Mating behaviour and reproductive success
in the reed bunting

_Emberiza schoeniclus_

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by
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Abstract

The reproductive behaviour and success of a number of male reed buntings were studied at Rutland Water (Leicestershire) during the breeding seasons of 1988, 1989 and 1990. A major constraint on reproductive success was the level of nest predation. Only 42% of nests survived to fledging, representing 41.5% of males' territories. The annual mean productivity for each male was estimated to be 0.77 offspring surviving to the following season. With these large constraints on male reproductive success, extra-pair paternity is seen as potentially highly advantageous to males. Females are constrained in their mate choice indicating a potential advantage in seeking sires from surrounding males of higher quality than their mate.

Rates of extra-pair paternity as derived from DNA fingerprinting were found to be high: 50% of chicks and 69% of broods. Approximately one-third of males were responsible for all cases of extra-pair paternity. However, 70% of males lost some paternity in their nests to other males with no correlation between levels of extra-pair and within-pair paternity. Copulations were observed to be frequent (estimated to be 4.06 per day) during the prelay period and continued through to the day of the third egg. A large between male variation in copulation rates was observed, with a diurnal peak immediately following the laying of an egg. Observed extra-pair copulations were rare (4.39%) with a significant disparity compared to extra-pair paternity levels, indicating that females may seek furtive extra-pair copulations.

Mate guarding as measured by time spent less than 10 m from the female, flights orientated towards the female and vigilance were observed to peak during the prelay period. These behaviours declined dramatically on the day of the first egg, indicating that peak fertility probably occurs prior to egg laying. No increase in mate guarding activity was observed to occur in response to an increased neighbour density, whilst neighbour status did not influence guarding. A diurnal pattern in guarding was recorded for vigilance and all three behaviours showed significant between male variation.

Territorial intrusions were mainly by neighbours; they peaked significantly during the prelay period and were responded to by an attack from the defending male. The seeking of extra-pair copulations was observed as excursions into neighbouring territories, followed by an attack from the territory holder. Excursions occurred primarily when males were not mate guarding, during egg laying and incubation, and were significantly directed towards territories with nests in the prelay stage.

Song was found not to function as a mate guarding behaviour, with a significant decline in output during the prelay period. Song output increased significantly in the presence of a neighbour with a "fertile" female, supporting the hypothesis that song functions as a measure of male quality to fertile females. Song of unpaired males was significantly different from that of paired males and is hypothesised to function in territorial maintenance (and to indicate male status), whereas the more complex song of paired individuals also functions to display male quality to their partners.

An analysis of the relationship between behaviour and reproductive success when analysed indicated that males which guarded most were more subject to losing paternity. Acquisition of extra-pair paternity was found to be significantly enhanced through increased song output and excursions. Song output was also recorded as significantly increasing total reproductive success, indicating that females may choose males on the basis of their song to obtain furtive extra-pair copulations.
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For Jo, Megan & Jason

“The nightmare ends”
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1. INTRODUCTION

The proposal that the males of species that invest heavily in parental care should adopt a "mixed reproductive strategy" was first put forward by Trivers 1972. Trivers argued that whilst still providing parental care to one female's brood a male should also take advantage of additional opportunities to fertilise other females (see Beecher and Beecher 1979). When two males are competing for fertilisations which has now been established as widespread (Smith 1984a), then subsequent physiological competition between sperm for fertilisations can follow. The evolution of sperm competition has produced not only physiological competition for fertilisations per se but also behaviours designed to protect paternity (see Birkhead & Møller 1992 for a review). For example, behaviour termed mate guarding (Birkhead 1979), is the development of behaviours associated with the prevention of cuckoldry such as extra-pair copulations (EPCs), by maintaining a proximity to females and preventing access of non-pair males. Obtaining EPCs may occur by a number of plausible routes (Fisch & Shugart 1984) i.e., by male selection, either with or without female consent, or by female selection of willing males (Birkhead & Møller 1992). In avian species females must usually be willing to enable cloacal contact for copulation and fertilisation (see Tienhoven 1983). Exceptions, however, do exist (Burns et al 1980) where intromittent organs are present or where copulations are used to assess male quality (Westneat et al 1990). Females are likely to resist extra-pair copulations where the costs outweigh the benefits, due for example to divorce or aggression from their partner (Birkhead et al 1985; Wagner 1991) or transmission of parasites or disease (Hart et al 1987; Hart 1990; Read 1990; see also Hamilton and Zuk 1982). If females can resist unwanted EPCs then it is predicted that females should exert a choice and accept copulations from those males which display characters associated with high fitness (Trivers 1972).

Females can obtain both direct and indirect benefits from participating in EPCs. Possible direct benefits include: (1) fertility insurance, where partners may be incapable of supplying sperm in sufficient quality or quantity (Drummond 1984; Walker 1980; Gibson & Jewel 1982; Mckinney et al 1984). The need to fertilise with more than one male to ensure fertilisation seems unlikely, since birds frequently copulate more than once (Birkhead et al 1987), only a small number of sperm are required for fertilisation (Wishart 1987; Balkar 1989) and the incidence of male sterility would have to be much higher than is known to account for promiscuous matings (Westneat et al 1990). (2) Provision of ejaculate nutrients is known to occur in insects (Thornhill 1976; Thornhill & Alcock 1983) but this is unlikely to occur in birds (Birkhead & Møller 1992). (3) Food provision and courtship feeding does occur in birds (Cronin & Sherman 1976; Wolf 1975; Lack 1940; Brown 1967) but there are no records of a link with EPCs (Birkhead & Møller 1992). (4) Provision of paternal care does occur through mating (Stacey 1982; Davies 1991) but there exist no evidence to support its occurrence in monogamous species. The direct benefits of extra-pair copulations therefore seem rather limited in birds.

To explain the indirect benefits of extra-pair copulations two major models have been proposed to explain female choice: (1) increased genetic diversity of offspring and (2) genetic quality,
encompassing selection for good genes and genes for secondary sexual characters. The former model proposes that females which mate with several males will produce genetically diverse offspring which buffers against environmental change. This has been largely refuted since meiosis will in itself provide diversity (Williams 1975) and secondly due to the negative effects of increased costs of competition between individuals which are not full siblings within a brood (Hamilton 1964; Trivers 1972; Sherman 1981; Parker 1984). The alternative good genes model assumes that females will choose males having genes which will provide genetic benefits to their offspring, for example allowing the female to produce “sexy sons”, with traits which make males attractive to females (Fisher 1930; Weatherhead & Robertson 1979). Alternatively the good genes may be related to general vigour, such as resistance to parasites (Zahavi 1975, 1977; Andersson 1982; Hamilton and Zuk 1982). Recent studies have shown that females will perform EPCs with males of higher quality than their partners (Møller 1991a), seeking higher ranking individuals (Afton 1985; Møller 1985; Frederick 1987a; Westneat 1987a; Smith 1988; Bollinger and Gavin 1991; Gowaty and Bridges 1991a) or with males with more attractive secondary sexual characteristics (Møller 1988a, 1991a, c; Burley and Price 1991; Houtman 1990). Females will also avoid EPCs with unpaired males (see McKinney et al 1983; Afton 1985; Møller 1988b), suggesting selection of those males which are of higher fitness and able to maintain a territory.

The route by which females select males was first identified by Darwin (1871). He recognised that character variation such as in plumage was associated with variation in reproductive success. The process was termed “sexual selection”, which as Darwin recognised can run counter to natural selection, the latter resulting in traits that enhance reproduction and survival, whereas the former results in traits that enhance sexual reproduction at the expense of survival. It therefore follows that females should select males with those traits which are reliable signals of quality. Trivers (1972), argued that the sex investing most should select most, consequently, as females have the higher gamete investment they should select males with those characters that are the best indicators of high fitness (see also Li & Owings 1987a, b; Petrie 1983a; Owens 1992; for reviews of the methods of action in monogamous species see Kirkpatrick et al 1990 and Møller 1991a).

This study examines reproductive behaviour and success of the reed bunting Emberiza schoeniclus schoeniclus (L) a primarily monogamous sexually dimorphic species in which both parents invest in offspring provision (Bell 1968). The sexual dimorphism is apparent both as a size difference and a plumage difference (Bell 1968). In this thesis the observed social reproductive success and actual variation in reproductive success, as measured by DNA fingerprinting (Burke & Bruford 1989; Wetton & Parkin 1989), are analysed in conjunction with physical and behavioural variation to determine which parameters best predict male reproductive success. Sexual selection can act on physical traits such as size and plumage (Andersson 1982, 1986) but song is also a trait subject to selection by female choice (e.g., reviews by Krebs 1977; Krebs & Kroodsma 1980; Dawson 1982). Therefore, both the morphological characteristics and the behaviour of individual males, were measured as the potential means through which females might select males. The plumage characteristics of male reed buntings have been shown to be fixed for life and are presumed to be genetic (Bell 1968), however song is an acquired and learned trait (Ewin 1977). It therefore follows that females of
monogamous species which do not pair with males of the highest quality (Westneat et al. 1990) or may not have the opportunities to do so (Møller 1991a), may select neighbouring males for EPCs (Westneat et al. 1990), according to observable traits linked to fitness.

I have divided this thesis into two sections. The first section is divided into three chapters, each with an introduction with associated hypotheses and predictions. In chapter 1, I examine patterns of territory occupation, mate fidelity and the observed “social” reproductive success. In Chapter 2, I examine the actual pattern of individual reproductive success as measured by DNA fingerprinting. The pattern of copulations is described in Chapter 3 to establish if reproductive success is a reflection of observed levels of sperm competition. Section 1 has been kept distinct to provide a measure of reproductive success and an examination of the constraints upon that success. In the second section I examine male variation in morphological characters and behaviours associated with sperm competition. There are three chapters which examine behavioural variation: Chapter 4 describes those behaviours which can be termed mate guarding and are essentially methods by which males prevent cuckoldry. Chapter 5 covers those behaviours which are linked to the acquisition of extra-pair paternity and Chapter 6 examines patterns of song output as a measure of levels of sperm competition and male quality. Both sections are drawn together in the final chapter which is an analysis of the link between behaviour, morphological variation and reproductive success.
CHAPTER I

Territory distribution, breeding chronology and observed breeding success

Abstract: Reproductive success of individuals may be constrained by environmental influences such as predation and nest failure. Reed buntings, Emberiza schoeniclus, showed a strong territory and mate fidelity over successive seasons with low mortality rates associated with the breeding season. A major constraint on social reproductive success in the reed bunting was the level of predation. Only 42% of nests survived to fledging and 41.5% of males achieved no fledging success. The annual productivity for each male was 0.77 offspring surviving to the following breeding season. With large constraints on social reproductive success, extra-pair paternity is seen as advantageous for males. Females are constrained in their mate choice and there is potentially a large advantage in seeking sites from surrounding males of higher quality than their mate.

1.1 INTRODUCTION

No recent reviews of reed bunting breeding habits exist and consequently this chapter is a review of data from previous studies and an introduction to the patterns of territory occupation and breeding success on my study site at Rutland Water.

From January onwards males spend progressively more time on territory, and may even begin to sing (Ewin 1977) although they survey an area prior to settlement. Males are difficult to age unless previously ringed, either as a juvenile or as a previously breeding adult (Bell 1968; Hornby 1971). Older males are the first to arrive and occupy territories they held in previous years, whilst younger males arrive somewhat later, and either occupy peripheral territories or areas made vacant due to mortality of previous breeders. The tenacity with which males return to the same site was recorded by Bell and Hornby, with older males returning to the same site with an average shift of only 38 m over six seasons. Females exhibit a similar tendency (observed mean = 106 m), although not as strong as in males and shifts were attributed to being displaced by other females. Similar results were obtained by Haukioja (1971). Hornby (1971) proposed that weather was the main determinant of territorial shifts, since in years of warm spring weather breeding started early and consequently both territory establishment and pair formation take place more rapidly than in years of inclement spring weather. Cold periods especially in March and April, cause interruptions in territorial behaviour, retardation of pair formation and a greater observed level of territory shift. These interruptions might promote settling in other areas because more time is spent away from the former territory and consequently produce a higher observed level of mate change.

Haukioja (1971), showed that the autumnal distribution of juveniles closely matches their breeding distribution the following season, suggesting some element of imprinting had taken place, so that when birds return the following spring to their previous site they will occupy vacant territories automatically, the tendency being weaker in females. Haukioja further concluded that population distribution is not density - dependent because five territories made
vacant by removal of the occupants were filled by four pairs (first years) plus the removed birds on their release.

The pattern of territory occupation through the season also varies. Ghiot (1976) observed that males were the first to return to occupy a bachelor territory. These territories were not adjoining and often two males would initially defend together an area twice the size of a normal bachelor territory. The arrival of females and the subsequent building of nests produced an expansion of the territory so that territories became contiguous with fixed boundaries. Hermann (1982, 1983) observed that early males occupy a large territory, the borders only becoming well defined during the main breeding season, with a dissolution of these borders as chicks became independent.

Ghiot (1976) noted that during egg laying and incubation the male occupies only an undefined pseudoterritory, rather similar to the earlier bachelor territory. During the period when the young are being fed in the nest, the surface area which the pair use increases a little, but once the young have fledged the male’s activity area increases again and exceeds previous territorial boundaries. Variations in territory distribution also reflect neighbour activities, with evictions occurring when the area becomes too compressed.

Ghiot (1976) found that reed buntings only occupy a small territorial area (0.20 Ha) and that they should be classified as “type B”, i.e., having a defended area within which all breeding activities occur but which is not the primary source of food (Hinde 1956). This is in fact a relatively rare situation, otherwise found only in the European nightjar Caprimulgus europaeus and the reed warbler Acrocephalus schoenobaenus, and contrary to the more usual expectation that the reed bunting would be a “type A” territory holder i.e., holding a territory in which all functions related to reproduction occur.

Within the British Isles the construction of nests usually occurs during the latter half of April and early May (Bell 1968, Hornby 1971) and the first eggs are laid soon after nest construction. In years with warm springs nesting begins earlier than in colder years (Bell 1968; Hornby 1971) with relatively synchronous initial laying in early May, followed by non-synchronous replacement and second clutches as the season progresses.

The choice of nest sites by reed buntings has been intensively reviewed (Bell 1968; Hornby 1971; Haukioja 1970; Kearton 1907; Witherby et al. 1943). Nest sites include; vegetation under small willows, in willow scrub, grass tussocks, dense clumped vegetation, nettles and brambles. The nests are usually well hidden within the vegetation and are most commonly within 30 cm of ground level, consisting of interwoven grasses, reeds and moss lined with fine grasses, hairs and Phragmites seed heads.

One egg is usually laid each day; the most frequently observed clutches are of 4-6 eggs, most commonly 5 eggs within the British Isles and occasionally 7 in Scandinavia. The eggs are of a variable colour pattern; purplish grey/ pale olive/ pale purple brown and streaked with rich dark brown purple (Bell, 1968; Hornby, 1971; Haukioja 1970).
The adaptive significance of clutch size in the reed bunting was extensively studied by Haukioja (1971), who proposed that clutch size should be adapted to time and place and should therefore have an element of heritability. He observed that clutch size varies during the season, with a mean = 5.3 in May and the first half of June, a mean = 4.0 after the middle of June. This pattern is typical of species with more than one clutch per year and is probably adaptive. Haukioja concluded that the size of the clutch is determined by the female and not the male and is a genetic trait, subject to extreme environmental pressure.

Replacement clutches are laid in response to nest loss and in Haukioja’s study in Finland only older females laid true second clutches although this is not true within the British Isles. The second clutch is usually smaller than the first, but this is dependent on the phase of the breeding season. Haukioja found female age to be an important determinant of clutch size, which was larger in older birds, even allowing for laying date.

Bell (1968), and Hornby (1971) considered the mean temperature in March and April to be the main factor determining the date of commencement of laying. Further north, Haukioja (1970) found that the mean temperature in May had a similar effect. Within the British Isles a cold May produces deserted and smaller than expected clutches, plus runt eggs and missed days in laying. Therefore the causes of clutch size variation are: laying date, age and weather.

The relative productivity of different clutch and brood sizes varies (in Lack 1948, 1954). The most common clutch size should be the one which leaves the most surviving offspring in a population. During the period up to fledging, early broods of different sizes showed no variation in mortality and weights, however after fledging the recapture rates for different size broods vary (Haukioja 1970). Proportionally fewer young are caught from large broods than from small ones. Brood size at fledging may affect the probability of survival of individuals subsequently. Initial independence is a period of significant mortality, however the sexes show no differences in survival rates or dispersion. Productivity indices (PI) reveal that a clutch size of 3 is the most common and the most productive (PI = 0.57), closely followed by 6 (PI = 0.46) and 4 (PI = 0.43); therefore clutch size is apparently adapted to produce the maximum number of surviving offspring. In later broods the commonest clutch size was 4 and consequently the most productive clutch size drops as the season progresses. Clutch size is also affected by age; older males and females produce more young from their first brood than young females. This evidence supported Lack’s theory of clutch size although other studies (Perrins and Moss 1975; Hogstedt 1980) have found no supporting evidence.

Incubation follows completion of the clutch and can last between 10 and 13 days (Howard 1929; Bell 1968; Hornby 1971; Hermann 1982/83; Haukioja 1970) and is mostly undertaken by females. Instances of failing to hatch are relatively uncommon (Wolfenden unpublished). Weight increase of chicks is very rapid after hatching up to day 8 whereafter the chicks may “explode” (leave the nest), but the age at departure is usually between days 11 and 13 (Bell 1968; Hornby 1971; Hermann 1982/83; Haukioja 1970). Within 2 weeks of hatching the fledglings are capable of short flights. Both parents feed and brood the chicks in the nest with the majority being performed by the female. Adults usually fly to the nest from particular observation points. Once fledged the chicks scatter and the brood is partitioned between the
parents, and can be fed until they are 30-35 days of age (Bell 1968; Hermann 1982) but never longer than 40 days.

One of the most important determinants of nest success is the level of predation. Ghiot (1976) recorded 82.7% predation of eggs and young, the main predators including weasels Mustela nivalis, magpies Pica pica, jays Garrulus glandarius, and cats Felis domesticus. Bell (1968) and Hornby (1970) recorded only a 20% nest success rate with an estimated average of 3.7 nests/ pair each season. In their study they regarded nest predation to be so severe that population levels were only kept stable by immigration of new individuals at the start of each breeding season. Hermann (1982) recorded nestlings from only 41.5% of eggs laid, however Wolfenden (unpublished) unusually did not find predation to be an important factor in affecting reproductive success and found hatching failure due to harsh weather to be more important, only 2 of 27 (7.4%) nests recorded were predated. Haukioja (1970) recorded only 48% of eggs laid as producing fledglings, but did not specifically attribute the failures to predation.

Prys-Jones (1977) compared the annual adult mortality rates of British and Swiss populations. No overall differences were detected (mean rate ± SE), Bell (1968) 43 ± 2.3%; Prys-Jones (1977) Great Britain 43 ± 5.0%, Switzerland 47 ± 4.4%; Batten (1971) 39–40%. However, in both British and Swiss populations a differential mortality rate was detected between the sexes. Males: Great Britain: 39 ± 3.0%, Switzerland 40 ± 5.9%; females: Great Britain 52 ± 5.0%, Switzerland 53 ± 6.9%. The maximum variation for males is 4% and for females 1%, unless recruitment is different for the sexes then a theoretical surplus of males should exist.

Observations of breeding reed buntings have shown them to be primarily socially monogamous (Bell 1968; Hornby 1971; Haukioja 1970), but this is variable and a few isolated records of polygyny and "promiscuity" have been recorded (Bell 1968; Hornby 1971; Haukioja 1969; Ghiot 1976). Bell (1968) and Hornby (1971) observed incidences of simultaneous polygyny, successive polygyny and successive polyandry. The latter two were observed only at a very low frequency and were probably due to normal occurrences of mate change after a failed breeding attempt (Ghiot 1976). Observations indicate that over successive seasons where both members of a pair return more than half will breed with their former partner (Bell 1968; Hornby 1971). Hornby (1971) observed that incidences of polygyny occur in years where breeding starts earlier, allowing males to form more rigid territories which may hold more than one female; this was reinforced by observations that polygyny occurred in larger more desirable territories.

The evolution of polygyny has been explained by models such as the polygyny threshold model (Orians 1969) with two major predictions (Orians 1969; Garson et al. 1981) (i) a male's territory quality will be correlated with his mating success and (ii) if differences in territory quality affect polygyny then higher levels should be predicted for patchy environments (Verner & Wilson 1966). For the reed bunting a number of factors may operate to limit the occurrence of polygyny. Firstly males are required to feed the chicks except in rare circumstances when food is super-abundant then males may seldom feed (Bell 1968; Hornby 1971). Secondly males are usually in excess (Bell 1968; Hornby 1971), constraining the operational sex ratio in favour of monogamy. Males should occupy available territories and attempt to obtain a mate, since
females are a limiting resource and initial fertility is relatively synchronous then the chance of polygyny is reduced. The final feature which limits the occurrence of polygyny is strong site fidelity over successive years. Individuals return to breed in the same locality over successive seasons so available breeding sites will be limited for first years. Consequently this must impose limits on the number of individuals which can participate in polygamy and since reed buntings can extend into new habitats for breeding when populations rise it seems likely that polygamy will be rare unless females become more numerous than males. This evidence agrees with hypotheses proposed for the evolution of social monogamy (Wittenberger & Tilson 1980), (i) monogamy occurs when male parental care is essential for female reproductive success and (ii) monogamy occurs in territorial species when pairing with an unmated male is always better than pairing with an already mated male. The data from previous studies shows that polygyny does occur but may be limited to seasons where breeding starts early (Hornby 1971), when breeding has failed (Bell 1968; Hornby 1971) or where partners are no longer present (Bell 1968; Hornby 1971).

The settlement pattern of reed buntings as described in previous studies (Bell 1968; Hornby 1971, Chiot 1976) have shown that females are constrained in their choice of mate by the availability of vacant territories and that subsequently females will remain in a territory over successive seasons and change males as they are removed (Bell 1968; Hornby 1971). Westneat et al (1990) suggest a number of reasons why females of monogamous species might pair with males that are not of the highest genetic quality in the population. Females may select males on the basis of (i) territory quality, (ii) his potential for providing parental care, (iii) familiarity with the territory or (iv) familiarity with a mate. It is probable, at least in the reed bunting that females may select areas on territory quality as shifts towards more “optimal habitats” are observed when vacancies arise (Bell 1968; Hornby 1971). Familiarity with a territory is also a plausible cause in the reed bunting as territory fidelity is strong across years, but this is not always separable from male familiarity which might also occur. The quality of parental care is not addressed in this thesis but may be subject to female selection. In reed buntings in which first year females arrive on territory last (Bell 1968), then choice may not be available (Møller 1991a) and consequently females will accept those males available. The data are analyzed to determine on what basis females select a partner and if there is sufficient evidence to support the above criteria proposed by Westneat et al (1990).

In this chapter I address some of the above topics and examine how they are linked to observed levels of breeding success. The topics in order of presentation are territory density, population age structure and return rates, territory fidelity, adult loss rates, breeding chronology, causes of nest loss and individual success rates. Causes of polygyny are examined to determine if their is any evidence to support the polygyny threshold model. Constraints on male reproductive success are examined in the hypothetical environment where no extra-pair paternity occurs. I subsequently use these data to test the hypothesis that the seeking of extra-pair paternity will be advantageous for males as it may be the only means by which they can ensure siring any surviving offspring. These data then provide the background to the the following chapter which examines the actual patterns of reproductive success.
1.2 METHODS

1.2.1 The study site and territory distribution

To determine territory density and distribution for 1988, 1989 and 1990, I recorded the occurrence of all nests and territories focusing my activity around the northern lagoon of the nature reserve at Rutland Water (near Oakham, England) (see Figure 1.1). This lagoon, "lagoon III", was split into two sections, block 1 and block 2, both of which contained a variety of habitats ranging from common reed (Phragmites australis) and great reedmace (Typha latifolia) beds to relatively dense tree plantations. Within this area I mapped the distribution of reed buntings and recorded males as territory holders if they were heard singing in one area on more than three different occasions spread over at least three days. This observational method overcame the chance recording of males setting up temporary or satellite territories. I made additional observations at two other lagoons located within the nature reserve which comprised block 3 (Figure 1.1). Block 1 formed the focus for observation studies in 1989 and 1990, although observations were also made in block 2 in 1989.

1.2.2) Trapping and marking

I captured all birds in mist nets, potter traps or Skokholm traps (Spencer 1974). Each bird received a British Trust for Ornithology (BTO) alloy ring and a combination of four coloured rings (one above the metal and three on the other leg) allowing with five different colours a total of 1250 combinations. I also ringed nestlings with a BTO alloy ring and with a possible two rings per leg one of which was a split colour permitting rapid identification of the year and brood of origin. I sexed and aged birds according to Svensson (1984); consequently if adults were not ringed as juveniles then it was impossible to reliably age them. The taking of morphological measurements is described in the methods section of Chapter 4.

1.2.3) Nest data and nest protection

I located nests either through observing the adults entering or exiting the site or by flushing the adult from the nest by gently tapping the surrounding vegetation. When I located nests during incubation or provisioning the date of the first egg was estimated using the method developed by Bell (1968), based on recorded times from laying to hatching and seasonal variations.

High predation rates on nests necessitated the use of nest cages to prevent larger avian and mammalian predators from getting close to the nest. I made the cages from 1.5 inch mesh chicken wire, which formed a dome shaped cover with approximately a 50 cm clearance around and 1 metre above the nest. The cage was supported by four vertical wooden posts embedded into the soil and supported when possible by guy ropes. The cage was only placed over the nest after the completion of a clutch. After a series of nests were predated despite the use of cages in 1989, a more rigid structure was used which included a metal frame. This could only be added in the latter stages of incubation for fear of nest desertion. The cages were coated in painted green to decrease the likelihood of observation by aerial predators or humans.
1.3 RESULTS

1.3.1 Territory numbers and density

The total numbers of territories in the study area for 1988, 1989 and 1990 are given in Table 1.1. Between 1988 and 1989 there was an overall 38% reduction in territory numbers and a 39% reduction in the main area of block 1. The territory numbers increased in 1990 to 64% of their 1988 level and in block 1 to 94% of their 1988 level.

Table 1.1 Territory numbers and density in Lagoon III study area (block 1 in parentheses)

<table>
<thead>
<tr>
<th>Year</th>
<th>N</th>
<th>Density /hectare</th>
</tr>
</thead>
<tbody>
<tr>
<td>1988</td>
<td>45 (18)</td>
<td>12.58</td>
</tr>
<tr>
<td>1989</td>
<td>28 (11)</td>
<td>6.91</td>
</tr>
<tr>
<td>1990</td>
<td>29 (17)</td>
<td>8.11</td>
</tr>
</tbody>
</table>

During the course of the study, a number of fields were altered as part of the reserve management programme, which either enhanced or detracted from the suitability of the habitat as a nest site, however, alterations were usually compensated for in other areas. The data show a 55% decrease in density between 1988 and 1989 with a subsequent 15% rise in 1990.

1.3.2 Population age structure and return rates of breeding individuals

The known age composition of males and females is given in Table 1.2, birds were only regarded as being in their first year when they were ringed as juveniles. Table 1.3 shows the return rates for both ringed males and females for 1989 and 1990.

The variation in age structure between 1989 and 1990 for males is due to the number of 1988 breeders failing to return in 1989. Only 27% of males returned from 1988 to breed in 1989, whereas 59% of the 1989 males continued to breed in 1990 producing a skew towards an older population, with most males being in their 3rd year of breeding.
Table 1.2  
Population age structure

Males

<table>
<thead>
<tr>
<th>Year of breeding</th>
<th>Percentage of males (N in parentheses)</th>
<th>1988</th>
<th>1989</th>
<th>1990</th>
</tr>
</thead>
<tbody>
<tr>
<td>on the study site</td>
<td></td>
<td>(45)</td>
<td>(28)</td>
<td>(29)</td>
</tr>
<tr>
<td>known 1st years</td>
<td></td>
<td>5</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>16</td>
<td>39</td>
<td>21</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>2</td>
<td>11</td>
<td>34\textsuperscript{a1}</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>2</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>unknown age</td>
<td></td>
<td>75</td>
<td>36</td>
<td>31\textsuperscript{a2}</td>
</tr>
</tbody>
</table>
\textsuperscript{a1} one male returned to breed after breeding within the study site in 1988 but not in 1989.
\textsuperscript{a2} a male ringed in 1988 appeared as a new breeder in the area in 1990.

Females

<table>
<thead>
<tr>
<th>Year of breeding</th>
<th>Percentage of females (N in parentheses)</th>
<th>1988</th>
<th>1989</th>
<th>1990</th>
</tr>
</thead>
<tbody>
<tr>
<td>on the study site</td>
<td></td>
<td>(25)</td>
<td>(23)</td>
<td>(25)</td>
</tr>
<tr>
<td>Known 1st years</td>
<td></td>
<td>4</td>
<td>17</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>12</td>
<td>43</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>4</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>unknown age</td>
<td></td>
<td>80</td>
<td>31</td>
<td>44</td>
</tr>
<tr>
<td>Additional</td>
<td></td>
<td></td>
<td></td>
<td>12\textsuperscript{a}</td>
</tr>
</tbody>
</table>
\textsuperscript{a} Rings were lost and so identification was not possible as birds were not recaptured to permit metal ring identification.

Table 1.3  
Return rates of ringed breeding male and females during 1989 and 1990

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>27</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>12</td>
<td></td>
<td>(15+2)\textsuperscript{a1}</td>
</tr>
<tr>
<td>% males ringed</td>
<td>91</td>
<td>100</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td></td>
<td>32</td>
<td>36</td>
</tr>
<tr>
<td>N</td>
<td></td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>% females ringed\textsuperscript{a2}</td>
<td>56</td>
<td>82</td>
<td>86</td>
</tr>
</tbody>
</table>

\textsuperscript{a1} Two males returned presumably from outside the study site after being ringed in 1988, and not breeding within the study area in 1989.
\textsuperscript{a2} these are estimated data assuming a 1:1 sex ratio with all territorial males.
The return rates of females to some extent run opposite to that of males ($\chi^2 = 26.71, p < 10^{-4}$) with a relatively high proportion of ringed females returning compared to the males in 1989 and a low rate in 1990. Why this difference exists is unclear and may be due to a large number of first year females ringed in 1988 surviving to 1989 but not surviving on into 1990. Certainly the expectation of equal annual mortalities or greater in females was not consistently supported and is therefore indicative of an underlying inequality in age structure or a bias in ringing.

It is difficult to estimate the number of first year breeding birds, as few birds were actually ringed as juveniles, however assuming new breeding males were first year males the data for 1989 and 1990 indicated that 50 and 48 percent of males respectively were first years i.e., an average of 49%, and 52 and 48 percent of females were first years i.e., an average of 50%. In any one year the breeding population was probably composed of approximately 50% first year birds.

1.3.3 Male territory fidelity

Many small territory shifts occurred between seasons with males occupying part of their former territories however, a few larger shifts also occurred when territories elsewhere became vacant. The extent of male territory fidelity is shown below, with shifts based on an estimated territory centre and separated into the following categories: no discernible shift or <50 m, 51-100 m, 101-200 m and >200 m.

<table>
<thead>
<tr>
<th>Distance of territory shift (metres)</th>
<th>N</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;50</td>
<td>(79)</td>
</tr>
<tr>
<td></td>
<td>51-100</td>
<td>(0)</td>
</tr>
<tr>
<td></td>
<td>101-200</td>
<td>(7)</td>
</tr>
<tr>
<td></td>
<td>&gt;200</td>
<td>(14)</td>
</tr>
</tbody>
</table>

Of the shifts which were >200 m two were from outside the study site whilst the others were of 300 m and 700 m occurring in 1989 and 1990 respectively.

The territory shift data appear to be bimodal with most males hardly moving at all and others moving much further, i.e., 21% to more than 100 m. None of the above shifts were the result of habitat loss; rather, they were the result of moving from what Bell (1968) and Hornby (1971) would have regarded as suboptimal habitat (dry tree plantations) into wetter more optimal habitat. No males which bred in cultivated fields surrounding the nature reserve ever returned to occupy territories within the nature reserve.

1.3.4 Female mate and territory fidelity

The females which returned over successive years were classified into: (i) females returning to
the same male and same territory, (ii) to a different male and the same territory and (iii) a different male and a different territory. No incidences of the same male and a different territory were recorded as occurring between years. The percentage of females in each category for 1989 and 1990 are shown in Table 1.5.

Table 1.5 Female fidelity to mate and territory between years

<table>
<thead>
<tr>
<th></th>
<th>Same male &amp; same territory (%)</th>
<th>Different male &amp; same territory (%)</th>
<th>Different male &amp; different territory (%)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1989</td>
<td>25</td>
<td>33</td>
<td>42</td>
<td>12</td>
</tr>
<tr>
<td>1990</td>
<td>56</td>
<td>11</td>
<td>33</td>
<td>9</td>
</tr>
</tbody>
</table>

In every case where a female obtained a different mate, the original male failed to return. Therefore as expected with the higher return rates of males in 1990 more females which returned were partnered to the same male despite the lower female return rates.

Table 1.6 Female territory shifts

<table>
<thead>
<tr>
<th>Distance of territory shift (metres)</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;50</td>
<td>8</td>
<td>(38)</td>
</tr>
<tr>
<td>51-100</td>
<td>0</td>
<td>(0)</td>
</tr>
<tr>
<td>101-200</td>
<td>8</td>
<td>(38)</td>
</tr>
<tr>
<td>&gt;200</td>
<td>5</td>
<td>(24)</td>
</tr>
</tbody>
</table>

As in the males, female territory shifts were of either very short distances or more considerable distances with no intermediates (50-100 m). The data do however show that females are significantly ($\chi^2 = 48.00 , 3$ df, $p <10^{-3}$) more likely to move greater than 100 m than males.

1.3.5 Composition of pairings.

I observed two cases of simultaneous polygyny involving two males during 1988 occupying adjacent territories. An additional female was identified as having been paired twice with one male and with a second in between, each time nest failure resulting in a shift. A further female was identified as having become the second female of a polygynous male after the disappearance of her original mate. Two males were identified as remaining unpaired from mid-May though it is not known if they had been paired earlier in the season.

In 1989 pairings were easier to follow because I had caught and marked a large number of birds prior to the start of nesting. I observed two cases of simultaneous polygyny, both arising from the loss of males. In each case the female remained on her nest site and became paired with an already paired neighbouring male. One of these cases involved a male who was paired to a total of three females during the season but only two simultaneously. The second case only lasted until shortly after a female’s third nest failed, when she moved to breed successfully with a nearby unpaired male whose mate had disappeared. There was one other instance where a male
became unpaired after losing his mate.

In 1990 I observed four cases of simultaneous polygyny, of which two were known to have arisen following the death of the female’s original partner. A single case of polygyny arose after an unpaired male (whose mate had been predated) acquired a new partner who had shifted from a territory some 600 m away following three nest failures. The male was subsequently removed for 24 hours (Dixon in prep) and on returning was unable to retain his original female, becoming single once more. The male who acquired the female was subsequently polygynous. One pairing involved a female with two males simultaneously, one of which was already paired to a second female.

The above data indicate that nest failure can be the cause of mate switching by the female during the season and that mate loss usually results in the female becoming the second female of a polygynous male.

1.3.6. Loss of breeding adults during the season

Loss of males and females was defined as the disappearance of individuals during either the prelay, egg laying, incubation, provisioning or early (first 10 days) fledging stages. Any individuals which stopped breeding early relative to the end of the season are thereby largely excluded. The results, Table 1.7, show losses were not large during the breeding season but did result in cases of single males from female loss and bigamous males from male loss. Loss rates are also given as the percentage of individuals per ten day period assuming that the data include the period from 1st April through to 30th June (91 days). Too few data exist to make any comparison between males and females but rates for both sexes are relatively low compared to the overall annual mortality. The two male losses in 1990 both occurred prior to the 1st April.

Table 1.7 Loss of breeding adults during the season

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>10 day⁻¹</td>
</tr>
<tr>
<td>1988</td>
<td>2° (4)</td>
<td>0.44</td>
</tr>
<tr>
<td>1989</td>
<td>2 (7)</td>
<td>0.77</td>
</tr>
<tr>
<td>1990</td>
<td>0 (0)</td>
<td>0.00</td>
</tr>
<tr>
<td>Mean</td>
<td>1.33 (3.67)</td>
<td>0.40</td>
</tr>
<tr>
<td>SE</td>
<td>0.66 (2.03)</td>
<td>0.22</td>
</tr>
</tbody>
</table>

° denotes the data are uncertain.

Predation on the nest accounted for at least two cases of female mortality.

1.3.7. The chronology of nesting

The total number of nests recorded in 1988, 1989 and 1990 varied little (Table 1.8) however the average number of nests I located per territory did vary considerably with noticeably higher rates in 1989 and 1990. The difference in nests located per territory between 1988 and subsequent years was significant (z > -2.349, p <0.02), but no significant difference was detected between 1989 and 1990.
The low density of nests detected in 1988 was probably the result of both observer inexperience (nests were more likely to have been predated before detection or not detected at all), and real year to year differences. It is impossible to know how many nests were not detected as complete records exist only for block 1. Previous studies by Bell (1968) and Hornby (1971) estimated an average of 3.7 nests per pair, but productivity per nest in their study was exceptionally low.

Table 1.8 Nests located, total and number / territory

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of nests located</th>
<th>Nests/ territory</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>lagoon III</td>
</tr>
<tr>
<td>1988</td>
<td>44</td>
<td>34</td>
</tr>
<tr>
<td>1989</td>
<td>47</td>
<td>42</td>
</tr>
<tr>
<td>1990</td>
<td>46</td>
<td>46</td>
</tr>
</tbody>
</table>

1.3.8. First egg dates

The data for each of the three years are shown in Figure 1.2. The proportion of nests started for each ten day period were significantly correlated ($r_s > 0.70$, $n = 11$, $p < 0.05$) but the patterns were different. The 1988 distribution shows an earlier and wider peak followed by a comparative lull in new nests and then a second peak. The lull is attributable to flooding on the study area during late April followed by continued cool weather and rain during early May, which caused the loss of some nests and restricted the building of new nests. In 1989 the early season peak was later, with a gradual decline as nests were being continually replaced. The season continued longer than during the other years as the weather permitted continued replacement nests. The distribution for 1990 shows a similar peak to the previous year, with low continued levels of new nests. The apparent shortness of the 1990 season was due to a reduced intensity of observations after day 180, rather than any natural phenomenon.

1.3.9. Causes of nest loss and success.

Nest loss through predation as described earlier can account for the majority of clutches laid during a season. Of those nests which had a known termination dates, I recorded the relevant causes of nest loss or if they were successful up to fledging. The data include both caged and uncaged nests as the latter still remained susceptible to predation. Table 1.9 and Figure 1.3 show the rates for 1988, 1989 and 1990. The data include all nests recorded and are therefore not an absolute representation of the failure rate as some nests will not have been recorded and not all nests were discovered during the prelay period. The nest data for block 1 have been included as a control sample for which exact start and termination dates are known. The data therefore provide a measure of the fates of nests during the season both overall and for those nests within block 1. The loss and failure rates for each year were not significantly different ($\chi^2 = 0.38$, $2 \text{ df}$, $p = 0.828$). Comparison of block 1 data for 1989 and 1990 showed no significant difference ($\chi^2 = 1.68$, $1 \text{ df}$, $p = 0.167$).
The individual stages show some degree of variation; no nests were destroyed during the prelay stage, although two examples of double constructions were recorded in 1989. No losses occurred during the egg laying period of 1988 but more than 10% of nests were lost at this stage in 1989, with half this level being lost in 1990. Flooding caused desertion and hatching failure in 1988, and in 1989 only one failure was ascribed to physical factors (chilling during persistent snow cover in late April). The overall prehatch loss rates for the three years varied little with only a minor rise in 1989. The levels of loss during provisioning did show a high degree of variation being highest during 1988 and accounting for more than a quarter of all nests with a lower level in 1989 and an intermediate level in 1990.

<table>
<thead>
<tr>
<th>Stage</th>
<th>1988</th>
<th>1989</th>
<th>1990</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All nests</td>
<td>Block 1</td>
<td>All nests</td>
</tr>
<tr>
<td>Prelay</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Egg laying</td>
<td>0.00</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>Incubation 1*</td>
<td>0.11</td>
<td>0.13</td>
<td>0.23</td>
</tr>
<tr>
<td>Incubation 2*</td>
<td>0.14</td>
<td>0.00</td>
<td>0.03</td>
</tr>
<tr>
<td>Incubation 7*</td>
<td>0.05</td>
<td>0.08</td>
<td>0.00</td>
</tr>
<tr>
<td>No Hatch</td>
<td>0.05</td>
<td>0.04</td>
<td>0.00</td>
</tr>
<tr>
<td>Prehatch Total</td>
<td>0.36</td>
<td>0.38</td>
<td>0.39</td>
</tr>
<tr>
<td>Provisioning</td>
<td>0.27</td>
<td>0.17</td>
<td>0.19</td>
</tr>
<tr>
<td>Success</td>
<td>0.39</td>
<td>0.45</td>
<td>0.42</td>
</tr>
<tr>
<td>N</td>
<td>44</td>
<td>47</td>
<td>31</td>
</tr>
</tbody>
</table>

\* incubation is divided into (1) as the first half (2) as the second and (?) as unknown.

The proportion of successful nests actually varied little over the three years but was lowest in 1988 with little difference between 1989 and 1990. The actual loss rate for 1988, was probably higher as some of the first nests would not have been located before they were lost during flooding of parts of the study site.
Figure 1.2 First egg dates

1988

1989

1990

Proportion of nests

Day of the year
Figure 1.3 Nest loss data

1988 N = 44

Proportion of failed nests

1989 N = 47

Proportion of failed nests

1990 N = 46

Proportion of failed nests

19
1.3.10. Observed nest success rates per male

Males varied greatly in their apparent reproductive success as measured in terms of the number of offspring fledged from their territories. The data for 1988 were less complete and are not considered here. The data for 1989 and 1990 (Table 1.10) provide an indication of the distribution of mean success of males.

**Table 1.10 Apparent productivity per male**

<table>
<thead>
<tr>
<th>Nests successful to fledging</th>
<th>Number of males (percentage in parentheses)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1989</td>
</tr>
<tr>
<td></td>
<td>N (%)</td>
</tr>
<tr>
<td>3</td>
<td>1 (5)</td>
</tr>
<tr>
<td>2</td>
<td>4 (17)</td>
</tr>
<tr>
<td>1</td>
<td>9 (39)</td>
</tr>
<tr>
<td>0</td>
<td>9 (39)</td>
</tr>
</tbody>
</table>

Chicks fledged/nest 1989 (N = 20) 1990 (N = 18)
Mean 3.88 3.93
SD 1.08 1.25

*1 only 23 males were analysed, insufficient data were available to support a claim for 5 individuals.

*2 only 25 males were analysed, insufficient data were available to support a claim for 4 individuals.

Based on the above data approximately 40% of males will not fledge offspring from their territories. The expectation from these data therefore is that the remaining 60% of males will successfully fledge an average of 5.0 chicks, based on an average fledging success of 3.9 chicks per nest which is identical to previous studies (Hornby 1971) and an average 1.29 successful nests per male. The average nest success rate of all individuals was 0.75 per male with the average chicks fledged being 2.93 chicks per male. Assuming that a best estimate based on Hornby’s work of 75% flying success then 2.20 chicks will actually survive to flying. Subsequently 65% of chicks will not survive to breeding (Hornby 1971) then the expected mean contribution per male per year to the next generation is 0.77 chicks. This type of data handling is dealt with in more detail by Hornby (1971) in which his best estimate was 0.55 chicks per pair per season resulting in a negative population balance and requiring recruitment from elsewhere. This not necessarily the case with this population as productivity was higher and mortality rates may differ.

1.3.11 Causes of nest failure.

As already mentioned, failure to hatch accounted for some of the losses. However this was only a small proportion (3%) of the total. Predation by a variety of possible predators accounted for the rest of the nests. The main species actually known to be accountable for some of the 1989 losses was the European badger, *Meles meles*. During the summer of 1989 a
severe drought in combination with a clay based soil produced an impenetrable surface, preventing the local badgers from digging for their normal food. During a period of 24 hours 6 nests under nest cages were destroyed, the destruction to nearby vegetation and the crushing, removal and ripping of the cages could only have been performed by badgers. Larger, more rigid and less penetrable cages were subsequently used.

Other possible predators known to be present on the study site included weasels Mustela nivalis (observed once removing chicks), magpies Pica pica, jays Garrulus glandarius, voles Microtus spp. (videoed chewing chicks, Dixon pers comm.), foxes Vulpes vulpes and grass snakes Natrix natrix. Fledglings were also observed being consumed by a family of four tawny owls Strix aluco, magpie and a hobby Falco subbuteo.
1.4 DISCUSSION

The results of the general population dynamics match those of previous studies (Bell 1968; Hornby 1971; Haukioja 1970), with the following general conclusions. Territory fidelity among adults, especially males, is very marked, although shifts do occur with some occasional shifts of larger distances to the wetter habitat identified by Bell (1968) as "optimal" habitat. Females also show a high degree of territory fidelity which (usually) results in mate fidelity if both individuals return (56%). Upon failure to return of their previous mate the female will usually obtain a new partner (11%) on her former territory or shift to an alternative territory to pair with a new male (33%). Both Bell (1968) and Hornby (1971) observed territory shifts by females whose former partner was still present but had been displaced by a second female, but no such instances were observed during this study.

Return rates of adults were 43% and 44% for males and females respectively, over the two years. These estimates are similar to those for males from previous studies, but are a little lower for females, (Bell 1968; Hornby 1971; Haukioja 1970; Prys-Jones 1977). However, the actual annual rate of return of previous breeders varied significantly between years for both sexes did not covary, being highest in 1989 and lowest in 1990 for males and the opposite for females. Adequate data on the survival and return of first year birds is not available, since few definite cases of known first year breeders were observed (four males and four females). The long term study by Bell and Hornby identified an annual loss rate of 65% for first year breeders prior to the second season of breeding.

Despite variable losses, the sex ratio observed in the population was approximately 1:1, with incidences of polygyny being approximately matched by the number of single males. Bell and Hornby in their study concluded that males were in excess at least to the extent that the annual mortality rates are higher for females, the available data on the breeding sex ratio would however indicate equal numbers of each sex. The inference from this section is that females are either produced in excess to account for the higher adult mortality rates or the immigration rates of females into the population are higher. Alternatively females may have a different dispersal pattern between years and do not return to areas where they have previously bred, for example when males are not available and therefore are forced to emigrate elsewhere.

The occurrences of polygyny were judged to have arisen from the loss of a partner and the female becoming the second female of a neighbouring male. Territory size was not related to the acquisition of females, rather the larger territories are located in what has previously been defined as "sub-optimal" reed bunting habitat. In "optimal habitats" territory size diminishes and neighbour density increases. Females are unlikely to benefit from polygyny as resources would appear to be defining the territory size and polygyny would certainly (and was observed to) result in increased competition for resources and conflict between females. Other reasons for polygyny being rare in the reed bunting were described previously. It is therefore unlikely that polygyny will be common in reed buntings unless male numbers decline sufficiently to the level where females are forced into being the second female of a polygynous male.

Concomitant with previous studies the main factor limiting nesting success is predation. The
average success rate for all nests over the three years was approximately 42% survival to fledging with an average of 3.9 chicks per nest. A crude productivity index is 0.77 chicks returning to breed per male per year, this compares to 0.55 estimated by Bell and Hornby who concluded this was too low a level to maintain a viable population. The data available for 1989 and 1990 showed that 41.5% of males achieve no success to fledging. Hornby (1971) estimated that only 20.1% of nests actually fledged young and Haukoja (1970) estimated 37%, based on this study’s level of 42% it is reasonable to conclude that fewer males actually obtained nesting success in Hornby’s study at least. These values are essentially crude estimates as a complete knowledge of breeding is not possible. The estimated loss of 25% of fledglings prior to flight will probably increase the level of male failure and with a further 65% loss prior to breeding the conclusion is that few males (approximately 30%) will actually achieve true productivity through to the next season.

The expectation from this discussion is that for many males there is a risk of not actually observing any reproductive success from their territorial nests. An alternative mating strategy (Trivers 1972) is that of seeking EPCs (see Birkhead and Möller 1992 for a review) with females. The estimated average success of nests to fledging for this and other studies is 33%, with the expectation that 70% or more of males will not actually achieve success in any one season. Assuming that males in their first year are representative of the overall population then 70% will not be successful of which 65% will not return consequently 46% of males may never achieve reproductive success through their territorial nests. Hornby showed an average of 0.80 nests/pair as successful, however assuming as in this study 15.5% of the nests are from double-brooded individuals then his data show that 64.5% of males achieved nest success compared with 58.5% in this study. The result is that males will only have approximately a 60% chance of achieving success in their territorial nest. The expectation is that any male who can pursue EPCs as part of a reproductive strategy may have a significantly increased chance of reproductive success.

Females have been identified previously (Bell 1968; Hornby 1971; Haukoja 1970) and in this study as showing strong territory and male fidelity. Westneat et al (1990) and Möller (1991a) suggested that females may not either have a choice of a mate or pair based on other selection criteria. The data collected here indicates that females will pair with males on available territories although observations have also shown that females do fight during settlement apparently over males. The collective data do indicate that opportunities for mate choice are limited and that females are unlikely to have much option in their final pairing. The fidelity of females to mates and territories over successive breeding attempts is high. Only when a former partner fails to return or breeding repeatedly fails during the season do females pair with new males. Mate choice although constrained may therefore be influenced strongly by mate and territory familiarity (Westneat et al 1990). Birkhead and Möller (1992) predict that females who pair later will have higher rates of extra-pair paternity (EPP). The expectation for reed buntings is that few females will have performed active selection so there is potentially a big advantage to seeking sires from surrounding males who are of higher quality than her mate. However, males should naturally guard against EPCs by their female (see Chapter 4) and females should seek extra-pair copulations subversively to avoid male punishment (Barash 1976) or withdrawal of parental care (Trivers 1972).
In summary this chapter suggests that the pursuit of EPCs may prove a worthwhile strategy for male reed buntings. With high observed rates of nest predation a male can greatly increase the probability of his reproductive success by obtaining fertilisations in the nests of females other than his mate. Females are constrained in their mate choice and there is potentially a big advantage in seeking sires from surrounding males of higher quality. The data from this chapter predict the occurrence of extra-pair paternity (EPP) as it has advantages for both sexes. The following two chapters will determine the extent of EPP and therefore whether EPCs are occurring and if so by which mechanism.
CHAPTER 2
Determination of actual reproductive success by DNA fingerprinting

Abstract: Analysis of paternity in the reed bunting, Emberiza schoeniclus, using DNA fingerprinting revealed a high rate of extra-pair paternity, 50% of chicks and 69% of broods. Approximately one third of all males are responsible for all cases of extra-pair paternity. However, 70% of males lost some paternity and no correlations were recorded between within-pair paternity and extra-pair paternity indicating no apparent relationship between male quality and success. The level of extra-pair paternity was significantly different from observed levels of extra-pair copulations.

2.1 INTRODUCTION
Multilocus DNA fingerprinting (Jeffreys et al 1985 a,b) has allowed the determination of parentage in a variety of animals including bird species (Burke & Bruford 1987, Wetton et al 1987). The technique permits the detection of individual specific genetic information through the visualisation of hypervariable tandem repetitive DNA regions called minisatellites (Jeffreys et al 1985 a,b). The techniques and literature related to DNA fingerprinting are not reviewed here but have been covered extensively in a review by Bruford et al 1992.

Multilocus DNA fingerprinting has been used successfully in a number of studies to provide accurate measures of within-pair paternity and extra-pair paternity and to document the occurrence of intraspecific brood parasitism (Burke et al 1989; Birkhead et al 1990; Davies et al 1992; Gibbs et al 1990; Gyllensten et al 1990; Hunter et al 1992; Morton et al 1990; Rabenold et al 1990; Westneat 1990; Jones et al 1991; Wetton & Parkin 1991; Pinxten et al 1992; see also Birkhead & Möller 1992 for a review). The level of extra-pair paternity detected in these studies ranges from 0% to 60%, whilst the percentage of broods containing extra-pair fertilisations varied within a similar range. The results show a wide disparity in the extent of extra-pair copulations with indications of inter-specific and inter-population variation.

Extra-pair young occur from forced, unforced or solicited extra-pair copulations (see Birkhead & Möller 1992) or alternatively through rapid mate switching (McKinney et al 1984; Birkhead et al 1990; Pinxten et al 1993). In Chapter 1 I analysed the observed distribution of reproductive success and discussed the potential advantages of extra-pair paternity in reed buntings from both the male and female perspectives. The form and frequency of copulations in reed buntings is dealt with in Chapter 3. This chapter deals with the identification of levels of extra-pair paternity in the breeding population of 1988 and 1989. The 1989 data and that of 1990 (A. Dixon, thesis in prep) are combined with individual behavioural observations in Chapter 7 to examine why and how individual variation in reproductive success occurs. Only a limited number of other studies have combined behavioural data and DNA fingerprinting from the same individuals to examine variation among individuals in reproductive success (Burke et al 1989; Birkhead et al 1990; Lifjeld et al 1991; Davies et al 1992; Hunter et al 1992; Kompengaers et al 1992).
2.2 METHODS

2.2.1 Sampling techniques

During the breeding seasons of 1988 and 1989 (see Chapter 1 and Chapter 4 for further details), I obtained blood samples from 16 and 24 families respectively. These comprised 32 families where both parents were sampled, two families where only the father was sampled, two where only the mother was sampled and four for which no parents were sampled. The average (± SD) number of chicks per brood were 3.88 ± 1.08 and 3.93 ± 1.25 respectively for 1988 and 1989. In no cases did a case of mate replacement occur which may have confounded paternity analysis.

I sampled each adult on capture taking between 50 and 200 µl of blood from the brachial vein at the point where it crosses the elbow joint. Blood samples were originally taken from chicks whilst they were still in the nest on or after the fifth day of provisioning, however during 1988 and the first half of the 1989 breeding season many broods were predated prior to sampling. Subsequently samples of 20 µl were obtained from 2-day old nestlings, from a visible vein near the distal end of the tarsus, with repeated bleeding on the fifth day if the brood survived predation.

I sampled broods both in the area in which behavioural observations were recorded and from an adjacent area in which marked birds were breeding but were not subject to intensive observation. This two-site sampling provided a control against the chance that the presence of observers would disturb birds sufficiently to alter the normal pattern of paternity.

2.2.2 Fingerprinting techniques

The protocols for multilocus DNA fingerprinting have been described by previous studies (Burke and Bruford 1987; Birlhead et al 1990) and the methodology has been reviewed in detail, with the inclusion of standard protocols (Bruford et al 1992). Some additional details specific to this study only will be provided here.

Blood was stored as 25 - 100 µl volumes in 400 µl of 1 x SSC, 0.1% EDTA solution at -70°C until required. DNA was extracted from a 50 µl volume of blood solution (Birlhead et al 1990; Bruford et al 1992) and digested with Alu I. Five µg of each digested DNA sample was electrophoresed in a 0.8% horizontal agarose gel in a TBE buffer (0.089 M Tris borate pH 8.8, 2 mM EDTA) until the 2 kb fragments of lambda cut with Hind III had migrated between 24 and 28 cm. The gels were then blotted onto nylon membrane (Hybond-N, Amersham) and the filters were probed with minisatellite multilocus probe 33.15 (Jeffreys et al 1985a). During initial probing trials 33.6 was found to be inappropriate, because strong cross-hybridisation of the probe with satellite-like sequences, preventing scoring of minisatellite loci. [32P]-labelled probe DNAs were prepared from M13 recombinants by primer extension (Jeffreys et al 1985a,b). The filters were prehybridised for 1 hour at 64 °C in a solution containing 7% SDS, 1 mM EDTA, 0.263 M Na phosphate pH 7.2 and 1% bovine serum albumin (Sigma fraction V) (Church and Gilbert 1984; Westneat et al 1988). Hybridisations were carried out in the same solution, overnight at 64°C. The filters were washed at low stringency at 64 °C once for 15 min.
in 0.263 M Na phosphate pH 7.2, 1% SDS, twice for 25 min in 2 x SSC, 0.1 % SDS and three times for 25 min in 1 x SSC, 0.1% SDS. Autoradiographs were obtained by exposure of the radioactive filter at -70 °C to Fuji RX or Amersham MP film for 2-14 days with one or two intensifying screens, as appropriate.

2.2.3 DNA fingerprint analysis

The band pattern produced with 33.15 was highly variable and relatively easy to score over the entire length of the gel. Band sharing values were obtained for unrelated birds in adjacent lanes on the same autoradiograph or for putative relatives by comparing lanes within family groups run together on the same gel. To be scored as identical, bands had to be regarded as being of similar intensity (less than two-fold difference) and of similar electrophoretic mobility (migration distances within 0.5 mm) (Birkhead et al 1990). Weakly hybridising bands present in an individual that may have been obscured if present by a stronger band in the individual with which it was compared were excluded from the analysis.

Calculation of band sharing probabilities was as described in Pinxten et al 1993. The mean probability of two unrelated individuals sharing a band of apparently similar mobility was estimated as the band sharing coefficient, \( \chi = 2n_{ab} / (n_a + n_b) \) where \( n_a \) and \( n_b \) are the number of bands present in individuals a and b respectively and \( n_{ab} \) is the number of bands shared by a and b. If all alleles are assumed to be of an equal frequency, and co-migrating bands shared between individuals are assumed to be identical alleles, the mean allele frequency, \( q \) is found from \( q = 1 - (1 - \chi)^{1/2} \) (Jeffreys et al 1985b). The proportion of bands expected to be shared between full siblings is found as \( (4 + 5q - 6q^2 + q^3) / 4(2 - q) \) (Bruford et al 1992), and for parent and offspring as \( (1 + q - q^2) / (2 - q) \) (Bruford et al 1992). For two second order relatives having a direct line of descent (such as aunt-nephew, but not double first cousins) the expected band sharing is \( (1 + 5q - 5q^2 + q^3) / 2(2 - q) \), and for third order relatives as \( (1 + 13q - 13q^2 + 3q^3) / 4(2 - q) \) (Bruford et al 1992). Expected band sharing values calculated between individuals having different degrees of relatedness are given in Table 2.1. The mean probability that an individual will share the same fingerprint pattern as the one observed in an unrelated individual, assuming that fingerprint bands are genetically independent is conservatively given by \( P = x^n \) where \( n \) is the mean number of bands scored in an individual (Jeffreys et al 1985a). The mean probability of false inclusion of an offspring arising from intraspecific brood parasitism (IBP) is \( (1 - (1 - x)^2)^n \), where \( n \) is the mean number of bands per fingerprint (this assumes that the actual parents are unrelated to the putative parents; Burke et al 1989). If the female shared significantly more bands with an offspring than expected for a nonrelative, then the assigned maternity was considered to be correct. Given this, the probability of falsely including an unrelated male as the actual parent is \( x^m \), where \( m \) is the minimal number of paternal-specific bands present in an offspring (Jeffreys et al 1985c). The probabilities of exclusion are equal to one minus the probabilities of false inclusion.

The calculations described above assume the independent segregation of the DNA fingerprint
fragments scored. It has previously been demonstrated that this assumption may be false in
birds and that it has to be tested for each enzyme / probe combination to be used (Brock and
White 1991; Hanotte et al 1992). In this study, independent segregation was tested using the
transmission of paternal- and maternal-specific bands in one large pedigree: male YMYGG and
female YMBWY with 9 offspring belonging to two different breeds in 1989.
2.3. RESULTS

2.3.1 DNA fingerprint analysis

Scorable DNA fingerprints were obtained from 11 broods in 1988, 20 broods in 1989 and from three chicks of a captive pair. DNA fingerprints, obtained using Alu I and multilocus probe 33.15 are shown in Figures 2.1 and 2.2 (Figure 2.1 of male YMYYGG 1989 brood 1 and brood 2, family JE 89, Figure 2.2 of GMBBR 1989, brood 1 and brood 2, family KW 89 indicating mismatches). The mean number of bands scored was 34.40 ± 8.45 SD (Table 2.1). The mean probability (x) of two unrelated individuals sharing a band of apparently similar mobility was estimated to be 0.21 ± 0.11.

Segregation analysis was performed on one large pedigree of 9 offspring (family JE89) from male YMYYGG and female YMYYGW obtained from two broods in 1989. Only a single mismatched band was obtained for one of the offspring and was consequently regarded as a mutant band.

In the segregation analysis the transmission of both paternal-specific (29) and maternal-specific (30) bands was recorded. Of 50 scorable bands recorded in all 12 individuals, 9 were shared by both parents and consequently excluded from the analysis along with the mutant but one case of apparent homozygosity was detected in the male (the probability of any heterozygous band being transmitted to all offspring by chance is 0.04). The female had two cases of apparent allelism with no observed cosegregation whilst the male had no allelism and one case of cosegregation between two bands. The probability of observing apparent cosegregation or allelism of any pair of bands in this sibship by chance (i.e., assuming no linkage) is 7.8 x 10^-4 and again is unlikely to arise as a chance event. The mean transmission frequency of the fragments was 0.58, close to the Mendelian expectation of 0.50 (G = 2.39, p >0.10). The results indicate that the fragments detected by 33.15 are mainly unlinked (one observed case), with a low level of allelism, and consequently are mainly inherited independently.

The segregation analysis for family JE89 is shown in Table 2.2. The deviation of the observed distribution from expectation (based on a binomial distribution with a mean of 0.50) for male and female was calculated using the Kolmogorov Smirnoff goodness-of-fit test, providing values of 0.06 and 0.12, indicating that the observed was not significantly different from expected (p >0.20).

Assuming independence, the mean allele frequency can be calculated for 33.15 and the proportion of band sharing between two first order relatives and second order relatives can be estimated (Table 2.1). The probability of two unrelated birds showing the same DNA fingerprint can be calculated (7.50 x 10^-24), as well as the probabilities of false inclusion of an incorrectly assigned pair (2.82 x 10^-15) - the probability of not detecting a case of intraspecific brood parasitism - or false inclusion of an incorrectly assigned father (1.36 x 10^-9) - the probability of not detecting a case of EPP. The exclusion probabilities (equal to one minus the false inclusion probabilities) are therefore close to one. The very low values obtained with probe 33.15 indicate that no case of falsely assigned parentage at least of the offspring of non-
relatives, is likely to be undetected in family analyses.
DNA fingerprints of family JE89 obtained with restriction enzyme Alu I and probe 33.15. The family comprises a pair of adults, male (M) YMYGG and female (F) YMBWY and their 9 offspring (brood 1 chicks 1-4, and brood 2 chicks 5-9). Fragments were scored in the size range 2.3 to 23.0 kb with only a single mismatching band recorded in chick 1.
Figure 2.2
DNA fingerprints of family KW89 obtained with restriction enzyme Alu I and probe 33.15. The family comprises a pair of adults, male (M) GMBBR and female (F) M/Own and their 10 offspring (brood 1 chicks 1-5, and brood 2 chicks 6-10). Fragments were scored in the size range 2.3 to 23.0 Kb. Male GMBBR was identified as the father of chicks 1 and 4 of brood 1 and chick 10 of brood 2. The father of the remaining chicks was identified as being the neighbouring male YMBWR as described in the text.
Table 2.1  DNA fingerprint statistics using multilocus probe 33.15

Similarities of DNA fingerprints between individuals:

<table>
<thead>
<tr>
<th></th>
<th>Estimated Mean Allele frequency</th>
<th>Mean number of bands ± SD</th>
<th>Mean shared bands ± SD</th>
<th>Observed band sharing of non relatives (N = 32)</th>
<th>Expected band sharing full sibs</th>
<th>Expected band sharing of parents and offspring</th>
<th>Expected band sharing of 2° relatives</th>
<th>Expected band sharing of 3° relatives</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.113</td>
<td>34.40 ± 8.45</td>
<td>7.39 ± 2.65</td>
<td>0.21 ± 0.11</td>
<td>0.59</td>
<td>0.58</td>
<td>0.40</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Probabilities of identity and false inclusion probabilities:

<table>
<thead>
<tr>
<th>Probe</th>
<th>Probability of identity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Parental false inclusion probability&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Paternal false inclusion probability&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>33.15</td>
<td>7.50 × 10^-24</td>
<td>2.82 × 10^-15</td>
<td>1.36 × 10^-9</td>
</tr>
</tbody>
</table>

<sup>a</sup> P = x^n where x is the mean band sharing coefficient and n is the mean number of bands scored per individual.

<sup>b</sup> P = (1-(1-x))^n

<sup>c</sup> P = x^m where m is the number of paternal-specific bands (m = 13.06 ± 3.50 SD, for 58 father-offspring combinations scored).
Within the population of adults analysed there is a chance that dispersion of nestlings over breeding territories will produce related individuals (primary relatives) breeding adjacently. One example of this was identified: two apparent sibling males YMWWY and YMBWR breeding on adjacent territories during 1988, 1989 and 1990. The band sharing between these two males was 0.69 which was very significantly higher than expected for unrelated individuals.
As shown in Table 2.3 cases of EPP arose within their broods and in neighbouring broods in which both males may have been the father. However, with sufficient scorable bands this presents no problems in assigning paternity between the two males, with the worst scenario being a minimum of 9 scorable bands which should indicate at least one band which is not present in the female and only present in one of the males. Both males were assessed in each case of paternity identification, when all putative paternal bands within an offspring were accounted for then paternity could be ascribed. In all the remaining cases one sibling had significantly more mismatches (5 or more) and was known not to be the father.

A large proportion of offspring had bands which were present in neither parent. The distribution of mismatches for Block 1 during 1989 (N = 63) is shown in Figure 2.3. The pattern of mismatching bands is similar to those reported by other studies (Lifield et al 1991; Birkhead et al 1990; Westneat 1990), although the actual frequency of each class is different. The majority of offspring (35) were recorded as having no mismatches, a few (6) mismatched for one or two bands and the rest (21) recorded more than four mismatches. A single case of three mismatches arose involving the two brothers (YMWYW and YMBWR) as previously mentioned. Assuming that up to two novel bands are the result of a mutation then there is a mutation rate of 0.003 per fragment per meiotic event. This value matches closely those observed in humans (Jeffreys et al 1985b) and other species of birds (Burke & Bruford 1987; Wetton and Parclin 1989; Westneat 1990; Hanotte et al 1992; Pinxten et al 1992). The observed distribution of offspring having 0, 1, or 2 novel bands is not different from that expected under a Poisson distribution (G = 1.036, p > 0.1) (see Burke & Bruford 1987). From the Poisson distribution, the probability of an offspring having 5 novel bands due to mutation alone will be $1.7 \times 10^{-6}$. Knowing that the fragments are essentially unlinked it can be assumed that the instances of five or more novel bands are not the result of one mutational event leading to a large haplotype. The offspring with 5 or more mismatches are therefore the result of a mismatch with at least one of the putative parents.
Figure 2.3
The distribution of mismatching bands observed with multilocus probe 33.155

![Bar chart showing the distribution of mismatching bands.](image)

- Extra-pair
- Within-pair
- Uncertain

Number of novel bands

Number of offspring

0 1 2 3 4 5 6 7 8 9 10 11 12 13
Table 2.3: Parentage summary for 1988 and 1989

<table>
<thead>
<tr>
<th>Year</th>
<th>Male</th>
<th>Female</th>
<th>Territory</th>
<th>Male status</th>
<th>Offspring</th>
<th>Extra-pair</th>
<th>Additional</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>WPP</td>
<td>EPP</td>
<td>males analysed</td>
</tr>
<tr>
<td>1988</td>
<td>RMBBB</td>
<td>YMRBG</td>
<td>JC88</td>
<td>M</td>
<td>5</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>YMWYB</td>
<td>YMBYW</td>
<td>L88</td>
<td>M</td>
<td>4</td>
<td>2</td>
<td>2 RMBBB</td>
</tr>
<tr>
<td></td>
<td>YMYYW</td>
<td>YMYYW</td>
<td>M88</td>
<td>M</td>
<td>4</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>RMBBB</td>
<td>YMGBR</td>
<td>YB88</td>
<td>M</td>
<td>4</td>
<td>0</td>
<td>4 URYS*</td>
</tr>
<tr>
<td></td>
<td>RMRWR</td>
<td>YMMYW</td>
<td>JDITCH1</td>
<td>B</td>
<td>5</td>
<td>1</td>
<td>4 YAMBWR</td>
</tr>
<tr>
<td></td>
<td>YMWYW</td>
<td>RMMGG</td>
<td>JDITCH2</td>
<td>M</td>
<td>4</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>RMRWR</td>
<td>YMBYB</td>
<td>JDITCH1</td>
<td>B</td>
<td>4</td>
<td>0</td>
<td>4 =YMBWR</td>
</tr>
<tr>
<td></td>
<td>YMYYB</td>
<td>YMBYB</td>
<td>JDITCH1</td>
<td>B</td>
<td>3</td>
<td>1</td>
<td>2 M/Osb</td>
</tr>
<tr>
<td></td>
<td>UR</td>
<td>YMBYB</td>
<td>JDITCH1</td>
<td>B</td>
<td>4</td>
<td>0</td>
<td>?</td>
</tr>
<tr>
<td></td>
<td>RMGGY</td>
<td>YMGWR</td>
<td>JDITCH1</td>
<td>M</td>
<td>4</td>
<td>0</td>
<td>4 RMRWW(3/4)</td>
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<tr>
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<td>UR</td>
<td>OSR89</td>
<td>M</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
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<td>3 YMBWR</td>
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<td>GMGRB</td>
<td>YS89</td>
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<tr>
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Percentage where the putative father was available 41.5 58.5
4.5 = monogamous, B = bigamous

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<th>Female</th>
<th>Territory</th>
<th>Male status</th>
<th>Offspring</th>
<th>Extra-pair</th>
<th>Additional</th>
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<td>EPP</td>
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<td>GMGYG</td>
<td>UR</td>
<td>OSR89</td>
<td>M</td>
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<td>1</td>
<td>-</td>
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<td>GMYYB</td>
<td>MMBB89</td>
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<td>0</td>
<td>5 ?</td>
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<tr>
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<td>GMRYB</td>
<td>OSPREY89</td>
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<td>1</td>
<td>1 M/Osb</td>
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<td>YMWGR</td>
<td>MEADOWS89</td>
<td>M</td>
<td>5</td>
<td>0</td>
<td>5 ?</td>
</tr>
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<td>YN89</td>
<td>M</td>
<td>4</td>
<td>3</td>
<td>1 YMBWR</td>
</tr>
<tr>
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<td>GMGGY</td>
<td>YC89</td>
<td>M</td>
<td>10</td>
<td>7</td>
<td>3 YMWWB</td>
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<td>GMGGB</td>
<td>MMGB89</td>
<td>M</td>
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<td>3</td>
<td>2 GMRGB</td>
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<tr>
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<td>GMYYB</td>
<td>GMW89</td>
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<td>9</td>
<td>0</td>
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<tr>
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<td>GMGBB</td>
<td>KW89</td>
<td>M</td>
<td>10</td>
<td>7</td>
<td>3 YMBWR</td>
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<tr>
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<td>YS89</td>
<td>M</td>
<td>5</td>
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<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>84</td>
<td>47</td>
<td>34</td>
</tr>
</tbody>
</table>

Percentage 56.0 44.0
4.0 = unknown individual
UR denotes unringed territorial male or female
? denotes unknown individual
§ fingerprint quality too poor to determine paternity
The offspring of male GMBBR (family KW89) were used in a goodness of fit test to examine if the putative parents was (i) a non relative, (ii) a close relative of the true parent, or (iii) the true parent. The expected numbers were calculated from the expected band sharing value for each category as estimated previously (see Table 2.4 a & b). The band sharing values were analysed for the putative mother, father, and two neighbouring males (YMWWYW and GMWYB). The putative female was found to be the mother of all 10 offspring, whereas the putative father was found to have sired only offspring 1 and 4 of the first brood, and offspring 5 of the second brood. Male GMBBR was unrelated to 7 of the 10 offspring but male YMWWYW was determined as being a second order relative of six of these seven, with the 7th being either a 2° or 3° relative. The overriding conclusion was that the sibling of YMWWYW male was the sire and this was verified as mismatching bands in these offspring are present in male YMBWR. The values for a neighbouring male (GMWYB) are also given (see Table 2.4 a & b) and in all cases he was shown to be unrelated (as were a further 5 neighbouring males). One chick had so few bands in common with male YMWWYW (11%) that although they were definitely not related this was still not significant. The same is true to an extent with other comparisons and may be due to only partial restriction digestion producing a lower than expected sharing level or else the scoring of a single band where two are actually present.

The results obtained from the use of the goodness of fit and mismatching band techniques were in agreement. However the use of mismatching bands does not permit the determination of second order or third order relatives, whilst the goodness of fit would appear to favour high quality autorads without partial digestion of the DNA and produce mismatches consistant with Type I errors.

### Table 2.3a Parentage data summary for 1988 and 1989

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total chicks identified as WPP or EPP (1988 + 1989)</td>
<td>122</td>
</tr>
<tr>
<td>% WPP identified</td>
<td>50</td>
</tr>
<tr>
<td>% EPP identified</td>
<td>50</td>
</tr>
<tr>
<td>Chicks where source of paternity was not ascribed</td>
<td>7</td>
</tr>
<tr>
<td>% Broods with EPP</td>
<td></td>
</tr>
<tr>
<td>1988 (7/10 broods)</td>
<td>70</td>
</tr>
<tr>
<td>1989 (13/19 broods)</td>
<td>68</td>
</tr>
<tr>
<td>average</td>
<td>69</td>
</tr>
</tbody>
</table>
Table 2.4 (a) Band sharing values for two broods from territory KW 89
Matches for putative father, mother and two neighbouring males

<table>
<thead>
<tr>
<th>Chick</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>GMBBR male obs</td>
<td>0.56</td>
<td>0.22</td>
<td>0.15</td>
<td>0.57</td>
<td>0.18</td>
<td>0.23</td>
<td>0.18</td>
<td>0.14</td>
<td>0.12</td>
<td>0.54</td>
</tr>
<tr>
<td>M/Own fem obs</td>
<td>0.51</td>
<td>0.58</td>
<td>0.53</td>
<td>0.55</td>
<td>0.56</td>
<td>0.54</td>
<td>0.53</td>
<td>0.54</td>
<td>0.52</td>
<td>0.60</td>
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<tr>
<td>GMWYB male obs</td>
<td>0.24</td>
<td>0.20</td>
<td>0.24</td>
<td>0.24</td>
<td>0.25</td>
<td>0.15</td>
<td>0.19</td>
<td>0.24</td>
<td>0.24</td>
<td>0.22</td>
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<tr>
<td>YMWYW male obs</td>
<td>0.17</td>
<td>0.39</td>
<td>0.36</td>
<td>0.11</td>
<td>0.35</td>
<td>0.41</td>
<td>0.46</td>
<td>0.49</td>
<td>0.41</td>
<td>0.14</td>
</tr>
<tr>
<td>expected 1°</td>
<td>0.58</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>expected 2°</td>
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</tbody>
</table>

The above table shows the observed band sharing values between adults YMWYW, M/Own, GMWYB and GMBBR and the 10 chicks. The expected band sharing values of primary (1°), secondary (2°), tertiary (3°) and unrelated individuals are also shown.

Male GMBBR and female M/Own are the putative parents, male GMWYB is a neighbour and unrelated to any chick, whilst male YMWYW is a primary relative of male YMBWR who was the extra-pair father of chicks 2, 3, 5, 6, 7, 8, and 9.
Table 2.4 (b). Q values indicating most significant relationships

<table>
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<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>male GMBBR</td>
<td>exp1°</td>
<td>0.35</td>
<td>54.11</td>
<td>79.47</td>
<td>0.24</td>
<td>67.8</td>
<td>51.00</td>
<td>67.80</td>
<td>83.66</td>
<td>92.55</td>
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<tr>
<td></td>
<td>exp2°</td>
<td>10.57</td>
<td>14.82</td>
<td>30.00</td>
<td>11.92</td>
<td>22.7</td>
<td>13.17</td>
<td>22.70</td>
<td>32.73</td>
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<tr>
<td></td>
<td>exp3°</td>
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<td>4.23</td>
<td>13.90</td>
<td>28.95</td>
<td>8.95</td>
<td>3.37</td>
<td>8.95</td>
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<td>0.44</td>
<td>0.78</td>
<td>3.46</td>
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<td>1.21</td>
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<td>0.85</td>
<td>1.21</td>
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<td>22.82</td>
<td>20.94</td>
<td>22.82</td>
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<td>43.88</td>
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<td>49.50</td>
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<td>52.43</td>
<td>49.50</td>
<td>52.43</td>
<td>46.65</td>
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<td>60.71</td>
<td>47.97</td>
<td>47.97</td>
<td>45.08</td>
<td>79.47</td>
<td>64.17</td>
<td>47.97</td>
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<td>18.51</td>
<td>11.60</td>
<td>11.60</td>
<td>10.17</td>
<td>30.00</td>
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<td>11.60</td>
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<tr>
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<td>2.59</td>
<td>1.95</td>
<td>13.90</td>
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<td>0.72</td>
<td>0.72</td>
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<td>0.46</td>
<td>0.72</td>
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<tr>
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<td>19.77</td>
<td>97.28</td>
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<td>6.01</td>
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<td>0.88</td>
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<td>0.93</td>
<td>4.65</td>
<td>10.03</td>
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<td>12.05</td>
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<td>10.60</td>
<td>20.61</td>
<td>31.24</td>
<td>38.58</td>
<td>20.61</td>
</tr>
</tbody>
</table>

Underlined text denotes either most significant or the lowest value between the adults and the chicks. Male GMBBR and female M/Own are the putative parents, male GMWYB is a neighbour and unrelated to any chick, whilst male YMWYW is a primary relative of male YMBWR who was the extra-pair father of chicks 2, 3, 5, 6, 7, 8, and 9.
2.3.2 Extra-pair paternity variation

Table 2.3 shows the distribution of paternity for families obtained in 1988 and 1989. Of the 29 broods analysed where the putative male was available, 20 contained cases of extra-pair paternity, i.e., 69% of broods contained one or more cases of extra-pair paternity. Within-pair paternity (WPP) varied between the two years with 41.5% (95% confidence limits, 24.7% - 52.8%) in 1988 and 58% (46.9% - 68.6%) in 1989. This represented levels of 50% WPP averaged over the two years where the putative male was available. The extent of extra-pair paternity within broods is shown in Figure 2.4. In the cases where the extra-pair father was detected (N = 39, 67%) he was either a neighbour or, in one case, was from one territory away. Cases where the extra-pair male was not identified were either due to poor fingerprint quality or believed to be from the lack of a sample from the appropriate neighbouring male. Although there is a large body of data showing that males move large distances between territories (Chapter 5), it is generally the neighbours that are achieving the actual fertilisation success.

Some males apparently do better than others in achieving reproductive success, as shown in Figures 2.5 (data exclude block 3, see Chapter 1). There is clearly a significant degree of heterogeneity ($\chi^2 = 44.2$, 13 df, $p < 10^{-4}$) with some males better than others at acquiring EPP (note that in some cases it is possible that EPP was achieved elsewhere but was not recorded) so that only 36% of males achieve EPP, while 70% lose some paternity to other males. Almost all males achieved at least some within-pair paternity except RMBRB with no recorded paternity and YMBWR with no within-pair paternity but 18 extra-pair offspring over the two years. Figure 2.6 shows the distribution of recorded paternity against lost WPP, with no significant correlation ($r = -0.365$, $n = 14$, $p > 0.10$). It is possible to hypothesise that: a) there is no relationship between the acquisition of EPP and maintenance of WPP, b) that there is a trade off, with a loss of WPP being the result of EPP acquisition and c) that acquisition of EPP and WPP are positively correlated as a result of male quality (selection for males with good genes). The data provide no evidence to substantiate the latter two scenarios, presumably not just due to variance in individual qualities but also attributable to the random nature of nest loss. The evidence therefore supports the hypothesis that there is no relationship between the acquisition of extra-pair paternity and the maintenance of within-pair paternity.

The data available away from the main study block included 15 offspring for which both parents were recorded, of which 12 were the result of extra-pair fertilisations i.e., 80%. These data were collected to examine if patterns of paternity were altered as a result of disturbance due to persistent observations. The indication from this is that rates of EPP ($\chi^2 = 5.82$, 1 df, $p < 0.02$) may be even higher elsewhere albeit from a small sample size. There was no evidence that observations caused an increase in the rate of EPP.
Figure 2.4
Distribution of extra-pair paternity with brood size

Chicks resulting from EPP

Figure 2.5
Recorded male success

Figure 2.6
Total recorded paternity and lost WPP
Levels of extra-pair paternity have been shown to vary markedly between species (Møller & Birkhead 1992). This study has shown the reed bunting to have one of the highest levels of extra-pair paternity yet studied with only 50% of offspring being the result of within-pair paternity and 69% of broods containing extra-pair offspring, and no identified cases of intraspecific brood parasitism.

The levels of EPP are not consistent with observed rates of EPCs which accounted for only 4.39% of observed copulations ($\chi^2 = 60.52$, 1 df, $p < 10^{-4}$). Reed bunting EPCs are therefore either infrequent but highly successful or else are much less observable than with pair copulations.

Explanations for EPCs have been reviewed by Westneat et al. 1990, and Birkhead & Møller 1992 (see also Colwell & Oring 1989). The genetic diversity hypothesis proposes that the female may actively engage in EPCs to increase the genetic diversity of the brood, whilst the good genes hypothesis states that females will seek to obtain fertilisations from a male of superior fitness. The good genes hypothesis has been supported by behavioural observations, although not paternity data in the swallow *Hirundo rustica* (Møller 1988a) and black capped chïcïcïdes *Parus atricapillus* (Smith 1988). Both behavioural and paternity data have provided evidence to support the good genes hypothesis in the blue tit *Parus caeruleus* (Kempenaers et al. 1992). Studies of the zebra finch (Birkhead et al. 1988a) show that the last copulation before egg laying can account for 80% of paternity. Consequently it would not be difficult for a male to achieve total paternity if a copulation was correctly timed. The two hypotheses of genetic diversity and genetic quality provide different predictions on the rates of EPP. The genetic diversity hypothesis predicts a high proportion of broods with EPP but with mixed within brood EPP consequently broods will contain two or more fathers with an evenly distributed overall level of paternity. The good genes hypothesis (Westneat et al. 1990) predicts that a few males should do better than others with broods fathered by a single male and consequently success will be skewed. It is also possible that both hypotheses may be correct and consequently broods will contain two or more fathers but paternity success will still be skewed.

The data show that 28% of the broods analysed were fathered by a single male, consequently the remaining 72% of broods contained extra-pair offspring but with a maximum of two fathers. Of the broods which contained extra-pair offspring, 64% comprised more than half EPP. The data therefore provide no conclusive evidence to support the genetic diversity hypothesis in that the majority of broods contained mixed paternity but no cases of more than two fathers in any brood. The evidence provided in this chapter indicates that approximately one third of males are responsible for all the cases of extra-pair paternity, indicating that females are seeking certain males above others, supporting the good genes hypothesis. However 70% of males lost some paternity and no correlations were recorded between within-pair paternity and extra-pair paternity indicating no apparent relationship between male quality and success. The data therefore provide no conclusive evidence to support or refute either hypothesis and rather indicates that both hypotheses may be valid. Conflicting results have been found in other species (Birkhead et al. 1990) supporting neither hypothesis whereas data from white crowned
sparrows *Zonotrichia leucophrys* (Sherman & Morton 1988) and indigo buntings *Passerina cyanea* (Westneat 1990) were in favour of the good genes model.

The previous chapter dealt with the hypothetical advantages from the male's perspective of acquiring EPP. The level of nest loss in reed buntings is such that EPP may be the only means by which a male achieves reproductive success. This is to some extent supported by the data from male YMBWR whose only reproductive success was achieved by EPP, due to no territorial fledging success in 1988 and no WPP in 1989. Most (88%) males eventually did achieve some reproductive success in 1989 but from many late nests which are known to have poor fledgling survival rates (Haukioja 1970), consequently any EPP from early nests will have considerable benefits compared to WPP from later nests.

Consequently, the expectation is that males may not only increase their reproductive success by acquiring EPP (Trivers 1972) but it may be their only route to success. Females will therefore potentially have access to a range of males all seeking to acquire EPP and therefore may be able to potentially select both good genes and genetic diversity if suitable males exist (however no cases of three father broods were recorded). Constraints however do exist for the female which include (a) the presence of a guarding partner, (b) the risk of reduced investment by the partner if an EPC is detected (c) male availability and (d) sperm precedence.

Subsequent chapters show that mate guarding does occur and how male behaviour varies in attempting to acquire and prevent EPP. This section has supported the expectation of EPP as a route to male success and providing some evidence to support the genetic quality hypothesis as a route to female success, whilst not refuting the genetic diversity hypothesis.

To what extent males of other species may offset the uncertainty of reproductive success from the pair nest via EPP is unclear but alternative strategies may exist, i.e., polygyny, polyterritoriality or lek breeding in which male genetic quality is the currency of success, rather than a random future event such as nest predation or weather-induced failure. Males therefore seek to achieve reproductive success through EPP whilst female are stuck with the necessity of rearing their offspring and select males based on characteristics of quality i.e., interspecific sexual selection resulting in some males doing better than expected.

To summarise; reed buntings show a high rate of extra-pair paternity, 50% of chicks and 69% of broods. These levels are significantly different from observed levels of extra-pair copulations with only one third of the males achieving extra-pair paternity. No evidence is found to support a correlation between acquisition of extra-pair paternity and maintenance of within-pair paternity.
CHAPTER 3

Copulation behaviour and indications of fertility

Abstract: Copulations in the reed bunting Emberiza schoeniclus are frequent (4.06 per day) during the prelay period continuing through to the laying the third egg. The diurnal pattern of copulations showed no significant early morning peak although on days when fertilisation can occur copulations peaked soon after egg laying. A large between-male variation in the rate of copulation was observed. Observed extra-pair copulations were rare (4.39%) although behaviours such as nest building and male displays clearly indicated the prelay stage. The observations support predictions indicative of species with high sperm competition, in which males copulate to reduce sperm competition and females apparently seek furtive EPCs.

3.1 INTRODUCTION.

Males should attempt to increase their reproductive success by pursuing mixed reproductive strategies in which extra-pair matings are one of the options (Trivers 1972). The frequency and distribution of copulations with respect to the breeding cycle in birds forms the basis for studies on sperm competition. In some species a single copulation is known to be sufficient to ensure fertilisation of a clutch (Lake, 1975) whereas in other species multiple copulations are more normal (see Birkhead et al. 1987; Birkhead and Møller 1992 for a review). The large variation in copulation frequency in birds has been reviewed by Birkhead et al. (1987), who proposed four sets of hypotheses to describe the variation in the timing and frequency of copulations. No strong evidence was found to support the idea that more copulations are (i) required to fertilise larger clutches, (ii) associated with the formation of pair bonds and the stimulation of follicle growth, or (iii) related to the risks of predation of the breeding individuals (Birkhead et al. 1987; Birkhead and Møller 1992 for a review). The fourth set of hypotheses concern the link with sperm competition in respect of which two separate hypotheses are analysed. The first is that copulation frequency can disguise the female’s fertile period and the second is that when the risk of extra-pair copulations (EPCs) is high, pair copulations will similarly occur at high frequency in order to devalue and displace a rival male’s sperm. The tests that Birkhead et al. used to determine the validity of these hypotheses were (i) where pairs are within view of neighbouring conspecifics copulations should occur at a constant rate over an extended period, and (ii) where species are unable to guard efficiently copulation rates should be high. The data reviewed supported the final hypothesis, in that the frequency and timing of copulation were as would be expected a response to the chance of sperm competition from competing males.

In this chapter, I examine the link between the occurrence of copulations and the fertile period, and the existence of associated and other behaviours which might provide an indication of the status of the pair female to non-pair males. Temporal patterns of copulations have been reviewed by Birkhead and Møller (1992). In a sample of 30 species, copulations were found to be most frequent 3 days before the first egg. Most species show a subsequent reduction or total cessation of copulations once the first egg has been laid, presumed to occur due to the inefficiency of sperm transfer once egg laying has commenced. Studies on poultry have shown that sperm are unable to travel up the oviduct once a hard shelled egg is present and that
subsequent uptake of sperm by the storage tubules is much reduced (Brillard and Bakst 1990). When sperm storage also occurs there is also no reason for further copulations during egg laying. One method by which sperm competition facilitates copulations throughout the fertile period is last male precedence. Birkhead et al (1988a & 1989) showed that the last male to copulate in the zebra finch Taeniopygia guttata obtained 80% of the fertilisations. However, studies on species where sperm competition is high have shown that it is reproductively advantageous for a male to continue copulating at a high rate through egg laying. This is found to occur in those simultaneously polyandrous species so far studied: the dunnock Prunella modularis, alpine accentor Prunella collaris, and Smith’s longspur Calcarius pictus (Nakamura 1990; Hatchwell and Davies 1991; J.V. Briskie in Birkhead and Møller 1992). Previous studies therefore would predict that in the reed bunting the temporal pattern of copulations should mainly occur prior to the day of the first egg, unless sperm competition is important, in which case they should continue on through egg laying.

Alternative hypotheses predict different effects on the diurnal pattern of copulations, studies having shown that they are not equally frequent through the day. Of 28 genera reviewed (Birkhead et al 1987), 54% copulated most frequently in the early morning, 25% mainly in the morning and the evening, 4% in the afternoon and 18% with no obvious pattern. In domestic bird species, fertilisation generally occurs within 30 minutes of ovulation, which in turn occurs within 2 hours of laying the previous egg (Howarth 1974; Sturkie 1976). After egg laying, some sperm can reach the site of fertilisation in the infundibulum within about 15 minutes of copulation (Mimura 1939; Bobr et al 1964; Howarth 1971), because the passage of spermatozoa is not obstructed by the presence of an egg. Cheng et al (1983) confirmed in mallards, Anas platyrhynchos, that there was the high probability of a copulation leading to fertilisation during this short period which was termed the ‘insemination window’. Thus for multiple egg clutches, any copulations immediately after laying should have a higher chance of resulting in successful fertilisations. Most birds are known to lay in the early morning (Skutch 1952; Schifferli 1979; Tullett 1985), and so both within-pair and extra-pair copulations are expected to occur during this time of day. Two diurnal patterns may be predicted by the insemination window hypothesis; from the day of the first ovulation and onwards an early morning peak in copulations should occur, whereas before egg laying early morning copulations are not as important and another pattern may arise. Most bird species copulate mainly prior to ovulation, and infrequently or not at all during the laying period (Birkhead et al 1987; Møller 1987c; Aguilera and Alvarez 1989; Birkhead and Møller 1992). There may however be other reasons for the early morning peak. If males are uncertain about when their female will ovulate it will pay them to copulate early on all days prior to the first egg to maximise their chances of coinciding with the insemination window.

When copulations occur they usually consist of a single copulation, but a number of species have bouts of multiple mounts. For example, a maximum of 30 mounts in quick succession were observed in the house sparrow (A.P. Møller personal observation in Birkhead & Møller 1992). Not all copulations may result in sperm transfer since studies of domestic species have demonstrated that the number per ejaculate decreases by about 50% following each copulation (Parker et al 1940, 1942, Lorenz et al 1955; McCartney et al 1958; McDaniel and Sexton 1977; Tan 1980; Bakst and Cecil 1981; Birkhead 1991b). In fact it is unlikely that sperm are
transferred during each copulation, and it is possible that frequent mounts serve to advertise male quality, which is reflected by the higher observed rates of copulation in colonial species than in solitary breeding species. Copulation frequency may advertise male status and also serve to dilute the sperm of competing males. Other methods exist by which sperm of competing males can be reduced in efficacy; for example, clausal pecking by the male dunnock results in expulsion of sperm from the female's oviduct (Davies 1983).

Interference of copulations by other males and its effects upon copulation rates are well documented in colonial and lek species where they can have a marked effect upon mating success (Foster 1983; Trail 1985; Trail and Koutnik 1986). In the fulmar, Fulmarus glacialis (Hunter 1991), interference either resulted in a failure to make cloacal contact or in a forced EPC. In guillemots, Uria aalge, interference was common amongst neighbours (Birkhead et al. 1987) however, contrary to the frequent copulation hypothesis, copulation rates were negatively correlated with colony size. It is possible that at high densities copulations are reduced in response to interference. Little data exist on copulation interference in territorial species. However, in the reed bunting it is possible to predict that when intrusions are low, copulations are likely to be more frequent since the chance of interference by other males is lower. Alternatively if sperm competition is high then the within-pair copulation frequency will be high in order to dilute the sperm of extra-pair males. Consequently the frequency of copulations by reed buntings is examined to determine if there is a relationship with intrusion levels.

Extra-pair copulations may occur either with or without female consent (Fitch & Shugart 1984; Trivers 1972). Refusal to comply with a male seeking an EPC may be costly and even result in death (Huxley 1912; McKinney et al. 1984) but can also result in injury (Frederick 1987a) or continued harassment (McKinney et al. 1983). Costs of disease transmission and retaliation from the pair male are also apparent (Barash 1972), with the additional risk of withdrawal of parental care (Trivers 1972). Proposed benefits include those predicted by the sexy son and good genes hypotheses (Walker 1980). The implication is that females should participate in extra-pair copulations but should be selective and sneaky about their acquisition to avoid negative genetic consequences and withdrawal of parental care. Extra-pair copulations may be frequent in reed buntings but any disparity in the frequency of EPP and EPCs may provide evidence of the route by which females acquire EPCs.

In this chapter I review behaviours linked to the prelay period as components by which other males might judge the status of a female. One or both of the sexes may solicit copulations (Birkhead and Moller 1992). In most species the female is involved in soliciting copulations, however displays by the male are also common and probably important in acquiring successful copulations (see Birkhead et al. 1989). Calls are also used by several species to advertise fertility status of the female to neighbouring males. This is seen in red backed shrikes, Lanius collurio in which females give copulation calls, as a result of which males from neighbouring territories rapidly approach and attempt to interfere (Durango 1956). Montgomerie and Thornhill (1989) hypothesise that calls by females immediately after ovulation also function to advertise fertility status and in their review of 18 species with multi-male mating groups suggested that calls produced male-male competition by which the female was able to select the
highest quality male. In the bearded tit *Panurus biarmicus* a monogamous species, females apparently incite male-male competition by a conspicuous vocal and aerial precopulatory display (Hoi 1989). Calls (other than song) may also be given, perhaps even more frequently by males (Birkhead and Möller 1992) possibly as a means of announcing the male's quality to neighbouring males and females.

A number of displays associated with the fertile period have been recorded in the reed bunting, as described by Andrew (1961). During courtship, males have been observed to display, and visit potential nest sites, repeatedly lowering their bill and making scrabbling movements with their legs. This is also associated with the collection and carrying of nest material by the male in a display in which the male repeatedly shows nest sites to the female (Andrew 1961; pers obs). During reproductive fighting between males, a wing fluttering display is given by the male when at a temporary disadvantage during a contest (Andrew 1961). Wing vibrating is also used by the male in courtship displays and this is also associated with high pitched 'see' notes. The raising of body feathers over the head and rump and spreading of tail feathers are also associated with courtship and fighting. Reed buntings are also recorded as holding the tail spread during reproductive fighting in between slow fights. A further trait described by Andrew (1961) is running with short steps, which forms part of a display in association with some other behavioural elements. Females also exhibit precopulatory displays, and these include the following components: the body lowered, the legs flexed, the bill and tail raised, the wings raised and vibrated, and the tail vibrated in a vertical plane. These visual behaviours may also be associated with soft high pitched calling. There is sufficient evidence to indicate that these displays are performed in clear view of neighbours (Andrew 1961) and serve to obtain copulations and also incite male-male competition between neighbours (Howard 1929; Andrew 1957, 1961).

Further visible signs of fertility or proximate fertility exist. For example nest building in many passerine species coincides with the period of copulations (Lack 1943). Females will also be carrying the burden of additional weight prior to and during egg laying; in the sand martin, *Riparia riparia*, females are apparently 20% heavier and this is detectable by males (Beecher & Beecher 1979; McKinney & Stolen 1982). The indications are that features of reproduction serve not to disguise the fertile period, but rather to advertise it and may provide temporal cues for optimising copulations via last male precedence (Birkhead et al 1988a & 1989).

To summarise, I will examine the pattern and frequency of copulations with respect to the sperm competition hypothesis. Sperm competition is important as demonstrated in Chapter 2 which would therefore predict extended copulations through egg laying. In response to the insemination window hypothesis two diurnal patterns of copulation are predicted, one prior to ovulation with no initial early morning peak and followed by a an initial peak and subsequent decline after ovulation. The frequency of copulations within a bout will be analysed for a link with intrusion activity which reflects high sperm competition. Female behaviours will also be analysed to determine the extent to which females may incite male-male competition. Both male and female behaviours are described as possible indicators of female fertility status.
3.2 METHODS

Observations were made during the breeding seasons of 1988, 1989 and 1990. The study site, distribution of territories and observed patterns of reproductive success are described in Chapter 1. The form of behavioural observations are described in Chapter 4 in more detail than is required for this chapter. The frequency of copulation and nest building behaviours are shown as the rate per hour calculated from those entire minutes of observation during which a male was visible, rather than the total time over which the observations were made. In this way, those periods during which the male could not be seen were excluded. Male reed buntings do not build nests but within this chapter rates of nest building are described, these are rates of nest building by the female of the focal male recorded during an observation bout. The data were relatively easy to collect as during the nest building period males maintain a close proximity to the female (Chapter 4) allowing collection of behavioural data for the male and female.

The diurnal data are presented as the time after sunrise to avoid seasonal influences associated with actual time. Nonparametric statistics were usually used to avoid problems associated with the non-normal distribution of some behavioural observations.

The data recorded during observation bouts include; successful, failed and forced within pair copulations as well as successful and failed extra-pair copulations. Copulation behaviours were also recorded outside the behavioural observation periods however, these are not included in the analysis of rates as none can be calculated for these data. Other behaviours including solicitation displays are also described.

Contact calls heard as 'tseep' style vocalisations (Andrew 1961) were recorded during behavioural observations. The occurrence and frequency of these vocalisations are described in this chapter as one possible indication of fertility status.
3.3 RESULTS

3.3.1 Copulations

The data set is composed of those copulations which were observed during the entire study and those which were observed during the timed observation periods. These data can then be further divided into copulations observed for the first nest and those for subsequent replacement nests. Table 3.1 shows the distribution of copulations for all nests. Of a total of 92 copulations, only 43 were actually recorded during the focal observations.

<table>
<thead>
<tr>
<th>Nest</th>
<th>Total</th>
<th>Focal observations</th>
<th>Bouts recorded</th>
<th>Copulations per bout (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>12</td>
<td>26</td>
<td>1-7</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>26</td>
<td>13</td>
<td>1-4</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>1-4</td>
</tr>
<tr>
<td>Unknown</td>
<td>9</td>
<td>0</td>
<td>6</td>
<td>1-3</td>
</tr>
<tr>
<td>Total</td>
<td>92</td>
<td>43</td>
<td>48</td>
<td></td>
</tr>
</tbody>
</table>

3.3.1.1. Frequency distribution of copulations

In this section I examine the frequency with which copulations occurred within a bout. A bout is defined in this chapter as a period in which the mountings observed occurred in discrete clumps not separated by behaviours other than those associated with mate guarding; for example the bout involving seven copulations involved a maximum gap of 190 seconds between mounts. The data presented in Figure 3.1 shows the copulation bout size and the frequency of occurrence. The modal bout size of one accounts for 34.8% of copulations, however the mean number of copulations per bout is 3.34 (95% confidence limits 2.36, 4.72). Clearly although most copulations encountered will be single, the average bout size is rather different. A comparison of intrusion rates during focal observation periods as a whole with that during the copulation bout ± 5 minutes revealed no significant difference (Student's paired t test, n = 20, p = 0.4809). Therefore there was no evidence that copulations occur in periods of reduced or increased intrusion activity.
Figure 3.1
Frequency distribution of copulations/bout
3.3.1.2. The temporal pattern of copulations

The pattern of copulations recorded for first nests have been analysed separately from those of replacement nests. This is due to the large difference in the prelay period associated with the two data sets. The prelay period of first nests extends from pairing frequently for greater than 20 days prior to the first egg. However, the prelay period of replacement nests following failure is most frequently only 5 days (n = 22, of 29 nests, 76%), and true repeat clutches were only observed on 2 occasions.

a) First nests

The number of copulations and pairs copulating are shown in Table 3.2. Unfortunately too few copulations were recorded during the behavioural observations to provide an indication of actual copulation rates per hour. However, the data are shown in Figure 3.2 as the proportion of the total pairs recorded copulating allowing the inclusion of all data.

<table>
<thead>
<tr>
<th>Copulations</th>
<th>Pairs copulating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>Focal observations</td>
</tr>
<tr>
<td>49</td>
<td>12</td>
</tr>
</tbody>
</table>

Copulations began as early as day -19 and continued through to the day of the second egg. The distribution is probably a good representation of the true distribution of actual copulation behaviour, since my observations were relatively constant throughout, only reducing in periods of bad weather.

b) Replacement nests.

The data for all replacement nests are presented in Table 3.3 and Figure 3.3. No copulations were recorded for nest 3 and only five were observed for nest 4. Only a single copulation was recorded prior to day -5 and that was between a female and her second male, (the first having disappeared earlier in the season) and just prior to her divorcing him and moving to breed with a third male. For all nests starting on day -5 (76%) no copulations were observed during the first 24 hours, this would suggest that once a nest has failed there is a lag before the female is ready to copulate again. The distribution of copulations therefore indicate that following a 24 hour lag when no copulations are observed there is copulation activity through to the third egg day. The distribution of copulations shows little variation apart from a minor increase in activity on days -1 and 0, however this variation was not significant (KW = 5.664, 6 df, p >0.10). The data in Table 3.3 are presented as the observed copulation rate per hour for replacement nests.

<table>
<thead>
<tr>
<th>Table 3.3 Summary of replacement nest copulation data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copulations</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Pairs copulating</td>
</tr>
<tr>
<td>Total time visible days -5 to +2</td>
</tr>
</tbody>
</table>
Figure 3.2
Distribution of copulations for first nests

Figure 3.3 Replacement nests
Copulation rate/visible hour
3.3.13. Diurnal patterns of copulations

Studies of other species as described in the introduction to this chapter found a general peak in activity during the early morning. Table 3.4 shows the distribution of copulations with respect to sunrise and minutes in which birds were visible, for days -19 to +2. For those days on which fertilisation is actually possible, days -1 to +2 the data have been separated to evaluate the insemination window hypothesis. Figure 3.4 shows the pattern of copulations for days -19 to -2. There is an absence of copulations in the hour after sunrise, after which the rate increases up to 4-5 hours post-sunrise, after which it steadily declines again until no more were recorded after 8 hours (despite 333 minutes of observations). The pattern of pairs recorded copulating is not similar indicating that copulations per pair change through the day whilst pairs copulating do not.

When possible, the timing of egg laying was recorded. The data fall into two categories, those where a near exact time was noted and those where the latest possible time was known, the remaining egg data was collected after observations had terminated for the day in order to minimise disturbance. The former was (mean ± SE) 42.25 ± 5.33 minutes post sunrise (n = 8 eggs from 5 females), and for the latter 87.70 ± 9.61 minutes post sunrise (n = 20 eggs from 9 females). Consequently I conclude that eggs are probably laid within the first hour after sunrise, however it would be wrong to exclude the possibility that eggs will still be laid during the second hour after sunrise. An analysis of the distribution of copulations on days -1 to +2 when fertilisation occurs (Figure 3.5) revealed no copulations during the first two hours after sunrise but as predicted by the insemination window hypothesis a peak in the hour following egg laying, and thereafter a decline until no copulation attempts were recorded beyond 8 hours after sunrise. When the pattern of copulations prior to day -1 is analysed (Figure 3.6), it follows a distribution similar to that of the overall data (days -19 to +2), peaking 4 - 5 hours post-sunrise. Although the distributions of copulations for days -19 to -2 and -1 to +2 are not significantly different (Kolmogorov Smirnov $\chi^2 = 2.00$, 2 df, p >0.20), they are not significantly correlated (Spearman rank correlation coefficient, $r_s = 0.306$, n = 9, p = 0.19). The data therefore indicate that the overall pattern of copulations across hours does not vary significantly on days -1 to +2 but it is not correlated with the levels on days -19 to -2.
Figure 3.4
The Diurnal pattern of copulation behaviour
days -19 to + 2.

![Graph showing the diurnal pattern of copulation behaviour from days -19 to +2.]

Figure 3.5
The diurnal pattern of copulation behaviour
days -1 to + 2

![Graph showing the diurnal pattern of copulation behaviour from days -1 to +2.]

55
Figure 3.6
The diurnal pattern of copulation behaviour
days -19 to -2

Hours after sunrise

Copulations/ hour

<1 1-2 2-3 3-4 4-5 5-6 6-7 7-8 8-12
Table 3.d. The diurnal distribution of copulations and copulation bouts

<table>
<thead>
<tr>
<th>Time post sunrise</th>
<th>Minutes observed</th>
<th>Bouts observed days -19 to +2</th>
<th>Copulations days -19 to +2</th>
<th>Copulation rate per hour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>visible</td>
<td>total</td>
<td>focal observations</td>
<td>per hour</td>
</tr>
<tr>
<td>&lt;1</td>
<td>109</td>
<td>2</td>
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<td>0.00</td>
</tr>
<tr>
<td>1-2</td>
<td>697</td>
<td>5</td>
<td>2</td>
<td>0.17</td>
</tr>
<tr>
<td>2-3</td>
<td>690</td>
<td>12</td>
<td>4</td>
<td>0.35</td>
</tr>
<tr>
<td>3-4</td>
<td>932</td>
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<td>6-7</td>
<td>630</td>
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<td>7-8</td>
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<td>3</td>
<td>0.38</td>
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<tr>
<td>&gt;8</td>
<td>333</td>
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<td>0.00</td>
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</table>

<table>
<thead>
<tr>
<th>Time post sunrise</th>
<th>total</th>
<th>focal observations</th>
<th>19 to +2</th>
<th>-1 to +2</th>
<th>-19 to -2</th>
</tr>
</thead>
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<tr>
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<td>1.12</td>
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<td>5-6</td>
<td>19</td>
<td>12</td>
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<td>0.55</td>
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<td>0.38</td>
<td>0.29</td>
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</tr>
</tbody>
</table>
3.3.1.4. Individual variation in copulation rates

Individual male rates of copulations are shown in Table 3.5. The average copulation rate was 0.58 per hour, of visible minutes. Males vary greatly in their copulation rates, for example, male PMRPG was visible for nearly 10 hours during 1990 up to and before day +3 and 19 successful copulations were recorded whereas male GMYB was visible for half as much time again in which no copulations were recorded. No relationship exists between time visible (>2 hours) and copulations recorded (Spearman rank correlation, \( r_s = 0.337, n = 17, p > 0.10 \)) suggesting that individual male or territorial variation exists. However, the differences between males were not significant (KW = 14.256, 18 df, p > 0.50). Figure 3.7 shows the distribution of copulation rates for males visible for more than 100 minutes (n = 13). Five males recorded no copulations, four males < 0.5 copulations per hour, one male for 0.50 - 1.00 and 1.01 - 1.50 and 2 males recorded 1.5 - 2.0 copulations per hour. Territorial variation cannot be ruled out as a cause; for example, male GMRGB and male PMRPG both occupied the same territory in 1989 and 1990 respectively and both were frequent copulators, however no similarity in neighbour copulation rates was apparent. It is probable that chance may play a large role in the recording of copulations and since they are rarely observed, then a few chance sightings can produce a relatively large copulation rate. For example, male GMBBR occupied a relatively densely wooded territory KW during 1989, a single observation period of 33 minutes yielded 9 minutes in which the male was visible and 6 copulations and one failed EPC attempt occurred. Further attempts at observations were terminated simply because the pair were seldom located and easily lost within the territory.
<table>
<thead>
<tr>
<th>Year</th>
<th>Focal Male</th>
<th>Copulations</th>
<th>Minutes</th>
<th>Copulations per hour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>observed</td>
<td>visible</td>
</tr>
<tr>
<td>1989</td>
<td>GMBBR</td>
<td>6</td>
<td>33</td>
<td>9</td>
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<tr>
<td></td>
<td>GMGWG</td>
<td>0</td>
<td>315</td>
<td>137</td>
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<tr>
<td></td>
<td>GMRBG</td>
<td>1</td>
<td>345</td>
<td>127</td>
</tr>
<tr>
<td></td>
<td>GMRGB</td>
<td>2</td>
<td>258</td>
<td>114</td>
</tr>
<tr>
<td></td>
<td>RMBYY</td>
<td>0</td>
<td>196</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>YMBWR</td>
<td>0</td>
<td>482</td>
<td>195</td>
</tr>
<tr>
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<td>141</td>
</tr>
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<td></td>
<td>YMYBG</td>
<td>0</td>
<td>131</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>YMYGG</td>
<td>1</td>
<td>599</td>
<td>136</td>
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<tr>
<td></td>
<td>YMYGR</td>
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<td>188</td>
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<td>1990</td>
<td>GMRBG</td>
<td>1</td>
<td>1103</td>
<td>748</td>
</tr>
<tr>
<td></td>
<td>PMFN</td>
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<td>351</td>
<td>122</td>
</tr>
<tr>
<td></td>
<td>PMRPG</td>
<td>19</td>
<td>1230</td>
<td>593</td>
</tr>
<tr>
<td></td>
<td>RMBYY</td>
<td>0</td>
<td>87</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>YMBWR</td>
<td>3</td>
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<td>842</td>
</tr>
<tr>
<td></td>
<td>YMWB</td>
<td>0</td>
<td>61</td>
<td>1</td>
</tr>
</tbody>
</table>
Figure 3.7
Distribution of male copulation rates
(time visible)

Number of males

0  1  2  3  4  5  6

Copulation rate / hour

0  <0.5  0.5-1.0  1.0-1.5  1.5-2.0
3.3.1.5. Calculation of actual copulation rates

Assuming that males copulate on average 0.58 times per visible hour and that during the period of copulations they can copulate during 7 hours, then an estimated 4.06 copulations occur per pair each day. This level of copulation places the reed bunting amongst species with high copulation rates (22 copulations/ female/ day Birkhead et al. 1987) although it is considerably lower than many species (range 1-40 copulations/ female/ day). However, the variation in observed copulation rates is large with a range of 0.00 to 1.94 copulations per hour of males visible for more than 100 minutes, therefore the assumption that all individuals copulate at the same rate may be false.

3.3.1.6. Failed copulation attempts

Failed copulation attempts occurred when the male attempted to mount the female but failed to make cloacal contact or when the male was physically rejected and was either rebuked vocally or deterred by the female moving away. Over the course of the study I witnessed 22 failed copulation attempts from 11 pairings, with a maximum of 4 failures for any single pair. Twelve of these failed attempts were associated with the first nest, 11 of which corresponded to the period of successful copulations (day -19 to day +2), the twelfth occurring on day -28. Of the 10 failed copulations that were associated with replacement nests four occurred on day -5 when no successful copulations were recorded. The remaining six failures were all between day -2 and +1. Overall 19.3% of all copulation attempts witnessed failed to make cloacal contact or resulted in rejection by the female.

A number of reasons may account for these failed attempts. For example, females may reject males as they are not yet receptive. This may account for the five observed failures which occurred outside the period of successful copulations. A single failed attempt was associated with displacement by an intruder who was apparently attempting, but failing, to gain an EPC. Twelve more were either closely preceded or followed by successful copulations. Three were from a pair with a known high rate of copulation (PMRPG, see Table 3.5), and two were for a pair where no successful copulations were observed. The main reasons for failure, therefore, would appear to be asynchrony between the male and female and attempts associated with other successes.

3.3.1.7. Extra-pair copulations

Extra-pair copulation attempts were recorded on four occasions, only two of which appeared to be successful. Of the failed attempts, the first (GMBBR, day -14), was when the pair male was knocked from his mate by an intruder (neighbour YMBWR), however before cloacal contact was made the pair male replaced the intruder on top of his mate. The second failed attempt occurred between a female (day -3) and a neighbouring male in the absence of the pair male, who was recorded as singing at least 20 m away; three failed attempts at mounting were made each resulting in a vocal rebuke and rejection by the female. The third and successful EPC event (YMWYB, day 2), was between the fertile female of a bigamous male and an unknown intruder and resulted in no male aggression and appeared to have been ignored.
despite having been witnessed by the pair male. It is possible that since only one pair were even recorded to copulate on day 2 that by this stage most males have ceased their copulation behaviour and regard EPC attempts as of no threat. The final apparently successful EPC (unknown day of the nest cycle) was observed by a male who had travelled more than 2 km from his own territory as described in Chapter 5. Extra-pair copulations and attempts represented 4.39% of successful + failed attempts.

3.3.1.8. Male response to EPCs and forced copulations

Forced copulations were recorded as such on only two occasions (2.1%) on adjacent territories and were within-pair following periods of intense neighbour intrusion. Both were observed for the first nest and consisted of the male flying onto the female and forcing her to the ground, then mounting whilst holding the female by her nape. Shortly after flying up the male was followed within a minute by the female. It is possible that this behaviour is more frequent than recorded here since rapidly growing ground vegetation cover makes it impossible to observe many activities. Both records were within the fertile period described above and occurred on day -5 and day 1.

Direct reaction of males to EPCs was observed to be within-pair copulation behaviour, and in 3 attacks on females which were also recorded on a number of other occasions (n = 14). The frequency of occurrence is extremely low and therefore not suitable for analysis in the following section. The attacks are limited to the prelay and egg laying periods with rates of 0.12 ± 0.08 and 0.06 ± 0.06 per hour respectively. No attacks were observed during incubation or provisioning, indicating that they are limited to the periods when copulations also occur. If the prelay data is extrapolated to a daily basis then females are attacked at least once a day.

3.3.2. Behaviours associated with the prelay (fertile) period

3.3.2.1 Grass display

As part of the pre-nest building process and associated with pairing a male will pick up dried grass and fly between the female and surrounding grass tussocks, presumably to encourage the female to commence nest building. It may well be that the areas in which the male performs this display are easily guarded areas and that a male will benefit if his female nests where he indicates. Grass displays were observed by nine males, four males were observed prior to their first nests and five were observed during the prelay period of replacement nests. The grass displays for the first nest were observed between days -33 and -12, the record for day -12 being the only one associated with copulations. For the replacement nests all grass displays were observed on days -6, -5 and -4. For four of these five displaying males the female was nest building and one of these males was also recorded copulating. The distribution of grass displays corresponds to the commencement of nest building.
3.3.2.2. Nest building

Nest building was observed between days -19 and -1 (n = 18 pair observations of known day) for first nests and between -7 and +2 (n = 40, pair observations of known day), for replacement nests. No visible peak in nest building was recorded for the first nest and the data indicate a low rate of nest building over an extended period. Figure 3.8 shows the number of females observed nest building per hour (minutes the male was visible) on all replacement nests starting on day -5 (females were not the focal individual). The data show a lag of one day prior to the commencement of nest building on day -4 which then peaks on day -2 and then all but ceases once the first egg has been laid. The extent to which copulations and nest building corresponded for individual pairs was quite low i.e., only 11.1% (2/18) of observation periods for first nests and 10.0% (4/40) occasions for replacement nests, so although the distribution of copulations and nest building correspond temporally they would appear to be largely exclusive activities. Analysis of copulation rates and nest building rates over the period from day -5 to +2 reveals no significant correlation (Spearman rank correlation, $r_s = -0.194$ n = 8, $p > 0.20$).
Figure 3.8
Temporal pattern of nest building for replacement nests
3.3.2.3. Diurnal distribution of nest building

Table 3.6 and Figure 3.9 shows the diurnal distribution of females recorded nest building and the minutes of nest building per hour (minutes of visible male activity). The data show a gradual rise in nest building activity after dawn until most females are recorded 3-4 hours post-dawn, whereas more time is actually spent nest building between 2-3 hours after sunrise. Rates then decline until they have ceased 8 hours after sunrise. A comparison of copulation rates per hour and minutes of nest building activity shows the two not to be correlated ($r_s = 0.370, n = 8, p > 0.20$)

| Time after sunrise hours | Females observed | Minutes of observed
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nest building</td>
<td>nest building per hour of time the male was visible</td>
</tr>
<tr>
<td>&lt;1</td>
<td>3</td>
<td>13.20</td>
</tr>
<tr>
<td>1-2</td>
<td>12</td>
<td>13.26</td>
</tr>
<tr>
<td>2-3</td>
<td>15</td>
<td>17.74</td>
</tr>
<tr>
<td>3-4</td>
<td>18</td>
<td>13.26</td>
</tr>
<tr>
<td>4-5</td>
<td>9</td>
<td>5.74</td>
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<tr>
<td>5-6</td>
<td>4</td>
<td>3.65</td>
</tr>
<tr>
<td>6-7</td>
<td>4</td>
<td>0.10</td>
</tr>
<tr>
<td>7-8</td>
<td>2</td>
<td>0.25</td>
</tr>
<tr>
<td>&gt;8</td>
<td>0</td>
<td>0.00</td>
</tr>
</tbody>
</table>

A range of additional behaviours were associated with copulations, these comprise solicitation displays by both males and females in which either one or both individuals flap their wings.
Figure 3.9
The diurnal pattern of nest building

![Bar chart showing the diurnal pattern of nest building.](image)

- **Rate / hour**
- **Hours after sunrise**

- **Females observed nest building**
- **Minutes nest building**
3.3.2.4. Male displays
A range of displays were exhibited by males and were classified by the behaviour observed.

Solicitation display
Very prolonged solicitation displays were observed twice during the prelay period performed by different males and involved a prolonged circling of the female whilst wing flapping and calling. Whilst the male performed this display the female continued to nest build, collecting material and returning to the nest and recommencing nest-building. When the female stayed in any one place for long enough the male would resume his very obvious and vocal solicitations. The ultimate result was that both pairs were subject to persistent intrusions from the surrounding males, whilst no copulations were observed. More usual was a simplified and short period of wing fluttering prior to a copulation (see section 3.3.2.5. female solicitation)

Male plumage display
This display by three males was observed between days -7 and +2 but was especially prevalent for one male (YMGYB) in which it was recorded between days -3 and +2 for two repeat nests. The display was performed in an area of open grass and involved the male erecting head and nape feathers, extending his chest and moving in a clearly exaggerated hop in clear view of his neighbours. The significance of this is unclear but was only recorded on days believed to be within the female’s fertile period and was a very obvious display apparently directed at neighbouring males. This display may have been more frequent than recorded since it relied on a clear view of the male whilst at ground level, which was usually not possible.

3.3.2.5. Female displays
Females were recorded as soliciting both visually and audibly. The visual solicitation involved a flattening of the female’s body and wing fluttering prior to a copulation attempt or refusal by the male. This solicitation was often extremely rapid and probably missed during many focal male observations. Female wing fluttering was recorded on 23 occasions (7 females) during focal watches all between days -19 and 0, of which 13 were associated with successful copulations. Male solicitation was less prevalent, only being recorded on 6 occasions (4 males), during observation bouts, three of which were followed by successful mountings. Two male solicitation displays as described above were elaborate, obvious and unsuccessful. Female displays could also be extremely obvious; two females (GMBBR 1989 and YMWHY 1989) were both recorded as soliciting with vigorous wing fluttering and loud vocalisations. In the case of GMBBR the male was dislodged from a mounting by an intruder who attempted an EPC as described previously.

Ten females were weighed both during and prior to egg laying and the prelay period. The average weight of females prior to breeding was 18.51 ± 1.53 g which increased to 21.63 ± 1.23 g, an average increase of approximately 17% not too dissimilar from the 20% recorded for sand martins (Beecher & Beecher 1979). The gain in weight may alter the flight pattern of females sufficiently for males to determine the stage of maturation.

3.3.2.6. Female absence
The apparent temporary loss of the female was recorded on 4 occasions, two in 1989 and two
in 1990. These may have been times when females were absent from their territory actively seeking EPCs. Combined with examples of females entering neighbours' territories whilst their pair male was in attendance, followed by subsequent absence may provide an indication of how EPCs actually occur. Rather than resulting from persistent attendance of intruders seeking opportunistic EPCs, females may deliberately select and seek copulations from males out of sight of their partners.

3.3.2.7. Occurrence of contact calls

'Tseep' style vocalisations defined as contact calls (Andrew 1961), were recorded during behavioural observations as described in Chapter 4. Analysis of their distribution shows no significant between-stage variation ($z < -1.200$, $p > 0.20$). However the frequencies in the prelay and egg laying periods are higher than in subsequent stages. The occurrence of tseep vocalisations showed a strong decline through the season ($r_s = -0.464$, $n = 123$, $p < 10^{-5}$). No significant difference in vocalisations occurred across days 98-150 (Kruskal Wallis anova, $KW = 8.2$, 4 df, $p > 0.05$) however, the difference was significant when days 151-200 are included ($KW = 31.71$, 8 df, $p < 10^{-3}$). The association was largely linked with the first nest during the period described in Chapter 7 during which song output declines. The level of contact calls for first nests was significantly greater than for subsequent ones (Wilcoxon signed rank, $z = 3.101$, $p < 0.002$).

Maximum and minimum temperature are both significantly negatively correlated with the occurrence of contact calls ($r_s > -0.135$, $n = 560$, $p < 0.002$), this may well be due to the seasonal change in output described previously. No correlations were found with the hours of sunshine, wind speed or rainfall ($r_s < 0.050$, $n = 560$, $p > 0.20$).

Neighbours within 100 m ($r_s = -0.586$, $n = 20$, $p < 0.02$) and the number of adjacent neighbours ($r_s = -0.453$, $n = 20$, $p < 0.05$) were significantly negatively correlated with the level of contact calls. Analysis of individual stages of the nest cycle showed that neighbours within 100 m were significantly negatively correlated with contact call output during incubation ($r_s = -0.582$, $n = 18$, $p < 0.02$), all other comparisons were not significant.

A comparison of contact call output by stage of the focal male and that of neighbouring males showed that only when in the prelay stage was output significantly higher when neighbours were also in the prelay stage (Wilcoxon signed rank, $z = -2.547$, $n = 11$, $p < 0.02$). This result may well be a reflection of the seasonal association with the first nest when pairs have a greater tendency to be synchronous.

Contact call output appears to have a strong seasonal element associated with first nests and probably the prelay period. Neighbour density is negatively correlated with contact calls indicating that on smaller territories which have a high density of surrounding neighbours males vocalise less than on larger territories with more distant neighbours. This may indicate that contact calls function primarily to contact mates when visual location becomes difficult.
3.4. DISCUSSION

The data indicate that in general reed buntings are frequent copulators based on the criteria devised by Birkhead et al. (1987). The average rate of 0.58 copulations per hour, gives a level of 4.06 copulations per day, which for replacement nests is spread from day -4 to day +2 giving an estimated 28.42 copulations per nest. Differences between males were not significant although most males were seldom or never recorded copulating. Copulations were also rapid and most frequently single which may also lead to the failure to observe a copulation.

Copulations were also rapid and most frequently single which may also lead to the failure to observe a copulation. The sperm competition hypothesis predictions that (a) copulations will occur at a high frequency (see Birkhead et al. 1987) and (b) will continue through egg laying. The first prediction is supported by the results which show the reed bunting to be a frequent copulator (Birkhead et al. 1987). The second prediction is only partly substantiated as copulations did occur until the day of the second egg, with one pair copulating on the day of the third egg. In comparison other species where sperm competition is important i.e., the dunnock, alpine accentor, and Smith's longspur copulations continue through to the day of the last egg (Nakamura 1990; Hatchwell and Davies 1991; Briskie in Birkhead & Möller 1992).

Of the other hypotheses there are few testable predictions which can be tested within a single species. No predictions are available from the fertilisation or social bond hypotheses. The one prediction from the predation hypothesis that can be examined is that in small birds where predation is a problem then copulations should be of a short duration. This is not rejected as copulations occur rapidly mainly within a single bout. However this may also be a response to intrusion pressure, although no correlation with intrusions was observed. It may also be argued that copulations need not be long to deliver sufficient sperm and that the low number of large bouts was due to sperm depletion. There is little evidence to support the sperm depletion hypothesis other than in species with exceptionally high copulation frequency (chicken Gallus domesticus: Parker et al. 1942, McDaniell and Craig 1959, McDaniell and Sexton 1977; turkey Meleagris gallopavo: Lorenz et al. 1955, McArtney et al. 1958, Balsat and Cecil 1981, Anah et al. 1984; duck Anas spp.: Tan 1980; see also Birkhead & Möller 1992). It is also probable that males store sperm, based on the size of the cloacal protrusion of male reed buntings which is only marginally smaller than that of the dunnock (personal observation) in which it serves to store sperm (Birkhead et al. 1991). It would therefore appear, that at least in part, the speed of copulation and bout size are consistent with the predictions the predation hypothesis.

The temporal pattern of copulations in reed buntings extends from day -19 to +2 in first nests and from day -4 to +2 in replacement nests. The extended period of copulations of first nests clearly doesn't serve purely to fertilise eggs (Birkhead 1988; Birkhead and Möller 1992), but it is possible that males cannot be exact in predicting the time of female fertility. Consequently males use the commencement of nest building as a predictor of fertility. For first and repeat nests the commencement of copulations is matched by that of nest building. It is possible to hypothesise that males, attempt to copulate through into egg laying and thereby maintaining a constant supply of sperm to the female, effectively reducing sperm competition and maximising chances of last male precedence. This however, cannot be substantiated as there is no evidence to whether the male or female dictates the pattern of copulations.
The diurnal pattern of copulations lends support to the insemination window hypothesis, i.e., as predicted the days on which ovulation occurs have an early morning peak (Møller 1987a), but the two patterns of copulations were not significantly different. The data therefore neither refute nor substantiate the insemination window hypothesis. No correlation was observed between nest building and copulations the two behaviours occurring over a similar time period but rarely occurring within the same observation bout. Consequently actual rates of nest building activity are not presumably an absolute guide to fertility status.

No relationship was found between copulation frequency and intrusion rates within the bout. This was not unexpected since most incidences of interference have been recorded in colonial breeders (Birkhead et al. 1985; Hatchwell 1988; Birkhead et al. 1987) and lek breeding species (Foster 1983; Trail 1985; Trail and Koutnik 1986).

Failed copulations (19.3%) do occur, but were often either being outside the period of successful copulations or were associated with the acquisition of successful copulations. Forced copulations were scarce (2.1%) and were in response to previous intrusion incidents. The true extent to which they occur may be greater since they are very rapid and furtive. Unforced pair copulations do occur in response to intrusions in other species (Melde 1984; Simmons 1990; Cramp and Simmons 1983; Morley 1949; Birkhead et al. 1989; Edinger 1988) and forced pair copulations also occur in response to interfering males (Barash 1977; McKinney and Stolen 1982; Derricke 1977 cited in Mock 1983; Afton 1985) and in response to EPCs (Wittenberg 1968; Goodwin 1955; Birkhead et al. 1989). Extra-pair copulations and attempts were infrequent in this study (4.39%), which is contrary to expectation from the actual pattern of reproductive success (Chapter 2). The levels of extra-pair copulations and extra-pair paternity are significantly different (χ² = 60.52, 1 df, p < 10⁻⁴). Extra-pair copulations must be occurring, but presumably are either infrequent but highly successful, or are occurring at a higher frequency but are relatively more difficult to observe than within-pair copulations. Evidence for the latter is supported by the periods during which males are observed as having misplaced or lost their partners. Excursions by females to other territories would be concordant with an active participation of females in EPCs (Fitch and Shugart 1984; Birkhead et al. 1985) and incidences of female-initiated EPCs. Female-initiated EPCs have been recorded in a number of species (Grant and Grant 1989; Birkhead et al. 1988b, 1989; Møller 1990a; Alatalo et al. 1987; Birkhead et al. 1985; Wagner 1991; Kempenaers et al. 1992) and be orientated to more dominant/attractive males (Fujikawa and Yamagishi 1981; Frederick 1987a; Smith 1988; Møller 1988a). Female disappearances and vocalisations in which observations were linked to broods with mixed paternity all give support to the conclusion that females are seeking EPCs. Active attacks on females by their pair male also support the hypothesis that females are involved in EPC acquisition; this type of behaviour has been witnessed in ring doves in which males act aggressively to their partners after periods of absence (Erickson and Zenone 1976).

The data collated in this chapter indicate that no attempt is made to hide the fertile period of the female, as can be inferred from nest building, visible solicitations and vocalisations. The patterns of these activities do however not provide a precise cue as to the closeness of egg laying, i.e., nest building continues on day -1 and female weight gain continues during the
prelay period. Consequently males are forced to continue copulating to reduce sperm competition and, if it is important, also achieve last male sperm precedence.

Male displays also occur presumably to firstly attract females (grass display) and then solicit copulations (wing fluttering) (see Andrew 1961 for a review). Males also have displays which may act to display male characters either to surrounding males or to females.

To summarise, reed buntings potentially can copulate frequently, with a large between-male variation, with copulations commencing with nest building and continuing through to the day of the third egg. These observations support predictions indicative of species with high sperm competition, in which males copulate to reduce sperm competition and females apparently seek furtive EPCs. The data support predictions of the sperm competition hypothesis (Birkhead et al 1987) but also the speed of copulation provides support for the predation hypothesis (Birkhead et al 1987).
2. Behaviour during the breeding season and its relationship with reproductive success

The acquisition and prevention of extra-pair copulations, with the objective of accruing greater reproductive success, has produced associated behaviours. Those behaviours concerned with paternity protection were defined as mate guarding (Beecher and Beecher 1979; Birkhead 1979). Behaviours may be split into defined categories, albeit with a degree of overlap, and these categories form the basis of this section. I discuss in Chapter 4 the guarding of females, which includes: vigilance, proximity to the female and the following/pursuit of the female. This has been kept distinct from male attempts at acquiring extra-pair copulations, which forms Chapter 5 and includes territorial intrusions, excursions and violence between males. In Chapter 6 song output is reviewed as a possible cue for females to use as an indication of male fitness and because of the wealth of data and research has been kept separate. The behaviours are drawn together in a final Chapter 7, which discusses the interrelationships among behavioural variables and using the reproductive success data obtained in section 1 to examine possible correlates of reproductive success.

In Chapter 4 the form of data collection and its distribution is described, prior to its use for analysis. The levels of observation are examined, by stage and day of the nest cycle, season, time, nest, environmental conditions, neighbour status and density. Additional to the behaviours observed, data was also collated on individual male morphometric variables i.e., plumage score, wing length, body weight, tarsus length and full head length. Male characters have in a few studies been shown to be reliable correlates of reproductive success (see Newton 1989). One such character is plumage, dimorphism between the sexes being a typical example of a secondary sexual character (Darwin 1871; Fisher 1915). Males are frequently brighter than females and have considerable elaboration as a result of sexual selection (Fisher 1930). Plumage serves a dual function in male competition and female acquisition, ultimately producing females which prefer the more elaborate individuals (Andersson 1982; Møller 1988a). Explanations for variation in character are as numerous as the hypotheses (see Owens 1992 for a review), but evidence does support their role as a signal during forms of competition. Relevant to this study is that these characters of plumage variance have been to be involved in mate choice (Eckert and Weatherhead 1987; Møller 1987b, 1988b; Røskaft and Rowher 1987; Norris 1990b; Norris & Bradbury 1992).

Reed buntings are sexually dimorphic, with males having a conspicuous black head and bib with white collar. Male variation in plumage does occur, showing variability after a prenuptial moult which continues whilst abrasion removes remaining buff tips during the breeding season (Fennell and Stone 1975; Bell 1970; Poulsen 1950). Reed buntings do not start their reproductive behaviours in a full breeding plumage, rather this develops through the season, providing visible and contrasting differences between males. Males also differ in their plumage development and rather than being a character which improves over successive seasons, males are fixed in their plumage brightness (Bell 1968) indicating that rather than being a measure of
current fitness (Fisher 1930) it is probably a fixed genetic character. Consequently males are fixed for life in a trait which might affect female preference. Reed buntings are also sexually dimorphic in other characters i.e., wing length, weight, and bill length (Bell 1968; Haukioja 1969; Fennell and Stone 1976; Wolfenden unpublished). Morphological characters such as these may potentially provide variation which may be selected through inter and intra sexual selection (O'Donald 1983). In this section male characters are examined as one means by which females may select males (see Andersson 1982; Barnard 1990; Møller 1988), and to determine the extent to which individuals vary and whether sufficient variance is present by which females may exhibit choice. If males are fixed for life in the traits which have been hypothesised to be indicators of genetic quality (Fisher 1930), then there may also be selection on females to assess those traits which might be a reflection of current vigour. Song is therefore discussed (in Chapter 6) as one sexually selected trait that may be a good indicator of actual health rather than genetic fitness (Hamilton and Zuk 1982).
CHAPTER 4.

Mate-guarding and female attendance behaviours

Abstract: Mate guarding is hypothesised to be a mechanism by which males protect their paternity. Three behaviours; time spent less than 10 m from the female, flights orientated to the female and vigilance were analysed for the link with guarding in the reed bunting, Emberiza schoeniclus. The guarding of females did occur, with the main phase arising shortly before first egg date (days -4 to -1 for replacement nests), after which it declined. An expected rise in guarding as a response to increased neighbour density did not occur, whilst neighbour status did not influence guarding. A diurnal variation in guarding was not apparent other than for a decline in vigilance during the day for the prelay period. Individual male variation in the levels of guarding was significant.

4.1. INTRODUCTION

Close following of the female by the male was first identified as a means of protecting paternity in the late 1970s. Studies by Beecher and Beecher (1979) and Birkhead (1979) originally defined the behaviour of mate guarding and along with subsequent studies have shown that males are responsible for maintaining the proximity of male to the female and are able to deter other males from obtaining extra-pair copulations attempt (see McKinney et al 1984 for a review and Fitch & Shugart 1984 for a critical analysis of requirements). A male will maintain his proximity to the female whilst she is fertile and by doing so protect his paternity.

With the risk of losing paternity and sperm storage by females enhancing the risk, males will adopt a strategy for reducing the chance of paternity loss. Close association of the female and the male has been shown to reduce the risk of cuckoldry. Removal of the male designed to mimic his short term absence has been performed on the bar-headed goose, Anser indicus (Lambrecht 1989); swallows, Hirundo rustica (Møller 1987a); great tit Parus major (Björklund et al 1991); pied flycatcher, Ficedula hypoleuca (Björklund and Westman 1983) and the zebra finch, Taeniopygia guttata (Birkhead et al 1989). The females of swallows, pied flycatchers and zebra finches were frequently chased and engaged in extra-pair copulations more than controls when their partner was temporarily removed. In the great tit the number of intruders increased with subsequent courting of the females but they failed to engage in extra-pair copulations. Similarly, female bar-headed geese did not engage in EPCs whilst their female was absent. Close following would at least in some species appear to prevent EPCs (see also Mumme et al 1983; Gowaty & Pliisner 1987).

The distance between males and females apparently also reduces the risk of extra-pair paternity. In the pied flycatcher, the risk of EPCs occurring increased linearly with increasing distance between mates. As long as the distance between pair members was less than 5 m, the male was always able to interfere successfully with would-be cuckolders (Alatalo et al 1987). Similar observations on the dunnock, Prunella modularis, and swallows also indicate that distances of less than 5 m between pair members are safe (Davies 1985; Møller 1987a). EPCs may potentially happen very rapidly and, at least in the swallow, males who spent a lot of time...
within 5 m were subject to fewer EPC attempts (Møller 1987a). The distance of 5 m may well be a habitat-related phenomenon and with a change in habitat visibility the safe guarding distance may increase or decrease accordingly. It may be hypothesised that within relatively closed habitat the male should attempt to remain closer to the female than within more open habitat, where a would be cuckold can be seen and intercepted with a greater margin of safety. Consequently, in the relatively open habitat occupied by the reed bunting a greater safe distance may be permitted.

An extension of maintaining proximity to the female is the pursuit of females, with females dictating movement patterns rather than the male. It should follow that the male who is required to guard will initiate fewer flights than the female and will pursue her when she moves beyond a safe guarding distance. For example, Birkhead (1982) showed that during the fertile period female magpies, Pica pica, initiated more flights than did males. Similar patterns have been observed in a number of other species, which overall show females dictating the patterns of movement rather than the male (see Birkhead and Møller 1992 for a review).

Males can also respond to the risk of cuckoldry by remaining alert to the presence of intruders, or solicitation of EPCs by the female (Lumpkin et al 1982). Increased intrusion threat dictates that a male should proportionally increase his level of vigilance, at the expense of other activities such as song.

It has been proposed that females should choose extra-pair partners based on either their quality of genes i.e., the 'good genes' hypothesis (Fisher 1930; Walker 1980), or they should select for males with a spectrum of genetic characters i.e., the genetic variety hypothesis (Williams 1975). A few studies have shown that some individuals have a disproportionate success in obtaining extra-pair copulations and paternity (Møller 1988a, 1990a, 1991a, 1991c). The expectation of the good genes hypothesis is that females should mate with males with secondary sexual characteristics which display male fitness (Andersson 1982, 1986). Consequently, in species where the opportunity arises for male fitness to be evaluated, females will select differentially, reinforcing behaviours which reflect these differences.

Three male behavioural traits are examined in this chapter these being proximity to the female, close following of the female and vigilance. It is hypothesised that these behaviours are associated with the protection of a male's paternity. The following predictions of this hypothesis will be tested: (i) mate guarding increases during the prelay stage, as copulations near to egg laying are more likely to result in fertilisation (Birkhead et al 1987, 1988 & 1989), (ii) the intensity will vary diurnally as individual eggs are fertilised separately 24 hours before laying (Mimura 1939; Bohr et al 1964; Howarth 1971) so copulations before laying will be more valuable. I will also investigate the evidence for individual variation and that these behaviours will be more pronounced (iii) where males are at a higher density and (iv) when neighbouring males are not having to guard their own females.
4.2. METHODS

The methods described in this chapter also refer to the two subsequent chapters. The methods are presented in the form of the behavioural (Y) variables followed by a description of the multivariate technique and subsequently the independent X variables.

4.2.1 Behavioural observations (Y variables)

Focal male watches were performed during the breeding seasons of 1989 and 1990. Behavioural variables were recorded for each minute of the observation bout, which usually lasted between 30 and 60 minutes being dependent on the level of activity observed.

(i) Male to female distance

The male to female distance was recorded at the end of each minute and collated as the time when the male was known to be: < 3 m, 3 - 5 m, 5 - 10 m, 10 - 15 m, 15 - 20 m, 20 - 30 m, 30 - 40 m, and > 40 m from the female. To avoid errors associated with infrequent sightings, the data were sorted as the time observed at a distance as a proportion of time the male was visible for more than 10 minutes. These data were then subsequently split between a) time less than 10 m and b) greater than 10 m. Additionally, to overcome the biases produced by the female being visually more obvious during nest building, the data were reanalysed as a proportion of the total minutes for which a distance was recorded, i.e., a) time <10 m /Σ(time of all recorded distances) and b) time >10 m /Σ(time of all recorded distances).

(ii) Male flight data

Flight data recorded for the focal male included: (i) unaccompanied flights, (ii) flights orientated towards the female and (iii) flights in which the male was followed by the female. The second grouping of female orientated flights were recorded as (a) flights to the female, (b) flights with the female and (c) flights with following the female. The data for analysis were taken as the total female orientated flights observed as a proportion of the total male flights (ii/(i+ii+iii)). Data for unaccompanied flights, all flights and female orientated flights are presented in the initial analysis of between stage variation. Subsequent analyses are performed on female orientated flights as a proportion of the total.

(iii) Male vigilance

Vigilance by the focal male was defined as those whole minutes when the male was not apparently engaged in any other behaviour and so apparently observing activity around him. Whole minutes of vigilance were used to provide values of the proportion of time spent vigilant whilst the male was visible. Periods for which the male was not visible for at least ten minutes were excluded to avoid bias associated with the disproportionate affects of behaviours observed over short bouts.

2.2. Multivariate analysis

The bivariate analysis of behavioural variables is limited in only providing information on the interaction of a single X variable with a behaviour. Stepwise regression permits analysis of the interaction of the X variables with a single behaviour. The results provide X variables which
significantly regress with a behaviour and explain a known amount of variation within the data set. The first model described here assesses the interaction between each Y variable and the X variables described below. The individual behavioural components were transformed ($\log_{10} (1 + x)$), thereby normalising the data and entered into a stepwise regression model with the X variables described subsequently. Data were entered using an F to enter of 2 ($p < 0.10$) to avoid the inclusion of spurious X variables into the model (Sokal & Rohlf 1981). Once the relevant X variables were entered into the model, the residuals were calculated and between male differences analysed for using the Kruskal Wallis Anova for three or more samples. The mean ranks of the residuals for the individual males were then compared with the morphometric data for each male using a further stepwise regression model (F to enter of 2) to determine their relationship with the observed behaviours. The residual data are the end point after the significant influences of X variables have been removed. In Chapter 7 these residual data are used to compare male behaviours with reproductive success.

The variance explained by the regression model is quoted as the adjusted $r^2$ which is a measure of the variance adjusted for the degrees of freedom of the final model. The contribution of the individual components is described by the F statistic with a sign of the correlation and associated probability values.

The independent X variables

4.2.3 Breeding chronology

(i) Stage of the nest cycle
The stage of the nest cycle was split into 5 major groupings: (i) the prelay period defined as the time prior to the first egg, of the first nest or prior to subsequent nests following failure or independence of the fledglings, (ii) the egg laying period, (iii) incubation, (iv) the period of provisioning and subsequent fledging and (v) unpaired males. For multivariate analysis the stages of the nest cycle were entered as categorical data, occurrence entered as 1 and non-occurrence as 0. Data for unpaired males was excluded from all final regression models. Heterogeneity across all stages was tested for using a Kruskal Wallis analysis of variance by ranks, whilst between stage variation was compared using a Mann Whitney two sample test.

(ii) Variation by day
The day of the nest cycle was calculated using a first egg date of 0, with ascending negative values prior to this date i.e., the day before first egg being -1, and ascending positive values after this date i.e., the day of the second egg being 1. When the nest was recorded without knowing the exact first egg date the days were determined using the method devised by Bell (1968). Bigamous males (n = 3) were given a hypothetical idealised nest cycle using data from females with priority in the order of stages (i) to (iv) and with proximity to the first egg date as the second criteria. The data for replacement nests were analysed for between day variation because sufficient data were available for each day, whereas the data for first nests were insufficient. The prelay stage for first nests, as described in Chapter 3, is far longer than for replacement nests; consequently the data are far more spread providing an incomplete set of observations. Days are shown as -5 through to +4, after which days are grouped as days 5 - 9.
and 10 - 15. Days 16+ have been omitted since after this time eggs hatch and provisioning takes place and visible data on males become sparse. The data were entered into the regression model, excluding all days for which a value could not be assigned. Bivariate analyses compared mean levels of behaviour for each day through the nest cycle (-16 to +26) using the Spearman Rank correlation test ($r_s$). Between day analyses were performed using the Mann Whitney U test; the number of observations and males recorded for each day (or grouping) are shown in Table 4.2.1.

<table>
<thead>
<tr>
<th>Day</th>
<th>Female orientated flights</th>
<th>Vigilance and time spent &lt; 10 m</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observation Males</td>
<td>Observation Males</td>
</tr>
<tr>
<td></td>
<td>Bouts n</td>
<td>Bouts n</td>
</tr>
<tr>
<td>-5</td>
<td>19 7</td>
<td>17 7</td>
</tr>
<tr>
<td>-4</td>
<td>24 10</td>
<td>23 9</td>
</tr>
<tr>
<td>-3</td>
<td>26 7</td>
<td>21 6</td>
</tr>
<tr>
<td>-2</td>
<td>33 12</td>
<td>26 9</td>
</tr>
<tr>
<td>-1</td>
<td>34 11</td>
<td>27 9</td>
</tr>
<tr>
<td>0</td>
<td>38 11</td>
<td>22 9</td>
</tr>
<tr>
<td>1</td>
<td>26 8</td>
<td>20 7</td>
</tr>
<tr>
<td>2</td>
<td>27 12</td>
<td>18 9</td>
</tr>
<tr>
<td>3</td>
<td>25 10</td>
<td>17 8</td>
</tr>
<tr>
<td>4</td>
<td>19 6</td>
<td>12 6</td>
</tr>
<tr>
<td>5-9</td>
<td>47 12</td>
<td>28 10</td>
</tr>
<tr>
<td>10-15</td>
<td>15 8</td>
<td>14 7</td>
</tr>
<tr>
<td>Total</td>
<td>333 245</td>
<td></td>
</tr>
</tbody>
</table>

(iii) Individual variation
Two approaches to analysing individual variation were performed. The first was a comparison of the level of behaviours for individual males during the prelay, egg laying and incubation periods using the Wilcoxon signed-rank test. This examines if a significant number of individuals are showing a shift in behaviour with a change in the stage of the nest cycle. The second measure of individual variation is described above in the multivariate analysis section, where residuals of individual males were compared.

4.2.4. Environmental variables
Weather records were obtained from a National Rivers Authority station adjacent to Rutland Water and provided values for: maximum and minimum temperatures ($^\circ$C), wind speed (mph), rainfall (mm) and hours of direct sunshine. The values corresponding to the day of the observation bout were entered into the regression model and bivariate correlations performed using the Spearman rank-order correlation coefficient $r_s$.

4.2.5. Neighbour status and density
(i) Status
Neighbour status was entered into the regression model as a categorical variable for adjacent neighbours present in the prelay, egg laying, incubation and provisioning/fledging periods,
using a 1 for a positive and 0 for a negative record for each of the four X variables. Bivariate comparisons of the four X variables were performed using the Wilcoxon signed-rank test. Consequently comparisons were made of the mean levels of behaviour for each male with and without a neighbour present in one of the four categories. These analyses were performed for the combined prelay, egg laying, incubation and provisioning data and for the prelay period only.

(ii) Density
Two values of neighbour density were derived: density within 100 m of a hypothetical territory boundary and the number of adjacent neighbours. Both variables were entered into the regression model and bivariate comparisons performed using the Spearman rank-order correlation coefficient ($r_s$) of the mean levels of behaviour for the individual males with their neighbour density. These analyses were performed for the combined prelay, egg laying, incubation and provisioning data and for each period separately.

4.2.6. Temporal effects
(i) Day of the year
The days of the year of the individual observation bouts were entered into the regression model as the seasonal variable, January 1st being day 1 etc. The first observation was made on day 98, the last on day 198, the observations therefore spanning 100 days. For the bivariate analyses the data were divided into the following categories: ≤ 110, 111-120, 121-130, 131-140, 141-150, 151-160, 161-170, 171-180 and >180. The analyses were performed using the mean male values for each of the day groups and then comparing across groups. The data were analysed across time using the Spearman rank correlation test ($r_s$) and between the groupings using the Kruskal Wallis Anova (KW)

(ii) Diurnal variation
The time of individual observation bouts was taken as the midpoint of the bout and transformed to provide a time after sunrise, consequently eliminating some of the seasonal component. The time was entered into the regression model for each bout. However, bivariate analysis was used to compare the mean level of behaviour for individual males across the following categories: 0-1, 1-2, 2-3, 3-4, 4-5, 5-6, 6-7, 7-8 and > 8 hours post sunrise. A significant decline or rise in behaviour was tested for using the Spearman rank correlation test ($r_s$) and the mean values for individual males across hours. This analysis was performed (unless stated otherwise) on the combined prelay, egg laying, incubation and provisioning data and for the prelay period alone.

(iii) Nest
The nest of the female was entered as a categorical variable into the regression model for first (1) or subsequent nests (0). Bivariate comparisons of behaviours between each nest were performed using the Wilcoxon signed-rank test, comparing the mean level of behaviour of individual males for first and subsequent nests. This analysis was performed (unless stated otherwise) on the combined prelay, egg laying, incubation and provisioning data and for the prelay period alone.
4.2.6. Observer influence
The duration (minutes) of each observation bout was entered into the regression model to examine if the extent of time for which individuals were observed influenced the level of behaviour recorded. Bivariate comparisons were performed using the Spearman rank-order correlation coefficient $r_s$.

4.2.7. Male status and morphometric variables
(i) Male status
Male status was entered into the regression model as a categorical variable: 1 = monogamous and 0 = bigamous; unpaired males were excluded. Bivariate comparisons of behaviour levels for monogamous and bigamous males were performed using the Mann Whitney two sample test.

(ii) Morphometric variables
Five morphometric variables were selected as the most repeatable based on ringing data collated in 1988. These were: wing length, body weight, tarsus length and full head length (Spencer 1984; Svensson 1988). The data used apply to individuals measured prior to the first egg date in the late March/early April. This period is essentially when territories and pairings have become fixed and inter-individual status is established (Bell 1968; Hornby 1971). This is the period most likely to behave as a cue to fitness for inter- and intra-sexual selection. A sixth morphological variable, plumage brightness, was also derived for the early season; later in the season plumage brightness increases and males become less differentiated. Plumage score was determined using a series of diagrams scaled from (1) resembling a female to (6) a breeding male with complete black head and nape with a distinctive white collar. The intermediate rankings were determined by the extent to which the black had developed or to which blemishes still remained i.e.,

1 = < 10% black plumage, male apparently a female, collar not visible, malar stripe not visible, female plumage pattern distinct.
2 = 15-45% black, buff tips still visible, bib now apparent, black also beginning to expand over the head, but still not linked to the bib and dispersed, dull areas around chin, eyes and bill.
3 = 45-60% black, malar stripe and collar still buff, bib connected to chin, although possibly still patchy around the eye, bill and head.
4 = 65-85% black, bib and head now showing clear definition, however the edges are not smooth, extensive buff patches still exist forming dull spots, malar stripe and collar has remaining buff tips.
5 = 85-99% black, small areas of grey, buff around eyes, behind beak, below chin and around the margins, essentially these are just a few feathers within black areas. Similarly one or two dull feathers within the collar and malar stripes.
6 = 100% black, textbook male reed bunting, distinct black head and bib, with bright malar stripe and collar.

A second multivariate analysis was performed as described previously using the mean residual rank for each male from the first model to enter as the Y variable for a second model using the morphometric data as the independent X variables. This overcame the problems of pseudo-replication which would arise if the data were entered into the first model. This second model
analyses the interaction between behaviour and morphology, examining if any morphological feature is a reliable correlate of behaviour. Bivariate analysis of the mean ranks was performed using the Spearman rank-order correlation coefficient $r_s$. Bivariate analysis was also performed using the mean behaviour values for individual males. This analysis was performed on the combined prelay, egg laying, incubation and provisioning data and for each separate period.
4.3. RESULTS

4.3.1. Male to female distance.
4.3.1.1. Variation in male- female distance: optimization of variables

Prior to the analysis of the relationship of male to female distance and the X variables a suitable representative distance measure was required. Consequently this first section examines the distribution of the different distance Y variables to determine the one most suitable for subsequent analyses. Table 3.1.1 shows the mean proportion of time ± SE of the distances recorded during the prelay, egg laying, incubation and provisioning periods. All distances below 15 m showed significant variation between the stages (Kruskal Wallis analysis of variance by ranks) but above this distance only one group (30-40 m) varied significantly. In all cases the prelay period had the greatest proportion of time in which the male was observed within a specific distance of the female. To avoid bias due to variable rates of female observation between stages, data were transformed to a proportion of time for which a female was observed with the male. The data in Table 3.1.1 show significant variation between the stages for distances less than 10 m but for >10 m no significant variation was found. Figure 3.1.1 shows the distribution of the means of all individual observations for the four stages below the 10 m point. The less than 10 m data show a definite decline in time spent with the female during the laying and incubation periods. This data set was subsequently used in further analyses.
Table 3.1.1. Distances between males and females for each stage of the nest cycle.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Distance</th>
<th>Prelay</th>
<th>Egg laying</th>
<th>Incubation</th>
<th>Provisioning</th>
<th>KW</th>
<th>p 3 df</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;3 m</td>
<td>0.092 ± 0.012</td>
<td>0.026 ± 0.006</td>
<td>0.011 ± 0.003</td>
<td>0.051 ± 0.014</td>
<td>32.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>3-5 m</td>
<td>0.092 ± 0.011</td>
<td>0.032 ± 0.007</td>
<td>0.019 ± 0.005</td>
<td>0.027 ± 0.010</td>
<td>34.85</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>5-10 m</td>
<td>0.080 ± 0.012</td>
<td>0.016 ± 0.004</td>
<td>0.012 ± 0.005</td>
<td>0.012 ± 0.007</td>
<td>43.56</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>10-15 m</td>
<td>0.017 ± 0.004</td>
<td>0.005 ± 0.002</td>
<td>0.003 ± 0.002</td>
<td>0.001 ± 0.001</td>
<td>20.00</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>15-20 m</td>
<td>0.024 ± 0.005</td>
<td>0.005 ± 0.002</td>
<td>0.006 ± 0.001</td>
<td>0.008 ± 0.012</td>
<td>6.88</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>20-30 m</td>
<td>0.019 ± 0.005</td>
<td>0.011 ± 0.005</td>
<td>0.004 ± 0.002</td>
<td>0.004 ± 0.003</td>
<td>5.34</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>30-40 m</td>
<td>0.013 ± 0.004</td>
<td>0.003 ± 0.002</td>
<td>0.001 ± 0.001</td>
<td>0.002 ± 0.001</td>
<td>10.83</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td></td>
<td>&gt;40 m</td>
<td>0.014 ± 0.005</td>
<td>0.005 ± 0.003</td>
<td>0.005 ± 0.002</td>
<td></td>
<td>3.11</td>
<td>&gt;0.50</td>
</tr>
</tbody>
</table>

Mean ± SE proportion of time observed at distances shown.

< 10m 0.264 ± 0.022 | 0.074 ± 0.011 | 0.042 ± 0.009 | 0.009 ± 0.022 | 66.44 <0.001
> 10 m 0.087 ± 0.012 | 0.030 ± 0.007 | 0.015 ± 0.004 | 0.026 ± 0.013 | 11.8 <0.01
< 10m/(Σ) 0.553 ± 0.034 | 0.427 ± 0.044 | 0.263 ± 0.043 | 0.498 ± 0.087 | 23.98 <0.001
> 10 m/(Σ) 0.181 ± 0.023 | 0.181 ± 0.032 | 0.243 ± 0.042 | 0.115 ± 0.051 | 3.73 >0.200
4.3.1.2. Multivariate analysis of male-female distance

Table 3.1.2. shows the results of stepwise multiple regression of time less than 10 metres from the female with the X variables described in the methods section. Seven variables were entered into the final model, accounting for 24.7% of the observed variance, of which five were significant; namely, a negative association with observations from the egg laying ($F = -50.6$) and incubation stages ($F = -40.7$), the day of the year ($F = -9.97$), the number of adjacent neighbours ($F = -19.2$), and nest ($F = -5.16$). Wind speed ($F = -2.29$) and the provisioning stage ($F = +2.61$) were both nonsignificant although they were entered into the model. The first variable entered into the regression model was a positive relationship with observations from the prelay stage this; was, however subsequently removed ($F = -0.058$).
Table 3.1.2. Stepwise regression model of time spent less than 10 m from the female

<table>
<thead>
<tr>
<th>Model 1</th>
<th>F(378,7)</th>
<th>Model 1 continued</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjacent neighbours</td>
<td>-19.217***</td>
<td>Minutes Observed +1.682</td>
</tr>
<tr>
<td>Neighbours in 100m</td>
<td>-0.175</td>
<td>Day of year -9.967***</td>
</tr>
<tr>
<td>Neighbours prelay</td>
<td>+0.382</td>
<td>Time after sunrise -1.931</td>
</tr>
<tr>
<td>Neighbours egg laying</td>
<td>-1.593</td>
<td>Wind speed -2.292***</td>
</tr>
<tr>
<td>Neighbours incubating</td>
<td>+0.309</td>
<td>Hours of sunshine -0.065</td>
</tr>
<tr>
<td>Neighbours provisioning</td>
<td>+0.040</td>
<td>Rainfall +0.650</td>
</tr>
<tr>
<td>Male status</td>
<td>-1.328</td>
<td>Maximum temperature +0.063</td>
</tr>
<tr>
<td>Nest</td>
<td>-5.160*</td>
<td>Minimum temperature +0.092</td>
</tr>
<tr>
<td>Prelay stage</td>
<td>-0.058</td>
<td></td>
</tr>
<tr>
<td>Egg laying stage</td>
<td>-50.595***</td>
<td></td>
</tr>
<tr>
<td>Incubation stage</td>
<td>-40.657***</td>
<td></td>
</tr>
<tr>
<td>Provisioning stage</td>
<td>-2.610***</td>
<td></td>
</tr>
<tr>
<td>Day of nest cycle</td>
<td>-0.079</td>
<td></td>
</tr>
<tr>
<td>Final Adj r squared</td>
<td>0.247</td>
<td></td>
</tr>
</tbody>
</table>

Model 2 Male morphometrics
(F0,0)

| Wing length | -0.184 |
| Weight | +0.174 |
| Plumage Score | -0.039 |
| Tarsus | +1.933 |
| Full head length | +0.347 |
| Final Adj r squared | 0.00 |

Total r squared = 0.247

Columns show the F values resulting from a stepwise multiple regression between each Y variable and the X variables (F-to enter for X variables not in the final model and F-to-remove of X variables introduced into the final model). X variables marked || were entered into the final model and those marked with one or more asterisk explained a significant amount of variation in the Y variable in the final model (*p < 0.05, **p < 0.01, ***p < 0.001). The degrees of freedom at the column head (V2, V1) are given as the number of observations and the number of significant variables entered into the model. The direction of the relationship between X and Y variables is given as - or +, indicating a negative or positive direction. The variance explained by the model is given as the adjusted r squared value which is corrected for the remaining degrees of freedom.
4.3.1.3 Breeding chronology

(i) Multivariate analysis
Data from the prelay stage were the first variable entered into the regression model, however this was subsequently removed with data from the egg laying ($F = -50.6$) and incubation ($F = -40.7$) periods remaining as significant predictors. The provisioning period was entered ($F = -2.6$) into the model but was not significant as was the day of the nest cycle.

(ii) Between stage variation
Across the four stages entered into the regression model the variation was significant ($KW = 43.89, 4$ df, $p < 10^{-4}$). The variation in the proportion of time spent above or below the 10 metre threshold is shown in Table 3.1.1. During the prelay period the male was seen within 10 m of the female more than during the egg laying ($z = -2.210, p < 0.05$) and incubation ($z = -4.917, p < 10^{-5}$) periods but not significantly more during the provisioning period ($z = -0.372, p > 0.20$). The egg laying stage was also significantly greater than in the incubation period ($z = -0.787, p > 0.20$) but again not significantly different from the provisioning period ($z = -2.604, p < 0.01$). The pair were least often close together during the incubation period and this was significantly lower than in all the other periods including provisioning ($z = -2.456, p < 0.02$). Of the time spent 11 m or more away from the female, no significant differences were observed between any of the stages.

(ii) a. Within stage variation in time spent <10 m and >10 m from the female
The data for each stage above and below the 10 m split were analysed to examine the difference in time spent near the female as shown in Table 3.1.3.

<table>
<thead>
<tr>
<th>Stage</th>
<th>$z$</th>
<th>$p$</th>
<th>Higher value</th>
</tr>
</thead>
<tbody>
<tr>
<td>prelay</td>
<td>- 6.523</td>
<td>$&lt; 10^{-5}$</td>
<td>&lt; 10 m</td>
</tr>
<tr>
<td>egg laying</td>
<td>- 4.447</td>
<td>$&lt; 0.001$</td>
<td>&lt; 10 m</td>
</tr>
<tr>
<td>incubation</td>
<td>- 0.696</td>
<td>$&gt; 0.20$</td>
<td>not significant</td>
</tr>
<tr>
<td>provisioning</td>
<td>- 2.889</td>
<td>$&lt; 0.01$</td>
<td>&lt; 10 m</td>
</tr>
</tbody>
</table>

For all but the incubation stage the male spent a greater proportion of time within 10 m of the female.

(ii) b. Variation in location <10 m from the female
To determine the most frequent distance a male was seen from the female <10 m, a Friedman analysis of variance by ranks was performed on the data. During the prelay, egg laying and incubation periods no significant difference exists between the distances but during the provisioning period the difference was significant due to the distances being ranked less than 3 m > 3-5 m > 5-10 m.
(ii) c. Variation in location >10 m

The data for time spent in the various categories >10 m away from the female were again analysed using Friedman analysis of variance by ranks. The data (Table 3.1.5) show a mixed distance dominance dependent on the stage. During the prelay period the male is most likely to be observed 11-15 m from the female, this increases to 21-30 m during the egg laying period, 16-20 m during the incubation period and returning to 11-15 m whilst the pair are provisioning.

Table 3.1.5. Variation in location >10 m

<table>
<thead>
<tr>
<th>Stage</th>
<th>$\chi^2$</th>
<th>p at 2 df</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prelay</td>
<td>11.11</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Egg laying</td>
<td>11.50</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Incubation</td>
<td>12.77</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Provisioning</td>
<td>8.71</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

Ranking of distances >10 m

<table>
<thead>
<tr>
<th></th>
<th>10-15</th>
<th>15-20</th>
<th>20-30</th>
<th>30-40</th>
<th>&gt; 40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prelay</td>
<td>*&gt;</td>
<td>&gt;</td>
<td>&gt;</td>
<td>&gt;</td>
<td></td>
</tr>
<tr>
<td>Egg laying</td>
<td>&lt;</td>
<td>&lt;</td>
<td>*&gt;</td>
<td>&lt;</td>
<td></td>
</tr>
<tr>
<td>Incubation</td>
<td>&lt;</td>
<td>&gt;</td>
<td>*&gt;</td>
<td>&lt;</td>
<td></td>
</tr>
<tr>
<td>Provisioning</td>
<td>&lt;</td>
<td>*&gt;</td>
<td>&gt;</td>
<td>&gt;</td>
<td></td>
</tr>
</tbody>
</table>

* = most frequent distance

(iii) Variation in time spent < 10 m from the female between individual days

The day of the nest cycle was not entered into the regression model but was negatively correlated ($r = -0.408, n = 39, p < 0.02$) with time spent < 10 m from the female, confirming the data from the above stage analysis. The distribution of the proportion of time the pair spent < 10 m apart is shown in Figure 3.1.2. Across all days the variation was significant (KW = 56.3, 11 df, p <0.001), however from days 0 through to 15 the time spent within 10 m of the female did not differ significantly (KW = 9.79, 6 df, p >0.10). The difference between days resulted from a significantly greater proportion of time spent close to the female during the prelay period. Days -5 to -1 (Table 3.1.6) show significant variation in time spent within 10 m of the female between days (KW = 9.79, 4 df, p <0.05). Day -2 is significantly greater than days -5, -4, and -1 and marginally nonsignificant for day -3. The data show that time spent close to the female peaks on day -2, whilst time spent within 10 m of the female for the other prelay days is greater than the egg laying or incubation periods.
Figure 3.1.1
Time spent < 10 m from the female

Figure 3.1.2 Time spent < 10 m from the female.
Day of nest cycle.
(iv) Individual variation in time spent <10 m from the female during the prelay, egg laying and incubation periods was analysed using the Wilcoxon signed-rank test. The difference between the prelay and egg laying periods was significant ($z = -2.556, p < 0.02$), as was the difference between the prelay and incubation periods ($z = -3.408, p < 0.002$). However no significant difference was found between the egg laying and incubation periods ($z = -1.334, p > 0.10$).

Analysis of the residual data from the multiple regression showed significant between male variation ($KW = 35.74, 18 \text{ df}, p < 0.02$) in the time spent <10 m from the female. No relationship exists between the number of observation bouts and the rank of individual males (Spearman rank, $r_s = 0.277, n = 19, p > 0.20$). The data for 1989 and 1990 are shown in Figure 3.1.3; differences between males are apparent, with five males above and seven below the 95% confidence limits of the mean (mean rank = 194.05, 217.98-170.13, $n = 19$). The 1990 individual males were analysed for day to day variability in the time spent <10 m from the female during the prelay period. Three of the five males: PMRPG, YMGYB and YMWYB showed a peak on day -2, with the former two males showing a significant variation across days ($KW = 9.45, & 10.73, 4 \text{ df}, p < 0.05$ respectively), whilst YMWYB was not significantly different ($KW = 6.97, 3 \text{ df}, p > 0.05$). RMRGB showed a peak on day -3, whilst YMBWR peaked on day -1, but both showed no significant variation over days ($KW = 3.96 & 1.43$ respectively, $p > 0.50$).
Figure 3.1.3
Mean ranks for individual males

1989
1990
4.3.1.4. Neighbour density and status

(i) Neighbour density

The density of adjacent neighbours was entered into the regression model as a significant variable \( F = -19.217 \), whereas neighbours within 100 m was not. During the incubation period, both neighbours within 100 m \( r_S = -0.558, n = 16, p < 0.05 \) and the number of adjacent neighbours \( r_S = -0.666, n = 16, p < 0.01 \) were negatively correlated with time spent < 10 m from the female. For males in the prelay and egg laying stages there was no significant correlation with neighbour density \( r_S < -0.376, n = 18, p > 0.05 \). Males therefore did not remain closer to their females as a response to increased neighbour densities. However, during the incubation period males spent less time close to their female at increased neighbour densities.

(ii) Neighbour status

(a) Response to neighbour status for all stages

Neighbours status was not entered into the regression model. Bivariate analysis (Wilcoxon signed-rank test) revealed significantly lower levels of time spent within 10 m of the female associated with neighbours in the prelay periods the converse being observed for neighbours in the provisioning stage:

- prelay \( z = -2.580, n = 17, p < 0.01 \), with \( 0.166 ± 0.024 \), without \( 0.103 ± 0.021 \),
- egg laying \( z = -1.285, n = 19, p > 0.10 \), with \( 0.124 ± 0.024 \), without \( 0.150 ± 0.022 \),
- incubation \( z = -1.810, n = 17, p > 0.05 \), with \( 0.104 ± 0.021 \), without \( 0.167 ± 0.031 \),
- provisioning \( z = 2.486, n = 17, p < 0.02 \), with \( 0.102 ± 0.022 \), without \( 0.162 ± 0.023 \).

(b) Response to neighbour status during the prelay period

Examination of males in the prelay period showed no significant effect on distance to the female with any of the neighbour status variables \( z < -1.800, p > 0.05 \). Contrary to expectations, therefore, males do not remain closer whilst neighbours are free to intrude.

4.3.1.5. Environmental Factors.

(a) Relationship for all stages

Wind speed \( F = -2.292 \) was the only environmental variable entered into the regression model. Three environmental factors showed no significant correlation with time spent less than ten metres, i.e., the hours of sun \( r_S = -0.034, n = 386, p > 0.20 \), rainfall \( r_S = +0.011, n = 386, p > 0.20 \) and maximum temperature \( r_S = -0.084, n = 386, p > 0.20 \). Wind speed \( r_S = -0.121, n = 386, p < 0.02 \) and minimum temperature did however show a significant correlation with time spent <10 m apart \( r_S = -0.154, n = 386, p < 0.001 \).

(b) Relationship for males in the prelay stage

No significant correlations with any environmental variable were recorded for males in the prelay stage \( r_S < -0.150, n = 154, p > 0.05 \), indicating that guarding males do not alter their proximity to the female with altered environmental conditions.
4.3.1.6. Seasonal and diurnal patterns

(a) Day
Day of year was entered into the regression model ($F = -9.97$), indicating a decline in time <10 m from the female during the season, agreeing with a negative correlation ($r_S = -0.320, n = 100, p < 0.002$).

(b) Time
Time was not entered into the regression model and no significant correlation or pattern was recorded across all stages ($r_S = -0.126, n = 123, p > 0.10$) or during the prelay period ($r_S = -0.163, n = 71, p > 0.10$).

(c) Nest
Nest was entered into the regression as a significant variable ($F = -5.16$) indicating an increase in time <10 m from the female across successive nests once seasonal factors have been regressed out. Bivariate comparison of nests shows that levels of time <10 m from the female were significantly (Wilcoxon signed-rank test, $z = -1.965, n = 16, p < 0.05$) higher for first nests ($0.155 \pm 0.026$) than subsequent nests ($0.114 \pm 0.025$). The variation in the male-female distance in the prelay period between first nest and replacements shows no significant difference (Mann Whitney U, $z = -0.189, p > 0.20$).

4.3.1.7. Observer influence.
The duration of the observation bout was not entered as a significant variable into the final regression model although a positive correlation ($r_S = 0.16, 386 \text{ df}, p < 0.002$) existed between the length of the observation period and time observed <10 m from the female.

4.3.1.8. Male status and morphometrics.

(i) Male Status
Male status was not entered into the regression model and bivariate analysis showed no difference (mean ± SE) between bigamous ($0.141 \pm 0.012$) and monogamous ($0.167 \pm 0.026$) males in the time spent less than 10 metres from the female ($z = -1.504, p > 0.10$). For males in the prelay stage, monogamous males ($0.276 \pm 0.025$) spent significantly more time within 10 m of the female ($z = -2.224, p < 0.05$) than did bigamous males ($0.175 \pm 0.044$).

(ii) Male morphometric variables
A second regression model (Table 3.1.1) of the mean residual ranks for the individual males and morphometric variables showed no significant relationship with time <10 m from the female. Bivariate analysis showed no significant correlations between the residuals and time <10 m from the female ($r_S = < 0.342, n = 19, p > 0.20$). Bivariate analysis of mean values for the prelay, egg laying, incubation and all observations showed no significant correlations with any of the male characteristics (Spearman rank, $r_S \leq 0.448, n = 19, p > 0.05$).
4.3.2. Flights and female orientated flights

4.3.2.1. Variation in observed flights
A total of 2496 (82.9%) flights were recorded as being by the male alone, and 431 (14.3%) were flights following the female, of which 345 were female initiated, 63 were to the female and 23 were of unknown initiator, whilst only 10 flights were considered to be male initiated with a female pursuit. Flights solely directed at the nest by the male numbered 67 (2.2%); since the female was not the focal bird data for females are not available. Flights ending with an attack on the female numbered 17 (0.6%), with 6 similar attacks by the female upon the male.

4.3.2.2. Multivariate analysis of female orientated flights
Table 3.2.1 shows the results of a stepwise multiple regression of female pursuit flights, 8 variables were entered into the final model, accounting for only 16.1% of the observed variance. Significant variables were the number of adjacent neighbours \( (F = -14.29) \), male status \( (F = -3.37) \), the prelay stage of the nest cycle \( (F = +43.62) \), the provisioning stage of the nest cycle \( (F = +3.02) \), nests \( (F = -15.28) \), day of the year \( (F = -6.55) \), minimum temperature \( (F = +4.78) \) and rainfall \( (F = -3.12) \). The number of neighbours within 100 m \( (F = +2.22) \) was entered into the model but was not significant.
Table 3.2.1. Stepwise regression model of female orientated flights

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1</th>
<th>F(512, 9)</th>
<th>Model 1 continued</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjacent neighbours</td>
<td>-14.293***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neighbours in 100m</td>
<td>+2.215†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neighbours prelay</td>
<td>-0.623</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neighbours egg laying</td>
<td>+0.029</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neighbours incubating</td>
<td>+0.939</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neighbours provisioning</td>
<td>+0.224</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male status</td>
<td>-3.373*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nest</td>
<td>-15.283***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prelay stage</td>
<td>+43.618***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg laying stage</td>
<td>+1.957</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incubation stage</td>
<td>-0.180</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Provisioning stage</td>
<td>+3.015*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day of nest cycle</td>
<td>-1.380</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final Adj r squared</td>
<td>0.161</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Model 2 Male morphometrics (F0,0)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 2</th>
<th>Total adjusted r squared 0.161</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wing length</td>
<td>-1.730</td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>+1.525</td>
<td></td>
</tr>
<tr>
<td>Plumage Score</td>
<td>+1.162</td>
<td></td>
</tr>
<tr>
<td>Tarsus</td>
<td>+0.481</td>
<td></td>
</tr>
<tr>
<td>Full head length</td>
<td>-1.225</td>
<td></td>
</tr>
<tr>
<td>Final Adj r squared</td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>

Columns show the F values resulting from a stepwise multiple regression between each Y variable and the X variables (F-to enter for X variables not in the final model and F-to-remove of X variables introduced into the final model). X variables marked † were entered into the final model and those marked with one or more asterisk explained a significant amount of variation in the Y variable in the final model (*p < 0.05, **p < 0.01, ***p < 0.001). The degrees of freedom (V2, V1) at the column head are given as the number of observations and the number of significant variables entered into the model. The direction of the relationship between X and Y variables is given as - or +, indicating a negative or positive direction. The variance explained by the model is given as the adjusted r squared value which is corrected for the remaining degrees of freedom.
4.3.2.3. Breeding chronology

(i) Multivariate analysis of female orientated flights
Both the prelay stage ($F = +43.62$) and the provisioning stage ($F = +3.02$) were entered into the regression model as significant variables, indicating a rise in female orientated flights during both these periods.

(ii) Between stage variation
Table 3.2.2 shows a summary of the flight data analysed by stages. All four groups show a significant degree of variation between stages (KW values shown in the top row, with significance levels.

(a) Solitary flights by the male
Figure 3.2.1a shows the distribution of solitary flights by the male. Data in Table 3.2.2 show that of all the stages only the provisioning period is significantly different, being lower than the others.

(b) Female orientated flights
Figure 3.2.1b shows the distribution of female orientated flights. Significantly more female orientated flights occurred during the prelay period compared to the lay or incubation periods, and the lay period was also significantly greater than the incubation period. The level of female orientated flights in the provisioning period was not significantly different from the prelay or egg laying periods but was when compared to the incubation period. The level of female orientated flights during the incubation period was therefore significantly lower than during any of the three other periods.

(c) All flights
Figure 3.2.1c shows the distribution of all flights. Data in Table 3.2.2 show the only significant variation is that the prelay period is greater than the both the egg laying and incubation periods.

(d) Female orientated flights as a proportion of all observed flights.
Figure 3.2.1d shows the distribution of this data set. Analysis shows that the level of flights during the prelay period is significantly greater than in both the egg laying and incubation periods and that the egg laying period is significantly greater than in the incubation period. The level of female orientated flights in the provisioning period, however, is intermediate between the prelay and egg laying periods being not significantly different from either but significantly greater than in the incubation period.
Table 3.2.2. Summary of flight data, between stage variation for male solitary, female orientated, all and female orientated flights as a proportion of all flights

<table>
<thead>
<tr>
<th></th>
<th>Solitary flights</th>
<th>Female orientated flights</th>
<th>All flights</th>
<th>Female orientated flights/all flights</th>
</tr>
</thead>
<tbody>
<tr>
<td>KW 3 df</td>
<td>11.655**</td>
<td>35.87***</td>
<td>16.081**</td>
<td>33.781***</td>
</tr>
<tr>
<td>(i) Prelay v egg laying</td>
<td>-0.687</td>
<td>-4.438****</td>
<td>-3.139**</td>
<td>-3.843**</td>
</tr>
<tr>
<td>(ii) Prelay v incubation</td>
<td>1.034</td>
<td>-4.953******</td>
<td>-3.305***</td>
<td>-5.166******</td>
</tr>
<tr>
<td>(iii) Prelay v provisioning</td>
<td>-3.293***</td>
<td>-1.710</td>
<td>-1.720</td>
<td>-1.627</td>
</tr>
<tr>
<td>(iv) Egg laying v incubation</td>
<td>-0.524</td>
<td>-2.418*</td>
<td>-1.036</td>
<td>-2.718**</td>
</tr>
<tr>
<td>(v) Egg laying v incubation</td>
<td>-2.907**</td>
<td>0.662</td>
<td>-0.122</td>
<td>-0.477</td>
</tr>
<tr>
<td>(v) Incubation v provisioning</td>
<td>-2.343*</td>
<td>-2.258*</td>
<td>-0.845</td>
<td>-2.449*</td>
</tr>
</tbody>
</table>

Mean ± SE

- Prelay: 0.294 ± 0.018
- Egg laying: 0.284 ± 0.019
- Incubation: 0.267 ± 0.026
- Provisioning: 0.190 ± 0.032

Comparison of flights by nest stages. Data are shown as values of z from Mann Whitney U (two tailed). Significance levels are denoted as * p <0.05, ** p <0.01, *** p <0.001, **** p <0.0001, p<0.10-4 and ****** p <10^-3.

96
Figure 3.2.1a
Solitary flights by the male

![Bar chart showing flights per minute by stage of the nest cycle with the following stages:
- Prelay
- Egg Laying
- Incubation
- Provisioning
The bars show a decrease in flights per minute from Prelay to Provisioning.]

Figure 3.2.1b
Female orientated flights by the male

![Bar chart showing female-oriented flights per minute by stage of the nest cycle with the following stages:
- Prelay
- Egg Laying
- Incubation
- Provisioning
The bars show a decrease in female-oriented flights per minute from Prelay to Provisioning.]

97
Figure 3.2.1c
All flights observed by the male

Figure 3.2.1d
Female orientated flights as a proportion of all flights
(iii) Variation in female orientated flights between days

The day of the nest cycle was not entered into the regression model as a significant variable but was significantly negatively correlated with female orientated flights ($r_s = -0.518$, $n = 40$, $p <0.002$). Figure 3.2.2 shows the distribution of female orientated flights. Across all the groupings described in the methods section the data were significantly different ($KW = 44.69$, $11$ df, $p <0.001$), however no difference lay within the prelay days ($KW = 5.70$, $4$ df, $p >0.20$), egg laying ($KW = 0.76$, $4$ df, $p >0.90$) or the incubation period ($KW = 4.91$, $6$ df, $p >0.50$). The difference between days actually arises at the boundary between the prelay and egg laying periods (Mann Whitney U, $z = -2.137$, $p <0.05$), and at the boundary of the incubation and the prelay periods (Mann Whitney U, $z = -2.871$, $p <0.01$). Despite the highest frequency occurring on day -2, no significant peak occurred during the prelay period (Mann Whitney U, $z$ max. = -1.87, $p >0.05$).
Fig 3.2.2
Female orientated flights
Day of the nest cycle

![Graph showing female orientated flights per minute over different days of the nest cycle.]
(iv) Individual variation in female orientated flights

Comparison of the mean levels of female orientated flights for the prelay, egg laying and incubation periods were made within individual males using the Wilcoxon signed rank test. The levels in the prelay period were significantly greater than during egg laying ($z = -2.663$, $p < 0.01$) and similarly during the incubation ($z = -2.166$, $p < 0.05$) periods. No significant difference was observed between the egg laying and incubation periods ($z = -1.334$, $p > 0.10$). No two periods showed any correlation in the levels ($p > 0.05$) of female orientated flights. A comparison of within stage variation showed significant between male differences in the prelay ($KW = 35.26$, 17 df, $p < 0.01$) and incubation ($KW = 28.27$, 15 df, $p = 0.02$) periods but no difference during the egg laying period ($KW = 21.56$, 15 df, $p > 0.10$). Analysis of the residual female orientated flight data from the multiple regression shows a significant degree of male variation in the number of female pursuits ($KW = 31.19$, 19 df, $p < 0.05$, mean rank = 250.45, 95% confidence limits, 231.45 - 269.45). The number of observation bouts and the rank were significantly correlated (Spearman rank, $r_s = 0.483$ n =19, $p < 0.05$), indicating an increased chance of a female orientated flight being observed with repeated observations. The mean residual rank data for 1989 and 1990 are shown in Figure 3.2.3. Individual males observed for every day of their prelay periods in 1990 were analysed for day to day variability in the time spent <10m from the female. Only one male, YMGYB, showed any significant variation ($KW = 10.67$, $p < 0.05$) during the prelay period, with a peak on day -4. The other males YmWyB ($KW = 4.28$, $p > 0.10$), RMRBG ($KW = 1.36$, $p > 0.20$), YMWBWR ($KW = 3.80$, $p > 0.20$) and PMRPG ($KW = 3.15$, $p > 0.05$) showed no significant difference across days. The difference among males (Figure 3.2.4) across days was very noticeable in the number of flights which were female orientated ($KW = 10.95$, $p < 0.05$); most noticeable is the high level by PMRPG twice as high as for the next male.
Figure 3.2.3
Female orientated flights
Mean rank for individual males

Figure 3.2.4
Female orientated flights for males
observed through their prelay stage in 1990
4.3.2.4. Neighbour density and status

(i) Neighbour density
(a) Neighbour density for all stages
The number of adjacent neighbours and the total within 100 m were both entered into the regression model. Neighbours within 100 m was not significant ($F = +2.215$), and is not significantly correlated with female orientated flights across all stages or during the egg laying and incubation periods ($r_s = -0.245$, $n = 20$, $p > 0.20$). The number of adjacent neighbours was entered as a significant variable ($F = -14.293$) and does show a significant negative correlation with female orientated flights, but only during the incubation stage ($r_s = -0.507$, $n = 16$, $p < 0.05$). It is therefore only during incubation that males apparently pursue females more but only in areas of low territory density.

(b) Neighbour density for males in the prelay stage.
Female orientated flights were not significantly correlated with neighbour density ($r_s < -0.216$, $n = 18$, $p < 0.20$), indicating that guarding of the female by close following does not alter significantly with increased neighbour density.

(ii) Neighbour status
(a) Response in of males to neighbour status for all stages
No neighbour status variable was entered into the regression model and no significant differences were found between any of the variables:
(i) prelay ($z = -0.388$, $n = 17$, $p > 0.20$, with = 0.043 ± 0.009, without = 0.041 ± 0.008),
(ii) egg laying ($z = -0.260$, $n = 19$, $p > 0.20$, with 0.062 ± 0.023, without = 0.045 ± 0.009),
(iii) incubation ($z = -0.142$, $n = 16$, $p > 0.20$, with = 0.042 ± 0.008, without = 0.05 ± 0.013) and
(iv) provisioning ($z = -0.596$, $n = 17$, $p > 0.20$, with = 0.044 ± 0.007, without = 0.047 ± 0.009).

(b) Response of males to neighbour status in the prelay stage
Males did not increase their following of the female in response to any neighbour status variable ($z = -0.889$, $p > 0.20$).

4.3.2.5. Environmental Factors
(a) Relationship for all stages
Minimum temperature was entered into the regression model as a significant variable ($F = +4.78$) indicating a rise in female orientated flights on the warmer days. Rainfall was also entered into the regression model ($F = -3.12$) as a significant variable; indicating a decline in female orientated flights on days with increased rainfall. No significant correlations were recorded for any of the variables, ($r_s \leq 0.051$, $n = 512$, $p > 0.20$ for rainfall, hours of sun, wind speed, maximum and minimum temperature).

(b) Relationship for males in the prelay stage
No significant correlations with any environmental variable were recorded for males in the prelay stage ($r_s \leq 0.095$, 183 df, $p > 0.20$), indicating that guarding males do not alter their
following of the female with altered environmental conditions.

4.3.2.6. Seasonal and diurnal patterns
(a) Day
The day of the year was entered into the final regression model ($F = -6.55$) indicating a seasonal decline in female orientated flights. Figure 3.2.5 shows the distribution of female orientated flights for which the difference across the periods is significant ($KW = 18.09$, 8 df, $p < 0.05$), however there was no significant correlation across the season ($r_s = -0.016$, $n = 125$, $p > 0.20$).

(b) Time
Diurnal differences in female orientated flights were not entered into the regression model and shows no significant ($r_s = -0.026$, $n = 136$, $p > 0.20$) correlation across the day. A comparison of time of day during the prelay stage shows no significant difference across hours ($KW = 3.27$, 7 df, $p > 0.80$); Figure 3.2.6 and no correlation across hours ($r_s = -0.108$, $n = 84$, $p > 0.20$). No diurnal patterns in female orientated flights was apparent across all stages or during the prelay period.
Figure 3.2.5
Seasonal distribution of female orientated flights

Figure 3.2.6
The diurnal pattern of female orientated flights during the prelay period
Nest was entered into the regression model \((F = -15.283)\) as a significant variable affecting female orientated flights. However, no significant difference (Wilcoxon signed-rank \(z = -0.189, p >0.20\)) was recorded in female orientated flights (mean ± SE) between first (0.035 ± 0.01) and replacement (0.035 ± 0.06) nests. Similarly, during the prelay period no significant difference (Wilcoxon signed-rank, \(z = -0.314, p >0.20\)) was recorded in female orientated flights between first (0.055 ± 0.018) and replacement (0.071 ± 0.018) nests.

4.3.2.7. Observer influence
Observation bout length was not entered into the final regression model, despite a positive correlation \((r = 0.092, 509 \text{ df}, p <0.05)\) with female orientated flights, reinforcing the results of the analysis of residual ranks.

4.3.2.8 Male status and morphometrics
(a) Male status
Male status was entered into the regression model \((F = -3.37)\) as a significant variable indicating higher levels of female orientated flights for bigamous males. However, bivariate analysis showed no significant variation \((z = -0.330, p >0.20)\) between levels of pursuit flights (mean ± SE) for monogamous (0.054 ± 0.008) and bigamous males (0.043 ± 0.004). Similarly, during the prelay period there was no difference between monogamous males (0.08 ± 0.013) and bigamous males (0.07 ± 0.008), \((z = -1.836, p >0.05)\).

(b) Male morphometric variables.
A second regression model (Table 3.2.1) of the mean residual ranks for each male and the individual morphometric variables showed no significant relationships with female orientated flights. Bivariate analysis showed no significant correlations between the residual data and female orientated flights \((r = -0.449, n = 19, p >0.20)\). Bivariate analysis of mean values for the prelay, egg laying, incubation and all observations showed no significant correlations with any of the male characteristics (Spearman rank, \(r = -0.441, n =16, p >0.05\)).
4.3.3. Vigilance

4.3.3.1. Multivariate analysis of vigilance

Table 3.3.1 shows the results of a stepwise multiple regression of time spent being vigilant. Eleven variables were entered into the final regression model, accounting for 21.5% of the variance. The significant variables were: the day of the year ($F = -20.07$), the prelay stage of nest cycle ($F = +32.83$), the provisioning stage of nest cycle ($F = +4.87$), time of day ($F = -4.91$), wind speed ($F = -6.38$), minimum temperature ($F = +8.58$), neighbours which were in the prelay stage ($F = +4.04$), neighbours which were incubating ($F = +8.55$), the number of adjacent neighbours ($F = -8.53$), male status ($F = -5.09$) and nest ($F = -5.16$).
### Table 3.3.1. Stepwise regression model of time spent vigilant

<table>
<thead>
<tr>
<th>Model 1</th>
<th>F(374,11)</th>
<th>Model 1 continued</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjacent neighbours</td>
<td>-8.531***</td>
<td>Minutes Observed</td>
</tr>
<tr>
<td>Neighbours in 100m</td>
<td>+0.404</td>
<td>Day of year</td>
</tr>
<tr>
<td>Neighbours prelay</td>
<td>+4.037**</td>
<td>Time after sunrise</td>
</tr>
<tr>
<td>Neighbours egg laying</td>
<td>-0.333</td>
<td>Wind speed</td>
</tr>
<tr>
<td>Neighbours incubating</td>
<td>+8.548***</td>
<td>Hours of sunshine</td>
</tr>
<tr>
<td>Neighbours provisioning</td>
<td>+0.035</td>
<td>Rainfall</td>
</tr>
<tr>
<td>Male status</td>
<td>-5.086**</td>
<td>Maximum temperature</td>
</tr>
<tr>
<td>Nest</td>
<td>-5.159**</td>
<td>Minimum temperature</td>
</tr>
<tr>
<td>Prelay stage</td>
<td>+32.831***</td>
<td></td>
</tr>
<tr>
<td>Egg laying stage</td>
<td>+0.001</td>
<td></td>
</tr>
<tr>
<td>Incubation stage</td>
<td>-0.030</td>
<td></td>
</tr>
<tr>
<td>Provisioning stage</td>
<td>+4.873**</td>
<td></td>
</tr>
<tr>
<td>Day of nest cycle</td>
<td>+0.406</td>
<td></td>
</tr>
<tr>
<td>Final Adj r squared</td>
<td>0.215</td>
<td></td>
</tr>
</tbody>
</table>

**Model 2 Male morphometrics**

(F 19,3)

| Wing length | -9.252* |
| Weight | +5.173** |
| Plumage Score | -0.193 |
| Tarsus | +2.734** |
| Full head length | -0.641 |
| Final Adj r squared | 0.303 |

Total adjusted r squared = 0.453

Columns show the F values resulting from a stepwise multiple regression between each Y variable and the X variables (F-to enter for X variables not in the final model and F-to-remove of X variables introduced into the final model). X variables marked ‖ were entered into the final model and those marked with one or more asterisk explained a significant amount of variation in the Y variable in the final model (*p <0.05, **p <0.01, ***p <0.001). The degrees of freedom (V2,V1) at the column head are given as the number of observations and the number of significant variables entered into the model. The direction of the relationship between X and Y variables is given as - or +, indicating a negative or positive direction. The variance explained by the model is given as the adjusted r squared value which is corrected for the remaining degrees of freedom.
4.3.3.2. Breeding chronology

(i) Multivariate analysis

The prelay stage was entered into the regression model as a significant variable \((F = 32.83)\) indicating increased levels of vigilance during the prelay period. The provisioning period was similarly associated with significantly raised levels of vigilance \((F = 4.87)\). Neither data from the egg laying and incubation periods or the day of the nest cycle were entered into the regression model.

(ii) Between stage variation.

A comparison of between stage differences shows a significant degree of variation between the stages \((KW = 75.33, 4 \text{ df}, p < 0.001)\). The means ± SE for the stages are shown in Figure 3.3.1. The pairwise comparison of stages are shown in Table 3.3.2. The prelay period had significantly greater levels of vigilance than all other periods, the egg laying period matched the provisioning period in having significantly greater levels of vigilance than the incubation period but they were not significantly different from each other.

Table 3.3.2. Between stage variation in vigilance

<table>
<thead>
<tr>
<th>Stage</th>
<th>Egg laying</th>
<th>Incubation</th>
<th>Provisioning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prelay</td>
<td>-4.059***</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Incubation</td>
<td>-6.578****</td>
<td>-2.895**</td>
<td>X</td>
</tr>
<tr>
<td>Provisioning</td>
<td>-4.168***</td>
<td>-1.378</td>
<td>-0.829</td>
</tr>
<tr>
<td>Unpaired</td>
<td>-5.257****</td>
<td>-3.794***</td>
<td>-2.543*</td>
</tr>
</tbody>
</table>

significant differences are *<0.05, **<0.01, ***<10^-3, ****<10^-4.

(ii) Variation in vigilance between individual days

Day of the nest cycle was not entered into the regression model but was negatively correlated with vigilance \((r_S = -0.341, n = 42, p < 0.05)\). Figure 3.3.2 shows the distribution of the proportion of time males were vigilant. Overall the variation between days was significant \((KW = 57.23, 11 \text{ df}, p < 10^{-5})\). Levels of vigilance rose on day -4, peak on day -2 and then declined steadily until days 5-9, after which there was a marginal rise. Pairwise comparisons of the data (Mann Whitney U) showed the rise from day -5 to -4 was not significant \((z = -1.535, p > 0.05)\). Vigilance levels on days -3, -2 and -1 were, however, all significantly greater than on day -5 \((z = -2.661, p < 0.01, z = -3.537, p < 0.001 \text{ and } z = -2.672, p < 0.01, \text{ respectively})\). Continuing through the sequence of days, vigilance on day -4 was only significantly greater than on day 4 and days 5-9 \((z = -2.163, p < 0.05, \text{ and } z = -3.335, p < 10^{-3} \text{ respectively})\). The subsequent increase in vigilance on day -3 produced significant differences with days 2, 3, 4 and day group 5-9 \((z = -2.148, p < 0.05, z = -2.271, p < 0.05, z = -3.138, p < 0.002 \text{ and } z = -4.594, p < 10^{-5} \text{ respectively})\). The peak day for vigilance was day -2, however this was not significantly greater than days -4, -3, and -1 \((z = -1.643, p = 0.10, z = -0.951, p > 0.20 \text{ and } z = -1.413, p > 0.10 \text{ respectively})\). Vigilance on day -2 was significantly greater than all other days \((z > 2.600, p < 0.01, \text{ for all days})\). Vigilance on day -1 was significantly greater than days 2,3,4 and days 5-9 \((z > 2.100, p < 0.05 \text{ for all days})\). The lowest level of vigilance was observed for days 5-9, and this was significant compared to all days except -5, 2,
4 and 10-15 (z > 2.000, p < 0.05). The data therefore showed a rise from day -4, which peaked on day -2, after which it declined to a low level during the first half of incubation and then showed a slight increase.
Figure 3.3.1
Proportion of time vigilant
Stage of the nest cycle

Figure 3.3.2
Vigilance. Day of the nest cycle
(iii) Individual variation in vigilance

The mean levels of vigilance for the prelay, egg laying and incubation periods were compared within individual males using the Wilcoxon signed rank test. The levels in the prelay period were not significantly greater than in the egg laying period \( (z = -1.874 \ p > 0.05) \), but were significantly greater than in the incubation period \( (z = -2.668 \ p < 0.002) \). The egg laying period was also significantly greater than the incubation period \( (z = -2.824 \ p < 0.01) \). No two periods showed any correlation in the levels \( (p > 0.05) \) of female orientated flights.

Analysis of the residual vigilance data from the stepwise multiple regression showed a significant degree of between male variation \( (KW = 32.99, 18 \ df, \ p < 0.05) \). No correlation of existed between the number of observation bouts and the mean rank \( (r_g = 0.206, 19 \ df, \ p > 0.20) \). Figure 3.3.3 shows the residual data for 1989 and 1990; differences between males are apparent with 6 males below and 7 males above the 95% confidence limits (mean rank = 192.16; 209.80-174.51). The males I observed on every day of their prelay period in 1990 were analysed for day to day variability in the time spent vigilant Figure 3.3.4. None of the five individual males from 1990 showed any significant difference between days. The expected distribution of a peak on day -2 was seen in three males: GMRBG \( (KW = 9.23, 4 \ df, \ p = 0.055) \), YMBWR \( (KW = 5.26, 4 \ df, \ p > 0.10) \) and YMWYB \( (KW = 2.25, 3 \ df, \ p > 0.50) \). The other two males were: PMRPG \( (KW = 5.10, 4 \ df, \ p > 0.10) \) who showed a decline in vigilance from day -4 to day -1 and YMGYB \( (KW = 8.56, 4 \ df, \ p > 0.05) \) who showed little change over a peak of days -4,-3 and -2. The difference between these males across days was very noticeable in the level of vigilance \( (KW = 23.29, \ p < 10^{-3}) \) and most noticeable is the low level by GMRBG, with other males showing greater but variable levels (see Figure 3.3.4).
Figure 3.3.3
Vigilance
Mean rank for individual males

Figure 3.3.4
Vigilance for males observed through their prelay stage in 1990
4.3.3.3. Neighbour density and status

(i) Neighbour density

(a) Neighbour density for all stages

The density of adjacent neighbours was entered into the regression model as a significant variable \( F = -8.53 \), indicating lower levels of vigilance at higher densities. Bivariate analysis showed no correlation between vigilance and neighbours within 100 m \( r_g = -0.207, n = 19, p >0.20 \) and numbers of adjacent neighbours \( r_g = -0.078, n = 19, p >0.20 \).

(b) Neighbour density for males in the prelay stage.

Vigilance was not significantly correlated with neighbour density \( r_g = -0.110, n = 17, p >0.20 \).

(ii) Neighbour status

(a) Response to neighbour status for all stages

Two neighbour behaviours were entered into the final model as significant, namely neighbours in the prelay stage \( F = +4.04 \) and incubating stage \( F = +8.55 \). Bivariate analysis revealed no significant (Wilcoxon signed-rank) effect of neighbour status upon vigilance.

(i) prelay \( z = -1.396, n = 16, p >0.20, \) with \( 0.154 \pm 0.018 \), without \( 0.120 \pm 0.023 \),

(ii) egg laying \( z = -0.065, n = 18, p >0.20, \) with \( 0.148 \pm 0.021 \), without \( 0.150 \pm 0.022 \),

(iii) incubation \( z = -0.973, n = 14, p >0.20, \) with \( 0.154 \pm 0.002 \), without \( 0.142 \pm 0.023 \),

(iv) provisioning \( z = -0.827, p >0.20, \) with \( 0.142 \pm 0.024 \), without \( 0.168 \pm 0.023 \).

(b) Response to neighbour status for males in the prelay stage

Males did not increase their levels of vigilance in response to any neighbour status variable \( z = -1.599, p >0.10 \).

4.3.3.4. Environmental Factors.

(a) Relationship for all stages

Wind \( F = -6.38 \) was entered into the regression model as a significant variable, indicating that vigilance declined with increased wind speed, whilst minimum temperature \( F = +8.580 \) was positively regressed with vigilance. Consequently, as minimum temperature increased so did the observed level of vigilance. Bivariate correlations showed wind speed to be negatively correlated with vigilance \( r_g = -0.181, 386 \text{ df}, p <10^{-3} \), whilst all other variables were not significantly correlated \( r_g < 0.046, n = 374, p >0.20 \).

(b) Relationship for males in the prelay stage

No significant correlations with any environmental variable were recorded for males in the prelay stage \( r_g < 0.112, n = 177, p >0.10 \), indicating that guarding males do not alter their levels of vigilance with altered environmental conditions.

4.3.3.5. Seasonal and diurnal patterns of vigilance

(a) Day

Day was entered into the final regression model \( F = -20.07 \), and was significantly negatively
correlated ($r_g = -0.258, n = 102, p < 0.01$) with vigilance, indicating a decline in vigilance through the season, (see song, Chapter 6, which shows a strong positive correlation with day). The time spent being vigilant during the season is shown in Figure 3.3.5. The distribution peaked between days 121-130 which approximates to the the first third of May, after which there was a gradual decline in vigilance.

(b) Time
Time was entered into the final regression model as significant ($F = -4.91$) indicating a decline in vigilance through the day. Bivariate analysis shows time is not significantly correlated with vigilance ($r_g = -1.876, n = 118, p > 0.05$). However, excluding the first hour the level of vigilance does significantly decline ($r_g = -0.196, n = 111, p < 0.05$) Figures 3.3.6 a,b & c show the diurnal patterns of vigilance for a) the prelay period, b) the egg laying plus incubation periods and c) the overall seasonal distribution. The pattern for the prelay period showed a general decline in vigilance during the day, excluding the data for the first hour the decline was significant ($r_g = -0.287, n = 70, p < 0.02$). The data for the combined egg laying and incubation periods showed no significant difference between hours ($KW = 8.94, 9 df, p > 0.30$) and no significant correlation between vigilance and hours after sunrise ($r_g = -0.079, n = 100, p > 0.20$). Figure 3.3.6 c closely resembles that of the prelay period and is probably due to the high ratesof vigilance from the prelay period. The difference between days is not significant ($KW = 12.59, 10 df, p > 0.20$), and as shown above no correlation existed between vigilance and hours after sunrise.
Figure 3.3.6 a  
The diurnal pattern of vigilance during the prelay period

Figure 3.3.6 b  
The diurnal pattern of vigilance during the egg laying and incubation periods

Figure 3.3.6 c  
The diurnal pattern of vigilance all stages
Nest was entered into the regression model as a significant variable \((F = -5.16)\) indicating a decline in vigilance after the first nest. Bivariate analysis revealed no significant (Wilcoxon signed-rank, \(z = -0.331, p >0.20\)) difference (mean ± SE) between first (0.159 ± 0.036) and subsequent nests (0.129 ± 0.016).

4.3.3.6. Observer influence

The duration of the observation bout was not entered into the regression model and no significant correlation was recorded \((r_S = 0.083, n = 386, p >0.10)\).

3.3.7. Male status and morphometrics

(a) Male status

Male status was entered into the final regression model \((F = -5.09)\), indicating lower levels of vigilance for bigamous males. However bivariate analysis showed no significant difference \((z = -0.707, p >0.20)\). Similarly for males in the prelay stage, no significant difference was recorded \((z = -0.663, p >0.20)\) between monogamous males and bigamous males.

(b) Male morphometric variables

Table 1 shows the results of the second regression model, 3 variables were entered into the model: tarsus length \((F = +2.73)\) and body weight \((F = +3.17)\) were not significant, however wing length was \((F = -9.25)\). This model accounted for an additional 30.3% of the 78.5% of variance remaining from the first model, accounting in total for 45.3% of the observed variance. Bivariate analysis showed no significant correlations between the residual data and vigilance \((r_S \leq -0.432, n = 19, p >0.20)\). Bivariate analysis of mean values for the prelay, egg laying, incubation and all observations combined showed no significant correlations with any of the male characteristics (Spearman rank, \(r_S \leq \pm 0.414, n = 16, p >0.05)\).
4.4 DISCUSSION

The three guarding Y variables of time spent less than 10m from the female, female orientated flights, and vigilance all had moderate levels of variance explained (excluding morphometric variables) by the range of X variables i.e., 24.7%, 16.1% and 21.5% respectively. Part of the reason why more variance was not explained is that the relationships between the X and Y variables may not be linear but rather showed peaks. Consequently, less of the variation is actually explained by linear regression and the patterns of behaviour may only appear using bivariate techniques.

In the introduction to this chapter a series of formal predictions were made based on the sperm competition hypothesis (Birkhead et al 1987). Each will be discussed in turn. (i) Mate guarding increases during the prelay period, as copulations near to egg laying are more likely to result in fertilisations. In Chapter 3, I delineated the prelay period and up to day 2 as the period of copulations, therefore the expectation is that guarding behaviour should increase during this period.

From the three multivariate analyses the results show for: (a) time spent < 10 m from the female, a negative association with the egg laying and incubation stages and therefore higher levels of time spent close to the female were recorded for the prelay and provisioning stages, (b) female orientated flights were significantly higher during the prelay and provisioning stages and (c) vigilance levels were also significantly higher during the prelay and provisioning stages. The day of the nest cycle was not entered as a significant variable into the regression model, but all three Y variables showed a significant decline through the nest cycle. The bivariate analyses of between stage differences identified the prelay period as the time of significantly increased levels of all three behaviours. When the data for replacement nests was analysed on a day to day basis the levels of behaviour were shown to rise after failure (day -5) to a peak on day -2, which subsequently declined to the approximate level prior to failure on day 0. All three Y variables show similar responses in their pattern of behaviour which indicate that the period of guarding and potentially the period when EPCs occur is restricted to the prelay period, but predominantly to days -3, -2, and -1, although some response is demonstrated on day -4 but little or none on day -5. Comparison of individual responses showed the prelay period again to have significantly higher levels of each behaviour compared to the egg laying and incubation stages. Individual variation in responses did occur with peaks not apparent for the individual 1990 males for female orientated flights, but peaks for the other two Y variables fall within the period of days -3 to -1.

The data therefore match the prediction that males should increase their guarding prior to egg laying. However, these data reveal a peak in response, which may reflect peak fertility and a decline in behaviour once egg laying commences, although copulations continue to the day of the third egg. This pattern resembles that of many other species which terminate mate guarding before the end of the fertile period (Birkhead 1982; Møller 1987a) although in many species it does continue into the egg laying stage. The guarding behaviours I observed ended rather abruptly on the day before first egg which may to a degree reflect some limited incubation by the female curtailing opportunities for guarding. However, the data show that males no longer
respond to female presence in a similar manner. It is possible that the success of EPCs declines once the first egg has been laid (Birkhead 1982), or a male may regard opportunities elsewhere as of greater benefit. Other factors include loss of condition (Birkhead 1982; Möller 1987a), age and experience (Möller 1987a) and differential values assigned to the first and subsequent eggs (Birkhead 1982). The data would indicate that a male reed bunting regards EPCs as no threat during egg laying, as witnessed by observations, and that he benefits from terminating guarding once laying begins.

(ii) The intensity of mate guarding will vary diurnally as individual eggs are fertilised separately 24 hours to laying. The data from prediction (i) have negated this prediction other than for day -1, since guarding stops abruptly with the beginning of egg laying. Neither time spent < 10 m from the female or female orientated flights showed any change across the day for the entire season or for the prelay period. Vigilance did however decrease significantly during the day for the prelay period and for the combined stages but not for the egg laying or incubation stages. It may be that as the day progresses the threat from other males decreases and so the need to be vigilant also declines. The pattern of intrusions is discussed in the subsequent chapter and a negative relationship of vigilance and song is discussed later. The data therefore point to no diurnal pattern in guarding other than for vigilance which matches the expectations from prediction (ii). The pattern of copulations during the prelay period, however, showed a gradual rise and decline through the day (see Chapter 3), therefore not agreeing with the hypothesis of a decline in behaviours through the day. Few studies have included the diurnal pattern of mate guarding, or the timing of mate guarding in relation to copulations. In the swallow copulations occur most frequently in the morning but guarding varies little in intensity over the course of the day (Möller 1987, unpublished in Birkhead & Möller 1992). Mate guarding in Smith's longspur Calura pictus occurred only when copulations took place in the morning or evening (J. V. Briske in Birkhead & Möller 1992). One hypothesis proposed which may explain diurnal patterns of guarding and copulations is the “copulation access hypothesis”. This proposes that males remain close to their mate simply to be able to copulate when the female solicits copulations (Gowaty & Plissner 1987). The hypothesis predicts that males should follow only when copulations are likely to occur, i.e., males should guard most intensively when copulations peak. The data from this study do show a day to day variation in intensity of guarding, but the pattern does overlap the period of copulations, although copulations occur after mate guarding has declined. The diurnal patterns of mate guarding during the prelay period were not correlated with the copulation pattern (r <0.613, n =9, p >0.05). The evidence therefore cannot be said to support the copulation access hypothesis as there is a disparity between the pattern of copulations and mate guarding.

(iii) Behaviours will be more pronounced when males are at a higher density. Males have been recorded as increasing their levels of guarding in response to increased population densities (Möller 1985, 1987a, 1991g; Birkhead et al 1987; Möller and Birkhead 1989, 1992). Examination of the data entered into the regression model revealed a significant negative relationship of the three Y variables to the number of adjacent neighbours. Bivariate analyses showed that only during the incubation period was there a negative correlation between density and time spent < 10 m from the female and female orientated flights. There was no evidence of a density effect during the prelay period. The copulation access hypothesis
predicts that mate guarding should remain constant even when the threat of EPCs increases, i.e., as density increases mate guarding does not increase. The lack of a response to density whilst mate guarding does lend support to the copulation access hypothesis.

iv) Mate guarding will be higher when neighbours are not having to guard their females. Asynchronisity in the population increases the risk of EPCs from non-guarding males (Morton et al 1990; Birkhead and Biggins 1987). Having established that guarding behaviours are mainly confined to the prelay stage then hypothetically the greatest threat of cuckoldry should come from males that are no longer guarding, i.e., those whose females are laying, incubating, or provisioning. The results from the regression models showed no definite behaviour pattern, i.e., neighbours in the prelay and incubation stages were regressed positively response with vigilance, but no neighbour status variables were entered for the two other Y variables. The bivariate analysis of the entire data again showed no significant effect on female orientated flights, vigilance and neighbour status. Time spent < 10 m from the female did significantly increase when neighbours were in the prelay stage but also decreased significantly when neighbours were provisioning. No effect was observed for any Y variable during the prelay period. The data do not support a link between increased mate guarding and neighbour status, with no clear pattern of response to neighbour status.

Only one of the four predictions has been satisfied by the data i.e., guarding should be confined to the fertile period. The lack of response in mate guarding intensity to neighbour density also lends support to the copulation access hypothesis. Variation in the guarding behaviour may be influenced by other factors as described previously. These additional factors are now discussed:

a) Seasonal influences of guarding behaviours. Guarding behaviours decline through the season which is probably a reflection of the decreasing frequency of prelay periods, from the initial seasonal peak through the replacement nests. Consequently negative correlations were recorded and entered as such into the models. This again is borne out by the lower levels of behaviours associated with replacement and repeat clutches.

b) Environmental influences on mate guarding. Time spent < 10 m from the female was not correlated with any environmental variable. Minimum temperature was found to have a positive association with female orientated flights whilst rainfall was negatively associated, albeit not significantly. No bivariate relationships were observed with female orientated flights. Across all stages wind speed was negatively correlated with vigilance whilst minimum temperature and hours of sunshine were both positively associated. However no significant relationships was found for the prelay stage. Why males are not so vigilant or close to the female on cold and windy days may be hypothesised as being due to them having to forage more or being unable to maintain these behaviours so efficiently. Those guarding males who were in the prelay stage show no response to altered environmental conditions. Consequently, the evidence indicates that when males are not constrained by guarding they decrease those behaviours which are normally associated with guarding in response to harsher environmental conditions. This is not wholly
unsurprising as the behaviours themselves are much reduced outside the prelay period and are therefore not constrained by the need to prevent EPCs.

c) Male status and morphometrics.
No significant differences were found between monogamous and bigamous males in their levels of guarding, except that the monogamous males in the prelay stage spent more time within 10 metres of their fertile female than did bigamous males, and vigilance was higher in bigamous males in the regression model. These results may be attributable to either individual differences or that bigamous males had females which may have required attendance simultaneously (at least two occasions) so although they remained vigilant and followed one female they may have been constrained sufficiently to not be able to remain close. Male morphometrics were only entered into the second regression model for vigilance, in which males with a shorter wing length had significantly lower levels of vigilance. Although there are significant between male differences in guarding this was the only significant variable.

To summarise this chapter, the guarding of females does occur in the reed bunting with the main phase arising shortly before first egg date (days -4 to -1 for replacement nests) after which it declines dramatically. The expected rise in guarding as a response to increased neighbour pressure did not occur, whilst neighbour status did not influence guarding as predicted. Diurnal variation in guarding was not apparent other than for a decline in vigilance during the day for the prelay period. Vigilance may decrease as the need to be able to respond to intruder threats declines whilst close guarding still occurs since the threat is not negated and copulations still occur. Individual male variation in the levels of guarding does occur but only vigilance was found to be related to any morphometric variable.
CHAPTER 5
Facilitation of EPCs and the aggressive response

Abstract: Males may increase their reproductive success through the acquisition of extra-pair copulations. In the reed bunting, Emberiza schoeniclus, territorial intrusions were mainly by neighbours and peaked significantly during the prelay period and were responded to with an attack from the defending male. No early morning peak in intrusion activity was observed to support the fertilisation window hypothesis. The seeking of extra-pair copulations was observed as excursions into neighbouring territories followed by an attack from the territory holder. Excursions occurred primarily when males were not mate guarding, during egg laying and incubation and were directed at territories with nests in the prelay stage. The data do not support either the non-territory holder or territory establishment hypotheses, whereas patterns of behaviour do support some predictions of the sperm competition hypothesis.

5.1. INTRODUCTION

The reed bunting during the breeding season is a territory holding species and although feeding does occur in other areas the majority of reproductive functions do occur within the territory boundaries. One theory for the existence of breeding territories is that with the evolution of guarding it became profitable for males to follow this by guarding an area which was the female’s designated range. Limits on the male’s ability to defend an exclusive area and with the advantage of proximate territories and the associated links of extra-pair paternity, provided a mechanism which defined territory size. The establishment of guarded areas around the female and the existence of preferred habitat types subsequently permits males to establish territories prior to female arrival. Advantages observed from the holding of territories would include reduced female harassment (Lumpkin 1983), resources associated with nest building, nest sites and food availability. With further selection on males, territory forms could become sexually selected (Price 1984) favouring the selection of optimal territories.

Territory formation promotes a system by which males can defend females and breeding requirements, but it also defines an area into which other males are required to enter to seek extra-pair copulations. Consequently these forays are observed as territorial intrusions. Three hypotheses exist to explain intrusions: (i) The territory establishment hypothesis, which proposes that intrusions will occur during the period of territory establishment when borders still remain unclear. Consequently an initial peak is followed by a decline as the limits become more defined. (ii) The non-territory holder hypothesis, which suggests that intruders are birds without territories and predicts a decline with territory establishment. (iii) As part of the sperm competition hypothesis in which intruders are males seeking extra-pair copulations; consequently intrusions should occur during the fertile period (see Buitron 1983; Ford 1983; Møller 1987g; Johnson and Kermott 1989; Rowley and Russel 1990). A second prediction is that intrusions should have a a peak which correlates with the insemination window (see Chapter 3). This prediction is separated from hypothesis (ii) in that males will only respond to the female’s fertile period (see Møller 1987g for a review). The third prediction is that intruders should be mainly male, and these should be territory-holding neighbours able to
predict the fertile period by surveillance of the pair. The result of the sperm competition predictions is that successive peaks should be observed during the season as replacement clutches occur and fertile periods arise (Møller 1987a).

In this chapter the timing and influences on intrusions are analysed for reed buntings and these results are then compared with the above predictions. Uncertainty of paternity may also have other counter-selective effects (Trivers 1972), including aggression towards mates (Barash 1976), and aggression towards intruding conspecifics (Zenone et al. 1979). One of the other ways in which intrusions are quantified is by the reaction of the guarding male. The obvious way to react is by aggression to remove the threat, which has been observed in studies on mountain bluebirds, Sialia currucoides, in which males reacted most aggressively whilst their female is fertile (Barash 1976), although other studies did not vindicate these results (Morton et al. 1978; Power and Doner 1978). In red-backed shrikes Lanius collurio (Jakober and Stauber 1989), male shrilces behaved most aggressively to intruder models during the nest building and egg laying periods, whilst females did not and the male attacks declined at other periods.

The sperm competition hypothesis therefore proposes that intrusions should occur most frequently during the fertile period (Chapter 3) and guarding males should react aggressively to intruders. An expectation of this argument is that males must leave their territories on excursions to be recorded as intruders and they must be recipients of aggression during these periods. This chapter examines the occurrence of intrusions, excursions, attacks upon other males and attacks upon the observed male. These behaviours are analysed for their fit with the sperm competition hypothesis, results on mate guarding obtained in Chapter 4, and how other external factors may influence their occurrence. The two predictions of the territory establishment and the non territory holder hypothesis will also be reviewed with respect to the results obtained in this chapter.

Predictions from the sperm competition hypothesis are: (i) intrusions should be most frequent during the fertile period, equating to the prelay period as defined in Chapter 3, (ii) as a response to intrusions, the defending male should attack males regarded as a threat to paternity, (iii) males should seek extra-pair copulations when not having to guard their pair female, and this should be observed as forays into surrounding territories outside the prelay period, (iv) males seeking extra-pair copulations will be the recipients of attacks, (v) intrusions will be most frequent early in the morning equating to the period of peak fertility (see Chapters 3 & 4) and (vi) intrusions will be more pronounced at higher densities.
5.2. METHODS

The methods of behavioural data analysis are explained in Chapter 4, consequently I am restricting this methods section to a description of the four behaviours associated with the acquisition and defence of paternity.

5.2.1 Intrusions

Intrusions were defined as the encroachment of one male into the territory associated with the focal male. Unfortunately unlike some other species reed buntings do not have rigidly defined boundaries and consequently intrusions were only recorded where certainty of territory ownership was discernible. This method of data collection may have therefore overlooked some intrusions but avoided the inclusion of normal within territory movements. The data are presented as the rate of intrusions per minute, the observed occurrences as a proportion of the time for which males were visible for more than 10 minutes. This avoided bias from the over estimation of behaviour during bouts of infrequent male visibility.

5.2.2 Aggressive attacks upon other males

The response of males to intruders was to displace them from their territory in a directed aggressive action. The occurrence of aggressive attacks was originally subdivided into three categories: (a) displacement of the intruding male by directed movement towards him but with no subsequent chase, (b) as described previously but followed by a chase which frequently involved removal of the intruder from the territory, (c) displacement of an intruder followed by physical violence, which in some cases was sustained. For ease of analysis the three categories were grouped to provide a rate of attacks per minute for the time focal males were visible for 10 minutes or more.

5.2.3 Excursions into surrounding territories

Excursions were defined as males visibly leaving their territory, i.e., being the converse of intrusions, and the actual data are presented as the proportion of time for which males were observed to be out of their territories, for the time males were visible for 10 minutes or more. This presentation of data avoids the assumption that if a male is witnessed leaving the territory and then not seen again that he has not actually returned to his territory. However, it may also underestimate the behaviour and relies on the location of focal males in adjacent territories.

5.2.4 Attacks received from other males

The response of defending territorial males as described above was to attack intruders, therefore focal males out of their territory were observed as being the recipients of attacks. However occasionally the defending focal territorial male was also subject to an attack. The original data were collected as the converse of (a), (b) & (c) of attacks on other males. These “attacks received” are presented in the same manner as attacks upon other males.

The results are presented in the same style as in the previous chapter with an initial data summary, multivariate analysis and bivariate analyses. The data set used in the analysis of between day variation was the same as that described for time spent < 10 m from the female and vigilance described in Chapter 4.
5.3. RESULTS
5.3.1. Intrusions
5.3.1.1. Data summary
(a) Duration of intrusions
During the observation bouts a total of 454 separate intrusions were recorded, of which 263
(58%) were identified as a neighbour holding an adjacent territory, whilst 191 (42%) were not
specifically identified as a neighbour or non-neighbour. The duration (mean ± SE) of an
intrusion was 1.498 ± 0.318 minutes; Table 3.1.1 and Figure 3.1.1 show the frequency
distribution for all observed intrusions. The distribution of intrusion shows a strong skew to
the modal class of less than 1 minute, the longest single intrusion being 19 minutes. Intrusions
were therefore most frequently very rapid forays into territories.

<table>
<thead>
<tr>
<th>Duration in minutes</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5-10</th>
<th>&gt;10</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>352</td>
<td>55</td>
<td>20</td>
<td>16</td>
<td>10</td>
<td>1</td>
<td>454</td>
</tr>
<tr>
<td>Proportion</td>
<td>0.782</td>
<td>0.113</td>
<td>0.044</td>
<td>0.031</td>
<td>0.026</td>
<td>0.006</td>
<td></td>
</tr>
</tbody>
</table>

(b) Movement of non-neighbours
Twenty eight non-neighbour captures were recorded during 1988 and 1989. Figure 3.1.2 and
Table 3.1.2 show the breakdown of captures by the stage of the male within whose territory
males were captured.

<table>
<thead>
<tr>
<th>Stage of territory at capture site</th>
<th>Numbers</th>
<th>Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg laying</td>
<td>10</td>
<td>0.357</td>
</tr>
<tr>
<td>Incubation</td>
<td>4</td>
<td>0.143</td>
</tr>
<tr>
<td>Provisioning/fledging</td>
<td>2</td>
<td>0.071</td>
</tr>
<tr>
<td>Unknown</td>
<td>3</td>
<td>0.107</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>0.321</td>
</tr>
</tbody>
</table>
Figure 3.1.1
The duration of intrusions

Figure 3.1.2
Stage of the breeding cycle at the site of capture of intruding males
The data show, for territories where the stage of breeding of the resident pair was known, that
the majority of captures were at sites where the female was in the prelay period. The spread of
captures was significantly different ($\chi^2 = 8.16, 3$ df, $p <0.05$) from an equal expected
distribution. Indicating that captures of non-neighbour males occurred significantly more in the
territories of pairs in the prelay period.

(c) Site of capture
The relationship of capture site and intruder was largely based on ringing effort, so although the
reed beds of lagoon III accounted for 32% of captures, it was also the site of the most
concentrated ringing effort during 1988 and 1989 which formed part of a constant effort site,
CES (Spencer 1984).

(d) Distance covered
Distance moved by the captured males is shown in Figure 3.1.3 and Table 3.1.3. The mean ±
SE distance covered was 447 m ± 116, however the modal class was less than 250 m (43%),
with a small decline in numbers travelling less than 500 m (32%) but only 11% travelling
greater than 500 m. Of the latter group two males were caught more than 1 km from their own
territory, the first was recovered 1.8 km away from his territory and the second was recorded
as copulating 2.6 km away from his known breeding territory. The distribution of males
differed significantly from an expected equal distribution ($\chi^2 = 23.90, 4$ df, $p <0.01$) with a
bias towards males travelling <500 m.

<table>
<thead>
<tr>
<th>Distance (metres)</th>
<th>Number</th>
<th>Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;250</td>
<td>12</td>
<td>0.430</td>
</tr>
<tr>
<td>250-500</td>
<td>9</td>
<td>0.320</td>
</tr>
<tr>
<td>500-750</td>
<td>1</td>
<td>0.040</td>
</tr>
<tr>
<td>750-1000</td>
<td>0</td>
<td>0.000</td>
</tr>
<tr>
<td>&gt;1000</td>
<td>2</td>
<td>0.070</td>
</tr>
<tr>
<td>unknown</td>
<td>4</td>
<td>0.140</td>
</tr>
</tbody>
</table>

Table 3.1.3. Distance from home territory of captured males
Figure 3.1.3
The distance covered by intruding males

![Bar chart showing the distance covered by intruding males](chart.png)
5.3.1.2. Multivariate analysis of intrusions

Table 3.1.4 shows the results of a stepwise multiple regression of intrusion rates. Six significant variables were entered into the first model accounting for only 10% of the observed variation. Significant variables were: presence of neighbours in the laying stage \( (F = +5.38) \), provisioning stage \( (F = -6.27) \), prelay stage \( (F = +7.65) \) and neighbours within 100 m \( (F = -4.59) \). The two non-significant variables entered into the model were: nest \( (F = +3.06) \), and the day of the nest cycle \( (F = -2.04) \). The model accounts for very little of the observed variation indicating that variation was either non-linear or due to unidentified variables not in the model.
### Table 3.1.4. Stepwise regression model of intrusions

<table>
<thead>
<tr>
<th>Model 1</th>
<th>F(380,6)</th>
<th>Model 1 continued</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjacent neighbours</td>
<td>-0.349</td>
<td>Minutes Observed</td>
</tr>
<tr>
<td>Neighbours in 100m</td>
<td>-4.592*</td>
<td>Day of year</td>
</tr>
<tr>
<td>Neighbours prelay</td>
<td>-0.870</td>
<td>Time after sunrise</td>
</tr>
<tr>
<td>Neighbours egg laying</td>
<td>+5.376*</td>
<td>Wind speed</td>
</tr>
<tr>
<td>Neighbours incubating</td>
<td>-0.031</td>
<td>Hours of sunshine</td>
</tr>
<tr>
<td>Neighbours provisioning</td>
<td>-6.273*</td>
<td>Rainfall</td>
</tr>
<tr>
<td>Male status</td>
<td>-0.487</td>
<td>Maximum temperature</td>
</tr>
<tr>
<td>Nest</td>
<td>+3.049†</td>
<td>Minimum temperature</td>
</tr>
<tr>
<td>Prelay stage</td>
<td>+7.651*</td>
<td></td>
</tr>
<tr>
<td>Egg laying stage</td>
<td>+0.186</td>
<td></td>
</tr>
<tr>
<td>Incubation stage</td>
<td>-1.041</td>
<td></td>
</tr>
<tr>
<td>Provisioning stage</td>
<td>+1.059</td>
<td></td>
</tr>
<tr>
<td>Day of nest cycle</td>
<td>-2.044‡</td>
<td></td>
</tr>
<tr>
<td>Final Adj r squared</td>
<td>0.100</td>
<td></td>
</tr>
</tbody>
</table>

#### Model 2 Male morphometrics

<table>
<thead>
<tr>
<th>F(17,1)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Wing length</td>
<td>-7.860*</td>
</tr>
<tr>
<td>Weight</td>
<td>+0.013</td>
</tr>
<tr>
<td>Plumage Score</td>
<td>-0.816</td>
</tr>
<tr>
<td>Tarsus</td>
<td>+0.604</td>
</tr>
<tr>
<td>Full head length</td>
<td>-1.756</td>
</tr>
<tr>
<td>Final Adj r squared</td>
<td>0.314</td>
</tr>
</tbody>
</table>

Columns show the F values resulting from a stepwise multiple regression between each Y variable and the X variables (F-to-enter for X variables not in the final model and F-to-remove of X variables introduced into the final model). X variables marked † were entered into the final model and those marked with one or more asterisks explained a significant amount of variation in the Y variable in the final model (*p < 0.05, **p < 0.01, ***p < 0.001). The degrees of freedom (V2,V1) at the column head are given as the number of observations and the number of significant variables entered into the model. The direction of the relationship between X and Y variables is given as - or +, indicating a negative or positive direction. The variance explained by the model is given as the adjusted r squared value which is corrected for the remaining degrees of freedom.
5.3.1.3. Breeding chronology

(i) Multivariate analysis

The level of intrusions during the prelay stage was entered into the regression model as a significant variable ($F = +7.65$), indicating a positive association with intrusion levels. No other stage was entered into the regression model.

(ii) Between stage variation

Variation in intrusion rates between the four stages was significant ($\chi^2 = 35.28, 4$ df, $p < 10^{-3}$), a comparison of the individual stages shows that the variation is accounted for by the high levels during the prelay period and the low levels during incubation. Figure 3.1.4 shows the distribution of intrusion rates per minute (mean ± SE) for the prelay ($0.099 ± 0.014$), egg laying ($0.047 ± 0.007$), incubation ($0.019 ± 0.006$), provisioning ($0.049 ± 0.018$) and unpaired ($0.021 ± 0.01$) stages. The pairwise comparison of stages are shown in Table 3.1.5. Intrusion rates during the prelay period were significantly greater than during all other periods and rates during the egg laying period were significantly greater than during the incubation period.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Prelay</th>
<th>Egg Laying</th>
<th>Incubation</th>
<th>Provisioning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prelay</td>
<td>-2.045*</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Egg Laying</td>
<td>-5.479***</td>
<td>-3.809***</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Incubation</td>
<td>-2.431*</td>
<td>-1.205</td>
<td>-1.642</td>
<td>X</td>
</tr>
<tr>
<td>Provisioning</td>
<td>-2.383*</td>
<td>-1.589</td>
<td>-0.396</td>
<td>-0.794</td>
</tr>
</tbody>
</table>

Significant differences are *<0.05, **<0.01, ***<10^{-3}, ****<10^{-4}

(iii) Variation in intrusion rates between individual days

The day of the nest cycle was entered into the regression model ($F = -2.04$), albeit not significantly and was also negatively correlated with intrusion rates ($r_s = -0.506, n = 39, p < 0.002$). Both analyses indicated a decline in intrusion rates as the nest cycle progressed. Figure 3.1.5 shows the distribution of intrusion rates for replacement nests. Overall the variation between days was significant ($\chi^2 = 40.99, 11$ df, $p < 10^{-3}$), however if days -3, -2 and -1 are removed then the variation was no longer significant ($\chi^2 = 9.98, 8$ df, $p > 0.20$). This is supported by the previous comparison between the stages; within the prelay period the difference between days was significant ($\chi^2 = 18.24, 4$ df, $p < 0.01$). However, within the egg laying period no significant variation was recorded ($\chi^2 = 4.56, 4$ df, $p > 0.30$) and similarly between the two incubation groups (Mann Whitney U test, $z = -0.892, p > 0.20$), or within the egg laying and incubation periods combined ($\chi^2 = 9.67, 6$ df, $p > 0.10$).

A comparison of intrusion rates between days (Mann Whitney U test) shows no significant difference between days -5 and -4 ($z = -0.283, p > 0.283$). Days -5 ($z = -2.903, p < 0.01$) and -4 ($z = -2.814, p < 0.01$) were significantly lower than on day -3. Intrusion rates on day -1 did not differ significantly from those on days -5 ($z = -1.907, p > 0.05$) and -4 ($z = -1.686, p > 0.05$).
Levels of intrusion activity on days -3 and -2 were not significantly different from each other ($z = -0.281$, $p > 0.20$) and day -1 ($z = -1.094$, $p > 0.20$ and $z = -1.394$, $p > 0.10$ respectively). The data showed a peak in intrusion rates on days -3, -2 and -1, with the highest rates associated with days -3 and -2.
Figure 3.1.4
Intrusion rates
Stage of the nest cycle

![Bar chart showing intrusion rates across different stages of the nest cycle.]

Figure 3.1.5
Intrusion rates. Day of the nest cycle

![Bar chart showing intrusion rates across different days of the nest cycle.]

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(iv) Individual variation in intrusion rates

Mean individual intrusion rates for the prelay, egg laying and incubation periods were compared using the Wilcoxon signed-rank test. The intrusion levels in the prelay period were significantly greater than the egg laying \((z = -2.198, p < 0.05)\) and the incubation \((z = -3.040, p < 0.002)\) periods. Intrusion rates during the egg laying period were significantly greater than the incubation period \((z = -1.957, p < 0.05)\) however, intrusion rates were significantly correlated \((r_s = 0.557, n = 13, p < 0.05)\).

Analysis of the residual data from the regression model shows the residual variation was not significant \((KW = 27.74, 18 \text{ df}, p > 0.05)\) between males. No correlation existed between the number of observations and the mean rank of the individual males \((r_s = 0.184, n = 19, p > 0.20)\). Figure 3.1.6 shows the distribution of residuals for all males, differences between males are apparent with 6 males below and 9 males above the 95% confidence limits \((\text{mean rank} = 182.16, 204.03 - 160.29)\). Comparison between 1990 males of the prelay daily rates, Figure 3.1.7, shows the means for the five males, the difference between individuals was significant \((KW = 25.15, 4 \text{ df}, p < 10^{-3})\). The intrusion rates \((\text{mean} \pm \text{SE})\) peaked on different days for the individuals: GMRBG had a very low intrusion rate with a slight peak on day -3 \((0.06 \pm 0.022)\), PMRPG showed a gradual increase with a peak on day -1 \((0.377 \pm 0.158)\), YMBWR suffered no intrusions other than on day -3 with a relatively high rate \((0.341 \pm 0.041)\), YMGYB had intrusions on all days with a peak on day -3 \((0.199 \pm 0.057)\), whilst YMWYB peaked on day -2 \((0.147 \pm 0.054)\).
Figure 3.1.6
Intrusions
Mean rank for individual males

Figure 3.1.7
Intrusion rates for males observed through their prelay stage in 1990
5.3.1.4. Neighbour density and status

(i) Neighbour density

(a) Neighbour density for all stages

Neighbour density within 100 m ($F = -4.59$) was entered as a significant variable into the regression model, indicating that intrusion level decline at higher densities. Bivariate analysis showed that during the incubation period was there a significant negative correlation with the density of adjacent neighbours ($r_g = -0.549, n = 16, p < 0.05$). All other correlations were not significant; egg laying ($r_g < -0.331, n = 16, p > 0.10$), and overall ($r_g < -0.210, n = 19, p > 0.20$).

(b) Neighbour density for males in the prelay stage

Neither the number of neighbours within 100 m ($r_g = 0.075, n = 17, p > 0.20$) nor the density of adjacent neighbours ($r_g = 0.093, n = 17, p > 0.20$) were correlated with intrusion rate. Intrusion rates were therefore not affected by neighbour density within the prelay period.

(ii) Neighbour status

(a) Response to neighbour status for all stages

The presence of a neighbour in the egg laying stage ($F = +5.38$) was positively regressed with intrusion rates, whilst the presence of a provisioning neighbour was entered into the regression model ($F = -6.27$) with a negative association with intrusions. Bivariate analysis (Wilcoxon signed-rank test) showed a significant decrease in intrusion rates with neighbours in the provisioning stage, all other comparisons were not significant:

(i) prelay ($z = -0.426, n = 17, p > 0.20$, with $0.046 \pm 0.010$, without $0.053 \pm 0.017$),
(ii) egg laying ($z = -1.811, n = 19, p > 0.05$, with $0.062 \pm 0.013$, without $0.034 \pm 0.009$),
(iii) incubation ($z = -0.454, n = 16, p > 0.20$, with $0.045 \pm 0.012$, without $0.053 \pm 0.014$),
(iv) provisioning ($z = -2.783, n = 17, p < 0.01$, with $0.034 \pm 0.008$, without $0.068 \pm 0.016$).

(b) Response to neighbour status for males in the prelay stage

Males in the prelay stage showed no significant difference in intrusion rates with neighbour status ($z \leq -1.067, p > 0.10$), indicating that no one group of males intruded more than others.

5.3.1.5. Environmental factors

(a) Relationship for all stages

No environmental variables were significant in the regression model and bivariate analysis showed no significant correlations with intrusion rates ($r_s < 0.105, n = 380, p > 0.20$).

(b) Relationship for males in the prelay stage.

For males in the prelay period only the hours of sunshine were significantly correlated ($r_s = 0.205, n = 155, p < 0.02$), indicating higher intrusion rates associated with brighter days. The other variables were all not significant ($r_s < 0.100, n = 155, p > 0.20$).

5.3.1.6. Seasonal and diurnal patterns

(a) Day

Day of year was not entered into the final regression model, bivariate analysis showed no
seasonal correlation with intrusion rates ($r_S = -0.092, n = 102, p > 0.20$), and no significant difference among the groupings shown in Figure 3.1.8 ($KW = 10.96, 8 \text{ df}, p > 0.30$), although towards the latter end of the season intrusion rates were visibly lower.

(b) Time

The time after sunrise was not entered into the regression model and the diurnal pattern showed no significant difference in intrusion rates among hours ($KW = 9.99, 8 \text{ df}, p > 0.30$) and no significant correlation across hours ($r_S = -0.119, n = 129, p > 0.10$). Similarly during the prelay period no significant difference in intrusion rates was found among hours ($KW = 8.87, 8 \text{ df}, p > 0.30$) and no significant correlation was recorded ($r_S = -0.012, n = 76, p > 0.20$). Figure 3.1.9a, shows a general plateau in intrusion rates between 1 and 8 hours post sunrise during the prelay period. The egg laying period (Figure 3.19.b), shows no significant difference between hours ($KW = 12.85, 8 \text{ df}, p > 0.10$), however, there was a significant negative correlation ($r_S = -0.281, n = 61, p < 0.05$), with a marked decline in intrusion activity occurring six hours post sunrise. The data show no obvious diurnal pattern in intrusion behaviour, although during egg laying intrusions when rates decline they were not spread over so many hours.
Figure 3.1.8
Seasonal distribution of intrusions
Figure 3.1.9a
The diurnal pattern of intrusions during the prelay period

Figure 3.1.9b
The diurnal pattern of intrusions during the egg laying period
Nest was entered into the regression model ($F = + 3.05$) but not as a significant variable, indicating intrusion rates are higher for first nests, albeit not significantly. Bivariate analysis showed that although the mean intrusion rate for first nests ($0.069 \pm 0.023$) was higher than for subsequent nests ($0.051 \pm 0.011$), this was not significant (Wilcoxon signed-rank test, $z = -0.341, n = 17, p >0.20$).

5.3.1.7. Observer influence

The duration of the observation bout was not entered into the regression model and no significant correlation was recorded ($r_s = -0.029, n = 386, p >0.20$).

5.3.1.8. Male status and morphometrics

(a) Males status

Monogamous and bigamous males were not entered into the final model as significant, however bivariate analysis (Mann Whitney U test) showed that bigamous males have significantly ($z = -2.243, p <0.02$) higher intrusion rates ($0.071 \pm 0.013$) than monogamous males ($0.060 \pm 0.007$). During the prelay stage no significant difference ($z = -0.318, p >0.20$) was recorded for intrusion rates despite the lower levels recorded for monogamous males ($0.064 \pm 0.016$) than for bigamous males ($0.108 \pm 0.018$).

(b) Male morphometric variables

Table 3.1.1 shows the results of the second regression model. Wing length ($F = -7.86$) was the only variable entered and was negatively regressed with intrusion rates, indicating that large winged males were subject to lower intrusion rates. The second model accounted for an additional 31.4% of the 90% of variance remaining from the first model, accounting for only 38.26% overall. Bivariate analysis of the residuals revealed wing length to be negatively correlated with intrusion rates ($r_s = -0.497, n = 19, p <0.05$). No other significant correlations were recorded ($r_s \leq -0.124, n = 18, p >0.20$). Bivariate analysis of mean values for the prelay, egg laying, incubation and all observations showed no significant correlations of intrusion rates with any of the male characteristics (Spearman rank, $r_s \leq 0.397, n = 16, p >0.10$).
5.3.2. Aggressive attacks upon other males

5.3.2.1. Data summary

Table 3.2.1 shows the number of attacks on other males for the three categories of attack defined in the methods section: (a) displacement of the intruding male, (b) displacement of the intruding male followed by a chase, and (c) displacement of an intruder followed by physical violence.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>%</th>
<th>rate min⁻¹</th>
<th>hour⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) displacement</td>
<td>97</td>
<td>19.52</td>
<td>0.008</td>
<td>0.480</td>
</tr>
<tr>
<td>b) displacement + chase</td>
<td>368</td>
<td>74.04</td>
<td>0.032</td>
<td>1.920</td>
</tr>
<tr>
<td>c) displacement + violence</td>
<td>32</td>
<td>6.44</td>
<td>0.003</td>
<td>0.180</td>
</tr>
<tr>
<td>Total</td>
<td>497</td>
<td>100.00</td>
<td>0.043</td>
<td>2.600</td>
</tr>
</tbody>
</table>

The majority of attacks on other males were followed by a chase, although approximately one fifth were not. Physical contact and actual violence were rare, accounting for only 6.44% of all attacks.

5.3.2.2. Multivariate analysis of attacks on other males

Six variables were entered into the final regression model, accounting for only 14.3% of the observed variance. Table 3.2.2 shows the results of the final regression model, significant variables entered were: the prelay stage ($F = 7.25.19$), nest ($F = -18.83$), provisioning neighbours ($F = -6.51$), neighbours egg laying ($F = + 6.01$) and the density of neighbours within 100 m ($F = -4.76$). Male status was also entered but was not significant ($F = -3.29$).
Table 3.2.2. Stepwise regression model attacks on other males

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 1 continued</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjacent neighbours</td>
<td>+0.049</td>
<td>Minutes Observed</td>
</tr>
<tr>
<td>Neighbours in 100m</td>
<td>-4.757*</td>
<td>Day of year</td>
</tr>
<tr>
<td>Neighbours prelay</td>
<td>-0.092</td>
<td>Time after sunrise</td>
</tr>
<tr>
<td>Neighbours egg laying</td>
<td>+6.010*</td>
<td>Wind speed</td>
</tr>
<tr>
<td>Neighbours incubating</td>
<td>-0.014</td>
<td>Hours of sunshine</td>
</tr>
<tr>
<td>Neighbours provisioning</td>
<td>-6.508*</td>
<td>Rainfall</td>
</tr>
<tr>
<td>Male status</td>
<td>-3.287*</td>
<td>Maximum temperature</td>
</tr>
<tr>
<td>Nest</td>
<td>-18.829***</td>
<td>Minimum temperature</td>
</tr>
<tr>
<td>Prelay stage</td>
<td>+25.194 ***</td>
<td></td>
</tr>
<tr>
<td>Egg laying stage</td>
<td>-0.317</td>
<td>Total r squared = 0.406</td>
</tr>
<tr>
<td>Incubation stage</td>
<td>+0.099</td>
<td></td>
</tr>
<tr>
<td>Provisioning stage</td>
<td>+0.077</td>
<td></td>
</tr>
<tr>
<td>Day of nest cycle</td>
<td>-0.042</td>
<td></td>
</tr>
<tr>
<td>Final Adj r squared</td>
<td>0.143</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Model 2</th>
<th>Male morphometrics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wing length</td>
<td>-6.454**</td>
<td>F(17,2)</td>
</tr>
<tr>
<td>Weight</td>
<td>+0.110</td>
<td></td>
</tr>
<tr>
<td>Plumage Score</td>
<td>+3.074**</td>
<td></td>
</tr>
<tr>
<td>Tarsus</td>
<td>-0.502</td>
<td></td>
</tr>
<tr>
<td>Full head length</td>
<td>-0.942</td>
<td></td>
</tr>
<tr>
<td>Final Adj r squared</td>
<td>0.307</td>
<td></td>
</tr>
</tbody>
</table>

Columns show the F values resulting from a stepwise multiple regression between each Y variable and the X variables (F-to-enter for X variables not in the final model and F-to-remove of X variables introduced into the final model). X variables marked ‡ were entered into the final model and those marked with one or more asterisks explained a significant amount of variation in the Y variable in the final model (*p <0.05, **p <0.01, ***p <0.001). The degrees of freedom (V2,V1) at the column head are given as the number of observations and the number of significant variables entered into the model. The direction of the relationship between X and Y variables is given as - or +, indicating a negative or positive direction. The variance explained by the model is given as the adjusted r squared value which is corrected for the remaining degrees of freedom.
5.3.2.3. Breeding Chronology

(i) Multivariate analysis
The prelay stage was entered into the regression model as a significant variable (F = +25.19) indicating increased levels of attacks on other males during this period. Data from the egg laying, incubation and provisioning stages were not entered into the model.

(ii) Between stage variation
Figure 3.2.1 shows the distribution (mean ± SE) of attacks for the prelay, (0.072 ± 0.009), egg laying (0.031 ± 0.006), incubation (0.030 ± 0.009), provisioning (0.032 ± 0.018) and unpaired (0.057 ± 0.027) periods. The differences between the stages were significant (KW = 42.60, 4 df, p <10^-3). Table 3.2.3 shows the comparison of the individual stages, and that a significant degree of variation was accounted for by the high levels during the prelay period. The prelay period had significantly higher levels of attacks than all other periods, but not compared to unpaired males. There was no significant difference between any of the other stages.

<table>
<thead>
<tr>
<th></th>
<th>Egg laying</th>
<th>Incubation</th>
<th>Provisioning</th>
<th>Unpaired</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prelay</td>
<td>-4.496***</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Egg laying</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Incubation</td>
<td>-5.740****</td>
<td>-1.883</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Provisioning</td>
<td>-3.092**</td>
<td>-0.129</td>
<td>-1.270</td>
<td>X</td>
</tr>
<tr>
<td>Unpaired</td>
<td>-1.731</td>
<td>-0.203</td>
<td>-1.145</td>
<td>-0.245</td>
</tr>
</tbody>
</table>

significant differences are *<0.05, **<0.01, ***<10^-3, ****<10^-4.

(iii) Variation in attack rates between individual days
Day of the nest cycle was not entered into the regression model but was significantly negatively correlated with attack rates (r5 = -0.345, n = 39, p <0.05), confirming the higher rates during the prelay period. Figure 3.2.2 shows the distribution of attacks on other males for the period defined in the methods section of Chapter 4. Across all the days there was a significant difference (KW = 36.19, 11 df, p <10^-3), however, no significant difference in attack rates occurred within the egg laying period combined with the incubation period (KW = 6.99, 6 df, p>0.30), addition of any one prelay day creates a significant difference. The variation was derived from the inclusion of the prelay period with the other days. However, within the prelay period there was no significant difference in attack rates (the only pair of days to be significantly different are -4 and -3, Mann Whitney U test, z = -2.154, p <0.05, all other comparisons were not significant z ≤ -1.900, p >0.05), despite rising to a peak on day -2.
(iv) Individual differences in attack rates

The mean attack rates for the prelay, egg laying and incubation periods were compared for individual males using the Wilcoxon signed rank test. The levels in the prelay period were significantly greater than the egg laying ($z = -2.795$, $p < 0.01$) and the incubation ($z = -2.292$, $p < 0.05$) periods. Attack rates for laying period were not significantly different from those of the incubation period ($z = -0.039$, $p > 0.20$). The means for the males were not correlated across periods ($r_g < 0.515$, $n = 13$, $p > 0.05$). The residual variation between males was significant ($KW = 37.78$, $18$ df, $p < 0.01$). Figure 3.2.3 shows the distribution of residuals for all males. Variation between males is apparent with 6 males below and 4 males above the 95% confidence limits (mean = 183.95, 207.30 - 160.59). Comparison of daily attack rates for males observed throughout their prelay periods in 1990, Figure 3.2.4, reveals the difference between individuals was significant ($KW = 10.139$, $4$ df, $p < 0.05$). The rates of attack (mean ± SE) of the males were not significant between days for all individuals: GMRBG with a low attack rate declined steadily from day -5 (0.061 ± 0.019), PMRPG showed no real pattern but peaked on day -1 (0.109 ± 0.021), YMBWR had a relatively low attack rate and no discernible pattern (0.028 ± 0.010), YMGYB had a peak on day -3 (0.089 ± 0.020), whilst YMWYB peaked on day -2 (0.058 ± 0.017). Clearly attack rates varied between individuals both in their levels and temporal patterns.
Figure 3.2.1
Attacks on other males
Stage of the nest cycle

Figure 3.2.2
Attacks on other males
Day of the nest cycle
Figure 3.2.3
Attacks on other males
Mean rank for individual males

Figure 3.2.4
Attacks on other males for males observed through their prelay period in 1990
5.3.2.4. Neighbour density and status

(i) Neighbour density

(a) Neighbour density for all stages

The number of neighbours within 100 m ($F = -4.76$) was entered into the regression model as a significant variable, however, throughout the season the number of adjacent neighbours ($r_s = -0.182, n = 19, p > 0.20$) and neighbours within 100 m ($r_s = -0.271, n = 19, p > 0.20$) were not significantly correlated with attack rates. Similarly, no significant correlations were recorded for the egg laying ($r_s = -0.479, n = 16, p > 0.05$) and incubation ($r_s = -0.463, n = 16, p > 0.05$) stages.

(b) Neighbour density for males in the prelay period

Within the prelay period there was no significant correlation with the number of adjacent neighbours ($r_s = -0.016, n = 18, p > 0.20$) or neighbours within 100 m ($r_s = -0.145, n = 18, p > 0.20$). The bivariate analysis showed no significant relationship between neighbour density and attack rates.

(ii) Neighbour status

(a) Response to neighbour status for all stages

Two categories of neighbour status were entered into the final model as significant variables: provisioning neighbours ($F = -6.51$), and neighbours egg laying ($F = +6.01$). Bivariate analysis showed that attack rates were significantly lower when neighbours were not in the egg laying stage (Wilcoxon signed-rank test); no other significant differences were recorded.

(i) prelay ($z = -0.085, n = 17, p > 0.20$, with $0.030 \pm 0.006$, without $0.035 \pm 0.010$),

(ii) egg laying ($z = -1.991, n = 19, p < 0.05$, with $0.040 \pm 0.007$, without $0.024 \pm 0.006$),

(iii) incubation ($z = -0.628, n = 16, p > 0.20$, with $0.028 \pm 0.007$, without $0.037 \pm 0.006$),

(iv) provisioning ($z = -0.540, n = 17, p < 0.20$, with $0.028 \pm 0.006$, without $0.039 \pm 0.010$).

(b) The prelay stage.

Whilst in the prelay stage no significant difference was found between any of the neighbour status variables (Wilcoxon signed-rank test, $z \leq -1.428, n = 11, p > 0.10$).

5.3.2.5. Environmental Factors

(a) Relationship for all stages

No environmental variables were entered into the regression model and no significant correlations were detected with attack rates ($r_s = <0.070, n = 386, p > 0.05$). The results show that attacks on other males were not controlled or influenced by environmental variation.

(b) Relationship for males in the prelay stage

No environmental variable was found to be correlated with attack rates ($r_s <1.628, n = 168, p > 0.05$), indicating that attacks during the prelay period are independent of weather conditions.

5.3.2.6. Seasonal and diurnal patterns

(a) Day

Day was not entered into the final regression model, and was not significantly correlated with
attack rates ($r_s = 0.015$, $n = 106$, $p >0.20$). Figure 3.2.5 shows the ten-day groupings of attack rates; no significant pattern was recorded and the difference between the blocks was not significant ($KW = 12.15$, $8$ df, $p >0.10$).

(b) Time
Time after sunrise was not entered into the regression model and no significant difference was recorded between hours for any stage ($KW \leq 8.74$, $8$ df, $p >0.30$) and no significant decline or rise in attacks was recorded ($r_s \leq -0.095$, $n \leq 123$, $p >0.20$).

(c) Nests
Nest was entered into the regression model as a significant variable ($F = -18.83$), indicating a rise in attack rates for replacement nests. This result was supported by the bivariate analysis which showed that attack rates were significantly lower (Wilcoxon signed-rank test, $z = 2.166$, $n = 17$, $p <0.05$) for first nests ($0.020 \pm 0.004$) than subsequent nests ($0.043 \pm 0.009$).

5.3.2.7. Male status and morphometrics
(a) Male status
Male status was entered into the final multiple regression model ($F = -3.29$) indicating that bigamous males were more likely to be observed attacking than monogamous individuals albeit not significantly. Bivariate analysis (Mann Whitney U test) showed that monogamous males ($0.041 \pm 0.004$) have lower attack rates again not significantly ($z = -1.823$, $p >0.05$) than bigamous males ($0.057 \pm 0.007$). During the prelay stage no significant difference ($z = -0.200$, $p >0.20$) was found between the attack rates of monogamous ($0.074 \pm 0.011$) or bigamous males ($0.063 \pm 0.017$).

(b) Male morphometric variables
Table 3.2.1 shows the results of the second regression model, wing length was entered into the model ($F = -6.45$) as a significant variable, but plumage ($F = +3.07$) which was also entered was not significant. The second model accounted for 30.7% of the 85.7% variance remaining from the first model, accounting in total for 40.6% of the observed variance. Bivariate analysis showed no significant correlation between the residuals and male morphometrics ($r_s \leq 0.330$, $n = 19$, $p >0.10$). Bivariate analysis of mean values for the prelay, egg laying, incubation and combined periods showed no significant correlations with any of the male characteristics (Spearman rank, $r_s \leq 0.405$, $n = 16$, $p >0.10$).
Figure 3.2.5
The seasonal distribution of attacks on other males
5.3.3. Excursions

5.3.3.1. Data summary

The data used for analysis in this section were of minutes observed outside the territory in observation bouts for which males were visible for at least ten minutes. Males were observed outside their territory for a total of 556 minutes during 193 observation bouts (47% of the total bouts for which males were visible for more than 10 minutes). The overall average excursion rate was 3.06 minutes/hour of visible male activity.

5.3.3.2. Multivariate analysis of excursion rates

Table 3.3.1 shows the results of a stepwise multiple regression of the excursion data. Six variables were entered into the final model, all were significant but accounted for only 12.3% of the observed variance. The variables entered into the model were: neighbours in the prelay stage ($F = +19.51$), neighbours in the egg laying stage ($F = +11.08$), the incubation stage ($F = +21.85$), the egg laying stage ($F = +9.55$), maximum temperature ($F = -6.12$) and the day of the year ($F = +7.35$).
### Table 3.3.1: Stepwise regression model of excursions

<table>
<thead>
<tr>
<th>Model 1</th>
<th>F(380,6)</th>
<th>Model 1 continued</th>
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<tbody>
<tr>
<td>Adjacent neighbours</td>
<td>+0.589</td>
<td>Minutes Observed</td>
</tr>
<tr>
<td>Neighbours in 100m</td>
<td>+0.404</td>
<td>Day of year</td>
</tr>
<tr>
<td>Neighbours prelay</td>
<td>+19.505***</td>
<td>Time after sunrise</td>
</tr>
<tr>
<td>Neighbours egg laying</td>
<td>+11.079**</td>
<td>Wind speed</td>
</tr>
<tr>
<td>Neighbours incubating</td>
<td>+0.969</td>
<td>Hours of sunshine</td>
</tr>
<tr>
<td>Neighbours provisioning</td>
<td>+0.803</td>
<td>Rainfall</td>
</tr>
<tr>
<td>Male status</td>
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<td>Maximum temperature</td>
</tr>
<tr>
<td>Nest</td>
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<td>Minimum temperature</td>
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<tr>
<td>Prelay stage</td>
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<tr>
<td>Egg laying stage</td>
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<td>Incubation stage</td>
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<td>Provisioning stage</td>
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<tr>
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<tr>
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<tr>
<td>Tarsus</td>
<td>-0.190</td>
</tr>
<tr>
<td>Full head length</td>
<td>-0.251</td>
</tr>
<tr>
<td>Final Adj r squared</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Columns show the F values resulting from a stepwise multiple regression between each Y variable and the X variables (F-to-enter for X variables not in the final model and F-to-remove of X variables introduced into the final model). X variables marked § were entered into the final model and those marked with one or more asterisks explained a significant amount of variation in the Y variable in the final model (*p <0.05, **p <0.01, ***p <0.001). The degrees of freedom (V2,V1) at the column head are given as the number of observations and the number of significant variables entered into the model. The direction of the relationship between X and Y variables is given as - or +, indicating a negative or positive direction. The variance explained by the model is given as the adjusted r squared value which is corrected for the remaining degrees of freedom.
5.3.3.3. Breeding Chronology
(i) Multivariate analysis
Data from the egg laying stage ($F = +11.08$) and incubation stage ($F = +19.51$) were entered as significant variables into the regression model. The results indicate that excursions increased after the prelay period rising in the egg laying stage, peaked during incubation and declined again during provisioning.

(ii) Between stage variation
Figure 3.3.1 shows the distribution of excursions (mean per minute ± SE) for the four nest stages and unpaired males: prelay stage ($0.022 ± 0.003$), egg laying ($0.046 ± 0.008$), incubation ($0.058 ± 0.009$), provisioning ($0.009 ± 0.003$) and unpaired males ($0.026 ± 0.011$). The differences between the stages were significant ($KW = 26.63, 4 \text{ df}, p < 10^{-3}$). The pair-wise comparison of stages are shown in Table 3.3.2. High levels of excursions occurred during the egg laying and incubation periods, which although not significantly different from each other, were significantly greater than the prelay and provisioning periods. The lowest period of excursions was during provisioning. Single males were not significantly different from other males during their breeding stages.

![Table 3.3.2. Between stage variation in vigilance](image)

(iii) Variation in excursions between individual days
Day of the nest cycle was not entered into the final regression model and was not correlated with excursion rate ($r_S = -0.044, n = 39, p > 0.20$). Figure 3.3.2, shows the distribution of excursions. Overall, the variation between the days was significant ($KW = 32.18, 11 \text{ df}, p < 10^{-3}$). Within the prelay period there was no significant difference in excursions between days ($KW = 8.33, 4 \text{ df}, p > 0.05$) and similarly within the egg laying period ($KW = 6.89, 4 \text{ df}, p > 0.10$). However, the addition of the incubation period produces a significant difference due to the low level of excursion activity associated with days 10 to 15 ($KW = 15.79, 6 \text{ df}, p < 0.02$). The data show that excursions peaked between days 2 and 9, with the lowest rates observed on day -2.
Figure 3.3.1
Excursions
Stage of the nest cycle

Figure 3.3.2
Excursions
Day of the nest cycle
(iv) Individual variation in excursion rates
Mean excursion rates for the prelay, egg laying and incubation periods were compared for individual males using the Wilcoxon signed-rank test. The level of excursions in the prelay period was significantly lower than the egg laying (z = -2.499, p <0.02) and incubation (z = -2.261, p <0.05). The excursion rate during the egg laying period was not significantly different from the incubation period (z = -0.028, p >0.20). The mean excursion rates for the males in the prelay and egg laying periods were significantly correlated (r_s = 0.570, n =16, p <0.05) and were marginally nonsignificant for the prelay and incubation periods (r_s = 0.50, n = 16, p >0.05). No correlation was found in the rate of excursions between the incubation and egg laying periods (r_s = 0.308, n = 14, p >0.20).

Analysis of the residual excursion data from the stepwise multiple regression showed variation between males was significant, (KW = 44.69, 18 df, p <10^-3). There was no correlation between the number of observation bouts and the mean rank (r_s = 0.144, n = 18, p >0.20). Figure 3.3.3 shows the distribution of residuals for males during 1989 and 1990, differences between males are apparent with 4 males above and 4 males below the 95% confidence limits (mean rank = 187.84, 211.76 - 163.93). The 1990 males were not compared for their variation between days during the prelay period because this was not the period of significant excursion activity.
Figure 3.3.3
Excursions
Mean rank for individual males

<table>
<thead>
<tr>
<th>Male</th>
<th>Mean rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>1989</td>
<td>1990</td>
</tr>
</tbody>
</table>

Legend:
- 1989
- 1990
5.3.3.4. Neighbour status and density

(a) Neighbour density

Neighbour density was not entered into the regression model. Bivariate analysis of neighbour density showed that number of adjacent neighbours ($r_s \leq 0.241, n = 16, p > 0.20$) and neighbours within 100 m ($r_s \leq 0.151, n = 16, p > 0.20$) were not correlated with excursion activity at any stages or for the combined data. The results show no relationship between observed excursions and higher neighbour densities.

(b) Neighbour status

Two measures of neighbour activity were entered into the regression model; neighbours in the prelay stage ($F = +19.51$) and neighbours egg laying ($F = +11.08$). Bivariate analysis revealed significantly (Wilcoxon signed-rank test) higher levels of excursion activity associated with neighbours only in the prelay stage. The data demonstrate that males are leaving their territories at times when neighbouring females were possibly fertile.

(i) prelay ($z = -2.369, n = 18, p < 0.02$, with $0.029 \pm 0.006$, without $0.019 \pm 0.005$),
(ii) egg laying ($z = -1.438, n = 18, p > 0.10$, with $0.031 \pm 0.007$, without $0.027 \pm 0.005$),
(iii) incubation ($z = -1.335, n = 16, p > 0.10$, with $0.025 \pm 0.005$, without $0.026 \pm 0.005$) and
(iv) provisioning ($z = 0.00, n = 17, p = 1.00$, with $0.025 \pm 0.005$, without $0.024 \pm 0.006$).

5.3.3.5. Environmental factors

Maximum temperature ($F = -6.12$) was entered into the regression model as a significant variable indicating a decline in excursion behaviour as temperature increased. Bivariate analysis showed no significant correlations between excursions and any environmental variable ($r_s \leq 0.080, n = 387, p > 0.20$).

5.3.3.6. Seasonal and diurnal patterns

(a) Day

Day was entered into the regression model as a significant variable ($F = +7.35$), but bivariate analysis revealed no significant difference between the day groupings ($KW = 11.03, 8 df, p > 0.10$) and no significant across-time ($r_s = 0.149, n = 106, p > 0.10$). However, if days 98-110 are excluded then there was a significant decline in excursion rates across time ($r_s = -0.218, n = 96, p < 0.05$). Figure 3.3.4 shows the seasonal distribution of excursions, which exhibit a gradual decline after day 130.

(b) Time

Time was not entered into the regression model, no significant difference existed in excursion rates between hours ($KW = 8.34, 8 df, p > 0.30$) and no significant correlation was associated with the time of day ($r_s = 0.014, n = 125, p > 0.20$). Figure 3.3.5 shows the diurnal pattern of excursions which were maintained at a relatively constant level throughout the day.

(c) Nest

Nest was not entered into the regression model and no significant difference in excursion rates (mean $\pm SE$, Wilcoxon signed-rank test) was found between first ($0.027 \pm 0.009$) and replacement ($0.024 \pm 0.005$) nests in the level of excursion activity.
5.3.3.7. Observer influence
Observation bout length was not entered into the regression model and no significant bivariate correlation was revealed for excursion levels ($r_s = -0.087, n = 387, p > 0.05$).
Figure 3.3.4
Seasonal distribution of excursions

Figure 3.3.5
The diurnal pattern of excursions
All stages
5.3.3.8. Male status and morphometrics

(a) Male status
Male status was not entered into the final model and bivariate analysis showed no significant (z = -1.588, p >0.10) differences in excursion activity between monogamous males (0.038 ± 0.004) and bigamous males (0.025 ± 0.06).

(b) Male morphometrics
A second regression model (Table 3.3.1) of the mean residual ranks for the individual morphometric variables showed no significant relationships, which was supported by no significant correlations (r$ < ± 0.209, n = 18, p >0.20). Bivariate analysis of mean excursion values for the prelay, egg laying, incubation and all observations showed no significant correlations with any of the male characteristics (Spearman rank, r$_s$ ≤ ± 0.281, n = 16, p >0.20).
5.3.4. Attacks received from other males

5.3.4.1. Data summary

The data used for analysis in this section were of minutes in which the focal male received an attack in observation bouts for which males were visible for greater than ten minutes. Males were attacked on a total of 81 occasions during 65 observation bouts (17% of the total bouts for which males were visible for more than 10 minutes). The overall average rate of attacks received was 0.44 hour\(^{-1}\) and therefore quite low. Due to the infrequent occurrence of attacks received the data analyses in the following sections are not as detailed as for behaviours analysed previously.

5.3.4.2. Multivariate analysis

Four variables were entered into the final regression model, accounting for only 3.4% of the observed variance. Table 3.4.1 shows the results of the final regression model; only one significant variable was entered into the final model: neighbours in the prelay stage \((F = +8.64)\). The three non-significant variables were time of day \((F = -4.46)\), male status \((F = -3.21)\) and the egg laying stage \((F = +4.59)\).
Table 3.4.1. Stepwise regression model of attacks received

<table>
<thead>
<tr>
<th>Model 1</th>
<th>F(31,4)</th>
<th>Model 1 continued</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjacent neighbours</td>
<td>-0.485</td>
<td>Minutes Observed</td>
</tr>
<tr>
<td>Neighbours in 100m</td>
<td>-0.488</td>
<td>Day of year</td>
</tr>
<tr>
<td>Neighbours prelay</td>
<td>+8.637*</td>
<td>Time after sunrise</td>
</tr>
<tr>
<td>Neighbours egg laying</td>
<td>-0.694</td>
<td>Wind speed</td>
</tr>
<tr>
<td>Neighbours incubating</td>
<td>-1.466</td>
<td>Hours of sunshine</td>
</tr>
<tr>
<td>Neighbours provisioning</td>
<td>+0.110</td>
<td>Rainfall</td>
</tr>
<tr>
<td>Male status</td>
<td>-3.209%</td>
<td>Maximum temperature</td>
</tr>
<tr>
<td>Nest</td>
<td>+0.101</td>
<td>Minimum temperature</td>
</tr>
<tr>
<td>Prelay stage</td>
<td>-0.024</td>
<td></td>
</tr>
<tr>
<td>Egg laying stage</td>
<td>+4.594%</td>
<td></td>
</tr>
<tr>
<td>Incubation stage</td>
<td>+1.021</td>
<td></td>
</tr>
<tr>
<td>Provisioning stage</td>
<td>-0.885</td>
<td></td>
</tr>
<tr>
<td>Day of nest cycle</td>
<td>+0.021</td>
<td></td>
</tr>
<tr>
<td>Final Adj r squared</td>
<td>0.034</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model 2 Male morphometrics</th>
<th>F(17,1)</th>
<th>Total r squared = 0.105</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wing length</td>
<td>+0.878</td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>+0.014</td>
<td></td>
</tr>
<tr>
<td>Plumage Score</td>
<td>+0.129</td>
<td></td>
</tr>
<tr>
<td>Tarsus</td>
<td>-0.120</td>
<td></td>
</tr>
<tr>
<td>Full head length</td>
<td>-2.173%</td>
<td></td>
</tr>
<tr>
<td>Final Adj r squared</td>
<td>0.073</td>
<td></td>
</tr>
</tbody>
</table>

Columns show the F values resulting from a stepwise multiple regression between each Y variable and the X variables (F-to-enter for X variables not in the final model and F-to-remove of X variables introduced into the final model). X variables marked § were entered into the final model and those marked with one or more asterisks explained a significant amount of variation in the Y variable in the final model (*p <0.05, **p <0.01, ***p <0.001). The degrees of freedom (V2, V1) at the column head are given as the number of observations and the number of significant variables entered into the model. The direction of the relationship between X and Y variables is given as - or +, indicating a negative or positive direction. The variance explained by the model is given as the adjusted r squared value which is corrected for the remaining degrees of freedom.
5.3.4.3. Breeding chronology

(i) Multivariate analysis
The egg laying stage was entered into the final regression model \((F = +4.59)\), albeit not as a significant variable, indicating that attacks were received more frequently during this period.

(ii) Between stage variation
A comparison of between stage differences showed significant differences across stages \((KW = 10.87, 4 \text{ df}, p < 0.05)\). The distribution of attacks received from other males is shown in Figure 3.4.1, with the pairwise comparisons shown in Table 3.4.2. Much of the variation was associated with the large number of attacks upon unpaired males, which includes data associated with the violent displacement of one of the unpaired males. Of the periods associated with paired males attacks received during egg laying period were noticeably higher but only significantly greater than the provisioning period.

<table>
<thead>
<tr>
<th></th>
<th>Egg laying</th>
<th>Incubation</th>
<th>Provisioning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg laying</td>
<td>-1.869</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Incubation</td>
<td>-0.135</td>
<td>-0.288</td>
<td>X</td>
</tr>
<tr>
<td>Provisioning</td>
<td>-1.924</td>
<td>-2.780**</td>
<td>-1.852</td>
</tr>
<tr>
<td>Unpaired</td>
<td>-2.026*</td>
<td>-0.826</td>
<td>-1.996*</td>
</tr>
</tbody>
</table>

Significant differences are *<0.05, **<0.01).

(iii) Variation in attacks received from other males between individual days
The day of nest cycle was not entered into the final regression model and was not correlated with attacks received from other males \((r_s = -0.174, n = 39, p > 0.20)\). Figure 3.4.2 shows the distribution of attacks received from other males. Across all the day groupings the level of attacks received were not significantly different \((KW = 12.21, 11 \text{ df}, p > 0.30)\), and despite the apparent variation only one pair of days was significantly different, i.e., day 3 was significantly greater than day -1, \((z = -2.240, p < 0.05)\), all other comparisons were not significant \((z < 0.185, p > 0.06)\).
Figure 3.4.1
Attacks received
Stage of the nest cycle

Figure 3.4.2
Attacks received
Day of the nest cycle
(iv) Individual variation in attacks received from other males
The individual male means were compared using the Wilcoxon signed rank test. The level of attacks received during the prelay period was not significantly different from the egg laying \((z = -1.938, p >0.05)\) or the incubation periods \((z = -0.178, p >0.20)\), however, levels during the incubation period were significantly lower than the egg laying period \((z = -2.002, p <0.05)\). Levels of attacks received for individual males were correlated in the three available comparisons \((z >0.600, >13 \text{ df}, p <0.05)\) indicating that males are consistent through time in the attacks they receive from other males.

Analysis of the residuals from the stepwise multiple regression showed a significant degree of between male variation \((KW = 29.16, 18 \text{ df}, p <0.05)\) and no correlation between observation bouts and the mean rank \((r = 0.158, n = 19, p >0.20)\). Figure 3.4.3 shows the mean rank for males observed in 1989 and 1990. Differences between males are apparent with 5 males below and 6 males above the 95% confidence limits \((\text{mean rank} = 193.69, 214.95 - 172.42)\).

5.3.4.4. Neighbour status and density.
(a) Neighbour density
Neither measure of neighbour density was entered into the final regression model and neither were correlated with attacks received \((r = -0.225, n = 16, p >0.20)\).

(b) Neighbour status
Neighbours in the prelay stage \((F = 8.64)\) was entered into the regression model as the only significant variable. Bivariate analysis (Wilcoxon signed-rank test) showed no significant difference in the rate of attacks received for any neighbour status variable.

(i) prelay \((z = -1.735, n = 17, p >0.05, \text{with} 0.006 \pm 0.002, \text{without} 0.004 \pm 0.001)\),
(ii) egg laying \((z = -0.393, n = 19, p >0.20, \text{with} 0.005 \pm 0.001, \text{without} 0.005 \pm 0.002)\),
(iii) incubation \((z = -1.329, n = 16, p >0.10, \text{with} 0.005 \pm 0.002, \text{without} 0.008 \pm 0.002)\) and
(iv) provisioning \((z = -0.551, n = 17, p >0.20, \text{with} 0.006 \pm 0.002, \text{without} 0.005 \pm 0.001)\).
Figure 3.4.3
Attacks received
Mean rank for individual males
5.3.4.5. Environmental Factors
No environmental factors were entered into the final model and bivariate analysis showed no significant correlations with attacks received ($r_s < ± 0.090$, $n = 386$, $p >0.05$).

5.3.4.6. Seasonal and diurnal patterns
(a) Day
The day of the year was not entered into the regression model as a significant variable and bivariate analysis shows no significant correlation ($r_s = 0.004$, $n = 105$, $p >0.20$) with attacks received. Figure 3.4.4 shows the levels of attacks upon the observed male, the distribution showed no distinct pattern and declined after day 170.

(b) Time
Time after sunrise was entered into the final regression model but was not a significant variable ($F = -4.46$). Bivariate analysis showed no significant correlation between attacks and time of day ($r_s = -0.034$, $n =126$, $p >0.20$) and no overall significant difference ($KW = 12.40$, 8 df, $p >0.05$) between hours despite an initial peak (Figure 3.4.5). Individual periods were examined for any differences and only the egg laying period showed a significant decline after the first hour ($r_s = -0.314$, $n = 59$, $p <0.05$) but no overall significant difference between hours ($KW = 14.16$, 8 df, $p >0.05$). Other periods showed no correlation ($r_s < ± 0.060$, $n ≤ 76$, $p >0.20$) and no significant difference between hours ($KW <10.00$, 8 df, $p >0.05$). In summary, the data demonstrated a decline in attacks received during the day for the egg laying stage only, which was previously shown to be the time of a significant rise.

(c) Nest
Nest was not entered into the regression model and bivariate analysis (Wilcoxon signed-rank test) showed no significant difference in attacks received ($z = -0.059$, $n = 17$, $p >0.20$) between first (0.006 ± 0.002) and subsequent nests (0.005 ± 0.002).
Figure 3.4.4
Seasonal distribution of attacks received

Figure 3.4.5
The diurnal pattern of attacks received
All stages
5.3.4.7. Observer influence
Observation bout length was not entered into the final regression model and no correlation was found with attacks received ($r_S = -0.013, n = 386, p > 0.20$).

5.3.4.8. Male status and morphometrics
(a) Male status
Male status was entered into the final regression model ($F = -3.21$) but was not significant. Similarly no significant difference in attacks received ($z = -0.090, p > 0.20$) was found between monogamous males ($0.006 ± 0.001$) and bigamous males ($0.010 ± 0.004$).

(b) Male morphometrics
A second regression model (Table 3.4.1) of the mean residual ranks and the morphometric variables entered full head length ($F = -2.173$) as a variable but this was not significant. This second model accounted for only 7.3% of the 96.6% of the variance remaining from the first model. Bivariate analysis showed no significant correlations between the residual data and morphometric variables ($r_S < ± 0.375, 18 df, p > 0.20$). Bivariate analysis of mean values for the prelay, egg laying, incubation and all observations showed no significant correlations in attacks received with any of the male characteristics (Spearman rank, $r_S ≤ 0.390, n = 16, p > 0.10$).
5.4. DISCUSSION

The four Y variables analysed in this chapter: intrusions, attacks on other males, excursions and attacks received, all had very low levels of variance explained by the range of X variables excluding morphometric variables, i.e., 10.0%, 14.3%, 12.3% and 3.6% respectively. Part of the reason why more variance was not explained, as discussed in Chapter 4 is that the relationships between the X and Y variables may not be linear. Consequently, some of the variation is not explained by linear regression and some of the patterns of behaviour only appear using bivariate techniques. The analyses do usually reinforce one another in identifying the same patterns of variation. I have divided the data for ease of discussion into two groups: firstly intrusions and attacks upon other males and then excursions and attacks received. The data are discussed with respect to the hypotheses and predictions discussed in the introductory section of this chapter.

Intrusions were by males, as predicted by the sperm competition hypothesis, which in more than half the observed cases were identified as being by neighbours and were frequently very rapid events. The data also showed that some intruders were from more distant territories and occasionally distances of more than 1 km were travelled. The sperm competition hypothesis predicts a response to the intruder by a territorial male when his paternity was threatened. This response was usually observed as displacement followed by a chase which rarely (6.44% of attacks) resulted in actual physical violence. No seasonal pattern of intrusions or attacks was detected. The territory establishment hypothesis proposes that intrusions will occur during the period of territory establishment when borders still remain unclear. Consequently, an initial peak is expected to be followed by a decline as the territorial limits become more defined. The data clearly do not support the prediction of the territory establishment hypothesis, rather there is a slight increase with the replacement nests and no overall seasonal pattern, except for a decline towards the latter stages. The non-territory holder hypothesis predicts a decline in intrusions after territory establishment, which is clearly not supported by the above results. Intrusions and attacks both had observed peaks during the prelay period, which was confirmed by captures, multivariate analysis, between stage comparisons and individual analysis. This is supported by the comparison of the days of the nest cycle which reveals a peak on day -2, albeit not a significant peak for attacks on other males. The pattern of intrusions and attacks during the nest cycle agreed with one of the predictions from the sperm competition hypothesis, that intrusions and the resultant attacks will peak during the fertile period. In Chapter 3 the fertile period was identified as being the prelay period and possibly early into egg laying. What is apparent from both sets of data is that there was an abrupt decline in both intrusions and attacks on the first egg date. This would indicate that the fertile period possibly terminates once the first egg has been laid, resulting in a decline in guarding (Chapter 4) and intrusions.

Another prediction of the sperm competition hypothesis was of a peak of intrusions during the early morning of the fertile period. The daily pattern of intrusions and attacks did not match this fertilisation window hypothesis, but rather intrusions and attack rates remained relatively constant during the days of the prelay period.

Contrary to the expectations of the sperm competition hypothesis at higher neighbour densities,
intrusion and attack rates do not increase, rather during incubation there was a negative
 correlation. It is possible to hypothesise that higher densities, rather than producing increased
 pressure, provide a stable neighbour boundary across which neighbours can observe rather
 than intrude. Whereas at lower densities males cannot observe from their territory and have to
 physically intrude permitting other males also to move into the area.

Neighbour status is apparently important for intrusions with lower levels when neighbours are
 provisioning. However during the prelay stage neighbour status apparently has no affect upon
 intrusion rates. Attacks on other males were significantly lower if neighbours were not egg
 laying, the time of most excursions. The prediction from the sperm competition hypothesis is
 for a rise in violence associated with times when the neighbouring males are not guarding
 (Chapter 4), but the relationship with intrusions was not as clear. Intrusions apparently were
 significantly higher only when neighbouring males were not constrained by provisioning.

Additional data are available from the results on intrusion and attack rates which were not
 directly linked to predictions made in the Introduction. Male status was not a significant
 influence on either of the behaviours during the prelay stage but bigamous males overall were
 significantly subject to higher intrusion rates and attacked more often but not significantly more
 than monogamous males. The overall differences may be a reflection of two females being
 potentially available and a degree of overlap observed in their nest cycles. From this evidence,
 although rates within the prelay period did not differ, there are potentially large negatives with
 guarding two females, of higher intrusion rates and the associated cost of defence.

Individual variation in intrusion rates and attack rates were significant with different temporal
 patterns also increasing the between male variation. This individual variation was significantly
 regressed with wing length for both intrusions and attacks. The data therefore indicated that
 males with larger wings were subject to lower intrusion rates and consequently attacked less.

The influences of observation bout length and environment were not significant, other than for
 a significant correlation between hours of sunshine and intrusion rates during the prelay stage.
 This result may be a reflection of the increased ease with which reed buntings can be viewed on
 brighter days.

The intrusion and attack data, although not wholly supporting the predictions of the sperm
 competition hypothesis, do agree with some of the predictions. Some of these predictions also
 have their converse when viewed as excursion behaviour. Due to the difficulty of following
 males outside their territory the data were not as extensive as for intrusions or attacks on other
 males and therefore may not provide as good an indicator of behaviour patterns. Both
 excursions and attacks received are to a degree linked, in that the attacks were frequently the
 result of an excursion into another males territory. Excursions occurred in nearly 30% of the
 observation bouts, with a rate of 3.06 minutes per hour, whilst attacks received only occurred
 in 17% of bout, at a rate of 0.44 per hour. Excursions were, as expected, most frequent
 outside the prelay period during egg laying and incubation, which was confirmed by
 multivariate analysis, between stage comparisons and individual analysis, with the highest
 levels associated with days 2 to 9. This agreed with the prediction from the sperm competition
hypothesis that excursions are most likely to occur when the male is no longer required to
guard. The resultant attacks upon the focal male, however, peaked when the male was in the
egg laying stage, confirming the rise in behaviours associated with the acquisition of extra-pair
paternity outside the prelay stage. What the results also show is that excursions decline again
during provisioning, when the male’s activities become focused on the feeding of the chicks.

Not in agreement with predictions is that excursions were not more frequent at higher densities.
The explanation for this discrepancy may simply be that at higher densities, males need not
enter neighbours territories as activities can be observed from within the safety of a males own
territory.

The sperm competition hypothesis predicts that males will focus their activities on territories
where females are most likely to be fertile. The excursion results show that rates were highest
when neighbours were in the prelay stage and that neighbours in the egg laying stage were also
the focus of some activity. The data therefore support the prediction that males leave their
territories more when fertile females are present.

No seasonal patterns of excursions or attacks received were apparent, other than a decline
towards the end of the season corresponding to the final replacement nests. The data did not
follow the patterns predicted by the territory establishment hypothesis or the non territory
holder hypothesis and confirm levels throughout the season whilst fertile females are available.

The sperm competition hypothesis predicts an early morning peak in intrusions, so therefore
excursions should also peak in the early morning. Excursion rates and attacks received showed
no apparent pattern. There is therefore no evidence for an early morning peak in activity, rather
activities rise during the day and thereafter show fairly constant levels.

The remaining results on excursions and attacks received showed no significant variation with
status, i.e., monogamous and bigamous males showed no difference in their propensity to
leave their territory or receive attacks. The regression data showed a significant amount of
between male variation, with very little of the actual variation explained and no morphometric
trait explaining any further significant variation.

Observation bout length did not affect the final results and environmental variables had only a
small effect, with a rise in excursions with increasing sunshine and a decrease with a rise in the
maximum temperature. These results confirm my field observations that as temperature
increased on hot summer days the frequency of sightings declined dramatically, to the extent
that during the drought of 1989 males became impossible to locate during the afternoon.

The data in this chapter do not support predictions of either the territory establishment or non-
territory holder hypotheses (Ford 1983, Møller 1987g) and the predictions available from the
sperm competition hypothesis are not all in agreement with the data. The pattern of intrusion
and excursion behaviour is of males leaving their territories when not guarding and focusing on
territories in the prelay stage. These data are in agreement with the prediction that fertile
females should be the focus of intrusion activity (Baitron 1983; Ford 1983; Møller 1987g;
Johnson & Kermott 1989; Rowley & Russell 1990). The sperm competition hypothesis also predicts a diurnal pattern of intrusions, with an early morning peak during the fertile period (Skutch 1952; Schifferli 1979; Tullet 1985). The data do not show any early morning peak in activity for the prelay period but do for the egg laying stage, when rates are much lower. The results would indicate no direct support for the prediction of an early morning peak in intrusions. Neighbours were identified as being the source of intrusions (Møller 1985; 1987g) and did so mainly during the fertile period, again lending support to the sperm competition hypothesis. No relationship of intrusion activity was found with density, which may have been a result of a decreased necessity to intrude when females can be observed from within neighbouring territories. The results from the analysis of aggressive behaviour confirm that it is a reflection of intrusion activity and a response to a paternity threat. Consequently, attacks rose during the prelay stage whilst females were fertile and decline dramatically on the day of the first egg. The intrusion threat and response behaviour of guarding males showed considerable between individual variation which was not linked to any morphological trait but does end abruptly with the commencement of laying.

To summarise, the results of intrusions, excursions and aggressive behaviour do not support either the non-territory holder or territory establishment hypotheses. The patterns of behaviour do support some predictions of the sperm competition hypothesis but fail to support the fertilisation window hypothesis. Territories of females in the prelay stage are the focus of activity until the first egg is laid, with males aggressively defending against intrusions by non-guarding males.
Abstract: Song output in the reed bunting, Emberiza schoeniclus, decreased significantly during the prelay period when females are most likely to be fertile. The increased level of activities associated with mate guarding prevented males from maintaining the song output associated with egg laying and incubation. The data failed to support the hypothesis that song functions as a mate guarding behaviour. This was further supported by a lack of a dawn chorus peak in song output during the prelay period. Song output increased significantly in the presence of a neighbour with a ‘fertile’ female supporting the hypothesis that song acts as a measure of male quality. Song bouts of unpaired males were more frequent than paired males but consisted of significantly fewer songs. Song in unpaired males is hypothesised to act in territorial maintenance and to indicate male status, whereas the more complex song of paired individuals functions to display male quality to their partners.

6.1. INTRODUCTION
In this introductory section I have divided the information available on song into three parts: an initial review of existing data on song in the reed bunting, a review of the function of song and finally how I intend to examine the function of reed bunting song.

a) The function of song in the reed bunting, a review
During the autumn and winter reed buntings are non-territorial. However, as early as January males move to some sites and begin to sing (Ewin 1977). Bell (1968), observed that the first males to exhibit territorial behaviour survey an area prior to the commencement of song. Both territoriality and song are under testosterone control which in turn is dictated by the available photoperiod. First songs were recorded by Ewin (1977) as early as 11th January, Howard (1929) observed that visits are initially confined to the early morning and gradually increase in duration over a number of days. Hermann (1982/83) records his population in Bavaria as arriving by late February to early March, with males usually appearing some fourteen days before females over a period of 36 days and the initial song being different from the territorial song. The end of July/beginning of August marks the end of the breeding season and a subsequent cessation of song, however at the end of September recrudescence of the gonads render the bird photosensitive and whilst light and day length are still strong and long enough, there is some return to reproductive behaviour. Ewin (1977) concluded that song was under photoperiod control but declined in response to pairing.

Reed buntings show a cyclical song pattern according to Bell (1968) and Hornby (1971), with a peak of song pattern before the arrival of females, a reduction thereafter and a resurgence during nesting. A number of authors have attempted to use song for census of the breeding density of reed buntings within an area. Bell et al (1968) compared results of the Common Bird Census, CBC (Snow 1965) with the actual breeding density of a colour ringed population. The census estimated 78% of all territories as errors arose where territories became diffuse and when song ceased during parts of an individuals breeding cycle. In a later paper, Bell et al. (1973) reviewed their original work, concluding that reliability increased with observer
experience, i.e., the original estimates of 78% increased steadily to 100% over successive years
and indicated that this form of census can monitor population fluctuations with reasonable
accuracy. Haukioja (1968) reported a similar study using a line survey method (described by
Merikallio 1946) with which he only recorded 40% (20% if only including singing males) of
his known colour ringed population. Haukioja also tested the line survey method, achieving
75% accuracy, subject to the same errors of diffuse territories and song output variation that
Bell et al (1968; 1973) observed. Song was also used by Jensen (1972), who recorded errors
due to individual variation in song output and the occurrence of bigamy.

Ewin (1977) in his study of reed bunting song found population output to be separated into two
peaks similar to that reported above by Bell (1968) and Hornby (1971). The initial smaller
peak lasts from the second half of February until late March, while the larger second peak lasts
from May to late July. The rise and fall of the initial peak reflects the different arrival times of
males and females onto territories. Males set up territories in late January and sing from then on
until late March, by which time females have arrived and pairing has occurred. March
represents a period of high song output for the first 4 hours of the day, just starting before
sunrise, with no song in the middle of the day but resuming prior to sunset. Failure to obtain a
mate eventually results in cessation of song (Andrew, 1961). During April and after pairing
the song is dramatically reduced and not until the end of April, when egg laying takes place,
does song increase again until the end of July. During May song output is heard regularly
throughout the day, however there is a large difference in the number of songs between
individuals. The amount of song per individual seemed according to Ewin to depend very much
on his input into incubation, but this is very misleading since males generally have little input.
The number of songs per bout varied little during the day but bout frequency and therefore song
output increased. With young in the nest the males song output declines, and in June many
territories become deserted as fledglings seek food.

Åstrom (1976) studied the abiotic factors affecting song and recorded paired males as singing
less than unpaired ones. The reed bunting in northern Scandinavia commonly sang for periods
for up to 20 hours each day, song commencing 2-3 hours before sunrise. Increase in
illumination is the main control of song but day length, date and stage of breeding season are all
very important. Fredriksson et al (1973) showed that activity rhythms based on captures match
closely those of song.

Ewin’s work at Rye Meads (Herts) deals with the seasonal variation in song and its role in
breeding. Individual differences in song output are hard to account for, however birds do tend
to follow the same daily rhythm of output. The song of reed buntings serves the purpose of
advertising, which is enhanced by the use of relatively conspicuous song posts. It is formed of
various components, making analysis comparatively complex. There is a large variation in
interval duration between songs of individuals, the same being true for phrases which comprise
the song. A bimodal distribution of interval durations between phrases is the most conspicuous
and unusual feature, the two peaks corresponding to rapid and slow songs. Songs may contain
from 2-7 phrases but 4 or 5 is more common. The arrangement of the phrase classes after the
initial introductory phrase is clearly non-random, i.e., preferential routes occur, trends can be
seen, note complexes normally occur in the central part of the song, whereas trills and multiple
note complexes occur towards the end of the song and are rarely repeated.

Only 35% of the time in a bout of advertising song is actually spent singing. Songs of individual males always begin with the same introductory phrase which may form a note complex of neighbouring birds. The introductory phrase is of a much shorter duration than following note complexes, which are elaborate with a wide frequency range composed of several notes. It is known that males can recognise the song of their neighbours, i.e., individual recognition is very important, but it is also used for species recognition, therefore the song is comprised of two aspects. Combinations of slight differences may result in noticeable individuality and neighbour recognition becomes rather complex where repertoires have over 20 phrase types.

The seasonal singing pattern was hypothesised by Ewin to correspond to a change in song function, i.e., before pairing the song is for establishing and maintaining a territory and attracting a mate, whereas later in the season territories are established and there are long periods when the female is incubating and the song may serve for pair-bond maintenance. It is therefore possible that changes in status bring about a changed reaction, which according to Ewin should vary with the threat level, i.e., it would be pointless to react to stimuli during incubation which pose no threat. Ewin evaluated this with a number of playback experiments and observed that during the first and second periods of song, males responded to song with silence and a resumption after cessation of playback. During these periods, contact calls are not produced during playback, however during the quiet phase of the season playback induces increased use of contact calls. Overall, a similarity of response was apparent, i.e., the playback represents a threat of similar magnitude. The basic response is to cease whatever was being done and confront the intruder with the aim of removing him. Since no visual stimuli were possible the male began to search or display aggressively, i.e., breast to breast fighting (Andrew 1957b).

The function of song is primarily in intraspecific territoriality, both to deter males and attract females (Ewin 1977). Advertising song involves the transmission of information about species identity, sex, breeding status and motivation (Marler 1956, Armstrong 1973), the relative importance of which depends on the habitat and social system of the species (Catchpole 1973). Evidence for the function of song of reed buntings in reproduction can be determined from its features: a very strong seasonality, aggression to non-neighbour songs, selection of the same phrase type by both members of a counter-singing pair, with matching also being demonstrated. Males have large repertoires of 20-30 phrase types, with very strong intra- and inter-individual variation in the arrangement. There is no evidence of first year males learning from their fathers, i.e., a varying number of phrase types are shared by territorial males with their neighbours, the maximum by close neighbours, and therefore a purely genetic basis to song acquisition is unlikely.

Bell (1968), Haukioja (1971) and Hornby (1971) all showed that individuals will return to a territory over successive seasons. First year males (Haukioja 1971) also return to their natal area but not the exact area, therefore neighbour song sharing will originate from the influence of old males on these novel breeders. The young males also arrive later and will have to
experience a period of intense song learning. Reed buntings isolated in experiments develop a normal song (Stewart 1955; Thorpe 1964), of which the introductory phrase appears to be genetic and the rest learnt subsequently. The introductory phrase seems to be species-specific, but the wide variety denotes the opportunity for individual identification.

A dichotomy exists in the song of reed buntings with the existence of both fast and slow types. Ewin hypothesised the learning of a fast type from chaffinches, Fringilla coelebs, in suboptimal habitat, since the song style is very similar. With first year birds arriving later they are forced into occupying the peripheral habitats where they are exposed to chaffinches and modify a basic template. This seems unlikely, since males actually return to the same site over subsequent season so that mixing would not occur and song would be split between two subpopulations.

(b) The function of song
Learning is important in species with large repertoires (Nottebohm 1975), with aspects of dialect formation and sexual selection being implicated. Dialect may play a role where adaptations to local conditions are important and one group may be favoured above another, and this certainly occurs in birds of a similar size. Three main groups of hypotheses explain the function of song repertoire. They propose that the existence of a repertoire (1) enhances individual recognition, (2) is a response to intersexual selection and (3) increases success in territorial competition (e.g., reviews by Krebs 1977; Krebs & Kroodsma 1988; Dawson 1982). These hypotheses are not mutually exclusive and all give a functional explanation for the existence of a repertoire in birds.

Sexual selection will favour the production of large diverse repertoires for the purpose of attraction, stimulation and retention of a mate. It is unlikely that a large repertoire exists purely to enhance neighbour recognition since time would only be spent in non-neighbour competition (Krebs 1977a). A large repertoire would be less likely to be similar to a neighbour’s and a single song would be unlikely to permit neighbour recognition, but some form of averaging over several may. However, song alone may not be the only cue and plumage may be important.

Large repertoires have been linked to sexual selection (Krebs 1976; Nottebohm 1972; Howard 1974). Krebs (1976) demonstrated that settlement was faster in habitats with smaller repertoires. However, Ewin (1977) suggested this is unlikely in the open visual habitat occupied by reed buntings. In the sedge warbler, Acrocephalus schoenobaenus, (Nottebohm 1972) song is only used to attract a mate (see Catchpole et al 1984; and Searcy 1984 for a review). Mocking birds Mimus polyglottos obtain a higher territory ranking and greater reproductive success through a larger repertoire (Howard 1974). In the great tit Parus major larger repertoires seem to be advantageous in territorial defence (Krebs et al 1978) and repertoire size may be related to lifetime reproductive success (McGregor et al 1981; Lambrechts & Dhondt 1986). In Ipswich sparrows, Passerculus sandwichensis princeps, both song and territory size are used as determinants of mate choice by females (Reid & Weatherhead 1990); both are used in combination as annual variability means the variables are not correlated, (see also Weatherhead & Robertson 1977; Thornhill 1983; Price 1984). Other
observations have also shown that females preferred males who sang the most vigorously (Gottlander 1987; Radesitter et al 1987, Reid 1987; Strain & Mumme 1988; Cosens & Sealy 1986), males who spend more time singing must have better energy reserves (Searcy 1979b), be better at foraging or have more food available (Davies & Lundberg 1984; Reid 1987; Strain & Mumme 1988). Therefore females who choose such males may be able to nest on a richer territory or mate with a male who will be better at provisioning nestlings, or both. Song rate predicts male nest defence and provisioning rates in stonechats, Saxicola torquata, (Greig-Smith 1982), but in general more research is needed. In addition to those material benefits, song may reliably signal something about male genetic quality (Andersson 1982; Kodric-Brown & Brown 1984).

The results obtained for the Ipswich sparrows are to some extent matched by those obtained for pied flycatchers, Ficedula hypoleuca. Alatalo et al (1986) demonstrated that female pied flycatchers selected males solely on the basis of territory quality. However, Lifjeld & Slagsvold (1988) subsequently minimised the variation in territory quality and showed in this case that females selected males based on quality. It is probable that a series of environmental factors may inhibit selection of males by quality by providing conditions where all males appear equal, the reverse being true where male variability becomes large and very apparent. Male song sparrows, Melospiza melodia, with small repertoires are replaced preferentially by floaters (Hiebert et al 1989), the males with larger repertoires held territories for longer and consequently achieved a greater reproductive success. Ewin (1977) found no evidence for the spring peak being involved in sexual selection or territory acquisition and found no evidence that larger repertoire size increased attractiveness or territory size. Both Åström (1976) and Ewin (1977) demonstrated song matching by neighbours indicative of male-male competition.

In most species of passerine birds, males possess repertoires of different song or phrase types (Hartshorne 1973), although several quite different hypotheses have been advanced as explanations (see reviews in Krebs & Kroodsma 1980 and Kroodsma 1982). Discrimination studies have been performed on species with relatively small repertoires i.e., one to five song types (e.g., Falls 1969; Krebs 1971; Goldman 1973; McGregor & Avery 1986), and on species with a larger repertoire (Kroodsma 1973; Ewin 1977; Falls & D’Agincourt 1981; Searcy et al 1981; Brindley 1991). There is a general trend towards a weakening of the discriminatory response with increased repertoire size. The robin, Erithacus rubecula, shows neighbour recognition based both on song and position of the male (Brindley 1991), with an increased response in countersinging when an unfamiliar aspect arose, especially when close to a territorial boundary. The ability to discriminate intruders clearly has advantages in avoiding wasted conflict with neighbours. In the asymmetric war of attrition hypothesis proposed by Maynard Smith (1982) and modified for the response to neighbour song (Ydenberg et al 1988), males should initially show high levels of aggression with a gradual scaling down over time, although this has been criticised by Getty (1989) who suggested that conflict is the only measure by which resources can be measured. Certainly individual recognition occurs (Krebs, 1971; Brooks and Falls 1975) and recent evidence (Weary & Krebs 1992) provides a mechanism by which individual great tits are able to differentiate the song and fit song types to other individuals. This was based on a previous study (Weary et al 1990) which showed that song can be separated on the basis of repertoires, individual specific song variants and voice.
traits. The only other studies which analysed how individuals differentiate song were based on a unpaired song type (Nelson 1989; Brooks and Falls 1975), which showed frequency was an important component. Countersinging, as well as a means of neighbour recognition may be a means of dominance assertion for example in the marsh wren, *Cistothorus palustris* (Kroodsma 1979), and apparently in testing unfamiliar neighbour-neighbour situations in the robin (Brindley 1991).

Differential responses to a song “context” have also been recorded, with often more aggressive responses to song coming from the territory centre rather than the boundary (e.g. Ickes & Ficken 1970; Melemis & Falls 1982), and differently to an alien than to individuals from the same population (McGregor 1983; Hansen 1984). Males of certain species also refrain from entering areas from which rival song is given (Krebs et al 1978; Smith 1979), indicative of some information exchange. Response may also vary with different song rates or versions, as shown by playback experiments on the willow warbler, *Phylloscopus trochilus* (Helb 1973; Järvi et al 1980), song learning in yellow warblers, *Dendroica petechia* (Spector et al 1989) and in the blackbird, *Turdus merula* (Dabelsteen & Pedersen 1990). Work on the latter species showed a positive relationship of song intensity and aggressive response during playback, however this is not the case in sedge warblers (Catchpole 1989), where all playback was responded to but with no observed intensity variation. Song as an honest fitness marker is adaptive in that it reduces the risk of injury (Tinbergen 1951) and may conserve energy (Van Rhijn 1980; Popp 1987). Bluffing in contests may not arise, due to its associated costs (Huntingford and Turner 1987), for example when a bluffer is attacked due to the high threat level instantly developed. Consequently, due to the constraints imposed by energetic (Greenewalt 1968) and combat costs an honest signal is the most efficient and safest.

The message implicit in a repertoire and conveyed as contrast is a result of switches, but few studies have actually examined these (Brenowitz 1981; Richards 1981; Horn & Falls 1988a). Detection of switches poses problems (Wiley & Richards 1978, 1982), due to alteration from producer to receiver, however it has been proposed that acoustic contrast may function as a suitable method (Horn & Falls 1988a). If contrast is important then it should be possible to arrange songs in such a way as to achieve the greatest effect (Nelson 1973; Whitney 1981b). The response of eastern meadowlarks, *Sturnella magna* (Falls et al 1990), is largest to songs with switches of greater contrast. It may be the greater degree of variety which is more stimulating (Falls & d’Agnicourt 1982; Kramer et al 1985; Horn & Falls 1988a,b) or alternatively it is the variety implied. The ability to detect switches supports the view that individuals use their own songs as references in evaluating the singing of others (see Margoliash & Konishi for neurophysiological correlates; and Falls et al 1982; McGregor et al 1983; McGregor and Falls 1984; and MacArthur 1986 for behavioural correlates). The familiarity of song will ultimately make switching more detectable (Falls et al 1990), facilitating mate or neighbour recognition, thus the perception of variety may be limited to only close neighbours.

(c) The function of song in the reed bunting, hypothesis testing

A dichotomy in song types in the reed bunting suggests a duality of function although Ewin discussed (1977) it as arising from habitat sharing with chaffinches. Where the two song types
can be identified then any function will be assessed with respect to mate attraction and territory maintenance/male competition (Searcy and Andersson 1986). If song functions to keep out males then when the threat of intrusion is greatest so should song be greater (Greig-Smith 1982; Möller 1988f, 1991e). In concordance with this theory male stonechats (Greig Smith 1982), great tits (Mace 1987a), and yellowhammers, Emberiza citrinella (Möller 1988), sang most frequently when their female was most fertile. Reed buntings, however, have already been described as having little or no song output during the fertile period, at least of first nests. Many species show a peak during the fertile period (Möller 1991e), with mainly close guardsers singing most during their partners fertile period. Möller hypothesises that if birds are close guardsers then song should peak during the fertile period. Further, where species breed asynchronously and extra-pair copulations are dependent on asynchrony (Birkhead and Biggins 1987) then again the data supports a peak during the fertile period (Möller 1991e). This may indeed explain why song is not heard during the fertile period of the first nest, where synchrony is high, so it may be further hypothesised that as pairs become asynchronous for the repeat clutches that song should peak during subsequent fertile periods.

An increase in song during the fertile period will clearly indicate the fertility status of the female. However, if guarding is apparent then nothing will be lost further by singing. Good males will stand to gain from singing when it provides an effective ‘keep out’ signal and intrusions will be reduced. However, poor males will be able to sing less and intrusion rates will be higher and in fact disproportionately higher. This may force them into singing more in order to reduce their share of intrusions (Möller 1991e). It is therefore possible that females can reliably use song as an indication of male fitness and increase sexual selection on males by female choice. Extending this argument, it may be possible to obtain an indication of male quality from song and that the more successful males should show the highest output in song relative to their neighbours in situations where asynchronisity is important.

Song of a paired male may act as a mate guarding behaviour or a courtship behaviour, soliciting the female to copulate with her male and no other (Greig-Smith 1982). The dawn chorus was implicated by Mace (1986) as a means by which males may protect paternity. Certainly, since song is also present at other times this is not its sole purpose. Evidence from studies on the collared flycatcher, Ficedula albicollis (Pärt 1991), suggested that dawn chorus singing was directed at the female, but unlike in great tits (Mace 1987a) and other species (Greig-Smith 1982; Möller 1988), the singing did not peak during the fertile period and apparently did not function as a paternity guard. Song would therefore appear to be implicated as a paternity guard at dawn and with an increase during the fertile period in some species such as stonechats, (Greig Smith 1982), great tits, (Mace 1987a), and yellowhammers (Möller 1988), but not in others, i.e., the collared flycatcher (Pärt 1991). Two other hypotheses also may be proposed to explain the dawn chorus, firstly as a means whereby males may perform a keep out message to would-be invaders (Kacelnik and Krebs 1982) a feature which would largely limit song to dawn when the chance of a take over is greatest. The second alternative is that males sing to females (Mace 1986; Pärt 1991) and that they start to sing when they lose contact with the female (Wasserman 1977; Krebs et al; 1981, Cuthill & Hindmarsh 1985). Song is known to attract females (Eriksson & Wallin 1986) and consequently song may serve as a means of locating the female, although this requires the probability of losing a female to be high, i.e., if
predation is a threat overnight. Costs of singing at dawn are probably slight as low light reduces foraging profitability (Kacelnik & Krebs 1982), so when a male is not in contact with a female song should occur during the dawn chorus.

Song can have both territorial and reproductive functions but presumably has associated costs. To explain declines in song rates of the great tit, Lambrechts and Dhondt (1988) proposed the anti-exhaustion hypothesis. Sound production presumably is costly (only shown in anurans, Wells and Taigen 1986), and if song types use different effecters then males may be able to maintain higher song output by switching between songs. Studies by Weary et al (1988) showed that song switches were due to motivational changes (Halliday & Slater 1983), rather than exhaustion. Lambrechts and Dhondt (1988) refuted this study but a later study, by Weary et al (1991), provided evidence which supported both hypotheses. However, the lack of influence of song switches on output argues against the anti-exhaustion hypothesis. Costs associated with singing may provide an indication of male fitness, in that in order to sustain a high song output males according to the anti-exhaustion hypothesis must have a larger repertoire and be able to switch. Providing this is valid, then male differences in quality may be assessable by the female based on his song output. In some species, females implanted with oestradiol respond more strongly to longer, more complex songs of conspecifics (e.g. Catchpole et al 1984; Clayton and Pröve 1989). Females (swamp sparrows, Melospiza georgiana) may also respond to aspects of song (Searcy et al 1981b), to which males do not respond (Peters et al 1980; Searcy et al 1981a). It has also been shown that female red-winged blackbirds, Agelaius phoeniceus (Searcy 1990), brown headed cowbirds, Molothrus ater (King et al 1980) and white-crowned sparrows, Zonotrichia leucophrys (Baker et al 1981; 1987; Baker 1983) respond preferentially to the elements of local male songs. Rejection of song not belonging to a conspecific is regarded as of importance (Searcy & Brenowitz 1988), as a mistake may result in a wasted hybrid mating.

Song can be regarded as a secondary sexual characteristic. Two major groups of models have been advanced as explanations, namely the ‘good genes’ and ‘arbitrary traits’ models (Bradbury & Andersson 1987). The good genes model suggests that song variability between males is a reliable estimate of their quality (Zahavi 1975, 1977; Andersson 1982; Hamilton and Zuk 1982; Kodric Brown & Brown 1984; Pomiankowski 1988). If the trait is open to variability then male condition will exert an influence, for example his energy status or parasite load. A negative correlation of blood parasite load and song as a secondary sexual characteristic was found by Hamilton Zuk (1982; see also Read & Harvey 1989 for a critical analysis of their techniques, and Read & Weary 1990 who refuted this correlation in their analysis). Möller (1991) took a far more direct, albeit rather drastic approach to parasite burden and manipulated parasite burdens of male swallows, Hirundo rustica. His results demonstrated that an increased mite burden (Ornithonyssus bursa) reduced song output. If singing is a reflection of the current food supply (Searcy 1979; Wilhelm et al 1980; Morton 1982; Davies & Lundberg 1984; Gottlander 1987; Reid 1987) or current energy reserves (Slagsvold 1973; Garson & Hunter 1979; Higgens 1979; Greig-Smith 1983; Gottlander 1987; Reid 1987), then output should be a reliable indication of health, including parasite burden (including the chance of transmission to a mate) and male quality.
The function of song in Scott’s seaside sparrow, *Ammodramus maritimus peninsulae* (McDonald 1989), was studied by muting of males. The first round of muting tested for mate and territory acquisition. Muted birds remained mateless and were late in obtaining territories. A second set of birds were muted midseason and all lost their mates and suffered a large territory attrition, but regained much once song was regained, a situation similar to results obtained previously for red-winged blackbirds (Peek 1972; Smith 1976). Other muting studies have shown no detrimental effect in caged monogamous duet singers, *Cossypha humilis* (Todt & Hultsch 1982), and an effect on dominance ranking in a captive flock of brown-headed cowbirds (Dufty 1986).

Song output in many species takes place from conspicuous points located around the individual’s territory. Song posts can be hypothesised as serving a number of functions; (i) a point from which to display territorial ownership, observing and deterring intruders, (ii) a point from which to display to and observe a partner and (iii) a point from which to display to and observe neighbouring females. To discern whether one or all of these functions are play a role, testable hypotheses are required. If song is a means by which a male conveys his quality to a female, then a male should endeavour to raise his success by displaying more openly to a female. Therefore, a shift in song post to a visible display point will be one means by which this may be achieved.

To summarise, previous studies on song have provided evidence that males are chosen based on song components and/or output. In this section I examine how sperm competition affects song output and in the subsequent chapter how this relates to reproductive success. The temporal pattern has been shown to be different from other species which experience a rise in song output during the prelay period, which indicates conflicting behaviours in the reed bunting. The influence of external factors will also be analysed with regard to how they influence song output and finally male morphological characters will be examined with respect to how they may correlate with song activity. The function of song in the reed bunting has therefore not been specifically identified. However, there are a number of areas which merit investigation with regard to sperm competition and reproductive success. A seasonal variation in song output for the population is observed, with a lull after pairing and recommencing with egg laying, the period when sperm competition would be most intense. It may therefore be hypothesised that if sperm competition is high then the need to guard and repel intruders will be great and reduce the time available for song during the prelay period. Reed buntings show individual-specific song introductory phrases and neighbour matching and counter singing. This is indicative of male-male competition and a need to recognise individuals. Individual reed bunting song output varies, which may be indicative of variable male quality. The data in this section are therefore used in the final chapter as a variable for assessing reproductive success.

The predictions and questions available for testing are: (i) if song is implicated as a paternity guard then males should increase their output of song during the fertile period (see Chapter 3) to advertise their quality, and as asynchrony between pairs increases then song output should correspondingly increase. However, existing data (Ewin 1977) suggests this is unlikely to be the case and that variation between stages will explain the inaccuracies associated with surveys. If the need to guard females imposes constraints upon singing then output should be most
reduced during the early morning and the prelay stage which are the hypothetical periods of peak fertility. If song is operates as a paternity guard then song output should be higher around dawn during the fertile period. (ii) Individual variation will be observed not in the rate of song output (songs per minute), but in the total amount of song and this should increase at higher densities as a response to counter singing. The between individual variation will be analysed to examine if differences are significant after other factors have been controlled for. (iii) If song is involved in advertising then males should respond with greater song output in the presence of their own female and neighbouring females if mate guarding permits. Males should therefore respond with song to fertile females, to optimise advertisement and display more vigorously when their own female cannot be located. (iv) Two song types occur in reed buntings, fast and slow. Do they have an observable territorial function or does the variation reflect differences in reproductive behaviour?
6.2. METHODS

Many of the methodologies for the data analysis were described in the methods section of Chapter 4, consequently I will confine this section to a description of how song data were collected.

Data were collected during behavioural observations and comprised whole and part minutes spent singing. The data were transformed to provide a measure of the proportion of whole minutes spent singing for the time a territory was observed. Song requires no visual observation of a male and so a proportion of the data collection was made without reference to the behaviours described in the previous chapters. Records of the number of songs per minute were also made for each male (minimum number of counts = 20) and records were made for neighbouring males during observations of the focal males. Territories were also surveyed for singing males throughout the season to provide a record of the number of males singing.

Additional data are mentioned in the text on a small sample of female removals, observations of song post shifts with regard to the nest cycle and using aviary birds to provide an indication of how male song output may relate to female acquisition.

The numbers of individual males and observations analysed for between day variation are listed shown in Table 2.1. The sample sizes used in the other analyses are quoted with the appropriate analytical results.

<table>
<thead>
<tr>
<th>Day</th>
<th>Number of observations</th>
<th>Number of males</th>
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<tbody>
<tr>
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<td>21</td>
<td>9</td>
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<td>-4</td>
<td>24</td>
<td>10</td>
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<td>-3</td>
<td>26</td>
<td>7</td>
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<tr>
<td>-2</td>
<td>37</td>
<td>12</td>
</tr>
<tr>
<td>-1</td>
<td>34</td>
<td>11</td>
</tr>
<tr>
<td>0</td>
<td>36</td>
<td>11</td>
</tr>
<tr>
<td>1</td>
<td>27</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
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<td>12</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>5-9</td>
<td>30</td>
<td>14</td>
</tr>
<tr>
<td>10-15</td>
<td>19</td>
<td>9</td>
</tr>
</tbody>
</table>
6.3. RESULTS

3.1. Data summary
A total of 23,334 minutes (389 hours) of territory observations (582 bouts) were performed of which 4117 minutes were recorded as whole minutes of song. The mean (± SE) percentage of time for which males were recorded singing within an observation bout was 18.30% ± 0.88%. The perspective this overall average figure provides is that males will on average spend less than one fifth of their day in song. This figure is however extremely coarse and the following results explain the causes of variation in male song output.

3.2. Multivariate analysis of song
Table 3.1 shows the results of a stepwise multiple regression of the proportion of time spent singing. Sixteen variables were entered into the model all of which were significant. The variables were: neighbours within 100 m ($F = +8.27$), neighbours in the prelay stage ($F = +11.06$), neighbours in the egg laying stage ($F = +10.63$), neighbours in the incubating stage ($F = -3.21$), neighbours in the provisioning stage ($F = +2.13$), male status ($F = +8.51$), nest ($F = +3.16$), the prelay stage ($F = -25.57$), the provisioning stage ($F = -11.62$), day of the year ($F = +20.49$), time after sunrise ($F = +41.08$), wind speed ($F = +5.31$), hours of sunshine ($F = -14.72$), rainfall ($F = -8.19$), maximum temperature ($F = +10.13$) and minimum temperature ($F = -3.23$). The model accounted for only 26.2% of the observed variance in song output, despite the large number of significant variables. The remaining variance must be due to factors not included in the model, such as between male variation.
Table 3.1. Stepwise regression model of time spent in song

<table>
<thead>
<tr>
<th>Model 1</th>
<th>F (322, 16)</th>
<th>Model 1 continued</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjacent neighbours +0.195</td>
<td>Minutes Observed +0.107</td>
<td></td>
</tr>
<tr>
<td>Neighbours in 100m +8.265***</td>
<td>Day of year +20.493***</td>
<td></td>
</tr>
<tr>
<td>Neighbours prelay +11.060***</td>
<td>Time after sunrise +41.083***</td>
<td></td>
</tr>
<tr>
<td>Neighbours egg laying +10.628***</td>
<td>Wind speed +5.312***</td>
<td></td>
</tr>
<tr>
<td>Neighbours incubating -3.205**</td>
<td>Hours of sunshine -14.720***</td>
<td></td>
</tr>
<tr>
<td>Neighbours provisioning +2.125*</td>
<td>Rainfall -8.194****</td>
<td></td>
</tr>
<tr>
<td>Male status +8.508***</td>
<td>Maximum temperature +10.133***</td>
<td></td>
</tr>
<tr>
<td>Nest +3.162**</td>
<td>Minimum temperature -3.234**</td>
<td></td>
</tr>
<tr>
<td>Prelay stage -25.568***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg laying stage +0.555</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incubation stage +0.810</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Provisioning stage -11.618***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day of nest cycle -0.878</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final Adj r squared 0.262</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Model 2 Male morphometrics
(F20,2)

| Wing length +9.931** | Total r squared = 0.522 |
| Weight +0.012 | |
| Plumage Score +1.444 | |
| Tarsus -4.871* | |
| Full head length -0.330 | |
| Final Adj r squared 0.352 | |

Columns show the F values resulting from a stepwise multiple regression between each Y variable and the X variables (F-to-enter for X variables not in the final model and F-to-remove of X variables introduced into the final model). X variables marked ‖ were entered into the final model and those marked with one or more asterisks explained a significant amount of variation in the Y variable in the final model (*p <0.05, **p <0.01, ***p <10^-3). The degrees of freedom (F=F1,FV) at the column head are given as the number of observations and the number of significant variables entered into the model. The direction of the relationship between X and Y variables is given as - or +, indicating a negative or positive direction. The variance explained by the model is given as the adjusted r squared value which is corrected for the remaining degrees of freedom.
6.3.3. Breeding chronology

(i) Multivariate analysis

Two stages of the nest cycle were entered into the regression model, indicating that song declined significantly during the prelay ($F = -25.57$) and provisioning ($F = -11.62$) stages, being more severe in the former.

(ii) Between stage variation

Variation between the stages including unpaired males was highly significant ($KW = 70.545, 4 df, p<10^{-3}$). Comparison among the individual stages, (Table 3.2 and Figure 3.1 means ± SE) reveals that song output during the prelay period was significantly lower than during all the other periods except provisioning. Song levels during the egg laying and incubation periods were not significantly different, but were significantly greater than during the prelay and provisioning periods. The unpaired males show a significantly higher level of song than the other stages, however the data is limited to only three males.

### Table 3.2 Between stage variation in song

<table>
<thead>
<tr>
<th></th>
<th>Laying</th>
<th>Egg laying</th>
<th>Incubation</th>
<th>Provisioning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laying</td>
<td>-5.341****</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Incubation</td>
<td>-7.130****</td>
<td>-1.480</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Provisioning</td>
<td>-1.364</td>
<td>-2.418*</td>
<td>-3.657***</td>
<td>X</td>
</tr>
<tr>
<td>Unpaired</td>
<td>-5.280****</td>
<td>-2.667**</td>
<td>-2.173*</td>
<td>-3.776***</td>
</tr>
</tbody>
</table>

Significant differences are *<0.05, **<0.01, ***<10^{-3}, ****<10^{-4}

(iii) Variation in song output between individual days

The day of the nest cycle was not entered into the regression model but there was a significant correlation between song and the day of the nest cycle ($r_g = 0.439, n = 39, p<0.01$). This was largely due to the seasonal effect associated with the prelay period of first nest (see below), but there is also an effect observed with replacement nests. Figure 3.2 shows the distribution of the proportion of time males were singing. The variation between days was significant ($KW = 59.02, 11 df, p<10^{-3}$), however no difference exists between days 0 through to 15 ($KW = 5.82, 6 df, p>0.30$). A comparison between individual days during the period 0-15 revealed no difference between any two days ($z <1.780, p>0.05$). The data for days -5 to -1 do show a significant level of difference between days ($KW = 20.26, 4 df, p<10^{-3}$). The song rate shows a definite decline from day -5 to day -2 and then a subsequent rise to the level associated with the egg laying and incubation stages. The levels on day -5 were not significantly different from the egg laying and incubation periods ($z = -0.941, p = 0.3736$), indicating that the response to the female’s change in status was not immediate. On day -4 there was a no significant decline in song rate ($z = -0.894, p = 0.376$), but by day -3 the decline was significant ($z = -2.243 p<0.01$) and remained significant on days -2 ($z = 3.864, p<10^{-4}$), and -1 ($z = 1.808, p<0.05$). On day 0, the day of the first egg, song rate resumes to levels equivalent to day -5 ($z = -0.166, p = 0.436$) and the egg laying and incubation periods. The change in the song pattern during the prelay period was probably the result of intensified alternative activities associated with sperm competition (see Chapters 4 & 5).
Figure 3.1
Song output
Stage of the nest cycle

Figure 3.2
Song output
Day of the nest cycle
(iv) Individual variation in song output

(a) Paired males

Sufficient song data were collected to allow the comparison (Wilcoxon signed-rank test) of the mean individual song output for the prelay, egg laying, incubation and provisioning stages. The level of song output was significantly lower during the prelay period compared to the egg laying \((z = -3.92, p < 10^{-3})\) and incubation periods \((z = -3.845, p < 10^{-3})\) and the egg laying and incubation periods were not significantly different \((z = -0.896, p = 0.187)\) from each other. The provisioning stage had a significantly greater song output compared to the prelay period \((z = -2.062, p < 0.02)\) but was significantly lower than in the egg laying period \((z = -1.647, p < 0.05)\). However, the levels of song output during the provisioning and incubation stages were not significantly different \((z = -1.503, p > 0.05)\). No two stages were correlated in their levels of song output \((r_s \leq 0.441, n = 19, p > 0.05)\).

(b) The special case of unpaired males

Unpaired males differed in their song output but only three of the observed males were recorded as being unpaired during the study, and consequently the Wilcoxon signed-rank test is not suitable. A comparison of the output of unpaired males by the Mann Whitney U test reveals that output is significantly greater than for all periods of the nest cycle: prelay \(z = -2.681, p < 0.01\), egg laying \(z = -2.342, p < 0.01\), incubation \(z = -2.401, p < 0.01\), and provisioning \(z = -2.091, p < 0.02\). The form of each unpaired male's song type matched that at the start of the season, typically being of four repeated phrases "tsip-tsap, tsip-tsap". Paired males' songs were complex and more extensive in duration and highly variable within and between individuals.

(c) Variation in song rates per minute

Song was recorded as the number of songs per minute for those males observed during 1989 and 1990. A comparison of the prelay, egg laying, incubation and provisioning stages revealed no significant difference (Wilcoxon signed-rank test, \(z = -0.089, p > 0.20\)). An overall average was obtained for 21 males, 11 males for the prelay period, 12 males for the lay period, 15 males during incubation, 6 males whilst provisioning and 3 unpaired males. The analysis of the data for unpaired males was performed using the Mann Whitney U test and the difference was found to be significant \((z = -2.75, p < 0.01)\). Figure 3.3 shows the song rate per minute for each stage (mean ± sd), the average for the unpaired males \((4.987 ± 0.35)\) is clearly different from the overall average excluding unpaired males \((11.94 ± 1.917)\). These results combined with those of the output above, show that although males are singing for more minutes whilst unpaired the overall rate of total songs was actually reduced for two males, PMPNR(1990) and GMRGB (1989) by 32% and 22% respectively. However, GMRGB (1989) had a 55% greater song output during his unpaired period compared to his subsequent egg laying and incubation periods. All males were presumed to have lost their original females due to predation (the legs and rings being found in one case), but only GMRGB obtained and successfully paired with a replacement female. GMRGB was also a male with a high reproductive success from both within-pair paternity and extra-pair paternity in 1989, whereas GMRGB achieved no EPP but was successful within his own nest and PMPNR achieved no reproductive success in 1990 (Dixon, thesis in prep).
(d) Residual variation
Analysis of the residual song data from the regression model shows a significant degree of male variation ($KW = 50.46, 20\, df, \, p < 10^{-3}$). No correlation existed between the number of observation periods and the mean rank of the individual males ($r_s = 0.027, n = 21, \, p > 0.20$).

Figure 3.4 shows the distribution of residuals of all males, differences between males are apparent with eight males above and ten males below the 95% confidence limits (mean rank = 270.62, 300.13 - 241.11).
Figure 3.3
Songs per minute
For all stages

Figure 3.4
Song output
Mean rank for individual males
6.3.4. Neighbour density and status
(i) Neighbour density
(a) Neighbour density for all stages

Neighbours within 100 m was entered into the final regression model as a significant variable (F = +8.27), indicating higher levels of song at higher densities. Bivariate analysis shows no significant overall correlation with song and neighbours within 100 m ($r_S = 0.435, n = 20, p > 0.05$) and the number of adjacent neighbours ($r_S = 0.345, n = 20, p > 0.10$). Song output within the egg laying stage was, however, significantly correlated with the density of adjacent neighbours ($r_S = 0.507, n = 19, p < 0.05$).

(b) Neighbour density for males in the prelay stage.
During the prelay stage there was no significant correlation between song and the number of adjacent neighbours ($r_S = -0.003, n = 20, p > 0.20$) and neighbours within 100 m ($r_S = -0.035, n = 20, p > 0.20$). Song was therefore not significantly influenced by neighbour density during the prelay period.

(ii) Neighbour status
(a) Response to neighbour status for all stages

Four neighbour behaviours were entered into the final model as significant. Positive relationships were observed between song and neighbours in the prelay ($F = +11.06$), egg laying ($F = +10.63$) and provisioning ($F = +2.13$) periods. Neighbours who were incubating ($F = -3.21$) were recorded as having a significant negative relationship with song. Males therefore sang more whilst neighbours were in the prelay and egg laying periods indicating a link between song and the fertile period. Song subsequently declined as neighbours commenced incubation. Bivariate analysis (Wilcoxon signed rank test) showed that song output was significantly higher only in response to neighbours in the egg laying stage:

(i) prelay ($z = -1.024, n = 18, p > 0.20$, with $0.152 \pm 0.02$, without $0.142 \pm 0.017$),
(ii) egg laying ($z = -2.173, n = 19, p < 0.05$, with $0.165 \pm 0.021$, without $0.110 \pm 0.015$),
(iii) incubation ($z = 0.00, n = 16, p = 1.00$, with $0.146 \pm 0.018$, without $0.137 \pm 0.026$),
(iv) provisioning ($z = -1.823, n = 17, p > 0.05$, with $0.166 \pm 0.020$, without $0.121 \pm 0.018$).

(b) Response to neighbour status for males in the prelay stage

Only neighbours in the egg laying period significantly affected song output ($z = -2.043, n = 16, p < 0.05$), with song being more frequent when neighbours were laying ($0.108 \pm 0.22$) than when not ($0.052 \pm 0.013$).

6.3.5. Environmental factors

a) Relationship for all stages

All environmental variables were entered as significant variables into the final regression model. Song was positively associated with maximum temperature ($F = +10.13$), although minimum temperature was negatively associated with song ($F = -3.23$). Bivariate analysis showed positive correlations between song output and maximum ($r_S = 0.09, n = 522, p < 0.05$), and minimum temperature ($r_S = 0.123, n = 522, p < 0.01$). Thus on warmer days males spent a greater proportion of time in song, this may to some extent be a reflection of the seasonal
influence. Wind speed was a significant variable from the stepwise regression ($F = +5.31$) but was not significantly correlated with song ($r_g = 0.076, n = 522, p >0.05$), so on windier days males spent more time in song once other variables had been controlled for. Sunshine and rainfall were both entered as significant negative associations ($F = -14.72$ and $F = -8.19$ respectively), neither of these variables were significantly correlated with song ($r_g = -0.062, n = 522, p >0.20$ and $r_g = -0.014, n = 522, p >0.20$, respectively). Therefore only once other influences have been corrected for can the influence of sunshine and rainfall be detected. Song is therefore positively influenced by temperature and wind speed but apparently on bright and wet days there is a decline in song output.

(b) Relationship for males in the prelay stage
Song during the prelay period has similar correlations to the overall pattern but only minimum temperature ($r_g = 0.226, n = 186, p = 0.002$) and maximum temperature ($r_g = 0.177, n = 186, p <0.02$) were significantly correlated. Therefore, temperature was the only influence on song of males during the prelay stage, with a rise on warmer days.

6.3.6. Seasonal and diurnal patterns
(a) Day
During 1988 and 1989 records were made of the proportion of males observed which were engaged in song. The distribution of song recorded during these seasons (Figure 3.5) matches that recorded by Ewin (1977), with an initial early season peak towards the end of February and the beginning of March, with a lull thereafter until the commencement of the laying of the first clutch. The lull in song activity was compared for those males during 1989, in which observation data was recorded for the prelay and the subsequent egg laying stages. The analysis (Wilcoxon signed-rank test) showed a significant decrease (sometimes cessation) in song for all males recorded ($z = -3.540, n = 14, p <10^{-3}$). The decrease preceding the first nest was generally larger than that prior to replacement nests. However this was not significant ($z = -0.980, n = 8, p = 0.1634$).

The observation data also was used to determine seasonal effects upon song output, day was entered into the regression model as highly significant ($F = +20.49$), and bivariate analysis shows a strong correlation between song and day of the year ($r_g = 0.330, n =114, p<10^{-3}$). The effect is as expected of a rise in the proportion of time spent singing corresponding (Figure 3.6) to the time of first eggs, with a peak corresponding to the first 10 days of June. The proportion of time spent singing subsequently declines to low levels at the end of July. The proportion between 10 day blocks was significant ($KW = 38.27, 8 df, p <10^{-3}$) and the proportion of time spent singing showed a different pattern from that of the proportion of males observed singing. The two distributions were not correlated ($r_g = 0.539, n =10, p >0.20$), with the difference being that the proportion of time spent singing shows a far sharper peak with a decline to the end of the season. The inference from the difference between the distributions is that towards the latter half of the season males were still singing, but for less of the time.
Figure 3.5
Males in song by survey

Figure 3.6
Seasonal distribution of song output
Time was entered into the final regression model as the most significant influence upon song ($F = +41.08$) and bivariate analysis showed a strong positive correlation ($r_S = 0.327$, $n = 136$, $p <10^{-3}$). The overall diurnal pattern of song is shown in Figure 3.7; the distribution shows a minor peak at dawn, a slight decline to 2-3 hours post dawn and a rise thereafter. The difference between hours was significant ($KW = 24.57$, 8 df, $p <0.01$). During the 1990 breeding season, the diurnal output of song was recorded for seven males; of these, data for five males was available throughout the prelay, egg laying and incubation periods. The diurnal pattern of song output during the prelay period was compared to that of the combined egg laying and incubation periods. Figure 3.8 shows the song output post sunrise (mean proportion of time observed singing ± SE) for these five males. The output for the egg laying/ incubation period was significantly greater throughout the day (Wilcoxon signed-rank test, $z = 2.345$, $p <0.01$). The two distributions followed different patterns for the first two hours of the day. During the prelay period the song level began at a low level increasing throughout the day and thereafter decreases to a minimum level between 2-3 hours post sunrise. The levels of song then increased attaining a plateau after 4-5 hours post-sunrise, which is similar to that observed during the prelay period. Figure 3.9 shows the patterns of song for the five males analysed for both their prelay and egg laying/ incubation periods. Although there were individual differences in the general patterns, there were trends which remained throughout. During the prelay period there was lower levels of song during the first three hours of the day, with an increase thereafter. This differed from the following periods in which 4 of the five males showed an early peak, followed by a lull and a subsequent rise. The one male (PMRPG) which showed no early morning peak had a gradual rise up to 4-5 hours post dawn. The prelay period had a slightly different pattern in the diurnal distribution of song compared to the combined data for the egg laying/ incubation periods. The difference occurred in the first part of the morning where no early morning peak, (dawn chorus), was observed for the prelay period. This may be ascribed to other activities associated with sperm competition (Chapters 4 and 5). Individual males showed differences in the level of song reduction during the prelay period and in the extent of change in their song distribution.

To assess the extent to which song changed diurnally during the prelay period a comparison of days -5 and -4 was performed against days -3 and -2. Figure 3.10 shows the mean song rates for each 2 day period ± SE; the two distributions are not dissimilar (Kolomogorov Smirnov, $z = 1.336$, $p = 0.09$), but the level of song was significantly greater for days -5 and -4 (Wilcoxon signed-rank $z = -2.197$, $p = 0.0143$).
Figure 3.7
The diurnal pattern of song output
All stages

Figure 3.8
The diurnal pattern of song output
for males observed in 1990
Figure 3.9a
The diurnal song output of male YMWB

Figure 3.9b
The diurnal song output of male YMGB

Figure 3.9c
The diurnal song output of male YMBWR
Figure 3.9c
The diurnal song output of male PMRPG

Figure 3.9d
The diurnal song output of male GMRGB
Figure 3.10
The diurnal song output for days
-5,-4 v -2,-1
Nest was entered into the regression model as a significant variable ($F = +3.16$) but bivariate analysis (Wilcoxon signed-rank test) revealed no significant difference between first ($0.113 \pm 0.021$) and subsequent nests ($0.169 \pm 0.017$).

6.3.7. Observer influence
The duration of the observation bout was not entered into the final regression model and no significant correlation with song output was recorded ($t_S = -0.063$, $n = 522$, $p > 0.10$).

6.3.8. Male status and morphometrics
(a) Male status
Male status was entered into the regression model as a significant variable ($F = +8.51$), agreeing with the bivariate analysis which showed that the monogamous males ($0.184 \pm 0.011$) sang significantly ($z = -2.341$, $p < 0.02$) more than bigamous males ($0.129 \pm 0.016$). However, during the prelay period no significant difference was found between monogamous and bigamous males ($z = -0.666$, $p > 0.20$). Unpaired males, as explained previously, had the greatest song output per minute.

(b) Male morphometric variables
Table 3.1 shows the results of the second regression model, two variables were entered into the model accounting for 35.2% of 73.8% of variation remaining from the first model. The regression models accounted for 52.18% of the observed variance in song output. The variables entered into the second model were wing length ($F = +9.93$) and tarsus length ($F = -4.87$) both of which were significant. Bivariate analysis of the residual data revealed no significant correlations with song output ($rs \leq \pm 0.390$, $n = 21$, $p > 0.05$). Bivariate analysis of mean values for the prelay, egg laying, incubation, and provisioning stages and all observations produced the following significant correlations: full head length and song output for the combined data (Spearman rank, $r_S = 0.481$, $n = 18$, $p < 0.05$), wing length and the combined data ($r_S = 0.475$, $n = 21$, $p < 0.05$), wing length and song output during the incubation stage ($r_S = 0.592$, $n = 19$, $p < 0.05$) and full head length and song output during provisioning ($r_S = -0.617$, $n = 12$, $p < 0.05$). All other correlations were not significant.

6.3.9. The function of song posts
Nine shifts were observed for males who had neighbours in the prelay period, whilst they were not recorded for the other stages. The shifts were always towards the territory with the fertile female, and increased the view of the singer. In two cases these shifts were observed as mini-satellite territories of the song post and the immediate area (less than 5 m surrounding it). No shifts away from the territory with the fertile female were observed. The data is different from an expectation of 0 shifts and supports the hypothesis that males display to neighbours rather than just watching from the territory borders.

6.3.10. Female removal / absence
A male was observed to temporarily lose track of his mate with reasonable certainty on only four occasions, two in 1989 and two in 1990. On two occasions this resulted in the male
circling the territory, in one case of which the female was relocated but in the second, a bigamous mating, the female was not recovered during the course of the observations. On two occasions the response of the male was to search the territory, and when no female was located, to commence singing until the female returned. The link between female absence and a switch to singing prompted a single temporary removal of a female (PMRF 90), the immediate response of the male was to search the territory, followed by a switch to singing. In the 24 hours prior to removal the mean percentage of time spent singing was 11.30%, and was 8.70% following the return of the female. During the period the female was absent the male spent 58% of the observation time in song, a five fold rise. Although this is not statistical evidence of a link between song and advertisement to the female it does provide an indication that males are singing to advertise to females.

6.3.11. Aviary studies
During the 1989 breeding season a study was performed on two pairs of aviary held reed buntings. The pairs were maintained in separate cages during 1989, no song was recorded for two months, until the individuals were all separated. This produced a unpaired male song from one of the males whilst nothing was recorded from the other. The singing male was removed and producing song in the other male until the former male was replaced at which stage the song reverted to the previous state. When paired again song once again ceased. The inference from this study was that males sing there more complex song when there is a need to attract females which are being competed for. Remove the male - male competition and song ceases, remove the female and song reverts to a basic territorial form. These observations are very simple and therefore it is not legitimate to make rigid conclusions, but the data do indicate possible functions associated with song.
6.4. DISCUSSION

The temporal pattern of song as predicted by its function as a mate guarding or courtship behaviour was that song should increase during the fertile period. The data for males recorded singing through the season corresponded to that of previous studies (Bell 1968; Hornby 1971; Ewin 1977), in which a lull was recorded after pairing and prior to the first egg date. The seasonal output subsequently increased after the initiation of the first clutch only decreasing toward the end of the season. The decline in song after the first third of June was associated with males still singing but less frequently. Song output when compared with the first egg dates shows a decline corresponding to a decline in replacement nests (Chapter 1). Individual males showed repeated and significant declines in song output during the prelay period and more specifically days -3, -2 and -1 for replacement nests. Previous chapters have shown that the prelay period is an intense period of mate guarding activity, with repeated intrusions and the need to deter intruders. The decline in song during the prelay period would therefore appear to be the consequence of increased activities associated with sperm competition. Møller (1991c) hypothesised that species which are close guards should show a peak in song output during the fertile period, especially where they breed asynchronously. The results from this study run counter to Møller's prediction, because the requirements of guarding detract from time available to sing.

Song output per minute did not vary through the nesting stages as observed by Ewin (1977), who recorded no between male difference in bout length or songs per minute but a significant between male difference in the frequency of song output. This is supported by examination of the residual data from the multiple regression, which showed significant between male differences in song output. The between male variation indicates that some males, as well as singing more often than others, also did not show such a marked decline as other males during the prelay period. Møller (1991c) hypothesised that poor males will be able to sing less and that only the good males will be able to sing sufficiently to reduce their share of the intrusions. The song data showed that all males suffer a decline in song during the prelay period, but that those males with a low overall song output sang less than some other males. Song may therefore still function as a mate guarding behaviour but for the majority of males the added pressure of intruders means that they are unable to benefit.

Song at dawn has been proposed to function as a paternity guard (Birchhead and Møller 1992), witnessed through its reaching a peak during the fertile period. In the reed bunting no dawn chorus peak was observed during the prelay period and rather there is a gradual rise in song through the day (Ewin 1977, Åström 1976). These data in fact lend support to the fertilisation window hypothesis (Birchhead et al 1987), in so far as the decrease in singing is also associated with a rise in intruder activity (Chapters 4 & 5). These activities effectively reduce song which although supporting sperm competition activities does not support the copulation link of male activity. Two other hypotheses have been proposed to explain the dawn chorus: firstly, that it may function as a keep out signal to other males (Kacelnik & Krebs 1982) and secondly to reaffirm contact with the female (Wasserman 1977; Krebs et al 1981; Cuthill & Hindmarsh 1985). The lack of a dawn chorus in reed buntings during the prelay period provides no support for these hypotheses, but female removal and a subsequent rise in song activity does
provide support to the hypothesis that song is a mechanism by which males may relocate a lost mate.

Counter singing by males was described by Ewin (1977) as occurring in reed buntings but also song is known to play a role in advertisement to females (Catchpole et al. 1984; Searcy 1984). The results presented here show that during the egg laying period song output was significantly greater with higher neighbour densities. This can be explained through the need to counter-sing with the increased requirement for neighbour recognition, but also it being advertisement to females. Ewin (1977) concluded that song serves both as advertisement and a mechanism of individual recognition. Unpaired male song shows that song need not be of elaborate form or high rates if neighbour recognition is important. The results in this chapter show that song of males which are not themselves guarding is apparently directed towards neighbours in the prelay (multivariate analysis only), and egg laying stages and decreases in the presence of incubating neighbours (multivariate analysis only). Song was directed at females which are most likely to be fertile and at periods when they were more evident. This was supported by evidence of song post shifts and the occurrence of satellite territories which were directed at fertile females, rather than being random shifts during the season. Previous studies have shown that song is involved in mate attraction (Catchpole et al. 1984; Searcy 1984) and that males who have large repertoires obtain a higher reproductive success (McGregor et al. 1981; Lambrechts & Dhondt 1986). The evidence from this study supports the results of the other studies, with males singing more to fertile females. If song is involved in female attraction for mates then presumably it acts also to attract females for extra-pair copulations, provided it is a reliable indicator of male fitness (Searcy 1979b; Davies & Lundburg 1984; Reid 1987; Strain & Mumme 1988; Greig Smith 1982). The opportunity for mate choice is very limited in reed buntings (Chapter 1), so consequently song may provide a reliable mechanism by which females can assess the fitness of neighbouring males.

Unpaired male song is one factor which was highly evident during the season. The song of unpaired males was greater in frequency but of a lower rate/minute than paired males. The dichotomy was as described by Ewin (1977) of a fast and slow song type. The data from aviary individuals provided indication that a territorial function may be implicated for the slow song, as only one of the caged males sang at any one time when separated. Additional circumstantial data supports a dichotomy of song function: unpaired males (n = 3) when presented with a female (in each case accompanied by a male guard) on territory resumed song to normal levels albeit for the short periods of intrusion. A further paired male exhibited a dichotomy of song within his territory; in the area adjacent to the nest he was recorded as singing song of a normal paired male, whilst at the other end of his territory approximately 100 m away his song reduced to that of a unpaired male. Although not conclusive evidence, it is possible to hypothesise that slow song operates as a territorial maintenance function and associated with this provides a cue to the female of male status, which is evidenced by the occurrence of female intrusions. To declare himself as unpaired has obvious benefits. Firstly, intrusions and territory conflict should be reduced, however, this was only true in the case of two of the unpaired males the third receiving attacks only after a neighbour had acquired his female and proceeded to eject him from his territory. Secondly, it should also advertise his availability to surrounding females. In 1989 and 1990 unpaired males paired females who had
left a nearby territories. Once paired a male is presumably required to advertise his fitness to the female which he does so through the higher song rate with which he competes with the surrounding neighbours, density being one potentially important correlate associated with counter-singing (Ewin 1977). This competition hypothetically is turned into an additional benefit which is evidenced in discernible differences in male quality which permits competition for extra-pair paternity.

The abiotic influences on song match those of the study by Åström (1976) in which he showed seasonal variation in the pattern and influences of the weather. Increased temperature and wind speed were both recorded as raising song output, whereas increased rainfall and hours sunshine both diminished song. The increase in song with increased temperature may be associated with a decreased requirement to forage (Seay 1979b), whilst increased wind speed is associated with decreased vigilance and perhaps is therefore a response where another behaviour is not suitable. The effect of rainfall is a marked feature of the need to take cover in such an open habitat as is the effect of direct sunshine which markedly reduced observations during 1989.

Male mating status was a significant factor influencing song output. Over the season, bigamous males sang less than their monogamous counterparts, whilst unpaired males spent most of the time singing. The difference between the former states is however not observed during the prelay period. Providing that these differences are not related to specific individuals one may hypothesise that bigamous males are subject to greater competition thus reducing song, but with all males’ song being reduced during the prelay period this difference disappears. No character variable was found to be correlated with song although a partial correlation with wing length was recorded.

The data presented in this chapter oppose some expectations of high advertisement rates during the prelay period but can be explained by counter-directional pressures associated with sperm competition. A link with advertisement is supported by data which show a positive increase with neighbour density and times when surrounding females are available to display to. Patterns of copulation support the effect of other sperm competition activities being a major influence, with significant between male differences.

To summarise, the constraints of sperm competition activities effectively reduce the ability of males to guard and sing. Song therefore does not appear to operate as a keep out message, but does apparently function as a means of advertisement to neighbouring females. Two forms of song were observed, a simple form linked to territory maintenance and a more complex form associated with female attraction. Individual variation in song output was significant, providing a further indication that it may be a reflection of variation in male fitness.
CHAPTER 7.
The link between behaviour and reproductive success

Abstract: Mate guarding is hypothesised to be a mechanism by which males can protect their paternity. Similarly, other behaviours are designed to enhance the acquisition either actively or through display. Analysis of these behaviours and reproductive success in the reed bunting Emberiza schoeniclus indicates that guarding during the prelay period failed to protect within-pair paternity, supporting the hypothesis that guarding occurs in low quality males who are more subject to losing their paternity. The acquisition of extra-pair paternity was found to be significantly enhanced through increased song output and excursions, indicating that song output may provide a significant cue to male quality. A male’s overall reproductive success is best predicted by song output. A low frequency of recorded extra-pair copulations and high a level of extra-pair paternity indicates that the active seeking of extra-pair copulations is not the sole route to their acquisition. Rather, females choose males on the basis of their song output to obtain furtive extra-pair copulations.

7.1. INTRODUCTION

In Chapters 1, 2 and 3, I examined the constraints upon social reproductive success, actual reproductive success and the pattern of copulations. To summarise: one of the main constraints on social reproductive success is the level of predation which accounts for the majority of nests prior to fledging (42%), levels of extra-pair paternity are high (50%) indicating a mixed reproductive strategy (Trivers 1972; Westneat et al 1990) with only one third of males achieving extra-pair paternity. Copulations are relatively frequent (4.06 per day) occurring predominantly during the prelay period but continuing through to the third day of egg laying. Observed extra-pair copulations were rare (4.39%) showing a significant disparity with the level of extra-pair paternity, indicating a potential for secretive EPCs. The data therefore show high levels of extra-pair paternity with the potential for behaviours associated with the acquisition and prevention of extra-pair paternity.

In Chapters 4, 5 and 6 behaviours were grouped on the basis of the prevention and acquisition of extra-pair paternity, whilst song was analysed separately. It is evident that many of the behaviours show similar temporal patterns and must apparently be linked. During the prelay period mate guarding occurs with significantly increased levels of vigilance, time spent close to the female and following of the female. These behaviours are associated with an increased attack rate upon intruding males apparently seeking extra-pair copulations. Outside the prelay period males increase the level of song output and spend more time out of their territory apparently seeking extra-pair paternity and as a result of this are attacked more frequently.

This chapter represents an analysis of the interrelationship of behaviour variables and how these are related to reproductive success. Previous studies have emphasised the use of lifetime reproductive success (LRS) as a measure of individual performance (see Newton 1989 for a review), but few have combined behavioural data with known levels of within-pair and extra-
pair paternity (see Chapter 2). The advantage of using LRS is that it reveals the extent of individual variation in reproductive success. Most studies (Newton 1989) suffer to some degree from gaps in records resulting from movements and incompletely recorded life spans and uncertainties over parentage. LRS provides a measure of variation but seldom examines behavioural mechanisms for increasing reproductive success. Factors such as age and physical characters are used as correlates of observed reproductive success but if EPP cannot be measured then mechanisms for maximising reproductive success cannot be analysed. Individual differences in LRS have been associated with the term “fitness”, which has genotypic connotations and avoids issues such as those influences known to be important in the reed bunting, such as predation. An individual of optimal fitness (Williams 1966, Grafen 1988) may therefore not actually achieve the largest number of offspring due to chance events, the converse arguments also being true. LRS only provides a measure of individual fitness in the circumstances prevailing within the current population but no actual measure of how individuals may influence behaviourally their own reproductive success. The behaviours of reed buntings have been analysed in the previous sections revealing temporal and individual variation. The evidence indicates that a mixed reproductive strategy (Trivers 1972) is employed with males guarding and seeking EPP. This, however, does not explain clear individual differences in paternity success and behaviour. In this chapter the behaviours are combined with reproductive success data to identify whether behaviour can increase reproductive success or whether the observed pattern is due to purely random influences.

The three guarding behaviours described in Chapter 4 were shown to peak during the prelay period and declined subsequently. The timing of behaviours were in agreement with the sperm competition hypothesis (Birkhead et al. 1987) with an observed increase during the `fertile` period. Two hypotheses can be proposed to explain variation in the extent of guarding: (i) males will guard to protect their paternity, therefore predicting that males which guard more will be subject to fewer cases of cuckoldry, and (ii) low quality males will be subject to greater intrusion pressure and forced to guard their paternity more, this predicts a negative correlation between guarding and within pair paternity. Levels of intrusion behaviour and subsequent attacks upon males also rose during the prelay period and again provide measures for individual variation in the potential for extra-pair paternity. The two hypotheses described for mate guarding should therefore also describe similar relationships for intrusions and defence against intruders, i.e., (i) males which defend more vigorously will be subject to lower levels of EPP and (ii) males which suffer higher levels of intrusion are of lower quality and will suffer higher levels of EPP.

The mechanisms for the acquisition of extra-pair paternity were described in Chapter 5. Higher levels of excursion behaviour and subsequent receipt of attacks from defending males were observed outside the prelay period. Alternative hypotheses for explaining variation in these behaviours are that males which spend more time seeking extra-pair paternity, as witnessed by excursion behaviour and attacks received, will (i) acquire more extra-pair paternity, consequently this predicts a positive correlation between these behaviours and EPP, and (ii) will spend less time within their territory defending their paternity, therefore predicting a negative correlation with within-pair paternity.
Song output (Chapter 6) was discussed and hypothesised to be a marker for male quality and a characteristic to display quality to surrounding females. This hypothesis predicts that males which sing more should be of higher quality and therefore achieve higher levels of EPP and WPP. Alternatively, males which sing more may guard less potentially losing WPP (although this conflicts with a marker of quality, but still acquire higher EPP.

To validate the above predictions this chapter examines the interrelationships among behaviours across all stages and within the prelay period to determine the extent to which behaviours occur together or not within the same observation bout. This analysis is not statistically robust but does provide an indication of behavioural interactions. I then examine the relationships of behaviour, density and morphometric variables with reproductive success. The reproductive success data includes males from 1989 and 1990 (A. Dixon thesis in prep.). The data from the regression models are also examined for their relationship with reproductive success. The relationships between behaviour and reproductive success are then combined to into a model which describes variation in that success.
7.2. METHODS

7.2.1 The relationship between behaviours
The links between behaviours are simplistically analysed for within-bout behavioural correlations (Spearman rank correlations, $r_s$) and then separated based on these into a chart of how the various behaviours are potentially related and interact. Two analyses are performed, firstly for the entire season and then for the prelay stage, which is now known to be a period of behavioural changes. These analyses are not statistically valid as between male variation is excluded, however, they do provide an important indication of associated behaviours within bouts.

7.2.2 The relationship between behaviours and reproductive success

Five measures of reproductive success are shown in Table 7.1.: (i) the number of chicks from within pair paternity, (ii) the total chicks within an individuals brood (WPP + EPP) (iii) category (i) as a proportion of category (ii), (iv) the number of offspring sired by a male through extra-pair paternity and (v) the total number of chicks sired (WPP + EPP). Of these five variables analyses were performed using: EPP, total chicks sired and the proportion of the male's own chicks which were WPP.
Table 7.1 Reproductive success data used in analysis with behavioural variable

<table>
<thead>
<tr>
<th>Male &amp; Year</th>
<th>(i) WPP</th>
<th>(ii) Total chicks within the nest</th>
<th>(iii) WPP as a proportion of (ii)</th>
<th>(iv) EPP</th>
<th>(v) Total chicks sired</th>
</tr>
</thead>
<tbody>
<tr>
<td>GMBBR 89</td>
<td>3</td>
<td>10</td>
<td>0.30</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>GMGWG 89</td>
<td>3</td>
<td>5</td>
<td>0.60</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>GMRGB 89</td>
<td>3</td>
<td>5</td>
<td>0.75</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>GMRGB 90</td>
<td>2</td>
<td>8</td>
<td>0.25</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>GMRGB 90</td>
<td>2</td>
<td>8</td>
<td>1.00</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>PMPNR 90</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PRMRG 90</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>YMBWR 89</td>
<td>0</td>
<td>3</td>
<td>0.00</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>YMBWR 90</td>
<td>3</td>
<td>6</td>
<td>0.50</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>YMGB 90</td>
<td>1</td>
<td>8</td>
<td>0.13</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>YMWYB 89</td>
<td>3</td>
<td>5</td>
<td>0.60</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>YMWYB 90</td>
<td>4</td>
<td>9</td>
<td>0.44</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>YMWYBW 89</td>
<td>3</td>
<td>7</td>
<td>0.43</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>YMWYBW 90</td>
<td>0</td>
<td>8</td>
<td>0.00</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>YMGB 89</td>
<td>7</td>
<td>10</td>
<td>0.70</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>YMGB 89</td>
<td>9</td>
<td>9</td>
<td>1.00</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>GMWYB 89</td>
<td>3</td>
<td>5</td>
<td>0.60</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

7.2.2.1. Male mean behaviour values and reproductive success

The mean values per male for the behaviours described in Chapters 4, 5 and 6, were acquired for the prelay, egg laying, incubation and provisioning stages (song only) along with the combined data for all stages. These values were subsequently combined with data on the density of neighbours and male morphometrics (Chapter 4) to determine significant correlates (Spearman rank correlation test, r_s) of reproductive success.

7.2.2.2 Analysis of residuals from the regression models

In the previous Chapters (4, 5 & 6) the behaviours discussed were analysed using stepwise multiple regression to remove variation associated with the nest cycle, season and environment. The residual data for the 8 behavioural variables are analysed to determine correlates (Spearman rank correlation test r_s) of reproductive success.

Following the analysis of these two data sets I have reappraised the multi-variate analysis and repeated it excluding the influence of density. This third data set is subsequently analysed with the reproductive success data to examine the relationship between behaviour and reproductive success.
7.2.2.3. A model describing the interaction of behaviours and reproductive success

The analysis of behaviour and reproductive success by bivariate techniques (Spearman rank correlation test, $r_s$) cannot reliably determine interactions between behaviours. Consequently, using the male behaviour means and the second set of residual data, excluding the influence of density, I have performed two further stepwise regression models to determine the extent to which behaviours, density and morphometric variables interact to describe the observed reproductive success. Unfortunately, due to the constraints upon the data, i.e., more X variables than the model permits with a relatively small reproductive success data set, I have only included those variables significant at $p < 0.10$ in the bivariate analyses of male behaviours means. This may mean the exclusion of some behavioural interactions but this is unavoidable. The multi-variate techniques other than this alteration remain the same as described in Chapter 4.
7.3. RESULTS

7.3.1 Correlation of data for the entire season

(a) All stages
Figure 7.1 shows the relationships between behaviours across all stages. Correlations (Spearman rank correlation coefficient $r_s$, $n = 380$, significant values $p < 0.05$) across the breeding season are split into two sectors, those which are linked to the guarding of females and repelling of intruders and those which are not. The former group of behaviours includes: vigilance, intrusions, attacks on other males, proximity to the female and female pursuit flights. All these behaviours are significantly correlated, therefore indicating that periods of high vigilance are periods of high female guarding, with high levels of intrusions and repulsion by attack. Consequently, this group of behaviour is boxed with attacks on other males being partitioned because it is also positively correlated with attacks from other males. Attacks from other males is positively correlated with excursions which is in turn positively correlated with song. The data suggest that males which spend a greater proportion of time in song are observed leaving their territory more. The behaviours are therefore apparently at opposite poles, with guarding behaviours not associated with either song or the seeking of EPCs.

(b) The prelay stage
Examination of the prelay stage data reveals that not all the correlations hold as exhibited in Figure 7.2. The behaviours are now still split into two basic groups but patterns of behaviour are more discrete. The evidence for guarding of females comes mainly from vigilance, which is correlated with time <10 m from the female, pursuit flights and intrusions. Attacks on other males, however, are now only apparently a response to intrusions. The second group of behaviours is still separated by attacks from other males but this is now positively correlated with vigilance. Song and excursions are both correlated and in turn are correlated with attacks from other males. The groupings therefore indicate that where song rates are high males are still leaving their territories and are frequently attacked, whilst guarding males, as expected attack intruders and may also be subject to attacks from the males in the other category.
Figure 7.1 The relationship between behaviours across all stages of the nest cycle.

- vigilance
- flights
- time < 10m
- Intrusions
- attacks on other males

attacks from other males

excursions

song
Figure 7.2 The relationship between behaviours during the prelay stage.
7.3.2 The relationship between behaviour and reproductive success

7.3.2.1. Male mean behaviour values and reproductive success

The results in this section are ordered as in their presentation in Chapters 4, 5 and 6. The results (Table 7.2) list significant values (p < 0.05) and also those correlated at p < 0.10, all other correlations are therefore not significant and not described. Significant correlations between neighbour density, morphometrics and reproductive success are also described.

The data show a number of significant correlations relating to the reproductive success variables. If the data are analysed as the proportion of chicks sired within a male's nest (WPP) then song during the incubation stage and attacks on other males during the prelay stage are negatively correlated. This indicates that males that sing more during incubation are subject to more WPP loss, and that having to repel males during the prelay period results in paternity loss.

A large number of variables are positively correlated with EPP. Song output during the egg laying, incubation and combined stages are all significantly correlated with EPP, indicating that males which sing more are more successful. Excursion behaviour during incubation and the combined stages are both positively correlated with EPP, therefore males which spend more time apparently seeking EPCs acquire the greatest success. Both density variables were positively correlated with EPP so levels of EPP therefore increase at higher densities. Time spent < 10 m from the female during incubation and vigilance during the combined stages were negatively correlated with EPP, indicating that staying within the territory and being vigilant rather than singing reduce the chance of EPP.

Three values were correlated with the total young sired: song during the prelay stage and plumage score were both positively correlated whereas time spent < 10 m from the female across the combined stages was negatively correlated. The males which sang more during the prelay period and have the brighter plumage achieve greater overall success, whereas remaining close to the female results in decreased success.
Table 7.2 Correlations between mean behaviour data and reproductive success

<table>
<thead>
<tr>
<th>Behavioural variable</th>
<th>Reproductive success measure</th>
<th>WPP</th>
<th>EPP</th>
<th>Total chicks sired</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Time spent &lt; 10 m</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>from the female</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i) All observations</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r_s = -0.590, n = 15^-</td>
<td>r_s = -0.460, n = 15^-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ii) Incubation</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r_s = -0.510, n = 14^-</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2) Female orientated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>flights</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3) Vigilance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i) All observations</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r_s = -0.632, n = 13^-</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4) Intrusions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5) Attacks on</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>other males</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i) Prelay</td>
<td>r_s = -0.602, n = 14^-</td>
<td>NS</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>ii) Excursions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i) All observations</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r_s = 0.519, n = 15^-</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ii) Incubation</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r_s = 0.559, n = 14^-</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6) Attacks received</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7) Song</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i) All observations</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r_s = 0.62, n = 17^-</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ii) Prelay</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r_s = 0.565, n = 15^-</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iii) Egg laying</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r_s = 0.591, n = 17^-</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iv) Incubation</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r_s = 0.516, n = 16^-</td>
<td>r_s = -0.54, n = 16^-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>v) Provisioning</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r_s = 0.704, n = 12^-</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9) Neighbour density</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i) Within 100 m</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r_s = 0.535, n = 16^-</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ii) Adjacent</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r_s = 0.600, n = 16^-</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10) Male morphometrics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i) plumage score</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r_s = 0.509, n = 17^-</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results marked ^ are significant at p<0.05, ^ = p<0.10, and NS = not significant.
Variables not shown are described in the methods section.

7.3.2.2 Analysis of the residual data
The mean residual rank obtained for each male as described in Chapters 4, 5 and 6, for the eight behavioural variables were not significantly correlated with any measure of reproductive success.

7.3.2.3 A reappraisal of the regression data
The bivariate analyses demonstrated a correlation between density and reproductive success. The inclusion of density values within the regression model will therefore tend to remove any variation which influences reproductive success. If the relationship between behaviour, density and reproductive success is significant then to include density in the regression model is
erroneous. The regression data have therefore been reanalysed excluding density to provide values which can be entered into a subsequent model reintroducing density. The following significant results were obtained as shown in Table 7.3.

**Table 7.3. Correlations between residual behaviour data and reproductive success**

<table>
<thead>
<tr>
<th>Behavioural variable</th>
<th>Reproductive success measure</th>
<th>Total chicks sired</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WPP</td>
<td>EPP</td>
</tr>
<tr>
<td>1) Time spent &lt; 10 m from the female</td>
<td>No significant correlations</td>
<td></td>
</tr>
<tr>
<td>2) Female orientated flights</td>
<td>NS</td>
<td>$r_s = -0.677$, $n = 16$, ** NS</td>
</tr>
<tr>
<td>3) Vigilance</td>
<td>No significant correlations</td>
<td></td>
</tr>
<tr>
<td>4) Intrusions</td>
<td>No significant correlations</td>
<td></td>
</tr>
<tr>
<td>5) Attacks on other males</td>
<td>No significant correlations</td>
<td></td>
</tr>
<tr>
<td>6) Excursions</td>
<td>No significant correlations</td>
<td></td>
</tr>
<tr>
<td>7) Attacks received</td>
<td>No significant correlations</td>
<td></td>
</tr>
<tr>
<td>8) Song</td>
<td>NS</td>
<td>$r_s = 0.503$, $n = 17$, *</td>
</tr>
</tbody>
</table>

Significant correlations are marked: * = p <0.05, ** p <0.001 and NS = not significant.

The above data show song to be positively correlated with EPP and female orientated flights are negatively correlated. Song is also positively correlated with the total number of young sired. The proportion of young sired within a males nest was not significantly correlated with any variable. The residual data provide useful bivariate results but the interaction between behaviours, density and morphometrics has been excluded.

7.3.3. Interactions between behaviour, density, morphometrics and reproductive success

The previous section demonstrate how bivariate correlations can be used to explained variation in reproductive success. However, behaviours have been also shown to interact, consequently this next section combines the mean behaviour values and residual ranks for individual males in a series of stepwise regression models. The first set of models examines interactions between the mean values and reproductive success shown in Table 7.2, combined with density measurements and morphometrics. This data set may be regarded as limited as external influences upon behaviour have not been accounted for.
7.3.3.1. Male mean behaviour values and reproductive success

The correlation data obtained in section 3.2.1 were entered with both density variables and plumage score into three separate stepwise regression models. The sections below describe: the degrees of freedom as described in Chapter 4, the variance ($r^2$) explained by each model and the significance of each variable.

7.3.3.1a Extra-pair paternity

$$r^2 = 0.926$$

The above model describes a very significant amount (92.6%) of the variation in male acquisition of extra-pair paternity. Increased levels of song during egg laying and excursions during incubation are positively regressed with EPP as is neighbour density. The two guarding behaviours of vigilance and time spent < 10 m from the female during incubation are both associated with decreased levels of EPP.

7.3.3.1b Total young sired

$$r^2 = 0.891$$

The variation explained by this model is again very significant (89.1%) with increased levels of success associated with the density of adjacent neighbours and song during the prelay stage, whereas increased levels of vigilance are negatively regressed with success.

7.3.3.1c Proportion of the males own young sired (WPP)

$$r^2 = 0.57$$

This model describes 57% of the observed variation in WPP, song during incubation and
attacks during the prelay stage are negatively regressed with WPP, whilst plumage score is positively regressed with WPP.

The above three models describe significant amounts of the variation in reproductive success but fail to account for the variation known to be associated with seasonal and environmental changes. The models must therefore be regarded as flawed although they do provide an indication of the interaction of behaviour and reproductive success. The next section is an analysis of reproductive success once the external influences on behaviour have been removed.

7.3.3.2 Analysis of the residual variables
The data analysed here are the residual values obtained for each behaviour and male from the regression model excluding density. The model combines the 8 behavioural variables, density and morphometrics. The sections below describe: the degrees of freedom as described in Chapter 4, the variance ($r^2$) explained by each model and the significance of each variable.

7.3.3.2a EPP

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Song</td>
<td>+15.032</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Density of neighbours</td>
<td>+4.472</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Adjusted $r^2$: 0.60

Song and neighbour density were both positively regressed with EPP, indicating increased levels at increased densities and song output. The model accounts for 60% of the between male variation in EPP.

7.3.3.2b Total young sired

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Song</td>
<td>+42.218</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Adjusted $r^2$: 0.746

Song again was entered into the regression model explaining 74.6% of the variation in male reproductive success.

7.3.3.2c Proportion of young sired

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excursions</td>
<td>+2.751</td>
<td>&gt;0.10</td>
</tr>
</tbody>
</table>

Adjusted $r^2$: 0.111

Although excursion data were entered into this model they were not significant and only 11.1% of the between male variation in WPP was explained. This model has therefore not adequately described between male variation in WPP, which may better explained by the above variation in overall paternity being the result of song output.
7.4 DISCUSSION

Correlation analysis identified two groupings of behavioural characters which were similar for both the prelay and all stages. These were the song, excursions and attacks received grouping i.e., EPC acquisition activities and the second group being defence activities associated with resisting EPCs, i.e., proximity to the female, vigilance, pursuit flights and attacks on intruding males. This separation of behaviours indicates the dichotomy of behaviours orientated to the acquisition of EPCs and those from necessity trying to resist EPCs. This data analysis is not necessarily valid but does provide an indication of how behaviours within a bout will occur together. The separation of data into two categories of paternity defence and paternity acquisition correlated with song output supports the analyses of the previous chapters. During the prelay period males spend time in defence of paternity (see Birkhead et al 1987), whereas when not defending paternity males spend time trying to acquire extra-pair paternity. The constraints imposed by guarding mean a decline in song during the prelay stage which subsequently rises during egg laying and incubation.

Mate guarding was predicted to (i) prevent loss of within-pair paternity and (ii) be a result of low quality males having to defend against intrusions but resulting in loss of WPP. Bivariate analyses of guarding behaviours from the prelay stage showed that only attacks on other males was negatively correlated with WPP. This supports prediction (i) that guarding occurs in low quality males who are susceptible to losing their WPP. Subsequent multivariate analysis shows that plumage score is significantly positively regressed with WPP, whilst it is negatively regressed with song output during incubation and attacks on other males. The analysis of residual data shows that excursions are positively regressed with WPP. The data therefore indicate that guarding fails to maintain WPP and that there may be a trade-off between seeking EPP through song and losing WPP. However, plumage score and excursion activity may somehow act to maintain WPP levels.

Behaviours positively linked to the acquisition of EPP were excursions during incubation, and across the combined stage data and song during egg laying, incubation and across all stages. The data show that both the seeking of EPP and advertising through song are significantly correlated with the successful acquisition of paternity. Negative correlates of EPP were time spent < 10 m from the female during incubation and the combined stages along with the level of vigilance. Males that maintained guarding behaviours outside the prelay period and therefore not actively seeking EPP failed to acquire EPP. Both neighbour density variables were positively correlated with EPP indicating raised levels at the higher densities. Bivariate analysis of the regression data supported the correlation between song output and EPP, but also showed a negative correlation between song and female orientated flights again indicating a trade-off between EPP and guarding. The regression model of mean behaviour data validated the positive correlations between, song, excursions neighbour density and the acquisition of EPP. Guarding behaviours outside the prelay period, i.e., vigilance and time spent < 10 m from the female were again negatively associated with EPP acquisition. The second model combining residual data showed that EPP acquisition is adequately explained by song output and neighbour density. The data therefore support the prediction that males seeking paternity through excursions and song increase their level of success, whilst the continuance of guarding...
type behaviours during egg laying and incubation prevent the acquisition of EPP.

The total young sired was positively correlated with song from both sets of bivariate analysis as well as with plumage score, and negatively correlated with the time spent < 10 m from the female across all stages. The first regression model of mean values supported the relationship with song but showed neighbour density to be a positive correlate. Vigilance across all stages was negatively correlated with total success. The second model using residual data showed a positive relationship with song output that explained a high level of the variation in total chicks sired.

To summarise: the analyses in this chapter show links with observation bouts between behaviours associated with the acquisition of EPP and those associated with the defence of paternity. Analysis of these behaviours and reproductive success indicates that guarding during the prelay period failed to maintain WPP, supporting the hypothesis that guarding occurs in low quality males who are more subject to losing their paternity. The acquisition of EPP through song and excursions is shown to provide a significant benefit indicating that song may provide a significant cue to male quality. A male's overall success is finally best predicted by song output. The data support the hypothesis that there is a mixed reproductive strategy of poor quality males seeking to defend their paternity, whilst high quality males endeavour to acquire further paternity through song and excursion behaviour. The finding of song output as a predictor of EPP success combined with the low frequency of recorded EPCs but high level of EPP indicates that the active seeking of EPCs is not the sole route to their acquisition. Rather, females choose males on the basis of their song output to obtain surrogate EPCs.
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