

**Breeding density, male age and mixed reproductive strategies in
the northern oriole (*Icterus galbula bullockii*)**

Thesis submitted for the degree of
Doctor of Philosophy
at the University of Leicester

by

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June 1997

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To my parents

Fieldwork blues.

They muck you about those Orioles.
They may not mean to but they do.
They build their nests and lay their eggs
and then desert, it makes you blue!

But they get banded in their turn,
by us field workers brave and true.
We bleed their wings and clamp their legs
with plastic bands red, green and blue.

and then we watch them all day long,
we think from them we'll wrest the plot.
but when the answers close at hand
alas predation takes the lot!

Hastings Reservation Fieldwork party 1994
(with apologies to Philip Larkin)

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ABSTRACT

Breeding density, male age and mixed reproductive strategies in the northern oriole (*Icterus galbula bullockii*)

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1) This study investigated the relationship between breeding density, male age and the reproductive strategies employed by northern orioles (*Icterus galbula bullockii*) in central coastal California, USA, 1994-1996.

2) A nesting association was found to occur between northern orioles and yellow-billed magpies (*Pica nuttalli*). Northern orioles often chose to nest close to magpie nests (< 50m) and, apparently as a result, suffered significantly lower nest predation (13.5%, 5/37) than did northern orioles nesting further away from magpies (43.3%, 13/30). The clumping of northern oriole nests that occurs in some areas is suggested to be largely a result of northern orioles attempting to nest close to the less abundant, semi-colonial magpies to gain from the predator protection they afford.

3) Single-locus DNA profiling revealed that overall 32.2% of chicks were extra-pair young (EPY) and that 46% of nests contained EPY. EPY was shown to be distributed across nests in a highly bimodal fashion. Paternity was assigned for 44.6% (29/65) of EPY. Low levels of intra-specific brood parasitism were also detected (1.0% [2/202] of chicks in 4.6% [2/48] of nests).

4) Male age/plumage status was shown to have a considerable affect on the frequency of extra-pair paternity. On average, first-year (subadult plumage) males lost 55.3% of their paternity while older (adult plumage) males lost only 20.6% of their paternity. Furthermore, paternity assignment indicates that it is nearly always adult males that gain the extra-pair paternity (EPP). The uneven distribution of paternity resulted in adult males having a significantly higher annual reproductive success rate than subadult males (4.3 v. 1.9 fledglings, respectively). The bimodal distribution of EPP and the evidence that adult males gain higher levels of both within and extra-pair paternity are, in the light of female control of extra-pair fertilisations (EPFs), best explained by the 'good genes' hypothesis. Females appeared to prefer fertilisations from older 'better quality' males and it is suggested that they base this choice on the plumage differences that occur between adult and subadult males. This result has considerable implications for the evolution of delayed plumage maturation (DPM) and new hypothesis (the honest plumage hypothesis) is suggested that may explain the evolution of DPM in the northern oriole and other bird species.

5) Breeding density also had an influence on the frequency of EPP. Both the number of nests that contained EPY, and the mean proportion of EPY in a nest, increased with decreased nearest conspecific neighbour distance. furthermore 'colonial' nests (internest distance < 100m) contained EPY significantly more often than 'solitary' nests (16/26 v. 6/22 respectively) and also contained a higher mean proportion of EPY (48% v. 16%). There was no evidence to suggest any relationship between breeding density and first egg date, male age, male body condition or breeding synchrony. Breeding density was shown not to have an overall effect upon mean male reproductive success. However it was not possible to determine if the variance of male reproductive success increased with breeding density.

Evidence from this study suggests that females mated to low quality mates (subadults) may prefer to nest at a higher breeding density (close to high quality adult male nests). It is, therefore, possible that the pursuit of extra-pair copulations by certain females is also actually a factor in determining the nesting pattern of northern orioles.

ACKNOWLEDGEMENTS

This study was supported by a SERC studentship. Equipment and facilities were provided by the Department of Zoology at Leicester University.

I would like to thank my supervisor Terry Burke for setting up this project, and for his help and guidance throughout. He has always been supportive while allowing me enough freedom to develop my own ideas.

I undertook fieldwork at Hastings Natural History Reservation, University of California, Berkeley. This reservation only exists due to the generous support of Fanny Hastings Arnold and I would like to thank her for helping create such an outstanding field centre. Furthermore, I wish to thank Mark Stromberg and Mark Johnson who manage and maintain the excellent facilities at Hastings. They often helped to dig me out of trouble (sometimes literally). I was also very fortunate to have the support and guidance of Walt Koenig and Janis Dickinson. They gave me invaluable help, both in fieldwork and in academic matters. They also helped to create an excellent social atmosphere at Hastings in which to both work and play. Sorry about the hot tub!

Life at Hasting was made stimulating, happy and fun by the many friends I made during my stay there. There are too many to mention here, however I would like to especially thank Andren 'the mad nanny', Ginger, Joey and Siri.

I was helped on fieldwork by three very able, hard working and enthusiastic field assistants, Mike Cant, Karl Evans and Andy Spellman. Their good nature, humour and friendship made it possible to cope with the gruelling hours sometimes required during fieldwork. Ginger Bolen and Joey Haydock were of great help to me during this project, not least in towing my car out of ditches. Furthermore, Ginger and I worked together on the northern oriole magpie association. Thank you all.

The majority of my work was, however, done back in Blighty and I owe a debt of gratitude to everyone in Leicester Zoology department who has helped me over the last four years. DNA profiling was explained to me by Mike Double and Andy Krupa; Douglas Ross, Graham Rowe, Ian Stewart, and Olivier Hanotte were always willing to give advice. Help in the lab, on computers, or in sorting out my finances was given by Ewen, Lesley, Jean, Joan, Penny, Ted, and Yvonne. I would also like to thank Drs Ross, Double, Siriwardena, Thomson, Hartley, and Burke as well as Ian Stewart, Magnus Johnson and Simon Griffith (all soon to be doctored we hope) for all their advice and discussion regarding this project.

As well as those mentioned above, many other people have also added to my enjoyment of working (and socialising) in Leicester and I'd like to thank them. Within the department these include; Lucy, Ems, Gaynor, Harrie, Louise, Debs, Sarah, Dee, Jules, Mark, Matt, Rory, Stans, Andy D, Dave C, Bev, Kate, Jeff, Jo, Helen, Carolyn

and many others. Outside the department, Bex helped me to get here and to survive the first couple of years. My housemates then managed to put up with me and my bad sense of humour while the Volleyball Club members (especially Sophie) have managed to cope with my incompetence.

Finally I'd like to thank my family and friends (both of them but in no particular order) for supporting me throughout my time at university, especially my mum and dad who have always encouraged me to do what I enjoy. Thank you.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

1.2 Thesis aims

1.3 Chapter summaries

1.4 The northern oriole

1.5 Study area

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CHAPTER 1

INTRODUCTION

1.1 GENERAL INTRODUCTION

This thesis examines the effects that breeding density and male age have upon the use of alternative reproductive strategies in a socially monogamous species. A northern oriole (*Icterus galbula bullockii*) population that exhibits a range of breeding densities and age-related plumage characteristics was chosen in which to investigate these variables. Even though this study focuses on birds, the theories and predictions explored are applicable to a wide range of animal species. It is therefore hoped that by setting this study against a background of evolutionary theory, and in context with the wealth of information from other studies, that a greater understanding of the general influence of these two variables will be gained.

The strict definition of monogamy is the “..prolonged association and essentially exclusive mating relationship between one male and one female” (Wittenberger & Tilson 1980). Recent research has shown that the social association between the sexes in monogamous species is not always reflected in an exclusive sexual relationship. Many studies have reported copulations occurring outside of the pair bond (reviewed in: Gladstone 1979, Birkhead & Møller 1992) and the strict definition of monogamy probably occurs very rarely. A more operational definition of monogamy has been accepted to help distinguish between the social system and the sexual exclusivity of pairs. The term “socially monogamous” indicates that a single male and female form a constant social pair-bond over the duration of a breeding attempt. (see Wickler & Seibt 1983). This bond does not have to involve an exclusive mating relationship with the result that pairs may not be “genetically monogamous”. Extra-pair copulations (EPCs) may occur within social monogamy and an EPC is defined as a copulation between a paired individual and an individual other than its social (pair bonded) mate. The evolutionary significance of EPCs within socially monogamous mating systems must, therefore, be considered.

A consequence of both natural and sexual selection (Darwin 1859, 1871) is that individuals are expected to behave selfishly in order to increase their own reproductive success (Dawkins 1976). Trivers (1972) suggested that fundamental differences occur between the relative amounts of parental investment by each sex as a result of anisogamous sexual reproduction (the fusion of two gametes of unequal size). The differing levels of investment are thought to result in different selection pressures upon the sexes (Parker *et al.*

1972, Trivers 1972, reviewed in Birkhead & Parker 1997). Males are potentially able to fertilise more eggs than any one female can produce and can, therefore, increase their reproductive success through finding and fertilising many different females. Conversely, females are constrained in the number of offspring they can produce and should attempt to improve the quality of their offspring by being more discriminating in their choice of partner. Thus the strategy for maximising reproductive success may not be the same for each sex. The resolution of the conflict between the sexes is reflected in the evolution of the different reproductive strategies displayed within the varied mating systems of animals (reviewed in Emlen & Oring 1977, Krebs & Davies 1993).

Social monogamy occurs commonly among birds where it has been recorded as occurring in 90% of species (Lack 1968). This mating system was originally suggested to have evolved because biparental care of the offspring increases the reproductive success of both the male and the female parent (Lack 1968). In this case, each sex is constrained from pursuing its optimal reproductive strategy of either polygyny (males) or polyandry (females). However, biparental care is not always essential to rear some offspring (Wright & Cuthill 1989; reviewed in Bart & Tornes 1989). It would therefore appear that either sex could increase their reproductive success by deserting their partner after clutch completion and remating elsewhere. When biparental care is not required other constraints may still lead to social monogamy. For males these include: the temporal distribution of females, the spatial distribution of resources and female-female aggression (reviewed in Emlen & Oring 1977, Wittenberger & Tilson 1980, Gowaty 1995). It may, alternatively, be the female that is somehow constrained to be socially monogamous, but this possibility has, as yet, received little attention (Gowaty 1995).

Even within the constraints of a socially monogamous system, individuals which exhibit behaviour to increase their reproductive success will be favoured through natural selection (Trivers 1972). Males may do this by taking every opportunity to obtain EPCs while also helping their partner to raise their offspring. These males are said to have adopted a “mixed reproductive strategy” (Trivers 1972). If EPCs are successful and result in extra-pair fertilisations (EPFs) then the male will gain by having manipulated another male into caring for an unrelated offspring and may thereby gain increased reproductive success. Conversely, being cuckolded (caring for unrelated offspring) is costly in evolutionary terms for males. This is thought to have resulted in behavioural and physiological mechanisms to prevent individuals from being cuckolded (i.e. mate guarding, frequent copulations, increased ejaculate size) evolving in tandem with parental care (Trivers 1972, reviewed in Birkhead & Møller 1992, Andersson 1994). Despite these paternity guards, EPCs and EPFs still occur, at least within a large proportion of socially monogamous birds. However some studies suggest that males may be able to assess their confidence of paternity in a brood and reduce their investment accordingly to reduce the cost of rearing unrelated offspring (Davies *et al.* 1992, Dixon *et al.* 1994, but see Westneat & Sherman 1993, Westneat 1995).

Originally, females were viewed as either unwilling or passive participants in EPCs, however they too may be engaging in a mixed reproductive strategy. The majority of more recent evidence suggests that females not only accept these EPCs, but may actively solicit matings from males other than their mates (Birkhead *et al.* 1988, Smith 1988, Kempenaers & Dhondt 1993, Wagner 1993). Furthermore, many studies have reported evidence to suggest that females largely control EPCs and therefore EPFs (Wagner 1991, Kempenaers *et al.* 1992, Lifjeld & Robertson 1992, Birkhead & Møller 1993b, Dickinson 1997). This in turn indicates that females must also benefit from participating in EPCs. However, while males may increase the quantity of offspring sired by mating with more than one female, females are ultimately restricted in the number of offspring they can produce and so must benefit from EPCs in other ways

The possible benefits to female of EPCs fall into the two main categories of direct benefits and indirect benefits according to their effect (reviewed in Westneat *et al.* 1990, Kirkpatrick & Ryan 1991, Birkhead & Møller 1992, Kempenaers 1993). The direct benefits that females may gain from EPCs include courtship feeding, foraging rights, paternal investment, future mate appraisal and avoidance of the costs of rejecting persistent males. Unlike indirect benefits (see below) direct benefits do not necessarily require that EPCs result in EPP.

Females may also (or alternatively) be gaining from EPCs through indirect benefits. For example, the ‘fertility insurance’ hypothesis suggests that females mate with more than one male to guard against male sterility, poor sperm quality or sperm depletion and thus insure that their eggs are fertilised and viable (Walker 1980, Gibson & Jewel 1982, McKinney *et al.* 1984, Gray 1997). Two main genetic benefits have also been proposed as possible sources of indirect benefits. The ‘genetic diversity’ hypothesis suggests that females perform EPCs to increase the genetic diversity of their offspring, thus increasing the likelihood of some surviving in an unpredictable environment (Williams 1975). Alternatively the ‘genetic quality’ hypothesis suggests that females benefit from EPFs by males that are of a higher quality than their social partner. The genes that they acquire for their offspring may be of higher quality for two reasons: (i) they may produce traits which make the male offspring more attractive to females both in social matings and in extra-pair matings (‘sexy son’ hypothesis: Fisher 1930, Weatherhead & Robertson 1979); or (ii) the female may acquire genes which result in higher general vigour, or greater parasite or disease resistance, of all offspring (‘good genes’ hypothesis: Zahavi 1975, 1977; Hamilton & Zuk 1982, Masden *et al.* 1992). There is a mounting body of evidence to suggest that females are choosy about extra-pair mates (Møller 1985, Kempenaers *et al.* 1992, Wetton *et al.* 1995), and that they may gain genetic benefits from choosing males on the basis of secondary sexual characteristics (Petrie 1994, Hasselquist *et al.* 1996, Sheldon *et al.* 1997).

Within certain animal groups (birds and fish especially) socially monogamous females may also include intra-specific brood parasitism (IBP) as part of a mixed

reproductive strategy. Females dump eggs within the nest of other conspecifics while rearing their own clutch, with the potential of achieving a direct increase in their reproductive success. Intra-specific brood parasitism has now been reported in a large variety of bird species (reviewed in; Yom-tov 1980, MacWhirter 1989, Petrie 1991, Yamauchi 1993), however it is still not known if this is an actual mixed reproductive strategy or just a 'best of a bad job strategy'. Counter-adaptations to IBP are also predicted to have evolved to avoid the costs of being parasitised. Possible adaptations include nest guarding, egg recognition and nest desertion. Nest guarding is, however, the only strategy that has been repeatedly documented (Gowaty 1989, Brown & Brown 1989).

The use and significance of mixed reproductive strategies is, perhaps, best investigated within bird species, where a multitude of studies have reported EPCs occurring in a wide range of socially monogamous species (Westneat *et al.* 1990, Birkhead & Møller 1992). However, for EPCs to be functionally adaptive they must, at least occasionally, result in EPP. DNA profiling is now considered the most reliable method of parentage analysis (Jeffreys 1985, Burke & Bruford 1987, Burke 1989, Queller *et al.* 1993) and has been used to determine the frequency of EPP (and IBP) within a wide range of bird species. The frequency of EPP reported in these studies varies widely between species, e.g. from 0% in fulmars (Hunter *et al.* 1992) to 76% in the superb fairy wren (Mulder *et al.* 1994) and even between populations of the same species e.g. in pied flycatchers (Ross 1997), and tree swallows (Lifjeld *et al.* 1992, Dunn *et al.* 1994b) (reviewed in Gowaty 1996). Most of these parentage studies have used a multilocus DNA profiling technique which can determine if a chick is genetically related to the putative parents (exclusion analysis) but does not allow the efficient identification of the chick's true genetic parents. More recently, the development of single-locus DNA profiling techniques has provided a method of identifying the true biological parents of these offspring (inclusion analysis). By this method the individuals gaining from EPP and IBP can also be determined, thus allowing studies to quantify individual reproductive success and investigate factors related to mixed reproductive strategies, such as female choice (Kempeneers *et al.* 1992, Wetton *et al.* 1995).

At present little is known about which factors determine the use of mixed reproductive strategies in monogamous bird species. Various ecological and demographic factors have been suggested to influence the levels of EPP. These include breeding density, breeding synchrony and the relative age or experience of the individuals concerned (Westneat *et al.* 1990, Birkhead & Møller 1992). However, the specific influence that these variables have upon EPP is still undetermined despite the large number of parentage studies undertaken on birds.

A greater breeding density of conspecifics has been predicted to increase the opportunities for EPCs and consequently for EPP (Alexander 1974, Hoogland & Sherman 1976, Gladstone 1979, Birkhead & Møller 1992, Wittenberger & Hunt 1985). Several different, non-exclusive, reasons have been suggested to explain this increase in EPC

opportunities. These include both the increase in density of birds *per se*, and also the ecological consequences of living at higher densities (reviewed in Westneat *et al.* 1990). The relationship between density and EPP is not predicted to be a necessarily linear one and may differ between territorial and non-territorial species. It may also depend upon 'the nature of interactions between neighbours' and how this varies in different species (Westneat *et al.* 1990).

Comparative studies have suggested that the proportion of females engaging in EPCs is positively related to the degree of sociality, and that EPCs are more frequent in populations or species nesting at higher densities (Birkhead & Møller 1992, Møller & Birkhead 1993). However, no correlation was found between observed levels of EPCs and levels of EPP in a comparison across socially monogamous species (Birkhead & Møller 1995). The frequency of EPCs has also been reported to increase with density in various intraspecific studies (Møller 1987, 1991b, Hatchwell 1988) and higher levels of EPP have been found to occur at higher breeding densities in some species (Gowaty & Bridges 1991a, Double 1995, Hoi & Hoi-Leitner 1997). Other studies, however, have failed to find a link between EPP and breeding density (Gowaty & Bridges 1991b, Dunn *et al.* 1994a).

The potentially higher levels of extra-pair activity and cuckoldry at higher densities were traditionally viewed as a costly consequence of sociality, at least for males (Alexander 1974, Hoogland & Sherman 1976, Gladstone 1979). However, although some males will suffer as a result of EPP, other must gain, and perhaps more importantly, potentially all females may benefit from increased EPCs. This has led to the recent suggestion that the increased opportunities for EPCs may be a pre-laying advantage of coloniality (Morton *et al.* 1990, Wagner 1993, Hoi & Hoi-Leitner 1997). In these hypotheses, the pursuit of EPCs by either males (Morton *et al.* 1990) or females (Wagner 1993, Hoi & Hoi-Leitner 1997), is a selective force that actually favours the formation and maintenance of higher breeding densities.

The pursuit of EPCs should, then, be considered along with (i) reduced predation risk, (ii) increased foraging efficiency and, (iii) restricted suitable nest sites or habitat (Alexander 1974) when attempting to determine the selective forces behind coloniality. It is also important to note that the selective forces proposed by Alexander can apply both between conspecifics and also between individuals of different species. Restricted suitable nest sites or habitat may account for the formation of many mixed-species colonies especially in seabirds (Orians & Wilson 1964). Alternatively, if habitat and nest sites are not limiting then mixed-species colonies may function as 'information centres' which increase the foraging efficiency of the birds within them (Ward & Zahavi 1973). Finally, reduced predation pressure may be an important factor causing aggregations of mixed species, as the birds within these aggregations may suffer lower rates of predation for the same reasons as in conspecific colonies (e.g. predator swamping, increased vigilance). Certain non-aggressive species may also benefit from nesting in close association with an aggressive

species that is more able to deter predators (Koskimies 1957: reviewed in Bogliano 1994). This may result in multi-species colonies, e.g. grebes nesting in gull colonies (Burger 1984), or in nesting associations where a non-aggressive species is nesting near to a solitary aggressive species e.g. azure-winged magpies and Japanese lesser sparrowhawks (Ueta 1994). It is, therefore, possible that the presence of one species may have an important effect upon both the distribution and the breeding density of another species. This may, in turn, have consequences on the use of mixed reproductive strategies within the affected species.

The influence of breeding density on EPP can also be obscured and confounded by the other factors such as breeding synchrony and the age, size and vigour, or experience of breeding individuals (Morton *et al.* 1990, Westneat *et al.* 1990, Wetton *et al.* 1995). Furthermore, the specific effects of increased breeding density will depend upon the adaptive significance of participating in EPCs for each of the sexes, and upon which sex is in control of EPCs. For example, if females are seeking EPCs for 'good genes' reasons, then one would predict EPP to increase directly as a result of there being a greater choice of available males at higher densities. However, if females are pursuing EPCs for 'genetic diversity' then an increase in density may not necessarily result in higher levels of EPP. All these variables must be examined and understood before the effect of breeding density can be isolated and examined.

As suggested above, age may influence the use of mixed reproductive strategies by breeding individuals. Older males have often been reported to be dominant to younger males (Holberton *et al.* 1990, Lyon & Montgomerie 1986). Hence they may be able to overcome the younger males' mate guarding efforts and obtain EPCs with their partners (e.g. Morton *et al.* 1990). Younger males may be less efficient at mate guarding because they are weaker or less experienced than older males. They may also be less efficient at foraging, thus restricting the amount of time they can engage in mate guarding and also reducing the amount of time they have to pursue EPCs. All or any of these factors above could result in age-related differences in levels of EPP suffered and gained by males and subsequently in their annual reproductive success. They do not, however, take into consideration the strongly supported idea that females largely control both EPCs and EPP (discussed above).

If females are in control of EPCs then, as with breeding density, the influence of age will vary depending upon the adaptive significance of EPCs to females. Neither the 'genetic diversity' hypothesis nor the 'fertility insurance' hypothesis (assuming that younger males do not have reduced fertility, but see Hilton 1968, Hill 1994) directly predicts there to be any effect of male age on EPP). On the other hand, if females are trying to obtain genetic quality benefits through EPCs, then females may choose to perform EPCs with older males which should, on average, be of better quality than younger males (Trivers 1972, Halliday 1983, Kokko & Lindstrom 1996). Evidence from some DNA profiling studies has also supported this prediction by showing that older males suffer less EPP in their own broods (Morton *et al.* 1990, Wagner *et al.* 1996) and also gain more EPY than younger males (Wetton *et al.*

1995, Sundberg & Dixon 1996). However, other studies have found no relationship between male age and EPP and so this idea remains controversial (Dunn *et al.* 1994b, Westneat *et al.* 1995, Hasselquist *et al.* 1996). Even if a link is found between male age and EPP it may be due to females seeking direct benefits from older males i.e. foraging rights, paternal care. This would benefit the female if older males have better quality territories or are better providers of paternal care. Therefore, the existence of direct benefits may cause male age-related differences in the levels of EPP and needs to be considered.

Female age may also influence the use of mixed reproductive strategies. Older females may be more able to pursue EPCs either because they are more experienced or because they have more time available due to more efficient foraging. Conversely, younger females may be expected to seek EPCs more often if, because of assortative mating, they are mated to lower quality males. There are, as of yet, very few studies (either theoretical or empirical) that have examined the possibility of female age affecting mixed reproductive strategies (but see Gowaty & Bridges 1991a, Westneat 1995).

1.2 THESIS AIMS

This thesis describes a case study of a population of northern orioles (*Icterus galbula bullockii*) that breed at Hastings reservation, Monterey County, California. The population exhibits a range of breeding densities from solitary pairs through to small, loose colonies of non-territorial pairs (Williams 1988). However, the selective forces responsible for this nesting pattern have not, as yet, been identified. Although levels of EPP, or IBP, have never been determined for the northern oriole, the occurrence of EPCs has been previously documented (Flood 1985, Edinger 1988, Butcher 1991). This species was therefore considered for investigating the influence of nesting density on reproductive success. The study attempts to consider and understand the effect of the other ecological and demographic factors that may influence mixed reproductive strategies (i.e. breeding synchrony, time of season) in order to isolate the influence of breeding density.

Northern orioles also display male delayed plumage maturation. The age/status of breeding individuals was examined to determine its influence on EPP and reproductive success. The results of this analysis could then be used to investigate the relationship between delayed plumage maturation and reproductive success, and to attempt to understand the evolution of delayed plumage maturation. Furthermore, the effect of male age/status can then be controlled for when investigating the influence of breeding density.

The specific aims of this study were as follows:

- (1) To determine the selective forces underlying the nesting pattern in this population of northern orioles.
- (2) To establish a DNA profiling system to provide an efficient method for the assignment of parentage in this species.
- (3) To quantify the frequency at which mixed reproductive strategies occur in this socially monogamous species.
- (4) To determine the influence of male age and the related delayed plumage maturation on EPP and reproductive success.
- (5) To investigate the relationship between breeding density and the use of mixed reproductive strategies, and the effect this has upon reproductive success.

1.3 CHAPTER SUMMARIES

This thesis is composed of six chapters, each (except the overall discussion) in the basic format of a scientific publication.

Chapter 1 introduces the theoretical background to the study, the study species, the study site and the general methodology used for the study.

Chapter 2 investigates the selective factors responsible for the semi-colonial nature of the northern orioles at Hastings, and examines if and why there is an active association between northern orioles and magpies.

Chapter 3 explains the identification and application of hypervariable minisatellite probes and reports the basic parentage results obtained for this population.

Chapter 4 presents data on the influence of male age/delayed plumage maturation. It then discusses the evolution of delayed plumage maturation in the light of these findings.

Chapter 5 investigates the influence of breeding density on EPP and reproductive success and then discusses the possibility that the pursuit of EPCs may actually be a selective force favouring higher breeding densities.

Chapter 6 attempts to bring together the results from each separate chapter and discuss how they interconnect, thereby presenting a coherent story relating the significance of age/delayed plumage maturation and breeding density within this northern oriole population.

1.4 THE NORTHERN ORIOLE

The northern oriole (*Icterus galbula*) is one of 240 species belonging to three subfamilies in the family Fringillidae (Sibley & Ahlquist, 1990). There are two subspecies. This study focuses on the western race (Bullock's oriole, *Icterus galbula bullockii*) which was formerly considered a separate species but is now considered to be conspecific with the eastern race (Baltimore Oriole, *Icterus galbula galbula*) (Bent 1958, Scott 1983). The northern oriole is a migrant passerine that overwinters in Mexico and central America, but breeds across North America. *Icterus galbula bullockii* (subsequently referred to simply as the northern oriole) breeds in western and central North America (Scott 1983).

An adult bird weighs 30-40 g and is 22 cm long (Pyle *et al.* 1987). The sexes are monomorphic for size, but sexually dichromatic with males having conspicuous yellow and black adult plumage (Figure 1.1) while females are a cryptic olive green (Figure 1.3). First year males exhibit delayed plumage maturation (Figure 1.2), although they are reproductively mature during their first potential breeding season (Flood 1980, Sealy 1980). Individual males can therefore be sexed and aged into subadult (first year) and adult (after first year) categories, using naturally occurring plumage characteristics. Female age cannot be determined (Williams 1988).

Figure 1.1 Adult male northern oriole



Figure 1.2 Subadult male northern oriole



Figure 1.3 A female northern oriole with nesting material



Figure 1.4 An adult male singing



The northern oriole is a short lived, insectivorous passerine with annual adult survival rates of approximately 50% (Butcher 1991). Individuals return to the breeding grounds in early spring (late March). Adult males arrive first and set up and defend territories or nesting sites from which they advertise for mates. Adult females arrive on average five days later and subadult males 16 days later than the adult males (Williams 1988). They pair up soon after arrival and have a single brood, although females may lay a replacement clutch if their first clutch fails (Williams 1988). The female builds the woven, pendulous, nest and incubates the clutch of 4 - 6 eggs (Bent 1958). Both the male and the female provision the nestlings and fledglings (incubation period 11 days, nestling period 14 days). During the breeding season the diet of both the adults and offspring is composed largely of forest tent moth (*Malacosoma disstria*) caterpillars and other invertebrates gleaned from the areas of oak woodland, oak savanna and broad-leaved riparian woodland that they inhabit. Sealy (1980) reported evidence that large fluctuations in the number of tent caterpillars can dramatically affect the population levels of northern orioles. Males average about 50% of chick feeds, although this varies greatly between pairs (Edinger 1988). The birds leave the breeding sites and disperse during mid-summer and migrate back to the overwintering grounds soon after. There is no evidence for male or female philopatry.

Although this species has been investigated previously, few extensive studies relating to the breeding behaviour have been undertaken (Flood 1984, 1985, Edinger 1988). It has long been considered to be territorial (Bent 1958) but more recently has been discovered to breed colonially (in nesting aggregates) in some areas (Pleasants 1979, Williams 1988). Variability between populations has been shown to range from nesting aggregations of up to 17 pairs per ha, in which nest sites only are defended, to pairs which nest solitarily and defend territories which include foraging areas (Pleasants 1979, Sealy 1980, Butcher 1991, Williams 1988). They usually form socially monogamous pair bonds, but there is evidence to suggest that polygyny occurs occasionally in some populations (Flood 1985, Williams 1988), and that male orioles pursue extra-pair copulations (EPC) as part of a mixed reproductive strategy (Flood 1985, Edinger 1988). Butcher (1991) suggested that males protect their paternity by mate guarding during the female's fertile period. However, previous to the study reported here there was no data on confirmed extra-pair fertilisations (EPFs), or on the occurrence of intra-specific brood parasitism (IBP).

1.5 STUDY AREA

Fieldwork was conducted between March - June in 1994-96 at Hastings Natural History Reservation, Monterey County, California and on adjacent ranch land, over an area of approximately 650 ha. Hastings is located in the middle elevations (467-953 m) of the Santa Lucia Range in central coastal California (Figure 2.1, Figure 2.2). The climate is

mediterranean, with dry hot summers (July mean daily max = 31°C) and only winter rains. Most of Hastings and the adjoining land use area is steep hillside with scattered open savanna as shown in Figure 2.6. The dominant trees are evergreen and deciduous oaks that form stands ranging from deciduous savannas to hardwood forests. There are also tracts of mixed riparian woodland adjacent to the three-season streams. In this area the northern oriole is classified as a common summer resident and shows the range of nesting densities described above.

Figure 1.5 The location of the field site within North America and California, USA.

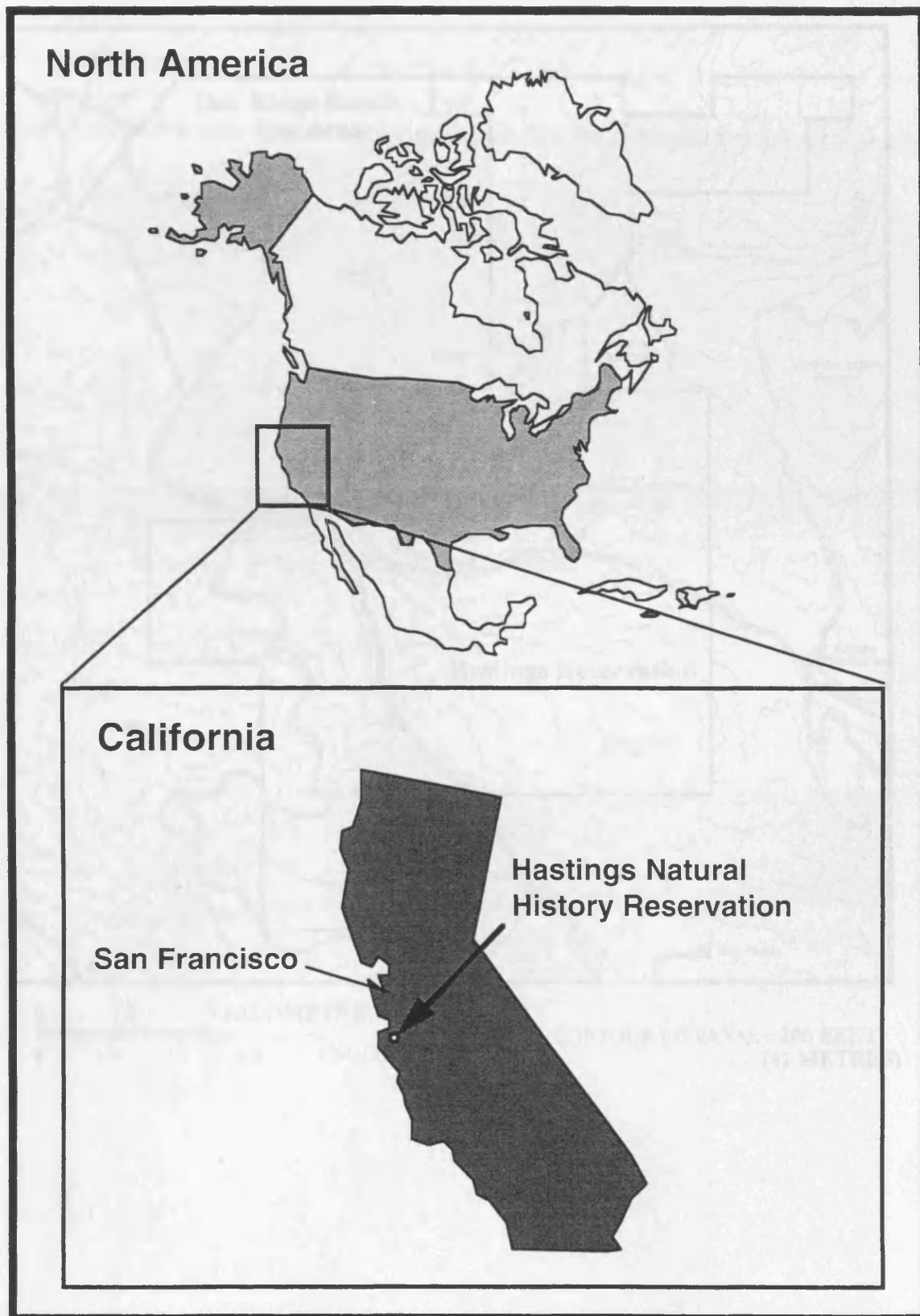


Figure 1.6 Hastings Natural History Reservation and the adjoining Oak Ridge Ranch use area.

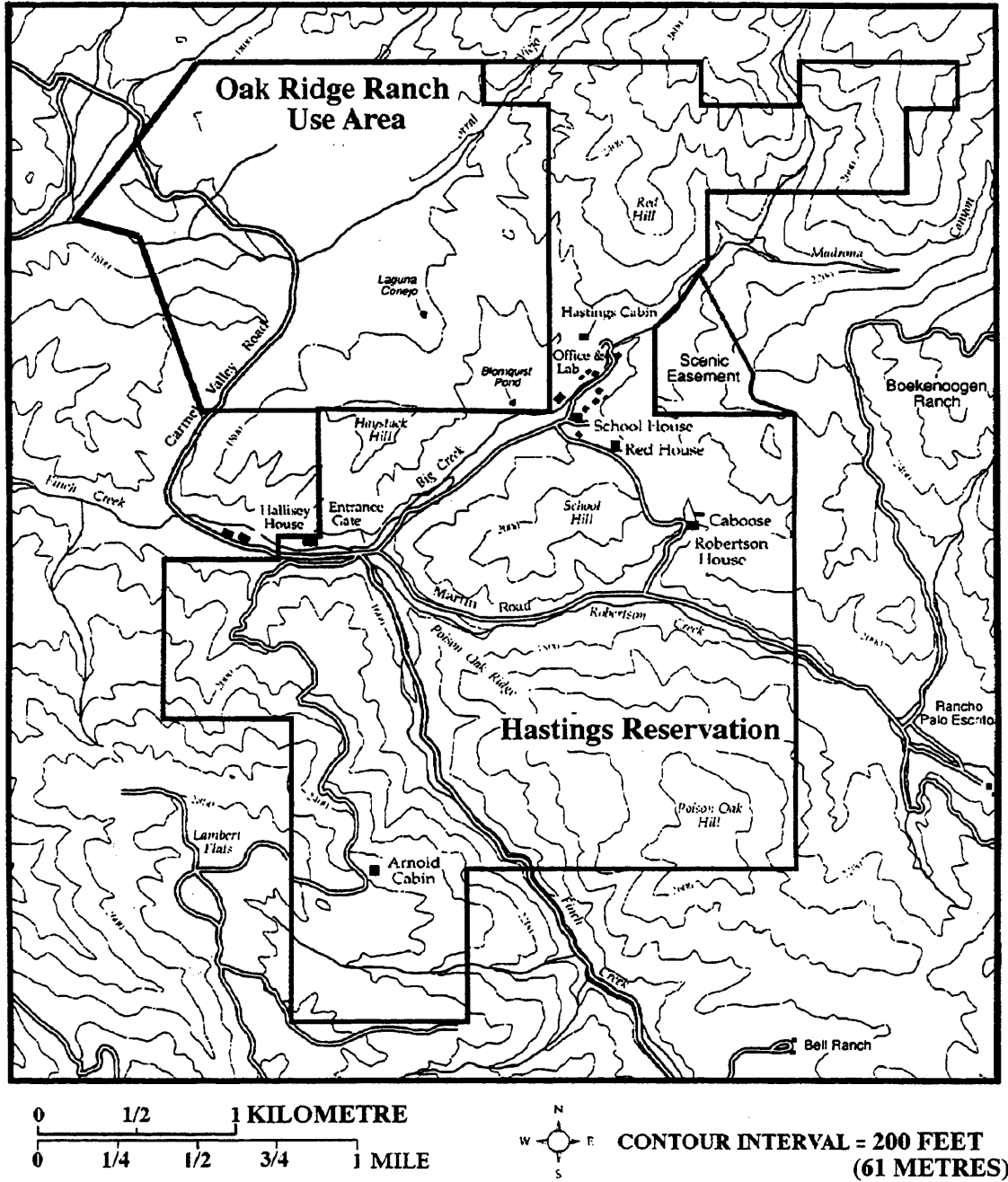


Figure 1.7 Oak savannah habitat at Hastings Reservation



Figure 1.8 Northern oriole nest with red wool incorporated



1.6 FIELD METHODS

The general methodology is described in the following section, while more specific methods are explained in the relevant chapters.

1.6.1 Nest finding

Nests are difficult to locate due to their being well concealed, often in lace lichen, (*Ramalina menzeisii*) or in bunches of mistletoe (*Phoradendron villosum*). Female birds were watched collecting nesting material and then followed back to the nest. In order to make nests easier to locate short strands of red wool were distributed around the field site; female northern orioles are attracted to these and often incorporated them into their nest as shown in Figure 1.8.

1.6.2 Capture methods

A variety of different methods were used to trap adult birds. Upon arrival at the breeding site unpaired birds were caught by two methods:

a) A series of up to 16 mist nets was situated around the main study area in places where male northern orioles were seen feeding and engaging in male-male chases. As the area is closed to the public these could be left *in situ* and furled overnight. Trapping in this way proved to be time-consuming and inefficient, but necessary to catch at least the first adult male of the season. The first adult male was kept in captivity (see below) for use as a decoy during future trapping attempts.

b) In areas of male chases a 'V' formation of mist nets was erected in a suitable position close to a woodland edge. A portable cage containing the decoy male bird was placed in a visible position in the middle of the 'V'. Occasionally a tape of an adult male song was played in conjunction with the decoy. Birds of both sexes were attracted to the decoy; males often became agitated enough to fly into the mist net, while female birds were more cautious, but could be startled into the net.

During pairing and nest building, birds were caught in mist nets strategically placed as close to the nest tree as possible. These were 'baited' with the decoy male and tapes of male song (as above). Females were also caught by placing drop-door sparrow traps, baited with red thread, close to the nest tree.

Any breeding individuals not caught by the time nest building was almost complete were left until the incubation or nestling period to avoid disturbance during the female's main fertile period. Female birds were not blood-sampled during the incubation period to avoid the

possibility of any stress or desertion. In these cases the birds were caught and bled late in the nestling period.

1.6.3 Decoy birds

In each of the three years the first adult male to be caught was kept in captivity and used as a decoy for future trapping attempts (see Section 2.3.2). The bird was housed in aviaries at the field site and placed in a smaller portable cage for use in the field. The portable cage contained food and water and was kept covered when not in use. The bird's weight and plumage condition were recorded at initial capture and then reassessed at regular intervals to check on the effect of captivity on the bird. The birds readily took to a diet of mealworms and wet puppy food, and this was supplemented with tent caterpillars taken from the field. In each year the weight of the captive bird increased over the period of captivity. The plumage often became slightly frayed due to abrasion against the cage, but on release all birds appeared to fly normally.

1.6.4. Processing

Each captured adult was uniquely ringed by using a combination of a metal U.S. Fish and Wildlife service aluminium band and three UV-resistant plastic colour rings. Two rings were placed on each leg, with the metal ring always on the left leg. Northern orioles were sexed using naturally occurring plumage characteristics (Pyle *et al.* 1987). Males were a conspicuous contrast of yellow and black, whereas females were a dull cryptic colour (olive/brown). In addition, the sexes were confirmed by the possession of either a protruding cloaca (male), or a brood patch (female). Males were assigned to adult or subadult status according to conspicuous changes in plumage which occur with age. All adult males have black wing feathers, acquired in a mid-winter moult, whereas those of first-year males are brown and often worn (Flood 1984). First-year females may also be distinguishable, as they are generally paler than adult females, with less yellow colouration on their breasts and less black on their throats (Williams, 1988). This could not be confirmed, however, due to the lack of banded females returning in successive years in this study and so females were not aged. Any banded birds that returned in subsequent years were assigned a minimum known age.

Biometrical data were taken for all birds. Tarsus length (± 0.1 mm) (from the posterior notch at the intertarsal joint to the front of the tarsal bone) was measured using Vernier calipers. Wing length (± 0.5 mm) was measured using a butted metal rule (maximum chord, flattened) and body weight taken using a 100 g Pesola spring balance (± 0.5 g).

1.6.5 Body condition index (BCI)

The condition of individual birds has been calculated using various phenotypic characters (i.e. tarsus, mass, wing chord) or combinations of these characters (i.e. $\text{mass}/[\text{wing}]^3 \times 100$; Møller 1988a). In the northern oriole older adult males have longer wings than first year males (Pyle *et al.* 1987). Thus wing chord length is correlated with age and is not therefore used as a factor in a body condition index for this species. Instead a body condition index (BCI) derived from the regression of weight on tarsus (both parameters were normally distributed) was calculated (Packard & Boardman 1987). The regression was calculated for each sex using all resident individuals sampled in 1994 and 1995. Each bird was only caught and processed once and so is represented only once in the regression (number of individuals: males = 46, females = 35). Individuals could not be caught and weighed on a standard date during the breeding season. The day of the season (male $F_{44,1} = 10.00$, $p > 0.01$; female $F_{33,1} = 4.39$, $p = 0.04$) and the time of day (male $F_{44,1} = 7.26$, $p = 0.01$; female $F_{33,1} = 3.98$, $p = 0.05$) upon which an individual was caught each had an effect on the weight and therefore on the BCI. These effects were removed by taking the residuals of the regression of weight against first the catch day, and then the catch time, of the individuals and then using the final residuals to determine the BCI.

1.6.6 Bleeding

Approximately 50 μl of blood was taken from the bird by piercing the brachial vein with a small hypodermic needle and then drawing off the blood using a graduated capillary tube. This was immediately mixed with at least five volumes of lysis buffer (4M Urea, 0.2 M NaCl, 100 mM Tris/HCL pH 8.0, 0.5% n-Laurylsarcosine, 10mM CDTA, filtered). Samples were stored at 4°C both in the field and in the laboratory at Leicester. Excess blood was collected and placed in 800 μl of 100% ethanol as a back-up stock. This sampling technique has been shown to have no ill effects on birds (Stangel 1986).

1.6.7 Nest monitoring

All individuals within the main study site were monitored during pairing. Once paired up, females were carefully watched for signs of collecting nesting material and nest building. They could then be followed to pinpoint the position of the nest. Other nests were found by a thorough search of all likely nesting sites within the study area. All pairs were observed every two days during the nest building stage in order to predict first egg dates (FED). Easily accessible nests were regularly examined for eggs. At less accessible nests, behavioural

clues (i.e. nest lining or termination of nest building) were used to predict when the female was laying, and the FED confirmed by one actual inspection of the nest. First egg dates were used as an index of arrival date as the detection of newly arriving birds was not always immediate, and thus settlement date based on first sightings may be inaccurate. The nests were inspected at the onset of incubation to determine the final clutch size and near the end to establish the hatching date. During provisioning, nests were checked at least once every three days to ensure they were still active. Nests were reached by using either a 5 metre step ladder, a 12 metre extension ladder, a system of climbing ropes, climbing harness and ascendeurs, or a combination of this equipment. Nestlings were ringed and bled (as per adults), and tarsus length and body weight measurements taken at nine days old. The nestlings fledged after approximately 14 days.

1.6.8 Synchrony index for individual nests

The amount of synchrony among breeding attempts may influence certain variables of potential importance in avian mating systems such as extra-pair paternity (EPP) reproductive success and predation (Birkhead & Møller 1992). A synchrony index (SI) was therefore calculated for each nest in which egg laying was initiated. This was based on the proportion of females fertile on the FED of the female at the focal nest (Based on Kempenaers 1993, Double 1995):

$$\text{Synchrony index} = \text{number of females fertile on day } i / \text{total number of females}$$

The fertile period of a female was defined as the period from day -4 to day +3, of the female's breeding cycle (day 0 is the day the first egg is laid). This period was chosen because it is a conservative estimate of a passerine's fertile period (Birkhead & Møller 1992) and it corresponded to the peak of mate guarding of the female by the male in the northern oriole (pers. obs.).

1.7 STATISTICAL METHODS

All the statistical methods used are described in Sokal & Rohlf (1981), Siegel & Castellan (1988), Wilkinson (1992) and Crawley (1993). The software package Systat 5.2TM (Systat Inc.) was used in the majority of the data analysis (Wilkinson 1992). All tests are two-tailed and corrected for continuity or tied ranks as appropriate. The data were checked for normality by performing the Lilliefors test (Crawley 1993). Where appropriate, transformations were applied in order to obtain normality within the data set. The majority of the data could not be normalised and therefore nonparametric tests were used. Whenever

parametric tests were used, residual values were checked for normality (this is an assumption of both regression and general linear model analysis). A probability of $p \leq 0.05$ was taken to indicate biological significance.

General linear model analysis was used to establish the relative influence of several factors on one dependent variable. The number of independent factors examined was kept to a minimum, each factor being investigated only if it was predicted to have an effect on the dependent variable. The minimum adequate model was presented following parsimonious streamlining of the original model in order to remove non-significant terms. F tests were carried out to establish the significance level of the change in deviance following removal of a term from the model. If a significant change was found then the term was replaced in the model.

The binomial form of some of the data also meant that logistic multivariate analyses were required. These were performed using the JMP 3.0.2™ software package (SAS Institute Inc). In the case of EPP, many nests contained all or no EPP, (see section 4.4.4). This meant that continuous multivariate analysis could not be used. The data were therefore placed into categories based on the percentage of EPP in a brood: 1 = 0%, 2 = 1-20%, 3 = 21-40%, 4 = 41-60%, 5 = 61-80, 6 = 81-100%, and ordinal logistic analysis was used. For both types of logistic analysis the minimum adequate model was presented following parsimonious streamlining of the original model to remove non-significant terms. The Wald chi-square test results were used to establish the effect each explanatory variable had upon the dependent variable.

CHAPTER 2

NORTHERN ORIOLES AND YELLOW-BILLED MAGPIES: A NESTING ASSOCIATION?

2.1 Introduction

- 2.1.1 Nesting associations
- 2.1.2 The nesting pattern of northern orioles

2.2 Aims and predictions

2.3 Methods

- 2.3.1 Habitat and the nesting association
- 2.3.2 Nest desertion and predation
- 2.3.3 Data analysis

2.4 Results

- 2.4.1 Available habitat and the nesting association
- 2.4.2 Nest desertion, predation and the proximity of nests to magpies and conspecifics
- 2.4.3 Logistical regression analyses of the factors effecting nest predation and desertion
- 2.4.4 The timing of nest predation in relation to the magpie breeding cycle

2.5 Discussion

- 2.5.1 The nesting association and the northern oriole nesting pattern
- 2.5.2 The benefits of the nesting association
- 2.5.3 Conclusion

CHAPTER 2

NORTHERN ORIOLES AND YELLOW-BILLED MAGPIES: A NESTING ASSOCIATION?

2.1 INTRODUCTION

2.1.1 Nesting associations

Colonial breeding has been recorded in many individual bird species and various selective forces have been suggested to favour coloniality (reviewed in Alexander 1974, Mock 1985, Wittenberger & Hunt 1985). These forces include increased foraging efficiency ('information centre' hypothesis, Ward & Zahavi 1973) and increased predator protection (Lack 1968). Alternatively, a shortage of suitable nest sites or habitat may oblige certain species to be colonial (Lack 1968). There are also many possible costs associated with coloniality. These include decreased feeding rates, competition for space, and increased intracolony predation (Alexander 1974, Mock 1985, Wittenberger & Hunt 1985). Recent work into mixed reproductive strategies also predicts that birds nesting at higher densities will suffer from higher rates of extra-pair paternity (EPP) and intra-specific brood parasitism (IBP) (see Chapter 6). This was originally seen as being a cost of coloniality (Alexander 1974, Hoogland & Sherman 1976, Wittenberger & Hunt 1985) but more recently it has been suggested that the pursuit of extra-pair copulations may actually favour colony formation (Morton *et al.* 1990, Wagner 1993, Hoi & Hoi-Leitner 1997).

Cases of inter-specific coloniality or mixed-species nesting associations of birds also exist (reviewed in Burger 1981; Smith 1985). The majority of these involve two or more colonial seabird (or shorebird) species (e.g. common terns and black skimmers [Erwin 1979], grebes nesting in gull colonies [Burger 1984], bar-tailed godwits and whimbrel [Larsen & Moldsvor 1992]) but may also occur between other bird groups (e.g. least bitterns and boat-tailed grackles [Post & Seals 1995]). Another form of nesting association occurs between a non-aggressive species nesting near to a solitary aggressive species (often a raptor) (e.g. fieldfares and merlins [Wiklund 1980, 1982], yellow warblers, gray catbirds and red-winged blackbirds [Clark & Robertson 1979], azure-winged magpies and Japanese lesser sparrowhawks [Ueta 1994]). Associations also occur between birds and species in other orders (e.g. rufous-naped wrens and wasps [Joyce 1993], see also Smith 1985 and

references therein). Although less is known about mixed-species associations of birds, it is probable that they are influenced by the same forces as in single-species colonies.

Nest site availability and habitat suitability may account for many mixed-species nesting aggregations of birds (Lack 1968). However there are situations where suitable, evenly distributed, nesting habitat is available and yet the birds nest in large aggregations in one area and leave similar adjacent areas uncolonised (Ward & Zahavi 1973, Erwin 1979, Wiklund 1982, 1994). Information sharing has also been suggested as a possible factor with mixed colonies functioning as 'information centres' for food finding (Ward & Zahavi 1973). This cannot, however, explain associations between species that feed in different locations or on different food types (Erwin 1979, Wiklund 1982, Ueta 1994).

Predation pressure may be an important selection pressure for mixed-species associations if birds within them suffer lower rates of predation for the same reasons as in monospecific colonies. It has also been suggested that non-aggressive species may gain a protective advantage by nesting in close association with an aggressive species that is more able to deter predators from the nesting site (Koskimies 1957, Walsh 1976, Clark & Robertson 1979, Erwin 1979, Dyrce *et al.* 1980, Wiklund 1982, Bogliano 1994, Ueta 1994). In addition, Nuechterlein (1981) suggested an expansion of the 'information centre' idea to include the transfer of information about potential predators, as well as food. He supported this suggestion with data on how western grebes parasitised the alarm calls of Forster's terns.

Although many studies have now reported mixed-species nesting associations, few have shown that an active association occurs, with one species choosing to nest close to the other species (but see Wiklund 1982, Larsen & Moldsvor 1992). Similarly, only a few studies have been able to test the predator protection hypothesis by directly comparing the success of associated and non-associated nests (Clark & Robertson 1979, Wiklund 1982, Post & Seals 1995).

2.1.2 The nesting pattern of northern orioles

The northern oriole has long been considered to maintain classic, type A, territories (Bent 1958) but more recently has been discovered to breed colonially (in nesting aggregations) in some areas (Pleasants 1979, Williams 1982). Williams (1988) investigated a population of northern orioles at Hastings Reservation, Monterey County, California, which exhibit a range of breeding densities from solitary to colonial (up to 20 pairs in a colony). No evidence was found to suggest that restricted nest sites or suitable habitat were causing the clustering of nests, while evidence for the 'information transfer' hypothesis was ambiguous. Nor was there any overall difference between the northern oriole breeding densities in terms of vegetation structure, nest predation or reproductive performance.

However, predators may limit the habitat suitable for nesting, and Williams (1988) suggested that the territories of nesting scrub jays and of northern oriole colonies do not overlap.

More recent observations have suggested that northern orioles may be nesting in association with the semi-colonial yellow-billed magpies (hereafter referred to simply as magpies) at Hastings Reservation (G. Bolen pers. comm.; pers. obs.). Nesting close to a magpie may afford the northern orioles some protection from the nest predators (e.g. scrub jays and crows) as the magpies aggressively repel these species from the vicinity of their own nests (G. Bolen pers. comm.; pers. obs.). The existence of a nesting association between magpies and northern orioles will, at least partially, determine the breeding distribution of northern orioles at Hastings Reservation.

2.2 Aims and predictions

This study tests the hypothesis that a nesting association occurs between northern orioles and magpies and that this is not the result of restricted nest sites or habitat. It is suggested that the association has evolved due to the predator protection afforded to northern orioles nesting near magpies. Therefore, it is predicted that predation will be related to northern oriole-magpie distance, and northern orioles nesting nearer to magpie nests will have reduced predation rates. The preference of northern orioles to nest near the semi-colonial magpies is also suggested to be an important factor in the formation of northern oriole breeding colonies in this area. Higher densities of northern orioles are predicted to occur around magpie nests, with northern orioles nesting closer to magpies than to conspecifics.

2.3 METHODS

2.3.1 Habitat and the nesting association

Fieldwork was conducted between March and June in 1995-96 at Hastings Natural History Reservation, Monterey County, California and on adjacent ranch land (see Section 1.2). Most of Hastings and the adjoining land use area is steep hillside with scattered open savanna. The dominant trees are evergreen and deciduous oaks that form stands ranging from deciduous savannas to hardwood forests. There are also tracts of mixed riparian woodland adjacent to the three-season streams. Magpies nest in a semi colonial fashion in the areas of open oak savanna characterised by large trees scattered through grassland (Reynolds 1990). Northern orioles utilize both the open oak savanna and the areas of riparian woodland with higher densities of trees and shrubs, though in both cases they prefer sites adjacent to

open grassland areas. Clusters of northern orioles have, however, only been recorded as occurring in the open savanna areas (Williams 1988; pers. obs.)

There is a possibility that the apparent association between northern orioles and magpies is merely due to similar habitat preferences and restricted habitat or nest sites. To evaluate this hypothesis the following methods were undertaken in each year. A grid of 100 m x 100 m squares was superimposed upon a composite aerial photograph of the entire study site. In each year, the exact positions of all the northern oriole and magpie nesting attempts that reached the egg laying stage were marked (to avoid the problem of pseudoreplication only the first nesting attempt to reach egg laying by each pair was used). It is important to note that the return rate of adult northern orioles was low in this study (18%) and that pair bonds were not maintained between years (pers. obs.). The few individuals that did return between years did not nest at the same location. All these points indicate that the northern oriole nests recorded are independent between years and not based on the same pairs nesting in the same places each year.

The number of times that a northern oriole nest occurred within the same 100 m x 100 m square as a magpie nest was then recorded. The probability of the observed number of cohabited squares occurring by chance (given the number of magpies and northern orioles in that year) was determined by comparison with an expected distribution model. This distribution model was based on the random redistribution of northern orioles over all the suitable habitat squares in the study area, given the original distribution of magpies. The distribution model was generated using 1000 Monte Carlo runs in a Fortran 90 program (courtesy of E. Bell). Suitable habitat was defined in two ways: (i) based on the human perception of which squares contained suitable habitat i.e. all squares which contained large trees (over 5 metres high) adjacent to an open area of grassland at least 50 m x 50 m in size, (ii) as all squares in which a breeding attempt by either species had been recorded (after Wiklund 1982, Reynold 1990). The second method of defining suitable habitat avoids the problem of squares being erroneously designated as suitable by human perception and was therefore used in testing for an association. This may have resulted in a conservative estimate of the total of suitable squares as magpie pairs frequently nest in the same territory in successive years (Verbeek 1970, G. Bolen pers. comm), however this only increases the validity of any positive results. The nesting data used to determine previously utilised habitat squares were obtained from this study on northern orioles from 1994-96, a study on magpies from 1994-97 (G. Bolen, pers. comm.) and two previous studies covering a smaller portion of the study area (magpies, 1981-1984 [W. Koenig pers. comm.], northern orioles, 1978-81 and 1984 [Williams 1988, pers. Comm.].

Both northern orioles and magpies have been reported as semi-colonial breeders at Hastings Reservation (Williams 1988, Reynolds 1990). The nesting distribution of each species was tested separately to determine if they were non-randomly distributed in relation to their conspecifics. This was accomplished using the same grid of 100 m x 100 m habitat

squares as for the nesting association. The number of observed nests within habitat squares was tested against an expected number of nests generated using the Poisson distribution. This analysis was repeated separately using both definitions of suitable habitat squares as defined above and each year was tested separately. This method of analysis treated each habitat square independently and did not take into account whether or not adjacent squares also contained conspecifics. Therefore it was not able to resolve the possibility of conspecific clumping occurring at a greater scale.

The study area was completely searched for northern oriole nests at least once every week throughout each field season and so it is unlikely that any northern oriole nests were overlooked. The locations of magpie nests were recorded as part of the northern oriole study and another study (G. Bolen, pers. comm.), and at least three complete searches of all areas were completed in each year. Although there is a possibility that a small proportion of solitary magpies may have been missed over the two years, it is unlikely that this constitutes a large enough effect to bias the results significantly. Seven northern oriole nests were on the boundary of the study site and were excluded from the analyses as it was impossible to search the area to determine the distance to the nearest conspecific or magpie nest. Nests that deserted before egg laying were not used in the nesting association analysis. After excluding these nests the 650 ha area of the field site contained breeding attempts by 32 and 37 pairs of northern orioles, and 29 and 33 magpie pairs (in 1995 and 1996 respectively).

2.3.2 Nest desertion and predation

Oriole nests were monitored once every two days up until egg-laying and then at least once every three days thereafter until fledging (see Section 1.3.7). Nests were counted as deserted when the breeding attempt at the nest site was aborted before egg-laying commenced. Adults were never observed to desert nests after egg-laying. Nests were counted as having been preyed upon when all the eggs, or all the nestlings, in the nest had disappeared and the nest was no longer attended by the adults. Partial nest losses were unlikely to be due to predation especially if the adult birds were still attending the nest. Therefore, the disappearance of single nestlings from active nests was attributed to the adults removing nestlings which had died from starvation or hypothermia. The three cases where the entire brood of nestlings was found dead in the nest were attributed to the severe stormy weather that had occurred over the previous days.

The distance between each northern oriole nest and both the nearest active magpie nest and conspecific nest was measured. Each northern oriole nest was then classified as either associated with a magpie (within 50m) or not (over 50 m) based on the inter-nest distances. Fifty metres was chosen as the measure of association as this was estimated to be the distance at which magpies protect their own nests (Verbeek 1970, G. Bolen pers.

comm). Both this categorical measure of association (magpie association) and the continuous inter-nest distances were used in the subsequent analyses. Northern orioles were also allocated to a conspecific density category, with a distance of 100 m between a northern oriole and its nearest conspecific being used to separate solitary breeding pairs from 'colonial' breeding pairs (see Section 5.2.3). All measurements were calculated on the first egg date (FED) of the northern oriole breeding attempt, except for deserted nests for which values were calculated on the day of nest desertion.

In 1996 the FED and fledging date of the magpie nests in the study area were recorded ($n = 20$). This was a subset of the total number of magpie nests, as limited time and resources meant that all nests could not be monitored. Nests for which the dates could not be determined were excluded. Nests involved in a separate magpie removal experiment were also excluded. The resultant data were then used to determine the relationship between the presence of active magpie nests and the timing of northern oriole predation. Too few predation events occurred on associated nests to allow for a separate statistical analysis of the effect of magpie fledging on the predation of the associated northern oriole nests ($n = 4$).

2.3.3 Data analysis

Data from both years were initially analysed separately. Data from both years were combined in the predation analysis. Analysis was performed as described in section 1.4 unless otherwise stated. Distances were transformed using the natural log function to normalise the data.

Logistic regression analysis, using the JMP software package (SAS Institute Inc), was performed to establish the relative influence of several possible explanatory variables upon: (i) nest desertion and (ii) nest predation. The number of explanatory variables examined was minimised, each variable being investigated only if it was predicted to have an effect on the dependent variable. The final logistic model was presented following parsimonious streamlining of the original model to remove non-significant terms. The Wald chi-square test was used to establish if each explanatory variable had a significant effect upon the dependent variable. The variables tested were: year (of study), male status (see Section 5.3.1), the northern oriole breeding synchrony index (see Section 1.3.8), magpie distance (see Section 2.3.2) and conspecific distance (see Section 2.3.2). The categories of 'magpie association' (see Section 2.3.2) and northern oriole 'conspecific density' (see Section 2.3.2) were also tested in place of 'magpie distance' and 'conspecific distance', respectively, for any models analysed.

2.4 RESULTS

2.4.1 Available habitat and the nesting association

The perceived association between magpies and northern orioles may have been due to similar habitat requirements. The overall area of suitable nesting habitat for magpies and orioles was estimated at 281 100 m x 100 m squares. The total number of habitat squares in which either species had been reported as previously nesting was 87. This was used as the conservative estimate of available habitat squares for the Monte Carlo simulation.

When tested using the conservative habitat estimate, the number of times that an oriole nested in the same square as a magpie was higher than the number expected under randomization in both 1995 (observed = 16 v. expected = 8.6; $p = 0.004$) and 1996 (observed = 18 v. expected = 11.7; $p = 0.017$) (Figure 2.1). If the human based estimate of 281 suitable habitat squares was used it further increased the strength of the result ($P < 0.001$ in both years). These results strongly suggests that the association between the two species was a positive response by the northern orioles to the presence of magpies and was not merely due to similarity of habitat requirements.

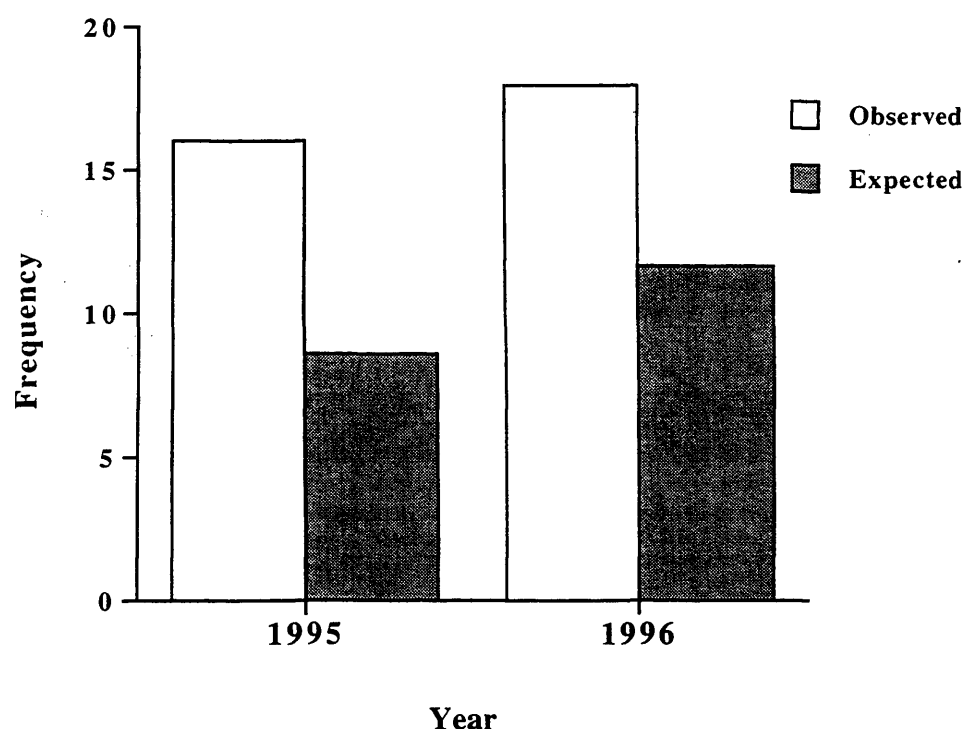


Figure 2.1 The frequency of squares containing both yellow-billed magpies and northern orioles. The observed number of squares (unshaded) is higher than the frequency of association expected under randomisation (shaded area) in both years (1995, $p = 0.004$; 1996, $p = 0.017$).

Each species was then separately tested to determine if the nests were randomly distributed in relation to other conspecifics. In this analysis (using 100 m x 100m squares) the distribution of both northern orioles and magpies did not deviate from a random distribution (G-test, $P > 0.1$ for all). Therefore, the distribution of northern oriole nests was less affected by the presence of conspecifics than it was by the presence of magpies.

Half of all northern orioles were found to be nesting in association (within 50 m) with a magpie nest (42/85). However, significantly more northern orioles associated with magpies were nesting at the higher 'colonial' conspecific density (28/42) than were non-associated northern orioles (17/43) ($\chi^2 = 4.33$, $df = 1$, $p = 0.04$). There was also a positive relationship between the nearest magpie distance and the nearest conspecific distance ($F_{1,83} = 6.55$, $p = 0.012$). This suggests that northern orioles nesting closer to magpies were also nesting at higher conspecific breeding densities, as shown in figure 2.2.

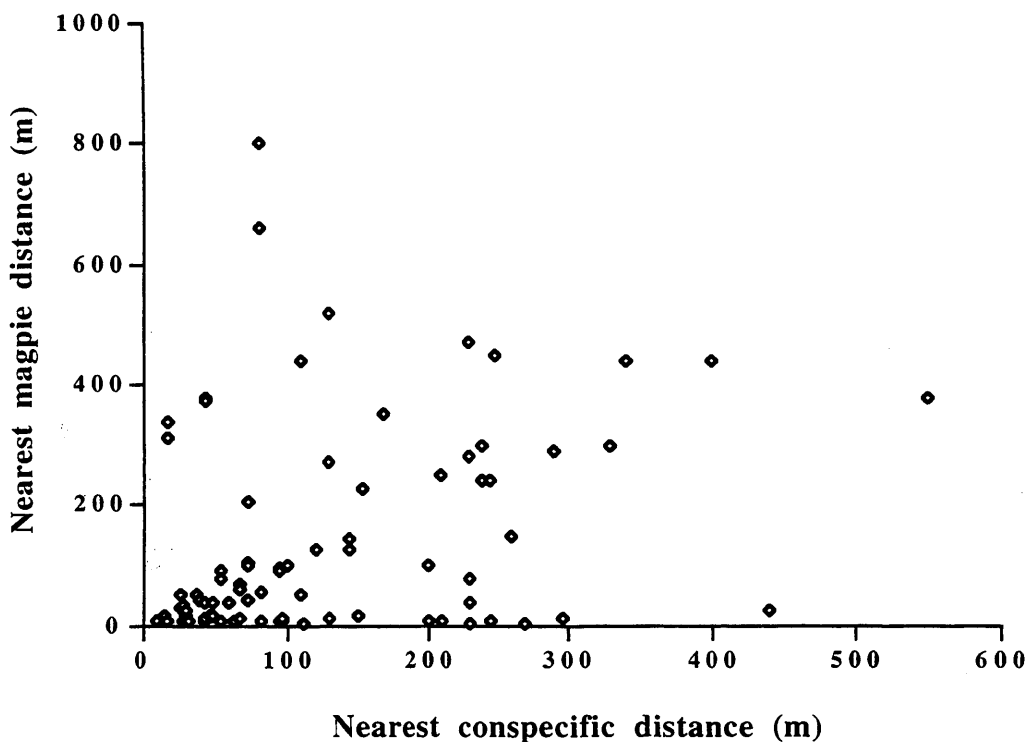


Figure 2.2 Nearest magpie distances in relation to nearest conspecific distances for northern orioles (log magpie distance v. log conspecific distance $F_{1,83} = 6.55$, $p = 0.012$)

Overall northern orioles were found to have nested closer to magpies more often than they did to conspecifics (Wilcoxon Sign test, $z = 2.75$, $p = 0.006$), despite there being more conspecifics than magpies breeding in the study area in each year (1995: 32 v. 29, 1996: 37 v. 33). Also, colonial northern orioles nested no closer to conspecifics than to magpies

(Wilcoxon Sign test, $z = 0.20$, $p = 0.84$). These results and that shown in Fig 2.2 suggest that the increased northern oriole density may have been a secondary effect of northern orioles attempting to nest near magpies.

2.4.2 Nest desertion, predation and the proximity of nests to magpies and conspecifics

Northern oriole nest desertion and nest predation rates were investigated in relation to the proximity of magpies and conspecifics. When tested, neither desertion rate ($\chi^2 = 2.57$, $df = 1$, $p = 0.11$) nor predation rate ($\chi^2 = 2.84$, $df = 1$, $p = 0.09$) differed significantly between years. However, because of the tendency for nest desertion and nest predation to be slightly higher in 1996, year was included as an explanatory variable in the final logistic models tested.

The mean and median distances between northern oriole nests and the nests of the nearest magpie and the nearest conspecific are given in relation to nest desertion in Table 2.1. However, no difference was found between deserted and undeserted nests for magpie distance (Mann-Whitney U-test, $z = 0.95$, $p = 0.33$), or for nearest conspecific distance (Mann-Whitney U-test, $z = 0.85$, $p = 0.36$). The continuous distance measures were then replaced with the categories: (i) magpie association (see Section 2.3.2) and (ii) conspecific density (see Section 2.3.2 and 5.2.3). There was again no effect of either the association with magpies ($\chi^2 = 0.78$, $df = 1$, $p = 0.38$) or conspecific density ($\chi^2 = 1.20$, $df = 1$, $p = 0.27$) on nest desertion.

Table 2.1 The distance between northern oriole nests and the nearest magpie and conspecific nest, in relation to: (a) nest desertion, (b) nest predation Significant results are highlighted in bold ($*p < 0.05$). means ($\pm$ SD) are shown.

	Distance to nearest magpie (m)		Distance to nearest conspecific (m)	
Nest desertion	Deserted v. undeserted		Deserted v. undeserted	
Mean	148 $\pm$ 142	134 $\pm$ 179	148 $\pm$ 96	129 $\pm$ 114
Median	90	41	140	95
Nest predation	Predated v. survived*		Predated v. survived	
Mean	201 $\pm$ 218	111 $\pm$ 162	120 $\pm$ 91	132 $\pm$ 124
Median	94	27	95	95

The analysis was then repeated using 'nest predation' as the dependent variable (see Table 2.1). The nearest magpie distance was found to have a significant effect on nest predation (Mann-Whitney U-test, $z = 5.03$, $p = 0.025$), with decreased nest predation occurring with shorter northern oriole-magpie distance. However, nearest conspecific distances had no effect on predation levels (Mann-Whitney U-test, $z = 0.008$, $p = 0.93$). When the continuous distance measures were then replaced with the categories of (i) magpie association and (ii) conspecific density, the results remained the same. Northern oriole nests associated with magpies were again less likely to be predated than nests without magpies nearby (associated = 5/37 v. unassociated = 13/30: $\chi^2 = 6.06$, $df = 1$, $p = 0.01$), while the local density of conspecifics had no effect on nest predation (colonial = 8/29 v. solitary 10/38: $\chi^2 = 0.00$, $df = 1$, $p = 1.00$).

There was no difference in the degree of nesting synchrony at the different northern oriole density categories ($z = 0.35$, $p = 0.55$). Additionally, no effect was found of northern oriole nesting synchrony (see Section 1.3.8) on either nest desertion (Mann-Whitney U-test, $z = 0.03$, $p = 0.86$) or on nest predation (Mann-Whitney U-test, $z = 2.43$, $p = 0.12$). Nor was there any effect of male status on either nest desertion ($\chi^2 = 0.36$ $df = 1$, $p = 0.55$) or on nest predation ($\chi^2 = 0.01$, $df = 1$, $p = 0.73$). There was, however, an effect of FED on nest predation: nests that were preyed upon had a mean FED six days later in the year than nests which survived (130 ± 7.8 [$n = 18$] v. 124 ± 9.0 [$n = 49$] respectively; Mann-Whitney U-test, $z = 6.21$, $p = 0.013$).

2.4.3 Logistic regression analyses of the factors affecting nest predation and desertion

None of the explanatory variables tested had a significant effect on nest desertion (Table 2.2 Nest desertion A and B). The logistic regression analyses were then repeated to determine which variables affect nest predation. Year, FED and magpie distance were the only variables tested that were found to have an effect on nest predation rates (see Nest predation A, Table 2.2). Levels of nest predation decreased with decreasing northern oriole-to-magpie nest distance, while the timing of the nesting attempt in the breeding season (FED) also had an effect on nest predation, with later nests being more susceptible to predation. In addition, variation occurred between years in predation rates. When magpie distance is replaced with the magpie association category (Nest predation B, Table 2.2) then the model shows that magpie association had an effect on predation, with FED and Year having a similar relationship with predation as in model A. Therefore, both models suggest that northern orioles suffered lower nest predation rates when nesting close to magpies.

Table 2.2 Nominal logistic regression models of the effects of certain variables on nest desertion and nest predation.

Independent variables	Dependent variable			
	Nest desertion		Nest predation	
	A	B	A	B
Male status	ns	ns	ns	ns
Synchrony index	ns	ns	ns	ns
Year	ns	ns	8.91**	7.00*
First egg date	-	-	9.34**	10.18**
Conspecific distance	ns	-	ns	-
Magpie distance	ns	-	4.74*	-
Density category	-	ns	-	ns
Magpie association	-	ns	-	5.94*
r^2			0.28	0.28

Models 'A' include the continuous variables of 'conspecific distance' and 'magpie distance', which are replaced by the categorical variable 'conspecific density' and 'magpie association' respectively in models 'B'. Wald chi-square values are shown for variables entered into the final model. * $p < 0.05$, ** $p < 0.01$ Final models N: nest desertion, $n = 85$; nest predation, $n = 67$.

2.4.4 The timing of nest predation in relation to the magpie breeding cycle

Northern orioles breeding later in the season showed higher levels of predation than earlier breeding attempts (see section 2.4.3). There was, however, no difference between the FED of northern orioles and their proximity to a magpie nest ($F_{1,65} = 0.62$, $p = 0.44$). Since FED is a reasonable indicator of settlement date and order, this result indicates that later breeding northern orioles were not restricted to nesting in areas without magpies.

The relation between FED and predation was then reanalysed separately for northern oriole nests with and without associated magpie nests (see Table 2.3). The FEDs of nests associated with magpies that were preyed upon, was 11 days later than for the surviving associated nests (Mann-Whitney U-test, $z = 6.45$, $p = 0.011$). No difference between FEDs was found relative to nest predation for unassociated nests (Mann-Whitney U-test, $z = 1.86$, $p = 0.17$). This indicates that the relationship between first egg date and predation is a result of the predation of associated nests.

Table 2.3 The first egg dates of northern oriole nests in relation to predation and magpie association. Mann-Whitney U-test z is shown, *p < 0.05.

	First egg date		
	N	mean ($\pm$ SD)	Z
Associated nests			
Preyed upon	5	135 $\pm$ 8.0	
Survived	32	124 $\pm$ 8.1	6.45*
Unassociated nests			
Preyed upon	13	128 $\pm$ 7.1	
Survived	17	124 $\pm$ 10.7	1.86

Personal observations suggested that the predation of associated nests often coincided with the fledging of magpie nests and the magpies leaving the area. This may result in the loss of the predation protection that seems to be afforded to the northern orioles by the magpies (see Section 2.4.2 and 2.4.3). Therefore, in 1996, the timing of nest predation was examined in relation to the magpie breeding cycle. Figure 2.3 indicates that the peak in northern oriole nest predation coincides with the decrease in active magpie nests. This in itself may not indicate a direct link between oriole nest predation and the fledging of magpie nests, however all four of the associated nests that were preyed upon in 1996 were preyed upon subsequent to the nearest neighbouring magpie nests fledging (all within eight days). Too few predation events occurred on associated nests to allow for a comparison between associated and unassociated nest predation events.

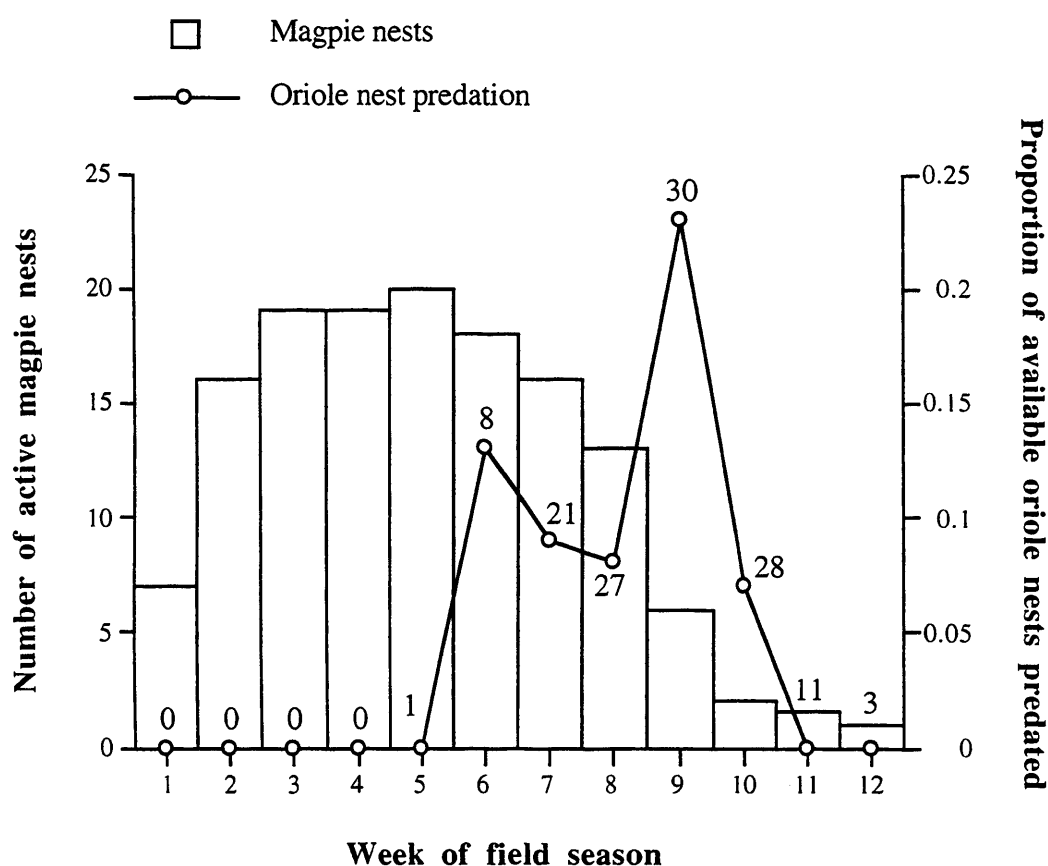


Figure 2.3 The timing of northern oriole nest predation in relation to the breeding cycle of magpie nests in 1996. (Total magpie nests, $n = 20$; total northern oriole nests preyed upon, $n = 14$). Numbers besides the nodes indicate the total number of active northern oriole nests in each week.

2.5 DISCUSSION

2.5.1 The nesting association and the northern oriole breeding distribution

Northern orioles have been reported to nest at a range of breeding densities in certain areas of their distribution (Pleasants 1979, Williams 1982). However, a study by Williams (1988) found no evidence to suggest that limited habitat or nest sites was forcing northern orioles to nest colonially at Hastings Reservation. Both Williams' study and the present study (on the same population of northern orioles) observed that sites of colony formation sometimes shifted between years, while the total area occupied by colonies was only a fraction of the overall area. Additionally, the number of colonies, rather than the size of colonies, increased when the overall population increased (Williams 1988). Therefore, factors other than the restricted availability of nest sites must explain the clustering of nests in

certain areas. Williams (1988) found no correlation between the colony sites and local food abundance, nor with any other habitat feature examined. She also found no direct relationship between conspecific density and nest predation, but did suggest that the territories of nesting scrub jays and of clusters of nesting northern orioles do not overlap. It is possible that increased conspecific density may be favoured by the prelaying advantage of increased EPCs at higher densities (see Chapter 5). This does not, however, exclude the possibility of northern orioles being associated with yellow-billed magpies.

In this study, northern orioles were found to nest near yellow-billed magpies more often than expected by chance, even when using a conservative estimate of the total area contained suitable nesting habitat. The nesting distribution of each species was not found to deviate from random when tested separately. This was thought to be due to the low resolution of the method of distribution analysis employed. It does not necessarily contradict the suggestion from other studies that both northern orioles and magpies are semi-colonial. The analysis method was, however, sufficient to show that each species was more associated with the other species than with conspecifics. Similar nesting associations have been suggested between many other species (e.g. Erwin 1979, Clark & Robertson 1979, Dyrce *et al.* 1980, Bogliano 1994, Ueta 1994) but very few studies have tested that this is an active association (Wiklund 1982, Larsen & Moldsvor 1992). It is possible that the habitat defined as suitable for nesting may have actually been unsuitable, either for some reason that was not apparent to humans, or because of habitat changes between years. However, this seems unlikely as suitable habitat was defined on the basis of previous site occupancy by either species and the habitat was highly conserved between years. The results of this study are consistent with the hypothesis that there is an active association between northern orioles and magpies.

Any active association that occurs must have been caused by northern orioles preferring to nest near to the magpies, as magpies are already nesting when northern orioles arrive at the study site. The preference shown by northern orioles for nesting close to the less abundant, semi-colonial magpies, appears to have resulted in the higher local conspecific densities which were observed around the magpie nests, and have led to loose 'colonies' forming. Therefore, the association between magpies and northern orioles may explain the semi-colonial nature of northern orioles in this area. However, the increased density of northern orioles may also have been influenced by the EPC benefits of nesting in close proximity to conspecifics (see Chapter 5). Evidence for this comes from the observation that northern oriole nest were sometimes closer together than expected if they were only trying to nest near magpies (within 5 metres of each other). Furthermore, on a couple of occasions two nests were clumped together (within 5 metres) despite the absence of magpies from the area (pers. obs.).

An alternative explanation for the association may be that northern orioles and magpies choose to nest in the same areas because of a separate, unrelated factor not yet taken

into account. One possible factor as suggested by Williams (1988) is the general presence of nest predators, e.g. scrub jays, which may restrict the suitable nesting habitat for both species. Northern orioles may, therefore, be choosing to nest in areas without scrub jays or crows, independent of the presence of magpies. Although this could not be totally ruled out, there is some evidence that cannot be explained by nest sites being preferred on the basis of having no scrub jays (or predators): some northern oriole nests were found within 100 m of scrub jay nests or crow nests, while large areas of apparently suitable nesting habitat which didn't seem to contain these predators were not utilized; northern orioles nest closer to magpies than they do to their more abundant conspecifics; and there is a strong relationship between magpie proximity and decreased nest predation, and between the fledging date of magpie nests and subsequent nest predation. However, to show convincingly that northern orioles actively choose sites because of magpies would require manipulative studies, e.g. the transfer of magpies between sites before the northern orioles arrived and the subsequent examination of whether the northern orioles prefer to nest near the new magpies nests, or in the vicinity of the original sites without magpies.

2.5.2 The benefits of the nesting association

An active nesting association will only have evolved if it benefits at least one of the species involved. In this case the nesting association is caused by northern orioles preferring to nest near magpies, therefore the suggestion is that northern orioles must benefit from this association in some way. The facilitated foraging benefits of the 'information centre' hypothesis (Ward & Zahavi 1973) can be discounted here as magpies and northern orioles feed both in separate locations and on different food types (Birkhead 1991, Bent 1958). There was, however, evidence to support the theory of predator protection (Koskimies 1957).

Magpies were observed to mob aggressively both scrub jays and crows which approached their nest sites (G. Bolen pers. comm., pers. obs.). Scrub jays and crows are probably the main nest predators of northern orioles in this area (pers. obs.), therefore the aggressive mobbing behaviour of magpies may also 'protect' northern orioles nesting in close proximity to magpie nests. Many other studies of nesting associations have suggested similar benefits (e.g. Erwin 1979, Nuechterlein 1981, Larsen & Moldsvor 1992, Ueta 1994, Bogliani 1994). However, fewer studies have been able to compare predation rates between nests with, and without, the associated 'aggressive' species (Clark & Robertson 1979, Dyrce *et al.* 1980, Wiklund 1982, Post & Seals 1995).

This study shows that the predation rate of northern oriole nests decreased in relation to the proximity of the nearest magpie nest, and that northern orioles suffered reduced nest predation when in close association with magpies. This was not due to the increased northern

oriole density that occurred in the proximity of magpie nests, as no direct relationship has been found between conspecific density and nest predation (Williams 1988, Section 2.4.2). Nor was it due to the predator-swamping effect of greater conspecific nesting synchrony (Lack 1968) in the higher density areas. No relationship was found between either nesting synchrony and nest predation or nesting synchrony and conspecific density. A further indication of the importance of the presence of the magpies comes from the increased predation that coincides with the cessation of nest site defence (i.e. at fledging) of the magpie nests. No evidence was observed to suggest that this increase in predation was due to the presence of juvenile magpies in the vicinity of the northern oriole nests.

Considering the considerable reduced predation benefits that seem to be associated with nesting in close proximity to magpies, it is maybe more puzzling to understand why some orioles do not nest near to magpies. There are, however, only a very limited number of magpies at Hastings and these are in semi-colonial clumps. It is therefore possible that some areas of habitat may be preferred for other reasons, i.e. prey availability, despite not containing magpies. Alternatively, some individuals may be excluded from nesting in close proximity to magpies by conspecific aggression. No evidence was found to suggest that either increased conspecific density or proximity to magpies had an effect on nest desertion. This is despite evidence from personal observations which indicated that higher levels of aggressive interactions occurred at higher conspecific densities. However, the nest desertion measure would not have picked up the effect of conspecific aggression on pre-breeding northern oriole pairs. The costs of conspecific aggression and competition for nest material (and possibly other costs, e.g. increased competition for space and food [reviewed in Wittenberger & Hunt 1985]) probably increased as a result of the increased conspecific density that occurred around magpie nests. Hence northern orioles may nest in other areas to avoid competition.

It has also been suggested that costs may occur directly as a result of the proximity of the aggressive protector species, e.g. predation by this species (Wiklund 1982, Bogliano 1994, Ueta 1994). However, in this study magpies were never observed to attempt to prey upon the northern oriole nests. Furthermore, northern orioles nesting close to magpies only repelled magpies that approached to within a couple of metres of their nest, and did so apparently easily, with a minimum of aggression and fuss. This contrasted sharply with the way northern orioles flew out of the nesting tree and furiously attacked passing scrub jays and crows (G. Bolen, pers. comm., pers. obs.).

It is, perhaps, surprising that the northern orioles nests are not preyed upon by the magpies, as magpies (both yellow-billed and black-billed) are often cited as avid nest predators (Birkhead 1991). However, orioles may just be large and aggressive enough to deter the magpie, especially the smaller yellow-billed magpie. This is illustrated by a observation made by Clarence Cottam (in Bent, 1958) of a black billed magpie alighting on an oriole nest; "A moment later the enraged male (northern oriole) came down with all his

force at the intruder, striking it on the crown of the head. The magpie dropped to the ground, stunned to such an extent that I was able to pick it up, and only after 10 minutes could it regain sufficient strength to fly away". Northern orioles may not be so able to deter the slightly larger or more aggressive crows and scrub jays (pers. obs.).

Other studies have reported that the 'protector' species may also benefit from the nesting association in some way (Wiklund 1979). Whether the magpies benefit in any way from northern orioles nesting in association with them is as yet unknown. However, the fact that northern oriole nests are tolerated within 3 metres of the magpie nests suggests that their presence is, at least, not detrimental to the magpies

2.5.3 *CONCLUSION*

This study suggests that there is a nesting association between northern orioles and yellow-billed magpies, and that this occurs as a result of northern orioles actively selecting to nest close to magpies. This preference may have evolved because nesting close to magpies seems to afford the northern orioles some protection from nest predators. At Hastings Reservation magpies nest semi-colonially and are less abundant than northern orioles. This appears to have resulted in the northern orioles also forming loose colonies as a secondary consequence of attempting to nest in close proximity to the magpies. However, northern oriole density may also be influenced by females seeking to benefit from the increased levels of EPCs that occur at the higher conspecific breeding densities. The final breeding distribution of northern orioles will, therefore, reflect a trade-off between the net benefits of associating with magpies, and the costs and benefits of breeding at different conspecific densities.

CHAPTER 3

DNA PROFILING METHODS AND BASIC RESULTS

3.1 Introduction

3.2 Aims

3.3 Methods

- 3.3.1 Identification of probes that cross-hybridise to hypervariable single-locus minisatellite loci in the northern oriole genome
- 3.3.2 Single-locus minisatellite markers
- 3.3.3 Single-locus DNA profiling
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3.4 Results

- 3.4.1 Identification of cross-hybridising hypervariable single-locus minisatellite probes
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- 3.4.4 Distribution of extra-pair paternity
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3.5 Discussion

- 3.5.1 Identification and characterisation of cross-hybridising hypervariable single-locus minisatellite probes
- 3.5.2 DNA profiling results and the distribution of extra-pair paternity

CHAPTER 3

DNA PROFILING METHODS AND BASIC RESULTS

3.1 INTRODUCTION

Evolution is driven both by natural and sexual selection (Darwin 1859, 1871), the underlying currency of which is reproductive success. Therefore, in evolutionary or behavioural studies, it is often essential to determine accurately the reproductive success of individuals if one is going to draw any meaningful conclusions.

Originally, calculations of individual reproductive success had to be based on behavioural observations or on simple counts of the number of offspring produced in a breeding attempt (see studies described in Clutton-Brock 1988). These studies do not take into account the existence of mixed reproductive strategies (i.e. extra-pair paternity [EPP] and intra-specific brood parasitism [IBP]) and may, therefore, be far from accurate.

More recent attempts to decipher biological relationships have been based on methods such as genetically inherited morphological characteristics e.g. plumage polymorphisms (Lank *et al.* 1989a) and heritability estimates of morphological traits (reviewed in Birkhead and Møller 1992). These methods have now been succeeded by more powerful and widely applicable molecular techniques such as single-copy nuclear restriction fragment length polymorphic loci (scnRFLP) minisatellite loci and microsatellite loci, the latter two of which have been found most useful for parentage studies (Burke & Bruford 1987, Burke 1989, Queller 1993).

Minisatellite and microsatellite loci are regions of DNA which contain short repeated sequences of bases. The number of these repeat sequences can vary markedly and result in many character states (allele sizes) per locus (Jeffreys *et al.* 1988). It is these alleles, and the fact that they are inherited in a mendelian fashion from the parents, that are utilised in DNA profiling.

In multi-locus minisatellite profiling, restricted genomic DNA is separated into different size fragments by electrophoresis and Southern blotted onto a nylon membrane. A radioactively labeled probe containing the 'core' sequence can then be hybridised to each allele of each locus containing the same core sequence (Jeffreys *et al.* 1985). This pattern can then be visualised by exposing the membrane to an autoradiogram, thus producing the 'fingerprint' pattern (see Burke 1989, Bruford *et al.* 1992) that, with the exception of

identical twins, is specific to an individual and can be used to determine parentage. There are, however, problems with using this technique as it is time-consuming and tedious with a large number of individuals. It is also impossible to assign bands to any particular locus and therefore to determine allele frequencies or individual genotypes. There are also difficulties in reliably comparing profiles across gels even when using DNA size markers.

In single-locus profiling, cloned minisatellite probes hybridised at high stringency recognise more specific DNA regions and a simple two-band pattern corresponding to the two alleles of a locus from a heterozygous diploid individual is produced. Identification and scoring of the alleles is thus rapid and straightforward. If DNA size-standard markers are utilized then the alleles can be measured and a genotype recorded for each individual which can be accurately compared across gels. The use of several single-locus probes in this way allows both parental exclusion and accurate parental assignment from the sample population. Usually the locus specific probes have to be cloned from the study species (see Bruford *et al.* 1992, Burke *et al.* 1996) but it is also possible, to save time and expense, to isolate suitable probes that cross-hybridise from other species (Gibbs *et al.* 1990, Hanotte *et al.* 1992, Hanotte *et al.* 1994).

Since its advent, DNA profiling has been used to investigate various problems in evolutionary and behavioural ecology. These range from studies investigating population and conservation genetics (Bruford 1992, 1993, Burke *et al.* 1996) to studies on mating systems and the use of mixed reproductive strategies (Packer *et al.* 1991, see also chapter 1 and references therein). In birds there has been a proliferation of parentage studies (reviewed in Birkhead & Møller 1992, Gowaty 1996). The rates of EPP reported in these studies vary widely between species; from 0% in wood warblers (Gyllenstein *et al.* 1990) corn buntings (Hartley *et al.* 1993) and eastern screech-owls (Lawless *et al.* 1997) to approximately 50% in the reed bunting (Dixon *et al.* 1994) and tree swallow (Lifjeld *et al.* 1993) and even higher in other species such as the superb fairy wren (76%; Mulder *et al.* 1994). Most of these studies have used multi-locus profiling. This method allows one to determine if the putative parents are the genetic parents of a chick, but is inefficient for finding the true genetic parents of the offspring from a large sample. The assignment of the actual parents through single-locus fingerprinting allows a more accurate estimation of the reproductive success of individuals, and a better investigation of problems such as female mate choice, male reproductive skew and other components of sexual selection (Gibbs *et al.* 1990, Wetton *et al.* 1995, Whittingham & Lifjeld 1995, Sundberg & Dixon 1996).

3.2 AIMS

In this chapter I will identify single-locus minisatellites, which have been isolated in a range of species, that will cross-hybridise with, and produce a suitable pattern for DNA profiling, in the northern oriole. These probes will then be used to investigate the levels of extra-pair paternity (EPP) and intra-specific brood parasitism (IBP) in a population of northern orioles and to assign parentage to individual chicks. This will allow a thorough investigation of the effects of breeding density and delayed plumage maturation/age on the use of mixed reproductive strategies by individuals.

3.3 METHODS

3.3.1 Identification of probes that cross-hybridise to hypervariable single-locus minisatellite loci in the northern oriole genome

A large number of single-locus minisatellite molecular markers have already been isolated in a wide range of bird species (Burke *et al.* 1996). Recent evidence shows that molecular markers isolated in one species will sometimes cross hybridize, and be useful in other species (Hanotte *et al.* 1992, Hanotte *et al.* 1994). The level of cross-hybridisation that occurs between species seems to correlate with the genetic distance between the species tested. Genetic marker probes for cross-hybridization were tested from available species within the same family (Fringillidae; Sibley & Alhquist 1990) as the northern oriole, i.e. the house sparrow and the reed bunting.

3.3.2 Single-locus minisatellite markers

The detailed methods for the isolation of single-locus minisatellite markers have been described elsewhere (Bruford *et al.* 1992). Single-locus, minisatellite markers have already been isolated in both the house sparrow and the reed bunting (Wetton *et al.* 1994, Dixon *et al.* 1994). Probes which were highly polymorphic, with good resolution, in the original species, were tested and characterised against the northern oriole genome. Minisatellite inserts were oligolabelled with [α ³² P]-dCTP, and used to probe a test strip membrane to which was bound electrophoresed DNA restricted with *Mbo*I (see section 4.3.3). On each test there were two independent clutches with three siblings in each clutch. Minisatellite

markers that exhibited a polymorphic single-locus pattern were selected for use in the parental analysis of this population.

3.3.3 Single-locus DNA profiling

Detailed methodology is presented elsewhere (Bruford *et al.* 1992) therefore only an abridged version, including the details of any adjustments made, will be given here.

Blood and tissue sampling

Blood was collected into both lysis buffer and 100% ethanol as described in section 2.3.6. Both can be stored at room temperature for many months and still yield high molecular weight DNA with no signs of degradation.

Tissue samples from dead pulli and unhatched eggs were stored in exactly the same manner. Brain tissue was extracted from the pulli by severing the neck at the base of the skull and squeezing the contents of the head half into lysis buffer and half into 100% ethanol. In the case of unhatched eggs, samples of whole embryos were placed in lysis buffer if they were less than half the volume of the 1.8 ml microfuge tube. For larger embryos, brain samples were taken as for dead pulli.

DNA extraction and digestion

DNA was extracted from both the blood and brain tissue samples in the same way. In the case of the lysis buffer samples approximately 80 µl of solution (containing approximately 20 µl of whole blood or brain tissue) was added to a 1.8 ml microfuge tube containing 500 µl of stock proteinasing solution (1 M TRIS-HCL, pH 8.0; 0.1 M NaCl; 1 mM EDTA) with 0.5% SDS and 5 units of proteinase K (Sigma). For ethanol samples, one small (2 mm x 2mm) flake of blood was air dried for 10 minutes and then added to the same proteinasing solution. This was incubated at 55 °C (or overnight at 37 °C) on a revolving rotisserie in a Hybaid oven. The protein was then removed from the homogeneous solution by two phenol/chloroform and one iso-amyl alcohol/chloroform extractions (Sambrook *et al.* 1990). The DNA was then precipitated in cold 100% ethanol and centrifuged for 5 minutes at 14000 RPM. The resultant pellet was washed in 70% ethanol and then dried for at least 20 minutes in a small vacuum dryer. The DNA was dissolved in 500 µl of ultrapure water by leaving it at room temperature overnight. The concentration and condition of the DNA was determined by electrophoresis on a 0.8% agarose gel, with DNA concentration standards for comparison (λ DNA; Life Technologies).

In the case of small unhatched embryos, the whole sample was macerated and a suitable amount of the resulting fluid was mixed with proteinasing solution and the DNA extracted as for blood/brain tissue.

Approximately 15 µg of each extracted DNA sample was then digested for 3 hours at 37 °C with 25 units of *Mbo*I in a buffer solution supplied by the manufacturer; Applied Biotechnologies, Medium buffer. The restricted DNA was then cleaned with phenol chloroform, precipitated with 100% ethanol and then washed in 70% ethanol and dried for 20 minutes in a vacuum dryer. The DNA was subsequently dissolved in 25 µl of ultrapure water at room temperature for one hour. The full restriction of the DNA was tested by electrophoreses and the concentration was then estimated using a DNA fluorometer (Hoefer).

Electrophoresis and southern blotting

Approximately 5 µg of *Mbo*I digested genomic DNA including 10 ng of DNA size standard markers (2 ng λ DNA digested with *Xho*I, 8 ng 1-kb DNA ladder [Life Technologies]) was loaded into the wells of a 30 cm x 20 cm 0.8% agarose gel (Sigma type I) and electrophoresed in 1 x TBE running buffer (0.089 M Tris, 0.089 M borate, 2 mM EDTA, pH 8.8) at 45 V for 48 h, until the < 0.5-kb DNA fragments were lost from the end of the gel. A marker ladder of λ DNA digested with *Mbo*I was run in the outermost lanes of the gel and was used to check the migration distance of the DNA after staining the gel with ethidium bromide following electrophoresis. These standard size markers were later used to help align autoradiographs revealing different minisatellite patterns. Up to 25 samples were run on a single gel.

Following electrophoresis, the DNA was transferred to a Hybond-Nfp (Amersham) nylon membrane by Southern blotting. Firstly, the DNA was depurinated by washing in 0.25 M HCl, then denatured by washing in 0.5 M NaOH, 1.0M NaCl, pH 7.4, and finally neutralised in 1 M Tris, 3 M NaCl, pH 7.4. The DNA was transferred to the nylon membrane by capillary action (blotting overnight in 20 x SSC). The membrane was then washed in 3 x SSC and the single-stranded DNA fixed by cross-linking with UV (irradiated for 90 seconds with a previously calibrated 254 nm UV transilluminator).

Probing and autoradiography

The nylon membranes were washed overnight at 65 °C in a hybridisation solution (0.5 M NaPO₄, 1 mM EDTA, 7% SDS and 1% bovine serum albumin, pH 7.2) that contained 10 - 20 ng of minisatellite probe DNA oligolabelled with [α ³² P]-dCTP and northern oriole competitor DNA at a concentration of 10 µg per ml of overall solution. One nanogram of oligolabelled λ *Hind*III was also added to the hybridisation solution. This

hybridised to the λ HindIII size standards on the outer lanes of each gel and aided with the alignment of autoradiographs when measuring allele sizes. After hybridisation the nylon membranes were washed at 65 °C in 0.1 x SSC, 0.01% SDS for up to three separate ten-minute periods. The level of radioactivity on the membrane was checked between each wash and the washes were stopped when the levels of activity detected on the filter reached between 10 - 15 counts per second in areas where DNA was present, and close to background in areas where no DNA was expected to be. The membranes were then rinsed in 3 x SSC and exposed to autoradiographs (Fuji RX film) for approximately 48 h with two intensifying screens. The films were then developed and if the image on the autoradiograph was still faint the membranes were exposed again for a longer period (up to 240 h).

Membranes were stripped of probe DNA before starting the next probing experiment. This was done by washing for 20 minutes in a solution of 0.1% SDS that had been boiled and then allowed to cool for 30 seconds. The removal of the probe DNA was complete when the level of activity on the filters was down to background levels.

3.3.4 Characterisation of minisatellite loci

The probability of false paternal inclusion (i.e. the probability of detecting extra-pair offspring assuming the mother is correctly assigned) was calculated following Jamieson (1965). The probability of false parental inclusion (i.e. the probability of detecting an offspring resulting from intra-specific brood parasitism) was calculated following Gundel & Reetz (1981). In both cases the probability is dependent upon the number and frequency of alleles at each locus and the total number of loci examined. Therefore the characteristics of the minisatellite loci used were quantified by measuring the allele sizes, for a subset of unrelated adult individuals.

There is an exponential relationship between the molecular weight of electrophoresed DNA fragments and their migration distance along the agarose gel. Consequently the separation (and therefore the degree of resolution) between the larger DNA alleles was less than for the smaller alleles. To account for this and other potential sources of error (e.g. slight misalignment of allele and marker autoradiographs, and differences in running distance of fragment between gels) alleles were allocated to 'size bins' (see Wetton *et al.* 1995). Alleles between 2 - 3 kb were split into 0.025 kb bins, 3 - 4 kb into 0.05 kb bins, 4 - 7 kb into 0.1 kb bins, and between 9 - 10 kb into 0.25 kb bins. The minimum size of a bin was greater than one mm at the lowest kb size and increased as the kb size increased so that the bin size was greater than the minimum distance between which two bands could be resolved at that kb size. The size of the alleles was calculated by determining their distance between the internal size markers. Measurements were taken from the centre of each band using vernier callipers.

The allele size range, the heterozygosity, the probability of identity, and the probabilities of false paternal and parental inclusion were calculated for each separate locus and for all loci combined. Allele frequencies were obtained from the genotype of a subset of 23 presumably unrelated adults caught on the field site in 1994. The actual allele frequencies, as opposed to the mean allele frequencies, were used for all calculations.

3.3.5 Assigning parentage

The offspring at a nest were run adjacent to the putative parents. The parentage of individual offspring was then assigned by matching the band pattern from the offspring genotype with the alleles from the putative parents. These alleles are inherited in a Mendelian fashion, thus one band from each parent will be present in the offspring. Bands from the same autoradiograph were considered as mismatched when they differed by over half a bin size. Intra-specific brood parasitism was identified if the offspring genotype mismatched that of both of the putative parents. Extra-pair paternity was identified if the genotype matched that of the putative mother but not of the putative father.

Two methods were used in an attempt to identify the true genetic parents of EPY or IBP. Firstly, neighbouring nests, including parents, were run on the same gels wherever possible. If this was not possible then at least the males from those nests were run on the same gels. The genotype of any extra-pair offspring could then be directly compared to the nearest neighbouring adults. Secondly, the genotype of every adult sampled at the site was entered into a Microsoft Excel data base. The database could then be searched for the genetic parents of mismatched offspring. An error of $\pm$ one 'bin' was allowed in the search, the kb size of which depended on the size of allele in question.

3.4 RESULTS

3.4.1 Identification of cross-hybridising hypervariable single-locus minisatellite probes

A total of six single-locus minisatellite probes was tested for cross-hybridisation: five derived from the reed bunting, and one from the house sparrow. They were tested against a membrane containing six individual chicks, three from each of two unrelated nests. All six probes produced patterns indicative of a single-locus minisatellite. One was rejected because it was monomorphic in all six unrelated individuals tested. Three other probes produced three bands instead of two due to an internal cutting site within the minisatellite of one of the alleles. One of these may still have been suitable for parentage analysis but was not optimal.

The two highly polymorphic single locus probes identified were subsequently used in the parentage analysis described in this thesis: one from the reed bunting (cEscMS6) and one from the house sparrow (cPdoMS14)

3.4.2 Characteristics of the minisatellite loci

The relative frequencies of alleles for each of the loci are displayed in Figures 3.1. Both loci have low mean allele frequencies and relatively little variation in frequency between alleles making them extremely suitable for parentage analysis. The power of this profiling regime was estimated using actual allele frequencies (rather than mean allele frequencies) in the probability calculations (see Table 3.1).

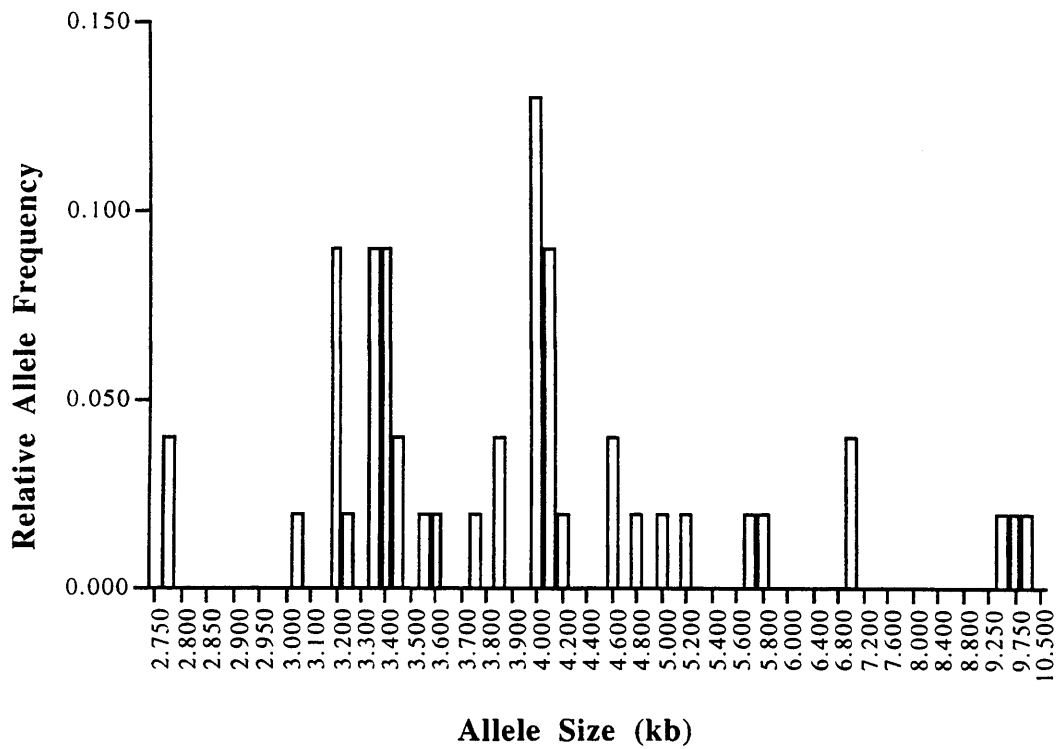
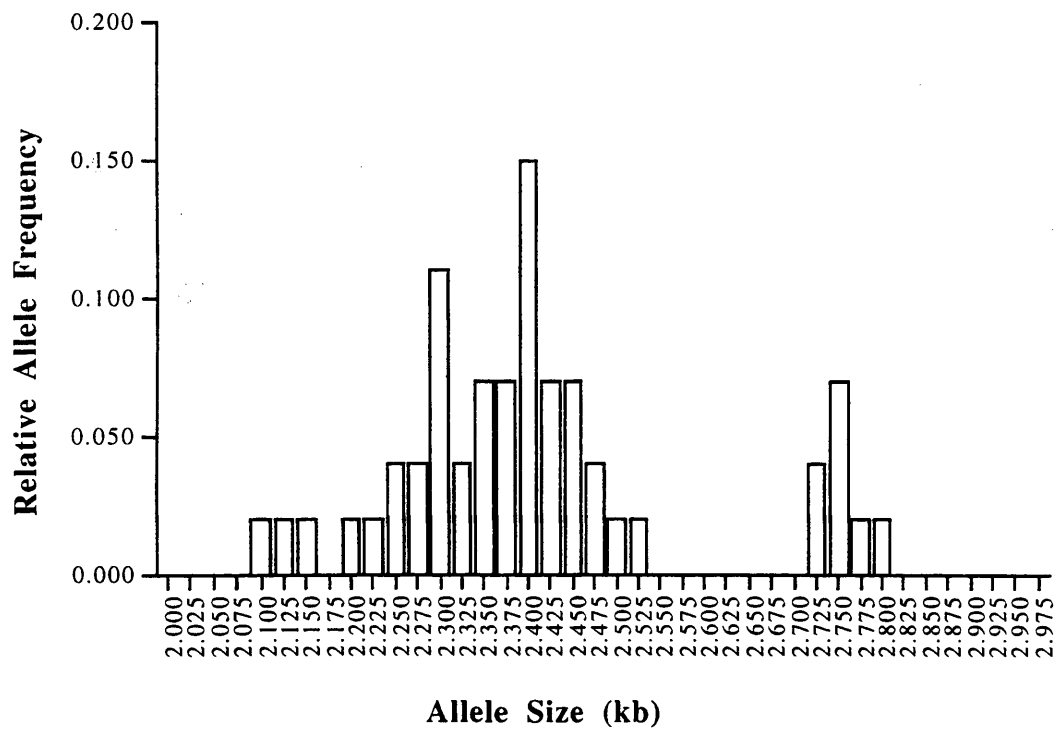
(a) Allele frequencies for probe *cEscMS6*(b) Allele frequencies for probe *cPdoMS14***Figure 3.1** Allele frequencies at minisatellite loci (N = 23)

Table 3.1 Characterisation of single-locus probes

Probe	Number of adults profiled	Allele size range (kb)	Number of alleles detected	Heterozygosity [#]	Probability of false paternal inclusion (P_{pat}) [†]	Probability of false parentage inclusion (P_{par}) [§]
<i>cEscMS</i>	23	2.8 - 10	24	95.7	0.144	8.70×10^{-5}
<i>cPdoMS14</i>	23	2.1 - 2.8	21	95.7	0.136	1.15×10^{-4}
Combined*					0.018	1.00×10^{-8}

[#] The heterozygosity for each locus was determined by counting the number of resolvable heterozygotes among the 23 adults.

[†] For a single locus the probability that a male will be falsely assigned as the true father (Probability of false paternal inclusion P_{pat}) is given by Jamieson (1965):

$$1 - \left(\sum_{i=1}^n q_i(1 - q_i)^2 - \sum_{i>j}^n q_i^2 q_j^2 (4 - 3 \cdot (q_i + q_j)) \right) n$$

[§] For a single locus the probability that the putative parents of a chick were falsely assigned as true genetic parents (P_{par}) was calculated from (Gundel & Reetz 1981):

$$1 - \left[1 + \sum_{i=1}^n (q_i^2(2 - q_i))^2 - 2 \left(\sum_{i=1}^n q_i^2(2 - q_i) \right)^2 + 4 \left(\sum_{i=1}^n q_i^3 \right)^2 - 4 \sum_{i=1}^n q_i^6 \right]$$

* The cumulative probabilities of false inclusion using both of the loci are calculated as the product of the two individual false inclusion probabilities.

3.4.3 Parentage results for the northern oriole

DNA profiling of 48 clutches containing 202 chicks was completed. Twenty-two clutches (46%) contained extra-pair young (EPY) and two clutches (4.2%) showed intra-specific brood parasitism (IBP) (Table 3.2). These samples were collected over two breeding seasons (1994 and 1995) but were combined as there was no significant difference between years either in number of clutches containing EPY ($\chi^2 = 0.00$, $df = 1$, $p = 1.00$), in the absolute number of EPY ($\chi^2 = 2.46$, $df = 1$, $p = 0.12$), or mean proportion of EPP in clutches (Mann-Whitney u-test = 1.20, $df = 1$, $p = 0.27$).

Table 3.2 Summary of parentage results from DNA profiling. EPY = extra-pair young, EPP = extra-pair paternity, IBP = intra-specific brood parasitism.

Year	Number of clutches (chicks)	Number of clutches containing EPY	Total number of EPY	Mean percentage of EPP in clutch	Number of clutches containing IBP	Total number of IBPs
1994	19 (76)	9 (47.4%)	30 (39.4%)	41.1%	1 (5.3%)	1 (1.3%)
1995	29 (126)	13 (44.8%)	35 (27.8%)	27.6%	1 (3.4%)	1 (0.8%)
Total	48 (202)	22 (46.0%)	65 (32.2%)	32.9%	2 (4.2%)	2 (1.0%)

3.4.4 Distribution of extra-pair paternity

Of the 202 chicks examined, 65 (32%) were sired by extra-pair males (EPMs). The distribution of EPY in clutches can be seen in Figure 4.2. Assuming a random distribution of EPY, the expected number of clutches containing EPY can be calculated from Lifjeld *et al* (1993):

$$\sum N_i (1-P)^i$$

where N_i is the number of broods of size i and p is the probability of any individual chick being extra-pair ($p = 0.322$). The expected number of clutches containing EPY (38) differed

significantly from the observed number of clutches containing EPP (22) ($\chi^2 = 10$, $df = 1$, $p = 0.005$). Therefore EPP is not randomly distributed across clutches, and appeared to show a bimodal distribution with most nests containing all or very little EPY (figure 3.2).

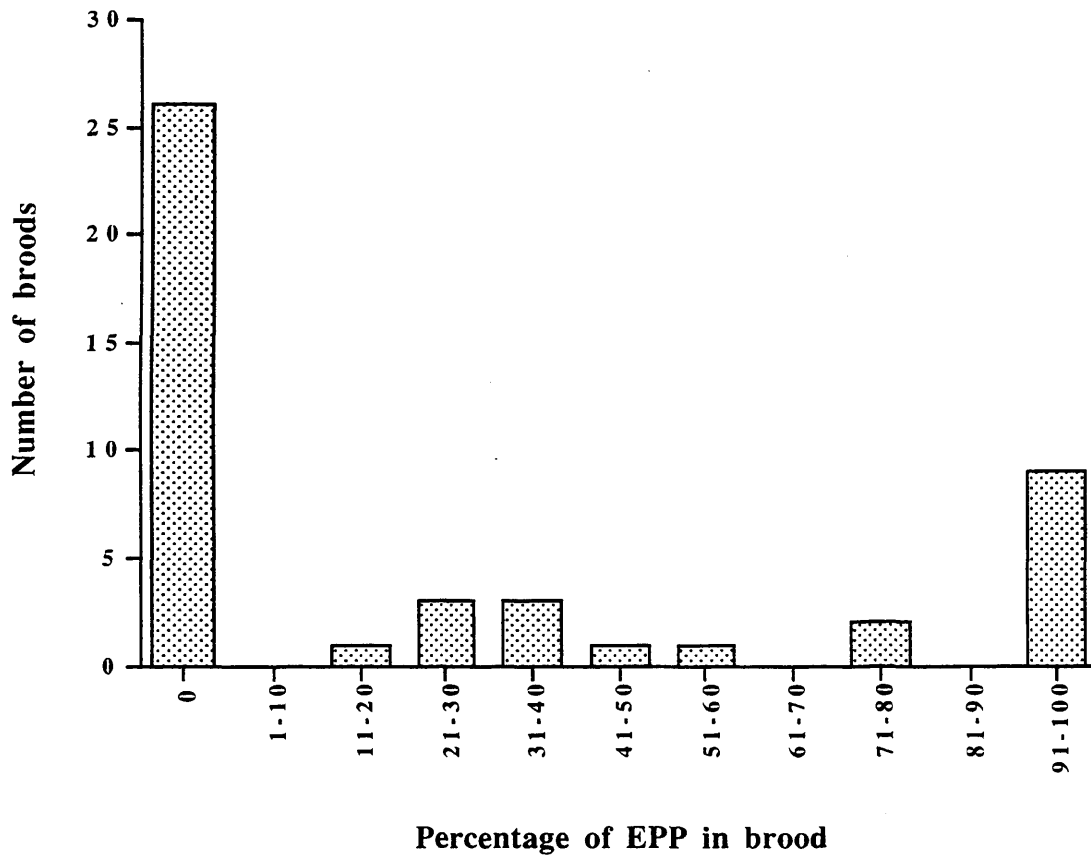


Figure 3.2 The frequency distribution of extra-pair paternity in clutches. (N = 48).

3.4.5 Assignment of extra-pair paternity

In 1994, a total of 22 out of 30 (73%) extra-pair young were assigned to their true, genetic fathers, while in 1995, only 7 out of 35 (14%) could be assigned. Therefore, the overall assignment level was 44.6% (29/65) of chicks.

3.5 DISCUSSION

3.5.1 Identification and characterisation of cross-hybridising hypervariable single-locus minisatellite probes

Through the use of cross-species hybridisation, two previously isolated hypervariable minisatellite single-locus probes were identified which had suitable characteristics for DNA profiling in northern orioles. One of these (*cPdoMS14*) was isolated from the house sparrow genome, and one from the reed bunting genome (*cEscMS6*). The size range of alleles at the loci recognised by these probes does not overlap and could therefore be used in a 'cocktail' allowing both distinct loci to be revealed in a single hybridisation experiment (Bruford *et al.* 1992). Both loci were highly polymorphic with the same level of observed heterozygosity (95.7%). This is similar to those from minisatellite loci isolated in other species (Double 1995, Burke *et al.* 1996)

The probability of false paternal inclusion (P_{pat}) (i.e. the probability of detecting extra-pair offspring assuming the mother is correctly assigned) and the probability of false parental inclusion (P_{par}) (i.e. the probability of detecting an offspring resulting from IBP) are presented in Table 4.1. Assuming statistical independence between the two loci then the combined P_{pat} and P_{par} values were 0.018 and 1.00×10^{-8} respectively. Therefore this combination of probes was considered sufficiently powerful to identify EPP and IBP, and to assign EPY to genetic parents in a parentage study.

3.5.2 DNA profiling results and the distribution of extra-pair paternity

The overall level (32.2%, 65/202) of EPY in this population of northern orioles is reasonably high for an avian species, but not exceptionally so (see section 3.1). This level of EPP should provide enough resolution to enable investigation of the effects of both breeding density and delayed plumage maturation/age on the use and success of mixed reproductive strategies. The bimodal distribution of EPY across clutches (see Figure 3.2) also suggests that certain parameters about the nest or attendant birds may influence the levels of EPP in a clutch. The level of IBP found in this study (2/202; 1.0%) was too low to be able to distinguish the factors affecting it. The following chapters will go on to investigate the effect of delayed plumage maturation/age (Chapter 4) and breeding density (Chapter 5) on the levels of extra-pair paternity revealed using DNA profiling. They will also investigate the characteristics of individuals gaining extra-pair paternity. Intra-specific brood parasitism will not be investigated further.

CHAPTER 4

MALE AGE, DELAYED PLUMAGE MATURATION AND EXTRA-PAIR PATERNITY

4.1 Introduction

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- 4.1.2 Male delayed plumage maturation
- 4.1.3 Extra-pair paternity and delayed plumage maturation
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4.2 Aims and predictions

4.3 Methods

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4.4 Results

- 4.4.1 Mating success
- 4.4.2 Body condition index
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CHAPTER 4

MALE AGE, DELAYED PLUMAGE MATURATION AND EXTRA-PAIR PATERNITY

4.1 INTRODUCTION

4.1.1 Age

Various possible benefits have been suggested for females engaging in extra-pair copulations (EPCs) (reviewed in Westneat *et al.* 1990, Birkhead & Møller 1992, Kempenaers 1993, Andersson 1994). One hypothesis is that females engage in EPCs to obtain better genes for their offspring than they would from their social partner ('genetic quality' hypothesis; Walker 1980). Such genes may increase either the attractiveness of their sons ('sexy son' hypothesis; Fisher 1930, Weatherhead & Robertson 1979) or the general viability of their offspring (good genes hypothesis; Zahavi 1975, 1977, Hamilton & Zuk, 1982). In a socially monogamous mating system, females seeking EPCs for 'good genes' reasons should, therefore, perform EPCs with males that are of 'better quality' than their own partner. One simple rule to ensure this would be to perform EPCs with older 'proven quality' males since older males, having survived longer, will on average, be of better quality than younger males (Trivers 1972, Kokko & Lindstrom 1996). Behavioural studies of several avian species have found that females paired to young males became involved in EPCs more frequently than females paired to older males (Birkhead & Møller 1992). Evidence from studies using DNA profiling indicate that younger males commonly suffer more losses from EPP than do older males (Morton *et al.* 1990, Wagner *et al.* 1996; but see Westneat *et al.* 1995, Hasselquist *et al.* 1996). Stronger support for female choice for older males though, comes from DNA profiling studies that actually assign the extra-pair young (EPY) to specific males. Several studies have reported that older males successfully gain EPY at the expense of younger conspecifics (Wetton *et al.* 1995, Sundberg & Dixon 1996; but see Dunn *et al.* 1994b). If females are choosing older males for EPCs what cues are they using to determine the age of individuals? There is evidence to suggest that females of some

species use age-related differences in plumage (i.e. brighter birds, bigger badges) in both social, and extra-pair mate choice (Enstrom 1993, Sunberg 1995, Sætre *et al.* 1995, Sundberg & Dixon 1996).

4.1.2 Male delayed plumage maturation

Delayed plumage maturation (DPM) is defined by Hill (1996) as: ...‘the delayed acquisition of definitive nuptial plumage until after the first potential breeding season such that the subadult plumage has a pattern distinct from that of males in definitive plumage but not necessarily from that of females.’

In North America there are many sexually dichromatic passerines in which the male displays DPM. In these species, although the males are reproductively mature, they do not acquire full adult breeding plumage until after their first potential breeding season (Rohwer *et al.* 1980, Lyon & Montgomerie 1986, Rohwer & Butcher 1988). They do, however, regularly or occasionally, breed successfully. Females of these species normally attain full adult plumage before their first breeding season, but this is usually considerably duller and less conspicuous than that of the adult male. During their first potential breeding season males showing DPM resemble mature females (Rohwer *et al.* 1980, Rohwer & Butcher 1988). Delayed plumage maturation is not, however, always restricted to males, e.g. in tree swallows (Stutchbury & Robertson 1987) cedar waxwings (Mountjoy & Robertson 1988) and orange breasted buntings (Thompson & Leu 1995).

If the adult male plumage of these species in some way facilitates the adult males ability to breed, then what is the function of the subadult plumage? A number of hypotheses have been proposed to explain the adaptive significance of DPM in passerines. These fall into two main sets: the ‘summer adaptation’ hypotheses and the ‘winter adaptation’ hypotheses (Rohwer *et al.* 1980, Lyon & Montgomerie 1986); these hypotheses all invoke selective forces favouring specific adaptive functions of subadult plumages in the respective seasons. A further two hypotheses pertain to the entire year. The ‘breeding-threshold’ hypothesis (Studd and Robertson 1985a) deals with life history characteristics that favour subadult plumage, without ascribing a particular function to the plumage, and as Rohwer & Butcher (1988) pointed out, these characteristics relate to the entire year. The ‘moult-constraints’ hypothesis (Chu 1994) suggests that a subadult plumage colour may occur because of phylogenetic or energetic moult constraints that prevent the complete transition from the juvenile plumage to the definitive adult plumage in the first year.

Several studies have investigated the significance of male DPM. Some have used comparative analysis between species (or subspecies) (Studd & Robertson 1985a, Lyon & Montgomerie 1986, Rohwer & Butcher 1988, Chu 1994, Hill 1996). Others have investigated individual species: e.g. American redstarts (Proctor-Gray & Holmes 1981),

northern orioles (Flood 1984), black-headed grosbeaks (Hill 1988), cedar waxwings (Mountjoy & Robertson 1988), orchard orioles (Enstrom 1992, 1993), and kestrels (Hakkarainen 1993). Despite all these studies, no general consensus of opinion for the function of DPM exists.

4.1.3 Extra-pair paternity and delayed plumage maturation

One major factor that has yet to be investigated in relation to DPM is the effect of extra-pair paternity (EPP). Until now studies have used mating success or number of young fledged at a nest as an estimation of reproductive success when investigating DPM (Clutton-Brock, 1988 Williams 1988, Lozano *et al.* 1996,). These estimations may be far from accurate due to EPP in the brood.

Females may use plumage differences associated with DPM as a signal of male age. If females prefer to engage in extra-pair copulations (EPCs) with older, adult males, then adult males may be increasing their reproductive success at the expense of subadults. This will affect the costs and benefits of DPM and have implications for the hypotheses relating to DPM. The use of DNA profiling to accurately assign paternity (see Chapter 3) can determine the true reproductive success of adults and subadults, thus allowing a better estimation of the costs of subadult plumage.

4.1.4 The northern oriole and delayed plumage maturation

Delayed plumage maturation occurs in the northern oriole (*Icterus galbula bullockii*), with yearling subadult males having no spring moult (Rohwer & Butcher 1988) and displaying a summer plumage that is intermediate between that of males and females (see Chapter 1). A previous study that focused on the pairing success of individuals supported the female mimicry hypotheses for DPM in northern orioles (Flood 1984). Another study, on the Hasting Reservation northern oriole population (Williams 1988), reported no difference between adults and subadults in observed fledging success, but did not account for EPP. DNA profiling evidence from this population (see Chapter 3) shows that 32.2% of young were extra-pair, and that 46% of all nests contained extra-pair young (EPY). It is, therefore, possible that female extra-pair mate choice occurs in this population, resulting in a skewed distribution of EPP and, consequently, of the annual reproductive success of males.

4.2 AIMS AND PREDICTIONS

This chapter will investigate the levels and distribution of EPP and reproductive success in relation to male age/status in a northern oriole population. These results will be

compared with the predictions from the 'good genes' hypothesis for the female benefits of EPCs to determine whether the data are consistent with this hypothesis.

The 'good genes' hypothesis predicts that females paired to subadult males will both seek EPCs more often than females paired to adult males, and prefer adult males when obtaining EPCs. This leads to several testable predictions:

- a) EPP will not be randomly distributed between broods.
- b) Subadult broods will contain EPY more often than adult broods
- c) Levels of EPP will be higher in subadult than adult broods
- d) Adults will have a higher annual reproductive success than subadults through the combined effects of higher within-pair paternity and increased EPP gained from other nests.

The implications of these results for the hypotheses of DPM will then be discussed, firstly in relation to northern orioles, and then for other species that display DPM.

4.3 METHODS

4.3.1 Arrival, capture, sexing and ageing

Work was conducted over the 1994 and 1995 breeding seasons at Hastings Natural History Reservation, Monterey County, California (for general field methods, see chapter 1). Daily inspections of the entire field site were made and the arrival dates of individual birds and of nest initiation were recorded. Birds were caught as soon after arrival as possible to minimize the chance of disturbance by encroaching in to the fertile period of the female. Captured birds were banded (see Section 1.3.4) and sexed using plumage characteristics (Pyle *et al.* 1987). Adult males were a conspicuous contrast of yellow and black, whereas females were a dull cryptic colour (olive/brown). The sexes were confirmed by the possession of either a protruding cloaca (male) or a brood patch (female). In addition, males were split into age categories (adult or subadult [first-year]) according to the conspicuous changes in plumage which occur with age (Pyle *et al.* 1987). All adult males have black wing feathers, acquired in a mid-winter moult, whereas those of first-year males are brown and often worn (Flood 1984). The sex and age category of uncaught individuals was determined after careful observation of plumage and behaviour using a 10 x 45 Bushnell Spacemaster telescope. All birds classified in this way and then caught, sexed and aged later in the season were found to be correctly assigned.

4.3.2 Nearest neighbour distances and breeding densities

The detection of newly arriving birds was not always immediate, and thus settlement dates based on first sightings may be slightly inaccurate. Therefore relative settlement dates

were estimated for all birds using first egg dates. All pairs were observed every two days during the nest building stage to help predict the exact first egg date. Easily accessible nests were regularly examined for eggs. At less accessible nests, behavioural cues (e.g. nest lining or termination of nest building) were used to predict when laying commenced, and the first egg date was confirmed by one mid-laying inspection of the nest.

The distances between each nest and the nearest active adult and subadult nests were measured (nearest neighbour distances) and the relative settlement distances of adults and subadults were subsequently compared. This could then be used to determine if subadult males were tolerated nearer to adult males than were other adult males. The effect of arrival date was controlled for by using comparisons of simultaneously settling males (within $\pm$ two days, based on settlement date estimates; Hakkarainen 1993).

Each northern oriole nest was also allocated to a breeding density category, with a distance of 100 m between a northern oriole and its nearest conspecific being used to separate solitary breeding pairs from clumped breeding pairs. This measure of breeding distribution was used in the analyses of EPP and reproductive success, as it was more likely to reflect the possibility of individuals interacting than was the overall nearest neighbour distance. (see Chapter 1). The numbers of adult and subadult nests in each density category were compared.

4.3.3 Mating success, predation and fledging success

The mating success of both categories of males was determined over the breeding season. The mating status of males was determined by inspection of all areas and known previously unpaired males at least once every three days during the breeding season. A previous eight-year study on this population (Williams 1988) determined the clutch size and fledging success rates for both adult and subadult northern orioles. Therefore, in order to keep nest disturbance to a minimum and due to the fact that climbing to nests was difficult and very time consuming, lower priority was given to measuring the final clutch size and the relative fledging success rate. The final clutch sizes and the relative fledging success rates determined by Williams (1988) will be considered in the discussion.

During provisioning, nests were checked at least once every three days to ensure they were still active. Unless the nest was subsequently preyed upon, the number of young fledged was assumed to be equal to the number of young banded.

4.3.4 Frequency distribution of extra-pair paternity

When tested using all males extra-pair paternity (EPP) was not randomly distributed throughout broods in this population (see section 3.4.4). The analysis was then repeated for

the different male status categories separately to see if the distribution of EPP was random in both categories. Assuming a random distribution of EPY, the expected number of clutches containing EPY can be calculated following Lifjeld *et al.* (1993). See section 4.4.3.

4.3.5 Extra-pair paternity and annual reproductive success

Blood samples were taken from all adults and chicks (surviving at day 9). DNA profiling techniques (described in Chapter 3) were used to identify EPY accurately in a brood, and to assign these EPY to their true genetic fathers. The mean levels of EPP in adult and subadult nests were calculated, as were various estimates of reproductive success. These include the mean number of offspring due to within-pair paternity fledged in the male's own brood (mean paternity fledged), the mean EPP gained from other broods (mean EPP gained) and the annual reproductive success (number of fledglings sired in own nest + the number of EPY sired that fledged from other nests).

It is unlikely that any one factor accounts for all the variation observed in a natural system, and so any one variable will be the effect of a combination of factors acting together. A stepwise general linear model was therefore constructed to determine which factors determine EPP and annual reproductive success, and the relative strength of their influence.

4.4 RESULTS

4.4.1 Mating success

In 1994 a total of 13 adults and 12 subadults established territories on the field site. All males obtained mates and initiated breeding attempts except for two subadult males. In 1995 there was a total of 28 adult males and 15 subadult birds, of which three subadults remained unpaired. The subadults remained unpaired throughout the season despite continuously advertising (singing) for a mate. From this, subadult males were found to have a lower mating success rate than adult males (Fisher Exact test, $p = 0.008$).

4.4.2 Body condition index

It is possible that adult males are in better condition than subadult males and that this directly influences both the mating success and levels of EPP. The body condition index (BCI; see Section 2.3.5) of adult males was no different to subadult males (ANOVA $F_{1,44} = 0.41$, $p = 0.58$), nor was there any difference between the BCI of females paired to adults or subadults (ANOVA $F_{1,33} = 1.02$, $p = 0.32$). There was, though, a relationship between a

male's BCI and that of their partner ($F_{1,33} = 6.43$, $p < 0.02$). This suggests that females and males in better condition pair together. Whether this is due to assortative mating by choice or a consequence of differential arrival times is unknown. There was, however, no relation between BCI and first egg date ($F_{1,44} = 2.02$, $p = 0.16$ for males: $F_{1,33} = 1.170$, $p < 0.29$ females), which suggests that it was not merely an effect of better condition birds arriving earlier and pairing up with those available at the time.

4.4.3 Nearest neighbour distances and breeding density

In order to investigate the effect of settlement date and nearest neighbour distances upon the levels of EPP and male reproductive success, this analysis was restricted to nests for which there were paternity results ($n = 48$).

The overall nearest neighbour distances for adult and subadult males were not significantly different (Mann-Whitney U-test, $z = 1.68$, $p = 0.20$). Subadults did nest closer to adult nests than did other adults (subadult to adult $99 \pm 98\text{m}$ v. adult to adult $266 \pm 260\text{m}$: Mann-Whitney U-test, $z = 4.16$, $p = 0.04$), but not closer to other subadults (subadult to adult $428 \pm 398\text{m}$ v. Adult to adult $440 \pm 406\text{m}$: Mann-Whitney U-test, $z = 0.09$, $p = 0.77$). The estimated mean settlement date of subadult males was 8.6 days later than for adult males (adults, 124.2 ± 11 days, subadults, 132.8 ± 11 days; ANOVA, $F = 6.72$, $df = 1$, $p = 0.01$). Therefore subadult males both arrived and initiated breeding later than did adult males.

It is possible then that subadult males arriving later are forced to breed near nests already occupied by adult males. Paired comparisons were made, relative to nearest adult male nest, of subadult males and adult males arriving simultaneously (laying date ± 0 -2 days). When controlling for arrival date, the mean distance between a subadult nest and an adult nest ($130.3 \pm 138\text{m}$) was again shorter than the mean distance between adults ($264.9 \pm 191\text{m}$) (Wilcoxon signed ranks, $z = 2.1$, $p = 0.035$) (figure 4.1).

No difference was found in the proportions of adults and subadults present at the different breeding densities, either between the years 1994 and 1995 (Fisher Exact test, $p = 1.00$ for both years) or within years (Fisher Exact test: 1994, $p = 0.65$; 1995 $p = 0.45$, respectively). Pooled data also showed no overall difference in the relative number of adult or subadult nests at the different densities (Fisher Exact test, $p = 0.37$). The overall nearest neighbours distance of adult and subadult male also did not differ (Mann-Whitney U-test, $z = 1.68$, $p = 0.20$). Therefore, the effect of male status on EPP is independent of breeding density.

The effect of male status on the distance to nearest-neighbour magpie was also tested. Subadults were nesting no closer to magpies than were adults (Mann-Whitney U-test, $z = 0.44$, $p = 0.51$), even when controlling for arrival date (Wilcoxon signed ranks, $z = 0.44$, $p = 0.50$).

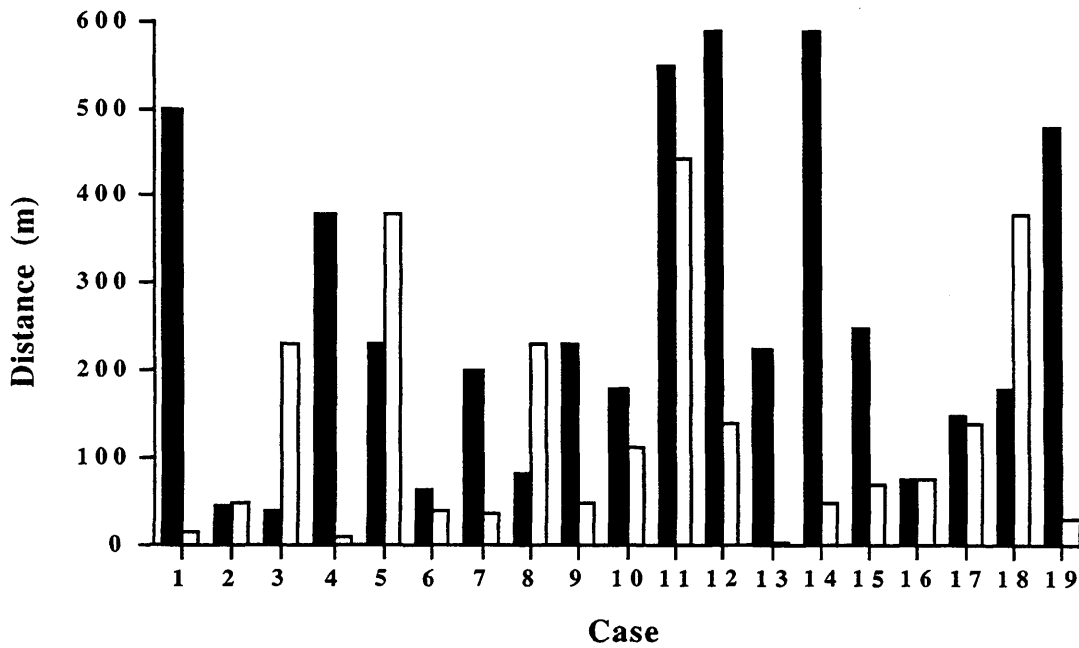


Figure 4.1 Settlement distance between adult males and simultaneously arriving (first egg date $\pm$ 0-2 days), (i) adult males (*filled bars*) and (ii) subadult males (*open bars*).

4.4.4 Extra-pair paternity

A total of 202 chicks from 48 clutches was analysed using DNA profiling techniques, 132 from 31 adult nests and 70 from 17 subadult nests (see Table 4.1). There was no difference between years in the numbers of EPY or in the mean proportion of EPP, either overall or in adult or subadult broods separately (all $p > 0.1$), therefore data from both years were combined.

Table 4.1. Summary of parentage results related to male age/status category

Male age class	No. of clutches (chicks) profiled	Total no. of clutches containing EPY	Mean proportion clutch lost to EPP
Adult	31 (132)	10/31 (32%)	20.6%
Subadult	17 (70)	12/17 (71%)	55.3%

Table 4.1 shows that more subadult broods contained EPY than adult broods (Fisher exact test $p = 0.016$). There was also a higher mean proportion of EPP in subadult nests (55.3%) than in adult nests (20.6%) (Mann-Whitney U-test, $z = 9.92$, $p = 0.002$). The

observed EPP distribution was then tested against the expected random distribution, calculated using the formula from Lifjeld *et al.* (1993):

$$\sum N_i (1-P)^i$$

(Where N_i is the number of broods of size i and p is the probability of any individual chick in a status category being extra-pair). The distribution of EPP was found not to be random in either the adult or the subadult broods ($\chi^2 = 6.35$, $df = 1$, $p < 0.02$; $\chi^2 = 38.25$, $df = 1$, $p < 0.001$, respectively) (see figure 4.2). Instead the distribution of EPP in subadult broods appears to show a bimodal pattern with broods having either zero, or low levels of EPP, while some have very high levels of EPP (all of the nine subadult broods in the 91-100% EPP category have 100% EPP). The distribution for adults is also non-random but does not appear bimodal.

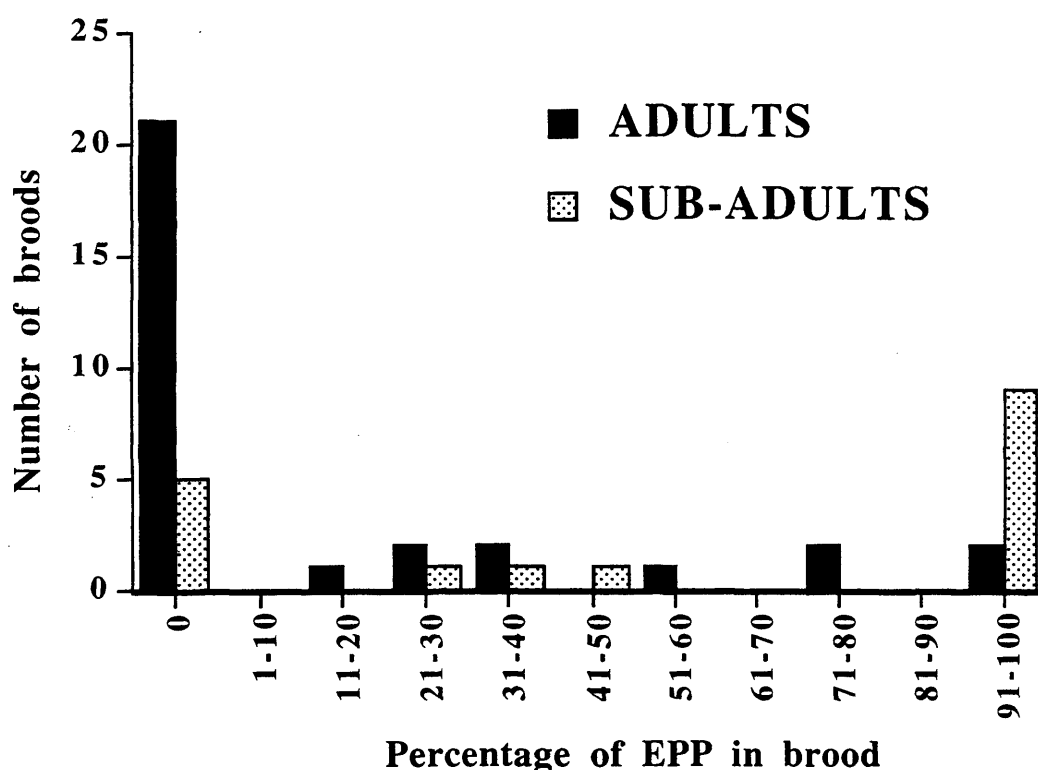


Figure 4.2 The distribution of EPP across broods for the two male status categories.

In both adult and subadult broods the distribution suggests that there are other factors that have an effect on which broods contain EPY. Therefore broods with and without EPY were investigated to see if there was any difference in certain variables. No difference was found for the variables: male body condition index, female body condition index, first egg

date, and synchrony index (Mann-Whitney U-test, $p > 0.5$ for all) (see section 4.4.6). However, when 'density category' was examined a significant effect was found between broods which contained EPY and those without (Mann-Whitney U-test, $z = 5.52$, $p = 0.019$) The effect of breeding density upon levels of EPP will be investigated and discussed in Chapter 5.

There was also a significant difference between the nearest neighbour distances for broods with and without EPY (Table 4.2). Overall, broods containing EPY had closer nearest neighbours than broods without EPY (Mann-Whitney U-test, $z = 4.64$, $p = 0.03$). This analysis was then repeated for nearest neighbouring adult and subadult nests separately. The results indicate that broods with EPY were nearer to an adult male nest than broods without EPY (Mann-Whitney U-test, $z = 5.52$, $p = 0.019$) while there was no difference in relation to the distance to subadult nests (Mann-Whitney U-test, $z = 2.44$, $p = 0.12$).

Table 4.2. The mean nearest neighbour distances for broods with and without EPY (Using all nests and for nearest-neighbour adult and subadult nests separately). Mann-Whitney U-test z values are shown (* $p < 0.05$, ** $p < 0.01$).

		Nearest neighbour distance (m)	Nearest adult male neighbour distance (m)	Nearest subadult male neighbour distance (m)
EPY brood	Mean	98	105	365
	Range	9 - 480	9 - 480	12 - 1000
No EPY brood	Mean	219	293	496
	Range	3 - 740	3 - 100	9 - 100
z		4.64*	7.93**	2.44

4.4.5 Annual reproductive success

The results of the different measures of breeding success and of the assignment of EPY are given in Table 4.3. Data from both years were pooled as there was no difference between years, overall, or for each of the different male categories, for any of the variables (Mann-Whitney U-test, all $p > 0.25$). However, EPP gained was close to being significantly different (Mann-Whitney U-test, $p = 0.06$), due to the different levels of EPY assigned in the different years. There was no difference between adults and subadults for original clutch size (Mann-Whitney U-test, $z = 1.05$, $p = 0.31$) or for clutch size at day nine (Mann-Whitney U-test, $z = 0.003$, $p = 0.95$).

Table 4.3. The effect of male status on measures of reproductive success

Male Status	Mean WPP gained	No. EPM assigned (all males)	Mean EPP gained	Mean annual reproductive success (n)
Adult	3.5 ± 1.6	9 [‡] (41)	0.71 ± 1.36	4.2 ± 1.7
Subadult	1.8 ± 2.1	1 (27)	0.11 ± 0.58	1.9 ± 2.0
z	6.95**		2.71§	10.70***

[‡] One of the adult males that obtained EPY did not breed in the area. Extra-pair male (EPM). Mann-Whitney U-test z values are shown (§ p < 0.1, * p < 0.05, ** p < 0.01, *** p < 0.001).

As shown in Table 4.3 adult males had a higher reproductive success than subadults based on their mean within-pair paternity in their broods (Mann-Whitney U-test, z = 6.95, p = 0.008). This, however, does not include the paternity gained by certain males through EPP. Assignment of all EPY was attempted in both years. In 1994, 22 out of 30 (73%) EPY were assigned to their true father, whereas in 1995 only 7 out of 35 (14%) of EPY were assigned. Complete assignment of EPY could not be achieved probably due to some extra-pair males coming from outside the sampled population. Furthermore, assignment of EPY proved more difficult in 1995 due to a combination of nests frequently being adjacent to the periphery of the study site, and to complications with the profiling techniques. A total of 31 EPY were assigned to 10 extra-pair males. Adult males were more likely to gain EPY than were subadult males (9 out of 41 adult males, 1 out of 27 subadult males; Fisher Exact test, p = 0.04). In the one case of the subadult who gained EPP, the EPY sired were in the brood of another subadult male. There was also a trend for adult males overall to gain a higher mean number of EPY (adults 0.71 ± 0.25, subadults 0.18 ± 0.18; Mann-Whitney U-test, z = 2.71, p = 0.1), despite the low levels of paternity assignment.

Table 4.3 shows that there was a higher mean annual reproductive success in adult male nests (4.2 ± 1.7) than in subadult male nests (1.9 ± 2.0) (Mann-Whitney U-test, z = 10.70 p = 0.001). This was a consequence of the combined factors of adult males showing higher within pair paternity and gaining more EPY in other nests than subadults.

4.4.6 Multivariate analyses

Multivariate analyses were undertaken to examine the effect certain factors had on levels of EPP (Table 4.3) and the annual reproductive success of males (Table 4.4). The variables examined were: First egg date (Section 1.3.7); Synchrony index (Section 1.3.8);

Male body condition index (Section 1.3.5); Male status (either adult or subadult male); Year, the breeding season year; breeding density category (Section 4.3.2); and nearest neighbour distance (log transformed) (Section 4.3.2). Female body condition was not included in this analysis as the data set for females was incomplete ($n = 30$) and would have considerably reduced the power of the analyses.

Logistical regression analysis was used to determine which factors influenced the presence and amount of EPP in broods (see Figure 4.4). The models 'EPY in brood A', and 'Proportion of EPP A' indicate that male 'status' and 'breeding density' both had a significant influence on the presence of EPY and the level of EPP in a brood. The continuous measure of 'nearest neighbour distance' was then entered into the models in place of 'breeding density' (see models; EPY in brood B, and 'proportion of EPP B'). 'status' still had a significant effect while 'nearest neighbour distance' showed a positive but non-significant tendency to effect both the presence and proportion of EPP in a brood. There was also a non significant positive relationship between first egg date and EPY in brood. Therefore, this study suggests that the status/age of a male at a nest had a considerable effect on levels of EPP in that nest, and that this effect occurs independently of breeding density.

Table 4.4 Logistical regression models of the effects of certain variables on (i) the presence of EPY in a brood and (ii) the proportion of EPP per brood.

Explanatory variables	Dependent variables			
	EPY in broods		Proportion of EPP	
	A	B	A	B
First egg date	3.07§	3.09§	ns	ns
Synchrony index	ns	ns	ns	ns
Male body condition	ns	ns	ns	ns
Year	ns	ns	ns	ns
Male status	6.72**	6.75**	9.20**	9.35**
Density category	4.35*	-	5.04*	-
Nearest neighbour	-	3.06§	-	2.20
df	3,44	3,44	2,41	2,41
r ²	0.23	0.21	0.13	0.14

The effect of EPY in brood was investigated using nominal logistical regression analysis. The proportion of EPP per brood was split into 6 categories and required ordinal logistical regression analysis. Models 'A' include the breeding density category, which in 'B' Models is replaced by the nearest neighbour distance (log transformed). Wald chi-square values are shown for variables entered into the final model. § $p < 0.1$, * $p < 0.05$, ** $p < 0.01$. $N = 48$.

General linear model analyses were undertaken to examine the effect that certain factors had on the annual reproductive success of males (Table 4.5). The annual reproductive success models suggest that male status/age was the only variable tested to have an effect male reproductive success ($F = 16.10$, $p < 0.001$). Density and nearest neighbour distance had no effect and this is discussed in chapter 5.

Table 4.5 Stepwise general linear model analyses to examine the effect of several variables on the annual reproductive success of males.

Explanatory variables	Annual reproductive success of males	
	A	B
First egg date	0.40	0.20
Synchrony index	0.91	1.09
Male body condition index	0.12	0.55
Year	0.06	0.35
Male status	16.05***	16.05***
Breeding density category	2.52	-
Nearest neighbour distance (m)	-	1.45
r^2	0.26	0.26

In model 'A' breeding density category was used as the measure breeding density, while in model 'B' nearest neighbour distance (m) was used. F-to-enter or F-to-remove values are shown. Independent values highlighted in bold type were entered into the minimal adequate models ($N = 46$, *** $p < 0.005$). r^2 indicates the amount of variation which was explained by the minimal adequate models.

The results of all the multivariate analyses concur with those from the univariate analyses. The evidence from this study therefore suggests that male status/age is an important factor in relation to both the loss of paternity in a brood and the gaining of EPP by males. There is also a very highly significant effect of male status on the annual reproductive success of males.

4.5 DISCUSSION

4.5.1 Female extra-pair mate choice and male EPP

The possible female benefits of EPCs fall into the two main categories of direct and indirect benefits (reviewed in Birkhead & Møller 1992, Kempenaers 1993, Andersson 1994). The direct benefits to females include foraging rights, paternal investment and future mate appraisal. These are probably unimportant in this northern oriole population since: (i) they do not defend foraging territories; (ii) there is no evidence of parental investment by extra-pair males; (iii) they only have one clutch per year; (iv) the average lifespan is short; and (v) the adult return rate between years is very low (Williams 1988, pers. obs.).

The indirect-benefit hypothesis gives different predictions about the levels and distribution of EPP, but none of them are mutually exclusive. The 'good genes' hypotheses predicts that females paired to low quality males will preferentially copulate with better quality males. If females use the simple rule of obtaining EPCs with older 'on average better quality' males than their social mate, then it can be predicted that: (i) the majority of broods of females paired to subadults will contain EPP; (ii) subadults broods will contain EPP more often and have higher levels of EPP, and (iii) adult males will be gaining the EPP lost by subadults. The 'genetic diversity' hypotheses predicts that: (i) EPP will occur in all broods independent of male status; (ii) will be at a low level in these broods, and (iii) that the loss and gain of EPP by males will be independent of male status. Finally, the 'fertility insurance' hypothesis predicts that, assuming infertility is rare: (i) only a few broods will contain EPP; (ii) that the proportion of EPP will be high in these broods, and (iii) that these are as likely in adult as subadult broods. These 'fertility insurance' predictions are only valid if subadult males are assumed to have the same levels of fertility as adult males. It is possible that there is reduced fertility in subadults. Evidence for this idea comes from Hilton (1968) who found that subadult starlings had seminal-glomera half the size of adult males and that smaller seminal-glomera contain less sperm. Furthermore, Hill (1994) found that subadult black-headed grosbeaks had significantly smaller testis than adult males. A reduced level of fertility through reduced sperm levels could also explain why subadults lose so much paternity to adult males and needs further investigation. However in the northern orioles some subadult do not lose any paternity in their own broods and the levels of infertile eggs in these broods are no higher than in broods with mixed paternity. furthermore one subadult which was totally cuckolded in his own brood managed to gain EPP in the brood of another subadult male. These observations suggests that subadults have sufficient sperm levels to fertilise an entire brood. However, subadults could still be at a disadvantage during direct sperm competition within the female if their sperm levels are relatively lower than adult males. further work is required to investigate the relative fertility of adult and subadult males.

The results from the DNA profiling showed that adult broods contained EPP less frequently than subadult broods (10/31 v. 12/17 respectively), and had a lower mean proportion of EPP (20.6%) than did subadult broods (55.3%). The distribution of EPY across subadult broods is nonrandom, and appears to be bimodally distributed, with broods containing either no EPY or very high levels of EPY. Finally, the GLM multivariate analysis indicates that the proportion of EPP in a brood was related to male status. These results are more consistent with the predictions from the 'good genes' hypothesis than the alternative hypotheses.

Showing that females actually prefer adult males (both in social and in extra-pair mate choice), and that it is the adult males that are preferentially gaining EPCs (and subsequently EPP) from the females mated to subadult males would be stronger evidence for the 'good genes' hypothesis. In this study the mating success of subadults was lower than that of adult males which may indicate female preference for adult males. This concurs with results from other studies, both of northern orioles (Flood 1984, Williams 1988) and in other species (Enstrom 1993). Adult males also seemed to gain EPCs more often; this is supported by limited behavioural data (all three of the successful EPCs seen during this study involved adult males), but more importantly by the assignment of paternity to males. The majority of extra-pair males assigned using DNA profiling were adult males, and they sired a total of 41 EPY in 21 adult and subadult broods, while the single subadult male sired three EPY in one subadult brood. This is similar to the results from studies on other species, including house sparrows (Wetton *et al.* 1995) and yellowhammers (Sunberg & Dixon 1996) (but see Westneat 1995, Dixon *et al.* 1994).

The pattern of both social and extra-pair mate choice, and the distribution of EPP in and between broods, is therefore consistent with the 'good genes' hypothesis for female benefits from EPCs, but not with the 'genetic diversity' nor the 'fertility insurance' hypotheses. This suggests that females participate in EPCs for qualitative genetic benefits. They achieve this by copulating with older, brighter adult males, when socially paired to younger, drabber, subadult males, since these older, brighter adult males will, on average, be of better overall genetic quality.

This study does not, however, directly test the hypothesis that a female's extra-pair mate choice is based on plumage differences. Females may be choosing males based on other cues that are linked to age and plumage, e.g. song repertoire ability (Hasselquist 1996). Male body condition, however, was discounted. The plumage differences linked to male age are honest cues of age, which are quickly identifiable and directly obvious to females. This makes them ideal as cues for female mate-choice. Support for this idea comes from a mate choice experiment carried out on the hooded oriole, a closely related species that also displays DPM. The results from this study suggested that females preferred adult male plumage over subadult male plumage (Enstrom 1993). However, Butcher (1991) found that reducing the colour contrast of male northern orioles by bleaching the black areas of the

plumage did not seem to reduce social mate attraction compared to the controls (EPP was not investigated). Female choice may, however, be based on the orange (carotenoid pigment) component of the male plumage, and female choice for more colourful males has been shown in other species, e.g. house finches (Hill 1990, 1991) and yellowhammers (Sundberg 1995). Similar mate choice studies, and further studies which manipulate the plumage of wild males, are needed to confirm the link between male plumage and female extra-pair mate choice in northern orioles.

4.5.2 Male reproductive success

Variation between adults and subadults in mean levels of paternity lost and gained, results in adult males having a mean annual reproductive success rate twice that of subadult males (4.2 ± 1.7 vs. 1.9 ± 2.0 , respectively). This is based on assigning only 48% of EPY and higher levels of assignment would be expected to result in a greater difference in annual reproductive success. The multivariate general linear model analysis also indicated that male status was very strongly related to annual reproductive success.

Age was also suggested to be linked to reproductive success through EPP in house sparrows (Wetton *et al.* 1995), and in purple martins (though this was inferred from levels of EPP lost and did not include EPY assignment [Morton *et al.* 1990]). However, this study on northern orioles is the first to assign paternity in a species with DPM, and to show that adults gained the reproductive success lost by subadult males. It also suggests that this is due to female extra-pair mate choice for the older, adult males possibly based on plumage differences (as opposed to forced EPCs as suggested in purple martins [Morton *et al.* 1990]).

4.5.3 The implications for delayed plumage maturation

All but one of the hypotheses for DPM assume that the plumage of subadult males has an adaptive function that is in some way beneficial to the subadult male (see Section 4.1.2). The summer hypotheses are based on the assumption that subadults are at a competitive disadvantage, independent of plumage, to adult males during their first breeding season. This may be due to factors such as dominance (Lyon & Montgomerie 1986) or later spring arrival (Slagsvold & Lifjeld 1988). The subadult plumage is therefore suggested to have evolved to enhance either the survival or reproductive success of the subadults in some way. Enstrom (1993), however, points out that "If the subadult plumage is itself a significant cause of that disadvantage and if no advantage for subadult plumage can be identified, then the argument becomes invalid, because subadult plumage can no longer be

viewed as an adaptation.” This study shows evidence to link the considerable disadvantage of reduced reproductive success due to EPP with the subadult plumage in northern orioles. However, for the adaptation hypotheses to be valid DPM must have some benefit that will ultimately be advantageous to males.

The ‘summer crypsis’ hypothesis (Selander 1965 [sexual selection hypothesis], Proctor-Gray & Holmes 1981) suggests that subadults have little chance of breeding and should, therefore, forgo breeding and maximise survival to the next year by wearing a cryptic plumage to reduce predation and intraspecific aggression. This hypothesis, though possible, seems unlikely for several reasons in this species: firstly, consistent with other studies on subadult passerines (i.e. Proctor-gray & Holmes 1981, Flood 1984, Enstrom 1992), a substantial number of subadult males do attempt to breed each year and in doing so often come into confrontation with adult males. Secondly, the predation of nesting adult birds, at least at this site, was very rare (pers.obs), and finally, the lifespan of northern orioles is very short. The breeding threshold hypothesis (Studd & Robertson 1985a) which suggests subadult plumage would evolve to reduce direct competition with older males and hence increase their survival chances is also unsupported because of the reasons just stated.

According to the female-mimicry hypothesis (Rohwer *et al.* 1980), the female-like plumage of the subadult deceives the adult males long enough to allow them to establish territories in better quality areas and subsequently to increase their reproductive success. This was supported by Flood (1984), who showed that subadults do suffer less aggression from adult males. In this study subadults did nest nearer to adults than did other adults. However, nesting in close proximity to adults resulted in higher levels of lost paternity through EPP. Furthermore, the increased density of conspecifics was positively related to increased levels of EPP and lower reproductive success. It is possible that by nesting close to adults, subadults suffered less predation, either directly because of the presence of the adults, or because adult males had already obtained the favoured nest sites near magpies (see chapter 2). However, subadults did not nest closer to magpies than did adults, even when controlling for arrival time (see chapter 2) and conspecific density had no effect upon predation levels. This evidence, and the evidence that female choice was based on plumage differences, both conflict with this hypothesis, which can probably therefore be rejected.

The ‘status signalling’ hypothesis (Lyon & Montgomerie 1986) similarly predicts that subadults gain as a result of reduced aggression from adult males. This, though, is because the subadult plumage is less preferred by the females (in social pair choice) and thus is an honest signal of subordination to males. The problem, then, is why would subadults signal their inferiority if females also use these signals on which to base both social and extra-pair mate choice? The benefits suggested for this hypothesis are similar to those associated with the summer crypsis hypothesis. Therefore it seems unlikely that there would be a net benefit to the subadult males and so the hypothesis can be discounted.

Therefore, none of the ‘summer hypotheses’ seem to explain the presence of DPM as, far from being adaptive in the breeding season, DPM appears to incur significant costs, in reproductive terms, upon the subadults.

Alternatively, the ‘winter hypotheses’ suggest that the benefits of DPM are achieved on the winter breeding grounds, either through crypsis (reduced predation) or status signalling (reduced intraspecific aggression), and may be maladaptive in the breeding season, but retained due to constraints on moulting (Rohwer *et al.* 1983, Rohwer and Butcher 1988). A modified version of these hypotheses, the moult-constraint hypothesis (Chu 1994), also suggests that the subadult plumage is maladaptive in the breeding season, but does not require it to be adaptive in the non-breeding season. Instead, the plumage difference may occur simply as a result of constraints (e.g. phylogenetic or energetic). This study, based in the breeding season, cannot determine the presence or extent of winter benefits for subadult northern orioles, and therefore cannot distinguish between these hypotheses. However, the reduction in paternity (and annual reproductive success) in subadult broods adds a considerable cost to retaining this plumage. The winter advantages, therefore, would have to be far greater than previously predicted to outweigh these costs and allow for DPM to be maintained.

I suggest another hypothesis, the ‘honest plumage’ hypothesis, which may explain the DPM in the northern oriole (and other passerine species). This is based on the differences in the resource-sequestering ability of males coupled to female mate choice (social, extra-pair or both) based on plumage differences. The plumage of a range of birds (including northern orioles) is based on carotenoid pigments or their precursors (Ralph 1969). The extent and colour of this plumage varies depending on dietary access to these pigments. This is similar to the situation in guppies (*Poecilia reticulata*) where the brightness of colour spots increases with the carotenoid content of the diet and may therefore be an indicator of feeding success and general vigour (Kodric-Brown 1989, Endler 1980; reviewed in Andersson 1994). In guppies there is also evidence to suggest that female choice occurs in respect to these carotenoid spots, with females preferring the males with the brightest spots (Kodric-Brown 1989). In northern orioles (and other passerines) younger males may be at a disadvantage in sequestering these pigments, due to less efficient foraging or because pigments can only be found in certain locations, e.g. the summer breeding grounds, thus resulting in a plumage that honestly signals male quality/age (Mountjoy & Robertson 1988, Hill 1991, 1992, Lozano 1994). Females may be basing social and/or extra-pair mate choice upon these age-linked plumage differences, as the evidence from several studies suggests (Enstrom 1993, Sunberg 1995, Sætre *et al.* 1995, Sundberg & Dixon 1996, this study). Therefore, younger males will be at a considerable reproductive disadvantage to older males, as they cannot compete in terms of the honest-plumage signal. It may, therefore, pay to reduce the costs associated with acquiring and maintaining an adult plumage by reducing or totally foregoing the spring moult (as is the case in this species and a large number of DPM passerines

[Rohwer & Butcher 1988]). This hypothesis may also be able to explain the correlation between life span and DPM that Studd & Robertson (1985) found in passerine birds as female choice for older males is likely to be more important in longer living species. Although the selective mechanism for DPM is different, the proximate functions of this hypothesis may concur with some of the other hypotheses, i.e. reduced intraspecific aggression and reduced predation. Further work will be needed to distinguish the exact predictions of the honest-plumage hypothesis, and to devise experiments to discriminate between this and other hypotheses.

4.5.4 Conclusion

In conclusion, this study does not support the summer hypotheses for DPM in the northern oriole. The winter and 'breeding threshold' hypotheses also seem unlikely due to the higher reproductive costs found to be associated with the subadult plumage through female extra-pair mate choice. An alternative hypothesis (the 'honest plumage' hypothesis) is suggested that is consistent with the results of this study.

Although these results and conclusions are based on an investigation of DPM in northern orioles, they have implications for all species showing DPM. The effect of EPP on the estimated reproductive success of adults and subadults must be taken into account when investigating DPM in any species, as must the possibility of female choice for gametic partner, based on plumage differences. The new 'honest plumage' hypothesis should be considered as a possible explanation for the evolution of DPM in addition to the other hypotheses.

CHAPTER 5

BREEDING DENSITY AND EXTRA-PAIR PATERNITY

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CHAPTER 5

BREEDING DENSITY AND EXTRA-PAIR PATERNITY

5.1 INTRODUCTION

The recent advent of DNA profiling has led to an abundance of studies investigating the use of mixed reproductive strategies in animals (see Chapter 3 and references therein). In monogamous birds the levels of extra-pair paternity (EPP) and intra-specific brood parasitism (IBP) have been reported to vary widely both between species and between populations of the same species (reviewed in Gowaty 1996). Several ecological and demographic factors, including breeding density, breeding synchrony and age or experience of breeders, have been suggested to influence the use of alternative reproductive strategies and therefore to explain some of this variation (Emlen & Oring 1977, Westneat *et al.* 1990).

It has been suggested that a greater density of breeding pairs increases the opportunities for extra-pair copulations (EPCs) (Alexander 1974, Hoogland & Sherman 1976, Birkhead 1978, Gladstone 1979) and, therefore, will lead to an increased frequency of EPP (reviewed in Wittenberger & Hunt 1985, Birkhead & Møller 1992; but see Westneat *et al.* 1990). This may be due to an increase in the density of birds *per se*, so increasing the number of potential EPC partners, or by affecting the operational sex ratio promoting EPCs. Alternatively, the different ecological factors and pressures occurring at the different densities may alter the cost and benefits for individuals either seeking EPCs or employing mate guarding behaviour (reviewed in Westneat *et al.* 1990, Birkhead & Møller 1992).

Various studies of single species have reported that the frequency of EPCs is positively related to density (Møller 1987, 1991; Hatchwell 1988). Birkhead & Møller (1993) tested this hypothesis in a comparative study across species and found that the proportion of females involved in EPCs was positively related to the degree of sociality. Another study examining the influence of density through intra- and inter-specific pairwise comparisons, also suggested that EPCs were more frequent in populations, or species, nesting at higher densities (Møller & Birkhead 1992). In some populations, the frequency of EPP has been reported to increase with an increase in the breeding density of conspecifics (Gowaty & Bridges 1991a, Double 1995, Hoi & Hoi-Leitner 1997). However, other studies have failed to find an effect of population density (Gowaty & Bridges 1991b, Dunn *et al.* 1994a). It should also be noted that Birkhead & Møller (1995) found a positive relationship

between levels of EPC and EPP in a pairwise comparison across various bird species (including polyandrous and polygynandrous species). There was, however, no relationship when the analysis was restricted to monogamous bird species.

Alexander (1974) suggested that the various selective forces that favour coloniality can essentially be grouped into three main categories: (i) reduced predation risk, (ii) increased foraging efficiency, and (iii) restricted suitable nest sites or habitat (see also reviews in; Mock 1985, Wittenberger & Hunt 1985, and Chapter 2). Originally, the higher predicted rates of EPC and subsequent EPP in colonies were seen as a consequence and cost of breeding at increased nesting densities (Alexander 1974, Hoogland & Sherman 1976, Gladstone 1979, Wittenberger & Hunt 1985). More recently, the assumed increased opportunities for EPCs in colonies have been seen as a potential pre-laying benefit that may actually favour the formation and maintenance of colonies (Morton *et al.* 1990, Birkhead & Møller 1992, Wagner 1993, Hoi & Hoi-Leitner 1997).

The benefits of EPCs and subsequent EPP will not be evenly distributed amongst individual members of a population. Furthermore, females and males acquire different benefits through EPCs (reviewed in Westneat *et al.* 1990, Birkhead & Møller 1992, Kempenaers 1993, Andersson 1994). Consequently, the distribution of benefits amongst individuals may also differ between the sexes. In theory, some males may suffer reduced reproductive success through loss of paternity at higher densities while others may realise an increase as a result of successfully gaining EPP (Birkhead 1979, Gladstone 1979, Wittenberger & Hunt 1987). Thus, there may be an increased variance of male reproductive success at higher breeding densities. The maintenance of higher breeding densities and coloniality may, therefore, be due to the behaviour of males of high phenotypic quality, since these individuals are likely to achieve a high net benefit in terms of reproductive success through EPP (Birkhead & Møller 1992). Evidence from a study on purple martins suggested that adult males obtained EPP through forced EPCs with the mates of nearby subadult males while losing little paternity in their own nests (Morton *et al.* 1990, but see Wagner 1996). This resulted in the adult males having a significantly higher annual reproductive success than the subadult males (see also Wetton *et al.* 1995, Sundberg & Dixon 1996). However, Wagner (1993) argued that males who suffer increased risks of cuckoldry should avoid breeding colonially if they can breed solitarily. If such individuals do not breed solitarily under these circumstances then this suggests there must be other factors selecting for coloniality.

Whereas only a proportion of males should achieve a net benefit through EPCs, it is possible that all or most females can potentially benefit through EPCs. Evidence to support this idea comes from the growing number of reports of females co-operating in, pursuing and/or controlling EPCs (reviewed in Birkhead & Møller 1993; Dunn *et al.* 1994a, 1994b; Gray 1997). Wagner (1993) proposed that the pursuit of EPCs by females may be a factor selecting for increased breeding density and coloniality in birds. He suggested that females

may nest at higher densities to increase the opportunities for EPCs. Males who gain EPCs will benefit from the higher breeding density, while other males who risk cuckoldry would be forced to breed at the higher densities if that is where the females choose to breed. Wagner (1993) supported this hypothesis with evidence from the literature and also from his own study on colonial razorbills which '....identified EPC as a pre-laying aim of females which may result in the clustering of males'. Evidence to support this hypothesis comes from work carried out on the bearded tit (Hoi & Hoi-Leitner 1997) which suggests that high quality females nest at high density to secure extra-pair fertilisations by high quality males. As yet no other studies have tested this hypothesis.

5.2 AIMS AND PREDICTIONS

The aim of this chapter is to investigate the potential influence that breeding density has upon levels of EPP and how this affects the annual reproductive success of males. At Hastings Reservation the northern orioles exhibit a range of breeding densities from solitary to varying degrees of coloniality (see Chapter 1). If higher breeding density increases the opportunities for EPCs, then it can be predicted that this will result in higher levels of EPP. Furthermore, if female choice for genetic quality is occurring (Fisher 1930, Zahavi 1975, 1977, Weatherhead & Robertson 1979, Hamilton & Zuk, 1982) then a greater variance in male reproductive success should be expected to occur at higher densities. This may also affect the relative frequency of EPP, and the annual reproductive success, achieved by adult and subadult males.

The semi-colonial nature of northern orioles at Hastings, combined with the evidence that males and females pursue alternative reproductive strategies (see Chapters 2 and 4), also provides an opportunity to investigate the 'EPC Hypothesis of colony formation' (Wagner 1993). This study aims to test whether the northern oriole data are consistent with the predictions of the 'EPC Hypothesis', and to determine if the female pursuit of EPCs may influence the local breeding density of northern orioles.

5.2 METHODS

5.2.1 General methodology, biometry and ageing

Work was carried out at Hastings Natural History Reservation, Monterey County, California, over the 1994 and 1995 breeding seasons (for general field methods see Chapter 1). Birds were caught as soon after arrival as possible to minimise the possibility of disturbance during the subsequent fertile period of the female (see Section 1.6.2). Captured birds were ringed and then sexed and aged using plumage characteristics (Pyle *et al.* 1987)

(see Sections 1.6.4 & 5.3.1). Biometric data were taken for all birds. These included measurements of tarsus length, wing length and body weight (see Section 1.6.4).

Individuals could not be caught and weighed on a standard date during the breeding season. Both the day of the season, and the time of day at which an individual was caught, had a significant effect upon the weight (see Section 1.6.5). These effects were removed by taking the residuals of the regression of weight against the catch day, and catch time of each individual. Body-condition indices were also calculated from the residuals of the regression of weight on tarsus (see Section 1.6.5)

The composition of the different breeding densities may vary with respect to factors such as the age, breeding experience or quality of the individuals within them. Any differences between breeding densities could greatly influence the use of reproductive strategies within the colonies (Morton *et al.* 1990). Therefore, the age and size/vigour (tarsus length, wing length, weight, and body condition index) of the birds within the different breeding densities, were investigated to determine if mean differences between the densities could have affected the frequencies of EPP.

5.2.2 Breeding synchrony and first egg dates

It has been predicted that the degree of breeding synchrony will affect the frequency of alternative reproductive strategies, and will be itself affected by breeding density (Westneat *et al.* 1990). Therefore, the influence of breeding synchrony must be considered in any investigation into the effect of breeding density on EPP. A synchrony index was therefore calculated for each nest in which egg laying was initiated (Kempnaers 1993; see Section 1.6.8) and included in subsequent analyses.

The arrival date of individual birds may affect the density at which they breed. This may occur if suitable nesting habitat or nest sites are restricted (see Chapter 2), or if later-arriving birds prefer to nest close to other conspecifics. First-egg dates are used as an index of arrival date as the detection of newly arriving birds was not always immediate and therefore settlement dates based on first sightings may be inaccurate.

5.2.3 Nearest-neighbour distances and breeding densities

The nearest-neighbour distance was measured between each nest and the closest active conspecific nest at the time of clutch initiation (first egg date). The first egg date was used in determining the density measures as this is the mid-point of the female's fertile period and is, therefore, the time during which the breeding density can affect EPP. Distances within 100 metres were measured directly (to the nearest metre), whereas distances over 100 metres were measured from an aerial photograph of the study area (accurate to

within 5 metres). Each northern oriole nest was also allocated to a breeding density category on the first egg date for that nest. A distance of 100 m between a northern oriole nest and its nearest conspecific was used to distinguish 'solitary' breeding pairs from 'colonial' breeding pairs (following Dunn *et al.* 1994a). The breeding density category was also included in the analyses of EPP and reproductive success as it may be a more accurate indicator of the possibility of individuals interacting than nearest neighbour distance. Table 5.1 shows the average nearest-neighbour distances for colonial and solitary nests. The 50 metre separation between the ranges of the 2 groups suggests that the classification of colonial and solitary nests reflects a natural pattern in the breeding dispersal of northern oriole nests.

Table 5.1 The nearest neighbour distances of northern oriole pairs breeding at the different density categories.

Breeding density	No. of pairs	Nearest neighbour distances (m)		
		Mean $\pm$ SE	Median	Range
Colonial	26	38 $\pm$ 5	39	3-80
Solitary	22	312 $\pm$ 26	230	130-740

5.2.4 Extra-pair paternity and annual reproductive success

Blood samples were taken from adults and from all chicks surviving at chick bleeding (approximately day 9). DNA profiling techniques were used to identify extra-pair young (EPY) accurately in a brood, and to attempt to assign these EPY to their true genetic fathers (see Chapter 3). The distribution of EPP between clutches was analysed in relation to the different measures of breeding density (see Section 5.2.3) and also to the other possible explanatory variables to determine whether they had an effect upon the frequency of EPP. The other explanatory variables included first egg date, breeding synchrony, male age/status, body condition, and year. Various estimates of reproductive success of broods were also calculated as dependent variables. These included the mean within-pair paternity fledged from the male's own brood (mean paternity fledged), the mean EPP gained from other male's broods (mean EPP gained) and the annual reproductive success (mean paternity fledged + mean EPP gained).

5.2.5 Data analysis

Unless otherwise stated, all results are based on the analysis of the 48 breeding pairs which survived until at least day 9, and for which DNA profiling data were obtained. The general linear model analysis and the logistic analysis were performed as described in Section 1.7.

5.3 RESULTS

5.3.1 Breeding densities

No difference between years for any of the variables examined below was significant and so data from both 1994 and 1995 were pooled.

5.3.2 Breeding density composition

Female age was not tested against breeding density in this study as it was not possible to determine (see Section 1.3.4). In males, however, it was possible to distinguish between subadult (first year) and adult males (at least second year) (see Section 1.3.4 and Chapter 4). The average nearest neighbour distance of adult and subadult males did not differ (Mann-Whitney U-test, $z = 1.68$, $p = 0.20$), nor did the relative number of adults or subadults nesting at the different breeding density categories (Fisher exact test, $p = 0.37$). Therefore, there is no evidence to suggest that there was any difference in the ages of males occupying the different breeding densities.

A series of phenotypic characters were then tested against breeding density and nearest neighbour distance in an attempt to identify any possible differences between individuals breeding at different densities. Tables 5.2 and 5.3 show that there were no differences in the male phenotypic characters in relation to either nearest neighbour distance or breeding density category. However, Table 5.2 indicates that females with closer nearest neighbours had significantly smaller tarsi ($F_{1,33} = 4.49$, $p = 0.041$) and shorter wings ($F_{1,33} = 4.81$, $p = 0.035$) than more isolated females, and the density category results (Table 5.3) also show that colonial females had smaller tarsi than solitary females (colonial = 27.2 ± 0.81 , solitary = 27.8 ± 0.69 ; t-test $t = 2.60$, $p = 0.015$). However, no difference was detected in wing length between the different density categories. There was also no difference in the weight or body condition index of females nesting at different densities.

Differences in the size of females did not appear to influence a female's participation in EPP; No difference was found in the female's wing length or tarsi length between females

with or without EPY in their broods, nor was there any relationship between these parameters and the frequency of EPP in a brood ($p > 0.1$ for all).

Table 5.2 The relationship between nearest neighbour distance and a series of phenotypic characteristics for both male and female birds. Regression F values are shown (§ $p < 0.1$, * $p < 0.05$).

Phenotypic character	Regression F-values	
	Males ($F_{1,44}$)	Females ($F_{1,33}$)
Wing length	2.73	4.49*
Weight	0.23	1.22
Tarsus	0.25	4.81*
Body condition index	0.21	1.02

Table 5.3 The relationship between breeding density category and a series of phenotypic characteristics for both male and female birds. t-test values are shown (§ $p < 0.1$, * $p < 0.05$).

Phenotypic character	t-test	
	Males (n = 44)	Females (n = 33)
Wing length	0.99	1.86
Weight	-1.64	-1.07
Tarsus	1.86§	2.60*
Body condition index	-1.23	-1.29

5.3.3 First egg date and breeding density

Section 5.3.2 suggests that colonially breeding females were smaller (with shorter wing lengths and tarsi) than solitary females. This may have been due to smaller female's arriving at the breeding grounds later than larger females and being forced to nest at higher densities as a consequence. There was, however, no relationship between either a female's wing length or length of tarsus, and her first egg date ($F_{1,33} = 0.80$, $p = 0.38$; and $F_{1,33} = 2.43$, $p = 0.13$; respectively). However, if the Bonferroni method correction for multiple comparisons is applied then a probability of 0.125 ($0.05/n$ where n = the number of

comparisons on the same data set) is required to indicate biological significance. In this case none of the variables tested above are significantly different between density categories. Additionally, no relationship was found between nearest neighbour distance and first egg date ($F_{1,46} = 0.12$, $p = 0.73$), nor was there a difference between the first egg dates of colonially or solitarily breeding pairs (Mann-Whitney U-test, $z = 0.16$, $p = 0.69$).

5.3.4 Breeding synchrony and breeding density

No relationship was found between nearest neighbour distance and breeding synchrony ($F_{1,46} = 1.70$, $p = 0.20$). Nor was there any difference in the breeding synchrony of solitary nests compared to colonial nests (ANOVA, $F = 0.22$, $p = 0.64$). The relationship between the mean proportion of EPP in a brood and breeding synchrony is shown in Figure 5.1. There was no effect of breeding synchrony on the mean proportion of EPP in a brood ($F_{1,46} = 0.23$, $p = 0.63$). The number of broods containing EPY was also unaffected by breeding synchrony (Mann-Whitney U-test, $z = 0.32$, $p = 0.57$).

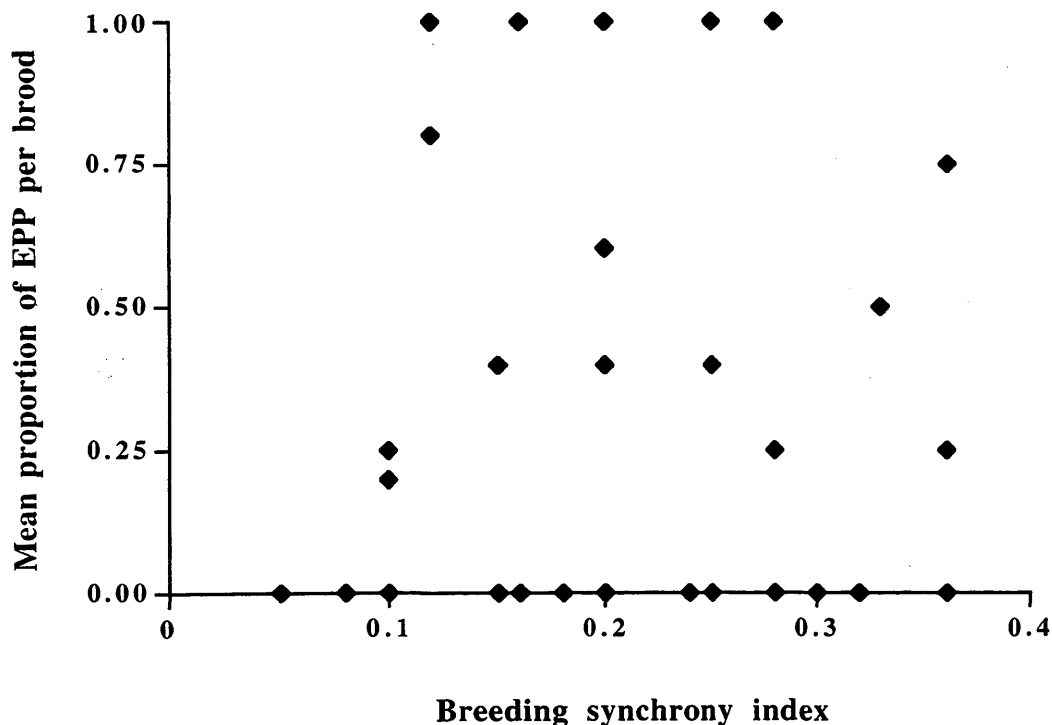


Figure 5.1 The proportion of extra-pair paternity in each brood in relation to breeding synchrony. ($F_{1,46} = 0.23$, $p = 0.63$).

5.3.5 Extra-pair paternity and breeding density

During this study DNA profiling of 48 clutches containing a total of 202 chicks was completed. Twenty-two clutches (46%) contained extra-pair young (EPY) and 65 (32%) of the 202 chicks were sired by extra-pair males. Two cases of intra-specific brood parasitism were identified in two separate colonial nests. The overall distribution of EPY was not random across broods, but appeared to show a bimodal distribution with most nests containing either all or very few EPY (see Chapter 3).

The distribution of EPP was then analysed in relation to the two different measures of breeding density. Broods containing at least one EPY had closer nearest neighbours than broods without EPY (median distance, EPY broods = 59 m, no EPY broods = 78 m: Mann-Whitney U-test, $z = 4.64$, $p = 0.031$). Furthermore, there was a negative correlation between the average proportion of EPP per brood and the nearest neighbour distance (Spearman rank correlation: $r = -0.32$, $p < 0.05$) (Figure 5.2).

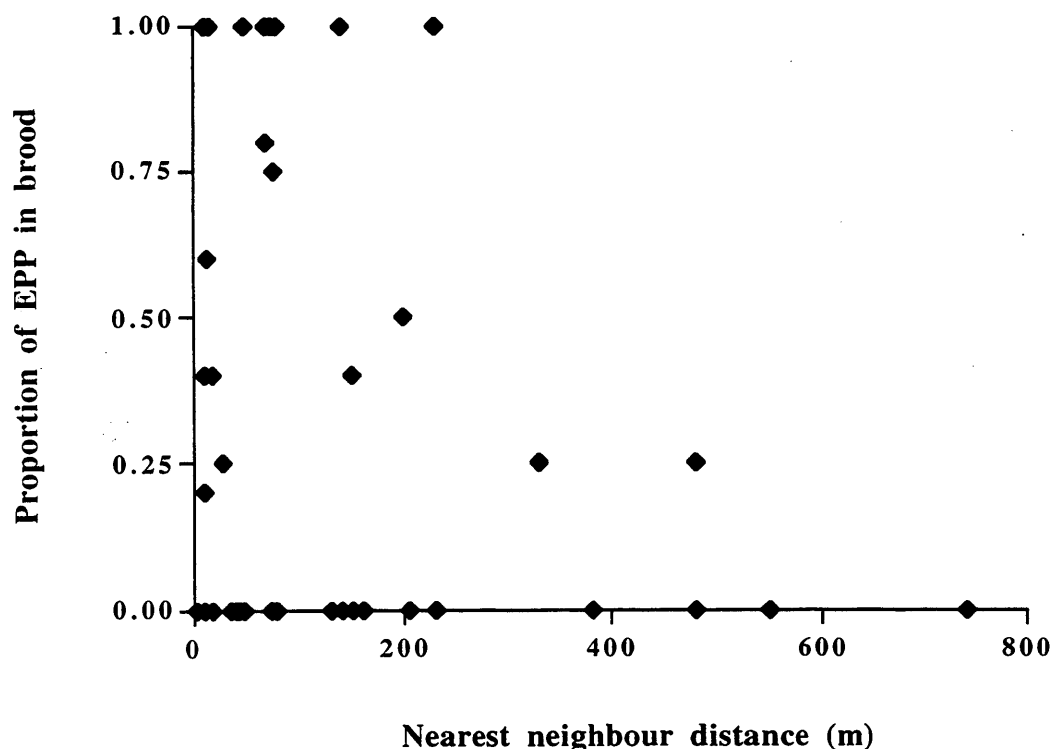


Figure 5.2 The proportion of EPP in a brood in relation to the nearest neighbour distance. Spearman rank correlation: $r = -0.32$, $p < 0.05$.

The analysis of EPP in relation to breeding density category supports the results of the nearest neighbour analysis. Figure 5.3 shows that the mean proportion of EPP per brood

was higher in colonial pairs than in solitary pairs for both adult and subadult male pairs. When tested separately, the proportion of EPP at the different density categories was significantly different for adult males (Mann-Whitney U-test, $z = 3.72$, $p = 0.05$) but not for subadult males (Mann-Whitney U-test, $z = 1.48$, $p = 0.23$). However, this test in subadult males was of low power due to the small sample size when the data are split into four subsets (see Figure 5.3).

The effect of status within each density category was also tested. The difference between adult and subadult males approached significance for solitary males (Mann-Whitney U-test, $z = 3.36$, $p = 0.067$) and was significant for colonially breeding males (Mann-Whitney U, $z = 5.27$, $p = 0.022$). Therefore, male age/status had an effect on EPP for both breeding density categories, and the relative difference between the two age categories appeared to remain the same (see Chapter 4). The analysis for solitary individuals was again of limited power due to the smaller sample sizes at this breeding density.

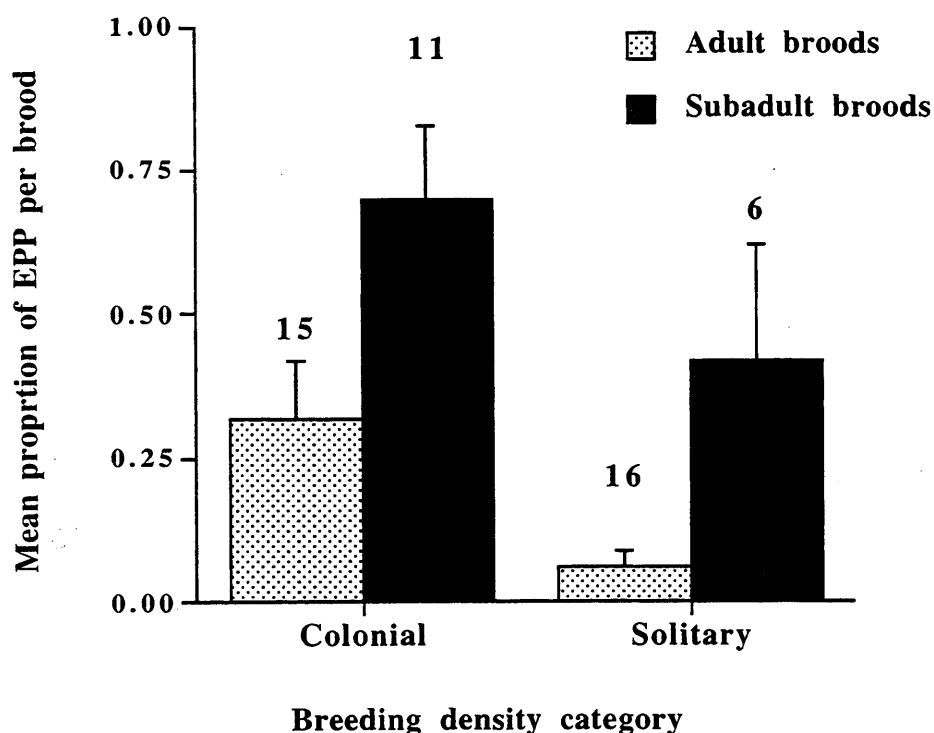


Figure 5.3 The mean proportion of EPP per brood in relation to breeding density for adult and subadult males separately. Columns indicate the mean ($\pm$ SE). Sample sizes are shown above bars. (Mann-Whitney U-tests, [solitary v. colonial] adults, $z = 3.72$, $p = 0.054$; subadults, $z = 1.48$, $p = 0.23$; [adults v. subadults] colonial, $z = 5.27$, $p = 0.022$; solitary, $z = 3.36$, $p = 0.067$).

As there was no difference in the numbers of adults and subadults nesting at each breeding density the data from both groups could be combined. Overall, significantly more

colonial broods contained at least one EPY (16/26) than did solitary broods (6/22) (Fisher's exact test, $p = 0.023$) and there was a higher proportion of EPP in colonial nests than in solitary nests (mean EPP; colonial = 0.48 ± 0.09 , solitary = 0.16 ± 0.07 : Mann-Whitney U, $z = 6.76$, $p = 0.01$) (figure 5.4). This indicates that higher frequencies of EPP occur at higher breeding densities.

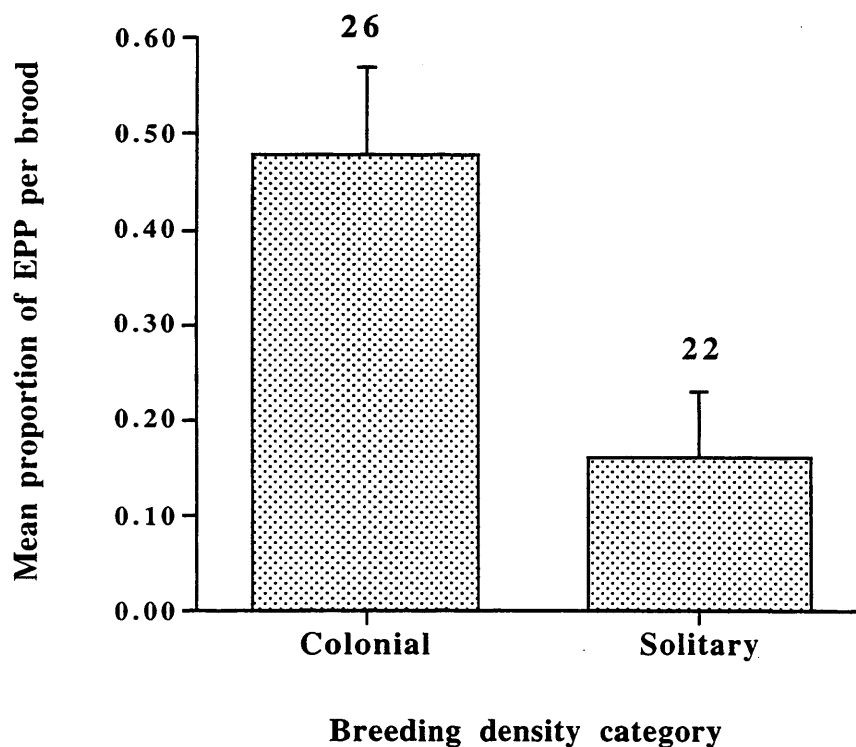


Figure 5.4 The mean proportion of EPP per brood in relation to breeding density. Columns indicate the mean ($\pm$ SE). Sample sizes are shown above bars. (Mann-Whitney U-test, $z = 6.76$, $p = 0.01$).

Logistic regression analyses were also used to determine which factors influenced the presence and amount of EPP in broods (Table 5.4). The models 'EPY in brood A', and 'Proportion of EPP in A' indicate that male status and breeding density both had a significant influence on the presence and proportion of EPP in a brood. The continuous measure of nearest neighbour distance was subsequently entered into the models in place of breeding density (see models: 'EPY in brood B', and 'Proportion of EPP in B'). In these models male status still had a significant effect while nearest neighbour distance showed a positive but non-significant tendency to affect both the presence and proportion of EPP in a brood. There was also a positive but non-significant relationship between first egg date and EPY in a brood that suggests that earlier broods were less likely to contain EPY.

The results from the multivariate analyses concur with those from the univariate analyses. Therefore, the evidence from this study suggests that, while male status has the largest effect on both dependent variables, higher breeding densities of pairs also resulted in more broods containing EPY and higher mean levels of EPP per brood. This effect is more pronounced when using the density category measure of breeding density rather than the nearest neighbour distances.

Table 5.4 Logistic regression models of the effects of potential explanatory variables on (i) the presence of EPY in a brood and (ii) the proportion of EPP per brood.

Explanatory variables	Dependent variables			
	EPY in broods		Proportion of EPP	
	A	B	A	B
FED	3.07§	3.09§	ns	ns
SI	ns	ns	ns	ns
Male BCI	ns	ns	ns	ns
Year	ns	ns	ns	ns
Male Status	6.72**	6.75**	9.20**	9.35**
Density category	4.35*	-	5.04*	-
Nearest neighbour	-	3.06§	-	2.20
df	3, 44	3, 44	2, 41	2, 41
r²	0.23	0.21	0.13	0.14

The presence of EPY in a brood was investigated using nominal logistic regression. The proportion of EPP per brood was split into 6 categories (see Section 1.7) and, therefore, required ordinal logistic regression analyses. Models 'A' include the breeding density category. In the 'B' Models this is replaced by the nearest neighbour distance (log transformed). Wald chi-square values are shown for variables entered into the final model. (§p < 0.1, *p < 0.05, **p < 0.01)

5.3.6 Annual reproductive success and breeding density

Overall, the assignment of EPP between males resulted in a significant difference between the number of offspring fledged and the real annual reproductive success of males in the population (Wilcoxon signed ranks test, $z = 2.83$, $p = 0.005$).

Breeding density had a significant influence upon the presence and levels of EPP in a brood, and this may subsequently have altered the distribution of annual reproductive success between males. The positive relationship between EPP and nearest neighbour

distance (Figure 5.2) inversely resulted in a negative relationship between within-pair paternity and nearest neighbour distance (Spearman rank correlation: $r_s = -0.33$, $p < 0.05$). No relationship was found between EPP gained and nearest neighbour distance (Spearman rank correlation, $r_s = -0.023$, $p > 0.5$), nor was there a relationship between annual reproductive success and nearest neighbour distance (Spearman rank correlation: $r_s = 0.27$, $p > 0.10$).

When the breeding density categories were compared no difference was found between colonial or solitary pairs, either in terms of the original clutch size (Mann-Whitney U-test, $n = 27$, $z = 2.33$, $p = 0.13$), or the number of chicks fledged (Mann-Whitney U-test, $z = 0.003$, $p = 0.96$) (Table 5.5). Colonially breeding males experienced higher levels of EPP than solitary males (Section 5.3.1) and consequently had lower levels of within-pair paternity (Mann-Whitney U, $z = 8.46$, $p = 0.004$). The effect that the relationship between breeding density and EPP had upon different measures of reproductive success is summarised in Table 5.5.

When both years were combined in the analysis the mean level of EPP gained by males did not differ significantly between colonial males and solitary males (Mann-Whitney U-test, $z = 1.04$, $p = 0.31$). However, for the two years together only 44.6% (29/65) of EPY were assigned to their true genetic fathers (see section 3.4.5). In 1994, 73% of EPY could be assigned to their genetic fathers while in 1995 only 14% (7/35) of EPY could be assigned. Using only the more complete 1994 data revealed that high density males did gain significantly more EPY compared with low density males (solitary = $0.11 \pm .33$ v colonial = 1.7 ± 3.8 : Mann-Whitney U, $z = 3.923$, $p = 0.048$). This was not because it is easier to assign the EPY from the higher density category, as the levels of assignment were no different for EPY from either density category ($\chi^2 = 0.17$, $p > 0.5$). Therefore, although colonial males on average lose more EPP they also appear to gain more EPP. When the data from both years were combined, the tendency for colonial males to gain more EPP resulted in there being only a non-significant tendency for colonial males to have a lower annual reproductive success than solitary males (Mann-Whitney U, $z = 3.38$, $p = 0.068$) (Figure 5.5). However, the separate analysis of the more complete 1994 data alone indicates that there was no difference in the reproductive success of solitary or colonial males (4.22 ± 3.94 v. 2.70 ± 2.50 , respectively: Mann-Whitney U, $z = 1.76$, $p = 0.17$). As many EPY were not assigned to males in 1995 it seems likely that the actual success of colonial males was greater than estimated, and that the difference between colonial and solitary males was even less than has been estimated.

Table 5.5 Summary of the reproductive success of colonial and solitary males using a range of measures.

Breeding density	Colonial	Solitary	z
Number of broods	26	22	
Clutch size (N = 12 v.15)	5.27 ± 0.18	4.83 ± 0.21	2.33
Number of fledglings	4.46 ± 0.21	4.27 ± 0.21	0.00
Within-pair paternity	2.10 ± 0.37	3.77 ± 0.35	8.46**
EPP gained	0.77 ± 0.30	0.23 ± 0.15	1.04
Annual reproductive success	2.85 ± 0.44	4.00 ± 0.38	3.38§

Mean ($\pm$ SE) and the Mann-Whitney U test statistic are shown. (§ $p < 0.1$, * $p < 0.05$, ** $p < 0.01$. Clutch size N = 27. Annual reproductive success = mean paternity fledged + mean EPP gained.

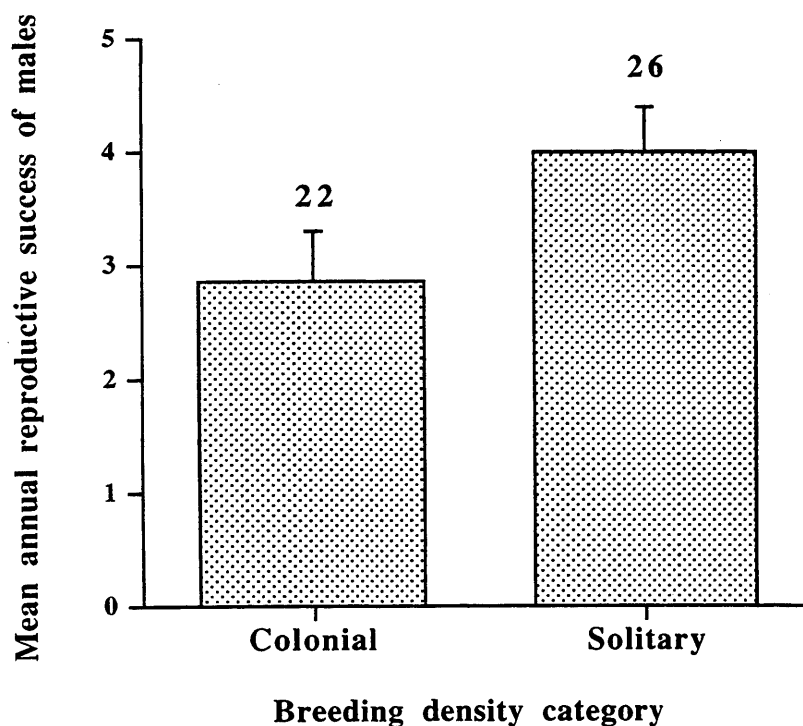


Figure 5.5 The mean annual reproductive success of males in relation to breeding density category. Columns indicate mean ($\pm$ SE). Sample sizes are shown above bars. (Mann-Whitney U, $z = 3.38$, $p = 0.068$).

Breeding density category may, however, affect adults and subadults differently. When analysed separately no significant difference was found between density categories for either adults (Mann-Whitney U, $z = 1.22$, $p = 0.27$) or subadults (Mann-Whitney U, $z = 1.04$, $p = 0.31$) (see Figure 5.6.). This suggests that there that there was no difference in the effect of breeding density on adult or subadult males.

The influence of status within each density category was also tested. The difference between adult and subadult males approached significance within the solitary density category (Mann-Whitney U-test, $z = 3.56$, $p = 0.059$) and was found to be significant for colonially breeding males (Mann-Whitney U-test, $z = 5.27$, $p = 0.019$). This indicates that male status had an affect upon reproductive success at both breeding density categories as result of adult males gaining EPP in the nests of nearby subadult males.

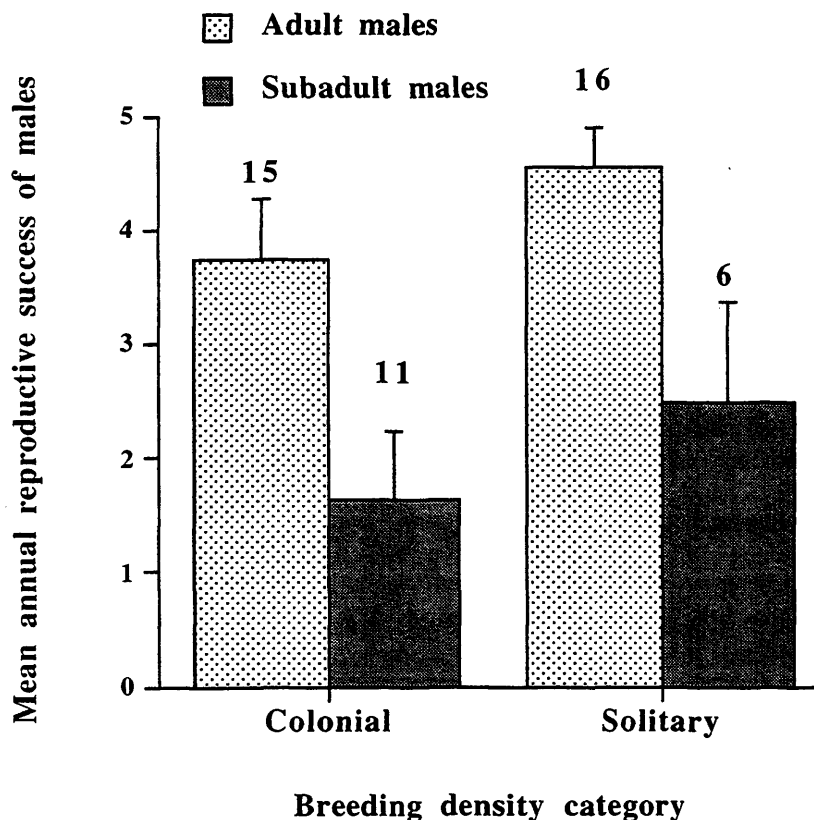


Figure 5.6 The mean annual reproductive success of adult and subadult males in relation to breeding density category. Columns indicate mean ($\pm$ SE). Sample sizes are shown above bars. (Mann-Whitney U-tests; [solitary v. colonial] adults; $z = 1.22$, $p = 0.27$; subadults, $z = 1.04$, $p = 0.31$; [adults v. subadults] colonial; $z = 5.27$, $p = 0.019$; solitary, $z = 3.56$, $p = 0.059$).

A general linear model analysis was undertaken in an attempt to reveal the influence that certain factors had on the annual reproductive success of males (Table 5.6). The variables examined were: first egg date (Section 1.3.7); synchrony index (Section 1.3.8); male body condition index (Section 1.3.5); calendar year; male status (either adult or subadult male); breeding density category (Section 5.3.2); and nearest neighbour distance (log transformed) (Section 5.3.2). Female body condition may have had an effect but was not entered as a variable because data were available for only a small subset of the population.

Figure 5.6 indicates that male status was the only variable tested to have a significant effect on male reproductive success ($F = 13.10$, $p = 0.001$). This is discussed in Chapter 4. The models also suggest that breeding density category (or nearest neighbour distance) did not significantly influence annual reproductive success independently of male status ($F = 2.52$, $p = 0.12$).

Table 5.6. Stepwise general linear models to examine the effect of several variables on the annual reproductive success of males.

Explanatory variables	Annual reproductive success of males	
	A	B
First egg date	0.40	0.20
Synchrony index	0.91	1.09
Male body condition index	0.12	0.55
Year	0.06	0.35
Male status	16.05***	16.05***
Breeding density category	2.52	-
Nearest neighbour distance (m)	-	1.45
r^2	0.26	0.26

($N = 46$). In model 'A' breeding density category was used as the measure breeding density, while in model 'B' nearest neighbour distance (m) was used. F-to-enter or F-to-remove values are shown. Independent values highlighted in bold type were entered into the minimal adequate models. (*** $p < 0.005$). r^2 indicates the amount of variation which was explained by the minimal adequate models.

It was not possible to determine whether the variance of male reproductive success increased with increasing density due to the non-normal distribution of the data and the small sample sizes

5.4 DISCUSSION

5.4.1 The effect of breeding density on extra-pair paternity

The breeding density of individuals is one of several factors predicted to influence EPP (Westneat *et al.* 1990). In birds, a higher density of breeding pairs has been predicted to increase the opportunities for EPCs and hence to increase the levels of EPP. This hypothesis was tested using two different measures of breeding density: the nearest neighbour distance and the breeding density category (solitary or colonial). Density categories were used in addition to nearest neighbour distance for two reasons: (i) these densities reflect the natural dispersion patterns of northern orioles at Hastings (Williams 1988); and (ii) the presence or absence of other nesting pairs within 100 m may be a better indicator of the opportunities for interactions than a continuous nearest neighbour distance scale. Once pairs of birds are over 100 m apart any further increase in the nearest neighbour distance may have little effect on opportunity to participate in EPCs (see Westneat *et al.* 1990).

In this study, broods containing EPY had closer nearest neighbours than broods without EPY, and there was a significant negative correlation between the proportion of EPP in a brood and nearest neighbour distance. Additionally, more colonial than solitary broods contained EPY (16/26 v 6/22, respectively) and there was a higher proportion of EPP in colonial rather than solitary nests (mean EPP per brood: colonial = 48%, solitary = 16%). However, other variables have been predicted to have an effect on EPP (Westneat *et al.* 1990, Birkhead & Møller 1992) and it is possible that the above results are a consequence of certain factors that may covary with breeding density.

Breeding synchrony is one factor that has been predicted to increase with breeding density and also to influence the frequency of EPP directly (Stutchbury & Morton 1995). However, EPP was not related to breeding synchrony and breeding synchrony was not related to breeding density. This variable cannot, therefore, explain the variation in EPP at the different breeding densities. First egg date might also be expected to influence the probability of EPP, but no relationship was detected between first egg date and EPP in a univariate analysis. When first egg date was entered into a multivariate analysis, there was a tendency ($p < 0.1$) for broods containing EPY to have later first egg dates than broods without EPY. Both male status and breeding density are controlled for in this analysis. One other factor that may increase EPP in later nesting birds is the operational sex ratio for EPCs which is suggested to be higher for later nests (Birkhead & Møller 1992). This was not investigated in this study due to the difficulty of determining the number of males available for EPCs with a female at any one time. This is because both males and females may forage at distances of over a kilometre from their nests and often outside the study area (Williams 1988). Therefore, individuals from both inside and outside of the area are able to interact

frequently. Furthermore, early in the season many males arrive at the study area only to move elsewhere subsequently. This made it impossible to determine the number of floater males in the area at any one point.

It is possible that the individual composition of the different breeding densities may influence the use of mixed reproductive strategies (Morton *et al.* 1990). Various studies have suggested that male age has a considerable influence on EPP (Wetton *et al.* 1995, Sundberg & Dixon 1996, see Chapter 4; but see also Dunn *et al.* 1994b). No difference was found in this study between the proportions of adults and subadults nesting at different breeding densities. Unfortunately it was impossible to distinguish female age.

There may be differences in the size and 'quality' of individuals nesting at the different densities. No relationship was found between any of a range of male phenotypic characters and density. This suggests that the size or quality of individual males did not differ with respect to breeding density. Secondly, an index of male body condition had no effect on EPP when included in a multivariate analysis. Consequently, male size and quality (as measured in this study) can be discounted as confounding factors influencing EPP. In females, individuals breeding at higher densities were found to have both shorter tarsi and wings (this result was not significant when a Bonferroni correction was applied). This relationship was not because smaller females arrived later in the season and consequently nesting at higher densities, as there was no relationship between first egg date and either female size or nesting density. There was also no relationship between a female's tarsi or wing length and EPP in her brood, thus female size and quality is unlikely to have affected the likelihood of EPP. In contrast to this study, Hoi & Hoi-Leitner (1997) reported that in bearded tits colonial females were of significantly higher quality in terms of body size and condition than solitary females. However, although they do suggest that higher levels of EPP occur at higher densities, they do not show a direct relationship between either the body size or condition of females and EPP.

None of the other variables that have been suggested to influence EPP had an effect upon EPP in this study. This was further confirmed by the multivariate analysis which indicated that breeding synchrony, male body condition and year had no effect upon EPP. This work, therefore, indicates that the frequency of EPP (and hence sperm competition) increased with increased breeding density. Other studies to report that nesting at a higher density was associated with an increased frequency of EPP include those of Gibbs *et al.* (1990) on red-winged blackbirds, Gowaty & Bridges (1991a) on eastern bluebirds, Double (1995) on starlings, and Hoi & Hoi-Leitner (1997) on bearded tits. Dunn *et al.* (1994a) were unable to find any relationship between density and levels of EPP in tree swallows. This lack of a relationship, they argued, was a consequence of the females being in control of EPCs. However, it seems likely that females are similarly in control of EPCs in other species, including those where a higher frequency of EPP has been found at higher densities (reviewed in Birkhead & Møller 1992, 1993; red-winged blackbirds [Gray 1997], eastern

bluebirds [Gowaty 1991], starlings [Double 1995] and bearded tits [Hoi & Hoi-Leitner 1997]).

In northern orioles there is evidence that females solicit EPCs from certain males while rejecting EPCs from other males. Females were observed to refuse extra-pair solicitations from both adult and subadult males. On six occasions they were also observed to actively solicit EPCs from adult males and on three occasions this resulted in successful EPCs. Females were never seen soliciting or performing EPCs with subadult males. Furthermore forced-copulation attempts were never observed. This evidence suggests that female northern orioles are in control of EPCs. This concurs with, and is supported by, the evidence from passerine bird studies, which indicates that females probably control EPCs and EPP through a number of behavioural and physiological mechanisms (reviewed in Birkhead & Møller 1993). If females are seeking EPCs then higher levels of EPP may be a result of there being more potential extra-pair mates and hence more opportunities for EPCs at the higher breeding densities. If females are seeking EPC for genetic benefits (as suggested in Chapter 4) any increase in the number of pairs will cause an increase in the number of females who are not paired to the 'best' male. More females may therefore seek EPCs and the levels of EPP will increase, thus explaining the relationship found in this study.

The use of mixed reproductive strategies resulted in high levels of EPP in the observed population of northern orioles (see Chapter 3). While all females may benefit from obtaining EPP, the benefits to males are likely to be unevenly distributed with some males benefiting at the expense of others. In this study EPP was not evenly distributed between males with adult males gaining paternity at the expense of subadult males (see Section 3.4.4 and Chapter 4). This resulted in the annual reproductive success of males being significantly different from the estimate of reproductive success based on the number of young fledged from a nest. The effect of EPP on the annual reproductive success of males was, therefore, investigated in relation to breeding density.

Any bias in the distribution of EPP between the males breeding in a population will be reflected in the annual reproductive success of individuals. If females are seeking EPCs for genetic quality then higher levels of EPP at higher densities should also lead to a higher variance of male reproductive success at the higher densities. This study was unable to test this hypothesis due to the small sample sizes and highly bimodal nature of the data. In contrast, extra-pair paternity is not expected to cause a difference in the mean annual reproductive success of males between breeding densities. At the different breeding densities any gain in the reproductive success of one male will be offset by the loss of reproductive success of another male. No relationship was found between annual reproductive success and nearest neighbour distance and there was only a non-significant tendency for colonial males to have a lower annual reproductive success than solitary males. This tendency for a lower annual reproductive success in colonial males could be a result of the low overall

levels of EPY assignment (46%). Because many EPY were not assigned to males in 1995 it seems likely that the actual success of colonial males was greater than estimated, and that the difference between colonial and solitary males was even less than has been estimated. This is supported by the separate analysis of the 1994 data, where 73% of all EPY were assigned, and no difference was found in the annual reproductive success of males breeding at the different densities. Furthermore, the multivariate analysis found no relationship between annual reproductive success and either of the breeding density measures.

On average, males breeding at higher densities suffered higher levels of EPP, but gained more EPP and so did not have a lower annual reproductive success than males at lower breeding densities.

5.4.2 The EPC Hypothesis of colony formation

The work done on purple martins by Morton *et al.* (1990) led them to propose that the benefits to males of attaining EPP at higher breeding densities may select for coloniality. Wagner (1993) agreed that EPP may be linked to colony formation, but suggested that it would be the benefits to female fitness rather than male fitness that would select for coloniality. He proposed the 'EPC hypothesis of colony formation' based on the idea that females nest at higher densities to increase the opportunities for EPCs and that males are forced to cluster in response to the females seeking EPCs. Hoi & Hoi-Leitner (1997) provided evidence to support Wagner's EPC hypothesis of colony formation. They showed that 'higher quality' females nest colonially and suggested that they do this to benefit from the higher levels of EPP by securing EPFs from higher quality males. Although their results were consistent with all the criteria for the hypothesis (see below), they did not show directly that females nested at high densities in order to pursue EPCs. Nor did they show that females actually gained EPP from higher quality males. Furthermore (as suggested by Hoi & Hoi-Leitner), the genetic benefit of securing EPCs would seem to be greater for low quality females as they were probably paired to low quality males. It is, therefore, difficult to understand why low quality females did not also attempt to nest at higher breeding densities to gain EPFs from higher quality males, especially "when there is no clear evidence to suggest that high quality females fare any better at avoiding the costs of coloniality" (Hoi & Hoi-Leitner 1997). No other studies have yet tested the EPC hypothesis of colony formation.

Wagner (1993) pointed out that in order for the EPC hypothesis to explain colony formation, certain conditions must exist. These are: (i) females must make themselves accessible to males for EPCs; (ii) females must benefit from EPCs; (iii) the spatial and/or temporal distribution of food must not require defence of feeding territories around the nest; and (iv) males settle in colonies despite the risk of cuckoldry.

Behavioural observations from this study suggest that female northern orioles not only cooperate with extra-pair males but also actively solicit copulations. As suggested earlier it seems likely that female northern orioles are in control of EPCs and EPP. The distribution of EPP in relation to male status therefore indicates a strong female preference for EPCs with older, adult males (see Chapter 4). This is supported by the behavioural observations of females only soliciting and accepting EPCs from adult males. It is difficult to show directly that females benefit from EPCs. However, female receptivity to, and control of, EPCs will probably evolve only if EPCs are beneficial to females. Although there is no direct evidence from this study, it seems likely that female preference for older males may benefit the female in some way i.e. through the acquisition of 'good genes' (see Chapter 4). Therefore, the first two conditions of the EPC hypothesis appear to be met in this population of northern orioles.

Various studies have reported that in some areas of the northern oriole's range, breeding pairs do not defend a feeding territory around the nest and often breed in nesting aggregations (Pleasants 1979, Williams 1982, 1988). At Hastings the food supply for northern orioles is spatially and temporally variable (see Section 1.4), and individual birds forage at sites up to 1 km from their nests (Williams 1988). Hence the third condition of the EPC hypothesis is also fulfilled.

The paternity data discussed in this chapter indicate that males are more likely to be cuckolded when breeding at higher densities and that the benefits of gaining EPP are not equally distributed amongst males. This satisfies the final criterion of the EPC hypothesis of colony formation and poses the question as to why certain males will breed at higher densities despite the fact that it greatly increases their risk of cuckoldry.

It is possible that males gain other benefits by nesting at higher densities, which result in a net benefit (see Chapter 2). This study suggests that reducing nest predation by nesting near to magpies may be a factor leading to the clumped distribution of northern orioles at Hastings. However, increased conspecific density alone does not reduce nest predation (Williams 1988, chapter 2) and pairs of nests in close proximity (within 5 m) have been observed even in the absence of magpies (pers obs.). There is also no evidence to suggest that individuals benefit through enhanced food finding when nesting at higher densities (Williams 1988). Furthermore, northern orioles are not philopatric and adults have low return rates between years (Williams 1988, pers. obs). Therefore, it is unlikely that any breeding experience gained from nesting at higher densities in one year will provide a significant benefit to a male to compensate for the loss of EPP.

Wagner (1993) suggested that males may suffer difficulty in finding a mate unless they acquire a territory which provides females with access to other males for EPCs. Personal observations during this study suggest that a slightly different scenario may occur in this population of northern orioles. Although females choose a male who is displaying at a specific site it is the female who ultimately determines the location of the nest site (pers.

obs.). This is supported by another study on northern orioles in which females frequently nested outside the male's advertising territory (Butcher 1991). In the present study late nesting females often appeared to select a male displaying at a site then subsequently nested closer to another conspecific nest. Since there is a male bias in the adult population, the male may be forced to move with the female rather than risk losing a mate.

In Chapter 2 it was suggested that northern orioles frequently nest close to magpies to avoid nest predation, and that the presence of magpies has a stronger effect upon the nesting distribution than the presence of conspecifics. This explanation does not necessarily exclude the possibility that at least some females may prefer to nest closer to other conspecifics. Northern oriole nests were occasionally closer together than expected if they were only trying to nest near magpies (within 5 metres of each other). Furthermore, on a couple of occasions two nests were observed clumped together (within 5 metres) despite the absence of magpies from the area (pers. obs). It is possible that only certain females will attempt to nest closer to other conspecifics to obtain EPCs. One prediction of the EPC hypothesis of colony formation would be that females paired to less preferred males (i.e. subadult males, see Chapter 4) would be more likely to nest close to more preferred (adult) males in order to obtain EPFs from these 'better quality' males. In this study there was good evidence that subadult pairs did indeed nest closer to adult nests than did other adults, and that subadult pairs also nested closer to adults than they did to other subadults. This was the case even after controlling for the arrival date of individuals (see Section 4.4.3). If females mated to low quality males attempt to nest in close proximity to both magpies and to high quality male pairs, then this would increase the clumping of northern oriole nests in the areas around magpies (as shown in Chapter 2).

Further work is required to determine whether females paired to subadult males move the nest site closer to other conspecific nests than the original displaying site of the subadult male. This result would provide strong evidence to support the EPC hypothesis of colony formation. However, the effect of the nesting association with magpies would also have to be taken into account and controlled for when investigating any conspecific clumping of nests.

5.4.3 CONCLUSIONS

This study revealed that the frequency of EPP increased with increased breeding density in the Hastings population of northern orioles. Furthermore, the proportion of nests which contained EPY (46%) suggests that the use of alternative reproductive strategies is an important part of the breeding system. The high frequency of EPP had a significant effect upon individual male annual reproductive success but, as expected, there was no difference between the mean reproductive success of males at different breeding densities. It was not

possible to determine whether the variance of male reproductive success increased at higher breeding densities.

This population of northern orioles not only appears to fulfil all the conditions of the EPC hypothesis (Wagner 1993), but also provides some evidence to suggest that the pursuit of EPCs by females may affect the nesting pattern of pairs. Female northern orioles mated to low quality (subadult) males may attempt to nest in close proximity to higher quality (adult) males in order to increase the genetic quality of their offspring through EPP. This, combined with pairs attempting to nest near magpies, may explain the loose clumping of northern orioles in certain areas. Therefore, the higher levels of EPP may be a factor selecting for, rather than a result of, higher breeding densities. Further work is necessary to test this hypothesis and to determine whether some northern orioles are attempting to nest close to conspecific nests and not just nearer to the yellow-billed magpies which seem to provide protection from predation.

CHAPTER 6

OVERALL DISCUSSION

The original aim of this study was to investigate the effects that breeding density and male age/status had upon the use of mixed reproductive strategies in northern orioles. During the first field season it soon became apparent (by virtue of constantly disturbing Ginger Bolen working on the yellow-billed magpies) that northern orioles and magpies inhabited not only the same areas, but often the same individual trees (pers. obs.; G. Bolen pers. comm.). Nesting associations between avian species have been documented before (reviewed in Smith 1985; see Chapter 2). I was therefore attracted to investigating any possible nesting association between magpies and northern orioles and whether this determined the breeding distribution and density of the northern orioles.

The results of this study suggest that a nesting association occurs between magpies and northern orioles as a direct result of northern orioles preferring to nest close to magpies (see Chapter 2). This association was not due to restricted suitable habitat or nest sites. There was no evidence to suggest that the association was because both species favour the same food-rich areas, or that it was due to increased foraging efficiency with mixed colonies functioning as 'information centres' (Ward & Zahavi 1973). Instead, the evidence strongly indicated that the association may have evolved as a result of the reduced rates of nest predation suffered by northern orioles when they nest in close proximity to the magpies. This was thought to have occurred as a result of magpies aggressively excluding scrub jays and crows from the vicinity of their nests and hence also from the nearby northern oriole nests.

There is still the possibility that the nesting association is merely a result of both species nesting in the same area because of a separate, as yet, untested factor, such as the reduced presence of nest predators in some areas, making such areas more attractive to both species. This explanation seems unlikely, however, as scrub jays and crows were frequently seen around the general areas that contained a high density of orioles. (see Chapter 2). The existence of an active association could be investigated experimentally by manipulating the nest sites of magpies. Magpie nests could be encouraged to move to a new location before the northern orioles arrived. It could then be determined if orioles nest closer to the actual magpies than to the original magpie nest positions.

At Hastings, magpies nest in a semi-colonial fashion (Reynolds 1990). They are also less abundant than northern orioles (G. Bolen pers. comm. pers. obs). The preference of northern orioles to nest near to magpies results in loose colonies of northern orioles

occurring in the same areas as the magpies. The nesting association, therefore, appears to be a major factor in determining the local breeding density of the northern orioles and may subsequently affect the frequency of mixed reproductive strategies (See Chapter 5).

DNA profiling was used to determine the frequency of mixed reproductive strategies in this population of northern orioles (See Chapter 3). Overall, 32% of chicks were extra-pair young (EPY) and 46% of nests contained EPY. Paternity was assigned for 45% of the EPY. Two cases of Intra-specific brood parasitism (IBP) were detected in two separate nests, but although these were both from 'colonial' high breeding density nests, no conclusions could be drawn from such a small sample size. The frequency of extra-pair paternity (EPP) found in this study is fairly high, but still falls well within the range of EPP frequencies found in other socially monogamous bird species (see Gowaty 1996). EPP was not randomly distributed across nests but appeared to show a highly bimodal distribution - some nests contained little, or no EPP, while other nests had high levels of EPP (often 100%). This study then went on to try to determine what factors caused the differences in levels of EPP between nests.

One factor that may affect the frequency of EPP is male age/status. Northern orioles are one of many passerines that exhibit male delayed plumage maturation (DPM) (reviewed in Rohwer & Butcher 1988). There is a conspicuous plumage difference between first-year males (which display a drab 'female-like' subadult plumage) and past first-year, full adult males (which show a bright, conspicuous adult plumage). The DNA profiling results show that, on average, sub-adult males lost 55% of their paternity while older, adult males lost only 21% of their paternity. Furthermore, paternity assignment indicates that it is nearly always adult males that gain the EPP. The combination of losing less paternity in their own nests, while gaining more paternity from other nests, results in adult males having a mean annual reproductive success rate over twice that of the subadult males (4.2 ± 0.7 v. 1.9 ± 2.0 respectively; see Chapter 4).

Recent work on passerines indicates that females are probably in control of both EPCs and any subsequent extra-pair fertilisations (EPF) (reviewed in Birkhead & Møller 1993b). Behavioural observations from this study suggest this to be the case in northern orioles with females being observed both to refuse EPCs with some males (both adult and subadult) while soliciting EPCs from other males (always adult males). The bimodal distribution of EPP linked to the female control of extra-pair copulations (EPC) may indicate that female choice is occurring. Female northern orioles appear to prefer to be fertilised by adult males. Similar preferences for older males have been found in studies on other species (Wetton *et al.* 1995, Sundberg & Dixon 1996; but see also Dunn *et al.* 1994b). Direct benefits can probably be ruled out for EPCs in northern orioles (but see Kirkpatrick & Ryan 1991). The distribution of EPP among nests, and the apparent female preference for adult males, are both consistent with the hypothesis that females are seeking EPCs to increase the genetic quality of their offspring ('good genes': Zahavi 1975, 1977, Hamilton & Zuk 1982).

The results are not consistent with the genetic diversity hypothesis (Williams 1975). They are also not consistent with the fertility insurance hypothesis (Walker 1980, Gibson & Jewel 1982; reviewed in Birkhead & Møller 1992), at least unless subadults are markedly inferior to adult males in terms of ejaculate quality (i.e. decreased sperm counts or reduced ejaculate levels see Hilton 1968, Hill 1994). This seems unlikely as some subadults gained all the paternity in their own nests, and higher levels of infertile eggs were not detected in these nests (infertile eggs were rare within the whole population). However, the possibility that subadult males may be less fertile than adult males will have to be tested in the future.

This study suggests that females prefer to be fertilised by adult males to increase the viability of their offspring. It did not, however, directly investigate which proximate cues females used to discern between males of different ages. The highly conspicuous plumage difference between adult and subadult birds seems a likely cue for females to use (see Chapter 4; see also Chapter 1, Figure 1.1 & 1.2). This idea is supported by evidence from mate choice experiments in the closely related orchard oriole, which indicates that females preferred adult plumage over subadult plumage (Enstrom 1993). Similar mate-choice experiments need to be done in the northern oriole to confirm that females are using plumage cues and not other factors linked to age such as song repertoire.

The results of this study have profound implications upon the hypotheses for the evolution of delayed plumage maturation (DPM). Up to this point, the majority of hypotheses have assumed that the subadult plumage has an adaptive function. As suggested above the subadult plumage may be used by females as a cue in extra-pair mate choice. The female preference for adult males results in subadults having substantially lower annual reproductive success than adults. The subadult plumage, therefore, appears to be a significant cause of the disadvantage suffered by first year males and seems unlikely to be adaptive, at least during the breeding season. The fact that no significant benefits could be determined for the subadult plumage supports the idea that DPM has evolved as a result of constraints on young males (i.e. the 'moult constraint' hypothesis; Chu 1994). A new hypothesis put forward in this study is also consistent with all the evidence (the 'honest plumage' hypothesis; see Chapter 4). Furthermore, it explains how the female preference for older males (based on honest plumage signals linked to carotenoid intake) may have influenced the evolution of DPM. Further work is needed to test some of the assumptions made in this hypothesis e.g. that female choice is based on the carotenoid-determined section of the male plumage. However, this hypothesis should now be considered as a possible explanation for the evolution of DPM, both in northern orioles and in other DPM species. One initial way to do this would be to complete DNA profiling studies on a range of species that exhibit full DPM. The 'Honest plumage' hypothesis predicts that there will be a relationship between male age/plumage status and EPP in these species.

In this study breeding density was the only other factor found to have an influence upon the frequency of EPP (see Chapter 5). Nests containing EPY had closer nearest

neighbours than nests without EPY, and the frequency of EPP increased with decreased nearest neighbour distance. Furthermore, 'colonial' nests contained EPY significantly more often than 'solitary' nests (16/26 v 6/22 respectively), and the mean proportion of EPY was higher in 'colonial' nests (48%) than in 'solitary nests' (16%). There was no difference in the number of adult and subadult males breeding at the different densities, hence the result was independent of male age/status (as confirmed by the multivariate analysis; see Section 5.4).

Breeding density was shown not to have an overall effect upon mean male reproductive success. The higher mean loss of within-pair paternity at higher breeding densities was counter-balanced by the higher mean gain of EPP, i.e. the paternity lost by one individual was normally gained by another individual also nesting at high density. It was, however, originally predicted that the variance of male reproductive success would increase at higher breeding densities. Some males were expected both to lose less within-pair paternity, while gaining more EPP, than other males. However, the strongly bimodal distribution of EPP and annual reproductive success made it impossible to test this hypothesis.

This study, therefore, suggests that the clumping of northern oriole nests at Hastings reservation may be influenced by the northern orioles' preference for nesting in close proximity to magpies (to gain predator protection; Chapter 2). This clumping causes higher breeding densities of conspecifics to occur in the areas around the semi-colonial magpies. The higher breeding density of northern orioles may also result in higher levels of EPP as a consequence of increased opportunities for EPCs and the greater level of extra-pair mate choice available to females (Chapter 5).

Occasionally northern orioles were observed to nest in close proximity even in the absence of magpies. Additionally northern oriole nests were sometimes closer than would have been expected if they were only trying to nest near magpies (within 5 metres of each other). The question therefore arises as to whether there is a further factor which also selects for the clumping of northern orioles. Limited suitable habitat was discounted as a possibility (Chapter 2). Predator protection and facilitated foraging were also unlikely as an increase in conspecific density does not reduce nest predation, nor does it increase the reproductive success of individuals (Chapter 5, Williams 1988). Another possibility is that the pursuit of EPCs by females may actually affect the nesting pattern of conspecifics (Wagner 1993). Females paired to inferior quality males (i.e. subadult males) may endeavour to gain better quality genes for their offspring by obtaining EPFs from superior males (i.e. adult males). The females with low quality mates may choose to nest in close proximity to the nests of higher quality males (and/or at higher densities). Hence, they may increase their opportunities to obtain EPCs, and consequently EPFs, from higher quality males. The evidence from this study appears to be consistent with all the conditions of the EPP hypothesis (Wagner 1993). Furthermore, subadult pairs nested significantly closer to adult

pairs than they did to other subadults, which was also significantly closer than adults nested to other adults. Behavioural observations from this study and Butcher (1991) indicate that the female determines the ultimate location of the nest, which is often outside the original advertising territory of the male. These findings, combined with the evidence that females prefer to obtain EPFs from adult males, suggest that the females mated to subadults may indeed be deliberately nesting close to adult males to obtain EPFs. Consequently, the pursuit of EPCs by some females may be influencing the nesting pattern and breeding density of northern orioles.

Further work is required to test the EPC hypothesis in northern orioles (see Chapter 5), and to unravel the influence of the nesting association with magpies from the influence of the female pursuit of EPCs. One possible experiment would involve waiting until the majority of northern orioles had started nesting and then recording the nearest neighbour distances between all the nests. Adult males could then be removed from a subset of the adult pairs. This would cause the females to desert and reneest. The majority of these females would remate with a subadult male (most adult males would be already being paired up and the majority of unmated birds at this time are subadults). The EPC hypothesis would predict that females remated to subadult males would reneest closer to adult male pair nests than they had originally been when mated to an adult male. Alternatively, populations of northern orioles, where there is no obvious nesting association with magpies (or any other animal), could be investigated in relation to the EPC hypothesis.

In conclusion, this study suggests that the nesting pattern of northern orioles at Hastings may be (at least partially) determined by two factors; (1) the nesting association between northern orioles and yellow-billed magpies and, (2) the pursuit of EPCs by females mated to low quality males (females appear to consider sub-adult males as 'low quality' mates and DPM may have evolved as an honest indicator of the lower mean quality of first year males). The increased levels of EPP that were observed at higher breeding densities may, therefore, be a factor selecting for the clumping of nests, rather than just a response to the increased opportunities for EPC that occur at these higher densities.

APPENDIX

SCIENTIFIC NAMES REFERRED TO IN THE TEXT

American redstart	<i>Setophaga ruticilla</i>
azure-winged magpie	<i>Cyanopica cyana</i>
bar-tailed godwit	<i>Limosa lapponica</i>
black-headed grosbeak	<i>Pheuticus melanocephalus</i>
bearded tit	<i>Panurus biarmicus</i>
black skimmer	<i>Rynchops niger</i>
boat-tailed grackle	<i>Quiscalus major</i>
cedar waxwing	<i>Bombycilla cedrorum</i>
cliff swallow	<i>Hirundo pyrrhonota</i>
common tern	<i>Sterna hirundo</i>
corn bunting	<i>Miliaria calandra</i>
American crow	<i>Corvus brachyrhynchos</i>
eastern bluebird	<i>Sialia sialis</i>
fieldfare	<i>Turdus pilaris</i>
forster's tern	<i>Sterna forsteri</i>
forest tent moth	<i>Malacosma disstria</i>
gray catbird	<i>Dumetella carolinensis</i>
guppy	<i>Poecilia reticulata</i>
hooded oriole	<i>Icterus cucullatus</i>
house finch	<i>Carpodacus mexicanus</i>
house sparrow	<i>Passer domesticus</i>
human	<i>Homo sapien</i>

Japanese lesser sparrowhawk	<i>Accipiter gularis</i>
kestrel	<i>Falco tinnunculus</i>
Lace lichen	<i>Ramelina menziesii</i>
lion	<i>Panthera leo</i>
merlin	<i>falco columbarius</i>
Mistletoe	<i>Phoradendron villosum</i>
orange breasted bunting	<i>Passerina leclancheri</i>
orchard oriole	<i>Icterus spurius</i>
purple martins	<i>Progne subis</i>
razorbills	<i>Alca torda</i>
red-winged blackbird	<i>Aelaius phoeniceus</i>
reed bunting	<i>Emberiza schoeniclus</i>
rufous-naped wren	<i>Campylorhynchus rufinucha</i>
scrub jay	<i>Aphelocoma coerulescens</i>
splendid fairy wren	<i>Malurus splendens</i>
starling	<i>Sturnus vulgaris</i>
superb fairy wren	<i>Malurus cyaneus</i>
tree swallow	<i>Tachycineta bicolor</i>
wasp	<i>Polybia rejecta</i>
western grebe	<i>Aechmophorus occidentalis</i>
whimbrel	<i>Numenius phaeopus</i>
wood warbler	<i>Phylloscopus sibilatrix</i>
yellow-billed magpie	<i>Pica nuttalli</i>
yellowhammers	<i>Emberiza citrinella</i>
yellow warbler	<i>Dendroica petechia</i>

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