments have a mean transmission frequency of 53.1%. Furthermore, the pattern of transmission of both paternal and maternal fragments is consistent with a binomial distribution and a probability of band inheritance of 0.5. These findings suggest that in house martins minisatellite bands are inherited in a Mendelian fashion.

Pairwise comparisons of the segregation of all paternal and maternal bands using a maximum likelihood analysis (Cavalli-Sforza & Bodmer 1971) were used to detect linkage or allelism between bands. Figure 3.4 shows that 4 paternal fragments (numbers 18, 20, 21 and 23) segregate as though linked, they are either all present or all absent in a given offspring. These bands may represent different minisatellites in close proximity on the same chromosome, or a large minisatellite fragment with internal HaeIII sites. Three instances of apparent allelism have been detected. Maternal bands 2 and 16 appear to segregate in an allelic fashion. Each offspring has inherited one or other of these bands, but no offspring fingerprint either contains or lacks both bands, suggesting that

Table 3.5 Segregation of parental fragments and summary of polymorphic fragments in a house martin family

Number of offspring with band	Paternal bands		Maternal bands	
	observed	expected	observed	expected
0	0	0.05	0	0.03
1	0	0.41	0	0.25
2	1	1.42	1	0.88
3	2	2.84	1	1.75
4	2	3.55	3	2.19
5	6	2.84	1	1.75
6	2	1.42	2	0.88
7	0	0.41	0	0.25
8	0	0.05	0	0.03
	X^2	5.73		2.95
	p	>0.05		>0.05
Number of parent specific bands scored	13		8	
Mean transmission frequency	55.8		53.1	
Number of allelic pairs (a)	1		2	
Number of linked bands minus linkage groups (b)	3		0	
Number of loci scored	9		6	
Estimated total loci (N)	23		8	

they represent alleles at a minisatellite locus. Other apparently allelic fragments are maternal bands 11 and 19, and paternal band 12 and the 4-band linkage group.

In a family of eight offspring, the probability that a given pair of paternal or maternal bands segregate as though linked or allelic by chance is $2x0.5^8 = 7.8x10^{-3}$ which means that, of 13 paternal fragments producing 78 possible pairwise comparisons, a total of 0.6 pairs are likely to show linkage or allelism by chance. Similarly for eight maternal fragments and 28 pairwise comparisons the value is 0.2. Thus in this family all instances of linkage and allelism observed are likely to be real, but analyses of families of six or fewer offspring are likely to produce inflated estimates of linkage and allelism due to chance factors (Jeffreys & Morton 1987).

The number of distinct loci scored (L) in each parent can be estimated by eliminating allelic and linked bands. This involves subtracting the number of allelic pairs (a), and the number of bands involved in linkage groups minus the number of linkage groups (b), from the total number of parent-specific bands scored. Table 3.5 indicates that about 75% of the scorable fragments in each parent represent distinct loci with no resolved alleles. This suggests that large size differences exist between different alleles at most house martin minisatellite loci. Most of the loci in the scorable range must have short alleles which are present in the poorly resolved low molecular weight region of the fingerprint. Similar low instances of allelism have been detected in humans (Jeffreys *et al* 1986), dogs and cats (Jeffreys & Morton 1987), house sparrows (Burke & Bruford 1987), dunnocks (Burke *et al* 1989) and mute swans (Meng *et al* 1990); though a number of species of bird and mammal seem to show a higher incidence of linked minisatellite fragments than humans (Burke & Bruford 1987, Jeffreys & Morton 1987, Gyllensten *et al* 1989).

An estimate of the total number of hypervariable loci detected in house martins by the human probe 33.6 can be obtained if it is assumed that the number of distinct loci scored (L) represent a random sample of N heterozygous loci and a total of 2N bands in a DNA fingerprint. N can then be estimated as follows (Jeffreys & Morton 1987):

$$N = \tfrac{1}{2}\left[\frac{(L+a)(L+a-1)}{2a}\right] + 1 \qquad 3.6$$

Thus the DNA fingerprint of the male parent consists of about 23 heterozygous loci, or 46 fragments, and that of the female parent eight heterozygous loci or 16 fragments. These figures are likely to represent underestimates of the true number of heterozygous loci present because five fragments shared by the two parents, representing a significant proportion of the number of scorable bands in the fingerprint of each parent, could not be included in the analysis.

A similar analysis of a sand martin family was not carried out because the largest family available with fingerprint tracks of reasonable quality contained only four offspring (Figure 3.5). In a family of four there is a probability of 0.125 that a given pair of bands will segregate as if allelic or linked by chance alone and estimates of linkage or allelism are likely to be inflated.

3.3.3. Use of DNA fingerprints to determine parent-offspring relationships

Scorable DNA fingerprints were obtained for parents and offspring from 22 house martin and five sand martin families. Fingerprints were first assessed visually. Potentially mismatched offspring were identified by the presence of two or more bands that were not derived from either of the parents, and a low incidence of band sharing with one or both parents (Figure 3.6). A total of 10 house martin offspring were found to contain an average of four mismatched bands (range 2-5) and additionally to share few or no bands with the male. These 10 young are likely to have resulted from extra-pair fertilizations, as is one additional offspring with a fingerprint containing only one mismatched band but no bands derived from the putative father. Single mismatched bands in the DNA fingerprints of five other offspring were assumed to represent mutations, since in each case the remainder of the offspring's fingerprint was derived from roughly equal numbers of male and female bands.

Results from visual assessment were compared with those of a statistical analysis using software written by David Parkin based on models developed by John Brookfield. The analysis calculates the probability of obtaining an observed fingerprint pattern for each offspring in a family, given the fingerprint patterns of the putative parents, under four different models: (i) that both adults are the true parents, (ii) the male is the parent but the female is not, (iii) the female is the parent but the male is not, (iv) that neither the male or the female is a true parent. For each offspring, the model with the highest probability is chosen.

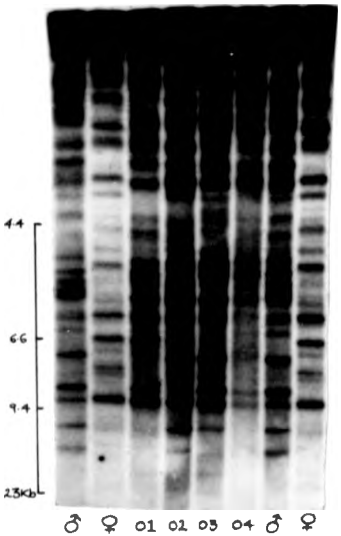


Figure 3.5 DNA fingerprint of a sand martin family (C16). The scale gives the approximate sizes of the DNA fragments (bands) in kilobases. O=offspring.

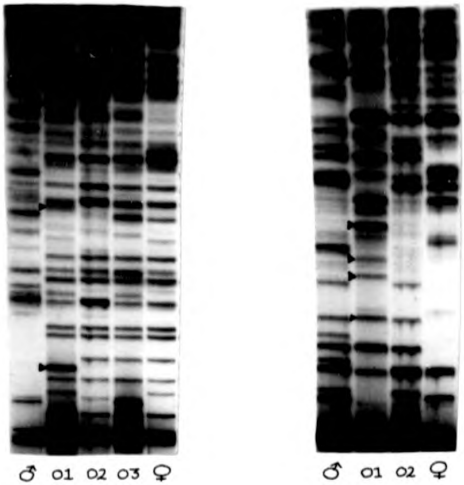


Figure 3.6 DNA fingerprints of two house martin families containing one offspring fathered by an extra-pair male (WN2 & C28). In each case offspring one (O1) is extra-pair. Non-parental bands are indicated by arrows.

The analysis is based on the following assumptions (Brookfield 1989):
(a) DNA fingerprints derive from a large number of unlinked genetic loci, the alleles at which are in complete linkage equilibrium in the population.
(b) None of the bands present in the scorable region of a fingerprint is linked or allelic but bands outside the scorable region always represent alleles of bands in the scorable region.
(c) Homozygotes and heterozygotes are indistinguishable at all loci.
(d) All bands have an equal gametic frequency (q) in the population.
(e) The mutation rate per gamete is constant for all alleles in both sexes and the gains and losses of scorable bands by mutation are independent processes but occur at an equal rate. Mutations always produce unique alleles.

The analysis of a house martin family suggests that assumptions (a) and (b) at least are not absolutely fulfilled because only about 75% of the total number of loci scored are inherited independently (section 3.3.2). However, in at least two families from which linked and allelic bands identified by maximum likelihood analysis had been removed (family in Figure 3.3 and another family for which data are not shown) there was no change in the results of the statistical analysis. Neither did the statistical interpretation of the results for any family conflict with the visual interpretation.

Table 3.6 contains details of parent-offspring relationships in 22 broods of house martins as determined by DNA fingerprinting. Excluding house martin brood G6(1), for which the data are inconclusive, evidence of at least one EPF was found in eight (38%) of 21 broods, and of a total of 72 offspring, 11 (15%) had been fathered by an extra-pair male. Time did not allow for the comparison of the fingerprints of mis-matched offspring with males from neighbouring nests to attempt to identify the true fathers. No case of intra-specific brood parasitism was identified.

Parent-offspring relationships in five families of sand martins as determined by DNA fingerprinting are given in Table 3.7. Because estimates for the average proportion of bands in the scorable region of a sand martin fingerprint that are inherited independently are not available, and because of the poor quality of the fingerprints obtained, the results for this species must be considered as preliminary, pending further study. Considering the five families screened, two (40%) showed evidence of EPF, with three of 17 offspring (18%) apparently fathered by an extra-pair male. In the case of family E15 it is tempting to suppose that the wrong male has been assigned. However, the male and female from this nest were colour marked and the subject of close observation during the egg-laying

Table 3.6 Parent-offspring relationships in 22 house martin families as determined by DNA fingerprinting

Nest (brood number)	Number of offspring		Comment
	Total	EPFs	
G1	2		
G2(1)	5		The male acquired a different mate
(2)	4	2	for the second brood
G3(1)	5		
(2)	3		
G5	4	1	
G6(1)	(3)2		Fingerprint of one offspring lost
(2)	3		
G7	2	1	Brood adopted by unrelated male after disappearance of pair male
G8	3	1	
G9(1)	3		
(2)	3		
G10(1)	(5)4	1	Fingerprint of one offspring lost
(2)	3		Male aquired new mate for 2nd brood
GN1	4	3	
GN2	3		
C4/5(1)	4		
(2)	2		
C28	2	1	
F4	4		
L34	4		
WN2	3	1	
22	72	11	Totals

Nests are coded with the initial of the colony name, followed by 'N' for a natural nest (no code letter implies a nest box). Nests without a brood number in brackets are first broods. EPF = extra-pair fertilisation. Unless otherwise stated, a male and female remained together to raise a first and second brood at the same nest.

Table 3.7 Parent-offspring relationships in five sand martin broods as determined by DNA fingerprinting

	Nest	Number of offspring	
		Total	EPFs
	E15	2	2
	E74	3	
	E75	3	1
	C14	5	
	C16	4	
Totals	5	17	3

Nests are coded according to subcolony (letter) and nest number. EPF = extra-pair fertilisation.

and early incubation period so their identity is certain. Of a clutch of five eggs laid, only two 'survived' until the time when the chicks were extracted from the burrow for ringing. The fate of the other three eggs is not known, so it is possible that some or all of them might have been fertilised by the pair male. As with house martins, no instance of IBP was found.

3.3.4 Correlates of paternity in house martins

The following chapter considers behavioural aspects of EPFs in house martins, including the relationship between the extent of male mate guarding and male brood provisioning and paternity. A number of other possible correlates of paternity are examined below.

(i) Attributes of male and female

Studies on a number of bird species have found correlations between attributes of an individual such as age and body size, and reproductive success (for example, Clutton-Brock 1988, Newton 1989). It seems likely that such attributes might also be related to the success of a male in achieving EPFs.

Table 3.8 shows the mean values of five body size parameters for males and females in broods where there were no extra-pair offspring compared with broods with at least one extra-pair offspring. In no case was there a statistically significant difference. Thus there is no apparent relationship between the body size of a male or female parent and the likelihood of a brood containing extra-pair offspring.

There was also no detectable relationship between the age of either parent and the likelihood that the brood would contain extra-pair offspring (Table 3.9).

(ii) Timing of breeding

Since the arrival of birds and the start of laying at house martin colonies is staggered, it might be expected that the nests of pairs that begin laying earliest are less likely to contain extra-pair offspring, since at this time there may be fewer extra-pair males to interfere with the laying females. An analysis of 15 first broods indicates that those that contain no extra-pair offspring were started earlier on average than those that contained at least one extra-pair offspring (respective dates of first egg 7th June, eight broods, and 19th June, seven broods), but the difference is not statistically significant ($T=-1.7$, $p>0.05$).

Table 3.8 Average body size of adult house martins related to the occurence of extra-pair offspring (EPO) in their nests

Sex & body size measure	No EPO (± se)	At least one EPO (± se)	t
Male parent			
mass	18.5 (0.179)	19.0 (0.346)	1.29ns
keel	19.7 (0.124)	19.7 (0.113)	0.21ns
wing	112.6 (0.694)	113.1 (0.833)	0.46ns
head & bill	26.7 (0.135)	26.7 (0.136)	0.19ns
tarsus	11.8 (0.111)	11.8 (0.139)	0.26ns
Female parent			
mass	19.0 (0.799)	19.3 (0.222)	0.73ns
keel	19.7 (0.186)	19.8 (0.168)	0.20ns
wing	114.4 (0.997)	112.9 (0.789)	1.06ns
head & bill	26.8 (0.179)	26.5 (0.149)	1.31ns
tarsus	11.9 (0.044)	12.0 (0.144)	0.63ns
Number of families	13	8	

Mass measured in grammes, all other body size measures in mm; ns = no significance

Table 3.9 The age of adult male and female house martins related to the occurence of extra-pair offspring (EPO) in their nests

Sex & age	Number of broods		X^2
	No EPO	At least one EPO	
Male parent			
1	11	6	0.0ns
2	2	2	
Female parent			
1	13	6	1.3ns
2	0	2	

Analysis based on a total of 21 families, ns = no significance

Table 3.10 The distribution of extra-pair offspring (EPO) in the first broods of house martins related to the occurence of a second brood

	First brood contains		p^+
	no EPO	at least one EPO	
pairs raising only one brood	3	6	<0.05
pairs raising two broods	4	0	

+ Fisher's exact test, one-tailed

(iii) Occurrence of second brood

Ignoring two nests where a second brood was attempted but one of the parents changed (in both cases there was a different female for the second brood), extra-pair offspring were never detected in the first brood of a pair that went on to attempt a second clutch. Of the 13 broods considered in Table 3.10, extra-pair offspring were found only in the broods of pairs that did not attempt a second clutch at the study colony, and this difference is significant (Fisher's exact test, one-tailed, $p<0.05$). All of the four pairs that went on to lay a second clutch were sucessful in raising the brood, and no extra-pair offspring were found in any of these second broods.

(iv) Colony size

It might be expected that the occurrence of extra-pair offspring would be higher in larger house martin colonies compared with small colonies, because in larger colonies there are more extra-pair males. It is difficult to draw any conclusions from the results of the present study, since most of the broods for which fingerprints were prepared came from a single colony. However, Table 3.11 does indicate that even in colonies as small as three breeding females, EPFs may occur.

3.3.5 Comparison of the frequency of EPFs in house martins and sand martins

For each species, the percentage of nests found to contain at least one offspring resulting from an EPF, and the percentage of nestlings that were not related to their putative fathers, are shown in Table 3.12. The preliminary indication is that there is a slightly higher incidence of EPFs in sand martins, but this result should be treated with caution in view of the small sample size and the poor quality of the sand martin DNA. Results of a more recent investigation into the mating system and social behaviour of sand martins (Alves, in prep) should enable a better comparison to be drawn between these two species.

Table 3.11 The number of broods containing at least one extra-pair offspring in house martin colonies of different sizes

Number of breeding females	Total families fingerprinted+	Families with at least 1 EPO	
		Number	%
12	18	6	33
4	1	1	1
3	2	1	50

+ includes first and second broods

Table 3.12 The frequency of extra-pair offspring in house martins and sand martins as determined by DNA fingerprinting

Species	Nests	% extra-pair fertilisations	
		nests	offspring
House martin	22	38	15
Sand martin	5	40	18

3.4 Discussion

3.4.1 Evaluation of the DNA fingerprinting technique

Human minisatellite probe 33.6 cross-hybridises to a number of dispersed loci in the DNA of house martins and sand martins to produce highly informative DNA fingerprints. Initial investigation has suggested that probe 33.15 hybridises to fewer loci than are recognised by probe 33.6 in both martin species, and that most of the loci detected by 33.15 are also detected by probe 33.6. Therefore, for the purposes of this study only probe 33.6 has been used.

A high level of variability is apparent in the DNA fingerprints of house martins and sand martins. Two unrelated individuals of either species share an average of 15% of the bands in the scorable region of their fingerprint. The calculated probabilities of identity suggest that for both species, there is a very small chance that even two closely related individuals such as siblings in a brood will have identical fingerprints (Table 3.4). Thus DNA fingerprinting can be used as a reliable means of characterising genetic relationships between individuals in both the study species.

The properties of DNA fingerprints of a number of species of bird and mammal are compared in Table 3.4. The average level of band sharing between unrelated individuals for birds probed with either or both of the human probes ranges from 9-47%. For two warbler species probed with a DNA sequence isolated from the willow warbler the equivalent band sharing probability was found to be 12%. Many of the bird species studied so far have a similar or slightly lower band sharing probability for unrelated individuals than has been found for humans. This is because human DNA fingerprints tend to contain more scorable fragments. The bird species in Table 3.4 that produces the least variable DNA fingerprints is the Rothschild's Mynah, a species that is now thought to be extinct in the wild and survives only as an inbred captive population derived from less than a dozen individuals.

Analysis of a family of house martins containing eight offspring suggests that approximately 75% of the scorable minisatellite fragments in a house martin DNA fingerprint are inherited independently with a transmission frequency of about 50%. The remainder of the fragments either represent pairs of alleles or groups of two or more linked bands. Thus the majority of minisatellite fragments are inherited in a Mendelian

manner and DNA fingerprinting can be used with confidence to investigate parent-offspring relationships in house martin families. A similar analysis of the pattern of inheritance of sand martin minisatellite fragments was not carried out because the largest available family of four offspring would have been likely to produce inflated estimates of linkage and allelism by chance. For the purposes of this study, Mendelian inheritance of minisatellite fragments in sand martins has been assumed and the results for this species are preliminary pending further investigation.

3.4.2 Evidence for a mixed reproductive strategy in house martins and sand martins

DNA fingerprinting provided no evidence for IBP in either sand martins or house martins. This is consistent with observations of nests of both species during the egg laying period, when no more than one egg per day was ever observed to be added to a clutch. In addition, Hoogland & Sherman (1976) noted an apparent absence of IBP in bank swallow (the American name for sand martin) colonies.

There was proof of EPFs in both of the study species. In house martins 38% of broods were found to contain at least one offspring that had been fathered by an extra-pair male, with 15% of all offspring mismatching with their putative father. Preliminary results indicated a slightly higher incidence of EPFs in sand martins (Table 3.12). This might be expected in view of the fact that sand martins live in larger colonies than house martins. The two sub-colonies from which sand martin nests were sampled contained approximately 30 and 100 nests, whereas the house martin colony from which most fingerprinted nests derived contained a maximum of 12 breeding females. Rates of EPF might be expected to be higher in larger colonies simply because a high density of nesting birds implies more opportunities for EPCs.

Thus in both house martins and sand martins DNA fingerprinting indicates that it is not safe to assume that all the offspring in a given nest are fathered by the male attending that nest, and that males may often waste parental effort by raising unrelated offspring. EPFs therefore represent significant fitness costs of reproduction for at least some males.

3.4.3 Correlates of paternity in house martins

No relationship was apparent between the body size of male or female house martins and the tendency of the brood to contain extra-pair offspring. Bryant (1988a, 1989) found that the apparent reproductive success (without considering paternity) of male house martins

was positively related to male body size, a result not convincingly corroborated in the present study (Section 2.4.2). Thus although larger male house martins may tend to raise more young on both an annual and a lifetime basis, there is no indication that large body size in males also confers a greater success in achieving paternity within the brood.

There was also no indication of an association between the age of the male or female parent and the tendency of the brood to contain extra-pair offspring. However, any effect of age may have been confounded by the relatively small sample size, and the fact that most parent birds were caught for the first time as breeding adults and assumed to be in their first year (section 2.2). Bryant (1988a, 1989) found that the apparent annual reproductive success of both sexes increased with age, seeming to peak in the second year of life. In a closely related species, the purple martin, Morton *et al* (1990) found that older males achieved 96% paternity in their nests, and increased their fecundity at the expense of younger males.

The only factor that did seem to be related to the occurrence of extra-pair offspring in house martins was the number of broods attempted. Males that remained paired to the same female for two broods in the same season were never found to be cuckolded. In two cases where males acquired a new mate for a second brood, extra-pair offspring were present in at least one of the broods. This may indicate that males of 'faithful', double-brooded pairs are more successful in ensuring that the female is not fertilised by extra-pair males, or that the female chooses not to participate in EPCs (Møller 1991a). The suggestion of Weatherhead & McRae (1990) that EPFs are more likely in second broods because the need to provision fledglings prevents the male from guarding his mate properly does not seem to hold for house martins, although I did observe parents feeding first brood fledglings up to six days before the first egg of a second clutch was laid.

The interval between pairing and laying is difficult to measure in house martins (section 4.3.1 ii), and the period for which females can store viable sperm after copulation has not been determined. It is possible that if a female copulates with a number of males before selecting a partner, and begins laying soon after pairing, her eggs may be fertilised by the sperm of more than one male, even if she has been 'faithful' to her eventual partner. Thus, as noted by Birkhead & Møller (1992) apparent EPFs might actually result from rapid mate-switching rather than EPC.

Behavioural and physiological aspects of mixed reproductive strategies in the two study species are the subject of the following chapter.

4. Time budgets of nesting house martins and sand martins

4.1 Introduction

4.1.1 Reproductive success, mating systems and sperm competition.

In birds the pre-laying and laying periods, or more precisely the fertile period of the female (the period during which copulations might result in the fertilisation of eggs), are the times when the maximum level of individual reproductive success from the current breeding attempt is set. Each fertilised zygote derives from one egg and one sperm and the theory of natural selection leads us to expect that both sexes should seek to contribute to as many fertilised eggs as possible, as long as this remains consistent with the maximization of lifetime reproductive success. Females could accomplish this by laying as many eggs as possible, perhaps in one or more nests; males by mating with very fecund females, or by mating with as many females as possible (Orians 1969), or both. In certain circumstances either sex might attempt to increase its own reproductive success at the expense of its partner, by deserting a current breeding attempt and acquiring a new mate (Trivers 1972), leaving the partner to raise offspring alone. The range of mating systems in bird species can be interpreted as different outcomes of this 'battle of the sexes' in differing ecological contexts (Orians 1969, Emlen & Oring 1977, Wittenberger & Tilson 1980, Oring 1982).

Bird mating systems can be divided into four principal categories: monogamy, polygyny, polyandry and promiscuity (Mock 1983, Perrins & Birkhead 1983). The definitions given here refer to consort patterns of the sexes within a breeding season. Monogamous mating systems are those in which a male and female form a pair bond and share parental care of the offspring, and this is the predominant mating system among birds (Lack 1968). In polygynous mating systems males acquire two or more mates, either simultaneously or successively, and often provide little or no parental care for their offspring. Polyandrous mating systems are those in which females mate successively or simultaneously with two or more males, and do not normally provide parental care. In promiscuous mating systems both males and females may mate with more than one member of the opposite sex, either simultaneously or in succession, and either sex may provide parental care. These definitions of mating systems are not hard and fast. House martins, for example, are normally described as monogamous, but about 10% of pairs split after the first brood,

with either the male or the female aquiring a new mate for a second brood (Bryant 1988a). There is also evidence that sand martins often switch mates for a second brood, and that females may leave their partners to care for first brood young in order to start a second brood earlier (Cowley 1983). Thus a proportion of the population of both of the study species could be described as promiscuous.

The term 'mating system' has previously been employed to refer to the general behavioural strategy employed in obtaining mates; encompassing features such as the number of mates acquired, the manner of mate acquisition, the presence and characteristics of pair bonds and the division of parental care between the sexes (Emlen & Oring 1977). Starch gel electrophoresis, and more recently DNA fingerprinting, provide evidence that the patterns of gamete transfer underlying many different mating systems are promiscuous. Even within the constraints of a monogamous breeding system individuals of both sexes have been shown to attempt to maximise their own reproductive success by pursuing a mixed reproductive strategy (MRS; Trivers 1972, Fitch and Shugart 1984), which might be viewed as an intermediate between the two extremes of monogamy and polygamy. For example, the occurrence of EPFs in house martins and sand martins demonstrated in the previous chapter is proof of promiscuous mating in two species that are classified as monogamous in terms of the consort patterns of the sexes. The advent of techniques for measuring genetic relationships between individuals means that there is a possibility of classifying mating systems at two distinct levels - the 'basic' level of gamete transfer, which might be defined as the 'mating system' proper, and the pattern of consort between males and females, which could be referred to as the 'breeding system'. Thus these terms, which have been used inter-changeably in the past, could now be considered as distinct.

In monogamous species that pursue a MRS sperm competition would be expected to occur. This involves competition between spermatozoa of different males to fertilize the eggs of a single female during one reproductive cycle (Parker 1970, Birkhead 1988, Birkhead & Møller 1992). A given male might be expected to maximise his reproductive success by (i) ensuring that he has fertilised all the eggs laid by his mate in his nest (and any eggs that his mate might dump in another nest) and (ii) copulating with as many extra-pair females as possible in an attempt to father extra-pair offspring which will be raised in the nest of another pair. Females might increase their reproductive success by mating with high quality males, either by choosing them as mates, or by selectively accepting EPCs from males of higher quality than their mates. Females could also increase their reproductive success by maximising the number of eggs laid, perhaps

laying some eggs in the nests of other pairs or females of the same species. Thus intraspecific brood parasitism (IBP) might be regarded as a component of a female MRS and the latter term is used to cover both EPFs and IBP in this thesis. Since no evidence for IBP was found in either of the study species (Chapter 3) it will not be discussed further here. Recent reviews of this subject are given in Yom-Tov (1980), Anderson (1984), Brown & Brown (1989) and Petrie & Møller (1991).

Evidence accumulated so far indicates that sperm competition occurs widely in bird species with monogamous breeding systems (Gladstone 1979, Ford 1983, Mckinney *et al* 1984, Birkhead 1987, 1988, Birkhead & Møller 1992) and has a profound effect on many aspects of avian ecology (Birkhead & Møller 1992).

4.1.2 Defence of paternity

If all males, or a substantial proportion of the males, of a given species, attempt extra-pair copulations (EPCs) then to fulfil condition (i) above a male will have to take steps to ensure that his partner is not fertilised by an extra-pair male. Males of many bird species remain in close proximity to their mates and may actively prevent the approach of extra-pair males during the period when the female is fertile. This behaviour, referred to as mate guarding (Birkhead 1979, 1982, Ford 1983, Birkhead & Møller 1992), occurs in a number of bird species and has been interpreted as a form of paternity defence.

Studies of mate-guarding have focused on the timing and duration of this behaviour, the reasons why it occurs in some species and not in others, and intra-specific variation in guarding intensity.

Birkhead (1982) considered the timing and duration of mate guarding in the magpie to be consistent with the hypothesis that males should mate guard only during the period that their mates are fertile, putting the emphasis on mate guarding as a behaviour that benefitted the male and raising the question of how males might perceive that their mates are fertile. However, since the fertile period is known for relatively few species of bird, and will vary with the length of time that a female can store viable sperm and the spread of laying (Birkhead 1988, Birkhead & Møller 1992), the hypothesis that a male only guards his mate while she is fertile is difficult to test. Lumpkin (1983) found that captive female ring doves solicit copulations from their mates and thereby induce mate guarding behaviour several days before the beginning of their fertile period, and the same may be true of willow ptarmigan (Martin & Hannon 1988). Lumpkin (1981, 1983) pointed out

that mate-guarding benefits females as well as males, for example by reducing the amount of time and energy 'wasted' by unguarded females in responding to EPC attempts, and suggested that female ring-doves were manipulating the anti-cuckoldry behaviour of their mates and deceiving them into mate-guarding for longer than was necessary. Morton (1987) found a high degree of variation in the intensity of mate-guarding and no correlation between guarding intensity and male parental effort in purple martins, and also suggested that mate guarding might serve to protect the female from harassment, rather than to defend the male's paternity. In extreme cases, forced EPCs, carried out by males of many waterfowl species (Mckinney *et al* 1983, 1984) can be exhausting, wounding or even fatal to females. Mate-guarding as an anti-harassment strategy will benefit both the female and the male, if it increases the number and/or quality of the eggs laid. In addition, two pairs of eyes are better than one, so mate guarding could increase the level of vigilance of a pair against predation, again benefitting both male and female.

Males of many species do not guard their mates continuously (Ford 1983), and it can often be difficult to decide if mate guarding occurs or not, as is the case with tree swallows. Lefelaar & Robertson (1984) suggested that the lack of mate-guarding in this species was related to a low probability of EPCs. However, a subsequent study of mating behaviour using coloured glass microspheres inserted into the cloacae of males to mark sperm transfer, indicated that EPCs were more frequent in tree swallows than had previously been suspected (Morrill & Robertson 1990), and the occurence of EPFs has been confirmed using DNA techniques (Table 12.2 in Birkhead & Møller 1992). Behavioural observations indicated that pair copulations (PCs) were very frequent, suggesting that males tree swallows attempt to ensure their paternity by copulating frequently with their mates in an attempt to dilute sperm introduced during EPCs (Morrill & Robertson 1990). In support of this, experiments on sperm competition indicate that when two or more males copulate with one female close together in time, the male that introduces the most spermatozoa is most likely to fertilise the eggs (Birkhead 1988, Birkhead & Møller 1992, and references therein). Males may in fact have a number of alternative options as far as paternity defence is concerned if other ecological factors such as the need to defend a nest site prevent them from mate guarding full time (Birkhead & Møller 1992). They may copulate frequently with their mates (see above), react aggressively towards a mate who has participated in an EPC in order to delay ovulation (Hutchinson & Lovari 1976), or even attempt to remove sperm deposited during an EPC as has been shown to occur in Dunnocks (Davies 1983).

4.1.3 Opportunities for extra-pair copulation

From the males' point of view, the opportunities for EPCs depend on the number of fertilisable females, other than mates, that are available in space (nesting dispersion) and time (breeding synchrony); on the amount of time that must be devoted to ensuring paternity of all the eggs laid by mates; on the availability of adequate resources to meet the extra demands on time and energy imposed by EPCs; and, in some species at least, on the willingness of females to participate in EPCs.

An EPC directed at a non-fertile female is a waste of time and energy, so the question of whether or not males can recognise fertile females arises once more. Female sand martins become heavier just before and during the egg-laying period, which is also the time that they are fertile. Jones (1986) demonstrated that male sand martins seem able to detect the slightly laboured flight of such females and apparently use this as a cue for the timing of EPC attempts. In other species males might use the occurrence of mate-guarding or PCs as an indication that a non-mate female is fertile, as is apparently the case with swallows (Møller 1987b). Results from several species indicate that males do time their EPCs to coincide with the presumed fertile periods of females, in that the pattern of occurrence of EPCs closely matches that of PCs (Birkhead & Møller 1992). There may also be an adaptive diurnal timing of EPCs to coincide with the 'fertilisation window' of a female (Cheng *et al* 1982, Birkhead 1988, Aguilera & Alvarez 1989, Birkhead & Møller 1992).

The amount of time and energy a male will be able to devote to EPC attempts may be constrained if he has to guard his own fertile mate or the nest site. Thus males might be expected to confine EPC attempts to the periods before and after their own mate is fertile.

Males of most bird species lack a penis (King 1981) and are probably unable to force females to accept copulations, with the exception of groups such as waterfowl (Mineau & Cooke 1979, Burns *et al* 1980, McKinney *et al* 1983). Published studies indicate a general tendency of females to avoid EPCs, especially in the presence of their mate, although on occasion females have been observed to accept (Møller 1985, Alatalo *et al* 1987, Martin & Hannon 1988, Aguilera & Alvarez 1989, Morrill & Robertson 1990) or even solicit (Buitron 1983, Hatchwell 1988, Wagner 1992) EPCs. If the ability of sperm from different males to compete to fertilise the egg varies, and sperm competitiveness is heritable, then it may be to the advantage of a female to promote sperm competition by

mating with two or more males during the fertile period. Her sons are thus likely to enjoy greater reproductive success (Fisher 1958). In an analysis of human behaviour, Bellis & Baker (1990) found that double-matings (a female mating with a second male while still containing fertile sperm from a previous male) were most frequent at the time in the menstrual cycle when conception was most likely to occur. This suggests that in humans at least, females actively promote sperm competition. A study of adders in Sweden found that the proportion of young that were stillborn was strongly negatively correlated with the number of matings, and the number of males that a female had mated with (Madsen *et al* 1992); providing evidence that multiple copulations with different partners increase offspring viability in this species. Females might also benefit from EPCs if they obtaining resources such as food for themselves or their offspring from extra-pair males (Thornhill 1984, Davies 1985). Participating in an EPC might reduce the risk of injury if an extra-pair male attempts to force copulation; decrease the risk of infanticide if the pair male dies (Møller 1988a, Crook & Shields 1985); and guard against total breeding failure if the pair male is sterile (Wetton & Parkin 1991). However, a female may risk producing 'unsexy sons' (Birkhead & Møller 1992) if males seeking EPCs are of low genetic quality; or desertion (Gladstone 1979, Trivers 1972), attack (Barash 1976) or reduced parental investment (Davies 1985) by her mate if she is detected. In addition, promiscuous mating probably has attendant costs of disease, parasite transmission, and possibly increased vulnerability to predators (Birkhead & Møller 1992).

4.1.4 Male removal experiments

Close proximity between the male and female of a pair during the pre-laying and laying periods is not conclusive evidence that the male is guarding his paternity. The male may alternatively remain close to the female so as to be available when she solicits copulations - minimising the effort of repeated mating by avoiding search time; to protect her from harassment (section 4.1.2) or predation; or to maintain the pair bond (Birkhead & Møller 1992). The question of what happens when a fertile female is not guarded arises. To simulate this situation, male house martins and sand martins were removed for periods of six hours early in the laying period, when females were presumed to be fertile. This allowed assessment of the frequency of attempted EPCs in the presence and absence of the pair male, and the responses of females to EPC attempts.

4.1.5. Provisioning of offspring

The feeding of offspring can be regarded as a form of parental investment. Trivers (1972)

defined parental investment as any investment by the parent in an individual offspring that increases the offspring's chances of survival (and future reproductive success) at the cost of the parent's ability to invest in further offspring. However, the pursuit of a MRS (Fitch & Shugart 1984, Trivers 1972) may allow parents to invest in further offspring at the same time as they care for the offspring of a current reproductive attempt. Thus parental investment and the pursuit of further matings need not be mutually exclusive activities. The term parental care, which may be defined as investment into offspring after fertilisation (Werren *et al* 1980) will thus be adopted. Parental care may still entail a cost to the parent as a result of raising offspring, but this term does not imply exclusivity between raising offspring and seeking further matings.

A central concern in the discussion of parental care in birds is the conditions under which one parent will abandon a current breeding attempt and attempt to acquire another mate (Trivers 1972, Dawkins & Carlisle 1976, Maynard-Smith 1977). It is often suggested that the male may be the sex that is most inclined to desert, since his initial investment in the clutch of eggs is less than that of the female (but see Dewsbury 1982, Gladstone 1979). In support of this, polygyny is more prevalent than polyandry in birds, but the majority of birds (over 90%) have a monogamous breeding system (Lack 1968), and it is common for both parents to feed the young. It has generally been assumed that in most species the maximum reproductive success is gained by both sexes staying to care for the offspring, rather than deserting and attempting to acquire another mate. However, in many bird species it seems that male assistance is not necessary to enable females to raise some young, although survivorship of young is usually higher when the male is present (Wittenberger & Tilson 1980, Bart & Tomes 1989). In some species such as the eastern bluebird (Gowaty 1983) and the sea-side sparrow (Greenlaw & Post 1985), females have been found to raise as many or more young alone than they did in the presence of the male.

Given the starting point of monogamy and bi-parental care, it remains possible that there may be inequalities in the amount of parental care given by the two sexes. If a male cannot be certain that the young in his nest are genetically related to him because his mate may have mated with an extra-pair male, he might be dis-inclined to invest heavily in the care of offspring, especially if the effort expended in parental care is likely to reduce his expectation of future reproductive success (Pianka 1976, Pianka & Parker 1975). Theoretical analyses of the influence of 'certainty of paternity' on paternal care have produced conflicting results. Werren *et al* (1980) predicted that paternity would influence the evolution of parental care only if a caring male sacrificed the opportunity

of promiscuous matings by opting to help raise the young in his nest. However, Knowlton & Greenwell (1984) and Winkler (1987) predicted that male parental care should be strongly related to certainty of paternity. In support of this, in a cross species comparison, Birkhead & Møller (1992) found a strong negative relationship between the amount of paterntal care and the extent of extra-pair paternity. In practise it is likely that the relationship between paternal care and certainty of paternity should depend on the relationship between male parental care and offspring recruitment, which varies between bird species (Whittingham *et al* 1992).

The relevant questions are (i) do males assess their likelihood of paternity and adjust their parental care accordingly and if so (ii) what cues do they use to assess their paternity? There is some evidence that male birds may be able to judge whether or not they are likely to have fathered at least some of the nestlings in a brood. Dunnocks have a very variable breeding system (Davies & Lundberg 1984) and it is often the case that two males occupy a territory with a single female. In such cases of polyandry, paternity of nestlings is often shared by both males. Burke *et al* (1989) found that males did not discriminate between their own young and those of another male when feeding multiply sired broods, but they apparently used the amount of exclusive mating access they had to the female to judge whether or not to feed the young. Males who had no exclusive access to the female rarely helped to feed her offspring. This was evidence of an all or nothing effect: no access = no feeding but access = feeding. Further work on this species has also demonstrated a relationship between the percentage of mating access to a female gained by a beta male (the subordinate of two males sharing a single female) and the percentage of male feeds provided by the same male (Davies *et al* 1992). The percentage of the brood fathered by the beta male was also related to the amount of mating access he had to the female, suggesting that males might monitor their paternity share by comparing the amount of exclusive access they had to the female with that of their rival, and adjusting their rates of brood feeding accordingly. However, this relationship may not be a causal one, it may reflect the fact that beta males who are better competitors both gain more mating access to the female, and are able to invest more in offspring.

The work on dunnocks described above provides evidence of relative adjustment of male feeding rates according to paternity in a polyandrous mating arrangement. There is, as far as I am aware, no direct evidence yet for an adjustment of male feeding rate relative to certainty of paternity in a monogamous species. Møller (1988b, 1991b) did find that male swallows decreased their brood feeding frequency and brood defence with experimentally induced increases in EPCs by male removal experiments. However, DNA

fingerprinting was not used to confirm the genetic relationships between parents and offspring. Further, this result has recently been questioned by Wright (1992), who suggested that the reduced feeding rate of experimentally removed males was in fact due to an increased level of nestling mortality and consequent reduced brood size in their nests.

This chapter presents an analysis of observations of the behaviour of sand martins and house martins during the nesting period. DNA fingerprinting has shown that EPCs occur in both species, and a number of questions arise from this finding. Where do PCs and EPCs occur; does mate guarding occur, and if so, is it organised so as to be effective as a strategy for paternity defence; and, how do males allocate their reproductive effort so as to ensure consanguinuity with their own brood whilst also attempting to obtain EPFs?

Bryant (1988b) found a correlation between the number of feeding visits made by adult house martins and their parental effort as measured by energy expenditure. The absolute and relative contributions of male and female house martins to the care of the offspring were examined, using the number of feeding visits made by each sex as a measure of the contribution to parental care. The specific question addressed was whether the rate of food delivery to the brood by the male was influenced by his paternity, as measured by DNA fingerprinting.

Mate removal experiments (section 4.1.4) provided an opportunity to investigate whether male house martins actively assessed their own likelihood of paternity. Removed males were separated from the nest and from their mates for periods of 5-8h. If this period of separation occurred while the pair female was fertile, the male might have cause to suspect that she had mated with an extra-pair male, and that one or more of the nestlings in the brood might not be fathered by him. It might therefore be predicted that males removed during the fertile period of their mate would show a reduced rate of brood feeding. This methodology assumes that the male believes his mate to be accessible to other males during his period of capture. Since the members of a pair were normally captured together at the nest, it is possible that males might assume that their partners were also being held away from the nest, and therefore would not be 'worried' about their paternity.

4.2 Methods

4.2.1 Catching and marking birds

Birds were caught as soon as possible after arrival at the nesting colonies with most captures being made at the nest site because this was the only place where birds regularly alighted and colour marked birds could be immediately assigned to a particular nest. Pairs of both species regularly roosted in the nest or burrow during the prelaying and laying periods, emerging about half an hour after first light. By setting up mist nets or nest traps (Figures 4.1a & b) at a colony just before dawn birds could be captured as they emerged. When attempts were made to catch several pairs of sand martins in a given morning the chances of success were improved by visiting the colony after dark. The targeted burrows were blocked with blind-ended cardboard tubes with holes punched in the ends to allow the free circulation of air into the burrow. Tubes were replaced with nest-traps at dawn the next day.

For both sand martins and house martins I delayed the beginning of intensive capture attempts and observations until the first few pairs had established themselves at a breeding colony. Capture of prelaying and laying birds at the nest site unfortunately carries with it a risk that one or both of the pair will desert and I did not want to cause birds to abandon a breeding colony. Laying females of both species seemed particularly prone to abandon a nesting attempt after a first capture. Thus for sand martins data for marked pairs was supplemented with observations of unmarked pairs for which capture was delayed until incubation had begun. Observations of unmarked house martin pairs were found to be less useful, but observations at two nests where a female deserted after capture and a marked male acquired another mate have been included in the analysis, assuming that an un-marked bird entering the nest was the female. In one of these cases the female was induced to mark herself by placing a pad of cotton wool soaked in red dye just inside the entrance to the nest box.

Captured birds were given individually numbered aluminium BTO rings and colour-marked using a combination of five colours of non-toxic water-based felt-tip pens (red, green, blue, brown and purple) and spots of 'tippex' liquid paper. For sand martins, colour marks on the throat (above the breast band), throat and chest (below and above breast band), and the vent and under-tail coverts were readily distinguishable, as were 'tippex' spots on the nape, the back and the upper marginal coverts of the wings.

Figure 4.1a House martin nest trap

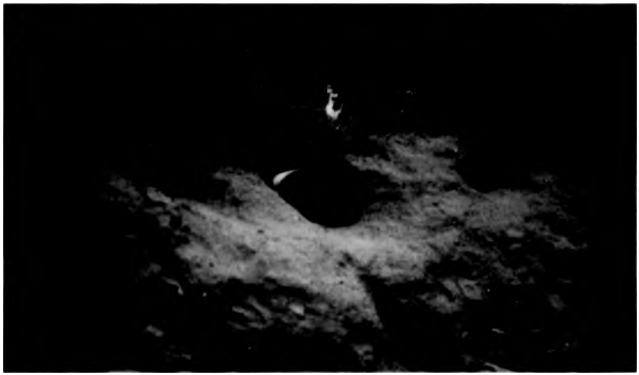


Figure 4.1b Sand martin nest trap

Combinations of marks for each bird were chosen so that identification could be made from the front and the rear, which was found useful when pairs entered or left a burrow in quick succession and only part of each bird was seen clearly. House martins were marked with combinations of colour marks on the throat-breast, the flanks, the rump and the vent and under-tail coverts. 'Tippex' spots rapidly wore off house martins and their use was abandoned. Colour marks were found to persist for several weeks, and they were renewed each time a bird was caught.

Some methods of marking individual birds have been shown to affect their subsequent behaviour, reproductive success, or survival (for example Kinkell 1989, Burley 1988, L Stader pers comm). I have no reason to suspect that colour marking as carried out in this study caused direct harm to any bird, or had any significant influence on its subsequent behaviour. There was no evidence, for example, of newly marked birds being chased or rejected by their mates, or being susceptible to increased predation risk. I did observe one sand martin male preening repeatedly after being marked on the breast, and tippex marks on sand martins sometimes wore off taking the feathers with them, leaving small bald patches, but these did not seem to affect the immediate future survival or breeding success of the birds concerned.

4.2.2 Time budget observations

Observations were made on the behaviour of house martins and sand martins during the prelaying and laying periods. Most of the data were collected in 1989, the final year of the study. Observations of sand martins were made at Barbush Sand Quarry (Figure 2.2, NN787026), concentrating on two subcolonies of respectively 100 burrows (about 70 pairs), and 30 burrows (20+ pairs), chosen because the burrows were accessible and observations could be related to events in the nest; and also because a car could be driven close to the colony and used as a hide. Most observations of house martins were carried out at Glendevon (Figure 2.2, Appendix A), which in 1989 was the largest colony with 12 breeding females. Here the birds were accustomed to the presence of humans and I was able to watch nests from 10-20m without causing discernable signs of disturbance.

Time budget data were collected for individual nests for periods of one hour. Depending on the number of nests available at a suitable stage, a given nest was observed for 1-3 hours per day. Behavioural events in and around the nest were recorded as they occurred within 1 minute 'observation windows'. No attempt was made to follow birds away from the nest because they rapidly flew out of sight or so far away that colour marks became

indistinguishable. Observations at a given nest were begun as soon as burrow digging was well advanced in the case of sand martins; or as soon as at least one of a pair of birds attached to a particular nest site had been marked for house martins. Data collection was continued until incubation behaviour was observed (the members of a pair regularly change-over at the nest). The list of events recorded for each hour time-budget session is given in appendix C.

For sand martins, observational data were usually collected in the morning (before 1200h) and the evening (after 1800h). Between 1200-1800hrs prelaying and laying pairs often spent long periods (more than 1h) away from the burrow, and afternoons were used for checking nests. In the case of house martins, most prelaying and laying observation sessions were begun between 0500h and 1200h, again using afternoons for nest checks, although late in the season I collected some prelaying and laying observations for house martin second broods after midday, whilst recording nestling feeds for late first broods.

In addition to data collected for target nests during time budget sessions, conspicuous events such as fights or matings were recorded opportunistically. Sand martins were regularly seen landing and pecking at the ground in certain areas of the quarry, and at this time a female may be vulnerable to mating attempts (which could be PCs or EPCs). Observations were made at such landing sites for 1-2 hours on four evenings, recording the number of birds landing and any interactions between birds. Male and female house martins co-operate to build a nest and often collect mud from the ground, so again this is a time that a female could be vulnerable to mating attempts. However, at the Glendevon colony most pairs nested in boxes, and as far as I was aware those pairs building nests collected mud exclusively from the gutters of the house. House martins may also alight to rest on telegraph wires, although I never observed this behaviour at Glendevon during the nesting period. Thus it seemed probable that females were on the wing all the time they were away from the nest-site, and that all PCs and EPCs would take place at the nesting colony.

4.2.2 Male removal experiments

Male removal experiments were carried out during the laying and incubation periods. Pairs were caught at dawn in the nest box or burrow, and weighed and marked if they had not been caught previously. The female was then released and the male kept in a cloth bird-bag for 5-8 hours. Retained males were fed at two hourly intervals with beef mince. Observations were carried out at the nest of the experimental pair for 2-3 hours

during the period that the male was held captive, normally for two hours after release of the female, and for a further hour before the male was released. In 1989 observations were continued for an hour after release of the male.

Male removals during the laying period were carried out on the day that the second or occasionally the third egg of the clutch was laid. Attempts to remove males earlier in the nesting cycle, for example the day the first egg of the clutch was laid, presented problems. It was found difficult to predicting the day on a, given pair would begin laying, and capture at this time carries the risk that the female will postpone the start of laying or desert (D M Bryant pers comm; pers obs). The two females (one sand martin, one house martin) that deserted after male removal experiments were both caught for the first time during the laying period. By delaying removal until after laying had started breeding was less likely to be disrupted and the experiment could be related to events in the nesting cycle even if either member of the pair deserted subsequently.

Females of both study species were thought to lay eggs between approximately 0600 and 0900hrs. So far as is known, each successive egg of a clutch is ovulated and fertilised within 15-75 minutes of the previous egg being laid (Lake 1975, Sturkie 1976). If there is last male sperm precedence (Birkhead & Møller 1992), then an EPC carried out within 75 minutes of laying should stand a good chance of fertilising the next egg and removing males for 5-8hrs from dawn (0400-0600hrs) should have provided opportunities for extra-pair males to gain fertilisations. In addition, if fertilisation of the next egg of a clutch occurs within 75 minutes of laying, a female should be fertile up until the day before the last egg of the clutch is laid. Since female sand martins and house martins normally lay clutches of at least three eggs, male removals on the day of the second egg should always fall within the fertile period of the female. If only three eggs are laid then a removal experiment carried out on the third day will not involve a fertile female.

Male removals during the incubation period were intended to allow comparison of the following in the presence and absence of the pair male: (i) the behaviour of fertile versus non-fertile females, and (ii) the behaviour of extra-pair birds towards fertile versus non-fertile females.

4.2.3 Measurement of feeding frequency

I made observations of the feeding frequency of male and female house martins for periods of three days at each of a total of 15 nests containing young between 10 and 20

days old. During this period the rate of food delivery by each sex is not influenced by nestling age (Bryant & Gardiner 1979, Gunten & Schwarzenbach 1962). Each nest was observed for a total of 12 hours, comprising four hours between 1000hrs and 1600hrs on three separate (usually consecutive) days. The daily timing of observations was chosen so as to fall within the period when feeding rate does not depend on the time of day (D M Bryant pers comm). Birds were colour marked so they could be identified when they landed at the nest to feed young. The list of behavioural and environmental variables recorded for each hour of observation is given in appendix D. Observations were made in all weather conditions except continous heavy rain when it was found that virtually no feeding trips were made.

4.3 Results

4.3.1 Arrival and pair formation

The following descriptions are based on general observations of nesting behaviour made during the three years of the study, supplemented by accounts in the literature.

(i) Sand martins

In Central Scotland, sand martins begin to arrive back from their wintering grounds during the last week of March or the first week in April, although they do not normally begin regular visits to breeding colonies until about 2 weeks after their first arrival (Asbirk 1976, pers obs). In 1989, the owners of Barbush Quarry reported that sand martins were first seen at the site on 29th March, the earliest arrival date they could remember. Occupation of the breeding colonies has normally begun by the third week in April, and laying of first clutches begins in the last week of April and continues through May and early June. The Barbush colony is composed of a number of subcolonies at different sand faces, with new subcolonies being occupied as the existing ones are settled (Jones 1987b). Older birds (2+ years) arrive on average 2-3 weeks before first year birds (Mead & Harrison 1979). Early arrivals often occupy and renovate burrows from the previous year, whereas later arriving birds more often have to dig their own burrows. Males occupy burrows first or initiate digging, and females appear to choose a male by accepting a nest site, thereafter helping with the burrow construction (Cramp 1988, pers obs). Burrows are normally at least a metre in length, and slope upwards for at least a short distance from the entrance, presumably to prevent rain water from running down into the nest. The nest chamber is usually, but not always, at the end of the burrow, and is normally lined with dry grasses before the eggs are laid, with feathers being added before and during incubation.

(ii) House martins

In Central Scotland, house martins begin to arrive at breeding colonies during the last week of April. Arrival is staggered, with new birds continuing to appear throughout May and June. Older birds (2+ years) arrive back before 1 year olds (Rheinwald *et al* 1976, Bryant 1979, Hund & Prinzinger 1985). House martins seem to pair very soon after arrival at the study colonies. First arivals in nest boxes in May were checked in 1988 and

1989 by visiting nesting colonies after dark or before dawn and shining a torch into the entrance to check for roosting adults. Of 21 nests thus found to be occupied, 18 (86%) contained two birds, although these may not always be a male and a female (D M Bryant, pers comm).

The start of breeding is staggered. Between 1987 and 1989 the overall spread of first clutches at study colonies was between 19th May and 15th August, with 81% of pairs laying during May and June (n=110 pairings). A few late first breeding attempts may have represented birds that had moved from another colony after a failed breeding attempt, but most late layers are birds that have been associated with colonies for some time (D M Bryant, pers comm).

Most of the birds in this study bred in artificial nest boxes, (Figure 2.3). Before egg-laying commenced, birds usually lined the entrance hole with mud and built a nest of dry grass, moss and often wool. Completed mud nests were also normally lined before egg laying commenced. Feathers were usually added to the nest lining before and during incubation.

The interval between pairing and laying was very variable, and difficult to measure accurately because the precise date of pairing was not easy to ascertain. At the Glendevon colony in 1989, the interval between the appearance of signs of occupation and the laying of the first egg was between 3-18 days for pairs nesting in boxes (n=9), and 11-22 days for pairs building natural nests from scratch (n=2). First clutch size varied from 3-5, with eggs normally being laid on successive mornings, between 0600 and 0900hrs, until the clutch was complete.

4.3.2. Pair copulation and extra-pair copulation

(i) Sand martins

It has been generally assumed that sand martins mate mostly in the burrow (Cramp 1988 and references therein). In support of this, Jones (1985) observed a pair mating on one occasion in a burrow with a viewing chamber, although Asbirk (1976) who observed pairs nesting in artificial glass-topped chambers never saw copulation take place within in the nest. During this study copulation was observed only once on a visit to Barbush on 5th May 1990, the year after the time-budget data were collected. Mating took place on the ground at 1700h. The attempt lasted about 30 seconds, but was not seen clearly

enough to be sure if cloacal contact was made. Both birds subsequently took off together. There are a number of accounts in the literature of sand martins mating on the ground, on wires, or even in the air (Cramp 1988, Jones 1985). In most cases the birds involved were unmarked so it was impossible to tell if the mating was a PC or an EPC, although observations involving elaborate pre- or post-copulatory behaviour (Watson 1946) probably indicate PCs, whereas multiple copulation attempts made on stuffed sand-martins placed on the ground near a nesting colony (Hoogland & Sherman 1976, Petersen 1955) indicated that EPC attempts may also take place on the ground.

No observations were made within nesting burrows, so I cannot comment on whether matings take place within the nesting chamber, although there seems no obvious reason why not. Sand martins landing on the ground in the vicinity of the nesting colony were often harassed, so it would seem likely that within the nesting chamber or at sites well away from the nesting colony are the only places where a pair might mate without disturbance.

A total of five hours on four separate evenings was spent watching sand martins landing on the ground near to the nesting colony during the laying period. During this time, 93 'bird-landings' were seen. No definite mating attempts were observed, although on one occasion a bird landed on the back of a sand martin on the ground. If this was a mating attempt it was quickly thwarted as the bird on the ground moved away and took off. Sand martins were frequently observed to land on the ground in pairs. Often one of the pair would peck at the ground whilst the other adopted a hunched posture with the feathers on its back raised and called continually, appearing quite agitated. This could be interpreted as a male guarding a female while she collected grit, but as no marked birds were observed this is unproven. Landings occurred sporadically, and once one bird had landed several would often follow suit. Birds on the ground were frequently swooped upon by other sand martins, although the only occasion contact was made was the possible mating attempt described above. A pair of house martins collecting mud in the vicinity of the sand martin colony were similarly harassed.

It seems therefore that PCs may take place within the nest chamber and on the ground, and that EPC attempts may also take place on the ground. Is there any evidence that EPCs may take place in the nest? Since sand martin males were found to mate-guard almost continually around the time that the first egg was laid a fertile female was rarely alone at the nest. It is possible that an extra-pair male might enter the nest and attempt to mate with a female whilst her mate is present, as occurs in house martins. However,

during 26 hours of observations of three marked pairs of sand martins during the pre-laying and laying periods, extra-pair birds were never observed to enter the burrow whilst the pair were inside, although mate guarding pairs were frequently chased back to the burrow entrance by one or more extra-pair birds. Beecher & Beecher (1979) found that identified chasers in bank swallows were always males. In addition, in 23 cases where captures were made at nests at the pre-laying or laying stages (including 10 captures at dawn before birds had emerged from roost), no more than two adults were ever caught in one burrow, implying that extra-pair birds are not present in burrows overnight.

(ii) House martins

Pair copulation was observed only once when a pair mated near the entrance of a nestbox with a particularly wide entrance hole. This took place at 0808h, five days before the laying of the first egg. Otherwise it was not usually possible to observe events in the nest, but I suspect that a gentle churring song sometimes heard from a nest occupied by a pair was an accompaniment to some stage of mating.

EPCs were never directly observed, but potentially occurred whenever an extra-pair male entered the nest when the pair female was present. Extra-pair birds were seen to enter nests under observation on 16 occasions (including nests where males had been experimentally removed), and the identity of the intruders and consequences of intrusion are summarised in Table 4.1. Only one of 10 identified intruders was a female, and this is significantly different to the 1:1 ratio that would be expected if both sexes showed an equal tendency to intrude (X^2=6.4, $p<0.05$). Sex ratios at breeding colonies were always close to 50/50, except for the first few days of the season.

In five out of six cases, intrusions in the presence of the pair male resulted in fights. Since it was normally impossible to see what was happening inside nests the success of EPC attempts could not be judged, though it seems unlikely that intruding males could sucessfully mate with females in the presence of the pair male. The single case where an intruder was tolerated in the presence of the pair male is difficult to explain. Song was heard from the nest after the intruder had entered, which suggests that mating could have taken place, but it was impossible to judge whether this was a PC or an EPC.

Mating was never observed outside the nest although there are accounts of matings taking place on roofs, on the ground, on wires and in the air (Cramp 1988 and references

Table 4.1 Identity of nest intruders and consequences of intrusion in house martins

Intruder sex	Duration of stay (mins)	DNC (DCC)	Present/absent		Outcome
			pair male	pair female	
M	<1	-5 (3)	A	A	Left within 1 minute
F	<1	-5 (3)	A	A	Left within 1 minute
?	<1	-4 (3)	A	A	Left within 1 minute
M	<1	-3 (3)	P	P	Expelled by pair male
M	<1	2 (6)	P	P	Expelled by pair male
M	<1	2 (6)	P	P	Expelled by pair male
?	<1	2 (3)	P	P	Expelled by pair male
M	<1	4 (3)	P	P	Expelled by pair male
?	2	5 (6)	P	P	Apparantly unchallenged song from nest = mating?
Fl	<1	1 (2)	A*	P	Left within 1 minute
M	<1	1 (2)	A*	A	Left within 1 minute
?	<1	1 (2)	A*	P	Expelled by pair female
M	4	1 (3)	A*	P	Song from nest = mating?
M	<1	1 (3)	A*	A	Left within 1 minute
?	18	2 (3)	A*	P	Female left nest but returned within 1 minute song = mating?
M	86	1 (2)	A*	P	mating?

Symbols: M=male, F=female, Fl=fledging, ?=unmarked bird, DNC=day of nesting cycle, DCC=day of clutch completion, P=present, A=absent, A*=pair male removed

therein). Since most accounts in the literature refer to un-marked birds there is no indication as to whether an observed mating might be a PC or an EPC, or whether both males and females, or indeed adults, are involved.

4.3.3 Mate guarding

(i) Sand martins

A few days before the first egg was laid the activity of a sand martin pair became highly synchronised, the members of the pair entering and leaving the burrow in rapid succession. Pair flights were defined as flights into or out of the burrow made by two birds within 10 seconds of each other. Figure 4.2 shows the mean percentage of all flights into and out of the burrow that were pair flights, against the day of the nesting cycle. This is based on 31 nest-hours observation of three pairs, one in which the male and female were marked, and two un-marked pairs. These were the only three pairs observed for which the date of first egg was known from inspection of the burrow, rather than back calculation from the estimated hatch date of nestlings.

Figure 4.2 illustrates that from four days before the first egg until the day that the third egg was laid, an average of 75-100% of flights to and from the burrow were made by two birds. Beecher & Beecher (1979) found that during a similar period of time around the laying of the first egg female bank swallows were accompanied on 100% of flights to and from the nest burrow, which might suggest a tighter degree of mate guarding than that observed in this study. However, since two pairs of un-marked birds have been used for this analysis I could not always identify females and had to use the percentage of pair flights as a measure of guarding. If a single bird was seen to enter or leave the burrow of an un-marked pair I could not tell if this was the pair male or female, or an intruder, and I may therefore have under-estimated the extent of mate guarding.

The direction of 'following' was observed for three colour-marked pairs. Males were observed to follow females on 43 of 49 pair-entry flights, and 41 of 44 pair-exit flights. A chi-square analysis of entry and exit flights indicates that males follow females significantly more than would be expected if following behaviour was random with respect to sex (X^2 for entry=27.94, $p<0.001$; X^2 for exit=32.8, $p<0.001$).

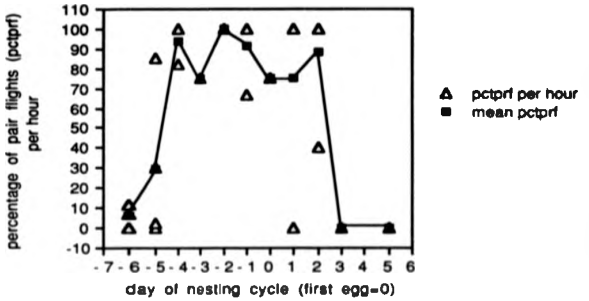


Figure 4.2 The percentage of sand martin pair flights (flights into or out of the nesting burrow made by two birds within 10 seconds of each other) recorded during 31 nest-hours of observation at three burrows during the prelaying and laying periods.

(ii) House martins

The following analysis is based on 54 hours of observations at nine nests during the pre-laying and laying periods. Both members of the pair were marked in seven cases, and only the male in the remaining two. For each nest, observational data have been included up until the day after laying had been completed, by which time incubation behaviour had begun (the members of the pair regularly changed over at the nest). This is hereafter referred to as the pre-incubation period. Overall, the data cover days -7 through to +6 of the nesting cycle (where day zero is the day the first egg is laid). Observed pairs laid clutches of four or five eggs on successive days, apart from one nest where laying was suspended (Bryant 1975a), with four eggs being laid over six days. Except where otherwise stated, data from nests where male removal experiments were carried out have been included only for observation sessions that took place before the removal experiment.

(a) Statistical analysis of data

The time budget data analysed here represent a 'haphazard' sample (in the terminology of Martin & Bateson 1986). Each hour observation period was arranged according to the availability of nests at a suitable stage with at least one of the pair colour-marked, and observations were sometimes made at the same nest for more than one hour per day. Statistical tests require that each value of a particular variable under consideration is independent, otherwise the nominal degrees of freedom can be inflated and interpretation of results may be incorrect. How independent are behavioural variables measured at the same nest on the same day? Specifically, is the behaviour of house martins in a given hour independent of their behaviour in previous hours? If this is the case, then hourly observations made at a given nest on the same day may be considered for treatment as independent data points.

This was assessed by correlating results obtained from the same nest on the same day. Table 4.2 shows the results of these correlations for all behavioural variables analysed, for observation sessions that ran consecutively at a given nest, and for sessions at the same nest separated by one hour. Data for male removal nests are included, except for data collected on the day of the removal experiment. None of the behavioural variables was found to be normally distributed. Spearman's non-parametric rank correlation coefficient was therefore calculated for variables with sample sizes greater than six, and Kendall's correlation coefficient for those with sample sizes between five and 10.

Table 4.2 Non-parametric correlation coefficients for the relationships between behavioural variables measured during hourly observation periods on the same day at the same nest

Variable	Observation periods	
	Consecutive	1 hour apart
MT	0.60	0.90
p	0.003**	0.001*
n	22	9
FT	0.78	0.90
	0.000***	0.001**
	22	9
MV	0.48	0.00
	0.024*	1.0
	22	9
FV	0.18	-0.16
	0.417	0.686
	22	9
MD	0.58	0.31
	0.014*	0.453
	17	8
FD	0.88	0.59
	0.000***	0.126
	16	8
PRF	0.09	0.20
	0.743	0.702
	10	4
PRTT	0.89	0.95
	0.000***	0.001**
	22	7
PRT	0.87	0.88
	0.000***	0.008**
	23	7
MTAN	0.60	0.92
	0.003**	0.003**
	22	7
FTAN	0.77	0.93
	0.000***	0.008**
	23	6

Symbols: MT,FT=time spent at nest by male and female respectively; MV, FV=number of male and female nest visits; MD, FD=mean duration of male and female nest visits; PRF=number of pair flights; PRTT=maximum time spent together by pair; PRT=time spent by pair together at nest; MTAN, FTAN=time spent alone at nest by male and female

Variables concerned with time spent at the nest (MT, FT, PRT, PRTT, MTAN, FTAN) measured at a given nest on the same day were found to be highly correlated and therefore not independent. In analyses using these variables I calculated a mean value per nest per day for those nests where more than one observation session took place on a given day.

Variables associated with movements to and from the nest (FV, PRF) showed either no correlation, or in the case of male visits per hour (MV) a correlation between consecutive hours only. In analyses using these variables I have assumed that each observation session represented an independent measurement, considering the correlation for MV to be an unrepresentative, chance, result.

(b) Pair flights

House martins do not show such complete synchrony of nest attendance as sand martins. Throughout the pre-incubation period males made significantly more and significantly shorter visits to the nest than females (Table 4.3).

The number of pair flights per hour showed an overall negative correlation with the day of the nesting cycle (Spearman rank correlation $r=-0.362$, $p<0.01$, $n=49$). Plotting the former variable against the latter (Figure 4.3a) indicates that most pair flights occurred during the 5 days before the first egg was laid, peaking around day -3. The mean percentage of flights into or out of the nest by the female on which she was accompanied by the male within 10 seconds is plotted in Figure 4.3b. This shows that on average, females were not escorted on more than 70% of flights.

The results pose a number of questions. Why do house martins not show the synchronised nest attendance of sand martins? Does the increase in the number of pair flights before the first egg is laid indicate that this is the time that the female is most vulnerable to EPC, and if so why does the male not accompany the female on all her flights from the nest? If, as already suggested, females are most vulnerable to EPC at the nest, why does the male bother to accompany the female at all?

It is possible that the female's behaviour makes her vulnerable to EPC on certain flights from the nest and the male is aware of this and follows her only at these times. She might for example land on the ground to collect mud, nesting material or calcareous grit for egg shell formation. I never saw house martins landing on the ground in the vicinity

Table 4.3 Comparison of the number and duration of nest visits made by male and female house martins per hour observation period

Median	Males	Females	Z*
nest visits per hour	6.5	2.0	-5.9 p<0.001
visit duration	3.4	7.8	-4.5 p<0.001

* Wilcoxon matched pairs signed ranks test

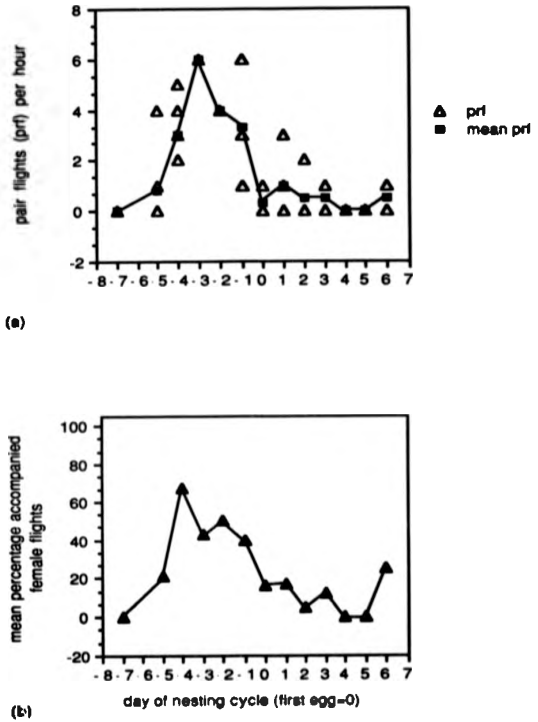


Figure 4.3a The number of house martin pair flights (flights into or out of the nest made by two birds within ten seconds of each other), and **b, the average percentage of accompanied female flights** (flights to or from the nest where the female is accompanied by the male) recorded during 54 nest-hours of observation at 9 nests during the prelaying and laying periods.

of the Glendevon colony, although at other colonies birds were seen to alight to collect mud and dry grass. Most observations involved just one bird, although on one occasion two birds landed and took off together in a manner that suggested they might have been paired. Since birds were not followed after they had left the nest it is not known whether they stayed together all the time they were away. Often, the male would leave the nest with the female and return to it several times during her absence, each visit lasting only about a minute, finally returning with the female.

An analysis of the direction of following reveals that of a total of 33 observed pair-exits, 28 (85%) were initiated by the female; however, of a total of 23 observed pair entries the female followed the male back into the nest on 17 (74%) occasions. Both of these results differ significantly from the 1:1 ratio that would be expected if following behaviour was random with respect to sex (X^2 for exits=16.03, $p<0.01$; for entries $X^2=5.26$, $p<0.05$). Thus during pair flights the male tended to follow the female away from the nest, but usually re-entered before his mate. This is rather different from the situation in sand martins, where the female usually entered and left the nest first. On a number of occasions extra-pair birds were observed to follow a pair of house martins into a nest, and it has been suggested that EPCs are most likely to take place in the nest. Thus a pair male may try to anticipate his mate's entry to the nest so that he is ready to expell any intruders that might attempt to follow her, or might already be in the nest.

(c) Time spent together by male and female

An alternative means of assessing mate-guarding is to look at the time spent together by the male and female. To be absolutely sure that a female is not fertilised by an extra-pair male it would be necessary for a male to stay with (and defend) his mate continually during the period that she is fertile, and this is apparently what male sand martins attempt to do. The maximum time that a pair of house martins spent together per hour observation period was estimated by adding the amount of time that the pair were together in the nest to time that the nest was empty; assuming that when both members of the pair were away from the nest they were together. The differences in nest visit behaviour between the sexes already described (Table 4.3) do not seem to support this assumption, although it is possible that the male splits from his mate for short periods while she is near the colony to return to the nest, so she is never far out of sight. Colour-marked birds have been observed together more than 1km away from a nesting colony (D M Bryant, pers comm).

Recorded values of maximum pair-time and pair time (the time spent by the pair together in the nest per hour observation period) are plotted against the day of the nesting cycle in Figure 4.4. In each case the raw data are values calculated per nest per day, and the mean values are from all nests together.

Both maximum pair time and pair time in house martins declined significantly with the day of the nesting cycle from day -7 to day six (Spearman rank correlations: maximum pair time $r=-0.77$, $p<0.001$; pair time $r=-0.43$, $p<0.05$; $n=32$). Examination of Figure 4.4 suggests that both maximum pair time and pair time remain fairly constant up until day one or day two, and thereafter decline to zero. Working backwards from day six, the maximum amount of variation in the relationship between both parameters and the day of the nesting cycle is explained when data from days two to six are considered alone (Table 4.4). Conversely, the decline in maximum pair time from day -7 does not become significant until data from day two are included (Table 4.5); and for pair time the 0.05 significance level is not reached until day four. It thus seems that the time that a house martin pair spend together per hour remains fairly constant up until the day the third egg is laid, and then declines to zero as incubation begins.

Although the male and female spent more time together before day two of the nesting cycle, they were not together all the time. Figure 4.4 shows that the mean maximum pair time rarely exceeded 50 minutes per hour and mean pair time never exceeded 45 minutes per hour. Thus male house martins do not attempt to stay with the female continually during the pre-incubation period.

If male house martins do not attempt to stay with their mates all the time during the pre-incubation period, they may try to remain in close proximity to females in situations where EPC might occur. It has been suggested that most PCs and EPCs take place in the nest. Males might guard their mates by ensuring that females spend little time alone in the nest during the period that they are vulnerable to EPC. It might therefore be predicted that females would spend little time alone in the nest during the pre-incubation period.

The change in mean time spent alone at the nest by males (MTAN) and females (FTAN) with DNC is plotted in Figure 4.5. Both variables were correlated with DNC (Spearman rank correlations on raw data: MTAN $r=0.4$, $p<0.05$, $n=32$; FTAN $r=0.51$, $p<0.01$, $n=32$). MTAN increased gradually with DNC, whereas FTAN remained fairly stable at around zero until around the day the first egg was laid, and increased thereafter. The greatest variation in the relationship between FTAN and day of nesting cycle is explained when

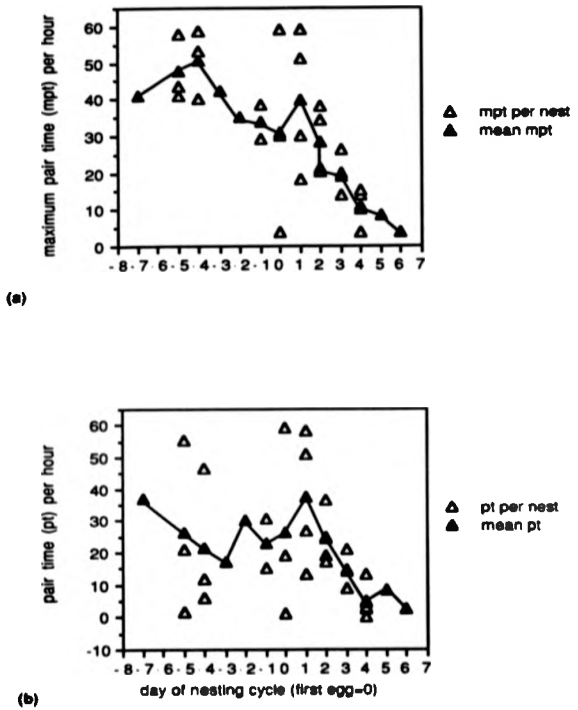


Figure 4.4a The maximum time spent together by a pair of house martins (at the nest and away from the nest), and **b, the time spent together by a pair at the nest** recorded during 54 nest-hours of observation at nine nests during the prelaying and laying periods. The open symbols represent values calculated per nest per day, closed symbols are means for all nests.

Table 4.4 Spearman rank correlation coefficients for the relationships between maximum pair time (MPT) and pair time (PT) with day of nesting cycle, working backwards from day six to day minus seven

Day range	MPT	PT	n
5 to 6	-	-	2
4 to 6	-0.58 0.168	-0.09 0.849	7
3 to 6	-0.79 0.007**	-0.66 0.037*	10
2 to 6	-0.88 0.000***	-0.84 0.000***	14
1 to 6	-0.86 0.000***	-0.83 0.000***	18
0 to 6	-0.65 0.001**	-0.61 0.003**	21
-1 to 6	-0.68 0.000***	-0.60 0.002**	23
-2 to 6	-0.69 0.000***	-0.62 0.001**	24
-3 to 6	-0.71 0.000***	-0.59 0.002**	25
-4 to 6	-0.76 0.000***	-0.46 0.013*	28
-5 to 6	-0.77 0.000***	-0.39 0.031*	31
-6 to 7	-0.77 0.000***	-0.39 0.031*	31
-7 to 6	-0.77 0.000***	-0.43 0.015*	32

Probability values are given below coefficients; *p<0.05, **p<0.01, ***p<0.001

Table 4.5 Spearman rank correlation coefficients for the relationships between maximum pair time (MPT) and pair time (PT) with day of nesting cycle, working backwards from day minus seven to day six

Day range	MPT	PT	n
-7 to -6	-	-	1
-7 to -5	0.54 0.456	-0.26 0.742	4
-7 to -4	0.23 0.614	-0.19 0.679	7
-7 to -3	0.15 0.721	-0.25 0.550	8
-7 to -2	-0.21 0.591	-0.16 0.690	9
-7 to -1	-0.56 0.074	-0.13 0.703	11
-7 to 0	-0.43 0.126	-0.09 0.750	14
-7 to 1	-0.25 0.311	0.14 0.585	18
-7 to 2	-0.48 0.025*	0.08 0.729	22
-7 to 3	-0.60 0.002**	-0.07 0.755	25
-7 to 4	-0.73 0.000***	-0.36 0.049*	30
-7 to 5	-0.75 0.000***	-0.39 0.031*	31
-7 to 6	-0.77 0.000***	-0.43 0.015*	32

Probability values are given below coefficients; *p<0.05, **p<0.01, ***p<0.001

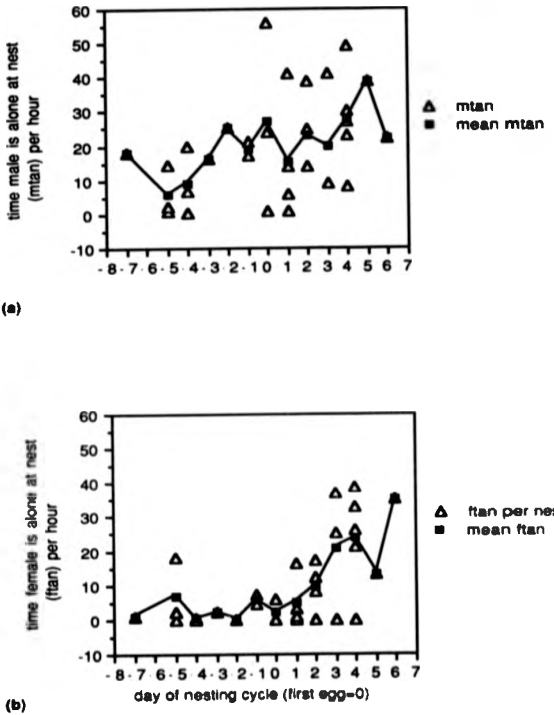


Figure 4.5 The time spent alone at the nest by a, male and b, female house martins during the prelaying and laying periods. Based on 54 nest-hours of observation at nine nests. Open symbols represent values per nest per day, closed symbols are means for all nests.

days zero to six are considered alone (Table 4.6), suggesting that day zero is the hinge point after which the former parameter begins to increase. Between days -7 and zero, there was no relationship between FTAN and day (Spearman rank correlation r=-0.08, p>0.05, n=14).

Since there was no relationship between the amount of time spent by males or females at the nest per hour with the day of the nesting cycle (Spearman rank correlation coefficients: male time, -0.12, p=0.512, n=32; female time 0.00, p=0.966, n=32), the data support the prediction that males mate guard by ensuring that their mates are rarely alone at the nest, at least until the first egg of the clutch has been laid. After laying has commenced, it seems that the male gradually slackens his mate guarding, and the average time the female is alone at the nest per hour increases as the clutch is completed.

The median amount of time that males and females spent alone at the nest until, and after day zero is shown in Table 4.7 (data from day zero are included in the first category). Day zero was used as the dividing point because it was the day that female time alone began to increase. There was no difference in the median amount of time spent at the nest by males and females either side of day zero (tests e and g), nor in the median amount of time spent alone by males up to or after day zero (test f). However, females spent significantly less time alone at the nest up to day zero than afterwards (test H), and up to day zero females spent significantly less time alone at the nest than males (test C). Before day zero females were alone for an average of less than one minute of the 28 spent per hour at the nest.

It thus seems that the behaviour of house martin pairs changes over the laying period from a pre-incubation state, when the members of a pair are together much of the time and the female is rarely alone at the nest, to incubation, where the pair spend almost no time together during daylight. A gradual transition in behaviour from one to the other seems to begin after a 'hinge point' that falls between day zero and day two of the nesting cycle (ie between the laying of the first and third eggs of the clutch). The exact hinge point may vary from pair to pair, perhaps depending on the clutch size, but I consider my sample size too small to look at this in detail.

(d) Do males slacken mate guarding before the fertile period ends?

Although the duration of the fertile period of female house martins is not known, it would seem likely that females of all bird species would be fertile up until 24 hours

Table 4.6 Spearman rank correlation coefficients for the relationships between the time spent alone at the nest by male and female house martins (respectively MTAN, FTAN) per hour observation period with the day of the nesting cycle, working backwards from day six to day minus seven

Day range	MTAN	FTAN	n
5 to 6	-	–	2
4 to 6	-0.09 0.849	0.09 0.849	7
3 to 6	0.15 0.677	0.09 0.814	10
2 to 6	0.16 0.581	0.45 0.109	14
1 to 6	0.31 0.211	0.52 0.026*	18
0 to 6	0.19 0.414	0.60 0.004**	21
-1 to 6	0.21 0.326	0.57 0.005**	23
-2 to 6	0.17 0.428	0.59 0.002**	24
-3 to 6	0.18 0.381	0.58 0.002**	25
-4 to 6	0.33 0.085	0.59 0.001**	28
-5 to 6	0.43 0.017*	0.52 0.003**	31
-6 to 6	"	"	"
-7 to 6	0.40 0.022	0.51 0.003	32

Probability values are given below coefficients; *p<0.05, **p<0.01, ***p<0.001

Table 4.7 Comparison of time spent at nest and time spent alone at nest by male and female house martins, up to and after day 0 of the nesting cycle (where day 0 is the day the first egg is laid)

Time in nesting cycle	Males		Females	
	median minutes per hour		median minutes per hour	
	at nest	at nest alone	at nest	at nest alone
Up to day 0 (n=14)	45.0	16.5	27.5	0.8
Test	A e	C f	A g	C H
Day 1 onwards (n=18)	43.5	22.5	36.6	14.5
Test	b e	d f	b g	d H

Tests: values joined by the same letters have been compared as follows (capital letters indicate variables that are significantly different): Wilcoxon matched pairs signed ranks test: A, Z=-1.06, p=0.296; C, Z=-2.59, p=0.009**; d, Z=-1.05, p=0.296. Mann-whitney U test: e, Z=-0.11, p=0.909; f, Z=-1.33, p=0.183; g, Z=-0.84, p=0.403; H, Z=-0.248, p=0.013*.

before the last egg of the clutch is laid (Lake 1975, Sturkie 1976). Despite this, time budget observations indicate that male house martins begin to slacken mate guarding around the day that the second egg is laid. Since females usually lay at least three eggs, and first clutches normally contain four or five eggs, it might be predicted that eggs laid later in the clutch might be more likely to be fertilised by extra-pair males.

Although the hatching order, and therefore relative ages, of house martin nestlings within broods was not established in this study, it is possible to estimate this with reasonable accuracy by comparing the relative wing lengths of chicks (Bryant 1978b). For the eight house martin broods that were known to contain at least one extra-pair offspring (Table 3.6, page 60) I estimated the relative ages of chicks by comparing wing lengths at 15-16 days, the time when nestlings were ringed, assuming that the rank of wing lengths from longest to shortest represented the hatching order from oldest to youngest. G10(1) was excluded because no fingerprint was available for the chick with the shortest wing, as was WN2 because two young were transfered to another (enlarged) brood and therefore the relative growth of the chicks may have been affected. For the remaining six broods (G2(2), G5, G7, G8, GN1 & C28), the positions of extra-pair chicks in the hatching order, as indicated by wing length measurements, is indicated in Figure 4.6. In the case of GN1, two chicks of equal wing length were separated by assuming that the lighter of the two was younger. The indication is that extra-pair nestlings also tend to be the youngest nestlings, and this is supported by a chi-square analysis of the relative proportions of 'last' (laid) and 'not last' chicks that resulted from EPFs (Table 4.8a). Thus in house martin broods that contain at least one extra-pair offspring, there is an increased likelihood that the last chick will not be fathered by the pair male, suggesting that the observed slackening of mate-guarding towards the end of the laying period does result in paternity loss.

It is possible to extend the above analysis to include all of the house martin broods in Table 3.6 that might potentially contain extra-pair offspring. It has already been suggested that some house martin pairing arrangements are probably not at risk of EPFs, specifically pairs that stay together and produce two broods in a season. However, males of single brooded pairs, or double brooded pairs where there is a change of partner, may be cuckolded. From the families listed in Table 3.6 all the 'faithful' double-brooded pairs were excluded (G3(1)&(2), G9(1)&(2), C4/5(1)&(2), and G6(1)&(2) assuming the offspring from which the fingerprint was missing was not extra-pair), as well as L34 as I do not know if this pair raised one or two broods, and G10(1) and WN2 for reasons given above. The remaining 11 broods contained a total of 36 offspring, of which 9

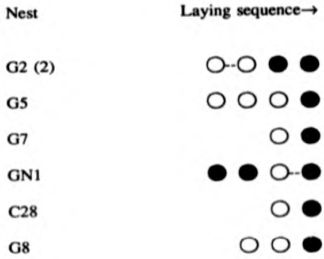


Figure 4.6 The sequence of laying in six house martin broods determined from relative winglengths of chicks. Offspring fathered by the pair male, according to DNA fingerprinting, are represented by open circles. Closed circles represent offspring fathered by an extra-pair male. Offspring joined by a dotted line have equal wing lengths.

Table 4.8 The paternity of house martin chicks with respect to laying order. The proportion of chicks fathered by extra-pair males has been compared between 'last' or youngest offspring (from the last egg of the clutch), and offspring that were 'not last'.

(a) All broods considered at risk of extra-pair fertilisations (see section 4.3.3 (d) of text)

		Position in laying order		X^2
		not last	last	
OFFSPRING	extra-pair	22	5	0.02*
	paternal	3	6	

* $p<0.05$

(b) All broods containing at least one extra-pair offspring

		Position in laying order		F
		not last	last	
OFFSPRING	extra-pair	10	0	0.003**
	paternal	3	6	

F = Fisher's exact test, ** $p<0.01$

resulted from EPFs. In six of these families the youngest chick apparently resulted from an EPF, and a chi-square analysis again indicates a significantly higher likelihood that the last chick will result from an EPF (Table 4.8b).

(e) Does the male actually guard the female or the nest?

Whether or not mate guarding in house martins slackens before the end of the fertile period of the female, the time budget data indicate that male house martins guard their mates by ensuring that females spend very little time alone at the nest before the day after the first egg has been laid. There is however an alternative explanation. During the pre-incubation period males returned to their nests for periods of about three minutes, about seven times per hour (Table 4.3), so that nests were actually rarely empty for more than a few minutes at a time. By doing this a male might have been (i) guarding the nest, or, as already proposed, (ii) guarding the female by ensuring that he was in or near to the nest when she returned, in case she was chased by an extra-pair male.

These alternative explanations can be assessed by looking at the tendency of the male to leave the nest. If he is guarding the female then he might be expected to stay in the nest with her while she is present. While she is absent he might leave the nest to feed, returning at regular intervals to check if she has returned. Thus the rate of male exits from the nest should be lower in the presence of the female. If the male is guarding the nest then his rate of exit would not be expected to change with the presence or absence of the female. Since I have previously shown that a transition from pre-incubation to incubation behaviour begins between day zero and day two of the nesting cycle, I have considered data collected until and including day one (the average 'hinge point') separately from day two onwards.

Until day one of the nesting cycle, the rate of male exit from the nest was significantly lower when the female was present than in her absence (Table 4.9). From day two onwards there was no difference in the rate of male exit in the presence or absence of the female. Thus up until the day after the first egg was laid males were less likely to leave the nest when their mates were present. This suggests that explanation (ii) is the most likely. In reality it is likely that the male guards both the nest and the female, but these results indicate that the female is the guarding priority.

Table 4.9 Comparison of the median rate of nest exit of male house martins in the presence and abence of the pair female up until, and after day one of the nesting cycle (where day 0 is the day the first egg is laid)

	median rate of male exit (per hour)		Z^+	n
	female present	female absent		
Until day 1	0.00	0.20	-4.0***	23
Day 2 onwards	0.07	0.04	-0.4ns	23

+ Wilcoxon matched pairs signed ranks test; ***$p<0.001$; ns not significant.

4.3.4 Sources of variation in behaviour in house martins

The preceeding analysis of time budget data has concentrated on patterns of behaviour common to all males and females. But behavioural differences between individuals are also of interest, since they may help to explain variation in reproductive success. The results of a Kruskal-Wallis one way non-parametric Anova for each of three possible sources of variation in the behaviour of house martins during the mate-guarding period are presented in Table 4.10. Median values for each behavioural parameter by paternity, pair and day of clutch completion are given in Table 4.11.

A major interest of this study is the factors that might account for differences in paternity. DNA fingerprinting has indicated that male house martins are sometimes cuckolded (chapter 3). A number of broods were found to contain 1-3 offspring not fathered by the pair male. There was no instance of a male mismatching with all the offspring in his brood, which indicates that no males were wrongly assigned, and that no males were infertile. Thus it must be assumed that mate guarding is not always effective in protecting a male's paternity. For the analysis, time budget data have been divided into two classes: that from pairs where there were no extra-pair offspring (EPO) compared with pairs with one or more EPO. The eight behavioural variables chosen are those which might be predicted to have some bearing on paternity, given the finding that EPCs in house martins are most likely to take place in the nest.

As far as the other factors were concerned, nine observed house martin pairs (PAIRS) were divided into three classes representing different days of clutch completion (DCC). The analysis has been confined to the period up to and including day one of the nesting cycle (the day after the first egg has been laid), since it has previously been shown that after this time a gradual change in behaviour takes place, from mate-guarding to incubation.

No behavioural variable was found to differ significantly with paternity. However, it is interesting to note that the lowest probability, verging on the 0.05 significance level, was that for female time alone at the nest (FTAN; Table 4.10). For nests with no EPO, the median value for FTAN was 0.00 minutes per hour; whereas for nests with at least one EPO, the corresponding value was 2.7 (Table 4.11). Although this result was not quite statistically significant, it suggests that females spending more time alone at the nest before day one of the nesting cycle were more likely to be subject to extra-pair fertilisations. This is an important finding because it suggests that mate guarding by male

Table 4.10 **Chi-square values for Kruskal Wallis one way non-parametric ANOVAs for the differences in behavioural variables with paternity, pair, and day of clutch completion in house martins**

	MTAN	FTAN	PRT	MT	FT	PRF	MEFA	MEFP
Paternity								
X^2	1.82	3.68	0.01	1.95	0.08	0.21	0.15	0.12
p	0.18	0.06	0.92	0.16	0.77	0.65	0.70	0.72
Pair								
X^2	10.80	11.12	10.41	9.74	9.71	5.74	13.51	11.24
p	0.15	0.13	0.17	0.20	0.21	0.57	0.04*	0.08
DCC								
X^2	9.91	0.51	3.79	0.36	4.59	2.39	9.19	1.54
p	0.01*	0.99	0.15	0.84	0.10	0.30	0.01*	0.46

Symbols: DCC=day of clutch completion; MTAN, FTAN, respectively the time spent alone by males and females at the nest; MT, FT the time spent by the male and female at the nest; PRF, the number of pair flights; MEFP, MEFA, respectively the exit rate of the male in the presence and absence of the female. All values are based on one hour observation sessions and are measured between day -7 up to, and including, day 1 of the nesting cycle. Paternity compares pairs raising extra-pair offspring with pairs that do not.

Table 4.11 Median values for a number of behavioural variables with paternity, pair, and day of clutch completion in house martins

	MTAN	FTAN	PRT	MT	FT	PRF	MEFA	MEFP	n
Paternity									
no EPO	18.0	00.0	30.0	55.0	30.0	01.0	04.0	00.0	13
EPO	10.4	02.7	23.0	42.0	34.0	01.0	04.5	00.0	15
Pair no									
358	01.0	18.0	21.0	22.0	39.0	00.0	-	-	2
410(1)	01.3	02.5	51.0	52.3	51.3	00.5	00.0	00.5	4
410(2)	10.5	01.3	06.7	17.3	08.0	02.0	06.0	00.0	7
413	24.0	06.0	19.0	43.0	25.0	00.0	06.0	02.0	1
415	06.0	03.0	51.0	57.0	54.0	00.0	02.0	02.0	1
417	17.5	11.5	21.0	38.5	32.5	01.5	21.0	02.5	1
418	17.0	00.0	30.5	55.0	35.0	01.0	04.0	00.0	11
419	48.5	00.5	07.0	55.5	07.5	01.5	15.0	01.5	2
DCC									
3	15.2	01.5	28.5	44.3	32.5	01.0	04.5	00.0	21
4	01.0	00.5	46.5	47.0	47.0	00.0	00.0	00.5	5
6	41.0	01.0	13.0	54.0	14.0	00.0	11.0	02.0	3

Symbols: DCC=day of clutch completion; EPO, extra-pair offspring; MTAN, FTAN, respectively the time spent alone by males and females at the nest; MT, FT the time spent by the male and female at the nest; PRF, the number of pair flights; MEFP, MEFA, respectively the exit rate of the male in the presence and absence of the female. All values are based on one hour observation sessions and are measured between day -7 up to, and including, day 1 of the nesting cycle. Paternity compares pairs with no extra-pair offspring with pairs that raise at least one extra-pair offsprng. The numbers listed under DCC refer to days in the nesting cycle (day of first egg = 0).

house martins is effective as a means of paternity defence. Males that were slacker at mate guarding and allowed their mates to spend more time alone at the nest were more likely to be cuckolded.

Significant differences were found between PAIRS and DCC in the rate of male exit from the nest in the absence of the female (MEFA) and, and between DCC in the time spent by the male alone at the nest (Table 4.9). Since all these differences apply to behaviour that took place in the absence of the female they are unlikely to be relevant to the mate guarding behaviour of the male.

4.3.5 Intrusions and fights

(i) Sand martins

A total of 12 fights was seen for which at least one of the following was known: identity of nest defender, identity of intruder, or stage of the nesting cycle of the burrow where the fight took place. All 12 fights took place at burrows at the prelaying stage. No intruders were colour-marked, but of nine identified nest defenders six were male and three female, and this ratio is not significantly different to the 1:1 ratio that would be expected assuming a 1:1 sex ratio and that males and females are equally likely to participate in nest defence ($X^2=1$, $p>0.05$). Although the sample is small, these results are in accordance with the statement in Cramp (1988) that in the early stages of nest excavation residents of both sexes vigorously defend the nest site against conspecifics.

(ii) House martin

The identity of intruders seen to enter observed nests has been discussed in section 4.3.2.(ii). A more general analysis of intrusions and fights is presented here.

An 'intrusion' to a nest under observation was recorded whenever an extra-pair bird (the intruder) swooped within five metres of, landed on the outside of, or entered a nest under observation. Fights almost always occurred when an intruder tried to enter an occupied nest when the pair male was present (Table 4.1). These usually ended in the intruder being forcibly expelled from the nest, sometimes being dangled from the entrance by one or both wings for up to a minute before being allowed to fly free by the nest defender. Alternatively the nest defender would attack the intruder and the two birds would fall from the nest entrance while fighting, normally separating before they hit the ground.

No relationship between the number of intrusion minutes (minute observation periods per hour when at least one intrusion was recorded) and day of nesting cycle was found (Spearman rank correlation r=0.02, p=0.897, n=54). The number of intrusion-minutes declined with the time of day (Spearman rank correlation r=-0.32, p<0.05, n=54). This ties in with the observation that activity at the colony was greatest during the 1-2 hrs after birds had emerged from roost in the mornings. It seems that birds other than pre-laying and laying pairs may use this time to monitor what is going on in neighbouring nests. A similar increase in activity at house martin colonies in the two hours before dusk is reported in Cramp (1988). At dawn and dusk, food availability is low and feeding relatively unprofitable, so time may be better spent in other activities.

On 46 occasions during time budget sessions the intruder was identified, and 42 (91%) were males. This is significantly different from the ratio that would be expected assuming a 1:1 sex ratio and no difference in the tendency of males and females to intrude (X^2=31.4, p<0.001). Of the male intruders 38 (90%) were from nests where laying had been completed (48% incubating, 43% with nestlings). Thus predominantly males rather than females showed an interest in events at nests other than their own, and these could have been males searching for opportunities for EPC. The fact that most of these males were from nests where laying was completed suggests that mate guarding and the pursuit of EPCs may be mutually exclusive activities for male house martins.

No relationship was found between the number of fights recorded during time budget sessions and the day of the nesting cycle for observations up to the day after the last egg of the clutch had been laid (Spearman rank correlation r=-0.03 p>0.816, n=54). Since fights are conspicuous events they were recorded whenever they were observed at any nest in the colony, and a more extensive analysis of 42 fights recorded throughout the breeding season both within and without time budget sessions (except for fights at nests where a male removal experiment was in progress) is shown in Tables 4.12 and 4.13.

In Table 4.12 'defenders' are birds associated with the nest where the fight took place, and 'intruders' are birds from another nest site. Males do most of the fighting. Of 47 identified participants 38 (81%) were males. A chi-square analysis indicates no significant difference in the tendency of either sex to be the defender or intruder in a fight (X^2=0.09 p>0.05). Even though females are less likely to fight, if they do fight they are apparently equally likely to intrude or defend. The two cases where females 'intruded' were both fights between a paired male and female. In each case the female has been classed as the intruder because it was she who entered the nest and was expelled by the male. Agonistic

Table 4.12 The sex and status of 47 identified house martins involved in fights

		SEX		X^2
		male	female	
STATUS	Intuder/ aggressor	13	2	0.09*
	Nest defender	25	7	

* $p<0.05$

Table 4.13 Comparison of the stage of the nesting cycle of defended nests (where fights took place) with intruder nests (from which identified intuders derived)

	PL	L	INC	N1	N2	?	X^2
Defended nest	19	15	2	1	5	0	19.67***
Intruder nest	2	1	4	1	8	26	

Symbols: PL=prelaying, L=laying, INC=incubating, N1=nestlings les than nine days old (brooding), N2=nestlings 9 days to fledging, ?=unknown, * $p<0.001$.

encounters between males and females may be common during the early stages of pair-formation, when the male may treat the female as a potential usurper of the nest site.

The nesting stages of defended nests (where fights took place) with the nesting stages of identified intruders in fights are compared in Table 4.13. A chi-square analysis indicates that there is a significant difference between the two groups (X^2=19.67, p<0.001, excluding the unknown category). Of the 42 defended nests, most (81%) were at the pre-laying or laying stage, whereas most intruders (81%, excluding unknowns) were from nests where laying had been completed. Thus most fights take place at nests that are at early stages of the breeding cycle, but aggressors tend to be males from nests where laying is complete, again suggesting that for male house martins mate guarding and the pursuit of a mixed reproductive strategy are mutually exclusive.

4.3.6 Male removal experiments

(i) House martins

Male removal experiments were carried out on 11 pairs, comprising eight removals during the laying period, and three removals during the incubation period, the latter being treated as controls. Data from one male removal experiment that was carried out on the day the final egg of the clutch was laid have been omitted from the following analysis, since it was felt that this could not be confidently assigned to either the laying or the incubation period.

(a) Behaviour of female

During male removals, a pair of birds was caught in the nest at dawn. The male was retained in a cloth bag, and the female released. Laying females took a mean of 15.3 minutes (range 1-46 minutes, n=6) to return to their nests after release, excluding one female not in the nest at dawn, but first seen to enter the nest 40 minutes after the capture of the male, and a second female which failed to return to the nest during the observation period, and subsequently deserted. Of the three incubating females, two were seen to return 46 and 64 minutes after release and a third returned between 120 and 265 minutes after release. The difference between experimental and control females is significant (Mann-Whitney U test U=17.5 p<0.05). Since six of the eight laying females laid an egg on the day of the removal experiment (after release) their rapid return to the nest was to be expected.

The behaviour of laying females did not seem to be significantly altered by the absence of the male in terms of time spent in the nest per hour or the number of visits made to the nest per hour (Table 4.14). Incubating females made fewer visits to the nest and spent less time per hour at the nest in the absence of the pair male. It is possible that this difference in behaviour was a result of capture and handling at dawn rather than the absence of the male. Incubating females can probably afford to be more cautious in their return to the nest than laying females, because eggs can survive without incubation for at least one day (D M Bryant, pers comm).

(b) Intrusions and fights

The occurrence of intrusions, intruder entries and fights at nests observed during the laying period (from day zero until and including the day before the last egg was laid) in the presence and absence of the pair male, and at incubating nests from which the male had been removed is shown in Table 4.15. There is thus the opportunity to compare events at nests where the female was presumed to be fertile in the presence and absence of the pair male, and also events at nests where the male was absent during the fertile, and non-fertile period of his mate.

Intrusion minutes are the number of minutes per hour observation period during which at least one extra-pair bird was observed to show interest in a nest under observation, by swooping within 5m, landing on or entering the nest. Since few observation sessions passed without at least one intrusion I have compared the average number of intrusion minutes in the presence and absence of the pair male. Intruder entries and fights were rarer events. I used binomial tests to compare the probability of at least one of these events occurring in an hour observation session in each category.

Comparing nests where the male had been removed at different times of the nesting cycle, there was a significantly higher chance that an intruder would enter a nest during a one hour observation period during the laying period compared with the incubation period, but no difference in the probability that a fight would occur (Table 4.15).

Comparing nests at the laying stage in the presence and absence of the pair male, there was no apparant difference in the average number of intrusion minutes per hour. However, in the absence of the pair male there was a significantly higher probability that an intruder would enter the nest, and a significantly lower chance that a fight would occur (Table 4.15). These results are consistent with the findings that male house martins

Table 4.14 Comparison of the behaviour of female house martins in the presence and absence of the pair male

Nest stage & behaviour	Male present	Male removed	M-W+ p
Laying:			
Median nest visits per hour	2.0	0.5	-1.8 0.08
Median time at nest (minutes per hour)	30.6	40.5	-1.1 0.27
Number of nests	6	6	
Hours observation	23	19	
Incubation:			
Median nest visits per hour	2.3	1.0	-2.4 0.02*
Median time at nest (minutes per hour)	45.0	25.0	-2.4 0.02*
Number of nests	3	3	
Hours observation	10	9	

+ Mann-Whitney U test

Table 4.15 **Comparison of the occurrence of intrusions, intruder entries and fights at house martin nests during the laying period in the presence and absence of the pair male, and at incubating nests where the male was removed**

	Male present	Male removed	
		Laying	Incubation
Total intrusion minutes	191	185	5
Median intrusion minutes (per hour)	7 a	3 a E	0 E
Total intruder entries per hour	4	6	0
Probability of intruder entry	0.13 B	0.37 B F	0 F
Median duration of intruder entries (minutes)	1.0 c	2.5 c	-
Total fights per hour	8	1	0
Probability of fight	0.26 D	0.05 D g	0 g
Number of nests	6	6	3
Hours observation	23	19	9

Letters indicate values compared as follows, capital letters indicating values that are significantly different: a, Mann-Whitnney U test z=-1.3, p=0.18; B, binomial test, p=0.02*; c, Mann-Whitney U test, Z=-1.08, p=0.28; D, binomial test, p=0.05*; E, Mann-Whitney U test, Z=-2.60, p=0.009**; F, binomial test, p=0.03*; g, binomial test p=1.0ns.

guard their females by ensuring that fertile females spend little time alone at the nest so as to prevent the entry of intruders, and that male house martins do most of the fighting. When males are absent fights are correspondingly scarce.

A Mann-Whitney U test indicated no significant difference in the median time that intruders remained in nests in the presence or absence of the pair male. However, Table 4.1 shows that when the pair male was present, intruders were rarely allowed to remain in the nest for more than one minute. In the absence of the pair male, intruders were sometimes allowed to stay longer, apparantly depending on the reaction of the female. One female expelled an unmarked intruder. In another case a female left the nest after the entry of an unmarked intruder, but returned within one minute. The intruder stayed in the nest with the female for 18 minutes, during which time a gentle churring song was heard, suggesting that EPC may have taken place. On two other occasions females allowed an extra-pair male to remain in the nest for extended periods. Thus it seems that female house martins vary in their response to approaches by extra-pair males, and may sometimes accept EPC in the absence of their mate.

(c) Behaviour of the male after release

Of the five laying nests that were watched after the release of the male, four males were seen to return within an hour, in a mean of 6.75 minutes. In the case of two incubating nests one male returned after 41 minutes and a second male failed to return within an hour. The sample size is too small for the use of a statistical comparison, but these results suggest that there was less urgency for a male to return to the nest during incubation than during the laying period, as was the case for females.

(d) Effects of male removal on paternity

The occurrence of extra-pair offspring (as indicated by DNA fingerprinting) in nests where a male removal was carried out during the laying period compared with nests where no laying male removal was carried out (including some nests where males were removed during incubation) is shown in Table 4.16. Statistical analysis indicates that there was no difference between the two groups (Fisher's exact test $p=1.0$), and that male removal experiments had no effect on paternity.

Table 4.16 Comparison of the occurrence of extra-pair offspring (EPO) between nests where a male removal was carried out during the laying period, and nests with no laying male removal

	No laying male removal	Male removed	p+
No EPO	9	3	
			1.0ns
At least one EPO	6	2	

+ Fisher's exact test, ns=not significant

(ii) Sand martins

Only three male removal experiments were carried out on sand martins because a decision was made to concentrate on house martins. In one case the female deserted after the experiment, and was not seen after release. The other two pairs continued breeding after the removal experiment. The day of the nesting cycle on which the removal was carried out was not precisely known for either nest, but back calculation of hatch date from measurements of young at ringing (using data from Turner & Bryant 1979) indicates that one experiment was carried out approximately two days before laying began, and the other on the day of the second egg. Thus both experiments probably fell within the fertile period of the female.

The two females that continued breeding first returned to the burrows eight and 21 minutes after release, and made a total of three and 13 visits to the nesting burrow during the following two hours. In each case, females were chased back to their burrows by one or more extra-pair birds on a number of occasions during the absence of the male, but no intruder was seen to enter a nesting burrow with the female. After release, neither male was seen to return to his burrow within one hour.

4.3.7 Nestling provisioning in house martins

(i) Independence of observations

Observations of feeding visits were made for four hours on three separate, usually consecutive, days at each of fifteen nests containing nestlings between 10 and 20 days old, comprising 12 first broods and three second broods. It is possible to split the data into feeding rates per hour per nest, per day per nest, or per nest. How independent are different observations made at the same nest on the same day, or different days?

The relationships between the number of feeding visits observed at the same nest in different hour observation periods on the same day, and between the mean daily feeding visit rate at the same nest on different days are shown in Table 4.17.

For both males and females observations at a given nest separated by one hour (time x with time x+1) were apparently unrelated, whereas feeding visits in consecutive observation periods, and observation periods separated by two or more hours (time x with time x+2, time x+3 or time x+4) were correlated. Despite this anomaly, which is difficult

Table 4.17 **Pearson correlation coefficients for the relationships between the numbers of feeding visits made by male and female house martins during different hour observation periods on the same day, and the mean feeding rates per hour on different days**

Number of feeding visits in 1 hour starting	Males Time x	Females Time x	n
Time x+1 hour	0.50 0.000***	0.40 0.005**	46
Time x+2	0.23 0.258	0.32 0.105	26
Time x+3	0.36 0.015*	0.37 0.014*	44
Time x+4	0.60 0.011*	0.63 0.005*	18
Daily feeding rate per hour	Day x	Day x	
Day x+1	0.60 0.049*	0.86 0.001**	11
Day x+2	-0.36 0.251	-0.25 0.437	12
Day x+3	0.59 0.414	0.79 0.205	4

Probability values are given below coefficients

to explain, it seemed safest to assume that observations of feeding rates of males and females measured at a given nest on a given day were not independent.

Mean daily feeding rates measured at the same nest on consecutive days (day x with day x+1) were also correlated for both males and females, although mean daily feeding rates measured at the same nest but separated by more than one day were not related.

Observations of parental feeding rate were usually made on three consecutive days at the same nest. The analysis above indicates that parental feeding rates on the first and second days, and the second and third days, cannot be considered independant. An average male and female feeding rate per nest was therefore used to investigate the variation in male feeding rates with paternity.

(ii) Relationships between feeding rates, brood size and environmental factors

No relationship was found between mean male feeding rate per nest and any brood size or environmental parameter (Table 4.18), although the increase in male feed rate with nestling age was nearly significant at the 5% level, which means that nestling age effects have not entirely been eliminated. Mean female feeding rates per nest were negatively correlated with mean peak nestling mass, mean ambient temperature and mean maximum temperature (temperatures averaged over the three day period). A previous analysis of feeding rates in house martins (Hails & Bryant 1979) found that both male and female feeding rates per hour were positively related to metabolic brood mass ($mass^{0.66}$), so the negative relationship between female feeding rate and brood mass found in this study is unexpected. A negative correlation between female feeding rate and temperature is also unexpected, because it implies that females feed less on warmer days when insect food would be expected to be more plentiful. However, Hails (1977) found a similar correlation. Since bolus size was not measured in this study, the possibility that on warmer days females bring back fewer, larger bolus' cannot be discounted, although Bryant & Turner (1982) found no correlation between bolus size and temperature in house martins, but an increase in bolus size with foraging distance.

Male feeding rates are seemingly less dependent on brood size and environmental factors than those of females. Overall, males made more feeding visits per hour than females (10.9 ± 0.98 (standard error) compared with 9.7 ± 0.92), but this difference was not significant ($T=0.99$, $p>0.05$, $n=15$). Hails & Bryant (1979) found that male house martins were more flexible in their response to brood demands than females. Males increased

Table 4.18 Pearson correlation coefficients for the relationships between feeding rates (per hour per nest) of male and female house martins at 15 nests with brood age, brood size, nestling weight, food supply and temperature

	MNA	BS	PWT	FWT	MFA	MINT	AMBT	MAXT
Mean male	0.42	0.18	-0.08	0.10	0.30	-0.00	-0.16	-0.09
feeding visits	0.061	0.256	0.391	0.355	0.141	0.498	0.282	0.370
Mean female	0.25	0.08	-0.64	-0.18	0.37	-0.24	-0.63	-0.52
feeding visits	0.180	0.387	0.005*	0.259	0.087	0.219	0.006*	0.023*

Symbols: MNA, mean nestling age; BS, brood size; PWT, mean peak nestling mass (15-16 days); FWT, mean fledging weight (25-26 days); MFA, mean daily food abundance; MINT, MAXT, respectively minimum and maximum temperature as recorded at the University of Stirling weather station, about 20km from the study site; AMBT, mean ambient temperature, measured in the shade at the time feeding observations were made.

their feeding rates more rapidly in response to increases in brood weight than females. The implication is that male house martins are more able, or more willing, to maintain a high rate of food delivery to the nestlings as brood weight increases, or environmental conditions deteriorate.

(iii) Variation of feeding rates with paternity

No difference was found between the feeding rates of males or females at nests with or without extra-pair offspring (Table 4.19). There was, however, a significant tendency for males to feed the brood at a higher rate than females at nests where at least one offspring was fathered by an extra-pair male. This finding is not consistent with the prediction that males that have been cuckolded should feed less than males that have not, and suggests that male house martins are not aware that they have been cuckolded; or, if they are then they make no response, or an inappropriate response.

There was also no difference in male feeding rates between the three classes of male removals: no removal experiment (10 nests), male removed during laying (three nests), or male removed during incubation (two nests; $F=0.22$, $p=.808$, degrees of freedom=14). This indicates that removal experiments had no effect on the subsequent feeding rate of the male.

The mean feeding rates of males and females with male age are given in Table 4.20. This shows that males with a minimum known age of one tended to feed nestlings at a higher rate than older males, although this difference was not significant. There was, however, a significant tendency for older males to feed the brood at a lower rate than their mates.

Combining the above findings, that males that have been cuckolded feed the brood at a higher rate than their mates, and that older males feed the brood at a lower rate than their mates, suggests that younger males may be more likely to be cuckolded. However, these results must be regarded as preliminary. No absolute differences between feeding rates of cuckolded versus non-cuckolded males have been demonstrated, only relative differences between paired males and females. No attempt has been made to control for female age (although all but one of the females in this analysis was classed as a one year old), or for the effects of brood mass and temperature on female feeding rate, mainly because of the limited sample size.

Table 4.19 The average number of feeding visits per hour made by male and female house martins at nests with and without extra-pair offspring (EPO)

	Mean feeding visits per hour (± standard error)		T p	number of nests
	Males	Females		
No EPO	9.8 (0.8)	10.6 (1.5)	0.60 0.568	8
At least 1 EPO	12.9 (2.0)	8.1 (1.0)	3.07 0.028*	6
T p	-1.59 0.137	1.36 0.200		

Table 4.20 The average number of feeds per hour delivered by male and female house martins at nests with one year old compared with older (2+) males

Male age	Mean feeding visits per hour (± standard error)		T p	number of nests
	Males	Females		
1	11.6 (1.0)	9.0 (0.9)	1.91 0.08	12
2+	8.5 (2.5)	12.4 (2.9)	4.95 0.039*	3
T p	1.29 0.219	-1.53 0.150		

(iv) Male adoption of unrelated young

The suggestion from time budget data that male house martins do not take paternity into account when assessing how much effort to put into raising the chicks is supported by the fact that males occasionally adopt unrelated young. This was observed once at the Naemoor colony before the present study began (D M Bryant pers comm), and at the Glendevon colony in 1989 (nest G7 in Table 3.6). In the latter case, the first egg was laid on 5th July, and the original male caught twice during the incubation period. He was not seen after 19th July, having died or deserted the nest. The three eggs of the clutch hatched on 22nd July. One nestling disappeared by the 24th July. The next day, a strange male was captured at the nest and observed to brood the two remaining young over the following three days. Between 1st and 3rd August the replacement male delivered 69% of a total of 208 feeds delivered during 12 hours observation, compared with 27% of feeds delivered by the female (in the remaining 4% of cases the feeding adult was not identified). His rate of food delivery to the brood, 12 visits per hour, compares favourably with the average male feeding rate of about 11 visits per hour (section 4.3.7.ii). The replacement male was still feeding at the nest 15th August, and both surviving young fledged successfully. DNA fingerprinting indicated that the first male caught at the nest was the father of one of the two surviving young, but that the replacement male was unrelated to either of the nestlings he helped to raise.

Adoption of young by male birds has been observed in a number of species, although it is rare for replacement males to provide the same level of parental care as pair males who have been with the female throughout a breeding attempt (Meek & Robertson 1991). Rowher (1986) suggested that adoption may enable a replacement male to acquire a female for renesting either within or between breeding seasons. Since house martins rarely remain with the same partner in successive years, it is unlikely that the adopting male was trying to acquire a mate for the next breeding season. He may have been attempting to acquire a mate for a renesting attempt, but as the brood that he adopted was started relatively late in the season it was unlikely that there would be time for a second brood, and in the event the female did not lay again. It is also possible that the replacement male had achieved EPC with the female at this nest while she was fertile, and therefore acted as though he had fathered at least some of the nestlings. The fact that the two surviving nestlings had different fathers indicates that the female had mated with at least two males during her fertile period.

The cause of death of the third nestling was unknown. It disappeared during the time that

male replacement must have occurred. In other hirundine species, replacement males that arrive well after clutch completion are said to kill nestlings (Crook & Shields 1985, Møller 1988a, Robertson 1990). In this case, it is hard to imagine that a single replacement male would selectively kill one nestling, and then help the female to raise the remainder of the brood.

(v) Feeding of second brood nestlings by fledglings from the first brood

In some house martin second broods, the parental effort of both males and/or females might be mediated by the contribution of first brood fledglings to nestling feeds. This was documented for one of the second broods entered in the analysis above (G3,2), where fledglings from the first brood were observed to help the parents feed nestlings during two of the three days on which feeding rate observations were made. On one day the fledglings provided 12% of 154 feeds delivered during four hours of observation, compared with 53% delivered by the female 32% by the male, and 3% by unknown birds (identity of feeder uncertain). On another day the fledglings provided 40% of 149 feeds over the same period of time, compared with 32% female, 26% male and 2% unknown. From a first brood of five fledged young at this particular nest, at least two fledglings were caught and identified while feeding second brood young. DNA fingerprinting confirmed that they were both the true offspring of the adults attending this nest. There were, in fact, no instances of extra pair paternity in either the first or second brood at this nest, and no change of adults between broods.

Feeding of house martin second broods by first brood fledglings has not been observed before in Central Scotland (D Bryant, pers comm), although it occurs quite regularly in the south of England. Bryant (1975) suggested that for his study colonies in Windsor Park, the apparent independence of growth of second brood young from environmental conditions might be due to the role of first brood fledglings in assisting with feeds. He noted that in one case fledglings contributed 41% of feeding visits.

4.4 Discussion

4.4.1 Differences in mate guarding between house martins and sand martins

Behavioural observations indicated that male sand martins and house martins guard their mates during the prelaying and laying periods, although the degree of guarding differs between the two species. Male sand martins attempt to stay with the female all the time from approximately four days before the first egg is laid, until the day that the third egg is laid, and follow her on all flights away from the nest. These findings are very similar to those of Beecher and Beecher (1979) for bank swallows. They found that mate pursuit flights began 3-5 days before the first egg was laid, and ended by the day the fourth egg had been laid.

Male house martins do not attempt to follow the female all the time, but ensure that she spends little time alone at the nest from about seven days before the first egg is laid, until the day that the second egg is laid. They also accompany the female on up to 70% of flights into or out of the nest during the 4-5 days before egg-laying commences.

These different mate guarding strategies may be linked to a differing risk of EPC in the two species. It seems probable that a female sand martin is vulnerable to EPC attempts all the time she is outside the nest burrow, whether she is in the air, or alighting on the ground or other places such as telegraph wires. Thus to prevent EPC, the male will need to accompany her continuously while she is away from the nest. There was no evidence to suggest that female sand martins were vulnerable to EPC while inside their nest, at least while the pair male was present, but in house martins the nest chamber was implicated as the place where EPC was most likely to take place, at least when the female was in the vicinity of the nesting colony. Thus it would make sense for the male to remain close to the nest to be on hand to intercept intruding males. Male house martins might additionally have accompanied females on flights away from the nest at the times when she was most at risk to EPC. This might have coincided with the time in the female reproductive cycle when copulations were most likely to result in the fertilisation of eggs. Or because at this time the behaviour of the female made her more vulnerable to EPC. She might for example need to land to collect grit for egg formation.

Another factor which may help to explain the observed differences in mate-guarding behaviour between the two species could be conflicting demands on the time and energy

resources of males. For example, males may face a trade-off between the need to guard the nest against usurpers or nest material robbers, and the need to guard the female. Male sand martins may guard the female more intensly because they have less need to guard the nest than in house martins. Alternatively, as discussed above, the risk of EPC and consequent harassment to the female may be higher in sand martins than in house martins, so male sand martins cannot afford the 'luxury' of guarding the nest. DNA fingerprinting results for sand martins, although preliminary, do suggest that the rate of EPF is slightly higher than for house martins (Table 3.12), lending some support to the second explanation.

The organisation of mate guarding in both sand martins and house martins suggests that it does function as a form of paternity defence. This is supported in house martins by the finding that males which allowed their mates to stay alone at the nest for longer periods during the prelaying and early laying period were more likely to be cuckolded, evidence of a direct link between paternity and the intensity of guarding. This does not rule out the possibility that mate-guarding also fulfils other functions, such as protection of the female from harassment, as discussed in section 4.1.2 of the introduction to this chapter. Yet fact that EPFs occur in both house and sand martins implies that mate guarding is not fully effective for paternity defence in either either species. In house martins this may be because of conflicting demands that prevent males from guarding their mates full time, leaving females alone to accept or perhaps seek EPCs. The apparently high occurence of EPFs in sand martins is less easy to explain, given that paired males and females seem to be together almost continually for much of the pre-incubation period, and that copulations were rarely observed.

4.4.2 The duration of mate guarding and the fertile period

Female birds of various orders are known to be capable of storing viable sperm for average periods of 6-42 days (Birkhead & Møller 1992), which indicates much variability between species in the length of the fertile period. The duration of the fertile period is not known for either house martins or sand martins, so it is impossible to judge whether the onset of mate guarding is timed to coincide with this. Whenever the fertile period begins, it would seem likely that in all bird species the female should be fertile up until the day before the final egg of the clutch is laid, assuming that eggs are fertilised approximately 24 hours before laying, and that there is last male sperm precedence (Lake 1975, Sturkie 1976, Birkhead & Møller 1992). Nevertheless, for both sand martins and house martins mate guarding seems to slacken before the day of the final egg, and there

is a gradual transition to incubation behaviour. This transition apparently begins on the day the third egg is laid in sand martins, where the first clutch normally contains five or six eggs. In house martins, where the first clutch usually contains four or five eggs, it begins around the day that the second egg is laid. Why does mate guarding apparently end before the fertile period of the female, leaving 1-2 eggs at risk of EPF?

If mate guarding does slacken before the end of the fertile period, then it would be predicted that the last egg(s) of the clutch would be at higher risk of fertilisation by an extra-pair male. In support of this, estimation of relative chick ages from wing length measurements suggested that in house martin families, the last-laid chick has a higher likelihood of resulting from an EPF.

Why should a male slacken his guarding before the fertile period of the female if this increases the risk that he will be cuckolded? It may be that once laying has begun, male house martins can no longer afford to guard the female because of the need to begin incubating the eggs. It may be necessary to begin incubation before the clutch is finished to promote asynchronous hatching and the possibility of brood reduction (Lack 1968, Bryant 1978a & b, Bryant & Gardiner 1979). Further, the youngest nestling(s) in the brood may be considered less valuable because they are most likely to suffer if environmental conditions deteriorate. Alternatively, males may be unable to sustain mate-guarding throughout the entire egg-laying period if their need to remain close to the nest to intercept intruders seeking EPCs interferes with self-feeding.

It is also worth considering whether all, or just some, house martins males slacken their guarding before the final egg of the clutch was laid. The pattern of variation in mate guarding with the day of the nesting cycle was an average result from observations of nine pairs. However, there were indications that males which left their mates alone at the nest for longer periods on average before the day the second egg was laid, were more likely to be cuckolded. This result might reflect the fact that some males tended to slacken their mate guarding earlier than others, and were therefore more likely to be cuckolded, and would be consistent with the finding that extra-pair offspring tended to come from the later eggs of the clutch. What might account for variation in mate guarding efficiency between house martin males? Individual variation in quality and/or condition might make some males less able to sustain guarding until laying has been completed. In addition, the period during which mate guarding is necessary may vary with the number of eggs laid, so that males mated to females laying larger clutches might need to mate-guard for longer. However, average clutch sizes at house martin nests with

and without extra-pair offspring (as determined by DNA fingerprinting) were not significantly different (Mann-Whitney U test, $Z=-0.67$, $p=0.5$, $n=21$).

In a study of paternity in dunnocks, Davies *et al* (1992) found that males removed for periods of three days after the day that the first egg of the clutch was laid never lost paternity, despite the fact that females were observed to mate with extra-pair males during this period, presumably before the last egg of the clutch was laid. This suggests that in dunnocks, copulations taking place after the first egg of the clutch has been laid have little or no chance of fertilising eggs. The evidence presented above suggests that the same is not true for house martins. Although male removal experiments were also found to have no effect on paternity in house martins this result must be viewed as inconclusive because of the small sample size. The implication is that different mechanisms of sperm storage and sperm precedence operate in different bird species.

4.4.3 Male removals and the response of female house martins to EPC attempts

Bjorklund & Westman (1983) removed male pied flycatchers for an average of 105 minutes, 1-3 days before the onset of egg-laying, and found that the number of extra-pair males visiting the territory increased during the absence of the male, as did the frequency of EPCs. The results of the present study indicate that in house martins during the laying period, there is no increase in the level of interest shown by extra-pair birds (as measured by intrusion minutes) to the nest (which might be considered as analagous to the territory of the pied flycatcher) in the absence of the male. There is however, an increased probability that an intruder will enter a nest when the pair male is absent and the female is laying (ie presumed fertile). Intruding birds that could be identified from colour marks were almost always males, presumably in pursuit of EPC. In the absence of their mates, laying females might attack and expel intruders, or they might allow the intruders to remain in the nest for extended periods, long enough for EPC to take place. Thus at least in the absence of the pair male, the occurrence of EPCs in house martins seems to be controlled by the female, as has been reported for a number of bird species (Wagner 1991).

The fact that intruders were more likely to enter house martin nests when the pair male was absent provided further evidence that mate guarding in this species does function as a paternity defence (see section 4.4.1).

Hogstad (1989) found that male willow warblers removed permanently during the periods of pair-formation, egg-laying and early incubation were rapidly replaced by floating males, whereas males removed after the middle of incubation were much less likely to be replaced. For only one of the house martin male removal experiments was there a suggestion that an extra-pair bird attempted to move in on the nest site. This was a removal experiment carried out during the laying period of a second brood in 1989. After the pair male was released he was forced to defend his nest from a particularly vigorous attack by an unmarked bird attempting to gain entry. The fight lasted for 3 minutes, during which time the participants pecked vigorously at each other, before the intruder was finally driven off.

4.4.4 Which male house martins are most successful in obtaining EPFs?

Time did not allow for random screening of DNA fingerprints to try to identify the fathers of mismatched offspring in either house or sand martins. Identified intruders at house martin nests were almost always males and these intruders tended to come from nests where laying had been completed. Thus in house martins the implication is that the pursuit of EPFs and mate guarding are mutually exclusive, and that males that breed earlier in the season will have more opportunities to obtain EPFs than those breeding later.

As well as identified intruders, I also observed many unmarked birds showing interest in the nests at the Glendevon colony throughout the 1989 breeding season, even after I had colour-marked all of the resident breeding adults. Of 16 extra-pair birds seen to enter house martin nests during the pre-incubation period, five (31%) were unmarked (Table 4.1), and the intruding bird in 42 fights recorded throughout the 1989 breeding season at Glendevon was unidentified in 26 (62%) occasions (Table 4.10). These unidentified birds may be breeding adults from different colonies, or unpaired floating adults.

On two occasions in 1989 I found breeding males from the Glenquey colony (Figure 2.2) dead in nests at Glendevon following periods of sustained bad weather. Presumably they had deserted their own nests in search of more favourable conditions on lower ground, but it seems possible that they may have visited nests at Glendevon previously in pursuit of EPCs.

The existence of floating males is suggested by the fact that a male that disappeared from one of the Glendevon nests in 1989 (G7) was replaced. Although the new male was not

the father of any of the offspring, he helped the female to raise the brood. He may have taken over in an attempt to gain a mate for a second breeding attempt in the same season, or because he had achieved EPC with the female at the nest and considered that he might have fathered some of the nestlings in her brood. Floating males may be pre-breeders that have not yet acquired mates, or males who's partners have died or deserted after a failed breeding attempt.

Thus it is possible that extra-pair offspring in house martins may be fathered by males from the same or different breeding colonies, or perhaps by males that are not attached to any particular colony, which complicates the measurement of realised male reproductive success. The question of how sperm competition will affect measurements of apparent male reproductive success in house martins and other species is discussed further in chapter 5.

4.4.5 Paternity and male parental care

Measurements of the rate of food delivery to house martin broods indicated no absolute difference in male feeding rates between nests where males had been cuckolded, and nests where males had fathered all the offspring. This finding does not support the prediction that males that have been cuckolded should reduce their parental effort (section 4.1.5). The only detectable difference was that males that had been cuckolded tended to feed the brood at a higher rate than their mates, whereas males that had not been cuckolded did not. Combining this result with the finding that older males tended to feed the brood at a lower rate than their mates suggests that older males are less likely to be cuckolded. Thus in house martins it may be age, rather than paternity, that effects the feeding rate of the male.

Few studies have so far compared the level of male parental effort with absolute knowledge of paternity from a genetic technique such as DNA fingerprinting, as opposed to knowledge inferred from behavioural observations such as the rate of EPCs. Morton *et al* (1990) found that male purple martins breeding for the first time suffered a higher rate of cuckoldry than older males. They also tended to feed the brood at a lower rate than their partners, whereas no difference was found between the feeding rates of older males and their mates. The authors suggested that the younger males were reducing their feeding rates in response to a low certainty of paternity. It is alternatively possible that young purple martin males are less efficient at feeding nestlings, or less prepared to pay the cost of increased parental effort, than older males. It is interesting that the results for

purple martins are almost the opposite of those reported above for house martins, indicating that different factors must account for the variation in male feeding rates with age and/or paternity in these two species.

It seems fairly clear that even if male house martins are aware that they have been cuckolded, they do not adjust their level of parental effort. Brood manipulation experiments (Chapter 2), which involve the successful fostering of young from one nest to another, provide evidence that house martins cannot distinguish between their own young and unrelated young, at least before fledging; or if they can distinguish they do not respond.

When parentage is uncertain due to EPCs and/or egg dumping, it would benefit parents to evolve means of recognizing genetic relatedness, and chicks to conceal it, at least if they were unrelated to one or both putative parents. Evidence accumulated so far suggest that the interests of chicks win out, and that parent birds are unable to distinguish offspring that are genetically related to them from offspring that are not (Beecher 1988). Thus even if a male suspects that he has been cuckolded he will be unable to selectively feed his own offspring. If he reduces his feeding rate to the whole brood then his own young are likely to suffer, as well as those fathered by extra-pair males. The finding that male house martins did not reduce their rate of chick feeding in response to paternity is therefore not unexpected.

It would however be useful if males could recognise when they were unlikely to have fathered any of the young in a brood. If they are unable to recognise offspring that are genetically unrelated to them, are there other cues that they could use to monitor their paternity? A male might have cause to suspect that he had been cuckolded if he observes his mate participating in EPCs, or if he is separated from her for extended periods during the time that she is fertile. Male removal experiments apparently had no effect on paternity, or male parental effort in house martins. Thus it is impossible to comment on possible male paternity cues in this species. Similar experiments on dunnocks were found to reduce the paternity of removed males (Davies *et al* 1992) in both monogamous pairs, and polyandrous systems where two males shared a female, provided the removal experiment took place on or before the day the first egg of the clutch was laid. In polyandrous systems, each male dunnock seemed to compare the amount of mating access he had to the female with that of the other, and use this to judge how much effort he should devote to chick feeding. Thus males that had less exclusive mating access to a laying female delivered a smaller proportion of male nestling feeds, and were also

found by DNA fingerprinting to have a smaller share in paternity of the brood. In monogamous systems, males that were forced to share mating access to their mate with extra-pair males by removal experiments did not reduce their rate of food delivery to the brood. Thus although male dunnocks are apparently able to monitor their paternity, they can only afford to reduce their parental effort in polygynous systems where they can be sure that another male will take up the slack.

Even though males of monogamous bird species may be aware that they have been cuckolded, they might only be expected to reduce the effort expended in raising the current, multiply sired brood, if this has a considerable effect on their chance of future reproductive success, or if they suspect that the brood has been entirely fathered by extra-pair males. Since male house martins raising two broods apparently do not bear any mortality costs compared with single-brooded males (Bryant 1979, 1988a), and males have been observed to adopt broods of young entirely unrelated to themselves, it is perhaps not surprising that paternity and male parental effort seem to be unrelated.

5. Discussion

5.1 Costs of Reproduction and breeding strategy in house martins

A major cost of reproduction previously identified in house martins is that the most productive (that is, double brooded) females suffer the highest mortality (Bryant 1979, 1988a). Because double-brooded pairs start breeding earlier and finish later than single-brooded pairs they are exposed to unpredictable conditions of food abundance at the beginning and end of the summer and these adverse conditions may disrupt breeding or affect migratory survival. Single brooded pairs on the other hand, confine breeding to the stable midsummer period. There are also indications that older females (2+ years) are more likely to attempt two broods (Bryant 1979, this study). Thus the oldest females, who are less likely than first year females to survive to the next breeding season, are seem to be prepared to expend more effort in reproduction. Unlike females, males apparently bear no mortality costs for an early start or two broods. Thus reproductive costs may act differently on the two sexes within a species, perhaps as a consequence of differing behavioural responses to adversity (Bryant 1979).

House martin females therefore face a trade-off between fecundity and future survival. This can be described as an interseasonal cost, in that reproductive activity in one breeding season will affect that in the next season. In the present study I concentrated on intra-seasonal costs, those acting within a given breeding season. In house martins, seasonal fecundity, or annual reproductive success, depends mainly on the date at which laying starts and the number of broods attempted. Pairs that lay earlier, and pairs that attempt two broods, enjoy higher annual reproductive success. They must also contend with unpredictable conditions of food supply at the beginning and end of the season, as described above. Experimental manipulations of reproductive effort show that there are potential costs associated with brood size in house martins. Increasing first brood size may have adverse affects on the growth and survival of offspring, and can influence the decision of a given pair to attempt a second brood, as has been shown for a number of other bird species (see introduction to Chapter 2).

An important fitness or fecundity cost of reproduction for male house martins identified in the present study is the possibility that they might be cuckolded. In addition, the pursuit of EPCs might entail costs in terms of sperm depletion and increased risks of cuckoldry, divorce, attack by other males, and predation (Birkhead & Møller 1992).

Previous attempts to identify reproductive costs in male house martins (Bryant 1979, 1988a) have used measures of apparent success. Until measures of realised reproductive success (taking genetic relationships into account) are available for male house martins then true costs of reproduction cannot be identified.

There has been much debate about how reproductive costs might influence the evolution of breeding systems (Reznick 1992, Partridge 1989). If the breeding strategy of a species is under genetic control, then true reproductive costs must act on the genotype. In practise, it is difficult to distinguish changes that are genetically based from those resulting from phenotypic plasticity (ie, a change in reproductive behaviour or output that does not have a genetic basis). Evidence from a number of species suggests that experimental manipulation of breeding strategy may produce both phenotypic and genetic changes (Partridge & Harvey 1988, Partridge 1989, Reznick 1992, Partridge 1992). Presumably species such as the house martin that are likely to be faced with changing environmental conditions during the breeding season, are equipped with sufficient phenotypic plasticity to allow them to respond. Experimental manipulations of brood size are likely to draw on this phenotypic plasticity, and tell us something about the capacity of, and the mechanisms whereby, this species responds to changes in the amount of effort that must be devoted to reproduction. It has been suggested here that the changes in the breeding strategy of house martins in Central Scotland observed over the period 1972 to 1989 have been caused by gradual, directional changes in the environment over the same time period. In the absence of information about the genetic control of breeding strategy in this species, it is impossible to say whether the changes have exploited the phenotypic plasticity of house martins, or if there have been underlying genetic changes. In a closely related species, the sand martin, a significant reduction in the mean keel length from one breeding season to the next was thought to be a result of selection for small size by severe drought conditions on the wintering grounds (Jones 1987a). Thus drastic short term environmental changes can apparently cause rapid genetic changes (O'Donald 1973, Grant & Grant 1989). The question of whether changing reproductive patterns in house martins at study colonies in Central Scotland reflects phenotypic plasticity or underlying genetic changes therefore remains unknown, although neither possibility can be dismissed at the present time.

5.2 Implications of sperm competition for measurements of Lifetime Reproductive Success

In house martins, double-brooded pairs have a higher annual reproductive success in

terms of the total numbers of offspring that fledge sucessfully (Bryant 1988a, 1989; this study). Lifetime reproductive success is the product of annual reproductive success and breeding lifespan (equation 1.1). Thus in this species where few individuals have a reproductive lifespan of more than two years (Bryant 1988a, 1989), variations in annual reproductive success are likely to have profound effects on the lifetime production of fledged young.

Previous work has found that the average lifetime output of fledged young for both male and female house martins was the same (a median of six offspring, Bryant 1989). However, the ranges in lifetime reproductive success differed between the two sexes, from 0-28 fledged offspring for females, and 0-42 for males. In addition, males showed a greater variance in lifetime reproductive success, with a higher frequency of successful individuals raising more than 15 young (Bryant 1989).

These results measure apparent lifetime reproductive success, assuming that all the young in a given nest were the genetic offspring of the attending adults. DNA fingerprinting of this species (Chapter 3) has shown that although this assumption holds for female house martins, males are sometimes cuckolded. As a result of sperm competition, an estimated 15% of nests contained at least one offspring not related to the male attending that nest (Table 5.1). What are the implications of sperm competition for the observed variation in lifetime reproductive success in house martins given the observed rate of EPFs? This will depend on the types of males that are successful in achieving EPFs.

In one extreme the population might consist of high quality males and low quality males. The former would start breeding early and pair with double-brooded females and therefore enjoy higher annual and lifetime reproductive success. In addition, they would succeed in achieving 100% paternity in their own clutches and in obtaining EPFs with females mated to low quality males. If this was the case then the upper limit of realised lifetime reproductive success for male house martins would be likely to exceed previously documented limits, since these figures do not include offspring from EPFs. The range of lifetime reproductive success for male house martins would be larger, and the variation would probably be different, to an extent depending on the relative proportions of high and low quality males present. A recent study on red-winged blackbirds provides some evidence for this kind of system. Gibbs *et al* (1990) found that there was a significant positive correlation between the percentage of young fathered by a male in his own nest(s) and the number of young he had fathered in the nests of other males. These findings are in accordance with the basic assumption of Trivers (1972), that individuals

should pursue a mixed reproductive strategy in order to increase their fitness.

An alternative hypothesis is that males that achieve the highest apparent lifetime reproductive success are not successful in achieving EPFs. They start breeding early and pair with double brooded females, and must devote all their attention to raising the young in their own nest. They therefore do not have sufficient time or resources to seek EPFs. Instead, males that start breeding relatively late in the season and pair with single-brooded females, and/or floating males who do not breed at all, have more opportunities to achieve EPCs, and thus increase their reproductive success at the expense of males paired with more productive, double-brooded females. In this case EPFs are seen as an complementary tactic for males that are less successful or unsuccessful breeders. Sperm competition would then tend to reduce the upper limit of LRS in male house martins, and also reduce the amount of variation between males. This may be the kind of system that operates in Coho salmon, where the two alternative male tactics of fighting for access to breeding females, or sneaking copulations opportunistically, may be of equal fitness value (Gross 1985).

Turning now to the main study species, the house martin, what conclusions can be drawn about the relationship between apparent and realised male reproductive success? Unfortunately the fathers of extra-pair offspring were not identified, so it is necessary to draw together information from different areas of the study to identify the kinds of males that are more likely to be successful in obtaining EPFs.

Behavioural observations indicate that male house martins tend not pursue a mixed reproductive strategy during the period that their partner is laying. They are more likely to seek EPCs, both at their own and at nearby breeding colonies, after laying has been completed at their own nests (Section 4.4.4). Since breeding at house martin colonies is not synchronous, males that start breeding earlier may have more opportunities to obtain EPFs. In addition, males that begin breeding earlier are more likely to pair with double-brooded females and to enjoy higher annual reproductive success (Chapter 1). Further, results from DNA fingerprinting suggest that double-brooded males that raise two broods with the same female are unlikely to be cuckolded (section 3.4.3). Thus there is evidence the house martin population in Central Scotland may contain some high quality males. What characteristics might account for male status? Perhaps high quality males are in fact older males. Measurements of the rate of food delivery to the brood hinted at the fact that older male house martins were less likely to be cuckolded than first year males (Section 4.4.5). Older house martin males may also tend to arrive and begin

breeding earlier (Bryant 1988a, but not this study, see section 2.3.2). Thus it may be, in house martins, that both the success of a male in achieving paternity in his own nest, and his success in obtaining EPFs, increases with age. This is certainly the case in a related species, the purple martin, where older males were found to increase their fecundity at the expense of younger males, as well as achieving 96% paternity in their own broods (Morton *et al* 1990).

Body size of male house martins has previously been shown to have a small positive effect on annual and lifetime reproductive success (Bryant 1988a, 1989), although these results were not corroborated by the present study (Section 2.4.2). Is there any evidence that characteristics of individual males might influence their ability to achieve full paternity in their own nest, or to obtain EPFs? There was apparently no relationship between any male body size measure and the tendency of the brood to contain extra-pair offspring. It is however possible that male attributes might influence success in obtaining EPFs through female choice. Female house martins apparently choose whether or not to accept EPC from males intruding into their nests (Section 4.4.3), and it may be that they base this decision on some male attribute which they percieve as a measure of male quality. Unfortunately it is not possible to speculate on the latter suggestion from data collected in the present study. Future work might usefully look at plumage variation, which has been implicated in female choice studies on other bird species (Andersson 1982, Cherry 1990, Møller 1988c).

There is therefore a suggestion that some male house martins may be more successful than others, both in achieving paternity in their own nest(s), and obtaining EPFs, and that these differences may be mainly a result of age. Thus as they grow older, male house martins might change from low to high quality males. Indeed, female house martins might preferentially accept EPCs from older males, using longevity as an indication of "good genes". If success in achieving paternity, both within and without the nest, does increase with age in male house martins then this may mean that the realised range in lifetime reproductive success of male is not very different from apparent success, but that there is a steep increase in reproductive success with age.

It also seems likely that at least some EPFs are perpetrated by unmated, floating, males (Section 4.4.4) who attempt to achieve some reproductive success at the expense of breeding males, and may opportunistically move in at nests where the pair male has disappeared. Further resolution of the relationship between apparent and realised male reproductive success should be a priority of future work on this species.

5.3 Sperm competition in wild bird populations, a review of results from studies involving DNA Fingerprinting

Table 5.1 summarises information from all the published studies of sperm competition in wild bird populations that were readily available at the time of writing. It is based on Table 12.2 in Birkhead & Møller (1992), but I have confined my attention to studies which used DNA fingerprinting to measure paternity, rather than including estimates of paternity from studies using electrophoresis or heritability measures, since the former method is the only one which allows genetic relationships between individuals to be characterised with certainty (see introduction to Chapter 3).

In the 15 bird species listed, the percentage of young that were fathered by extra-pair males ranges from 0-35%, with 0-50% of broods containing at least one extra-pair offspring. In the case of species which have polygamous breeding systems, or breed in social groups, I have extended the definition of 'extra-pair' to refer to offspring that are sired by males outside the breeding unit. Thus estimates of extra-pair paternity in some species, such as the dunnock, may differ from those given by Birkhead & Møller (1992).

Considering EPFs to be negligable in species where less than an arbitrary 10% of broods were affected, no difference was found in the tendency of extra-pair offspring to occur in nests of colonial or territorial bird species (Fisher's exact test p=0.59, n=15). Similarly, Birkhead & Møller (1992) also found no difference in the percentage of extra-pair offspring in colonial or solitary species. They noted that this finding was perhaps unexpected because EPCs are more frequent in colonial species, and a cross species comparison indicated that the percentage of extra-pair offspring is positively related to the percentage of copulations that are extra-pair. In contrast, within individual species, greater density of breeding individuals may lead to a higher incidence of extra-pair paternity, as is the case in red-winged blackbirds (Gibbs *et al* 1992). It may be that cross species comparisons are confounded by factors such as the varied intensity of mate-guarding or the response of females to EPC attempts, thereby obscuring any trends between coloniality and EPFs.

There was also no tendency for extra-pair offspring to be more frequent in the nests of monogamous or polygamous species (Fisher's exact test p=0.21, n=19, counting species such as the dunnock that exhibit both monogamous and polygamous systems twice). Thus breeding system apparently has no influence on the tendency of offspring to be sired outside the breeding unit.

Table 5.1 The occurrence of extra-pair offspring in wild bird populations as revealed by DNA Fingerprinting

Species	% Extra-pair offsp (n)	nests (n)	Breeding system	Mate guarding	EPCs	IBP	References
Fulmar	0 (85)	0 (85)	M C	Female guarded at nest	Mainly unforced Male or female solicited	No	Hunter *et al* 1992 Hatch 1987
Shag	18 (28)	33 (15)	M C 3-5% Pg	Male guards nest?	Female solicited	No	Graves *et al* 1992
House martin	15 (72)	38 (22)	M C	Female guarded almost continuously at nest, and accompanied on 0-60% of flights away	Male solicited Female accepts or or refuses	No	This study
Sand martin	18 (17)	40 (5)	M C	Male follows female on 70-100% of flights away from nest	Not observed	No?	This study Beecher & Beecher 1979
Purple martin	35 (52)	50 (14)	M C	Variable Male follows female on 0-100% trips for nest material	Forced	Yes	Morton *et al* 1990 Morton 1987

Table 5.1 continued

Species	% Extra-pair offsp (n)	nests (n)	Breeding system	Mate guarding	EPCs	IBP	References
Dunnock	0 (49)	0 (15)	M T	Male spends c.80% of time within 10m of female	Unforced, female solicited?	No	Burke *et al* 1989 Hatchwell & Davies 1992
	3 (34)	9 (11)	Pa				
	0 (50)	0 (19)	Pga				
Willow warbler	0 (120)	0 (19)	M T	Male follows female around territory	Observed	No	Gyllensten *et al* 1990
Wood warbler	0 (56)	0 (13)	M T 23% Pg	- - -	-	-	- - -
Pied flycatcher	7 (46)	25 (8)	M T	Male stays on average 2.6-2.9m from female but often leaves her unguarded	Male solicited Female passive or tries to escape	No	Lifjeld *et al* 1991 Alatalo *et al* 1987
	3 (89)	11 (19)	P				
Stripe-backed wren	1 (69)	3 (34)	SG T	No, dominant male guards nest?	No records	No	Rabenald *et al* 1990 Rabenald 1985

Table 5.1 continued

Species	% Extra-pair offsp (n)	% Extra-pair nests (n)	Breeding system	Mate guarding	EPCs	IBP	References
Blue tit	11 (314)	40 (25)	M T	Male spends 55-91% of time within 25m of female	Mainly female solicited	No	Kempenaers *et al* 1992
		9 (11)	Pg				
Indigo bunting	35 (63)	48 (25)	M T	Male spends 41% of time within 30m of female	Male solicited Females accepted 21% of 43 EPCs, remainder forced	No	Westnest 1990 Westneat 1987b
Red-winged blackbird	28 (111)	47 (36)	Pg T	No?	No records?	No	Gibbs *et al* 1990
Zebra finch	2 (92)	8 (25)	M C	Male follows female on 98% of flights away from nest	Male solicited, forced or unforced	Yes	Birkhead *et al* 1990 " " " 1988
House sparrow	14	27	M C	Yes?	Forced, unforced or female solicited	No	Wetton & Parkin 1991

Breeding system: M=monogamy, Pa=polyandry (1 female, 2+ males), Pg=polygyny (1 male, 2+ females), Pga=polygynandry (2 males, 2 females), SG=social group, C=colonial, T=territorial. In polygamous species I have extended the definition of 'extra-pair' to refer to offspring sired by males that are not part of the breeding unit. Other abbreviations: EPC=extra-pair copulations, IBP=intra-specific brood parasitism. A question mark indicates information not yet fully substantiated by data, or inferred from rather than stated in the literature.

What factors might account for the observed differences in the proportion of extra-pair offspring between the bird species in Table 5.1? Where possible, I have included an estimate of the intensity of mate-guarding from published studies of behaviour, and it is notable that in species such as the zebra finch and the dunnock, where guarding seems to be relatively intense, extra-pair offspring are rare. Species in which mate guarding seems to be variable or non-existent, such as the purple martin, shag, and red-winged blackbird, have higher rates of EPFs. These rules are obviously not hard and fast, as is shown by sand martins. In this species males attempt to follow their mates almost continually during the fertile period (Section 4.4.1), yet preliminary results from DNA fingerprinting suggest that at least 40% of nests contain extra-pair offspring.

The willingness of females to participate in EPCs might also be predicted to influence the percentage of extra-pair offspring. Table 5.1 indicates considerable interspecific variation in female behaviour. EPCs may be forced, as in purple martins; solicited by the male but apparently requiring female co-operation, as in house martins; or female solicited, as in blue tits. Female behaviour seems, however, to have no clear implications for extra-pair paternity. For example, female solicitation of EPCs has different outcomes in different species. In blue tits and shags it leads to a high incidence of extra-pair offspring, whereas in fulmars no cases of extra-pair paternity were recorded.

Intra-specific brood parasitism (IBP) might be regarded as a female equivalent of EPFs since it imposes a cost on a non-relative. It was documented in only two of the 15 species in Table 5.1, suggesting that it may occur more rarely than EPFs in birds. In zebra finches, 11% of offspring in 36% of nests were found to result from IBP (sample sizes as in Table 5.1, Birkhead *et al* 1990); and in purple martins the equivalent rates were 19% of offspring and 36% of nests (Morton *et al* 1990). It is perhaps not surprising that IBP may be rare in birds, it represents a cost of reproduction to both the male and female who raise the parasitised offspring, therefore both males and females would be expected to guard against brood parasites (Petrie & Moller 1991). EPFs, on the other hand, only represent a genetic cost to males and may often represent a genetic gain to females who obtain EPFs from high quality males.

5.4 Mixed Reproductive Strategy in the hirundines

Table 5.2 lists a number of behavioural and ecological aspects of the breeding biology of six hirundine species for which estimates of the percentage of nests containing non-kin offspring are available. Interspecific comparisons within the swallows and martins (family

Table 5.2 Mixed reproductive strategy, breeding ecology and behaviour of six hirundine species

Species	% of nests		Breeding system	Colony sizes		Nest type	Guarding		Sex differences	Refs
	EPO (n)	IBP (n)		study	average (range)		Mate	Nest		
House martin	38 (22)	0 (22)	M C	3-12	4-5 (2-1000+)	Closed, mud pellets, under eaves & rock ledges Use nest boxes	Yes	Yes?	Females slightly duller	This study Turner & Rose 1989
Sand martin	40 (5)	0 (5)	M C	30-100	38 (1000+)	Burrow in sandbank	Yes	Yes	Sexes similar in size & plumage	This study Turner & Rose 1989
Cliff swallow	7 (105)	43 (105)	M C	2-3500	2-3500	Closed, mud pellets, under eaves & rock ledges	No	Yes	" "	Brown & Brown 1988, 1989 Butler 1982

Table 5.2 continued

Species	% of nests		Breeding system	Colony sizes		Nest type	Guarding		Sex differences	Refs
	EPO	IBP		study	average		Mate	Nest		
Tree swallow	47 (15)	Yes	M T	-	-	Tree cavaties Use nest boxes	No	Yes	Female duller	Birkhead & Møller 1992 Lefebvre & Roberson 1984 Lombardo 1988 Turner & Rose 1989
Barn swallow	(22*)	0-40	M V		<5 (2-30)	Open cup, mud pellets, inside farm buildings etc	Yes	Yes	Female duller Male has longer outer tail feathers	Wellbourn *et al* unpub Møller 1987a-d Turner & Rose 1989
Purple martin	50 (14)	36 (14)	M C	30	6-8 (300)	Tree cavaties Use nest boxes	Yes	Yes	Females and subadult males duller than adult males	Morton *et al* 1990 Morton 1987 Turner & Rose 1989

Notes: EPO=% of nests with at least one extra-pair offspring, IBP=intraspecific brood parasitism; breeding system: M monogamous, C colonial, T territorial; * = percentage of offspring, rather than nests, that are extra-pair

Hirundinidae) are of particular value because of the strong similarities between species. All are morphologically similar, share the habit of catching insects on the wing, and nest in sites that are generally inacessible to predators.

Extra-pair fertilisations have been documented for all the species listed in Table 5.2, affecting a high proportion of nests in all but the cliff swallow. In house martins, sand martins, swallows and purple martins, high rates of EPF were observed despite the occurence of mate guarding. The results of this study indicate that in house martins, variation in the level of guarding may be linked to the success of individual males in defending their paternity (section 4.3.5). There is evidence for similar levels of variability in other hirundine species. In swallows, the male guards by following the female for about 70% of the time from about 10 days before the first egg is laid, until about 2 days before the clutch is completed. Males from colonial nesting pairs begin guarding earlier and guard more intensely than males from solitary pairs, indicating that the risk of EPC (which increases with colony size) may affect the effort that the male puts in to guarding his mate (Møller 1985, 1987c & d), and that male swallows percieve guarding as effective in paternity defence. Male purple martins also guard, accompanying their mates on trips from the nest to collect nesting material from the ground. However, the intensity of mate guarding varies enormously between males, from 0-100%, and it seems that its main function in this species is to prevent harassment of the female while she builds the nest, rather than to defend paternity (Morton 1987, Morton *et al* 1990). The absence of mate guarding in cliff swallows and tree swallows may indicate that the nest site has to be the male guarding priority. In cliff swallows this seems to be due to the high risk of intraspecific brood parasitism (Brown & Brown 1989); and in tree swallows because of the limited availability of natural nest sites (Lefelaar & Robertson 1984).

It might be predicted that IBP would occur at a higher rate in species with open nests compared with those using closed nest chambers, because in the former case eggs could be dumped into the nest more easily. However it seems that high rates of egg dumping may occur in species such as cliff swallows, which build closed nests with narrow entrances, as well as barn swallows which use open cup nests. The high incidence of IBP in cliff swallows, which occurs despite nest guarding, has been explained as a risk spreading strategy (Brown & Brown 1989). Since many nests fail completely because of parasite infestations, inclement weather, or crumbling of nest substrate, it might benefit birds to spread their offspring over several nests. In support of this, a positive correlation was found between the percentage of unsuccessful nests and the level of IBP in cliff swallow colonies (Brown & Brown 1989).

The Hirundinidae are generally classified as colonial breeders, although a few species, such as the tree swallow, which defends a substantial area around its nest from conspecifics (Robertson & Gibbs 1982), are better described as territorial. Coloniality is seen as an evolutionary trend in the hirundines, the development of which paralleled the development of the ability of birds to build their own nests (Snapp 1976, Shields *et al* 1988). Both EPFs and IBP might be expected to occur at higher rates when many birds nest in close proximity, thus an increased chance of raising non-kin offspring might be regarded as a cost of coloniality (Møller 1987d). This prediction was not supported by a comparison of the bird species in Table 5.1, does it hold across species within the hirundine family? Table 5.2 indicates that both EPFs and IBP may occur at equivalent rates in species forming large and small colonies, giving no indication that the frequency of either form of MRS increases with colony size between hirundine species. What about the effect of colony size within a species? Møller (1987d) found that the percentage of swallow nests being parasitised increased with colony size, whereas Brown & Brown (1989) found no effect of colony size on the incidence of brood parasitism in cliff swallow colonies with 10 or more nests. There was however, a significantly lower incidence of IBP in colonies with less than 10 nests, suggesting that in cliff swallows there is a threshold colony size above which IBP occurs at a fairly constant rate. Preliminary results from house martins suggest that EPFs may be equally prevalent in colonies ranging in size from 2-12 breeding females (Section 3.3.4 iv).

Sexual dimorphism in birds, in particular male tail length, is presumed to influence male mating success via female choice, and to be most extreme in polygynous species (Darwin 1901, Cherry 1990, Anderson 1982). In monogamous species female preferences for male ornaments or appearance might also increase male reproductive success by enabling the most attractive males to breed earlier and produce more, better quality offspring (Darwin 1901, Fisher 1958). Additionally, the most atractive males might be most successful in obtaining EPFs, and it might be predicted that high rates of EPF would be correlated with a high degree of sexual dimorphism. In swallows, for example, artificial elongation of male tail feathers enabled males to obtain mates more quickly and to enjoy increased seasonal reproductive success, as well as increased success in obtaining EPCs (Møller 1988c, 1989c). However, Table 5.2 shows that high rates of EPFs occur both in species such as the sand martin, where the two sexes are alike in size and plumage, and in purple martins where there are clear differences in appearance between males and females. Thus in the hirundines at least, sexual dimorphism seems to be unrelated to the prevalence of EPFs. It is notable that the two most monomorphic species, cliff swallows and sand martins, are also those that form the largest colonies. In these species it might be

advantageous for females to look like their partners, to minimise the level of harassment from extra-pair males. however, in spite of sexual monomorphism, male sand martins are apparantly able to recognise laying females by their laboured flight pattern (Jones 1986).

5.5 Implications for future research on reproductive success in birds

This preliminary review of consanguinuity in wild birds indicates that it is very difficult to generalise on the basis of data presently available. For each species the observed rates of EPF and IBP, if they occur, seem to be a result of unique interactions between behavioural and ecological factors. What is clear, however, is that EPCs probably occur in the majority of bird species. For example, they have been documented from behavioural and/or genetic evidence in all fifteen species listed in Table 5.1, even though in some, such as the Fulmar and the willow warbler, they are rarely if ever successful in fertilising eggs. IBP seems to occur at a lower rate than EPF, being documented in only two of the 15 species in Table 5.1. It does however seem to occur at a higher rate in the hirundine family (in four of the six species in Table 5.2), and it is worth bearing in mind that within a species there may be considerable variation in breeding behaviour. For example, IBP has not been observed in barn swallows breeding in Central Scotland (S Ward, pers comm), but has been documented in larger colonies on continental Europe (Møller 1987d).

The importance of variation between individuals cannot be overstated. It seems that, within a species, some males may be more successful at obtaining EPFs, or more susceptible to cuckoldry. For example, male house martins that raise two broods with the same female are unlikely to be cuckolded. Combining observations of behaviour with results from DNA fingerprinting suggests that male house martins that guard their mates more closely are also less likely to be cuckolded. Unfortunately few hints were obtained on the attributes of individual males that might account for their increased success in paternity defence, although there was a suggestion that older males might be less likely to be cuckolded. For sand martins too few broods were fingerprinted to make any judgement on possible individual variation in paternity defence.

A priority for future work on the study species should be the identification of the fathers of extra-pair offspring, which might be most sucessfully achieved with single locus fingerprints (Burke 1989). Only then can realised male reproductive success be compared with apparent reproductive success. Preliminary results from work on red-winged blackbirds suggest that the two may be very different (Gibbs *et al* 1990), and that the

pursuit of EPCs may result in increased lifetime reproductive success for some males. This has important implications for studies seeking to identify reproductive costs in birds, since both EPFs and IBP represent fecundity costs to breeding adults, and, conversely, the pursuit of a MRS by males and females may itself incur reproductive costs. Studies attempting to measure annual or lifetime reproductive success must also measure consanguinuity between adults and offspring. It is no longer possible to 'ignore' the effects of sperm competition or IBP, and DNA fingerprinting should become as integral a part of studies of wild bird populations as metal identification rings.

6. Summary and Conclusions

1. Both at an individual and a population level double-broodedness is implicated as the annual breeding strategy with the highest fitness benefits for house martins, in terms of total annual output of fledged young. Pairs that attempted two broods in a season fledged significantly more young on average than those that attempted only one brood, and in years when higher proportions of the study population attempted a second brood the average annual reproductive success per pair was also higher. In this species where few individuals have a reproductive lifespan of more than two years (Bryant 1988a, 1989), variations in annual reproductive success are likely to have profound effects on the lifetime production of fledged young.

2. An analysis of the relationships between individual characteristics of house martins (size and age) and their annual reproductive success produced only one clear result, that older females laid earlier than first year females. In contrast with earlier work on the same species (Bryant 1979, 1988a), there was a hint that small size in males might be associated with increased annual reproductive success, and no relationship was found between the age of males or females and any component of annual reproductive success. Because of the uncertainty of age estimates for most of the breeding adults in the present study it is difficult to draw any firm conclusions. It is however possible that changing environmental conditions, either on the breeding grounds or in the wintering areas, have brought about changes in the observed relationships between individual size and reproductive success in house martins; perhaps via a change in the age structure of the population whereby fewer birds survive beyond their first breeding year.

3. Experimental manipulations of first brood size in house martins were found to have effects on the interval between breeding attempts, the growth and mortality of nestlings, and the probability of a second clutch. Clutch intervals were shortest for pairs raising reduced first broods and longest for pairs raising enlarged broods. The increased breeding interval asociated with larger broods seems to be due to an extended period between the fledging of first brood nestlings and the laying of the second clutch. The increased effort required in raising a larger brood and/or an extended period of post-fledging feeding of young may affect the body condition of the female so she requires more time to recover condition and lay again. Nestlings in enlarged broods seemed to grow more slowly for the first 15-16 days after hatch, but were not on average lighter at fledging than those in smaller broods which may have been at least partly due to an inceased rate of nestling

mortality in enlarged broods. Using nest records from the present study, combined with data collected between 1972-83 by D M Bryant, manipulation of first clutch size in house martins was found either to reduce the chance that pairs raising enlarged first broods would produce a second clutch, or to increase the chance that reduced first broods would be followed by a second clutch, depending on the timespan of data analysed. These different effects may be due to the decline in the proportion of pairs attempting a second clutch that has occurred between 1972 and 1983.

4. Over the time period 1972-1989, house martins breeding at study colonies in Central Scotland have undergone a decline in annual reproductive success. Variation in food abundance apparently had some effects on the timing and success of breeding, but could not fully account for the observed changes. It is debatable whether this decline is real, or a result of changes in food availability, nest site preference, and possibly age structure of the house martin population in Central Scotland over the same period.

5. DNA fingerprinting was found to be a reliable method of characterising genetic relationships between individuals in both house martins and sand martins. There was no evidence of intraspecific brood parasitism in either species. In house martins 38% of broods were found to contain at least one offspring that had been fathered by an extra-pair male, with 15% of all young being unrelated to their putative father. Preliminary results indicated a slightly higher incidence of extra-pair offspring in sand martins. Extra-pair fertilisations therefore represent fitness or fecundity costs of reproduction for at least some males in both of the study species.

6. Observations of behaviour indicated that male sand martins and house martins guard their mates during the prelaying and laying periods, although the degree of guarding differs between the two species, probably due to differing risks of EPCs. It seems probable that female sand martins are vulnerable to harassment and EPC attempts all the time that they are out of the nesting burrow, and males attempt to follow females continuously from approximately four days before the first egg is laid, until the day the third egg is laid. In house martins, EPCs are apparently most likely to take place at the nest. Males do not try to follow females continuously, but ensure that females spend little time alone at the nest from about seven days before the first egg is laid, until the day that the second egg is laid. They also accompany the female on up to 70% of flights into or out of the nest during the 4-5 days before egg laying commences. Male house martins that allowed their mates to stay alone at the nest for longer periods during the prelaying and early laying period were found more likely to be cuckolded, and intruders were more

likely to enter a nest where the female was fertile when the pair male was absent, providing evidence that mate guarding does function as a form of paternity defence in this species. In both house martins and sand martins mate guarding seemed to slacken 1-2 days before the last egg of the clutch was laid, which theoretically puts the last 1-2 eggs at risk of fertilisation by extra-pair males. This prediction was supported in house martins by the finding that, in broods containing at least one extra-pair chick, the youngest nestling had a higher than expected chance of being fathered by an extra-pair male.

7. In the absence of the pair male, female house martins apparently choose whether or not to accept EPCs from males intruding into their nests. This decision may be based on some male attribute percieved as an indication of quality.

8. House martin males that have been cuckolded do not reduce their parental effort in terms of rate of food delivery to the brood. Although there is evidence that males of at least one bird species, the dunnock, are able to assess their certainty of paternity (Burke *et al* 1989, Davies *et al* 1992), it seems that birds in general are unable to distinguish young that are genetically related to them, because nestlings can be successfully fostered between nests (as evidenced by the brood manipulation experiments described in Chapter 2). Males of monogamous bird species such as the house martin might therefore not be expected to reduce their level of parental effort if they suspect that they might have been cuckolded, because their own young are likely to suffer alongside extra-pair offspring.

9. Former estimates of apparent male reproductive success in house martins (Bryant 1988a, 1989) must now be revised because males are not necessarily the genetic fathers of all the offspring in their brood, and at least some males increase their fecundity by obtaining EPFs. Unfortunately it was not possible to identify the fathers of extra-pair offspring. There was evidence from behavioural observations that males were more likely to seek EPCs after laying had been completed at their own nests, suggesting that males that begin breeding earlier may be more successful in obtaining EPFs. Males that breed earlier are also more likely to raise two broods and enjoy high annual reproductive success, and are unlikely to be cuckolded themselves if they stay with the same female partner. Thus there may be some high quality male house martins that achieve full paternity in their own nests and EPFs at the expense of other males, and there was some evidence that these differences might be related to male age. There were also indications that at least some EPFs might be perpetrated by non-breeding, 'floating' males who might opportunistically move in at nests where the pair male had vanished. Thus some

male house martins may increase their annual and/or lifetime reproductive success through the pursuit of EPCs, although if older males increase their fecundity at the expense of younger males then realised lifetime reproductive success in house martins which survive for more than one year is unlikely to be very different to apparent success. It is also possible that the pursuit of EPCs represents an alternative reproductive tactic for males that are for some reason without a mate.

10. A preliminary review of the occurrence of sperm competition in wild bird populations was undertaken using results for 15 species that have been investigated with DNA fingerprinting, including the two study species (Table 5.1, based on Table 12.2 in Birkhead & Møller 1992). EPCs were documented in all 15 species, although in some they apparently rarely if ever result in EPFs. The percentage of young fathered by extra-pair males varied from 0-35, with 0-50% of broods containing at least one extra-pair offspring (defined as an offspring sired outside the breeding unit in polygamous species, or species breeding in social groups). Cross-species comparisons indicated that EPFs were equally likely to occur in solitary/territorial versus colonially breeding species, and in monogamous versus polygamous breeding systems. The intensity of mate guarding varied considerably between species, but tight guarding did not necessarily result in a low rate of EPFs. EPCs were forced by males in some species, and actively sought by females in others, again with variable success in terms of rates of EPF. Intraspecific brood parasitism was proven in only two species, suggesting it occurs more rarely than EPFs in birds. This is not unexpected since IBP represents a genetic cost to both males and females raising 'parasite' offspring and both males and females would be expected to guard against parasites. EPFs on the other hand only represent a genetic cost to males and may actually benefit females who obtain EPFs from high quality males.

11. Comparisons of six hirundine species for which consanguinuity between parents and offspring has been investigated (Table 5.2) indicated generally high rates of EPFs (affecting 30-50% of nests) in all but cliff swallows; despite the occurence of mate guarding in sand martins, house martins, swallows and purple martins. Variability in the intensity of mate guarding has been documented in the latter three species (this study, Møller 1985, 1987c & d, Morton 1987, Morton *et al* 1990, and in the house martin at least, males that guard less intensely may be more likely to be cuckolded. The absence of mate guarding in tree swallows and cliff swallows probably indicates that the nest must be the guarding priority. The frequency of non-kin offspring, resulting either from EPFs or IBP, did not seem to increase with colony size between species, nor consistently within individual species. The prediction that high rates of EPF would be correlated with

a high degree of sexual dimorphism was also unsupported.

12. This preliminary review of consanguinuity in wild birds has failed to isolate any consistent themes. For each species, even closely related species within the hirundine family, the observed rates of EPF and IBP seem to be a result of unique interactions between behavioural and ecological factors. What is clear is that EPCs probably occur in the majority of bird species, even though in some, such as the fulmar (Hunter *et al* 1992) and the willow warbler (Gyllensten *et al* 1990) they rarely if ever lead to EPFs.

13. Future work on the study species could usefully concentrate on the identification of fathers of extra-pair offspring and the measurement of realised male reproductive success. This should allow true costs of reproduction to be identified in male house martins, taking into account the possible costs attendant on the pursuit of extra-pair copulations. Individual variation in mate guarding intensity in house martins merits further investigation, as does the role of the female in the success of EPCs, and possible cues by which female house martins might accept or reject copulations from extra-pair males. In sand martins, the preliminary results obtained here must be substantiated, the places where pair copulations and EPCs occur established, and possible variation in mate guarding intensity investigated, and this work is already in hand (Santos Alves, in prep). It would also be useful to investigate the duration of the fertile period, and the mechanics of sperm competition.

14. It is clear that it is no longer possible to ignore the effects of sperm competition and IBP, and no study of individual reproductive success in wild bird populations can now be considered complete unless it incorporates DNA fingerprinting.

References

AGUILERA E. & ALVAREZ F. (1989)
Copulations and mate guarding of the spoonbill (*Platalea leucorodia*)
Behaviour 110:1-22

ALATALO R.V. GOTTLANDER K. & LUNDBERG A. (1987)
Extra-pair copulations and mate guarding in the polyterritorial pied flycatcher, *Ficedula hypoleuca*
Behaviour 101:139-155

ALATALO R.V. GUSTAFSSON L. & LUNDBERG A. (1984)
High frequency of cuckoldry in pied and collared flycatchers
Oikos 42:41-47

ALATALO R.V. GUSTAFSSON L. & LUNDBERG A. (1989)
Extra-pair paternity and heritability estimates of tarsus length in pied and collared flycatchers
Oikos 56:54-58

ALVES M.A.S. (in prep)
Costs of coloniality in sand martins
PhD Thesis, University of Stirling

AMOS W. & DOVER G. (1990)
DNA fingerprinting and the uniqueness of whales
Mammal Review 20:23-30

ANDERSSON M. (1982)
Female choice selects for extreme tail length in a widowbird
Nature 299:818-820

ANDERSSON M. (1984)
Brood parasitism within species
In, Barnard C. (ed, 1984). Producers and scroungers: strategies of exploitation and parasitism:195-228
Croom Helm, London

ASBIRK S. (1976)
Studies on the breeding biology of the sand martin *Riparia riparia* (L.) (Aves) in artificial nest sites
Videnskabelige Meddelelser Fra Dansk Naturhistorisk Forening 139:147-177

ASKENMO C. (1979)
Reproductive effort and return rate of the male pied flycatcher
American Naturalist 114:748-753

BARASH D.P. (1976)
Male response to apparent female adultery in the mountain bluebird (*Sialia currucoides*): an evolutionary interpretation
American Naturalist 110:1097-1101

BART J. & TORNES A. (1989)
Importance of monogamous male birds in determining reproductive success
Behavioural Ecology & Sociobiology 24:109-116

BEECHER M.D. (1988)
Kin recognition in birds
Behavior Genetics 18:465-482

BEECHER M.D. & BEECHER I.M. (1979)
Sociobiology of Bank Swallows: reproductive strategy of the male
Science 205:1282-1285

BELLIS M.A. & BAKER R.R. (1990)
Do females promote sperm competition? Data for humans
Animal Behaviour 40:997-999

BIRKHEAD T.R. (1979)
Mate guarding in the magpie *Pica pica*
Animal Behaviour 27:866-874

BIRKHEAD T.R. (1982)
Timing and duration of mate guarding in magpies, *Pica pica*
Animal Behaviour 30:277-283

BIRKHEAD T.R. (1987)
Sperm competition in birds
Trends in Ecology & Evolution 2:268-272

BIRKHEAD T.R. (1988)
Behavioural aspects of sperm competition in birds
Advances in the Study of Behaviour 18:35-72

BIRKHEAD T.R. & BIGGINS J.D. (1987)
Reproductive synchrony and extra-pair copulation in birds
Ethology 74:320-334

BIRKHEAD T.R. & MOLLER A.P. (1992)
Sperm competition in birds. Evolutionary causes and consequences
Academic Press, London

BIRKHEAD T.R. ATKIN L. & MOLLER A.P. (1987)
Copulation behaviour of birds
Behaviour 101:101-138

BIRKHEAD T.R. CLARKSON K. & ZANN R. (1988)
Extra-pair courtship, copulation and mate guarding in wild zebra finches *Taeniopygia guttata*
Animal Behaviour 36:1853-1855

BIRKHEAD T.R. PELLATT J. & HUNTER F.M. (1988)
Extra-pair copulation and sperm competition in the zebra finch
Nature 334:60-61

BIRKHEAD T.R. BURKE T. ZANN R. HUNTER F.M. & KRUPA A.P. (1990)
Extra-pair paternity and intraspecific brood parasitism in wild zebra finches *Taeniopygia guttata*, revealed by DNA fingerprinting
Behavioural Ecology & Sociobiology 27:315-324

BJORKLUND M. & WESTMAN B. (1983)
Extra-pair copulations in the pied flycatcher (*Ficedula hypoleuca*). A removal experiment
Behavioural Ecology & Sociobiology 13:271-275

BROOKFIELD J.F.Y. (1989)
Analysis of DNA fingerprinting data in cases of disputed paternity
IMA Journal of Mathematics Applied in Medicine & Biology 6:111-131

BROWN C.R. & BROWN M.B. (1988)
Genetic evidence of multiple parentage in broods of cliff swallows
Behavioural Ecology & Sociobiology 23:379-387

BROWN C.R. & BROWN M.B. (1989)
Behavioural dynamics of intraspecific brood parasitism in colonial cliff swallows
Animal Behaviour 37:777-796

BRYANT D.M. (1972)
The breeding biology of the house martin (*Delichon urbica* L.) in relation to aerial insect abundance
PhD Thesis. University of London

BRYANT D.M. (1973)
The factors influencing the selection of food in the house martin (*Delichon urbica* L.)
Journal of Animal Ecology 42:539-564

BRYANT D.M. (1975a)
Breeding biology of house martins (*Delichon urbica*) in relation to aerial insect abundance
Ibis 117:180-216

BRYANT D.M. (1975b)
Changes in incubation patch and weight in the nesting house martin
Ringing and migration 1:33-36

BRYANT D.M. (1978a)
Establishment of weight hierarchies in the broods of house martins *Delichon urbica*
Ibis 120:16-26

BRYANT D.M. (1978b)
Environmental influences on growth and survival of nestling house martins
Ibis 120:271-283

BRYANT D.M. (1979)
Reproductive costs in the house martin (*Delichon urbica*)
Journal of Animal Ecology 48:655-675

BRYANT D.M. (1988a)
Lifetime reproductive success of house martins
In, Clutton Brock T.H. (ed). Reproductive success: studies of individual variation in contrasting breeding systems:173-188
University of Chicago Press

BRYANT D.M. (1988b)
Energy expenditure and body mass changes as measures of reproductive costs in birds
Functional Ecology 2:23-34

BRYANT D.M. (1989)
House martin
In, Newton I. (ed). Lifetime reproduction in birds:89-106
Academic Press

BRYANT D.M. & GARDINER A. (1979)
Energetics of growth in house martins (*Delichon urbica*)
Journal of Zoology 189:275-304

BRYANT D.M. & TURNER A.K. (1982)
Central place foraging by swallows (Hirundinidae): the question of load size
Animal Behaviour 30:845-856

BRYANT D.M. & WESTERTERP K.R. (1980)
The energy budget of the house martin (*Delichon urbica*)
Ardea 68:91-102

BRYANT D.M. & WESTERTERP K.R. (1982)
Evidence for individual differences in foraging efficiency amongst breeding birds: a study of house martins *Delichon urbica* using the doubly labelled water technique
Ibis 124:187-192

BRYANT D.M. & WESTERTERP K.R. (1983a)
Short-term variability in energy turnover by breeding house martins *Delichon urbica*: a study using doubly-labelled water (D218O)
Journal of Animal Ecology 52:525-543

BRYANT D.M. & WESTERTERP (1983b)
Time and energy limits to brood size in house martins *Delichon urbica*
Journal of Animal Ecology 52:905-925

BUITRON D. (1983)
Extra-pair courtship in black-billed magpies
Animal Behaviour 31:211-220

BURKE T. (1989)
DNA fingerprinting and other methods for the study of mating success
Trends in Ecology & Evolution 4:139-144

BURKE T. & BRUFORD M.W. (1987)
DNA fingerprinting in birds
Nature 327:149-152

BURKE T. DAVIES N.B. BRUFORD M.W. & HATCHWELL B.J. (1989)
Parental care and mating behaviour of polyandrous dunnocks *Prunella modularis* related to paternity by DNA fingerprinting
Nature 338:249-251

BURLEY N. (1988)
Wild zebra finches have band colour preferences
Animal Behaviour 36:1235-1237

BURNS J.T. CHENG K.M. & MCKINNEY F. (1980)
Forced copulation in captive mallards 1. Fertilization of eggs
Auk 97:875-879

BUTLER R.W. (1982)
Wing fluttering by mud-gathering cliff swallows: avoidance of rape attempts?
Auk 99:758-761

BUTTERFIELD (1953)
[house martin mating obs]
British Birds 46:65-66

CAVALLI-SFORZA L.L. & BODMER W.F. (1971)
The genetics of human populations
W.H. Freeman, San Francisco

CHAKRABORTY R. MEAGER T.R. & SMOUSE P.E. (1988)
Parentage analysis with genetic markers in natural populations I. The expected proportion of offspring with unambiguous paternity
Genetics 118:527-536

CHARNOV E.L. & KREBS J.R. (1974)
On clutch size and fitness
Ibis 116:217-219

CHENG K.M. BURNS J.T. & McKINNEY F (1982)
Forced copulation in captive mallards Anas platyrhynchos II. Temporal factors
Animal Behaviour 30:695-699

CHERRY M.I. (1990)
Tail length and female choice
Trends in Ecology & Evolution 5:349-350

CLUTTON-BROCK T.H. (ed. 1988)
Reproductive success: studies of individual variation in contrasting breeding seasons
University of Chicago Press

COMPTON M.M. VAN KREY H.P. & SEIGEL P.B. (1978)
The filling and emptying of the uterovaginal sperm-host glands in the domestic hen
Poultry Science 57:1696-1700

COULSON J.C. & PORTER J.M. (1985)
Reproductive success of the kittiwake *Rissa tridactyla*: the roles of clutch size, chick growth rates and parental quality
Ibis 127:450-466

COWLEY E. (1983)
Multi-brooding and mate infidelity in the sand martin
Bird Study 30:1-7

CRAMP S. (1988)
The birds of the western palearctic vol V
Oxford University Press

CRONMILLER J.R. & THOMPSON C.F. (1980)
Experimental manipulation of brood size in red-winged blackbirds
Auk 97:559-565

CROOK J.R. & SHIELDS W.M. (1985)
Sexually selected infanticide by adult male barn swallows
Animal behaviour 33:754-761

DAAN S. DIJKSTRA C. DRENT R. & MEJER T. (1988)
Food supply and the annual timing of avian reproduction
Proceedings of the XIX International Ornithological Congress
Ottowa

DARWIN C. (1901)
The descent of man and selection in relation to sex
New edn. John Murray, London

DAVIES N.B. (1983)
Polyandry, cloaca-pecking and sperm competition in dunnocks
Nature 302:334-336

DAVIES N.B. (1985)
Cooperation and conflict among dunnocks (*Prunella modularis*) in a variable mating system
Animal Behaviour 33:628-648

DAVIES N.B. & LUNDBERG A. (1984)
Food distribution and a variable mating system in the dunnock *Prunella modularis*.
Journal of Animal Ecology 53:895-912

DAVIES N.B. HATCHWELL B.J. ROBSON T. & BURKE T. (1992)
Paternity and parental feeding in dunnocks *Prunella modularis*: how good are male chick-feeding rules?
Animal Behaviour 43:729-745

DAWKINS R. & CARLISLE T.R. (1976)
Parental investment, mate desertion and a fallacy
Nature 262:131-133

DE LAET J.F. & DHONDT, A.A. (1989)
Weight loss of the female as a factor influencing second brood initiation in great tits *Parus major* and blue tits *P. caeruleus*
Ibis 131:281-289

DE STEVEN D. (1980)
Clutch size, breeding success, and parental survival in the tree swallow (*Iridoprocne bicolor*)
Evolution 34:278-291

DEWSBURY D.A. (1982)
Ejaculate cost and male choice
American Naturalist 119:601-610

DHONDT A.A. (1971)
The regulation of numbers in Belgian populations of blue tits
Proceedings of the Advanced Study Institute on the Dynamics of Numbers in Populations (Oosterbeek 1970):532-547

DIJKSTRA C. BULT A. BILJSMA S. & MEIJER T. (1988)
Brood manipulations in the kestrel: effects on offspring and parent survival
PhD Thesis, Rijksuniversiteit Gronigen, Chapter 4:63-84

DIXSON A.F. HASTIE N. PATEL I. & JEFFREYS A.J. (1988)
DNA 'fingerprinting' of captive family groups of common marmosets (*Callithrix jacchus*)
Folia Primatologica 51:52-55

DRENT R.H. & DAAN S. (1980)
The prudent parent: energetic adjustments in avian breeding
Ardea 68:225-252

EMLEN S.T. & DEMONG N.J. (1975)
Adaptive significance of synchronised breeding in a colonial bird: a new hypothesis
Science 188:1029-1031

EMLEN S.T. & ORING L.W. (1977)
Ecology, sexual selection and the evolution of mating systems
Science 197:215-223

EVANS P.G.H. (1987)
Electrophoretic variability of gene products
In, Cooke & Buckley (eds) Avian Genetics. A population and ecological approach:105-163
Academic Press

EVARTS S. & WILLIAMS C.J. (1987)
Multiple paternity in a wild population of mallards
The Auk 104:597-602

FAULKES C.G. & ABBOTT D.H. (1990)
Investigation of genetic diversity in wild colonies of naked mole-rats (*Heterocephalus glaber*) by DNA fingerprinting
Journal of Zoology 221:87-97

FINKE M.A., MILKINOVICH D.J. & THOMPSON C.F. (1987)
Evolution of clutch size: an experimental test in the house wren (*Troglodytes aedon*)
Journal of Animal Ecology 56:99-114

FISHER R.A. (1958)
The genetical theory of natural selection
Second edn, Dover, New York

FITCH M.A. & SHUGART G.W. (1984)
Requirements for a mixed reproductive strategy in avian species
The American Naturalist 124:116-126

FORD N.L. (1983)
Variation in mate fidelity in monogamous birds
In, Johnson R.F. (ed) Current Ornithology 1:329-356

GAVIN T.A. & BOLLINGER E.K. (1985)
Multiple paternity in a territorial passerine: the bobolink
The Auk 102:550-555

GARNETT M.C. (1981)
Body size, heritability and influence on juvenile survival among great tits *Parus major*
Ibis 123:31-41

GEORGES M. COCHAUX P. LEQUARRE A-S. YOUNG M.W. & VASSART G. (1987)
DNA fingerprinting in man using a mouse probe related to part of the *Drosophila* 'Per' gene
Nucleic Acids Research 15:7193

GEORGES M. LEQUARRE A-S. CASTELLI M. HANSET R. & VASSART G. (1988)
DNA fingerprinting in domestic animals using four different minisatellite probes
Cytogenetics & Cell Genetics 47:127-131

GIBBS H.L. WEATHERHEAD P.J. BOAG P.T. WHITE B.N. TABAK L.M. & HOYSAK D.J. (1990)
Realized reproductive success of polygynous red-winged blackbirds revealed by DNA markers
Science 250:1394-1397

GILL P. JEFFREYS A.J. & WERRETT D.J. (1985)
Forensic application of DNA 'fingerprints'
Nature 318:577-579

GLADSTONE D.E. (1979)
Promiscuity in monogamous colonial birds
The American Naturalist 114:545-557

GOWATY P.A. (1983)
Male parental care and apparent monogamy among eastern bluebirds *Sialia sialis*
American Naturalist 121:149-157

GOWATY P.A. & KARLIN A.A. (1984)
Multiple maternity and paternity in single broods of apparently monogamous eastern bluebirds (*Sialia sialis*)
Behavioural Ecology & Sociobiology 15:91-95

GRAFEN A. (1988)
On the uses of data on lifetime reproductive success
In, Clutton-Brock T.H. (ed, 1988). Reproductive success: studies of individual variation in contrasting breeding systems:454-471
University of Chicago Press

GRANT B.R. & GRANT P.R. (1989)
Natural selection in a population of Darwin's finches
American Naturalist 133:377-393

GRAVES J. HAY R.T. SCALLAN M. & ROWE S. (1992)
Extra-pair paternity in the shag, *Phalacrocorax aristotelis* as determined by DNA fingerprinting
Journal of Zoology (London) 226:399-408

GREENLAW J.S. & POST W. (1985)
Evolution of monogamy in seaside sparrows *Ammodramus maritimus*: tests of hypotheses
Animal Behaviour 33:373-383

GROSS M.R. (1985)
Disruptive selection for alternative life histories in salmon
Nature 313:47-48

GUNTEN K. VON & SHWARZENBACH F.H. (1962)
Zur ernährungsbiologie der Mehlschwalbe *Delichon urbica*: quantitative untersuchungen am nestlingsfutter
Orn Beob 59:1-22

GUSTAFSSON L. & SUTHERLAND W.J. (1988)
The costs of reproduction in the collared flycatcher
Nature 335:813-815

GYLLENSTEN U. JAKOBSSON S. & TEMRIN H. (1990)
No evidence for illegitimate young in monogamous and polygamous warblers
Nature 343:168-170

GYLLENSTEN U. JAKOBSSON S. TEMRIN H. & WILSON A.C. (1989)
Nucleotide sequence and genomic organization of bird minisatellites
Nucleic Acids Research 17:2203-2214

HAILS C.J. (1977)
Energy expenditure in free living house martins *Delichon urbica* during the breeding season
PhD Thesis, University of Stirling

HAILS C.J. & BRYANT D.M. (1979)
Reproductive energetics of a free-living bird
Journal of Animal Ecology 48:471-482

HANKEN & SHERMAN (1981)
Multiple paternity in Belding's ground squirrel litters
Science 212:351-353

HARVEY P.H. & BRADBURY J.W. (1991)
Sexual selection
In, J.R. Krebs & N.B. Davies (eds), Behavioural ecology, an evolutionary approach
Blackwell

HATCH S.A. (1987)
Copulation and mate guarding in the northern fulmar
Auk 104:450-461

HATCHWELL B.J. (1988)
Intraspecific variation in extra-pair copulation and mate defence in common guillemots *Uria aalge*
Behaviour 107:157-185

HATCHWELL B.J. & DAVIES N.B. (1992)
An experimental study of mating competition in monogamous and polyandrous dunnocks *Prunella modularis*: I. Mate guarding and copulations
Animal Behaviour 43:595-609

HAYMES G.T. & MORRIS R.D. (1977)
Brood size manipulations in herring gulls
Canadian Journal of Zoology 55:1762-1766

HEGNER R.E. & WINGFIELD J.C. (1987)
Effects of brood-size manipulations on parental investment, breeding success, and reproductive endocrinology of house sparrows
Auk 104:470-480

HILZ H. WIEGERS U. & ADAMIETZ P. (1975)
Stimulation of Proteinase K action by denaturing agents: application to the isolation of nucleic acids and the degradation of 'masked' proteins
European Journal of Biochemistry 56:103-108

HOGSTAD O. (1989)
The presence of non-territorial males in willow warbler *Phylloscopus trochilus* populations - a removal study
Ibis 131:263-267

HOGSTEDT G. (1981)
Should there be a positive or negative correlation between survival of adults in a bird population and their clutch size?
American Naturalist 118:568-571

HOOGLAND J.L. & SHERMAN P.W. (1976)
Advantages and disadvantages of bank swallow (*Riparia riparia*) coloniality
Ecological monographs 46:33-58

HUND K. & PRINZINGER R. (1985)
(The influence of age on breeding parameters in the house martin)
J Orn 126:15-28

HUNTER F.M. BURKE T. & WATTS S.E. (1992)
Frequent copulation as a method of paternity assurance in the northern fulmar
Animal Behaviour 44:149-156

HUTCHINSON J.B. & LOVARI S. (1976)
Effects of male aggressiveness on behavioural transitions in the reproductive cycle of the barbary dove
Behaviour 59:296-318

JARMAN A.P. & WELLS R.A. (1989)
Hypervariable minisatellites: recombinators or innocent bystanders?
Trends in Genetics 5:367-371

JEFFREYS A.J. (1987)
Highly variable minisatellites and DNA fingerprints
Biochemical Society transactions 15:309-317

JEFFREYS A.J. & MORTON D.B. (1987)
DNA fingerprints of dogs and cats
Animal Genetics 18:1-15

JEFFREYS A.J. BROOKFIELD J.F. & SEMEONOFF R. (1985)
Positive identification of an immigration test-case using human DNA fingerprints
Nature 317:818-819

JEFFREYS A.J. NEUMANN R. & WILSON V. (1990)
Repeat unit sequence variation in minisatellites: a novel source of DNA polymorphism for studying variation and mutation by single molecule analysis
Cell 60:473-485

JEFFREYS A.J. WILSON V. & THEIN S.L. (1985a)
Hypervariable 'minisatellite' regions in human DNA
Nature 314:67-73

JEFFREYS A.J. WILSON V. & THEIN S.L. (1985b)
Individual-specific 'fingerprints' of human DNA
Nature 316:76-79

JEFFREYS A.J. WILSON V. THEIN S.L. WEATHERALL D.J. & PONDER B.A.J. (1986)
DNA "fingerprints" and segregation analysis of multiple markers in human pedigrees
American Journal of Human Genetics 39:11-24

JONES G. (1985)
Parent-offspring resource allocation strategies in birds: studies on swallows (Hirundinidae)
PhD Thesis. University of Stirling

JONES G. (1986)
Sexual chases in sand martins (*Riparia riparia*): cues for males to increase their reproductive success
Behavioural Ecology & Sociobiology 19:179-185

JONES G. (1987a)
Selection against large size in the sand martin *Riparia riparia* during a dramatic population crash
Ibis 129:274-280

JONES G. (1987b)
Colonization patterns in sand martins *Riparia riparia*
Bird Study 34:20-25

JOSTE N. LIGON J.D. & STACEY P.B. (1985)
Shared paternity in the acorn woodpecker (*Melanerpes formicivorus*)
Behavioural Ecology & Sociobiology 17:39-41

KEMPENAERS B. VERHEYEN G.R. VAN DEN BROECK M. BURKE T. VAN BROECKHOVEN C. & DHONDT A.A. (1992)
Extra-pair paternity results from female preference for high quality males in the blue tit
Nature 357:494-496

KING A.S. (1981)
Phallus
In, King A.S. & McLelland J. (eds). Form and function in birds Vol 2:107-147
Academic Press, London

KINKEL L.K. (1989)
Lasting effects of wing tags on ring-billed gulls
The Auk 106:619-624

KLUYVER, H.N., van BALEN J.H. & CAVE A.J. (1977)
The occurrence of time-saving mechanisms in the breeding biology of the great tit *Parus major*
In, B. Stonehouse & C. Perrins (eds), Evolutionary Ecology

KNOWLTON N. & GREENWELL S.R. (1984)
Male sperm competition avoidance mechanisms: the influence of female interests
In, R.L. Smith (ed), Sperm competition and the evolution of animal mating systems
Academic Press, Orlando

KUHNLEIN U. DAWE Y. ZADWORNY D. & GAVORA J.S. (1989)
DNA fingerprinting: a tool for determining genetic distances between strains of poultry
Theoretical & Applied Genetics 77:669-672

LACK D. (1947)
The significance of clutch size in birds, part I & II
Ibis 89:302-352

LACK D. (1954)
The natural regulation of animal numbers
Clarendon, Oxford.

LACK D. (1968)
Ecological adaptions for breeding in birds
Methuen London

LAKE P.E. (1975)
Gamete production and the fertile period with particular reference to domesticated birds
Symp Zool Soc Lond 35:225-244

LANK D.B. MINEAU P. ROCKWELL R.F. & COOKE F. (1989)
Intraspecific nest parasitism and extra-pair copulation in lesser snow geese
Animal Behaviour 37:74-89

LEFFELAAR D. & ROBERTSON R.J. (1984)
Do male tree swallows guard their mates?
Behavioural Ecology & Sociobiology 16:73-79

LIFJELD J.T. & SLAGSVOLD T. (1989)
How frequent is cuckoldry in pied flycatchers *Ficedula hypoleuca*? Problems with the use of heritability estimates of tarsus length
Oikos 54:205-210

LIFJELD J.T. SLAGSVOLD T. & LAMPE H.M. (1991)
Low frequency of extra-pair paternity in pied flycatchers revealed by DNA fingerprinting
Behavioural Ecology & Sociobiology 29:95-101

LIND E.A. (1960)
(On the behaviour and ecology of the housemartin)
Ann Zool Soc 'Vanamo' 21:1-126

LINDEN M. (1988)
Reproductive trade-off between first and second clutches in the great tit *Parus major*: an experimental study
Oikos 51:285-290

LINDEN M. & MØLLER A.P. (1989)
Cost of reproduction and covariation of life history traits in birds
Trends in Ecology & Evolution 4: 367-371

LOMBARDO M.P. (1983)
Evidence of intra-specific brood parasitism in the tree swallow
Wilson Bull 100:126-7

LUMPKIN S. (1983)
Female manipulation of male avoidance of cuckoldry behaviour in the ring dove
In, Wasser S.K. (ed) Social behaviour of female vertebrates: 91-112
Academic Press, New York

LUMPKIN S. (1981)
Avoidance of cuckoldry in birds: the role of the female
Animal Behaviour 29:303-304

LYNCH M. (1988)
Estimation of relatedness by DNA fingerprinting
Molecular Biology & Evolution 5: 584-599

McCLEERY R.H. & PERRINS C.M. (1989)
Great tit
In, Newton I. (ed). Lifetime reproduction in birds:35-53
Academic Press

McGILLIVRAY W.B. (1983)
Intersexual reproductive costs for the house sparrow (*Passer domesticus*)
Auk 100:25-32

McKINNEY F. CHENG K.M. & BRUGGERS D.J. (1984)
Sperm competition in apparently monogamous birds
In, Smith R.L. (ed, 1984) Sperm competition and the evolution of animal mating systems:523-545
Academic Press

McKINNEY F. DERRICKSON S.R. & MINEAU P. (1983)
Forced copulation in waterfowl
Behaviour 86:250-294

MADSEN T. SHINE R. LOMAN J. & HAKANSSON T. (1992)
Why do female adders copulate so frequently
Nature 355:440-441

MAGNHAGEN C. (1991)
Predation risk as a cost of reproduction
Trends in Ecology & Evolution 6:183-186

MANIATIS T. FRITSCH E.F. & SAMBROOK J. (1982)
Molecular cloning. A laboratory manual
Cold Spring Harbor Laboratory

MARTIN K. & HANNON S.J. (1988)
Early pair and extra-pair copulations in willow ptarmigan
The Condor 90:245-246

MARTIN P. & BATESON P. (1986)
Measuring behaviour. An introductory guide
Cambridge University Press

MAYNARD-SMITH J. (1977)
Parental investment: a prospective analysis
Animal Behaviour 25:1-9

MEAD C.J. & HARRISON J.D. (1979)
Sand martin movements within Britain and Ireland
Bird Study 26:73-86

MEEK S.B. & ROBERTSON. R.J (1991)
Adoption of young by replacement male birds: an experimental study of eastern bluebirds and a review
Animal Behaviour 42:813-820

MENG A. CARTER R.E. & PARKIN D.T. (1990)
The variability of DNA fingerprints in three species of swan
Heredity 64:73-80

MINEAU P. & COOKE F. (1979)
Rape in the lesser snow goose
Behaviour 70:280-291

MOCK D.W. (1983)
On the study of avian mating systems
In, Brush A.H. & Clark G.A. Jr. (eds), Perspectives in Ornithology:55-84
Cambridge University Press

MØLLER A.P. (1985)
Mixed reproductive strategy and mate guarding in a semi-colonial passerine, the swallow *Hirundo rustica*
Behavioural Ecology & sociobiology 17:401-408

MØLLER A.P. (1987a)
Behavioural aspects of sperm competition in swallows (*Hirundo rustica*)
Behaviour 100:92-104

MØLLER A.P. (1987b)
Mate-guarding in the swallow *Hirundo rustica*
Behavioural Ecology & Sociobiology 21:119-123

MØLLER A.P. (1987c)
Extent and duration of mate guarding in swallows *Hirundo rustica*
Ornis Scandinavica 18:95-100

MØLLER A.P. (1987d)
Advantages and disadvantages of coloniality in the swallow *Hirundo rustica*
Animal Behaviour 35:819-832

MØLLER A.P. (1988a)
Infanticidal and anti-infanticidal strategies in the swallow *Hirundo rustica*
Behavioural Ecology & Sociobiology 22:365-371

MØLLER A.P. (1988b)
Paternity and paternal care in the swallow
Animal Behaviour 36:996-1005

MØLLER A.P. (1988c)
Female choice selects for male sexual tail ornaments in the monogamous swallow
Nature 332:640-642

MØLLER A.P. (1989a)
Parasites, predators and nest boxes: facts and artefacts in nest box studies?
Oikos 56: 421-423

MØLLER A.P. (1989b)
Frequency of extra-pair paternity in birds estimated from differential heritability of tarsus length: reply to Lifjeld and Slagsvold's critique
Oikos 56:247-249

MØLLER A.P. (1989c)
Viability costs of male tail ornaments in the swallow
Nature 329:132-135

MØLLER A.P. (1991a)
Double broodedness and mixed reproductive strategies by female swallows
Animal Behaviour 42:671-679

MØLLER A.P. (1991b)
Defence of offspring by male swallows *Hirundo rustica* in relation to participation in extra-pair copulations by their mates
Animal Benaviour 42:261-267

MØLLER A.P. (1992)
Nest boxes and the scientific rigour of experimental studies
Oikos 63: 309-311

MØLLER A.P. & BIRKHEAD T.R. (1992)
Validation of the heritability method to estimate extra-pair paternity in birds
Oikos 64:485-488

MORRILL S.R. & ROBERTSON R.J. (1990)
Occurrence of extra-pair copulation in the tree swallow (*Tachycineta bicolor*)
Behavioural Ecology & sociobiology 26:291-296

MORTON E.S. (1987)
Variation in mate guarding intensity by male purple martins
Behaviour 101:211-224

MORTON E.S. FORMAN L. & BRAUN M. (1990)
Extrapair fertilisations and the evolution of colonial nesting in purple martins
The Auk 107:275-283

MUMME R.L. KOENIG W.D. ZINK R.M. & MARTEN J.A. (1985)
Genetic variation and parentage in a California population of acorn woodpeckers
The Auk 102:305-312

NAKAMURA Y. LEPPERT M. O'CONNELL P. WOLFF R. HOLM T. CULVER M. MARTIN C. FUJIMOTO E. HOFF M. KUMLIN E. & WHITE R. (1987)
Variable number of tandem repeat (VNTR) markers for human gene mapping
Science 235:1616-1622

NEWTON I. (ed, 1989)
Lifetime reproduction in birds
Academic Press

NOORDWIJK A.J. VAN & BALEN J.H. VAN (1988)
The great tit *Parus major*
In, Clutton-Brock T.H. (ed). Reproductive success: studies of individual variation in contrasting breeding systems:119-135
The University of Chicago Press

NUR N. (1984a)
The consequences of brood size for breeding blue tits I. Adult survival, weight change and the cost of reproduction
Journal of Animal Ecology 53:479-496

NUR N. (1984b)
The consequences of brood size for breeding blue tits II Nestling weight, offspring survival and optimal brood size
Journal of Animal Ecology 53:497-517

NUR N. (1988a)
The cost of reproduction in birds: an examination of the evidence
Ardea 76:155-168

NUR N. (1988b)
The consequences of brood size for breeding blue tits III Measuring the cost of reproduction: survival, future fecundity, and differential dispersal
Evolution 42:351-362

O'DONALD P. (1973)
A further analysis of Bumpus' data: the intensity of natural selection
Evolution 27:398-404

ORIANS G.H. (1969)
On the evolution of mating systems in birds and mammals
American Naturalist 103:589-603

ORING L.W. (1982)
Avian mating systems
In, Farner D.S. King J.R. & Parkes K.C. (eds, 1982). Avian Biology VI
Academic Press

PARKER G.A. (1970)
Sperm competition and its evolutionary consequences in the insects
Biological Reviews 45:525-567

PARKIN D.T. (1987)
The value of genetic fingerprinting to the breeding and conservation of birds of prey
In, Hill D.J. (ed). Breeding and management in birds of prey
Proceedings of conference held at Bristol University
January 1987:81-86

PARKIN D.T. HUTCHINSON I. & WETTON J.H. (1988)
Genetic fingerprinting and its role in bird research and law enforcement
In Cadbury C.J. & Everett M. (eds, 1988) RSPB Conservation Review 1988:22-24

PARTRIDGE L. (1989)
Lifetime reproductive success and life history evolution
In I. Newton (ed), Lifetime reproduction in birds
Academic Press

PARTRIDGE L. (1992)
Measuring reproductive costs
Trends in Ecology & Evolution 7:99-100

PARTRIDGE L. & HARVEY P. (1988)
The ecological context of life history evolution
Science 241:1449-1455

PAUL A. KUESTER J. & ARNEMANN J. (1992)
Maternal rank affects reproductive success of male barbary macaques (*Macaca sylvanus*: evidence from DNA fingerprinting
Behavioural Ecology & Sociobiology 30:337-341

PAYNE L.F. & KAHRS A.J. (1961)
Competitive efficiency of turkey sperm
Poultry Science 40:1598-1604

PAYNE R.B. & PAYNE L.L. (1989)
Heritability estimates and behaviour observations: extra-pair matings in indigo buntings
Animal Behaviour 38:457-467

PERRINS C.M. (1965)
Population fluctuations and clutch size in the great tit *Parus major* L.
Journal of Animal Ecology 34:601-647

PERRINS C.M. (1970)
The timing of birds' breeding seasons
Ibis 112:242-255

PERRINS C.M. & BIRKHEAD T.R. (1983)
Avian Ecology
Blackie

PETRIE M. & MØLLER A.P. (1991)
Laying eggs in others' nests: intra-specific brood parasitism in birds
Trends Ecol Evol 6:315-320

PETTIFOR R.A. PERRINS C.M. & McCLEERY R.H. (1988)
Individual optimization of clutch size in great tits
Nature 336:160-162

PETERSEN A.J. (1955)
The breeding cycle in the bank swallow
Wilson Bulletin 67:235-286

PIANKA E.R. (1976)
Natural selection of optimal reproductive tactics
American Zoologist 109:773-784

PIANKA E.R. & PARKER W.S. (1975)
Age-specific reproductive tactics
American Naturalist 109:453-463

PINKOWSKI B.C. (1977)
Breeding adaptations in the Eastern Bluebird
Condor 1979:289-302

PRICE D.K. COLLIER G.E. & THOMPSON C.F. (1989)
Multiple parentage in broods of house wrens: genetic evidence
Journal of Heredity 80:1-5

QUINN T.W. & WHITE B.N. (1987)
Identification of restriction-fragment-length polymorphisms in genomic DNA of the lesser snow goose (*Anser caerulescens caerulescens*)
Molecular Biology & Evolution 4:126-143

QUINN T.W. QUINN J.S. COOKE F. & WHITE B.N. (1987)
DNA marker analysis detects multiple maternity and paternity in single broods of the lesser snow goose
Nature 326:392-394

RABENOLD K.N. (1985)
Cooperation in breeding by nonreproductive wrens: kinship, reciprocity and demography
Behavioural Ecology & Sociobiology 17:1-17

RABENOLD P.P. RABENOLD K.N. PIPER W.H. HAYDOCK J. & ZACK S.W. (1990)
Shared paternity revealed by genetic analysis in cooperatively breeding tropical wrens
Nature 348:538-540

REZNICK D. (1985)
Costs of reproduction: an evaluation of the empirical evidence
Oikos 44:257-267

REZNICK D. (1992)
Measuring the costs of reproduction
Trends in Ecology & Evolution 7:42-45

RHEINWALD G. & GUTSCHER H. (1969)
Dispersion and ortstreue der mehlshwalbe (*Delichon urbica*)
Vogelwelt 90:121-140

RHEINWALD G. GUTSCHER H. & HORMEYER K. (1976)
(Influence of age in house martins on their breeding biology)
Die Vogelwarte 28:190-206

RICKLEFS R.E. (1968)
Weight recession in nestling birds
Auk 85:30-35

RISING J.D. & SOMERS K.M. (1989)
The measurement of overall body size in birds
Auk 106:666-674

ROBERTSON R.J. (1990)
Tactics and counter-tactics of sexually selected infanticide in tree swallows
In: Blondell J., Gosler A., Lebreton J.D., & McCleery R. (eds). Population Biology of Passerine birds, an integrated approach
New York. Springer-Verlag

ROBERTSON R.J. & GIBBS H.L. (1982)
Superterritoriality in tree swallows: a reexamination
Condor 84:313-316

ROBINSON K.D. & ROTENBURY J.T. (1991)
Clutch size and reproductive success of house wrens rearing natural and manipulated broods
The Auk 108:277-284

ROSKAFT E. (1985)
The effect of enlarged brood size on the future reproductive potential of the rook
Journal of Animal Ecology 54:255-260

ROWHER S. (1986)
Selection for adoption versus infanticide by replacement 'mates' in tree swallows
Current Ornithology 3:353-395

SHERMAN P.W. (1981)
Electrophoresis and avian genealogical analysis
The Auk 98: 419-422

SHERMAN P.W. & MORTON M.L. (1988)
Extra-pair fertilizations in mountain white-crowned sparrows
Behavioural Ecology & Sociobiology 22:413-420

SHIELDS W.M. CROOK J.R. HEBBLETHWAITE M.L. & WILES-EHMANN S.S. (1988)
Ideal free coloniality in the swallows
In Slobodchikoff (ed), The ecology of social behaviour, Academic Press

SLAGSVOLD T. (1984)
Clutch size variation of birds in relation to nest predation: on the cost of reproduction
Journal of Animal Ecology 53:945-953

SMITH C.C. & FRETWELL S.D. (1974)
The optimal balance between size and number of offspring
American Naturalist 108:499-506

SMITH J.N.M. (1981)
Does high fecundity reduce survival in song sparrows?
Evolution 35:1142-1148

SMITH H.G. KALLANDER H. & NILSSON J-A (1987)
Effect of experimentally altered brood size on frequency and timing of second clutches in the great tit
The Auk 104:700-706

SMITH H.G. KALLANDER H. & NILSSON J-A (1989)
The trade-off between offspring number and quality in the great tit *Parus major*
Journal of Animal Ecology 58:383-401

SMITH J.N.M. & ROFF D.A. (1980)
Temporal spacing of broods, brood size and parental care in song sparrows (*Melospiza melodia*)
Canadian Journal of Zoology 58:1007-1015

SNAPP B.D. (1976)
Colonial breeding in the barn swallow (*Hirundo rustica*) and its adaptive significance
Condor 78:471-480

STAMPS J., CLARK A., ARROWOOD, P. & KUS, B. (1985)
Parent-offspring conflict in budgerigars
Behaviour 94:1-40

STANGEL P.W. (1986)
Lack of effects from sampling blood from small birds
Condor 88:244-245

STEARNS S.C. (1976)
Life-history tactics: a review of the ideas
Quarterly Review of Biology 51:3-47

STURKIE P.E. (1976)
Avian physiology
Springer-Verlag, New York (3rd edn)

TAYLOR L.R. (1973)
Monitoring change in the distribution and abundance of insects
Rep Rothamsted Exp Stn 1973 (2):202-239

TAYLOR L.R. & PALMER J.M.P. (1972)
Aerial sampling
In, van Emden (ed), Aphid Technology:189-234. London: Academic Press

TEGELSTROM H. (1989)
Cold room storage of blood may be better than storage at -70°C
Fingerprint News 1(4):5-6

TEGELSTROM H. & SJOGREN P. (1990)
Monomorphic "DNA-fingerprints" in northern pool frogs (*Rana lessonae*)
Fingerprint News 2(1):7-9

THEIN S.L. JEFFREYS A.J. GOOI H.C. COTTER F. FLINT J. O'CONNOR N.T.J. WEATHERALL D.J. & WAINSCOAT J.S. (1987)
Detection of somatic changes in human cancer DNA by DNA fingerprint analysis
British Journal of Cancer 55:353-356

THOMPSON M.L.P (1992)
Reproductive success and survival of Swallows *Hirundo rustica*: effects of age and body condition
PhD Thesis, University of Stirling

THORNHILL R. (1984)
Alternative hypotheses for traits believed to have evolved by sperm competition
In, Smith R.L. (ed). Sperm competition and the evolution of animal mating systems:151-178
Academic Press, New York

TINBERGEN J.M. (1987)
Costs of reproduction in the great tit: intraseasonal costs associated with brood size
Ardea 75:111-122

TINBERGEN J.M. & VAN BALEN J.H. (1988)
Food and multiple breeding
Acta XIX Congressus Internationalis Ornithologici Volume 1:380-391
University of Ottowa Press

TINBERGEN J.M. VAN BALEN J.H. DRENT P.J. CAVÉ A.J. MERTENS J.A.L. & DEN BOER-HAZENWINKEL J. (1987)
Population dynamics and cost-benefit analysis. An attempt to relate population dynamics via lifetime reproductive success to short-term decisions
Netherlands Journal of Zoology 37:180-213

TRIVERS R.L. (1972)
Parental investment and sexual selection
In, Campbell B. (ed, 1972), Sexual selection and the descent of man
Aldine, Chicago

TURNER A.K. & BRYANT D.M. (1979)
Growth of nestling sand martins
Bird Study 26:117-122

TURNER A. & ROSE C. (1989)
A handbook of the swallows and martins of the world
Christopher Helm, London

VAN NOORDWIJK A.J. & VAN BALEN J.H. (1988)
The great tit *Parus major*
In, Clutton-Brock T.H. (ed), Reproductive success: Studies of individual variation in contrasting breeding systems
University of Chicago Press

VASSART G. GEORGES M. MONSIEUR R. BROCAS H. LEQUARRE A-S. CHRISTOPHE D. (1987)
A sequence in M13 phage detects hypervariable minisatellites in human and animal DNA
Science 235:683-684

WAGNER R.H. (1991)
Evidence that female razorbills control extra-pair copulations
Behaviour 118:157-169

WAGNER R.H. (1992)
The pursuit of extra-pair copulations by monogamous female razorbills: how do females benefit?
Behavioural Ecology & Sociobiology 29:455-464

WASER P.M. & RABENWOLD P.P. (1990)
How to estimate bandsharing when lanes are not equally scoreable
Fingerprint News 2(2):10-13

WATSON (1946)
Display of sand martin
British Birds 39:282

WEATHERHEAD P.J. & MCRAE S.B. (1990)
Brood care in American robins: implications for mixed reproductive strategies by females
Animal Behaviour 39:1179-1188

WEISS M.L. WILSON V. CHAN C. TURNER T. & JEFFREYS A.J. (1988)
Application of DNA fingerprinting probes to Old World monkeys
American Journal of Primatology 16: 73-79

WERREN J.H. GROSS M.R. & SHINE R. (1980)
Paternity and the evolution of male parental care
Journal of theoretical biology 82:619-631

WESTNEAT D.F. (1987a)
Extra-pair fertilizations in a predominantly monogamous bird: genetic evidence
Animal Behaviour 35:877-886

WESTNEAT D.F. (1987b)
Extra-pair copulations in a predominantly monogamous bird: observations of behaviour
Animal Behaviour 35:865-876

WESTNEAT D.F. (1990)
Genetic parentage in the indigo bunting: a study using DNA fingerprinting
Behavioural Ecology & Sociobiology 27:67-76

WESTNEAT D.F. FREDERICK P.C. & WILEY R.H. (1987)
The use of genetic markers to estimate the frequency of successful alternative reproductive tactics
Behavioural Ecology & Sociobiology 21:35-45

WETTON J.H. & PARKIN D.T. (1991)
An association between fertility and cuckoldry in the house sparrow *Passer domesticus*
Proc Roy Soc Lond B 245: 277-233

WETTON J.H. CARTER R.E. & PARKIN D.T. (1987)
Demographic study of a wild house sparrow population by DNA fingerprinting
Nature 327:147-149

WHITTINGHAM L.A. TAYLOR P.D. & ROBERTSON R.J. (1992)
Confidence of paternity and male parental care
American Naturalist 139:1115-1125

WITTENBERGER J.F. & TILSON R.L. (1980)
The evolution of monogamy: hypotheses and evidence
Annual Revue of Ecology & Systematics 11:197-232

WILLIAMS G.C. (1966)
Natural selection, the cost of reproduction, and a refinement of Lack's principal
American Naturalist 100:687-690

WINKLER D.W. (1987)
A general model for parental care
American Naturalist 130:526-543

WREGE P.H. & EMLEN S.T. (1987)
Biochemical determination of parental uncertainty in white-fronted bee-eaters
Behavioural Ecology and Sociobiology 20:153-160

WRIGHT J. (1992)
Certainty of paternity and paternal care
Animal Behaviour 44:380-381

YOM-TOV Y. (1980)
Intraspecific nest parasitism in birds
Biological Reviews 55:93-108

ZAHAVI A. (1989)
Arabian babbler
In, Newton I. (ed). Lifetime reproduction in birds:253-275
Academic Press

Appendix A

Location and sizes of house martin colonies used in the present study

Colony	Map reference	Distance from Stirling University (km)	Number of pairs		
			87	88	89
Braeleny	NN642128	23	10	5	3
Frandy	NN943045	15	5	2	3
University Library	NS807965	0	Sampled for nestling growth & DNA fingerprints only		
Middlehall	NN994007	19	5	0	-
Naemoor	NO016013	20	-	1	0
Powmill	NT020982	21	3	3	4
Glendevon	NN989046	19.5	7	6	12
Wellhall	NS978963	17	-	9+	4+
Glenquey	NN983034	18.5	-	-	6+

The number of pairs is the number of first broods that were raised at least to hatching stage, and therefore entered in the nest record analysis for Chapter 2. Colonies that were not visited in a particular year are marked '-'. A '+' implies that there were some inaccessible nests not included in the total.

Appendix B

Variables used in analysis of annual reproductive success in house martins

Nes.dat

par	pair number
si	site code, 1=Braeleny, 2=Frandy, 3=University library, 4=Middlehall, 5=Naemoor, 6=Powmill, 7=Glendevon, 8=Wellhall, 9=Glenquey
doe	date of first egg, first clutch
cc	first clutch code, 1=lay date known, 2=lay date estimated
nt	nest type, 1=natural, 2=box
sfc	first clutch size
hat	hatch date, first nestling first clutch
hc	hatch code, 1=date known, 2=date estimated
hap	first clutch hatching period
bros	first brood size at hatch
mc	manipulation code, 1=unmanipulated, 2=enlarged, 3=reduced
brom	first brood size after manipulation
fle	number fledged, first brood
sec	second clutch code, 1=no second clutch, 2=second clutch laid, 3=first clutch abandoned, 4=interference may have caused breeding failure
doe2	date of first egg, second clutch, first relay
cc2	second clutch code, 1=lay date known, 2=estimated
ssc	size of second clutch
dofe2	date of first egg, second clutch, second relay/hatching relay
scc2	second clutch code
fec2	female code, 1=female recaptured during second breeding attempt, 2=female not recaptured/assumed to be the same
mac2	male code, 1=same male for second breeding attempt, 2=different male, 3=male not recaptured/asssumed to be the same
nc2	nest change code, 1=same nest for second brood, 2=different nest
nt2	nest code, second brood, 1=natural, 2=nest box
hat2	hatch date, first nestling second clutch
hc2	hatch date code, second clutch, 1=known, 2=estimated
hap2	hatch period, second brood
bros2	size of second brood at hatch
fle2	number fledged, second brood

ci	clutch interval, the number of days between the laying of the last egg of the first clutch, and the first egg of the second clutch
bi	breeding interval, the number of days between the fledging of fist brood young and the first egg of the second clutch
yr	year
nes	nestling period, first brood; the number of days between the hatching of the first, and the fledging of the last nestling
pem	mean peak nestling mass first brood, measured at 15-16 days after hatch (g)
flem	mean peak fledging mass, measured at 25-26 days after hatch
pew	mean peak nestling wing length (mm)
flew	mean fledging wing length
tam	mean fledging tarsus length (mm)
kem	mean fledging keel length (mm)
hebim	mean fledging head and bill length (mm)
mpr	male parent ring
mpm	male parent mass
mpw	male parent wing length
mpk	male parent keel
mphb	male parent head and bill
mpt	male parent tarsus
mpa	male parent age
fpr	female parent ring
fpm	female parent mass
fpw	female parent wing
fphb	female parent head and bill
fpt	female parent tarsus
fpa	female parent age

Insec.dat

yr	year
psc	percentage of pairs raising unmanipulated first broods that laid second clutches
mem	mean daily suction trap volume, May
mej	mean suction trap, June
mejl	mean suction trap, July
mea	mean suction trap, August

mes	mean suction trap, September
mdoe	mean date of first egg, first clutch, unmanipulated pairs
mdoe2	mean date of first egg, second clutch, unmanipulated pairs
mci	mean clutch interval, unmanipulated pairs
fct	mean first clutch threshold, unmanipulated pairs
sct	mean second clutch threshold, unmanipulated pairs
mfle	mean number fledged from first brood, unmanipulated pairs
msfc	mean first clutch size, unmanipulated pairs
totfle	mean annual reproductive success, unmanipulated pairs

Appendix C

Variables used in time budget analyses during the prelaying and laying periods

Hmtb.dat & Smtb.dat (respectively house and sand martins)

dat	date, May 1st=1
tem	ambient temp, °C
rai	rainfall estimate, 1=dry - 5=pouring
win	wind speed estimate, 1=still - 4=gale
clo	cloud cover estimate, % sky
par	pair number
buc	burrow position code, 1=central - 4=peripheral (sand martins)
nt	nest type, 1=natural, 2=box (house martins)
dnc	day of nesting cycle, day of first egg=0
dcc	day of clutch completion
hr	start of time budget session, to nearest hour
mrc	male removal code, 1=no removal, 2=pre-removal, 3=male away, 4=post release (on day of experiment), 5=post removal
et	empty time, time nest burrow is empty during 1h observation period, minutes
mt	male time, time spent at nest burrow by male during 1h observation period
md	mean duration of male nest visits
ft	female time, time spent at nest burrow by female
fd	mean duration of female nest visits
prt	pair time, time spent by pair together at nest
men	number of male entries to nest burrow in 1h
fen	number of female entries
pen	number of entries by male and female (2 birds) within 1 minute of each other
pren	pair entries, number of entries by male and female (2 birds) within 10 seconds
fmen	number of pair entries where female follows male
mfen	number of pair entries where male follows female
me	number of male exits from nest burrow
fe	number of female exits
pe	number of times male and female (2 birds) leave nest within 1 minute
pre	pair exits, number of times male and female leave within 10 seconds

fme	number of pair exits where female follows male
mfe	number of pair exits where male follows female
cmi	number of changeovers (where one partner leaves and the other enters the nest burrow) within one minute
cse	number of changeovers within 10 seconds
im	intruder minutes, the number of minute observation periods during which at least one extra-pair bird landed at the entrance to, or swooped within 5m, of nest or burrow
ie	intruder entries, the number of extra-pair birds seen entering the nest burrow
fm	the number of fights recorded
pc	paternity code 1=brood contained no extra-pair offspring, 2=at least one extra-pair offspring
bc	brood code, 1=first, 2=second
yr	year
mkc	mark code, 1=both birds colour marked, 2=unmarked pair
tm1	time that one bird is at nest (unmarked pairs)
en1	number of entries by one bird in one minute
ex1	number of exits by one bird in one minute
prd	mean duration of visits by two birds simultaneously
mefp	number of male exits while female is in the nest
mefa	number of male exits in the absence of the female

Appendix D

Variables used in the analysis of brood provisioning in house martins

Bp.dat

par	pair number
bn	brood number, 1=first, 2=second
nt	nest type, 1=natural 2=box
na	nestling age, days after first hatch
bs	brood size
pwt	average peak nestling mass, 15-16 days after first hatch
fwt	average fledging mass, 25-26 days after first hatch
pc	paternity code, 1= no extra-pair offspring, 2=at least one extra-pair offspring
ma	male parent age
mac	male age code, 1=known age, 2=age estimated
fa	female parent age
fac	female age code
mfv	number of male feeding visits during 1h observation period
ffv	number of female feeding visits
ufv	number of 'unknown' feeding visits, identity of feeding adult missed
mt	male time in nest
ft	female time in nest
mrc	male removal code, 1=no removal experiment at this nest, 2=laying removal, 3=incubation removal
fffv	number of feeding visits by first brood fledglings
dat	date
hr	time
wd	wind score
rn	rain score
tp	ambient temperature
cd	cloud cover
wnd	wind speed, knots*
rain	daily rainfall, tenths of a mm*
mtp	minimum daily temperature*
matp	maximum daily temperature*
cld	cloud cover*

su1	suction trap volume 1, the volume of insects collected at 1000 on the morning of day n+1, where n is the day on which observations were made
su2	suction trap volume 2, for days [(n+1)+n]/2
su3	suction trap volume 3, for days [(n+1)+n+(n-1)]/3

* Data from Stirling University weather station

Appendix E

Scientific names of species mentioned in the text

Birds	
Arabian babbler	*Turdoides squamiceps*
Bewick's swan	*Cygnus columbianus*
Budgerigar	*Melopsittacus undulatus*
Blue tit	*Parus caeruleus*
Cliff swallow	*Hirundo pyrrhonota*
Dunnock	*Prunella modularis*
Eastern bluebird	*Sialia sialis*
Fulmar	*Fulmarus glacialis*
Great tit	*Parus major*
House martin	*Delichon urbica*
House sparrow	*Passer domesticus*
House wren	*Troglodytes aedon*
Indigo bunting	*Passerina cyanea*
Kestrel	*Falco tinnunculus*
Lesser snow goose	*Chen caerulescens*
Magpie	*Pica pica*
Mute swan	*Cygnus olor*
Pied flycatcher	*Ficedula hypoleuca*
Purple martin	*Progne subis*
Ring dove	*Streptopelia risoria*
Red-winged blackbird	*Agelaius phoeniceus*
Rook	*Corvus frugilegus*
Rothschilds mynah	*Leucospar rothschildi*
Sand martin / bank swallow	*Riparia riparia*
Seaside sparrow	*Ammodramus maritimus*
Shag	*Phalacrocorax aristotelis*
Song sparrow	*Melospiza melodia*
Stripe-backed wren	*Campylorhynchus nuchalis*
Swallow	*Hirundo rustica*
Tree swallow	*Tachycineta bicolor*
Whooper swan	*Cygnus cygnus*
Willow warbler	*Phylloscopus trochilus*
Wood warbler	*P. sibilatrix*
Zebra finch	*Taeniopygia guttata*
Reptiles	
Adder	*Vipera berus*
Fish	
Coho salmon	*Oncorhynchus kisutch*