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The Influence of Actual Paternity and Assessment of Paternity on the Parental

Care of Male Chestnut-collared Longspurs (*Calcarius ornatus*).

by

Dorothy P. Hill

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Abstract

This study examined the influence of actual paternity and assessment of paternity on the parental behaviour of male Chestnut-collared Longspurs (*Calcarius ornatus*). Mate attendance patterns in this species are consistent with the mate-guarding hypothesis: mate attendance was promoted by the male, declined when the female was not fertile, and increased when there was a higher risk of extra-pair paternity (EPP). However, first and second broods overlapped, so that males were feeding fledglings when the female became fertile for the second brood. Thus, males could not mate-guard effectively during the second brood fertile period. Weatherhead and McRae (1990) hypothesized that this should result in males assessing a higher risk of EPP in second broods and, therefore, males should reduce their parental care to second broods. In the current study, I distinguished between second broods, which are initiated after a successful first brood, and replacement broods, which are initiated after nest predation. This is important because, as there are no young to feed after nest predation, males are able to mate-guard during the replacement brood fertile period. Therefore, males should assess a high risk of EPP in second broods and a low risk in both first and replacement broods. Male parental care patterns should reflect this assessment. This hypothesis was supported. Both male nest defence and feeding rate were less at second broods. Second and replacement broods did not differ with respect to mean clutch initiation date, which controls for seasonal effects. DNA fingerprinting revealed that second broods were more likely to contain extra-pair

young than first or replacement broods. An experiment to manipulate male assessment of paternity was conducted at replacement broods using either a conspecific decoy and song (experimental treatment) or a heterospecific decoy and song (control treatment). Males reduced their nest defence at experimental broods compared to control broods. The manipulation did not alter male feeding rates. In summary, the results of this study support Weatherhead and McRae's (1990) hypothesis and found evidence that there is a greater risk of EPP in second broods. It also demonstrated experimentally that males alter their parental care in response to their assessment of paternity.

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For Lorne

Table of Contents

List of Tables

List of Figures

CHAPTER 1: GENERAL INTRODUCTION AND METHODS

Theoretical Basis And Approach of Study

Before Trivers (1972) predicted that males should adopt a mixed reproductive strategy of aiding one female in raising her offspring while seeking matings with additional females, social monogamy was widely accepted as equivalent to sexual monogamy (Gowaty 1996). Since then, matings outside recognized pair-bonds (extra-pair copulations and extra-pair fertilizations) have been documented in many socially monogamous species (e.g. Baker and Bellis 1993), particularly in birds (reviewed in Birkhead and Møller 1992). In a recent review, Gowaty (1996) found that extra-pair paternity is the rule, rather than the exception, among avian species. Documentation of extra-pair fertilizations has become possible through the development of molecular genetic techniques that allow paternity exclusion and/or assignment (e.g., Jeffreys et al. 1985). Among the more important findings of several of these studies is the discovery that females often seek extra-pair copulations actively, possibly to secure good genes for their offspring (e.g., Kempenaers et al. 1992). Thus, both males and females may adopt a mixed reproductive strategy that involves extra-pair fertilizations (Westneat et al. 1990). However, in most studies, many more females participate in extra-pair behaviour than do males (reviewed in Birkhead and Møller 1992). Consequently, socially monogamous males vary more with respect to reproductive success than previously appreciated (Mock 1985).

Trivers (1972) further predicted that selection should favour individuals that avoid investing in offspring to which they are not related. A logical extension of this prediction is that parentage should influence parental care, and several models have been developed to predict this relationship. Some of these models, such as Maynard Smith's (1978) model, predict no relationship, while others predict that parentage should influence parental care, although the exact nature of the relationship is debated (e.g., Whittingham et al. 1992, Westneat and Sherman 1993, Gowaty 1996). Yezerinac et al. (1996) found a common set of conditions for these various models when they relaxed all except the most basic of assumptions. These assumptions are 1) that offspring survival increases from increased parental care and 2) that providing parental care is costly to individuals in terms of their own survival (e.g. Daan et al. 1996), future reproduction (e.g. Verhurlst and Hut 1996), and/or lost opportunities for additional or alternative matings (Westneat et al. 1990). According to Yezerinac et al.'s synthesis, male paternal care is influenced by paternity when:

(1) Paternity varies between breeding attempts. For example, if paternity is predictably greater in subsequent or alternative breeding attempts than in the current brood, then males should decrease their parental care in the current brood. If paternity is predictably less in the subsequent brood, then males should increase their parental care to the current brood.

(2) Males can assess their paternity in the current brood reliably.

(3) The benefits of reducing parental care to the current brood must

   outweigh the costs incurred by endangering genetic young within

   the brood. Because female birds ovulate each egg separately (see

   Birkhead and Møller 1992), mixed-parentage broods containing

   both extra-pair young and young genetically related to the social

   male often result.

In this study, I take an empirical approach to examine whether male

Chestnut-collared Longspurs (*Calcarius ornatus*) adjust their parental care in

accordance with their paternity. Although the models mentioned above are

important in interpreting the results of this study, I do not discuss the merits of

these models here. Throughout this study I refer to a male's genetic contribution

in a brood as his 'paternity' or 'actual paternity', whereas his 'assessment of

paternity' is his perception of that genetic contribution (see Schwagmeyer and

Mock 1993). While I do not examine the benefits of extra-pair paternity to

females, I assume that females must benefit from seeking and accepting extra-pair

copulations because otherwise they would not do so.

Study Area

I conducted this study in the Remount Community Pasture near Bindloss,

Alberta, Canada (50°40' N, 110°10' W). This native mixed-grass prairie pasture

comprises approximately 12,000 ha dominated by blue grama grass (*Bouteloua

gracilis*) and needle-and-thread grass (*Stipa comata*). This pasture is grazed

annually from June to October at a density of 1 cow-calf unit per 22 ha (D. Major, pers. comm.).

The study site was a 600 m x 700 m area along the east boundary of the pasture. Grid stakes were located every 100 m from east to west and from north to south. To the east of the study site is a cultivated field and a north-south vehicle trail. An east-west vehicle trail runs through the middle of the study site. Crested wheatgrass (*Agropyron cristatum*), a domesticated grass, grows along a buried gas pipeline running south-west to north-east and in small patches (< 0.25 ha) that were rehabilitated after gas-well removal. Small, shallow depressions in the landscape provide seasonal ponds.

The study area contained approximately 50 breeding territories of Chestnut-collared Longspurs at a mean density of 1.2 breeding pairs/ha (range 1.1 to 1.4 pairs/ha; during 3 years).

Study Species

Chestnut-collared Longspurs are small (20 g) socially monogamous, dichromatic passerines native to the central plains of North America. Their preferred breeding habitat is grazed native grassland (Maher 1973; Anstey et al. 1995) and breeding territories are clumped in large aggregations (Fairfield 1968). This species is a native prairie specialist and is found in greater abundance in native pastures than in those pastures planted with domesticated grasses (Anstey et al. 1995). Longspurs opportunistically breed at sites recently burnt, mowed, or

grazed (Owens and Myres 1973). Cultivation eliminates this species from a breeding site (Owens and Myres 1973) and, as a result, breeding populations have been drastically reduced in the eastern Great Plains (e.g., in Kansas, Minnesota, and Nebraska; Hill and Gould 1997).

Longspurs arrived on the study site in mid- to late April. The first arrivals were males, but not all males arrived prior to all females. Birds traveled and foraged on the study site in groups typically of five or six birds. Within one to two weeks after arrival, males began singing and interacted aggressively with one another. Male Chestnut-collared Longspurs perform aerial song displays in which they fly upward with undulating flight and, after the peak of ascent, descend while singing with their tail spread (Sibley and Pettingill 1955). They also sing from low perches such as shrubs, rocks, and fences (Harris 1944). Singing seems to function in territory defense because males counter-sing (Fairfield 1968) and, in my study, song playbacks elicited aggressive responses from territorial males. Estimated territory size on my study site ranged from 0.25 ha to 4 ha.

Longspurs nest on the ground in a depression excavated by the female (Bailey and Niedrach 1938). Females construct the nest of grasses, which a female collects within 20 m of the nest site (Fairfield 1968). When completed, the nest is flush to the ground.

Female longspurs lay 3-6 eggs on consecutive days (Harris 1944). In my study population, mean clutch size was 4.06 ($\pm$ 0.62 SD; range 2-6; n = 247 clutches). Three clutches contained only two eggs each but I could not determine whether this was the full complement of eggs or the result of partial clutch loss.

Incubation lasts 10 to 12.5 days and is by the female only (DuBois 1935; Harris 1944), although Wyckoff (1983) recorded an unusual case of a male incubating. In my study, mean incubation duration, measured from the day the penultimate egg was laid to hatch, was 12.0 days ($\pm$ 0.8 SD, range: 10-13 days, n = 29). Hussell (1972) noted that the interval between the first and last hatched nestlings can be up to 49 hrs, suggesting that females begin incubation prior to clutch completion. Because I visited nests only once daily, I could estimate hatch interval only within 24 hr intervals. Nonetheless, I found a hatching interval of 24 hrs or more was common, especially in clutches containing four or more eggs (Table 1.1). Both parents brood the young and in extreme heat, either parent may shade the young by standing over them with wings slightly spread. When flushed from the nest, incubating or brooding females perform a distraction display that involves hopping and fluttering through the grass with wings spread (Fairfield 1968). Brooding males also perform this display.

Hatchlings are altricial, with eyes closed, and are covered with buffy gray down (Harris 1944). Nestlings utter faint peeping by Day 4 (hatch day = Day 1) and call in response to parents bringing food by Day 7 (Fairfield 1968). Both parents feed nestlings. Nestling diet at a site in Saskatchewan consisted of grasshoppers (30-66 % of diet), larvae of butterflies and moths (9-27%), leafhoppers (8-25%), and spiders (3-8%) (Maher 1979). Young leave the nest on approximately nestling Day 10 (range: Day 9 to Day 14; Harris 1944, Moriarty 1965) at a mean mass of 15.1 g (74.5 % of adult mass; Sadler and Maher 1974). In my study, young left the nest at a mean age of 10.2 days ($\pm$ 0.9 SD, range: 9-13

days, n = 96 broods). When they leave the nest, young cannot fly and remain crouched in the grass waiting for parents to deliver food (Harris 1944). They continue receiving food from their parents for about 14 days after leaving the nest (24 days after hatch; Harris 1944; also see Chapter 2). Both parents feed the fledglings immediately after they leave the nest, but the female reduces or ceases her care of the young within a few days if she initiates a subsequent brood (Harris 1944). Based on growth rates of laboratory-fed fledglings, Sadler and Maher (1974) suggested that juvenile longspurs may be as old as 60 days (50 days after leaving the nest) before attaining adult mass.

Because Chestnut-collared Longspurs breed from May to the beginning of August, several authors suspected that the species was double-brooded (e.g., Harris 1944, Fairfield 1968, Maher 1973). My study confirmed that longspurs commonly double-brood, and that annual reproductive success of females increases with the number of broods they raise (Table 1.2). As discussed later (Chapters 3 and 5), male reproductive success is confounded by extra-pair paternity.

Predation on eggs and young is the single greatest cause of nest failure in this species (O'Grady et al. 1996). In my study, 47.2% (127/269) of all nests failed and 94.4% (120/127) of these failures were due to predation (Table 1.3). There is greater predation on nestlings than on eggs (Maher 1973, O'Grady et al. 1996) and in my study, 71.7% (86/120) of all nest predation was at the nestling stage. Other causes of nest failure included exposure of nestlings to extreme

weather (1.5%), nest desertion (0.4%), predation on incubating females (0.7%),
and trampling by cattle (0.4%).

Male singing declines by late July to early August. Flocking begins from
mid-July to mid-August, with juveniles flocking prior to adults (Harris 1944). By
early September flocks of 20-50 longspurs may be observed on the breeding
grounds (Maher 1973). All populations of Chestnut-collared Longspurs are
migratory and winter in the southern United States and northern Mexico (Hill and
Gould 1997).

General Field Methods

I conducted this study between the end of April and first week of August
from 1993-1995. I found nests by dragging an unweighted 30-m rope between
two people to flush incubating females off their nests, or by observing female
behaviour. Once found, nests were marked by placing a dried cowpat 1-2 m on
either side of the nest. A small piece of blue flagging tape (approximately 1 cm^2)
was secured to each cowpat with a nail. Nests were checked every day in 1994
and 1995. As part of another study, nests in 1993 were assigned randomly to a
visitation schedule of once every one, two, or four days (O'Grady et al. 1996).

I captured adult longspurs using mist-nets and marked them with a unique
combination of three plastic coloured and one numbered aluminum U. S. Fish and
Wildlife leg bands. Most females were captured during incubation by flushing
them off their nests into mist-nets. I attempted to capture every female whose

nest I had found to facilitate assigning nests as first, second, or replacement (after nest predation) broods. Males were more difficult to capture. Five mist-nets set up in various patterns were placed on the territory of the male I intended to capture. A tape recorder playing a longspur song to lure the male was then placed somewhere in the maze of mist-nets. In 1995, I only used tape recordings at the beginning (i.e. during first broods) of the breeding season to avoid interfering with an experiment involving decoy presentations and song playbacks (Chapter 4). Walk-in Potter traps were ineffective in capturing this species. I did not attempt to capture individuals at the nest during the nestling stage because I thought this could subsequently affect the birds' behaviour during nest observations (Chapter 3) or predator model presentations (Chapter 4).

I measured the right tarsus length (to the nearest 0.1 mm), right flattened wing length (to the nearest mm), and mass (to the nearest g) of every adult captured (Table 1.4). A blood sample (approximately 100 μL) was taken by pricking the metatarsal vein with a 26 gauge needle and collecting blood into three 40 μL heparinized microhematocrit tubes. These tubes were immediately placed into an Eppendorf tube containing 1 mL of Queen's Lysis Buffer (Seutin et al. 1991) and stored at 4°C until DNA was extracted (see Chapter 3 for methods of DNA extraction and analysis). A new needle was used for every bird to avoid disease transfer. To stop bleeding, I applied pressure with a clean cotton ball to the needle puncture. If necessary, ice was also applied to the vein. Birds were not released until bleeding stopped fully. Adult longspurs resumed normal activity more quickly when blood was taken from the metatarsal vein as opposed to the

brachial vein. Longspurs have very prominent metatarsal veins, perhaps because they spend much time walking while foraging on the ground.

I banded nestlings on Nestling Day 7 (Day 1 = hatch day). Similar to the adults, they received a unique combination of three coloured and one aluminum leg band and I measured their tarsus length, flattened wing length, and mass (Table 4.1). Blood samples were taken from nestlings that had attained a minimum mass of 12 g. If a nestling weighed less than this, I returned to the nest the following day to obtain the blood sample. Blood samples were collected and stored as above, except that nestling blood samples were taken from the brachial vein and only 50 uL of blood was collected from each nestling. Special care was taken to clean off any blood on the feathers so that predators would not be attracted to the newly banded nestlings. I avoided banding nestlings in extremely hot or cold weather. In broods containing more than one offspring, nestlings were removed from the nest for banding one or two at a time so that the parents would continue feeding the remaining offspring. Nestlings were banded away from the nest (usually in a vehicle) to avoid trampling vegetation around the nest and thus attracting predators.

During the three-year study, I captured and banded 98 adult females, 40 adult males, and 532 nestlings. Males were more likely than females to return to the study site: 66.7% (20/30) of banded males and 32.3% (21/65) of banded females were resighted on the study site in the following year (Yates' corrected χ^2 = 8.53, df = 1, p < 0.005). Of resighted individuals, males were also more likely to return to the same breeding territory than were females (males: 85.0% (17/20),

females: 42.9% (9/21); Yates' corrected $\chi^2 = 6.13$, df = 1, p < 0.01). This suggests that female longspurs may have lower site fidelity than males, and it may explain the differences in male and female return rates. It is also possible that over-winter mortality is higher for females than for males, but this aspect of longspur biology has not been examined. I did not resight any of the 312 individuals banded as nestlings in 1993 or 1994 in the following year.

I assigned nests as first, second, or replacement broods. All nests at the beginning of the breeding season were assumed to be first broods. The next breeding attempt was assigned as a second brood if young had fledged from the first nest, or as a replacement brood if the first brood had been preyed upon (eggs and young disappearing prior to Nestling Day 9 or nestling remains found near the nest). I classified a breeding attempt after a successful replacement brood as a second brood. I confirmed nest ownership by recapturing females on their second or replacement nests or by flushing them from the nest and observing their bands with binoculars.

Table 1.1. Interval between first and last hatched longspur nestlings by clutch size. Numbers are number of clutches per hatch interval; percentages of clutches per hatch interval are shown in brackets.

	Clutch Size (Percent)				
	2	3	4	5	6
Hatching Spread*					
0-24 hr	2 (100)	7 (43.7)	18 (15.4)	0 (0)	0 (0)
24-48 hr	0 (0)	9 (56.3)	88 (75.2)	25 (75.8)	0 (0)
48-72 hr	0 (0)	0 (0)	11 (9.4)	8 (24.5)	1 (100)
Number of Clutches	2	16	117	33	1

*Nests were visited every 24 hr

Table 1.2. Number (and percentage) of females that successfully raised one, two, or three broods in a single breeding season, and mean ($\pm$ SD) number of young raised by females fledging one, two, or three broods.

Number of broods raised	Number of females (Percentage) n = 156 female years*	Mean number of young raised ($\pm$ SD)
1 successful brood	78 (50.0%)	3.6 ($\pm$ 0.9)
2 successful broods	26 (16.7%)	6.9 ($\pm$ 1.3)
3 successful broods	2 (1.3%)	9.0 ($\pm$ 0.0)

* Females are counted once for each year in which they bred.

Table 1.3. Numbers and percentages of successful and unsuccessful nests by year and cause of nest failure.

Year	Preyed upon during egg stage	Preyed upon during nestling stage	Total preyed upon	Other nest failure[1]	Successful nesting attempts
1993	11 (13.8%)	27 (33.8%)	38 (47.5%)	4 (5.0%)	38 (47.5%)
1994	10 (12.2%)	25 (30.5%)	35 (42.7%)	3 (3.7%)	44 (53.7%)
1995	13 (12.0%)	34 (31.5%)	47 (43.5%)	1 (0.9%)	60 (55.6%)
Total	34 (12.6%)	86 (32.0%)	120 (44.6%)	7 (0.3%)	142 (52.8%)

[1] See text for other sources of nest failure.

Table 1.4. Mean ± SD mass, tarsus length, and flattened wing length of male, female, and nestling Chestnut-collared Longspurs. Range is shown in brackets.

	Mass (g)	Tarsus length (mm)	Flattened wing length (mm)
Males (n = 40)	19.8 ± 1.2	19.2 ± 0.5	87.1 ± 1.9
	(18-22)	(17.6-19.8)	(82-90)
Females (n = 98)	20.1 ± 1.1	18.8 ± 0.6	81.8 ± 2.9
	(17-23)	(17.0-19.4)	(78-88)
Nestlings[1]	13.7 ± 1.9	16.6 ± 1.2	30.8 ± 5.1
(n = 532)[2]	(7-18)	(11.4-19.5)	(12-48)

[1] Mean age at banding: 7.1 days ± 0.7.

[2] n = 524 for nestling mass.

CHAPTER 2: DIVISION OF LABOUR AND EVIDENCE OF MATE-GUARDING

INTRODUCTION

Parker (1970) recognized that competition between males for mates occurs not only in male-male combats, but also at the gametic level, within the female reproductive tract. This form of competition, termed "sperm competition", occurs when females mate with multiple males. Males compete at this level based on the number and quality of sperm transferred (e.g., Briskie and Montgomerie 1992; Birkhead and Petrie 1995) and/or by preventing females from receiving sperm from other males (Parker 1970; Olsson et al. 1996). The latter tactic is termed "mate-guarding" and is exhibited by a wide range of species.

Mate-guarding can occur before and/or after copulation. Many Crustaceans, such as amphipods and isopods, exhibit pre-copulatory mate-guarding in which the pair remain in amplexus for a long period prior to copulation (Jormalainen and Merilaita 1995). Female receptivity to copulation is brief in these species, and this time limitation has been suggested as a selective pressure for pre-copulatory guarding (Parker 1974). Post-copulatory mate-guarding is beneficial whenever delaying the female from a second mating increases the fertilization success of the first male. For example: male mating success increases with the duration of post-copulatory guarding in water striders (*Geris lacustris*; Jablonski and Kaczanowski 1994); male tiger beetles (Coleoptera: Cicindelidae) guard longer in the presence of other males (Shivashankar 1994); and male Formosan

squirrels (*Callosciurus erthraeus thaiwanensis*) elicit alarm calls after copulation which induces conspecifics to remain immobile and thus, delays the female from receiving additional copulations (Tamura 1995).

In many bird species, mates remain in close proximity to one another during the breeding season. This mate attendance behaviour is common among socially monogamous species and was, until recently, interpreted either as a way of strengthening the pair-bond, or as vigilance behaviour. However, since the discovery of extra-pair copulations and fertilizations across a wide range of socially monogamous species (reviewed in Birkhead and Møller 1992) this behaviour has been recognized as mate-guarding, which promotes paternity protection for the male (Beecher and Beecher 1979; Birkhead 1982). Generally, the last ejaculate of sperm to enter the female bird's reproductive tract prior to ovulation fertilizes the egg (Birkhead et al. 1988; Briskie and Montgomery 1993; but see Oring et al. 1993), so that male birds can benefit from guarding their mates after copulation to prevent subsequent copulations with other males.

Mate-guarding is only one of several competing hypotheses proposed to explain mate attendance behaviour in birds. These hypotheses and their associated predictions have been summarized by Gowaty et al. (1989) and Birkhead and Møller (1992) and are listed in Table 2.1. It should be noted that these hypotheses are not mutually exclusive and that mate attendance can serve more than one function simultaneously (Dickinson and Leonard 1996).

Several hypotheses propose that both sexes benefit from mate attendance. For example, the Social Foraging hypothesis proposes that individuals forage more efficiently in pairs than alone, whereas the Predator Avoidance hypothesis suggests that mate attendance increases vigilance against predators. These hypotheses predict that mate attendance should be initiated by both members of the pair, and that mate attendance should not decline when the female is infertile (e.g., during incubation). For example, Gowaty et al. (1989) suggested that if mate attendance functions as predator avoidance, then it should increase during the incubation stage when the female is especially vulnerable to predation. Similarly, because females have limited foraging time during incubation, mate attendance should increase during this stage if it functions to enhance foraging efficiency. The Strengthening the Pair-bond hypothesis (see Birkhead and Møller 1992) proposes that mate attendance functions to strengthen the bond between the male and female, and that this bond reduces the chances that either sex will desert its mate. This rather vague hypothesis proposes that mate attendance enhances the breeding success of the pair, rather than of the individuals (e.g., an individual might enhance its fitness by deserting its mate in favour of other mating opportunities). Thus, it predicts that mate attendance should be initiated by both sexes and should not decrease as the breeding cycle progresses. The Strengthening the Pair-bond hypothesis is applicable only for species in which biparental care is essential for breeding success (i.e., those species in which neither the male or female benefits from deserting their mate). However, mate attendance has been recorded in many species which are facultatively polygynous or in which females can successfully raise offspring alone,

which calls into question the general applicability of this hypothesis (e.g., Gowaty et al. 1989). A variation of this hypothesis, which would make more sense, is that mate attendance should be initiated by the sex more vulnerable to mate desertion (e.g., see Trivers 1972), and that it should increase with the cost of being deserted. In most passerines birds, females are more vulnerable to desertion because, by building nests and producing eggs, they make a greater initial investment in reproduction. Most female birds incubate the eggs alone and, therefore, the cost of being deserted should increase with their investment as incubation progresses.

The Changes in Territory Use hypothesis (see Birkhead and Møller 1992) proposes that mate attendance does not serve an adaptive function but, rather, close proximity between the pair at the beginning of the breeding season results passively as a consequence of contracted territory use at that time. Thus, neither the male or female initiate mate attendance. This hypothesis predicts that mate attendance will decrease as the breeding cycle progresses if the breeding territory expands during that time. This hypothesis does not address why territory use might change throughout the breeding cycle.

The Courtship Feeding hypothesis (see Birkhead and Møller 1992) suggests that pairs remain in close proximity to allow the male to feed the female during courtship. This hypothesis applies only to those species with courtship feeding and predicts that mate attendance may or may not decrease when the female is infertile, depending on whether incubation feeding occurs.

Birkhead and Møller (1992) classified all hypotheses which propose that

mate attendance functions to benefit the female as the Female Benefits Hypothesis.

This idea stems from Lumpkin's (1981) assertion that females play an active role in

mate attendance. The Female Benefits hypothesis proposes that females should

promote mate attendance (Birkhead and Møller 1992), and that the benefits

females derive should vary with their proximity to their mate (Kempenaers et al.

1995). Birkhead and Møller (1992) have suggested that in order to promote mate

attendance, females should follow their mates, rather than the reverse. However, if

males follow females during the fertile period, then females must signal their

fertility to the males (Lumpkin 1981), and this may be an active way in which

females promote mate attendance. Two benefits for females have been proposed:

reduced harassment from conspecific males and predator avoidance. It is unclear

how predator avoidance for females differs from the Predator Avoidance

Hypothesis above, unless it applies primarily when females are particularly

vulnerable to predation (e.g., during incubation; Gowaty et al. 1989). If mate

attendance reduces conspecific male harassment of females, then the Female

Benefits Hypothesis predicts that mate attendance should increase when the risk of

harassment is highest (e.g., when the female is fertile).

Two hypotheses propose that mate attendance primarily benefits males.

The Copulation-access hypothesis proposes that males maintain close proximity to

their mate in response to the female's readiness to copulate (Gowaty and Plissner

1987). Mate-guarding, a closely related hypothesis, proposes that mate attendance

functions as a male paternity guard. Both the Mate-guarding and Copulation-access hypotheses predict that mate attendance is initiated by the male and that it declines when the female is infertile. Gowaty et al. (1989) suggested that these hypotheses can be differentiated because the Mate-guarding hypothesis further predicts that mate attendance behaviour should increase when there is a higher risk of extra-pair copulations (e.g., at higher breeding densities; Gowaty et al. 1989). Therefore, increased opportunity for females to mate with extra-pair males is predicted to increase mate attendance under the mate-guarding hypothesis, but not under the copulation access hypothesis.

Mate-guarding circumvents female mate-choice and, therefore, females should resist male-guarding efforts. For example, when female isopods (*Idotea baltica*) are experimentally hindered from resisting mate-guarding, mate-guarding duration increases significantly (Jormalainen and Merilaita 1995). Female birds actively choose their mates by accepting or rejecting copulations (Lifjeld and Robertson 1992; Gowaty 1994; Gray 1996; Wagner 1992). However, female resistance to mate-guarding may be confounded if mate attendance serves multiple functions (Dickinson and Leonard 1996). For example, while male Ring-necked Pheasants (*Phasianus colchicus*) mate-guard and protect their paternity, females benefit through decreased harassment from conspecific males and, subsequently, an increased feeding rate (Ridley and Hill 1987). Similarly, Gauthier and Tardif (1991) suggested that male mate attendance allowed female Snow Geese (*Chen caerulescens*) to feed for longer periods during the pre-laying period and therefore,

we would not expect them to resist male mate-guarding efforts. Thus, female behaviour does not always facilitate interpretation of mate attendance.

Finally, mate-guarding is costly to males in terms of time and lost opportunities for additional matings, and it often conflicts with other activities such as nest-guarding (e.g., Schleicher et al. 1993; Beasley 1996), territory defence (e.g., Hanski and Laurila 1993), singing (e.g., Rodrigues 1996), foraging (e.g. Mace 1989; Alberts et al. 1996), and fledgling care (Weatherhead and McRae 1990).

In this study I examined the mate attendance behaviour of Chestnut-collared Longspurs. I predicted that if mate attendance functions as Mate-guarding in this species, then it should be promoted by the male, should be more intense during the fertile period than during incubation, and should increase when there is a higher risk of extra-pair paternity. I also documented the daily time-budgets of males and females to examine whether mate attendance conflicts with other behaviours, and I documented extra-pair behaviour of females and the influence it has on mates.

METHODS

Focal Observations

I conducted both focal-bird and focal-pair observations to quantify changes in mate attendance during the nesting cycle and to determine the division of labour

between breeding longspur pairs. In 1993, I conducted focal observations of individual males and females. Individuals were identified by their colour bands and location of their territory. I began observations from a distance of 20 m or more and recorded all behaviours for 30 continuous minutes. At a distance of 20 m, most longspurs habituated to my presence and resumed their normal activities. For individuals that were disturbed by my presence, I increased my distance and did not begin an observation until the bird(s) had resumed its normal activity. I did not conduct observations on the same day on which I attempted to capture the focal birds in mist-nets. Timing during focal observations ceased when the focal bird was out of view and resumed again when the bird was resighted. I attempted to observe all individuals once during each stage of the nesting cycle designated as: pre-egg/egg-laying, incubation, nestling, and fledgling. The pre-egg/egg-laying stage lasted from seven days before the first egg was laid until the laying of the penultimate egg. I assumed that this period corresponded to the female's fertile period (see Birkhead and Møller 1992). Incubation lasted from the day the last

egg was laid until the first nestling hatched. The nestling and fledgling stages corresponded to the periods during which offspring received care from parents in and out of the nest, respectively. In 1994, I conducted focal pair observations for 60 continuous minutes during the pre-egg/egg-laying and incubation stages only.

I recorded all copulation behaviour. Female longspurs solicit copulations by lifting their tails and rapidly fluttering their wings. They often precede solicitation by flying low over the male and landing close to him. I recorded the female as soliciting copulation if she exhibited this behaviour towards a male.

Male longspurs attempt copulations by trying to mount the female. Females resist copulations by flying away from the male and by sitting with their tail pressed firmly on the ground. Resistance to copulation often results in the male chasing the female aggressively. I refer to these sexual chases as copulation coercion attempts. It was difficult to see cloacal contact during copulation attempts; therefore, I assumed that if the female did not resist a copulation attempt, she had accepted the copulation and cloacal contact had resulted. I recorded female solicitation (see description above), acceptance of copulations from both within-pair and extra-pair males, and whether these copulations occurred on or off the territory. Similarly, I recorded whether female resistance to copulations and male copulation coercion attempts involved pair or extra-pair males and where these attempts occurred.

Male longspurs often intrude onto neighbouring territories, perhaps to attempt extra-pair copulations. These intrusions usually result in chases between the resident male and the intruder(s). Therefore, during focal observations, I also recorded the number of territory intrusions by neighbouring males.

I quantified whether mate attendance was initiated by the male or female by recording the proportion of flights in which the female followed the male or the male followed the female. An individual was recorded to follow only if it flew in the direction of its mate within 5 s of its mate leaving. I compared following behaviour between the four nesting-cycle stages to determine whether this behaviour changed after the fertile period.

During focal-pair observations, I quantified mate attendance intensity by recording the proportion of time individuals within a pair spent within 10 m of one another. I estimated the distances between the pair to the nearest 1 m for distances of 10 m or less and to the nearest 5 m for distances greater than 10 m. I compared mate attendance intensity between the pre-egg/egg laying and incubation stages to detect changes as the nesting cycle progressed. I also examined whether territory intrusions by neighbouring males during the pre-egg/egg-laying stage influenced mate attendance behaviour. I recorded male singing rate and calculated the mean rate during the pre-egg/egg-laying and incubation stages. I recorded female incubation behaviour as the proportion of time females spent on and off their nests during the incubation stage and calculated the mean length of incubation bouts and incubation breaks.

I recorded male and female brooding behaviour and number of feeding trips during 1-h long focal nest observations in 1994 and 1995. I conducted nest observations on Nestling Day 6 (Day 1 = hatch day) to control for nestling age, and between 2.5 and 5.5 hrs after sunrise to control for time of day. I recorded brooding behaviour as the proportion of time an individual was brooding on the nest and calculated the mean brooding rate for males and females. I also recorded the number of feeding trips individuals made during the focal nest observation and calculated mean male and female feeding rates to nestlings.

During the focal-bird observations in 1993, I recorded the proportion of food deliveries to fledglings by the male or female. I also calculated mean male and female feeding rates to fledglings. I conducted these focal-bird observations

1-4 days after the young left the nest. During the fledgling period, I continued to visit the territory every 1-4 days and recorded whether the fledglings still received parental care. I found fledglings by flushing them as I walked through the territory, by listening for their vocalizations (see Hill and Gould 1997), or by observing parents delivering food to them.

Interval Between Broods

I recorded the interval between broods as the number of days from nest failure or the young leaving the nest until the first egg of the next nesting attempt was laid. Longspur females build a new nest for each nesting attempt and lay eggs on consecutive days (Hill and Gould 1997). Therefore, clutch initiation date was determined either by finding the nest before the end of the egg-laying period or by back-counting 12 days (mean length of incubation; see Chapter 1) from hatch to the laying of the penultimate egg. I confirmed nest ownership by recapturing banded females on their new nest during incubation.

Statistical Analyses

I compared the proportion of flights in which the male followed the female with the proportion of flights in which the female followed the male using a chi-square test. I also used chi-square tests to examine whether the proportion of flights in which the male followed the female was independent of nesting stage. I used linear regressions to examine the relationships between mate attendance intensity and number of territory intrusions, and the number of territory intrusions

and male singing rate. For these analyses, percentages were transformed using an arcsine-square-root transformation and numbers of intrusions were log transformed. I compared among both nesting stages and nesting attempts with respect to mean male singing rate, mean territory intrusions, and mean percentage of time pairs spent in close proximity using a Kruskal-Wallis test in each case. I used a Type I error rate of 0.05 and present means $\pm$ standard deviations, unless otherwise specified.

RESULTS

Division of Labour

Only females built nests and incubated the eggs. During 1994, females spent 45.7% of their time on their nests during the incubation period (n = 20 females observed for 20.55 h). Mean incubation bout length was 15.23 min ($\pm$ 9.8 min; n = 37 bouts), while mean incubation break was 13.67 min ($\pm$ 11.78 min; n = 49 breaks).

Both males and females brooded nestlings, but 95.3% of all brooding was by females (n = 109 pairs). During nest observations on Day 6, females spent 14.8% of their time brooding and mean female brooding rate was 8 min, 52 sec/hr ($\pm$ 8 min, 26 sec; range: 0-30 min, 35 sec/hr; n = 109 hr of observation). In contrast, males spent < 1% of their time brooding and mean male brooding rate was 26 sec/hr ($\pm$ 1 min, 46 sec; range: 0-11 min, 57 sec, n = 109 hr).

Both parents fed nestlings. Mean male and female feeding trips during nest observations on Day 6 in 1994 were 6.2 trips/hr ($\pm$ 3.5, range 0-14, n = 52 pairs) and 5.5 trips/hr ($\pm$ 2.7, range 0-13, n =52 pairs), respectively. Mean total feeding trips (i.e., male and female trips combined) was 11.6 trips/hr ($\pm$ 4.5, range 1-21, n = 52 pairs).

During first broods, males made 78.0% (39/50) of all observed food deliveries to fledglings (n = 14 pairs). Males made a mean of 4.8 trips/hr ($\pm$ 3.4 trips/hr, n = 14 males) to fledglings, whereas females that fed fledglings made a mean of 2.3 trips/hr ($\pm$ 1.3 trips/hr, n = 10 females). Four of 14 (28.6%) females did not feed fledglings at all. The fledgling period overlapped with the pre-egg/egg-laying stage of the next brood (see below). I commonly observed parents feeding fledglings for as long as 16 days after the young had left the nest. I could not determine the range in duration of the fledgling feeding period because it was impossible to distinguish between undetected predation on fledglings and early fledgling independence. Once, I observed a male feeding a fledgling 22 days after the young had left the nest; the female was incubating the second brood at the time. Although I often saw parents together with fledglings more than 20 days after nest leaving, this was the only time I observed a fledgling this old being fed. On two occasions, I observed males aggressively chasing older fledglings (29 and 39 days after leaving the nest); in both cases, the second brood had hatched and the male was feeding second brood nestlings.

Number of Broods and Interval Between Broods

In 1993 and 1994 combined, 54 pairs raised a first brood to fledging and all pairs that did so initiated a second clutch (Table 2.2). One pair in 1993 and one pair in 1994 initiated a third clutch after raising two successful broods (n = 21 pairs raising two successful broods). In contrast, in 1995 only 11 pairs successfully fledged a first brood and four of these pairs then initiated a second clutch; no pairs attempted a third clutch in 1995 (Table 2.2). The percentage of pairs that initiated a second clutch after a successful first brood in 1993 and 1994 differed significantly from the percentage in 1995 (Yates' corrected $\chi^2 = 32.17$, df = 1, p < 0.001). Longspur pairs initiated clutches later in 1995, which may have influenced whether they initiated a second brood. Earliest clutch initiation dates were 1 May and 27 April in 1993 and 1994, respectively, and 12 May in 1995. Longspur pairs attempted as many as four clutches after successive nest failures (i.e., first brood plus three replacement clutches).

Females took significantly longer to initiate clutches after a successful brood than after nest failure (t = 6.22, df = 39, p < 0.001). Mean interval between nest failure and initiation of the replacement clutch was 5.5 days ($\pm$ 1.7 SE, range: 4-12 days, n = 34), whereas mean interval between young leaving the nest and initiation of a second or third brood was 9.7 days ($\pm$ 3.2 SE, range: 6-18 days, n = 28). Nonetheless, these short intervals between broods resulted in overlap between successive broods, because fledgling care typically extended for up to 16

days after the young left the previous nest (see above) and females are fertile as early as 7 days prior to clutch initiation (Birkhead and Møller 1992).

Mate Attendance

Males followed females significantly more than females followed males (Yates' corrected $\chi^2 = 217.5$, df = 1, p < 0.001). Occasionally (n = 4 of 38 pairs observed) females circled directly over their mate if he did not follow immediately. Following behaviour of males was not independent of nesting stage ($\chi^2 = 26.9$, df = 3, p < 0.001; Fig. 2.1). The male followed the female relatively more often when the female was fertile (pre-egg/egg-laying stage) than when she was not fertile (incubation and nestling stages pooled; Yates' corrected $\chi^2 = 16.68$, df = 1, p < 0.001). The incidence of following behaviour by males was lowest during the fledgling stage (Fig. 2.1) and differed significantly from that during the pre-egg/egg-laying stage (Yates' corrected $\chi^2 = 10.68$, df = 1, p = 0.001). Because the fledgling stage overlapped with the pre-egg/egg-laying stage of second broods (see above), I analyzed the proportion of time pairs stayed within 10 m of one another by both stage and nesting attempt (Table 2.3). Pairs spent significantly more time close to one another during the pre-egg/egg-laying stage of first or replacement broods compared to incubation or the pre-egg/egg-laying stage of second broods (Kruskal-Wallis, F = 20.5, df = 1, p < 0.001). Despite the close proximity of males and females, I never observed courtship feeding during any stage of the nesting cycle during the three years of study,

Territory Intrusions

During the pre-egg/egg-laying stage, the number of territory intrusions increased significantly with the percentage of time pairs spent within close proximity (y = 0.58 + 0.35x, r^2 = 0.16, df = 1,25, p = 0.04; Fig. 2.2). Intrusions occurred significantly more often during the pre-egg/egg-laying stage than during incubation (pre-egg/egg-laying $\bar{x}$ ± SE = 6.1 ± 0.7 intrusions/hr, incubation $\bar{x}$ ± SE = 1.6 ± 0.3 intrusions/hr; Kruskal-Wallis, F = 16.9, df = 1, p = 0.001).

Number of intrusions declined as the territorial male's singing rate increased (y= 0.50 - 0.02x, r^2 = 0.13, df = 45, p = 0.01; Fig. 2.3). Singing rate was similar between all breeding attempts and nesting stages (Kruskal-Wallis, F = 0.77, df = 3, p = 0.52; Table 2.4).

Copulation Behaviour

During the pre-egg/egg-laying stage, females vocalized frequently on their territories using the general contact call of this species (Hill and Gould 1997). Females solicited copulations from six days before they laid their first egg until the day on which they laid their third egg (Fig. 2.4). Females solicited and accepted copulations from extra-pair males when they were off their territories, but tended to reject extra-pair copulation attempts on their territory (Fisher exact test, p = 0.009; Table 2.5). Of 17 copulations for which I observed cloacal contact had been made, one (5.8%) was an extra-pair copulation.

Copulation coercion attempts involving social mates occurred on 15 occasions. Prior to five of these attempts, the female had left the territory and interacted with an extra-pair male during the pre-egg/egg-laying stage. On another occasion during the pre-egg/egg-laying stage, a male had been detained in a mist-net and, when released, immediately attempted to coerce his mate to copulate with him. Seven social-mate coercion attempts occurred during the fledgling stage (pre-egg/egg-laying stage of second broods), and I could not determine the context of the other two attempts.

DISCUSSION

Mate Attendance

The patterns of mate attendance in Chestnut-collared Longspurs are consistent with the Mate-guarding hypothesis. Three predictions distinguish mate-guarding from competing hypotheses: males initiate the close proximity between the pairs, mate attendance declines when the female is no longer fertile, and mate attendance increases when there is a greater risk of extra-pair paternity (Gowaty et al. 1989). My data are consistent with all of these predictions. Male longspurs initiated and maintained close proximity by closely following their mates. Both this following behaviour, and the proportion of time pairs spent within 10 m of one another, declined sharply during incubation when the female was no longer fertile. These two observations are also consistent with the Copulation-access hypothesis

which proposes that males maintain close proximity to their mates in response to female readiness to copulate (Gowaty and Plissner 1987). Thus, the same mate attendance patterns could arise as a result of female copulation behaviour. Indeed, I observed female longspurs soliciting copulations only during the pre-egg/egg-laying stage. Nonetheless, during this stage there was also a significant positive relationship between mate attendance and the number of territory intrusions by neighbouring males. This suggests that males increase their mate attendance in the presence of potential extra-pair males and it supports the prediction that mate attendance increases when the risk of extra-pair copulations is greatest, which is unique to the mate-guarding hypothesis (Bjorklund and Westman 1986, Gowaty et al. 1989).

Other hypotheses were not supported by the data. The Courtship Feeding hypothesis was not considered because I never observed longspurs courtship feeding. Mate attendance declined during incubation, which is inconsistent with the Social Foraging, Predator Avoidance, and Pair-bond hypotheses. Because incubating females are particularly vulnerable to predation, Gowaty et al. (1989) suggested that the predator avoidance hypothesis predicts that mate attendance should increase, rather than decrease, during incubation. Similarly, if mate attendance facilitates social foraging, then females would especially benefit when their foraging time is limited to incubation breaks. Thus, the social foraging hypothesis also predicts that if mate attendance changes at all, it should increase during incubation. Finally, male longspurs actively initiated and maintained mate

attendance, which does not support the hypothesis that mate attendance patterns passively arise as a result of changes in territory use over the nesting cycle.

The hypothesis that females may benefit from mate attendance (Lumpkin 1981, Aguilera and Alvarez 1989, Mace 1989) cannot be dismissed. If mate attendance functions strictly as a male paternity guard, then it circumvents female mate choice and females should resist male mate-guarding efforts. Instead, female longspurs appeared to promote male following behaviour by occasionally circling over their mate when he did not immediately follow. The mate-guarding hypothesis alone cannot explain such behaviour. Dickinson and Leonard (1996) pointed out that mate attendance can have more than one function. Therefore, mate attendance may simultaneously benefit males and females for different reasons.

The most likely benefit of mate attendance for female longspurs is reduced harassment by conspecific males (Lumpkin 1981). On their home territories, females typically rejected extra-pair copulation attempts by male intruders. Resistance to copulations is costly in this species because it usually results in a copulation coercion attempt in which the female risks injury and expends energy by fleeing from the male. Social mates may reduce this cost by chasing intruders away from the female. This situation resembles that found in blue tits (*Parus caeruleus*; Kempanears et al. 1995). Male blue tits chase intruding males off their territories before they can interact with the female, and temporary removal of territorial males results in increased harassment of females by intruders (Kempenaers et al. 1995).

Female Extra-pair Behaviour

Females left their territories and accepted extra-pair copulations from neighbouring males. However, on their home territories, female longspurs typically rejected extra-pair copulation attempts. This indicates that female longspurs actively choose their extra-pair partners. This pattern of copulation acceptance and rejection is predicted when females choose only high quality males as extra-pair mates (e.g. Kempanears et al. 1992), but all males, regardless of quality, attempt extra-pair copulations.

Females also vocalized frequently on their territories during the pre-egg/egg-laying stage. In some avian species, females advertise their fertility to several males by producing loud, distinctive calls during their peak fertility (Montgomerie and Thornhill 1988). This was not the case in this study. Female vocalizations were the typical contact call (see Hill and Gould 1997) for the species. It is unlikely that these vocalizations were directed towards potential extra-pair males given that females reject extra-pair copulations on their territories, while actively seeking extra-pair matings off territory. Therefore, there appears to be little benefit to females in attracting extra-pair males to their territory. Furthermore, the risk of retaliation by social mates (see below) suggests that females should seek extra-pair copulations covertly. Thus, it is more likely that females directed these vocalizations towards their social mate, and this may be a way in which females promote mate attendance (see above).

Males responded to their mate's potential extra-pair behaviour by trying to coerce them to copulate. In one third of these cases, the female had left the territory apparently to seek extra-pair copulations with a neighbouring male. Upon returning to the home territory, female rejection of copulation attempts by the social male resulted in a copulation coercion attempt. A similar situation occurred when a male was detained in a mist net. Again, the female rejected the copulation attempt by her mate which resulted in a copulation coercion attempt. This suggests male Chestnut-collared Longspurs protect their paternity in two ways. The first line of defence is mate attendance, which males use as mate-guarding to prevent their mates from obtaining extra-pair copulations or, if that fails, to assess the risk of extra-pair paternity. When all else fails and there is a high risk of extra-pair paternity, males may resort to copulation coercion.

Mate-guarding Conflicts

Møller (1991a) suggested that males may use song as a paternity guard by increasing their singing rate when the female is fertile, and thereby deterring conspecific intruders. This hypothesis makes two predictions: (1) singing rate peaks during the female's fertile period and (2) males that sing at a higher rate experience fewer territory intrusions. However, there has been little support of this hypothesis. For example, Rodrigues (1996) found that singing rate by male Chiffchaffs (*Phylloscopus collybita*) declined when they were mate-guarding, suggesting that singing conflicts with guarding. In Chestnut-collared Longspurs there was no indication that singing either complemented or conflicted with mate-

guarding: singing rate did not vary between nesting stages or nesting attempts, which is inconsistent with the first prediction. Nonetheless, there was a significant negative relationship between singing rate and number of territory intrusions, which supports the second prediction. However, in my study it is unclear whether higher singing rates inhibited neighbouring males from intruding on the territory or whether males that experienced fewer territory intrusions had more time to sing.

Mate-guarding conflicted with fledgling feeding. Male following behaviour was lowest during the fledgling stage which overlapped with the pre-egg/egg-laying stage of the second brood. Intensity of mate attendance during this stage was significantly less than during the pre-egg/egg-laying stage of first or replacement broods, but did not differ from mate attendance levels during incubation when the female was not fertile. One reason for this low mate attendance is that males provided most of fledgling care during this time, while females prepared for the next brood. Weatherhead and McRae (1990) suggested that this situation may lead to males having lower confidence of paternity in their second broods. This may explain why almost half of the observed copulation coercion attempts involving social mates occurred during the fledgling stage, and it may have profound influences on subsequent male parental care (Weatherhead and McRae 1990).

Table 2.1. Summary of hypotheses for mate attendance behaviour in birds and associated predictions (modified from Birkhead and Møller 1992).

Hypothesis	Who Benefits?	Predictions Concerning Mate Attendance Pattern:
1. Strengthening the Pair-bond	Both male and female	1. Initiated by female as often as male. 2. Does not decline as breeding cycle progresses.
2. Social Foraging	Both male and female	1. Initiated by female as often as male. 2. Does not decline as breeding cycle progresses or may increase during incubation when time constraints limit female foraging.
3. Predator Avoidance	Both male and female	1. Initiated by female as often as male. 2. Does not decline or may increase during incubation with increased risk of predation for female (Gowaty et al. 1989).
4. Female Benefits	Female	1. Initiated by female more than male. 2. May or may not decline as breeding cycle progresses, depending on when females benefit. 3. Benefit to female varies with proximity to male (Kempenaers et al. 1995).

Table 2.1 (cont.)

5. Courtship Feeding	Female	1. Initiated by male feeding female and/or female soliciting food from male.
		2. May or not decline as breeding cycle progresses depending on whether courtship feeding continues into incubation.
6. Changes in Territory Use	Neither	1. Results passively as a consequence of contracted territory.
		2. May decline as breeding cycle progresses if territory use expands over time.
7. Mate-guarding	Male	1. Initiated by male.
		2. Declines as breeding cycle progresses when female is infertile.
		3. Intensity increases when there is a greater risk of loosing paternity (Gowaty et al. 1989).
8. Copulation-access	Male	1. Initiated by male.
		2. Declines as breeding cycle progresses when female is infertile.

Table 2.2. Numbers and percentages of pairs raising successful first broods and initiating second and third broods in 1993/1994 and 1995.

	Pairs raising a successful 1st brood	Pairs attempting a 2nd brood after a 1st brood	Pairs attempting a 3rd brood after 2nd brood*
1993/1994	54/96 (56.3%)	54/54 (100%)	2/21 (9.5%)
1995	11/60 (18.3%)	4/11 (36.4%)	0/7 (0%)

* Includes second broods initiated after successful replacement broods.
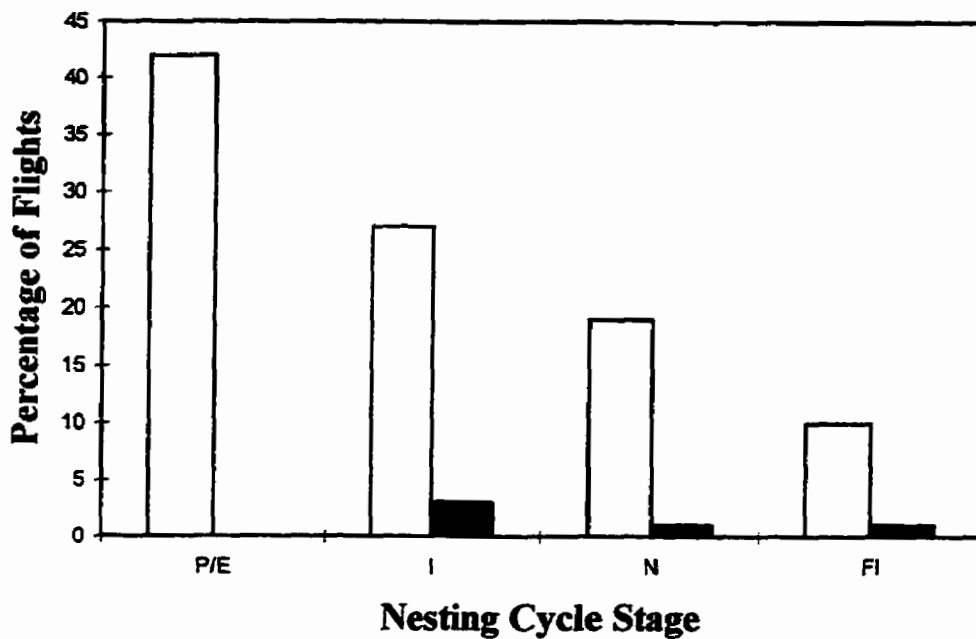
Figure 2.1. Percentage of flights during which the male followed the female □ or the female followed the male ■. Nesting cycle stages are: pre-egg/egg-laying (P/E; n = 117), incubation (I; n = 240), nestling (N; n = 259), and fledgling (Fl; n = 37).

Table 2.3 Proportion of time social mates spent within 10 m of one another.

Nesting Attempt and Stage	Percent of time within 10 m	N (No. of pairs)
First Broods Pre-egg/egg-laying	90.4%	6
Replacement Broods Pre-egg/egg-laying	94.9%	6
Second Broods Pre-egg/egg-laying	36.0%	15
All Attempts Incubation	27.0%	20
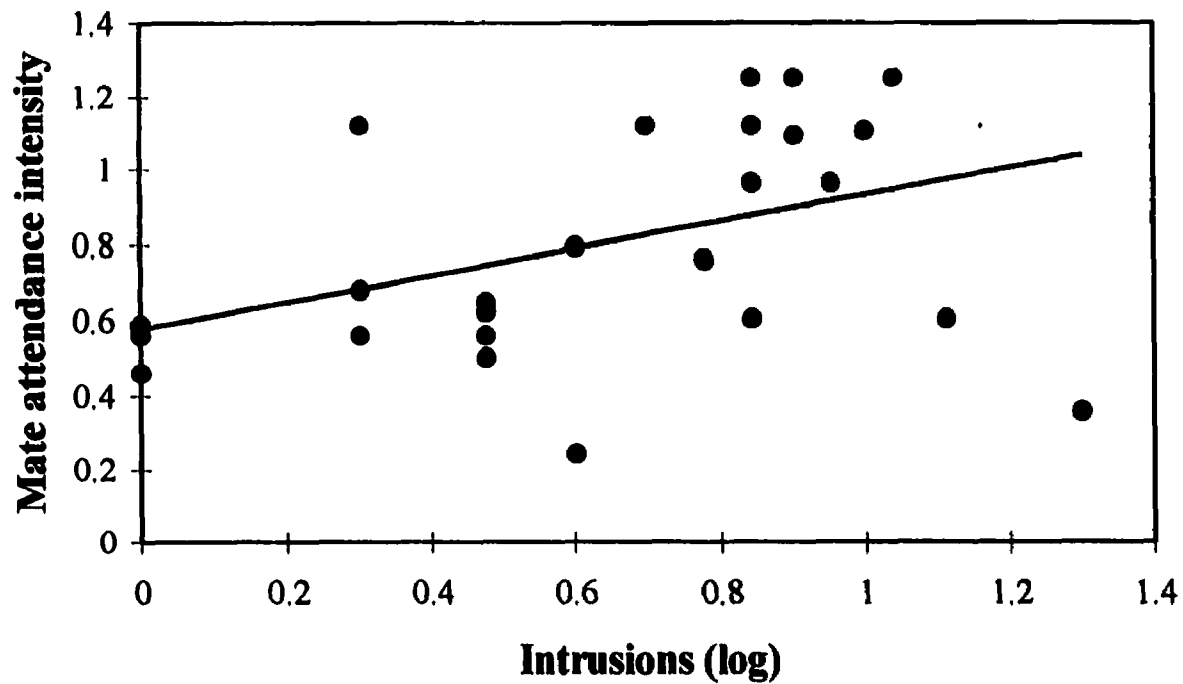
Figure 2.2. Relationship between mate attendance intensity (arcsine-square root transformation of percentage of time pairs spent within 10 m of one another) and number of territory intrusions by neighbouring males during the pre-egg/egg-laying stage ($y = 0.58 + 0.35x$).
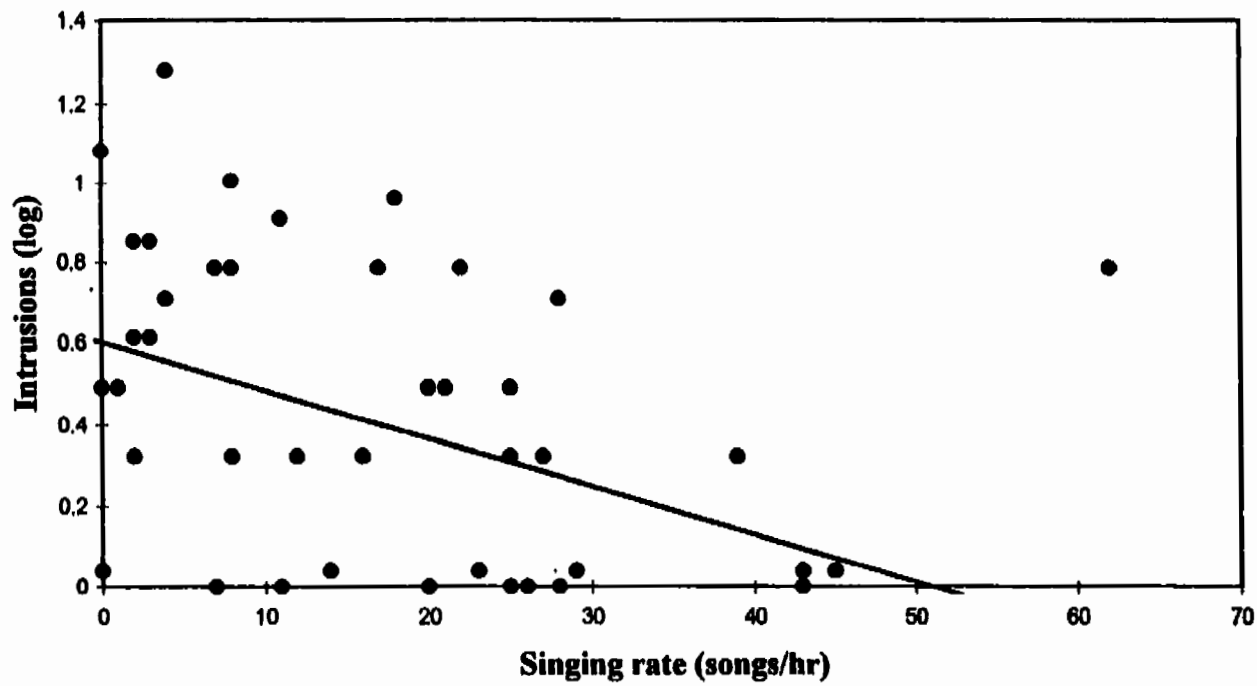
Figure 2.3. Relationship between singing rate and number of territory intrusions by neighbouring males ($y = 0.50 - 0.02x$).

Table 2.4. Mean male singing rate (songs/hr) $\pm$ SE by nesting attempt and stage.

Nesting Attempt and Stage	Song/hr $\pm$ SE	N (No. of pairs)
First Broods Pre-egg/egg-laying	18.0 $\pm$ 9.3	6
Replacement Broods Pre-egg/egg-laying	11.0 $\pm$ 3.6	6
Second Broods Pre-egg/egg-laying	16.3 $\pm$ 3.8	15
All Attempts Incubation	21.2 $\pm$ 3.2	20
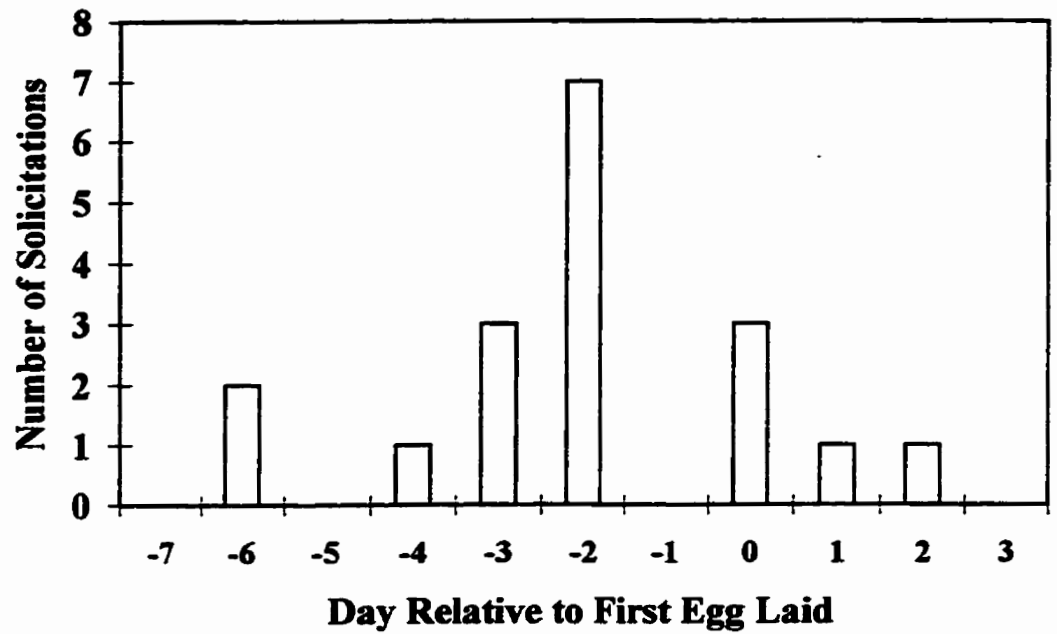
Figure 2.4. Timing of copulation solicitations by females relative to day first egg was laid (= Day 0). The fertile period was assumed to extend from Day -7 to +2 (see text).

Table 2.5. Female acceptance or rejection of extra-pair copulations on and off the territory.

Location	Female Accept EPC	Response Reject EPC
On territory	3	6
Off territory	8	0

CHAPTER 3: MALE PROVISIONING AND EXTRA-PAIR PATERNITY

INTRODUCTION

Extra-pair mating is one of the most common alternative reproductive strategies adopted by male and female birds (reviewed by Birkhead and Møller 1992). As a result, males often face the possibility of caring for unrelated offspring. Selection should favour males that avoid investing in young they did not sire (Trivers 1972), either by preventing their mates from obtaining extra-pair fertilizations (e.g., via mate-guarding; Parker 1970) or by assessing their paternity and adjusting their parental care accordingly.

Both theoretical and empirical studies examining the influence of paternity on male care have produced conflicting results. Some models predict that parentage should affect parental care (e.g., Winkler 1987; Møller 1988; Whittingham et al. 1992; Westneat and Sherman 1993), whereas others predict no such relationship (e.g., Maynard Smith 1978; Gross and Shine 1981). In general, models that assume that parentage is consistent from one breeding attempt to the next predict no relationship between parentage and parental care (e.g., Maynard Smith 1978; see Westneat and Sherman 1993; Houston 1995). When this assumption is relaxed and parentage varies among breeding attempts, several models predict a positive relationship between paternity and parental care (see Westneat and Sherman 1993).

Another factor influencing the paternity-parental care relationship is the importance of parental care for offspring survival and subsequent recruitment. As the importance of male care to offspring survival increases, the influence of paternity on male care decreases (Westneat and Sherman 1993). For example, when male Dunnocks (*Prunella modularis*) were temporarily removed to alter their assessment of paternity, experimental males in polyandrous trios decreased their parental care but monogamously mated males did not (Davies et al. 1992). In polyandrous trios, the parental care of one male is not vital to offspring survival because the other male is there to compensate for reduced parental effort. Key to Whittingham et al.'s (1992) model is the relationship between male care and offspring survival. When offspring benefits increase in a decelerating manner with increased parental care to a maximum benefit, their model predicts a continuous relationship between paternity and male care. When the relationship between male care and offspring survival is sigmoidal (i.e., same as above but with few or no benefits at low levels of parental care), then Whittingham et al.'s (1992) model predicts a threshold response in which males do not reduce their care until their paternity within a brood is very low.

Other factors that influence the relationship between male care and paternity are the costs of providing care (e.g., reduced male survival and/or lost opportunities for additional matings; Whittingham et al. 1992; Westneat and Sherman 1993) and whether males can discriminate among their own and extra-pair young within the brood. Because female birds ovulate each egg separately (see Birkhead and Møller 1992), each nestling can be sired by a different male and,

therefore, mixed-parentage broods can result. If males can recognize their own offspring, then they are predicted to reduce care to young sired by others and increase care to their genetic young (Westneat and Sherman 1993). However, no evidence indicates that male birds can discriminate among within-pair and extra-pair young (Kempenaers and Sheldon 1996). Most models assume that, males cannot discriminate among offspring within a brood, but they can use indirect cues to estimate their paternity (e.g., Houston 1995). Variation in female ability to raise offspring alone and/or to manipulate male assessment of paternity have rarely been considered in these models (but see Gowaty 1996).

There has also been no concensus among empirical studies examining the relationship between paternity and male parental care. Some studies have found a positive relationship between paternity and male care (e.g., Møller 1988; Burke et al. 1989; Møller and Birkhead 1993; Dixon et al. 1994), whereas several others have found no relationship (Gavin and Bollinger 1985, Westneat 1988, Wagner 1992, Whittingham et al. 1993, Yezerinac et al. 1996; also see Chapter 4). The interpretation of the latter case is ambiguous. Such results may mean that paternity does not influence male parental care or that males are unable to assess their paternity accurately.

Extra-pair paternity rates often vary between breeding attempts within the same year and thus several recent studies have used this natural variation to re-examine the paternity-parental care relationship (e.g., Dixon et al. 1994, Freeman-Gallant 1996, Yezerinac et al. 1996). Weatherhead and McRae (1990) were

among the first to suggest the existence of within-season variation in extra-pair paternity. They predicted that in species exhibiting brood overlap in which the male cares for first brood fledglings while the female is fertile and prepares for the second brood, males should assess a higher risk of extra-pair paternity in their second broods. This is because caring for fledglings prevents males from guarding their mates during the second brood fertile period. Thus, Weatherhead and McRae (1990) predicted that males should provision second broods less than first broods. However, recent empirical studies have not supported this hypothesis: male American Robins (*Turdus migratorius*) do not feed second broods less (Weatherhead and McRae 1990), extra-pair paternity is just as likely to decrease as increase from first to second broods in Reed Buntings (*Emberiza schoeniclus*; Dixon et al. 1994), and in Savannah Sparrows (*Passerculus sandwichensis*) there are fewer extra-pair offspring in second broods than in first broods (Freeman-Gallant 1996). One possible explanation for these results is that none of these studies distinguished second broods, which follow successful first broods, from replacement broods, which follow nest predation. This distinction is important because there are no young to feed following predation and, therefore, males can mate-guard effectively during the fertile period of the replacement brood.

In this study, I examined changes in extra-pair paternity and male provisioning rates from first to second broods in Chestnut-collared Longspurs. I distinguished second broods from replacement broods and found clutch initiation dates are similar for second and replacement broods, which controls for changes in male behaviour resulting from seasonal changes in offspring value or renesting

potential (see Montgomerie and Weatherhead 1988). Elaborating on Weatherhead and McRae's (1990) suggestion, I hypothesized that males should assess a low risk of extra-pair paternity in both first and replacement broods because they can mate-guard during the replacement brood fertile period (see Chapter 2). Thus, I predicted that males should provision first and replacement broods at similar rates and provision second broods less. Using multi-locus DNA fingerprinting, I determined actual paternity in first, second, and replacement broods.

METHODS

Paternity analysis

I collected all paternity data within a single year (1994) to avoid between-year effects (e.g., Krokene et al. 1996). I assumed that all broods initiated at the beginning of the breeding season were first broods and then determined whether subsequent broods were second or replacement broods by monitoring the first broods until the young fledged or the nest failed. By recapturing females on their new nests during incubation I was able to confirm nest ownership.

I determined paternity by multi-locus DNA fingerprinting. Blood samples (50-100 µL) were taken from either the brachial vein (nestlings) or metatarsal vein (adults; see Chapter 1) and stored them in Queen's lysis buffer (Seutin et al. 1991) at 4°C until DNA was extracted. Following the methods of Lifjeld et al, (1993), I used a phenol-chloroform extraction to extract the DNA. DNA was precipitated and then resuspended in 0.3-1 mL of 1 X TNE_2 buffer overnight on a rotating

wheel at 37°C. I tested the concentration and integrity of the DNA by digesting 4-6 μL genomic DNA with 1 μL EcoRI enzyme and separating it by electrophoresis on a 0.8% agarose gel in 1 X TBE buffer at 80-100 V for 3-5 hours. After staining the gel with ethidium bromide, I photographed it under ultra-violet light. I used these photographs to estimate the concentration of DNA by comparing each sample to a standard. This procedure was then repeated using ALU-I as the enzyme, allowing me to determine the volumetric equivalent of 5 μg of DNA for each sample.

For the fingerprint gels, I separated 5 μg ALU-I-digested DNA from each individual by electrophoresis on a 0.8% agarose gel in 1 X TBE buffer at 40-60 V for 48 hours. I allowed the DNA to migrate a total of 13-14 cm. I ran putative parents and their offspring on the same gel. I depurinated the gel (see Lifjeld et al. 1993) and Southern blotted it onto an Immobilon (Millipore) transfer membrane. The membrane was dried, baked, rehydrated, and then placed into a hybridization bottle with 20 mL of Westneat's prehybridization solution (see Westneat et al. 1988) for 2 hours at 65°C. I labeled the minisatellite *per* probe (see Shin et al. 1985) with α^{32}P-*d*CTP by primer extension and added it to the prehybridization solution. The membrane was hybridized with the probe overnight at 65°C. I then washed the membrane (see Lifjeld et al. 1993), sealed it in plastic, and placed it in an autoradiograph cassette with a piece of Cronex X-ray film. The film was placed between the membrane and a built-in intensifying screen. Autoradiography proceeded at -70°C for 1-8 days. For 10/25 broods, I stripped the membrane and repeated the hybridization process using the Jeffreys 33.15 probe (see Jeffreys et

al. 1985), again labeled with α^{32}P-dCTP. I used the second probe to confirm results obtained from the first probe.

DNA fragments that hybridize with the probe become visible as dark bands on the X-ray film. Electrophoresis separates these fragments according to size, with larger fragments migrating more slowly than smaller fragments. Thus, both the location and morphology of the bands identifies different DNA fragments. I scored these bands by placing an acetate overlay over the exposed X-ray film and marking all bands with permanent coloured markers. I considered bands in different individuals as identical if their density and morphology were similar and their centres were less than 1 mm apart. I scored each band in a nestling lane as shared with the female if it was identical to a band in the female lane, shared with the male if it was identical to a band in the male lane, shared with both if it was identical to a band in both the male and female lanes, or novel if neither the male nor female had an identical band. This allowed me to calculate the parent-nestling band-sharing coefficient (D-statistic) which is calculated as $2(N_{AB})/(N_A + N_B)$, where N_{AB} is the number of bands shared by a putative parent and offspring and $(N_A + N_B)$ is the sum of bands scored for the two individuals (Wetton et al. 1987). I excluded an individual as a genetic parent if the D-statistic was < 0.30 and the number of novel nestling bands was > 2. If only one of these criteria was met, I assumed the adult was the genetic parent. I re-probed the membrane with Jeffreys 33.15 whenever a nestling had a low band-sharing coefficient (< 0.30) with either parent, but had less than three novel bands. In one family, I was unable to obtain DNA for the female. In this case, novel bands could not be scored and paternity

assignment/exclusion was based on male-nestling band sharing alone, after probing with both *per* and Jeffreys 33.15.

Feeding rate

I collected provisioning and paternity data in the same year. On Nestling Day 6 (hatch day = Day 1), I conducted observations at each nest and recorded the number of feeding trips made by the male and female parent during one hour. I made observations from a distance of 20 m or more and, whenever possible, I made observations from a vehicle to minimize disturbance to the birds . To control for time of day, all nest observations were conducted between 2.5 and 5.5 hours after sunrise. I recorded ambient temperature at the beginning and end of each nest observation and used the average of these two numbers in the calculation of mean temperatures. At the end of the observation period, I recorded the number of nestlings and their masses (to the nearest g) using a Pesola 50 g scale and also recorded total brood mass.

Statistical Analyses

I used a linear regression to examine the relationship between parent-offspring band-sharing coefficients and number of novel nestling bands. To examine differences in the proportion of first, second, and replacement broods containing extra-pair young, I used a Fisher exact test. I used Mann-Whitney U-tests to test for differences in mean feeding rates between second and replacement

broods and second and first broods. I used analysis of variance to detect differences in mean number of nestlings, mass per nestling, total brood mass, and ambient temperature between the different types of broods. Finally, I used a Spearman's rank correlation to examine the relationship between individual male feeding rates and paternity. In all cases, I used a Type I error rate of 0.05.

RESULTS

Extra-pair paternity

Analysis of 85 offspring from 25 broods, and their putative parents, revealed moderately-high levels of extra-pair paternity in my study population of Chestnut-collared Longspurs. Intraspecific brood parasitism was not detected as only males were excluded as genetic parents.

Probing with *per* resulted in a mean of 15.6 ($\pm$ 0.7 SE) scorable bands per nestling. The mean number of scorable bands for males and females was 18.5 ($\pm$ 1.9 SE) and 15.0 ($\pm$ 1.6 SE), respectively. Band sharing coefficients and numbers of novel nestling bands were negatively correlated for males ($r^2 = 0.48$, df = 75, p < 0.001), but not for females ($r^2 = 0.002$, df = 75, p = 0.90; Fig. 3.1).

I identified one extra-pair young in the family (three consecutive broods belonging to the same pair) for which I could not obtain DNA for the female. The extra-pair young had a band-sharing coefficient with the male of 0.08 using *per* and 0.15 using Jeffreys 33.15. Mean male-nestling band-sharing coefficient for the

other nestlings was 0.60 ($\pm$ 0.07 SE; range: 0.23-0.83; n = 7) using *per* and 0.48 ($\pm$ 0.04 SE; range: 0.30-0.64; n = 7) using Jeffreys 33.15. The nestling that had a band-sharing coefficient with the male of 0.23 using *per*, had a band-sharing coefficient of 0.30 using Jeffreys 33.15. Therefore, I decided that this nestling was a genetic offspring of the male.

A total of 15/85 (17.6%) nestlings were extra-pair young and 8/25 (32.0%) broods contained one or more extra-pair offspring. Percentage of extra-pair young per brood varied from 0-100% (Fig. 3.2). Six of 14 (42.9%) males lost paternity in their nests and two of these males lost paternity in two broods. The mean percentage of extra-pair young in broods with mixed paternity was 60.5% (SD = 34%, n = 8 broods).

Of all extra-pair young, 73.3% (11/15) were in second broods, while 26.7% (4/15) were in first broods (Table 3.2). Replacement broods contained no extra-pair young. The proportion of first and replacement broods containing extra-pair young did not differ (Fisher Exact Test, p = 0.52); therefore, these data were pooled for comparison with second broods. The proportion of second broods containing extra-pair young differed significantly from first and replacement broods (Fisher Exact Test, p = 0.03).

Feeding rate

Male longspurs fed second broods significantly less frequently than either first broods (Mann-Whitney U-test, Z = 2.06, df = 26, p = 0.04) or replacement broods (Mann-Whitney U-test, Z = 2.36, df = 21, p = 0.02; Fig. 3.3). Total

feeding trips to second broods were similar to those at first (Mann-Whitney U-test, $Z = 0.77$, df $= 26$, p $= 0.44$) and replacement broods (Mann-Whitney U-test, $Z = 0.22$, df $= 21$, p $= 0.82$; fig. 3.4) because females compensated and fed second broods more (Fig. 3.5). Females fed second broods significantly more frequently compared to either first (Mann-Whitney U-test, $Z = 1.96$, df $= 26$, p $= 0.05$) or replacement broods (Mann-Whitney U-test, $Z = 2.17$, df $= 23$, p $= 0.03$). There was no significant difference in mean Julian date between second and replacement broods (second broods $\bar{x} = 190.30 \pm 13.0$ SD, n $= 10$; replacement broods $\bar{x} = 182.85 \pm 16.7$ SD, n $= 13$; Mann-Whitney U-test, $Z = 0.93$, df $= 21$, p $= 0.35$). First, second, and replacement broods did not differ in mean number of offspring, mean total brood mass, mean mass per offspring, or mean ambient temperature during nest observation (Table 3.3).

Individual male feeding rate was not correlated with the proportion of extra-pair young in his nest (Spearman's rank correlation, $r_s = -0.29$, p $= 0.15$, n $= 25$ broods; Fig. 3.6); even those males that did not lose paternity in their second broods tended to feed these broods less.

DISCUSSION

As predicted, second broods of Chestnut-collared Longspurs contained more extra-pair young than did first or replacement broods and males made fewer feeding trips to these second broods. To my knowledge, this is the first study to

support Weatherhead and McRae's (1990) hypothesis that males should assess a higher risk of extra-pair paternity in second broods and thus provision second broods less than first broods. I would not have found these results had I not distinguished between second and replacement broods. This distinction is important because, as there are no young to feed after nest predation, males can mate-guard during the replacement brood fertile period; whereas, their guarding is hindered during the female's fertile period for the second brood because they are caring for first brood fledglings. These results support my prediction that males should assess a low risk of extra-pair paternity in both first and replacement broods and provision these broods similarly: I found no difference in actual paternity between first and replacement broods and males fed these broods at similar rates.

The results reported here are not due to seasonal effects. If this were the case, then extra-pair paternity and provisioning rates should be similar in second and replacement broods, because these broods did not differ with respect to date. Other factors also do not appear to contribute to the lower male feeding rate at second broods: mean number of offspring, mean total brood mass, mean mass per offspring, and mean ambient temperature during nest observation were similar for first, second, and replacement broods.

Overall, the rate of extra-pair paternity in Chestnut-collared Longspurs is moderately frequent. The percentage of extra-pair young (17.6%) and number of nests containing extra-pair offspring (32.0%) is comparable to that found for Bluethroats (*Luscinia svecica*; Krokene et al. 1996), but is not considered exceptionally high (see Gowaty 1996, Krokene et al. 1996). However, extra-pair

paternity was common in second broods: 60.0% of second broods contained extra-pair young and 36.7% of second brood nestlings were sired by extra-pair males. Thus, there should be strong selective pressure on males to invest less in their second broods and, indeed, males provisioned these broods less.

Male longspurs appear to assess their paternity and adjust their parental care accordingly. However, this assessment is imperfect. These results are based on average extra-pair paternity and average male feeding rates. There was no significant correlation between an individual male's paternity and his feeding rate, and males tended to feed their second broods less even if they did not lose paternity. Why might this be so? The answer probably lies in the males' ability to assess their paternity (see Davies et al. 1992; Westneat and Sherman 1993). Male birds are not known to discriminate between their own and extra-pair offspring within a brood (Davies et al. 1992; Kempenaers and Sheldon 1996) and thus, they likely assess their paternity by using indirect cues during their mate's fertile period (Davies et al. 1992; Dixon et al 1994). Male longspurs, like many other passerines, feed their first brood fledglings while their mate becomes fertile for the second brood, which hinders male mate-guarding ability (see Chapter 2). Weatherhead and McRae (1990) suggested that inability of males to guard should lead to lower confidence of paternity in second broods. Mate-guarding traditionally has been viewed as a male paternity guard: the male maintains close proximity to his fertile mate to prevent extra-pair males from inseminating her (see Birkhead and Møller 1992). However, this behavior could also allow males to assess their paternity. As a result, females might be expected to manipulate male

confidence of paternity by "cooperating" with mate-guarding efforts (e.g., see Chapter 2) and seeking extra-pair copulations covertly.

According to the assessment hypothesis (above), when males cannot mate-guard during the second brood fertile period, two things happen. First, male ability to assess paternity decreases, leading to uncertainty of paternity regardless of whether paternity is actually lost. Second, females can seek extra-pair copulations undetected by their mates, which leads to higher extra-pair paternity in second broods, at the population level. However, not all females that have the opportunity to extra-pair copulate do so, because extra-pair matings are not advantageous to all females (e.g., those females already socially paired to high quality males; see Kempenaers et al. 1992; Otter et al. 1994). Thus, both female manipulation and female mate choice (e.g., see Yezerinac and Weatherhead, in press) can lead to discrepancies between a male's perceived and realized paternity.

Why is there a relationship between male care and paternity in Chestnut-collared Longspurs whereas several other empirical studies have failed to find any association (e.g., Gavin and Bollinger 1985, Westneat 1988, Wagner 1992, Yezerinac et al. 1996)? Male longspurs may not face the same costs associated with reducing care as do other species. Extra-pair offspring in birds usually occur in mixed-parentage broods (i.e., both within-pair and extra-pair young in the same nest) and, therefore, it has been suggested that withholding care may be costly to males because of the adverse effects on young they sired within the brood (Whittingham et al. 1992, Westneat and Sherman 1993). Male longspurs did not have this constraint because females compensated for reduced male care and fed

second broods more. Thus, male longspurs could spend less energy on broods in which they assessed a higher risk of extra-pair paternity, without endangering their own young.

Second, if the patterns of paternity I recorded within a single year are typical, then male longspurs should have predictably higher paternity in the first brood of the subsequent breeding season than in the current second brood. Therefore, males may reduce parental care in the current (second) brood to increase their chances of over-winter survival and obtaining greater benefits from future (first brood) breeding opportunities the following year (Westneat and Sherman 1993). Yezerinac et al. (1996) suggested that the low between-season survival rate (30%) of Yellow Warblers (*Dendroica petechia*) contributed to a lack of parentage-dependent paternal care in that species. In my study population, male longspurs survive much better: 66.7% (20/30) of males returned for more than one breeding season (see Chapter 1).

Finally, although male longspurs seem unable to assess their individual paternity accurately, they can respond to population changes in the paternity rate from first to second brood. Thus, by responding to a higher probability of extra-pair paternity in second broods, they predict their own paternity situation accurately more than 50% of the time. In Chapter 4, I examine male ability to assess paternity more closely.

I predict that the mechanisms male birds use to assess their paternity vary between species and between habitats (see Chapter 5) and, therefore, further examination of these mechanisms may provide insight as to why males of some

species adjust their parental care in accordance with their paternity, whereas in

other species they do not.

Table 3.1. Mean (± SE) band-sharing coefficients between social fathers and nestlings and social mothers and nestlings, and mean (± SE) number of novel nestling bands for within-pair young and extra-pair young.

	Within-pair young	Extra-pair young
Social father-nestling band-sharing coefficient	0.51 ± 0.01 (range: 0.19-0.75)	0.12 ± 0.02 (range: 0.0-0.27)
Social mother-nestling band-sharing coefficient	0.46 ± 0.02 (range: 0.25-0.72)	0.53 ± 0.03 (range: 0.30-0.64)
Number of novel nestling bands	0.3 ± 0.08 (range: 0-2)	6.6 ± 0.9 (range: 3-16)
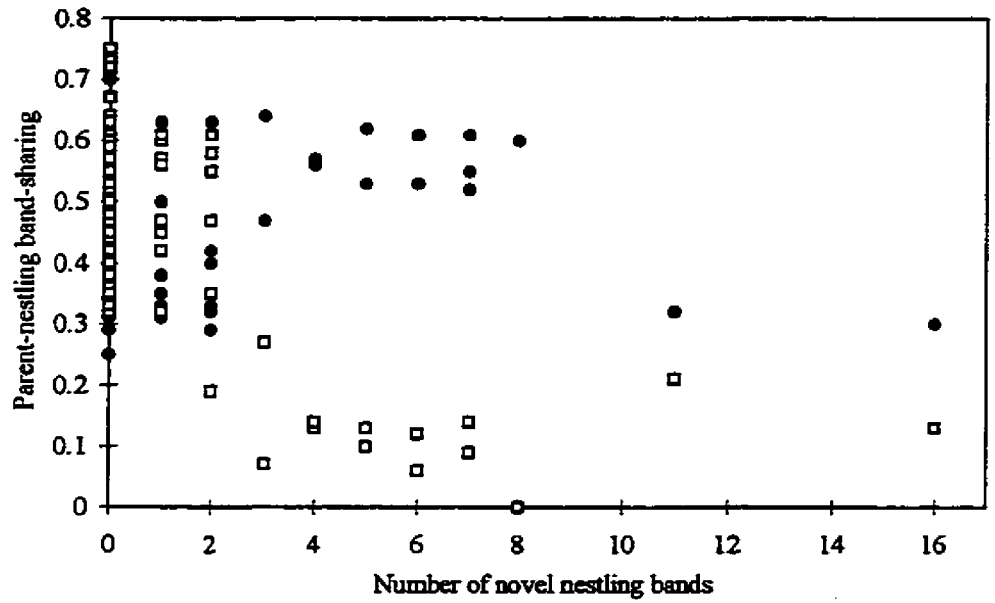
Figure 3.1. Band-sharing coefficients (D-statistic) between social mothers and nestlings (●) and social fathers and nestlings (□) and numbers of novel nestling bands.
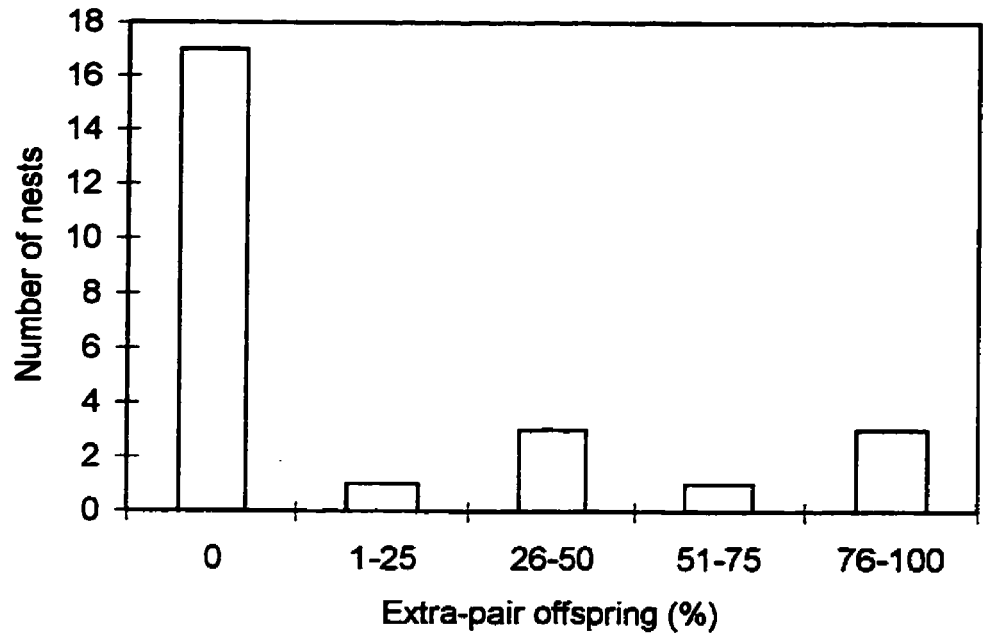
Figure 3.2. Percentage of young per nest that were extra-pair young.

Table 3.2. Percentages of nests containing extra-pair young and percentages of nestlings that were extra-pair young by nesting attempt.

	Percentage of nests containing extra-pair young	Percentage of nestlings that were extra-pair young
Nesting Attempt:		
First broods	20.0% (n = 10 broods)	11.8% (n = 34 nestlings)
Replacement broods	0.0% (n = 5 broods)	0.0% (n = 21 nestlings)
Second broods	60.0% (n = 10 broods)	36.7% (n = 30 nestlings)
Total	32.0 % (n =25 broods)	17.6% (n = 85 nestlings)
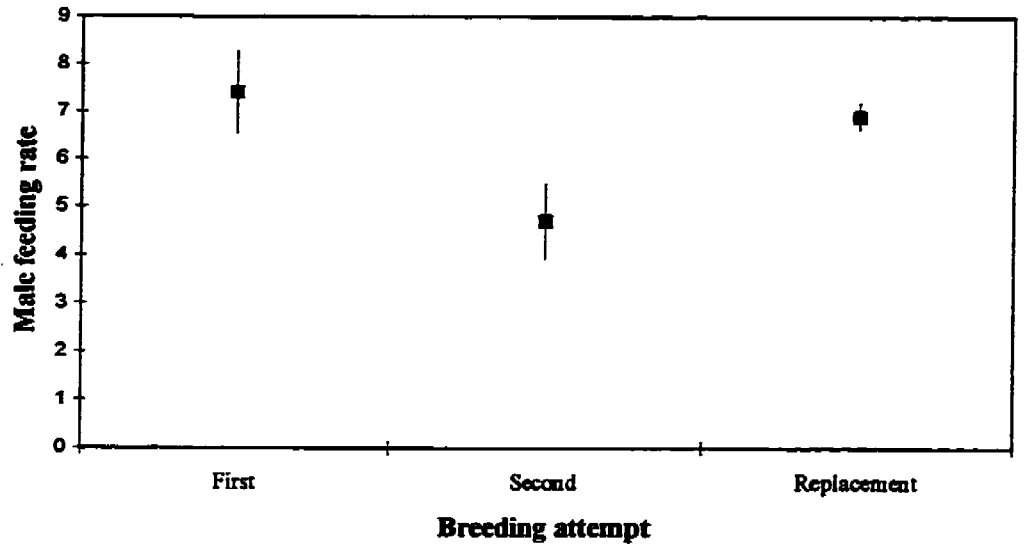
Figure 3.3. Mean (± SE) male feeding rates (feedings per hour) to first (n = 18), second (n = 10), and replacement (n = 13) broods.
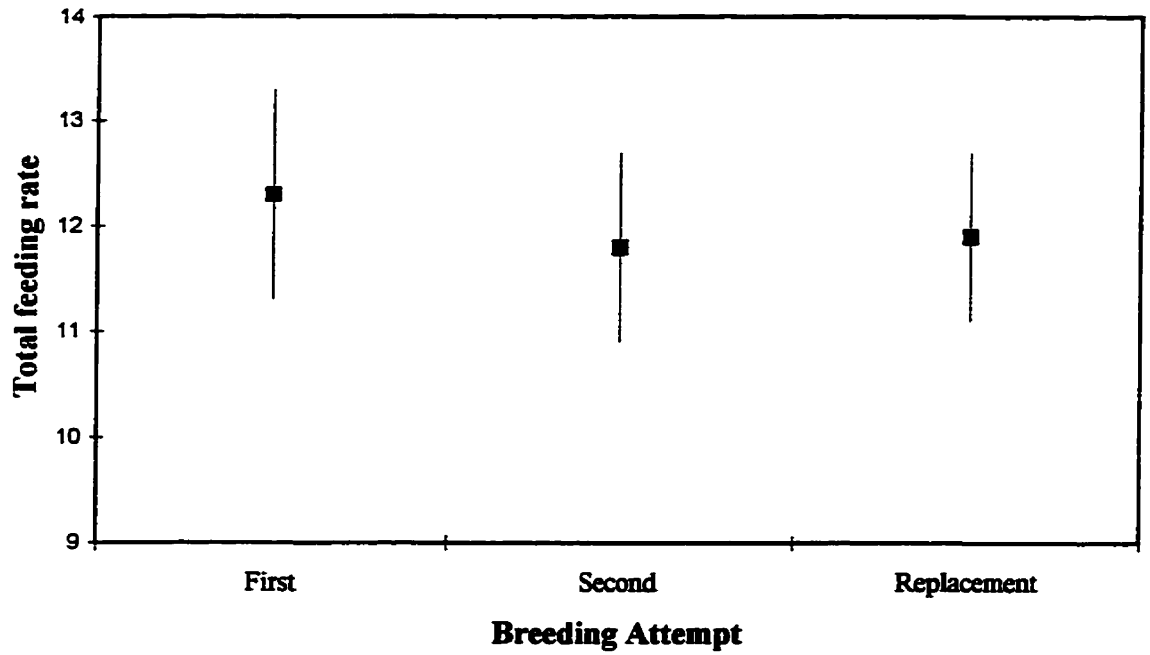
Figure 3.4. Mean (± SE) total feeding rates (feedings per hour; male and female feedings combined) to first (n = 18), second (n = 10), and replacement (n = 13) broods.
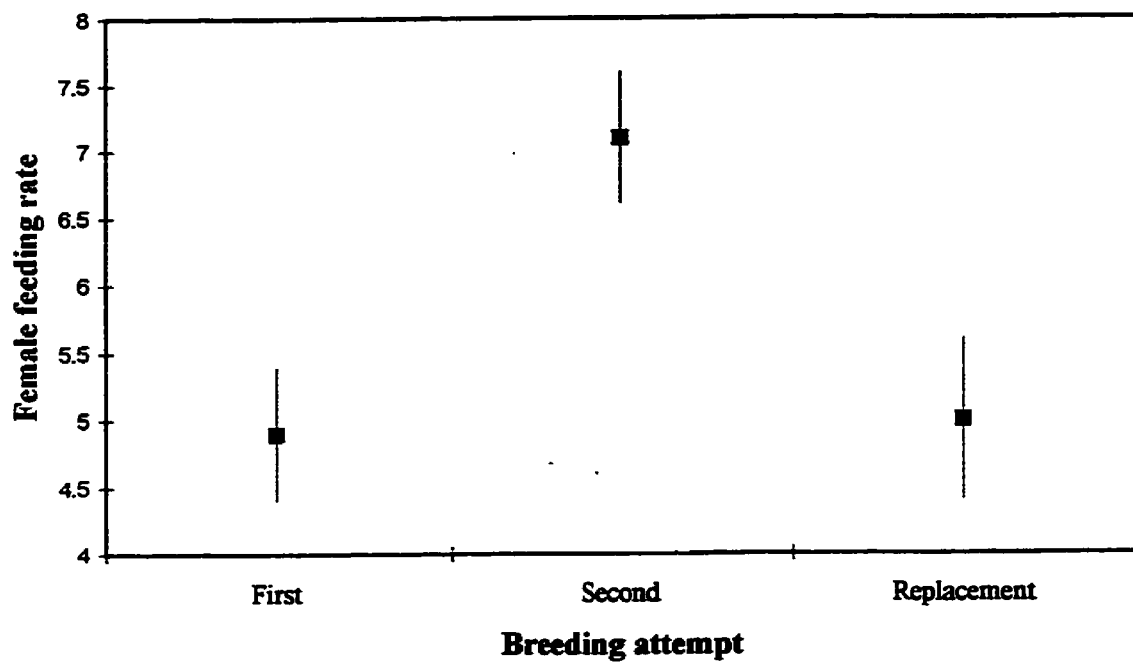
Figure 3.5. Mean (± SE) female feeding rates (feedings per hour) to first (n = 18),

second (n = 10), and replacement (n = 13) broods.

Table 3.3. Means ± SE for number of nestlings, mass per nestling, total brood mass, and ambient temperature during nest observations.

	First (n = 18)	Second (n = 10)	Replacement (n = 13)	ANOVA results
Number of nestlings	3.8 ± 0.2	3.6 ± 0.2	3.5 ± 0.2	F = 0.40; p > 0.60
Mass per nestling	12.3 ± 1.0	11.8 ± 0.9	11.9 ± 0.8	F = 0.09; p > 0.90
Total brood mass	38.3 ± 1.9	36.3 ± 1.4	38.2 ± 3.3	F = 0.18; p > 0.80
Ambient Temperature	19.8 ± 1.0	20.2 ± 1.5	21.8 ± 1.0	F = 0.91; p > 0.40

71
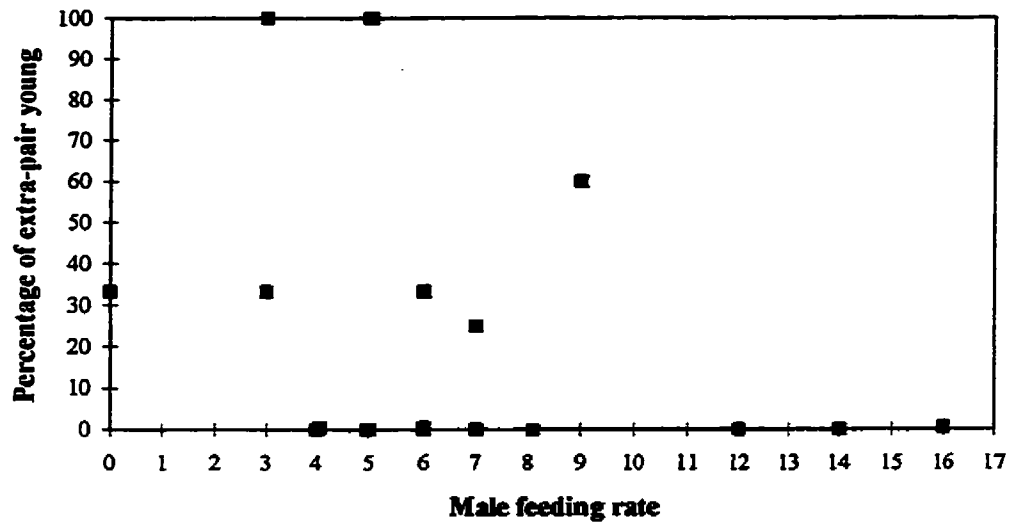
Figure 3.6. Percentage of extra-pair young per brood and male feeding rate.

CHAPTER 4: NEST DEFENCE AND MANIPULATION OF MALE ASSESSMENT OF PATERNITY

INTRODUCTION

Providing parental care can reduce an individual's survival (e.g. Daan et al. 1996) and future breeding opportunities (e.g. Verhulst and Hut 1996). Thus, selection should favour individuals that avoid investing in unrelated offspring (Trivers 1972). Multiple mating by females has been documented across a wide range of species, including every class of vertebrates (e.g. Parker 1970; Smith 1984; Birkhead and Møller 1992; Galbraith 1993; Stockley and Purvis 1993). In species that provide paternal care and biparental care, multiple mating often results in males directing their parental efforts towards unrelated young. Despite theoretical models predicting that male parental care should vary with paternity (e.g., reviewed in Westneat and Sherman 1993; also see Chapter 3), empirical evidence of a such a relationship is mixed (e.g. Whittingham et al. 1993; Dixon et al. 1994). Kempenaers and Sheldon (1997) argued that observational data may be inadequate to test these models (see also Wright and Cotton 1994). They suggested that different males within a population likely confront different tradeoffs and, as a result, variation in individual optimization of parental care can obscure the paternity-parental care relationship. Hence, they argued that an experimental approach is preferable.

Experimental studies generally manipulate a male's perception of relatedness to putative offspring (paternity assessment) as opposed to his realized paternity (see Schwagmeyer and Mock 1993). However, this does not alter the validity of using experimental studies to examine the relationship between paternity and parental care. Among birds, males are not known to discriminate between their own and extra-pair offspring within a brood (e.g. Davies et al.1992; Kempenaers and Sheldon 1996). Therefore, parental care decisions are based on the male's assessment of paternity, rather than his actual paternity (Davies et al. 1992; Kempenaers and Sheldon 1997).

To investigate the relationship between paternity and parental care, most experimental studies on populations of wild birds have involved temporary removal of males during their mate's fertile period (e.g., Whittingham et al. 1993). Recently this approach has been criticized because such experiments manipulate several proximate factors simultaneously, which can lead to uninterpretable results (Schwagmeyer and Mock 1993). For example, changes in male behaviour after temporary removal may result from captivity and/or social isolation, rather than from altered assessment of paternity (Wright and Cotton 1994). Moreover, because female birds often leave their territories to seek extra-pair copulations actively (Kempenaers et al. 1992; Wagner 1992; Gray 1996; Gowaty 1996; Chapter 2), male removals do not mimic a realistic situation (Gowaty 1996). Another problem with these experiments has been that the "control" treatment typically is male removal during incubation when the female is no longer fertile. This "control" relies on the assumption that males have accurate information

concerning their mate's reproductive condition. However, this may not be the case. For example, female European Starlings (*Sturnus vulgaris*) sometimes solicit copulations from their mates throughout incubation, possibly to prevent their mates from becoming polygynous (Eens and Pinxten 1995). This could render incubation-period controls ineffective if males use female behaviour to assess their mate's fertility. Finally, the success of male removal experiments in influencing male care has been limited largely to species with cooperative (Koenig 1990) or polygynandrous (Davies et al. 1992) mating systems; most experiments on monogamous species have been unsuccessful (Wright and Cotton 1994; but see Møller 1988, 1991b).

Wright and Cotton (1994) took another approach to manipulating male assessment of paternity by capturing fertile female starlings and placing them in cages with live male decoys in view of their mates. Males observing their mates "interacting" with another male subsequently reduced their parental care. Another method of altering male assessment of paternity is the use of male decoys. Wright and Cotton (1994) suggested that males may perceive that their paternity is never really threatened by such an experiment. However, if the decoy attracts the attention of the female, as well as the male, then the manipulation may mimic female interactions with a potential extra-pair male.

The aim of this study was to manipulate male assessment of paternity experimentally and examine its influence on subsequent male parental care in a population of Chestnut-collared Longspurs. In a previous study (Chapter 3), I found that second broods of longspurs are more likely to contain extra-pair young

than are replacement (after nest predation) broods, and that males feed these second broods significantly less. Clutch initiation dates of second and replacement broods are similar (Chapter 3), which controls for changes in male behaviour resulting from a seasonal decline in offspring value or renesting potential (see Montgomerie and Weatherhead 1988). In this study, I manipulated pairs only during the female's fertile period of replacement broods using a combination of a mounted male decoy and tape-recorded song. This method attracted the female, as well as the male, to the decoy. The control was a heterospecific decoy which poses no threat to the male's paternity. I examined the effect of the manipulation on both male feeding rates and nest defence. By engaging in nest defence, parents presumably increase the survival probability of their eggs or nestlings by distracting predators away from the nest (Montgomerie and Weatherhead 1988), but often risk injury in doing so (reviewed in Sordahl 1990). Although both offspring provisioning and nest defence can affect adult survival (Sordahl 1990; Daan et al. 1996), the risk of immediate injury may render nest defence a more costly behaviour. Thus, I predicted that both male feeding rates and nest defence should be less for experimental broods compared to control broods. I also compared nest defence at unmanipulated second and replacement broods. I predicted that, like male feeding rates, male nest defence should be less at second broods than at replacement broods because males can mate-guard effectively during the fertile period of replacement broods, but not of second broods (Chapter 2) and, therefore, they should assess a higher risk of extra-pair paternity in their second broods.

METHODS

Nest Defence

I quantified nest defence by placing a taxidermic mount of a Richardson's ground squirrel (*Spermophilus richardsonii*) near the nest. I chose to use this species because it is diurnal, resident on the study site, and I had previously observed longspurs aggressively responding to live ground squirrels near their nest. Circumstantial evidence, including ground squirrel tooth imprints on plasticine eggs placed in longspur nests (unpubl. data), indicate that Richardson's ground squirrels may be opportunistic nest predators. When they are attacked by longspurs, live ground squirrels often remain motionless (pers. obs.); thus, I reasoned that a motionless ground squirrel model was relatively realistic. The ground squirrel was mounted standing on all four legs with tail erect and was placed five m from the nest such that it faced the nest. I conducted all nest defence trials when nestlings were between the ages of Nestling Day 3 and 6 (hatch = Day 1) to control for age of young, and between 2.5 and 5.5 hours prior to sunset to control for time of day. I did not conduct these trials during inclement weather, which could have jeopardized the nestlings by preventing parents from brooding. At each trial there were two observers to ensure that all behaviours were recorded. I made observations from a distance of 20 m or more from the nest. From this distance, I was able to observe the longspurs interacting with the model without disturbing them.

I recorded the period from the time the model was in place until the male and female encountered it (hereafter, "response time"). I assumed that the bird had encountered the model if it flew directly over the model, dove at or passed over the model (see descriptions below), or landed within 10 m of the model. I allowed the birds to interact with the model for 5 min after the first individual of a pair had responded. If the second bird did not respond during the 5 min trial, I recorded the response time of the second bird as five min plus the response time of the first bird. Thus, this estimate was conservative and provided a minimum response time for the second bird. If neither bird responded within 15 minutes, I terminated the trial and repeated it the following day. If the model elicited no response from either parent on two successive days, I did not include the nest in my analyses.

In addition to response time, I recorded the following behaviours for both the male and female: (a) closest ground distance to the model, (b) closest height above the model, (c) percentage of time spent within 3 m of the model (on the ground and in the air, combined), (d) number of dives at (i.e., descended towards) the model, and (e) number of passes (i.e., circled; no change in vertical height) over the model. When an individual did not respond during the 5 min trial I recorded their closest ground distance and closest height as 50 m. I assumed that the intensity and, therefore, risk of nest defence increased with increasing frequency of dives and closeness to the model (see Montgomerie and Weatherhead 1988). I also assumed that response time indicated an individual's nest vigilance and willingness to defend the nest. I predicted that males would have a longer

response time, make fewer dives at and passes over the model, spend less time within 3 m of the model, and remain further from the model at second and experimental broods compared to replacement and control broods. I used an arcsin-squareroot transformation to transform the percentages in (c) for statistical analyses. At each nest I also recorded the number and age of nestlings.

I collected nest defence data at unmanipulated second and replacement broods in both 1994 and 1995 and estimated the clutch initiation date by back-counting 12 days from hatch to the laying of the penultimate egg (see Chapter 2). I converted clutch initiation dates to Julian dates for the purposes of statistical analyses. Because there were considerable between-year effects (e.g., mean age of young was significantly older in 1994; most second broods followed a replacement brood, rather than a first brood, in 1995; see Chapter 2), I analysed the data from the two years separately (see Statistical Analyses below).

Manipulation of Male Assessment of Paternity

I used a combination of decoy presentation and song to manipulate male assessment of paternity. I conducted manipulations during the female's fertile period for the replacement brood and then examined the effects of the manipulation on subsequent male nest defence and feeding rate. I assumed that the fertile period extended from seven days prior to the laying of the first egg until the laying of the penultimate egg (see Birkhead and Møller 1992). After a nest was preyed upon, I alternately assigned the pair to either the experimental or control

treatment. The experimental treatment involved presentation of a male Chestnut-collared Longspur decoy with accompanying longspur song, whereas the control treatment was a Horned Lark (*Eremophila alpestris*) decoy with accompanying Horned Lark song. I chose this control because Horned Larks are resident on the study site, are approximately the same size as longspurs, and pose no threat to the male longspur's paternity. Each decoy was a freeze-dried specimen and was mounted in a perched position on a 1-m high perch. I used song recordings from the Peterson Field Guides: Western Bird Songs (1992). The Chestnut-collared Longspur songs were recorded at North Dakota and Manitoba, whereas the Horned Lark songs were recorded at Colorado, Texas, and California (Peterson Field Guides: Western Bird Songs 1992). I recorded 1 min segments of song separated by 30 s of blank tape on a 30 min cassette tape (Sony HF) for each species. During the trials, I played the appropriate cassette tape on a Realistic AM/FM stereo cassette recorder/player, which I placed directly under the decoy. I set the volume control at ¾ maximum, which to my ear produced songs of similar volume to that of male longspur songs.

Female longspurs build a new nest for each nesting attempt and mean distance between nests of the same female is 31.5 m (range: 0.9-68.0 m, n = 55; Hill and Gould 1997). Therefore, I placed the decoy 10-20 m from the recently preyed upon nest. I began decoy presentations between two and five days after nest predation and presented the same decoy to the pair for five successive days. Previously, I found that longspur females initiate replacement clutches a mean of 5.5 days after nest predation (see Chapter 2). Therefore, by presenting the decoy

during the 5-day trial period, I ensured that each pair was exposed to the decoy during at least part of the female's fertile period. I conducted all trials between 0.5 and 2.5 h after sunrise. I recorded the period from the time the decoy and tape-recorder were in place until the male had discovered the decoy. Once the male responded, I allowed him to interact with the decoy for a total of 3 min, after which the decoy was removed. I made observations from a distance of 20 m or more. From this distance, the birds were not disturbed by my presence. Two observers attended all trials.

After the 5-day trial period finished, I searched the territory for the replacement nest. I recaptured the female on her nest during incubation to confirm nest ownership. I then monitored the nest daily until the young hatched. Once the young had hatched, I estimated clutch initiation date by back-counting 12 days from hatch to the laying of the penultimate egg (see Chapter 2). This allowed me to determine the female's fertile period relative to the decoy presentations. I assumed that the fertile period extended from Day -7 to + 2 (Day 0 = day first egg was laid, Chapter 2).

On Nestling Day 3, 4, or 5, I presented the pair with the ground squirrel model as described above. I presented the model to seven experimental pairs and eight control pairs. On Nestling Day 6, I conducted an hour-long nest observation and recorded the number of feeding trips made by the male and female parent, as described in Chapter 3. I made nest observations at eight experimental and eight control nests. These observations were conducted between 2.5 and 5.5 h after sunrise to control for time of day. At the end of the observation period, I recorded

the number and masses (to the nearest g) of nestlings, and total brood mass (see Chapter 3). I made observations from a distance of 20 m or more and, whenever possible, I made observations from a vehicle to minimize disturbing the birds .

Statistical Analyses

I predicted that males would provide less parental care at second broods compared to unmanipulated replacement broods, and less care at the experimental broods compared to the control broods and, therefore, I analyzed the male behavioural data using one-tailed tests. I did not make predictions regarding differences in the behaviour of females, age of nestlings, number of nestlings, or clutch initiation dates and, therefore, I analysed these data using two-tailed tests. I analysed differences in responses at second and replacement broods using an analysis of variance. In 1995, second and replacement broods differed with respect to date (see Results) and, therefore, I included date as a variable when I analysed the 1995 data. I analysed differences in responses to the experimental and control broods using analysis of variance for both feeding rate and response to the predator model. Because several of the nest defence variables were correlated, I used a stepwise discriminant function analysis to confirm analysis of variance results. I used a t-test to compare male latency time at experimental and control broods. In all cases, Type I error rate was 0.05. Unless otherwise indicated, I present means $\pm$ SE.

RESULTS

Influence of Nesting Attempt on Nest Defence

(a) 1994 data

During 1994, second and replacement broods did not differ with respect to mean clutch initiation date (Julian dates: $\bar{x}$ second = 171.9 $\pm$ 5.5, n = 8, $\bar{x}$ replacement = 169.8 $\pm$ 7.0, n = 6; t = 0.23, df = 12, p > 0.81). In this year, males spent a significantly smaller percentage of time within 3 m of the ground squirrel model at second broods compared to unmanipulated replacement broods (Table 4.1). With the exception of number of passes, the direction of difference in male nest defence between second and replacement broods was as I had predicted (Table 4.1), although only percentage of time within 3 m of the model was significant. Stepwise discriminant function analysis confirmed these results. Female nest defence did not differ between second and replacement broods (Table 4.2). Second and replacement broods did not differ with respect to age ($\bar{x}$ second = 4.8 days $\pm$ 0.5, n = 8, $\bar{\bar{x}}$ replacement = 4.5 days $\pm$ 0.3, n = 6, t = 0.41, df = 12, p > 0.68) or number of young ($\bar{x}$ second = 3.8 young $\pm$ 0.3, n = 8, $\bar{x}$ replacement = 3.5 young $\pm$ 0.2, n = 6, t = 0.72, df = 12, p > 0.48).

(b) 1995 data

During 1995, mean clutch initiation date differed significantly between replacement and second broods (Julian dates: $\bar{x}$ second = 186.2 $\pm$ 1.9, n = 12, $\bar{x}$

replacement = 161.7 ± 1.1, n = 16 ; t = 11.93, df = 26, p < 0.001). Second and replacement broods did not differ with respect to age ($\bar{X}$ second = 3.6 days ± 0.2, n = 12; $\bar{X}$ replacement = 3.4 days ± 0.1, n = 17; t = 0.89, df = 16.9, p = 0.39) or number of young ($\bar{X}$ second = 3.6 ± 0.3, n = 12; $\bar{X}$ replacement = 3.7 ± 0.19, n = 17; t = 0.19, df = 27, p = 0.85).

The direction of difference in male nest defence was as I had predicted for all the variables measured (Table 4.3). Initial analysis of variance indicated a significant difference for male response time, closest distance to the model, and number of dives at the model (Table 4.3): males took longer to respond to the model, made fewer dives at the model, and did not approach the model as closely at second broods compared to replacement broods. However, stepwise discriminant function analysis showed that almost all of the variation in male nest defence could be attributed to date, rather than breeding attempt. The final model included only date and male response time. Female nest defence did not differ between second and replacement broods (Table 4.4).

Manipulation of Male Assessment of Paternity

(a) Model Presentations

The longspur and Horned Lark decoys were presented to 15 and 12 pairs, respectively. Seven nests in the experimental treatment and four nests in the control treatment were preyed upon prior to testing with the ground squirrel model, leaving a sample size of eight in each treatment. All of the 5-day trials at

least partially over-lapped with the female's fertile period (Fig. 4.1). In two experimental treatments the female was no longer fertile on the last day of model presentation, and in one control treatment the female was no longer fertile on the last two days of model presentations. All other decoy presentations were made while the female was fertile.

Males typically responded to the longspur decoy by performing song displays, singing from nearby perches, and diving at the model. If the female was near the decoy (within 15 m), the male often landed between the decoy and his mate and, on four occasions, the male chased the female away from the decoy. One pair copulated during the decoy presentation and another pair copulated within two minutes after I had removed the decoy. On five occasions during the decoy presentation, the male chased an intruding male off the territory. Males often exhibited agitation during decoy presentations by ruffling their nape feathers or bill-wiping. Females showed little interest in the Horned Lark decoy and males did not chase females during these presentations. However, during Horned Lark presentations, males did perform song displays and were involved in chases with intruding males.

For all decoy presentations, there was no significant difference between experimental and control broods in the mean male latency time to encounter the model ($\bar{x}$ experimental = 333.7 sec $\pm$ 46.0, $\bar{x}$ control = 301.2 sec $\pm$ 34.3; ANOVA, F = 0.93, df = 1; p = 0.53). Experimental broods and control broods did not differ with respect to mean age of young ($\bar{x}$ experimental = 3.1 days $\pm$ 0.1, n = 7, $\bar{x}$ control = 3.3 days $\pm$ 0.2, n = 8; t = 0.49, df = 13, p > 0.31) or mean number of

young ($\bar{x}$ experimental = 4.0 young $\pm$ 0.3, n = 7, $\bar{x}$ control = 3.9 young $\pm$ 0.4, n = 8; t = 0.24, df = 13, p > 0.81).

(b) Influence on Parental Care

Mean male feeding rates at experimental and control nests were not significantly different ($\bar{x}$ experimental = 5.5 trips/hr $\pm$ 0.7, n = 8 , $\bar{x}$ control = 6.3 trips/hr $\pm$ 1.1, n = 8; ANOVA, F = 0.34, df = 1, p > 0.57), but power of the test was low (1 - β = 0.14). There was also no significant difference in female feeding rates ($\bar{x}$ experimental = 6.6 trips/hr $\pm$ 0.8, n = 8, $\bar{x}$ control = 6.9 trips/hr $\pm$ 1.2, n = 8; ANOVA, F = 0.03, df = 1, p > 0.86) or total (male and female combined) feeding rates ($\bar{x}$ experimental = 12.1 trips/hr $\pm$ 1.2, n = 8, $\bar{x}$ control = 13.1 trips/hr $\pm$ 1.7, n = 8; ANOVA, F = 0.23, df = 1, p > 0.63).

In every measure of male nest defence, the direction of difference between control and experimental broods was as I predicted (Table 4.5). The male responded to the ground squirrel before the female slightly less often for the experimental broods compared to the control broods (3 of 7 vs. 7 of 8, Fisher Exact Test, p = 0.119). This difference was not significant, but power of the test was low (1 - β = 0.05). Males tended to spent less time within 3 m of the model at experimental broods compared to control broods (Table 4.5). Closest distance approached by males to the ground squirrel model was significantly further at experimental broods compared to control broods (Table 4.5). No other measures of nest defence differed significantly. Stepwise discriminant function analysis confirmed these results.

With the exception of closest height and number of passes, the direction of difference in female nest defence between control and experimental broods was opposite to that for male nest defence (Table 4.6). There were non-significant trends that females approached the model closer and made more dives at the model at experimental broods compared to control broods (Table 4.6).

With respect to percentage of time males spent within 3 m of the model, experimental broods were similar to unmanipulated second broods in 1994 (ANOVA, $F = 0.18$, $p > 0.67$), whereas control broods were similar to unmanipulated replacement broods in 1994 (ANOVA, $F = 0.02$, $p > 0.89$; Fig. 4.2). Experimental broods differed significantly from replacement broods (ANOVA, $F = 5.25$, $p > 0.05$; Fig. 4.2). There was a trend that control broods differed from second broods (ANOVA, $F = 2.32$, $p > 0.15$; $1 - \beta = 0.42$; Fig. 4.2)

DISCUSSION

Influence of Nesting Attempt

Some of the measures of nest defence suggest that males defended their second broods less than unmanipulated replacement broods: at second broods, males spent less time close to the model (1994 data) and responded more slowly to the model (1995 data) compared to replacement broods. Additionally, with one exception, the direction of difference in male defence was as one would predict if males invested less in their their second broods. These results are compatible with

my results that male Chestnut-collared Longspurs feed their second broods significantly less than replacement broods (Chapter 3). In Chapter 3, I reported that second broods are more likely to contain extra-pair young than are replacement broods, and I implicated male assessment of paternity as the underlying factor contributing to the variation in male behaviour. In the current study, both female responses to the predator model were similar at second and replacement broods, suggesting that these broods are of similar value to females. This is not unexpected given that second and replacement broods did not differ with respect to either brood size or age of young, both of which can influence parental nest defence (e.g., Montgomerie and Weatherhead 1988, Kacelink and Cuthill 1990, Onnebrink and Curio 1991). Clutch initiation date can also influence variation in parental behaviour, because survival and recruitment of offspring is often less for young fledged later in the breeding season (e.g., Arcese and Smith 1985). Therefore, parents may invest less in late offspring (Weatherhead 1989) because they are less valuable to them. Alternatively, parents may invest more in later broods because they have less opportunity to renest if the brood fails (Montgomerie and Weatherhead 1988). In 1994, there was no difference in clutch initiation date between second and replacement broods, so this was not a source of variation in parental nest defence. When date was controlled for in the 1995 data, I found that breeding attempt influenced one measure of the nest-defence behaviour of males (response time), but not of females. As is the case in many other species (reviewed in Neudorf and Sealy 1992), female longspurs typically did not respond as intensely to the ground squirrel model as did males; in several trials

they did not respond at all. This lack of response decreased sample sizes in many of the female behaviour categories. Nonetheless, the results of this study provide further evidence that male longspurs invest less in their second broods, and suggest that second broods are less valuable to males than are replacement broods.

Influence of the Manipulation

There was some indication that males presented with a conspecific decoy responded less to the predator model than did males presented with a heterospecific decoy. In all measures of male nest defence, the direction of difference was as I predicted and suggest that the experimental manipulation was successful in altering male assessment of paternity. I assumed that nest defence risk increased with increasing closeness to the model (see Montgomerie and Weatherhead 1988). At experimental broods, males did not approach the predator model as closely, as they did at control broods. Males also tended to spend less time within 3 m of the model at experimental broods compared to control broods, and in this respect, experimental broods differed significantly from unmanipulated replacement broods. In contrast, males at control broods spent a similar amount of time near the ground squirrel model as did those males at unmanipulated replacement broods. This is expected because all manipulations were conducted on replacement broods. Therefore, this result indicates that the control treatment had little influence on male behaviour. Male response at experimental broods was similar to that at second broods, suggesting that males perceived experimental and second broods to be less valuable than control and replacement broods.

There was some evidence that female nest defence was more intense at the experimental broods compared to the control broods. At experimental broods, females tended to approach the ground squirrel model more closely and made more dives at it. These results indicate that females may have partially compensated for the reduced male nest defence at experimental broods. Incomplete compensation in response to a reduction in a mate's parental contribution is predicted by optimality models of biparental care (Kacelink and Cuthill 1990), and has been demonstrated in birds by experimentally handicapping either males (Wright and Cuthill 1989) or females (Markman et al. 1995) using tail-weights. In each of these experimental studies, the non-handicapped parent increased their care in response to their partner's reduced care, but did not compensate fully for the reduction. In contrast, results from Chapter 3 indicated that female longspurs compensated fully for reductions in male feeding at second broods.

Although experimental manipulation successfully influenced male nest defence, it had no effect on male feeding rates. Whittingham et al. (1993) experimentally manipulated male assessment of paternity in Tree Swallows (*Tachycineta bicolor*) by placing territorial males in glass cages such that they could observe their social mate copulating with extra-pair males. Their experiment did not alter male feeding rates, but there was a non-significant trend that some measures of male nest defence were reduced at experimental broods (see Whittingham et al. 1993). It is likely that nest defence is potentially more costly than provisioning offspring, because it can result in immediate injury or death to

the defending parent (reviewed in Sordahl 1990; but see Daan et al. 1996 for mortality resulting from provisioning). As a result, male nest defence may be more sensitive to changes in assessment of paternity than male feeding rates, which may explain the results of my study.

A survey of the descriptive and experimental studies examining the influence of actual paternity or assessment of paternity on male parental care (Table 4.7) reveals that most studies in which an effect has been found have been limited to species which are not strictly socially monogamous. One study on Dunnocks (*Prunella modularis*) found that only males in polyandrous or polygynandrous groups reduced their feeding rates after being held captive during the female's fertile period; monogamously paired males undergoing the same treatment did not alter their parental effort (Davies et al. 1992). Most other species in which paternity or assessment of paternity is correlated to male care are facultatively polygynous (Table 4.7), which requires that females are able to raise at least some of their brood to independence without male assistance. In most species which are strictly socially monogamous, males do not alter their parental care in response to paternity. This may indicate that females in these species cannot raise offspring alone and, therefore, the costs to males of reducing their feeding rates and potentially endangering young they sired within the brood may outweigh the benefits (Gowaty 1996). Chestnut-collared Longspurs are an exception to this. This species always pairs monogamously (Hill and Gould 1997), but males reduce both their feeding rate (Chapter 3) and nest defence (this study) at second broods, apparently in response to their assessment of paternity. Male

ability to reduce care at second broods may be related to female ability to compensate fully for reductions in male feeding (Chapter 3). Thus, the cost to males of reducing their provisioning rate does not explain why the experimental manipulation was successful in altering male nest defence but not male feeding rates.

Experimental studies necessarily manipulate male assessment of paternity as opposed to actual paternity (Kempenears and Sheldon 1997). However, little information exists on how males assess their paternity. Because male birds are not known to discriminate between genetic and extra-pair young within a brood (Kempenears and Sheldon 1996), they most likely base their assessment on cues obtained when the female is fertile (Davies et al. 1992). Although experimental manipulations attempt to mimic a natural increased risk of extra-pair paternity (e.g., via female removal and "interaction" with another male; Wright and Cotton 1994), they are unable to control the cues males receive directly from the female. If extra-pair fertilizations increase female fitness, but males reduce their parental care when they assess a high risk of loosing paternity, then selection should favour females that can deceive males concerning their paternity status. Thus, females should overtly "cooperate" with male paternity guard/assessment mechanisms, such as frequent copulations (Petrie 1992) and mate-guarding (Chapter 3), while covertly seeking extra-pair copulations. Therefore, female behaviour may diminish the influence of experimental manipulations designed to alter male assessment of paternity.

Table 4.1. Nest defence responses by males at second and replacement broods in 1994.

	Second broods Mean ± SE	Replacement broods Mean ± SE	ANOVA results
Response time (sec)	173. 8 ± 75.4 n = 8	168.7 ± 53.7 n = 6	$F < 0.01, p > 0.40^+$
% time spent within 3 m	28.1 ± 12.5 n = 8	63.8 ± 13.3 n = 6	$F = 3.95, p < 0.04^{+ *}$
Closest distance (m)	19.9 ± 8.8 n = 8	4.3 ± 3.1 n = 6	$F = 2.13, p > 0.08^+$
Closest height (m)	7.5 ± 0.5 n = 8	1.3 ± 0.6 n = 6	$F = 0.77, p > 0.19^+$
Number of passes	10.4 ± 4.1 n = 8	5.8 ± 3.2 n = 6	$F = 0.68, p > 0.21$
Number of dives	1.6 ± 0.7 n = 8	3.2 ± 1.0 n = 6	$F = 1.67, p > 0.11^+$

* Denotes significant difference.

+ Denotes difference in direction predicted

Table 4.2. Nest defence responses by females at second and replacement broods in 1994.

	Second broods Mean ± SE	Replacement broods Mean ± SE	ANOVA results
Response time (sec)	288.9 ± 25.5 n = 8	358.7 ± 53.2 n = 6	$F = 0.03, p > 0.86$
% time spent within 3 m	5.8 ± 3.9 n = 8	16.5 ± 12.9 n = 6	$F = 0.47, p > 0.50$
Closest distance (m)	40.1 ± 6.7 n = 8	33.7 ± 10.3 n = 6	$F = 0.30, p > 0.59$
Closest height (m)	31.6 ± 9.0 n = 8	33.5 ± 10.4 n = 6	$F = 0.02, p > 0.89$
Number of passes	2.5 ± 1.9 n = 8	1.3 ± 1.3 n = 6	$F = 0.23, p > 0.64$
Number of dives	0.5 ± 0.4 n = 8	1.0 ± 0.7 n = 6	$F = 0.47, p > 0.50$

Table 4.3. Nest defence responses by males at second and replacement broods in 1995.

	Second broods Mean ± SE	Replacement broods Mean ± SE	ANCOVA results
Response time (sec)	360.7 ± 82.6 n = 12	131.7 ± 47.1 n = 17	$F = 6.63, p < 0.01^{+}$ *
% time spent within 3 m	35.4 ± 12.3 n = 12	30.6 ± 7.9 n = 17	$F = 0.01, p > 0.45^{+}$
Closest distance (m)	21.7 ± 7.2 n = 12	16.2 ± 4.7 n = 17	$F = 0.44, p = 0.26^{+}$
Closest height (m)	21.5 ± 7.2 n = 12	5.8 ± 3.0 n = 17	$F = 4.94, p < 0.02^{+}$ *
Number of passes	3.7 ± 2.0 n = 12	7.1 ± 1.9 n = 17	$F = 1.51, p > 0.10^{+}$
Number of dives	0.8 ± 0.4 n = 12	4.2 ± 1.6 n = 17	$F = 3.30, p < 0.05^{+}$ *

* Denotes significant difference.

+ Denotes difference in direction predicted.

Table 4.4. Nest defence responses by females at second and replacement broods in 1995.

	Second broods Mean ± SE	Replacement broods Mean ± SE	ANCOVA results
Response time (sec)	332.0 ± 39.1 n = 12	302.0 ± 39.0 n = 17	F = 0.28, p > 0.60
% time spent within 3 m	21.2 ± 9.5 n = 12	19.1 ± 5.7 n = 17	F = 0.04, p > 0.84
Closest distance (m)	21.1 ± 6.6 n = 12	26.5 ± 5.7 n = 17	F = 0.38, p > 0.54
Closest height (m)	18.7 ± 6.7 n = 12	24.5 ± 6.0 n = 17	F = 0.41, p > 0.52
Number of passes	1.2 ± 0.7 n = 12	1.5 ± 0.7 n = 17	F = 0.05, p > 0.83
Number of dives	0.4 ± 0.2 n = 12	0.8 ± 0.3 n = 12	F = 1.07, p > 0.31
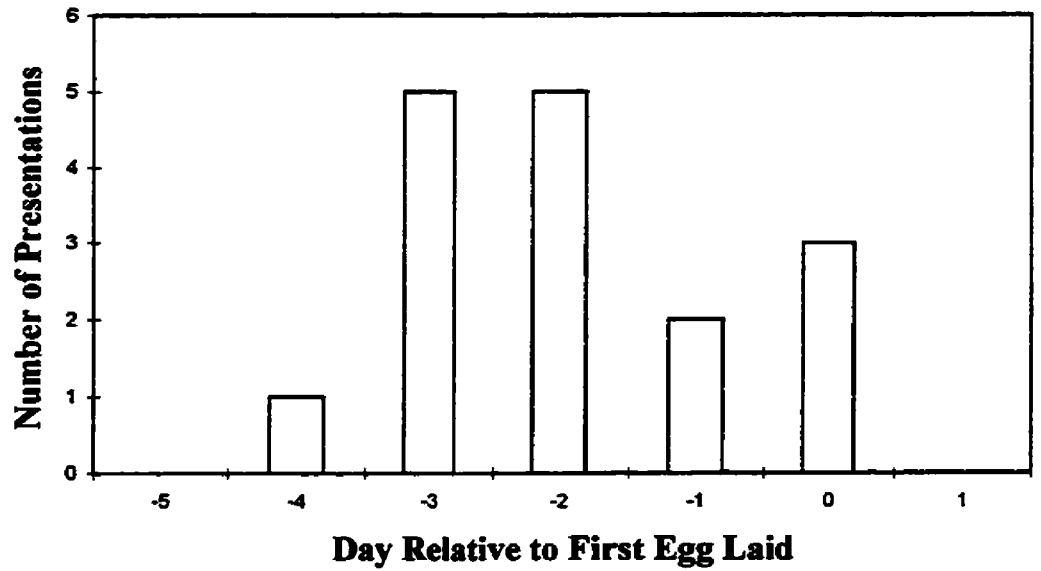
Figure 4.1. Day on which the 5-day decoy presentation began relative to day first egg was laid (= Day 0). The fertile period extended from Day -7 to +2.

Table 4.5. Nest defence responses by males at experimental and control broods.

	Experimental broods Mean ± SE	Control broods Mean ± SE	ANOVA results
Response time (sec)	288.1 ± 104.1 n = 7	164.1 ± 67.3 n = 8	$F = 1.05, p > 0.32$[+]
% time spent within 3 m	21.7 ± 13.5 n = 7	60.9 ± 13.6 n = 8	$F = 3.41, p < 0.10$[+]
Closest distance (m)	19.5 ± 8.2 n = 7	1.5 m ± 0.5 n = 8	$F = 5.49, p < 0.04$[+] [*]
Closest height (m)	9.4 ± 6.8 n = 7	7.4 ± 6.1 n = 8	$F = 0.05, p > 0.82$[+]
Number of passes	1.6 ± 0.8 n = 7	2.1 ± 0.9 n = 8	$F = 0.22, p > 0.64$[+]
Number of dives	1.3 ± 0.5 n = 7	2.3 ± 0.7 n = 8	$F = 1.10, p > 0.30$[+]

* Denotes significant difference.

+ Denotes difference in direction predicted.

Table 4.6. Nest defence responses by females at experimental and control broods.

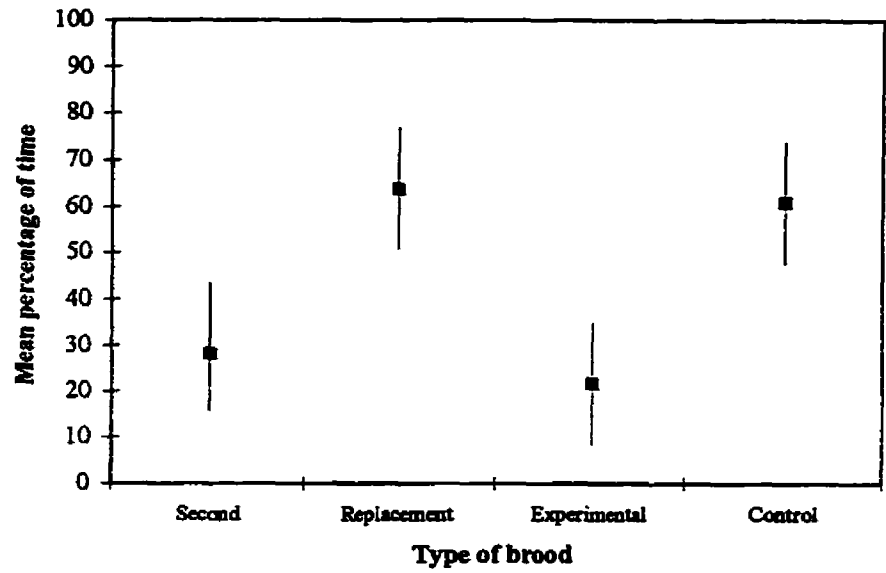|	Experimental broods Mean ± SE	Control broods Mean ± SE	ANOVA results
Response time (sec)	248.0 ± 70.9 n = 7	273.9 ± 74.3 n = 8	F = 0.06, p > 0.80
% time spent within 3 m	37.6 ± 15.3 n = 7	16.6 ± 11.3 n = 8	F = 1.18, p > 0.29
Closest distance (m)	9.9 ± 6.7 n = 7	28.9 ± 7.4 n = 8	F = 3.52, p > 0.08
Closest height (m)	15.4 ± 6.7 n = 7	14.9 ± 7.7 n = 8	F < 0.01, p > 0.96
Number of passes	2.6 ± 1.4 n = 7	2.4 ± 1.2 n = 8	F = 0.01, p > 0.91
Number of dives	1.7 ± 0.7 n = 7	0.3 ± 0.2 n = 8	F = 4.54, p > 0.05

Figure 4.2. Mean percentage of time (± SE) males spent within 3 m of the
ground-squirrel model at unmanipulated second and replacement broods,
experimental broods, and control broods.

Table 4.7: Summary of descriptive and experimental studies examining the effect of actual paternity and assessment of paternity on male parental care.

SPECIES	EFFECT OF ACTUAL PATERNITY	EFFECT OF ASSESSMENT OF PATERNITY	MATING SYSTEM	SOURCE
DESCRIPTIVE STUDIES				
White Ibis		none (feeding)	monogamous	Frederick 1987
Razorbills		none (feeding)	monogamous	Wagner 1992
Reed Buntings	+ve correlation (feeding)		monogamous/ polygynous	Dixon et al. 1994
Indigo Buntings	none (feeding)		monogamous	Westneat 1988
Bobolinks	none (feeding)		polygynous	Bollinger and Gavin 1991
Purple Martins	none (feeding)		monogamous	Wagner et al. 1996
Hooded Warblers	none (feeding)		monogamous	Stutchbury et al. 1994
Yellow Warblers	none (feeding)		monogamous	Yezerinac et al. 1996
Red-winged Blackbirds	none (feeding) +ve correlation (nest defence)		polygynous	Westneat 1995 Weatherhead et al. 1994
Great Tits	+ve correlation (nest defence)		monogamous	Lubjuhn et al. 1993
Barn Swallows	none (male incubation)		monogamous	Smith and Montgomerie 1992

Table 4.7 (cont.)

SPECIES	EFFECT OF ACTUAL PATERNITY	EFFECT OF ASSESSMENT OF PATERNITY	MATING SYSTEM	SOURCE
EXPERIMENTAL STUDIES				
a) male removals				
Barn Swallows		+ve correlation (feeding) +ve correlation (nest defence)	monogamous	Møller 1988 Møller 1991b
Tree Swallows		none (feeding) (+ve but non-significant for nest defence)	monogamous/ polygynous	Whittingham et al. 1993
Dunnocks		+ve (feeding)	polygynandrous	Davies et al. 1992
		none (feeding)	monogamous	Davies et al. 1992
Acorn Woodpeckers		+ ve (feeding)	cooperative	Koenig 1990
b) female removals				
European Starlings		+ ve (feeding)	monogamous/ polygynous	Wright and Cotton 1994

CHAPTER 5: CONCLUSIONS

Conditions For a Relationship Between Paternity and Male Parental Care

After relaxing all assumptions except that offspring survival increases from increased parental care and that providing parental care is costly to individuals, Yezerinac et al. (1996) synthesized the various models used to describe the relationship between parentage and parental care. Three conditions that determine whether paternity influences male care are: (1) the variability and predictability of paternity in subsequent broods, (2) the ability of males to assess their current paternity reliably, and (3) the costs to males of withholding care to genetic, as well as non-genetic, young in the brood. These conditions can explain why male Chestnut-collared Longspurs reduced care at second broods. First, male longspurs have predictably higher paternity in the first brood of the following season than in the current second brood, assuming that extra-pair paternity patterns are consistent between years. By reducing or withholding care at second broods, males may increase their chances of over-winter survival so that they can take advantage of greater genetic benefits the following year. Second, assessing a higher risk of extra-pair paternity in a second brood is reliable at the population level because most extra-pair young are found in second broods. This may result from constraints on when females can leave the territory to seek extra-pair copulations undetected (see below). Third, the cost to males in withholding care at a second brood, and potentially to some genetic young, is minimized because females compensated fully for reductions in male feeding rate. Thus, by reducing care to

second broods, males are more likely to influence female fitness than offspring survival.

Assessment of Paternity

Male Chestnut-collared Longspurs can apparently assess their paternity, albeit imperfectly. Although the exact mechanisms by which they do this remain unknown, this study provides some insight as to how males of this and other avian species may assess parentage. First, mate attendance patterns were consistent with the mate-guarding hypothesis, but showed variation between breeding attempts. Intensity of mate attendance was high during the female's fertile period of both first and replacement broods, but was reduced to non-fertile (incubation) levels during the female's fertile period of second broods when males fed their first brood fledglings (Chapter 2). Second broods were more likely to contain extra-pair young and males provided less parental care to these broods (Chapter 3). However, even those males that did not loose paternity tended to reduce their care to second broods. This suggests that both those males that did and those that did not loose paternity in second broods used a similar means of assessing their paternity. It seems likely that mate attendance provides such an assessment mechanism. Males should assess a higher risk of extra-pair paternity when (a) there is a high degree of uncertainty in their assessment (e.g., when they are unable to remain close to the female during the second brood fertile period; Weatherhead and McRae 1990; Chapter 2) or (b) by means of mate attendance, they directly

observe their mate interacting with other males (e.g., when the female leaves the territory and accepts extra-pair copulations; Chapter 2).

Conditions in (b) might explain the success of my experiment in apparently altering male assessment of paternity and subsequent male nest defence (Chapter 4). The tape-recorded longspur song attracted the female, as well as the male, to the conspecific decoy. Intensity of mate attendance is high during the replacement brood fertile period, so males could observe their mates near the decoy and this may have lead them to assess a higher risk of extra-pair paternity in the experimental broods. I observed males chasing their mates away from the decoy and landing between the decoy and their mate (Chapter 4), which suggests that males may have perceived the decoy as a threat to their paternity.

Female behaviour also plays an important role in male assessment of paternity. Although females can compensate fully for a reduction in male feeding rate at second broods (Chapter 3), doing so may negatively impact their own survival or future reproduction (e.g., Daan et al. 1996). Thus, it is in a female's best interest to deceive males concerning their paternity status when the brood contains extra-pair young, or to provide accurate information when their mate has full paternity in the brood. Females may manipulate a male's assessment of paternity by soliciting copulations from their mates (e.g., Petrie 1992; during decoy presentations in Chapter 4) or by 'cooperating' with male mate-guarding strategies (e.g., Mace 1989; Chapter 2). Gowaty (1996) predicted that variation in female quality should result in variation in female extra-pair behaviour, such that low-quality females should seek extra-pair copulations covertly, whereas high-

quality females (i.e., able to raise offspring unaided) can seek extra-pair copulations overtly without reducing their reproductive success. However, even a high-quality female should benefit from male assistance in raising offspring because assistance should allow her to reduce her own reproduction effort and, potentially, increase her survival and opportunity for future reproduction. Therefore, I predict that all females should manipulate male assessment of paternity. I further predict that variation in female quality expresses itself as variation in the ability of females to manipulate male assessment of paternity and, therefore, in the level of male assistance they can obtain.

Female Chestnut-collared Longspurs appear to have low success manipulating male assessment of paternity, and it is likely that the breeding habitat plays a role. The preferred breeding habitat of this species is recently grazed, mowed, or burnt native prairie with vegetation 20-30 cm in height (Hill and Gould 1997). The openess of the habitat not only facilitated my observations of longspur behaviour, but possibly also assisted males in their ability to mate-guard and assess their paternity. Females may be constrained in their ability to seek extra-pair copulations undetected when they become fertile for their first and replacement broods. Males responded to their mate's potential extra-pair behaviour through copulation coercion attempts (Chapter 2) and by reducing their parental care (Chapters 3 and 4). Therefore, the costs to females of seeking extra-pair copulations during first and replacement broods may outweigh the benefits. As a result, under these constraints females may choose to obtain extra-pair copulations

only during second broods when males cannot mate-guard effectively, and this may explain the pattern of extra-pair paternity I observed (Chapter 3).

If habitat plays a role in the ability of males to assess their paternity and females to manipulate that assessment, then I predict that patterns of extra-pair paternity and male parental care will vary with the habitat. For example, in forested or riparian habitats, females should have greater opportunity to leave the territory and seek extra-pair copulations undetected by their mate. In such a case, I predict that extra-pair young will be distributed more evenly between breeding attempts (i.e., not skewed towards second broods). Visually obstructed habitats may also hinder male ability to assess their paternity reliably so that in such habitats there may be no discernable relationship between paternity and parental care. On a continuum from visually obstructed to open habitats, I predict that male ability to assess paternity should improve, the relationship between paternity and parental care should be strengthened, and the distribution of extra-pair young should become increasingly skewed towards second broods as female ability to manipulate assessment of paternity becomes more constrained.

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IMAGE EVALUATION
TEST TARGET (QA-3)

1.0
2.8
2.5
1.1
3.2
2.2
3.6
2.0
4.0
1.8
1.25
1.4
1.6

|← 150mm →|

|← 6″ →|
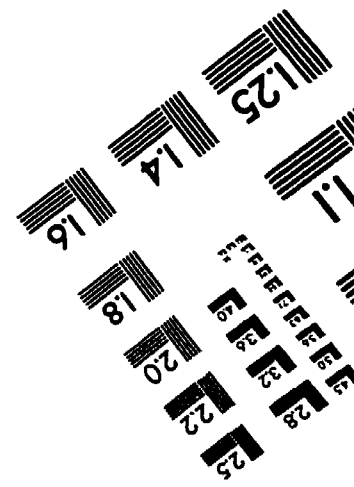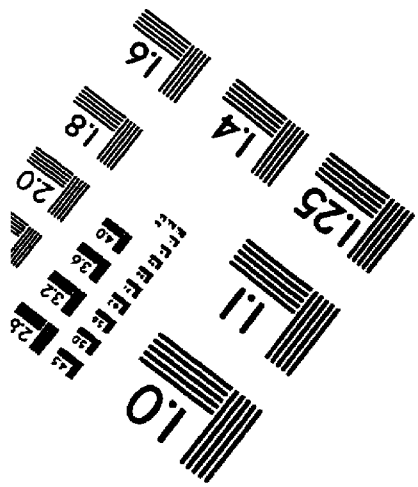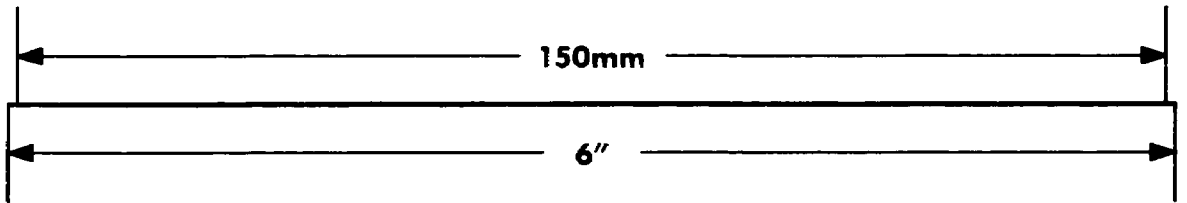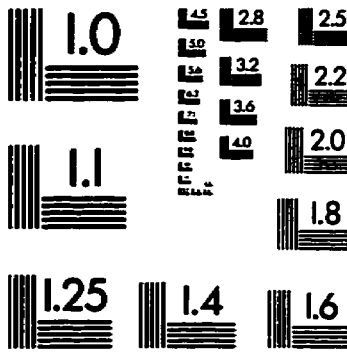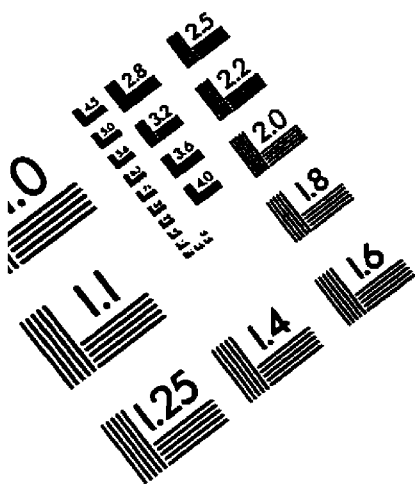
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1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989

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