A genetic and behavioural investigation of extra-pair copulation in stitchbirds *(Notiomystis cincta)* breeding on Tiritiri Matangi Island

by

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Abstract

Minisatellite DNA fingerprinting was used to assign parentage to nestlings produced by stitchbirds breeding on Tiritiri Matangi Island. Analysis revealed that 35% of nestlings were the result of extra-pair copulation (EPC) and that extra-pair young were present in 80% of nests. These results show that an individual's realised reproductive success is very different than that predicted from social relationships alone. Approximately half of the extra-pair fertilisations were by unpaired males. This is in contrast to the general trend in bird literature, which suggests extra-pair paternity is the result of copulations by males paired with other females.

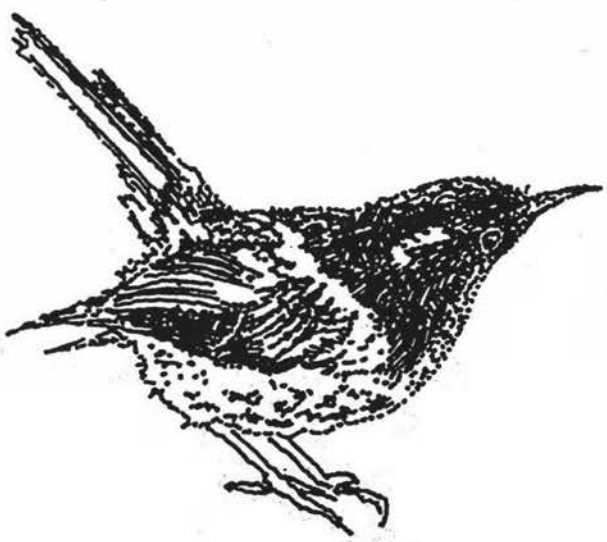
EPCs are resisted by females, hence EPC is assumed to be a male-driven reproductive behaviour. Extra-pair males concentrate their copulation attempts at peaks in female fertility. Regular visits made to nest boxes by extra-pair males may provide a cue to female fertility. Behaviour of extra-pair males suggests they also focus attempted EPCs on females at nest sites. Paired males attempt to defend their paternity by defending an area around the nest site by territorial calling and displacing intruding males. These paired males spend a majority of their time near the nest site, both when the female is present and absent.

The frequency of EPC attempts varied substantially between nests, and these attempts were often witnessed by the paired male. This variation mirrored closely the variation in the percentage of extra-pair paternity. The level of nest provisioning by males was strongly correlated with the frequency of attempted EPCs, and was less strongly correlated with actual paternity. This suggests that paired males assess their paternity using behavioural cues rather than actually discriminating related from unrelated offspring.

Although this thesis focuses on fundamental research, it is closely aligned to stitchbird conservation. The final chapter details management protocols used while monitoring stitchbirds for the first 18 months following translocation to Tiritiri Matangi. It details all management techniques believed to be important for gaining knowledge about the success/failure of this translocation, increasing public participation, and increasing the chances of success in establishing a self sustaining population.

Table of contents

GENERAL INTRODUCTION



General introduction.

EXTRA-PAIR COPULATION AND EXTRA-PAIR PATERNITY

The principle assumption underlying most theory of animal behaviour is that individuals act in ways that maximise their reproductive success (Maynard Smith 1988). In breeding pairs, the male and female are both trying to maximise their own reproductive success. One apparent outcome of this is that both males and females may seek extra-pair copulations (EPCs). By obtaining EPCs males can achieve increased reproductive success, and females can manipulate paternity of their offspring.

While about 90% of bird species were formerly considered to be monogamous (Lack 1968), recent research using molecular techniques such as minisatellite DNA fingerprinting have shown that social monogamy does not necessarily imply genetic monogamy (reviews in Birkhead & Møller 1992, 1995). Due to the apparent prevalence of EPC, it is now thought to be an important factor in the evolution of: (1) reproductive behaviors such as mate guarding and copulation (Birkhead et al. 1987), and (2) provisioning of young (Møller 1988; Westneat 1988; Burke et al. 1989).

This thesis documents both EPC and extra-pair paternity in stitchbirds breeding on Tiritiri Matangi Island, and tests the effect of EPC on breeding behaviours in this population.

THE STITCHBIRD

The stitchbird, or hihi (*Notiomystis cincta*), is a medium sized, sexually dimorphic, forest dwelling passerine. It is one of three endemic honeyeaters (Meliphagidae) found in New Zealand and is the most subordinate (Craig et al. 1981; Craig 1984; Rasch & Craig 1988). The male has a black head, golden shoulders and breast band, white erectile 'ear' tufts and a white wing bar (Rasch et al. 1996)(Figure 2). In comparison, the relatively dull female is predominantly olive brown, while also having a distinctive white wing bar (Craig 1985)(Figure 3). Males weigh about 40 g on average, and females are 28 g on average (Craig et al. 1982).

Figure 1. Male stitchbird

Figure 2. Female stitchbird

Stitchbirds are one of two honeyeaters that use cavities as nest sites, the other being the endangered Hawaiian O'o (Rasch 1989). On Kapiti Island, stitchbirds have a highly variable breeding system, including monogamy, polyandry, polygyny and polygynandry (Castro et al. 1996). Characteristic of birds with such mating systems, stitchbirds have large testes (4.2% of body mass), large cloacal protuberances, large seminal glomera and large numbers of sperm in the seminal glomera (Castro et al. 1996). On other islands, stitchbird breeding has been described as monogamous (Little Barrier Island Rasch 1985; Craig 1985), with records of many male-male and male-female chases during the breeding season (Kapiti Island, Lovegrove 1985; and Little Barrier Island, Angehr 1984). Stitchbirds copulate in two distinct positions. The male-on-the-females' back position common among birds (Castro et al. 1996), and the face-to-face position, also recorded in red-headed woodpeckers (Southern 1960). Face-to-face copulations were first recorded among captive birds (Anderson 1993), and later observed in the wild (Castro et al. 1996). This face-to-face positioning is thought to be a form of forced copulation in stitchbirds (Castro et al. 1996).

Prior to European settlement stitchbirds were common throughout the North Island of New Zealand, Great and Little Barrier Islands and Kapiti Island (Oliver 1955). However by 1894, in the space of a few decades, stichbirds became restricted to a single island population on Little Barrier. This rapid decline in range and numbers has been attributed to a variety of factors, not necessarily exclusive, including: (1) predation, (2) disease, and (3) loss of habitat (Rasch et al. 1996). Beginning in 1980 a series of translocations to other islands were attempted in an effort to expand the stitchbird's range. There have so far been 12 translocations to 5 islands (Rasch et al. 1996). Stitchbirds were translocated to Tiritiri Matangi Island in 1995, and I studied the population for the first 18 months after the translocation.

TIRITIRI MATANGI ISLAND

Tiritiri Matangi is an island situated 3 km off Whangaparaoa Peninsula, 25 km north of Auckland, New Zealand. The 220 ha island has a gentle topography with broad ridges sloping away from the main longitudinal ridge (60-80 m altitude). These ridges mostly end in steep cliffs. The island has a long history of human occupancy, which brings

Figure 3. Tiritiri Matangi Island, located 3 km off Whangaparaoa Peninsula, 25 km north of Auckland, New Zealand.

with it characteristic changes in vegetation structure. By 1940 the once forested island was estimated to be only 6% forest, the remainder made up of 92% pasture and 2% bracken (Mitchell 1985). The island began its reclamation in 1970 when stock was removed from most of the island. The entire island became a reserve by 1980, and the classification of open sanctuary was added in 1983 (Galbraith & Hayson 1995). Natural regeneration occurred very slowly because bracken and matting grass inhibited establishment of other vegetation (West 1980). This resulted in a revegetation program, initiated in 1984 (Cashmore 1995). During the ten year replanting program a total of 280 000 trees were planted by volunteers (Galbraith & Hayson 1995). This reforestation was partially to enhance nectar-providing species in anticipation of a future stitchbird translocation (Drey et al. 1982). Vegetation currently covers 52% of the island, mostly young regenerating coastal forest (Wilson 1997)(Figure 1).

The only mammalian predator on the island was the Kiore (or Polynesian rat). Kiore were eradicated in 1992 using an aerially distributed poison Talon® 20P (Eason & Spurr 1995). In a country where introduced mammalian predators have decimated native species (Craig 1997), islands lacking such pests are regarded as important refuges for native species. At the time Tiritiri Matangi Island was made a reserve, the native avifauna consisted largely of the more resilient and common species also present on the adjacent mainland (Galbraith & Hayson 1995). As the revegetation program progressed a series of native bird translocations were made, with the aim of restoring the island's depleted biodiversity. Other taxa, including insects and lizards, are proposed for future translocations (Hawley 1995). The open sanctuary classification has become the principle vision, promoting public awareness through involvement in the restoration project and allowing people to directly observe endangered species which are otherwise largely inaccessible (Craig 1997; Craig 1990; Galbraith & Hayson 1995).

STITCHBIRDS ON TIRITIRI MATANGI ISLAND

In August 1995, 20 male and 18 female stitchbirds were translocated to Tiritiri Matangi Island from Little Barrier Island. The birds were difficult to observe in the initial weeks following translocation making assessment of post-release mortality difficult. However,

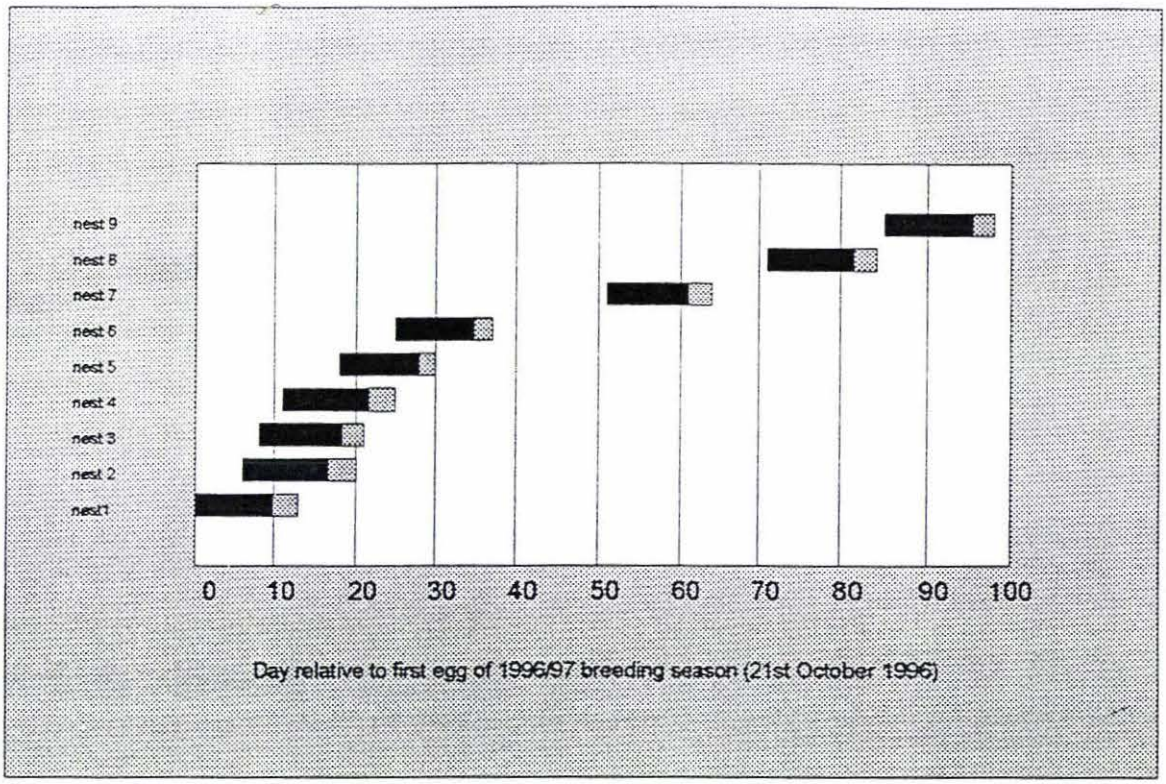


Figure 4. Breeding asynchrony of stitchbirds breeding on Tiritiri Matangi Island during the 1996/97 breeding season. Days are relative to the date the first egg was laid in the season. Each band represents a single nesting attempt. The black portion represents a period of ten days prior to the onset of egg laying, and the grey portion represents the period of egg laying.

by the beginning of the breeding season only 12 males and 4 females were alive. Reasons for this extreme mortality remain unclear with only two dead birds recovered. One was found with a severe head wound and the other had been observed looking exhausted after being chased by tui and bellbirds around the nursery area. In an attempt to bolster this population and even the sex ratio an additional 4 males and 9 females were translocated in August 1996. Three of these males and 2 females survived to breed in the 1996/97 breeding season. As a consequence, the sex ratio of the population was heavily biased toward males in both breeding seasons (12:4 in 1995/96 and 12:6 in 1996/97).

Due to the young stature of the forest on Tiritiri Matangi Island artificial nest boxes were distributed in bush patches over the island. This is because natural cavities form only in large trees. Previous research has shown stitchbirds successfully nest in boxes if provided (Rasch 1989; Castro et al. 1994; Armstrong et al. 1997). Nest boxes may allow stitchbirds to reside in otherwise unsuitable habitat (Perrott 1997).

Breeding started relatively late in the 1995/96 breeding season, the first egg being laid on 15 November. No second clutches were attempted. In comparison the first egg of the 1996/97 breeding season was laid on 29 October and three of the six females attempted second clutches. Egg laying was highly asynchronous in both years. There was no overlap in egg laying days during the 1995/96 season, and only limited overlap during the 1996/97 season (Figure 4). Stitchbirds lay one egg per day, on consecutive days until the clutch is complete (3-5 eggs). The first egg is laid near dawn and all other eggs follow an approximate 25 h cycle.

Castro et al. (1996) noted it was difficult to observe matings on Kapiti because stitchbirds lived in dense forest located in steep terrain. In contrast the gentle topography on Tiritiri Matangi Island, and use of artificial nest boxes, made it possible to do an intensive study of stitchbird breeding behaviour.

AIMS AND STRUCTURE OF THIS THESIS

The primary aims of this thesis are (1) to document the presence of EPC in the Tiritiri Matangi stitchbird population, (2) to provide accurate measures of the reproductive success achieved by males in this population, and (3) to investigate the significance of EPC and extra-pair paternity in observed behaviours by paired males, extra-pair males and females throughout the nest building, egg laying and nestling provisioning stages of reproduction.

Chapter 1 investigates the realised reproductive success of male stitchbirds breeding on Tiritiri Matangi, using minisatellite DNA fingerprinting. This allows me to measure: (1) the extent to which paired males' adopt mixed reproductive strategies, by pairing with one female while also seeking EPCs with other paired females, and (2) the extent to which unpaired males gain reproductive success from seeking EPCs.

The presence of extra-pair paternity in nests (Chapter 1) indicates that EPC is a successful reproductive strategy. Behaviours associated with this success are described and quantified in Chapter 2. Extra-pair males are predicted to behave in ways that will maximise the chances of fertilising eggs. Therefore, I investigate whether these males time their reproductive efforts to coincide with peaks in female fertility. This is important because timing copulations to coincide with peaks in female fertility is predicted to influence effectiveness of EPC in fertilising eggs (Birkhead 1987). I also assess the behaviour of paired males to determine whether they respond to the presence of EPC by attempting to defend their paternity, by guarding mates, guarding nest sites, and/or copulating frequently. It is important to assess the level of paternity defense because previous research has suggested that the presence of EPC has resulted in the evolution of such behaviours (Birkhead et al. 1987).

In many birds, feeding young is the most costly investment males make to reproduction (Walsberg 1983 cited in Møller & Birkhead 1993). Trivers (1972) developed a theoretical framework indicating that provisioning unrelated young was selectively disadvantageous. Subsequently, some models have predicted that parental investment should be related to paternity (Winkler 1987; Whittingham et al. 1992; Westneat &

Sherman 1993; Maynard Smith 1977) and others predict it should not (Werren et al. 1980). Very few studies have documented levels of both EPC and extra-pair paternity, and related them to parental investment of young (Westneat 1988; Wagner et al. 1996; Lifjeld et al. 1993). In Chapter 3, I investigate the affects of reduced paternity and reduced certainty of paternity on paternal provisioning of nestlings. This is important because so few studies have actually documented the effects of all these parameters, and because models vary in their predictions.

Although this thesis focuses on fundamental research, it is closely aligned to stitchbird conservation. Information on stitchbird breeding behaviour is fundamentally important for improving management of this species. In addition, in the course of this work, I provided detailed monitoring of reproduction, survival, and movements of Tiritiri Matangi Island stitchbirds for the first 18 months after release. The final chapter of my thesis details recommended management protocols for this population. Recommendations come from monitoring and management techniques used by myself while conducting field work, and discussions with Shaarina Boyd (Head of Threatened Species, Auckland Conservancy of the Department of Conservation). Isabel Castro and Doug Armstrong initially developed many protocols I have used for stitchbird management.

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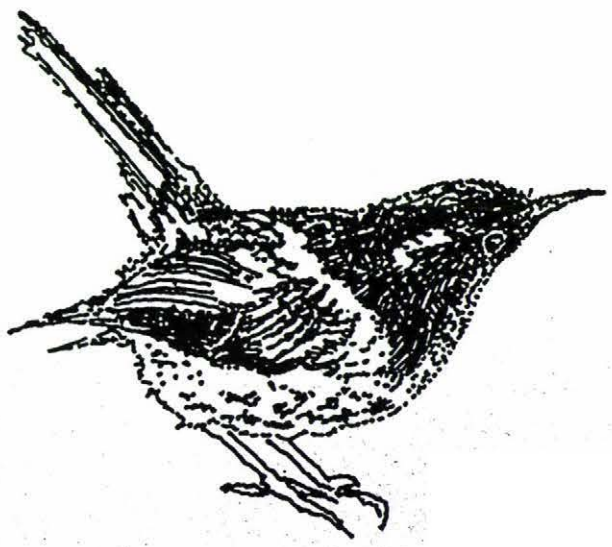
CHAPTER ONE



A genetic investigation of extra-pair fertilisations.

ABSTRACT

Minisatellite DNA fingerprinting was used to assign parentage to all but one nestling produced by stitchbirds breeding on Tiritiri Matangi. Analysis revealed that 35% of nestlings were the result of extra-pair copulation and that extra-pair young were present in 80% of nests. These results show that an individual's realised reproductive success is very different than that predicted from social relationships alone. Approximately half of the extra-pair nestlings were the result of unpaired males gaining reproductive success. This is substantially higher than predicted from literature suggesting extra-pair paternity is predominantly the result of already paired males pursuing a mixed reproductive strategy. The presence of extra-pair paternity also provides direct evidence for sperm competition, previously predicted in this species.

INTRODUCTION

With the discovery that human derived minisatellite probes could also detect highly variable regions in avian DNA (Burke & Bruford 1987; Wetton et al. 1987), a new tool was made available that could provide an accurate measure of reproductive success. Many species traditionally categorised according to social associations have needed re-defining because genetic analysis of parentage revealed either extra-pair fertilisations (e.g house sparrow, Wetton et al. 1987; great reed warbler, Hasselquist et al. 1995) or intra-specific brood parasitism (e.g zebra finch, Birkhead et al. 1990; purple martin, Morton et al. 1990).

Even where extra-pair copulations (EPC) have been observed, measures of reproductive success by observation alone have been misleading. For example, in socially polygynous red-winged blackbirds infrequent EPCs have been observed (Westneat 1992). Use of minisatellite DNA fingerprinting, however, has shown more than 20% of nestlings were the result of extra-pair fertilisations (28% Gibbs et al. 1990; 25% Westneat 1993). Similarly, observation of socially monogamous indigo buntings has revealed occasional intrusion of neighboring males and attempted extra-pair

copulations, which were usually resisted by the female (Westneat 1987; Westneat 1990). Again minisatellite DNA provided an accurate measure, revealing 35% of nestlings were the outcome of extra-pair copulations (Westneat 1990).

Results from such studies suggest that there is great variability in the extent to which extra-pair parentage can affect calculations of reproductive success, and therefore impact on the processes of sexual selection. Other genetic studies show that sexual monogamy is prevalent in many populations (e.g silvereye, Robertson 1996; North Island robin, Ardern et al. 1997; and common loon, Piper et al. 1997). In such species, behavioural observations alone can be used to measure reproductive success gained by individuals. The extent to which extra-pair parentage occurs varies greatly between species and populations. Documented levels of extra-pair parentage range from under five percent, for populations of great tit (Verboven & Mateman 1997) and willow tit (Orell et al. 1997), to extreme values of around 55% for reed buntings (Dixon et al. 1994). Great tits also provide an example where variation is recorded between populations, from as low as 3.5% recorded by (Verboven & Mateman 1997) to 15% recorded by (Gullberg et al. 1992).

Research to date indicates that EPCs are often part of mixed reproductive strategies, with paired males seeking copulations with other already paired females (Westneat et al. 1990). Females may also be involved in mixed reproductive strategies, by similarly seeking EPCs. Use of DNA fingerprinting allows direct quantification of reproductive success resulting from different strategies of both males (e.g red-winged blackbirds, Gibbs et al. 1990) and females (e.g great reed warbler, Hasselquist et al. 1996; tree swallows, Lifjeld & Robertson 1992).

One restriction in the use of minisatellite DNA fingerprinting is the number of individuals that can be run together on a gel. It is relatively easy to sample both putative parents at a nest and test for genetic relationships with any nestlings (e.g. great tit, Verboven & Mateman 1997; zebra finches, Birkhead et al. 1990; reed bunting, Dixon et al. 1994). Due to the nature of running gels, and the generally high mobility of animals being studied, it quickly becomes impractical to attempt assigning maternity and

paternity to those cases where extra-pair parentage was revealed. Some studies attempt to partially address this question by re-examining extra-pair young in comparison to neighboring adults (e.g house sparrow, Wetton et al. 1987; tree swallows, Lifjeld et al. 1993; indigo bunting, Westneat 1990). Such studies have revealed that extra-pair fertilisation is often by other paired males usually resident on neighboring territories. Very few studies have sampled all possible parents and been able to assign parentage to all nestlings. These include Hasselquist et al. (1995) and Gibbs et al. (1990). This study also sampled all possible parents and aimed to assign paternity to all nestlings.

Documenting the presence of extra-pair paternity also provides direct evidence of sperm competition. Sperm competition is defined as the competition between sperm from two or more males to fertilize the eggs of a single female (Parker 1970). It has been recognised as a powerful selective force, shaping life-history characteristics such as body size, morphology, physiology and behaviour (Birkhead & Parker 1997) and is thought to be ubiquitous in the animal kingdom (Birkhead 1995).

Social breeding associations in stitchbirds are thought to include all forms of polygyny, as well as monogamy (Castro et al. 1996). Extra-pair copulations have been observed among birds breeding on Kapiti Island (Castro et al. 1996), and multi-male chases of females have also been recorded by other researchers (Kapiti Island, Lovegrove 1985; Little Barrier Island, Angehr 1984). This variability in the breeding system is shared by three other unrelated passerines: the dunnock, Smith's longspur, and aquatic warbler (Birkhead & Hunter 1990). Minisatellite DNA fingerprinting has revealed that intense sperm competition occurs in these three species (dunnocks, Burke et al. 1989; Smith's longspur, Schulze-Hagen et al. cited in Birkhead 1995; aquatic warbler, Birkhead 1993). Although sperm competition had not been confirmed in stitchbirds, male reproductive anatomy includes, large cloacal protuberances, large testes, and large numbers of sperm (Castro et al. 1996), all of which are indicative of species where sperm competition is intense (Møller 1991; Briskie 1993).

This chapter addresses two aims. Firstly I aim to document the presence of extra-pair parentage in this population. Previous research on stitchbird breeding has reported: (1)

the presence of EPC, (2) the observation of multi-male chases of females during the breeding season, and (3) a reproductive anatomy indicative of a species with intense sperm competition. However no previous study has investigated the presence of extra-pair parentage in this species. Bandsharing coefficients and novel bands will be used to determine the presence of extra-pair paternity and/or intraspecific brood parasitism. The presence of extra-pair paternity suggests competition between the sperm of the paired male and the extra-pair male who was the genetic father of the offspring. Results presented in this chapter will substantiate Castro et al.'s (1996) prediction that sperm competition is intense in this species.

Secondly I aim to provide an accurate measure of realised reproductive success among males in this population. Gels are designed to maximise the chances of running genetic parents with their offspring. Realised reproductive success will be reported in three categories: (1) paired males gaining reproductive success by copulating with their mates, (2) paired males gaining reproductive success by achieving EPCs with already paired females, and (3) unpaired males gaining reproductive success by achieving EPCs with paired females. Very few studies have been able to sample an entire population and attempt to assign parentage to all offspring.

METHODS

Determination of putative parentage

The stitchbird breeding season occurs from October through to the end of February (Oliver 1955; Craig 1985). During this time, nest box checks and behavioural observations were used to locate breeding females in order to determine putative parentage.

Nest box checks

Stitchbirds are unique among the New Zealand honeyeaters in that they nest in cavities (Rasch 1985). This potentially restricts stitchbirds to mature bush, at least when breeding. Due to the lack of mature forest on Tiritiri Matangi, artificial nest boxes were

provided in all large bush patches. Throughout the breeding season all nest boxes were checked daily. This continued until all females on the island had commenced nest building. Checks were re-initiated as each female neared the completion of brooding in case a second clutch was attempted.

Behavioural observations

During systematic island searches of nest boxes, and all other movements around the island, any stitchbirds seen or heard were followed for as long as possible. As the birds are highly mobile this was never for longer than 30 mins. During nest building and egg laying stitchbirds in other populations regularly perform male-male, male-female, and female-female chases (Castro 1995; Lovegrove 1985, for Kapiti Island; Rasch 1985, for Little Barrier Island). These chases are accompanied by increased vocalization from males and females. In the present study, instances of such behaviour were used as indicators of the initiation of nest building, and birds were followed until the nest site was found.

Once nest sites were discovered, behavioural observations were conducted daily through nest building and egg laying (see Chapter 2) and every third day through brooding (see Chapter 3). All birds in the Tiritiri Matangi Island population were identifiable by unique combinations of coloured plastic bands and a numbered aluminum band. These bands were attached as birds were captured on Little Barrier Island before translocation, or while nestlings on Tiritiri Matangi.

Female stitchbirds generally have sole responsibility for nest building and incubation (Oliver 1955; Craig 1985), and they also do most of the provisioning to nestlings (see Chapter 3). Putative females were therefore assumed to be the female involved with such behaviours. More than one female has been observed nest building and incubating at one nest in the Kapiti Island population (Castro 1995). This was not a problem on Tiritiri Matangi because only a single female was ever observed building and incubating in each nest. Between 1-11 male stitchbirds were observed near each nest site. These males were involved in male-male and male-female chases (see Chapter 2). Despite this, a single male always spent a greater proportion of time with each female and attempted to defend a 'territory' around the nest site (see Chapter 2). These males also

contributed to the provisioning of nestlings, albeit at a lower level than the females (see Chapter 3). Such birds were termed 'paired' males and were assigned as putative fathers at each nest. All other males observed in the vicinity of the nest (within 30 m, see Chapter 2) were recorded as putative extra-pair males and ranked in relation to the number of times they were seen and the number of EPCs they attempted. This was necessary because there are limits to the number of individuals that can be run on a fingerprint gel (see below).

Collection of blood and tissue samples

Techniques employed for collection of blood are as detailed in Ardern et al. (1994). Blood samples were obtained through wing venipuncture of the brachial vein, using a 13-mm 27-gauge needle. Blood was collected in hematocrit tubes (mean blood volume 66 µl ± 10 µl) and then placed into screw topped 'nunc' tubes. Each tube was labeled with the bird's band combination, number and the date of blood collection, then placed into liquid nitrogen before being stored permanently at -80°C in the laboratory at the earliest opportunity. Gauze patches were placed over the puncture wound and birds were placed in banding bags while blood was processed. Prior to release the gauze patches were removed and the wounds were checked to make sure bleeding had ceased. All used gauze swabs were dabbed with used hematocrit tubes to remove any remaining blood, then placed into sterile 1.5 ml eppendorf tubes. Eppendorf tubes were labeled as above, and had holes pierced in the lid by puncture with the 27G needle to prevent explosion on thawing, and placed into liquid nitrogen. Eppendorfs provided additional samples in case of loss or contamination of the main blood sample.

Sampling adult birds

All adult birds were sampled upon capture on Little Barrier Island. Birds were mistnetted along three main ridge systems, covering an altitudinal gradient of 0-800 feet above sea level. Two trips were made to the island in order to obtain birds for transfer to Tiritiri Matangi. In 1995 a total of 18 females and 19 males were sampled, and in 1996 a further 4 males and 9 females were sampled. Both trips were made in August in

an attempt to coincide with the flowering of *Alseuosmia macrophylla*, common along the ridge systems of Little Barrier Island. Stitchbirds are known to cluster around seasonally variable resources (Rasch et al. 1996; Angehr 1984), and range widely when foraging (Ewen pers. obs. Tiritiri Matangi). I therefore assume that a random sample of the Little Barrier Island population was taken each year for transfer to Tiritiri Matangi and for subsequent genetic analysis. This was important to minimise the background bandsharing in the Tiritiri Matangi population, as minisatellite DNA fingerprinting losses power as the background bandsharing increases.

Sampling of broods

Once eggs had hatched, nests were routinely checked every 2-3 daylight hours (see Chapter 4). This was continued until all surviving chicks had been banded and blood samples collected. Female stitchbirds typically remove dead chicks from the nest and discard them away from the nest soon after discovery. Dead chicks found in the nest were collected for genetic analysis and post-mortem examination. If the female had removed chicks, an area of about 5 m in radius was searched in an effort to recover them. Typically white eggshells gave the location of nest refuse away and subsequently the location of any dead chicks. All chicks recovered had their wings and legs removed and placed in a –20°C freezer for between 0.5 and 2 months. Ideally, liquid nitrogen would have been used for tissue storage, but the short life of liquid nitrogen in the field, in addition to the unpredictability of nestling mortality, made this impractical. At the earliest opportunity these samples were permanently stored at –80°C. All broods with surviving young were bled at 21-25 days after hatching. Stitchbirds typically brood for 28-30 days. Therefore, sampling birds at this stage allowed for maximum chick growth with little chance of chicks fledging before sampling.

A total of ten families were sampled for parentage analysis: the single successful clutch of four young in the 1995/96 breeding season, and nine clutches comprising 36 young in the 1996/97 breeding season.

Minisatellite DNA fingerprinting

Background to method

The majority of an organism's DNA is made up of non-genic sequences, i.e those that are non-coding for specific proteins and therefore appear not to be influenced by natural selection (Jeffreys et al. 1985c; Burke et al. 1991). These regions often occur in sequences of tandemly repeated units varying anywhere from two to millions of repeats. Minisatellite DNA constitutes simple tandem repetitive regions with arrays of up to several hundred 15-60 base pair units (Jeffreys et al. 1985c; Bruford et al. 1991). These minisatellite loci are dispersed widely through the genome of many animals and plants (Jeffreys et al. 1985c; Jeffreys et al. 1985b; Bruford et al. 1991) and the majority are highly polymorphic due to allelic variation in repeat copy number (Jeffreys et al. 1985c). The hypervariable nature of repeats in minisatellite regions is thought to arise from mutation processes such as unequal crossover or replication slippage in the germline (Jeffreys et al. 1988). Hybridisation of probes consisting of tandem repeats of the core sequence can produce banding patterns of these minisatellite 'families' (Jeffreys et al. 1985a; Nakamura et al. 1987). By probing with variant core sequences, additional sets of minisatellite 'families' can be detected (Jeffreys et al. 1985b). Such minisatellite DNA patterns are completely specific to an individual or identical twins (hence the term 'DNA fingerprint') and provide a powerful tool for identifying genetic relationships among individuals.

Extraction

DNA extraction follows techniques detailed in Ardern & Lambert (1997). High molecular weight nuclear DNA was extracted from both blood and tissue samples. Fifteen μL of blood was mixed with 0.5mL of lysing solution (144 mM NH_4Cl; 10 mM NH_4HCO_3) by inversion. This lysate was centrifuged at 13000 rpm for five minutes then the supernatant was discarded. The remaining pellet was re-suspended in 400 μL of SET buffer (0.1 M NaCl, 1mM EDTA, 0.1 M Tris-HCl pH 8.0) by vortexing, 20 μL of 10% SDS and 20 μL of proteinase K (20 mg/ml) was added, and it was incubated overnight at 55°C. A series of washes was then required to remove proteins from samples. Firstly

400 µL of Tris-buffered Phenol was added and rocked for 30 minutes. Tubes were then centrifuged at 13000 rpm for five minutes and the bottom layer was removed with a 200 µL pipette. Four hundred µL of Phenol/chloroform/isoamyl was then added and rocked for 30 minutes, spun at 13000 rpm for five minutes, and the bottom layer removed. This Phenol/chloroform/isoamyl wash was repeated. Finally a single wash of 400 µL chloroform/isoamyl was rocked for 30 minutes, then centrifuged and the bottom layer removed as above.

DNA from tissue samples was extracted similarly with the following additions. A piece of tissue approximately 6×6×3 mm was removed from each dead chick. This was sliced into small pieces with a sterile scalpel blade and added to SET buffer as above. Thirty µL of 10% SDS and 30 µL of proteinase K were added and incubated at 55°C for three hours. A further 10 µL of proteinase K was then added and samples returned to 55°C overnight as above. Also an extra Tris-buffered Phenol wash was performed to remove additional proteins present in tissue.

DNA from both blood and tissue samples was precipitated in 40 µL 3M NaOAc pH 5.2 and 1 mL of 100% ethanol (room temperature) by rocking for 15 minutes, followed by vigorous shaking. Tubes were spun at 13000 rpm for five minutes, before the supernatant was removed. The pellet was then washed twice with 70% ethanol by gentle inversion. Ethanol was decanted off and the pellet was left to dry at room temperature for approximately half an hour. All DNA pellets were re-suspended in 60 µL of milli-Q water and stored at 4°C.

Digestion

Twenty µL of genomic DNA was digested with the restriction enzyme *Hae*III by the addition of: 4 µL 10× Buffer (react 2), 2 µL 2mg/mL BSA, 1 µL 160 nM Spermidine, 1 µL *Hae*III and 11 µL milli-Q water. This mixture was incubated overnight at 37°C. Another 1 µL of *Hae*III was added the following day and incubated at 37°C for a further hour. Digested samples were then stored at -20°C.

Quality of genomic and digested DNA

The quality of all samples of genomic and digested DNA were checked by running 1 µL of sample on a 0.8% agarose gel (70 mA) for about 1 h. High quality genomic DNA appeared as a solid single band of high molecular weight. Any streaking suggested degradation of the DNA sample, meaning that samples needed to be discarded and the extraction process repeated. Digested DNA appeared as a uniform elongated smear indicating even digestion of the entire sample. Any streaking again suggested incomplete digestion. Such samples were further digested (2 h) with the addition of 1µL of *Hae*III. If the sample again failed to completely digest it was discarded and a new sample was digested from the genomic stock.

The concentration of digested samples was tested using a Hoefer DyNA Quant 200 flourometer. One hundred ng/µL calf DNA was used as a standard to calibrate the flourometer before testing samples. The standard was checked every six samples to confirm calibration.

Electrophoresis

Approximately 5 µg of digested DNA was loaded in each lane. DNA fragments were resolved on a 0.8% agarose gel (19×27 cm) in 1× TBE running buffer at 55 V for 72 h. The DNA was then denatured by washing each gel for 15 minutes in 0.25 M HCl and then 45 minutes in 0.5 M NaOH, 1.5 M NaCl. Gels were then neutralized by two 15 minute washes in 1.5 M NaCl, 0.5 M Tris-HCl pH 7.2, 1 mM EDTA. Southern blot techniques were used to transfer DNA from agarose gels to nylon membranes (Hybond-N, Amersham) in 6×SSC. Membranes were then dried for 10 minutes at 37°C before being baked at 80°C for 2 h.

Baked membranes were soaked in prehybridisation mix (75 mL 0.5 M di-sodium hydrogen orthophosphate pH 7.2, 75 mL milli-Q water, 300 µL 0.5 M EDTA pH 8.0, 10.5 g SDS) for 2 h at 65°C. Firstly, Jeffreys 33.15 probe (Jeffreys et al. 1985b) was labeled with α-^{32}PdCTP by random priming with Amersham radprime kit. Unincorporated label was removed using a G50 sephadex column. Hybridization of Jeffreys 33.15 to membranes was at 65°C for a minimum of 18 h. Membranes were

then washed twice with 5×SSC, 0.1% SDS at 65°C. DNA fragments hybridized to the 33.15 probe were exposed on X-ray film at either -80°C with one intensifying screen or at room temperature for 1-6 days. After adequate exposure, membranes were stripped (see below) and re-probed with CA probe. CA probe was similarly labeled with α-^{32}PdCTP by random priming with Amersham radprime kit, and unicorporated label was removed using a G50 sephadex column. Hybridisation to membranes was at 55°C for a minimum of 18 h. Membranes were washed twice with 5×SSC, 0.1% SDS at 55°C, and then exposed as detailed for Jeffreys 33.15 probe.

Membranes were stripped of hybridised probes after adequate exposures were made. Membranes were firstly rocked in 150-200 mL of pre-warmed 0.4 M NaOH at 45°C for 45 minutes. This was followed by two 15 minute washes with 150-200 mL of neutralising solution (0.1%SSC, 0.1%SDS, 0.2M Tris HCl pH 7.5).

Data analysis

Scoring minisatellite DNA fingerprints

Samples from two clutches were loaded onto each gel. All chicks from a clutch were loaded with their putative parents on either side. Remaining lanes, situated in the center of the gel, were loaded with all putative extra-pair males for those families (Figure 1). This sequence of loading samples minimized distance for between-lane comparisons, a source of error in reading gels (Burke et al. 1991).

All gels contained molecular weight markers in the outermost lanes (1 & 20). This was important to determine even running within each gel and allow approximation of band sizes. In addition, gels included one common individual that acted as a genomic control to provide a means of standardization for hybridization conditions and the distance run between gels (Miller et al. 1994). Band positions were transposed on to the acetate overlays by dotting band centers with a permanent marker pen (Galbraith et al. 1991). Bands were considered identical in two individuals if there was no more than a two-fold difference in intensity estimated by eye (Bruford et al. 1991; Hasselquist et al. 1995) and

they fitted into bin sizes of no more than 1.5mm (Finch & Lambert 1996; Lambert et al. 1994).

Assigning parentage

Parentage was assigned through the identification of novel bands (Westneat 1990; Westneat 1993) and the bandsharing coefficients of putative parents (Wetton et al. 1987; Bruford et al. 1991).

As offspring inherit half their nuclear genome from each parent, combined parental fingerprints should be able to explain all bands present in their chicks. Therefore potential extra-pair paternity or intra-specific brood parasitism can be detected by investigating presence of novel bands. A novel fragment can arise from three sources: (1) scoring error, (2) mutation, or (3) descent from at least one adult other than the putative parents (Westneat 1990). Strict protocols for scoring gels (see above) were employed to minimize the chance of error. Previous workers have shown that mutation rates are sufficiently high to be directly measurable (e.g. humans, Jeffreys et al. 1985b; Jeffreys et al. 1988; dunnocks, Burke et al. 1989; indigo bunting, Westneat 1990). All are in agreement that this rate is in the order of 10^{-3} per gamete. Despite this, extra-pair fertilizations and/or intra-specific brood parasitism should result in many more novel fragments than can be explained by mutation alone.

Investigation for the presence of novel fragments was also compared to bandsharing coefficients between all putative parents and their offspring. Bandsharing coefficients were calculated using the formula:

$$D= 2N_{AB}/(N_A + N_B)$$

where N_{AB} is the number of bands shared by both individuals A and B, N_A is the total number of scorable bands in individual A and N_B is the total number of scorable bands in individual B (Wetton et al. 1987; Lynch 1990). Values can range from zero, where there are no common bands, to one, where both individuals share an identical fingerprint. All possible two way comparisons (dyads) between chicks and adults were

calculated in this way for each gel/probe combination.

Assuming Mendelian inheritance of DNA fragments, bandsharing coefficients between first-order relatives should be approximately 0.5. Furthermore, bandsharing coefficients for non-parent dyads should be somewhat less because fewer bands are common to both. Typically bandsharing values for unrelated individuals in outbreeding populations are approximately 0.2-0.3 (e.g North Island robin, Ardern et al. 1997; brown skua, Miller et al. 1994; willow tit, Orell et al. 1997). This technique losses power as the background bandsharing increases. Such is the case when individuals are highly related (e.g pukeko, Lambert et al. 1994), or have been through a recent population bottleneck (e.g black robin, Ma &Lambert 1997). Bandsharing distributions in an outbred population for related versus unrelated individuals should be distinctly bimodal in distribution.

RESULTS

Banding Profiles

Both probes produced variable banding profiles for most individuals (Figure 1). Five tissue samples probed with 33.15 and six tissue samples probed with CA were severely degraded and did not produce scorable profiles. These individuals were ignored for all subsequent analyses. Bands were scored in the approximate size range of 5-23 kb for probe 33.15 and 5-12 kb for probe CA. Samples extracted from whole blood produced banding profiles with more resolvable bands than those extracted from tissue. An average of 21.14 ± 3.19 (SD) and 12.29 ± 3.4 (SD) bands were scored from blood and tissue respectively for probe 33.15 and 19.39 ± 4.15 (SD) and 15.2 ± 5.07 (SD) from blood and tissue respectively for probe CA. Each probe provided a different set of fragments, although there was some degree of overlap: mean overlap (X)= 0.348 ± 0.098 (SD), n=11 for 33.15 vs CA; X = 0.291 ± 0.087 (SD), n=11 for CA vs 33.15.

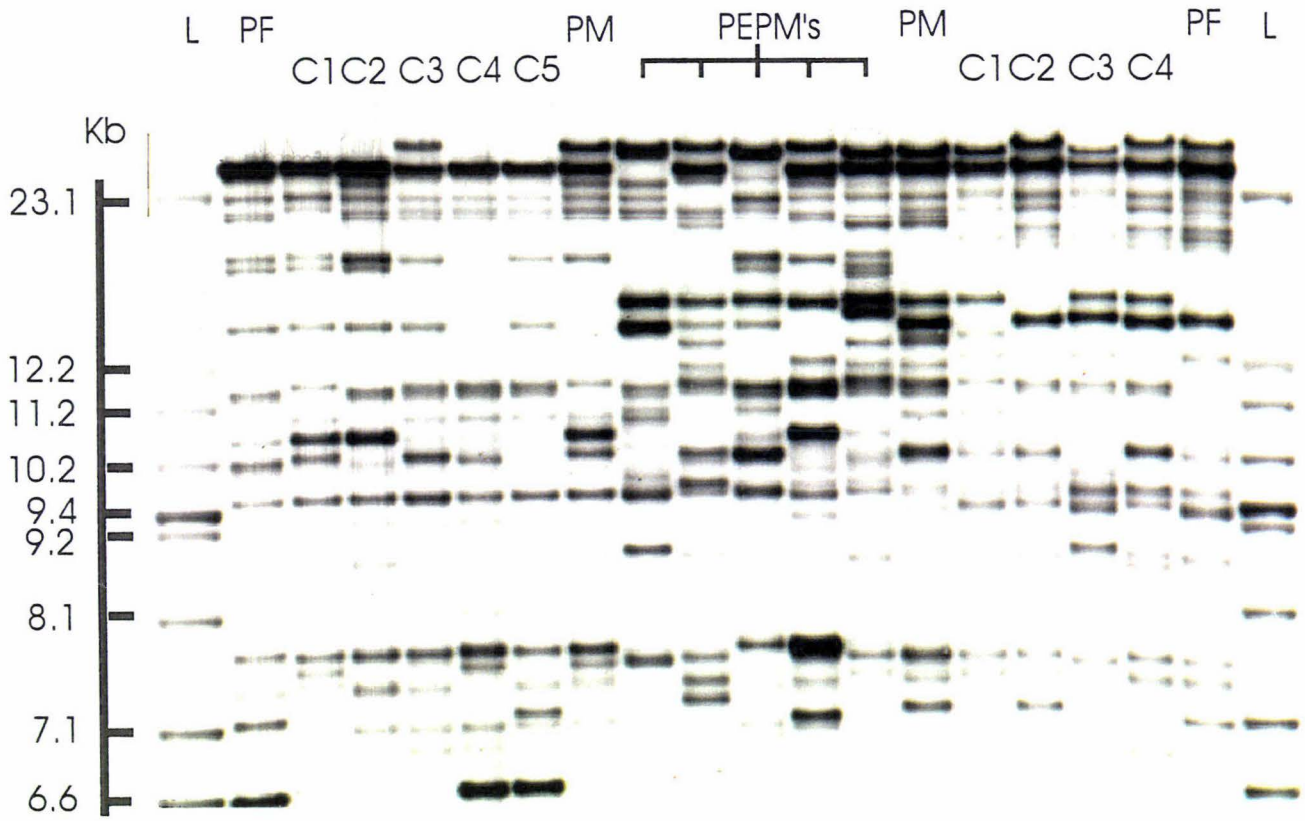


Figure 1. DNA fingerprint of two clutches of stitchbird offspring (C1-C5 and C1-C4), with their putative mothers (PF) and putative fathers (PM) positioned on either side. Remaining lanes in the center of the fingerprint are putative extra-pair males to both clutches. Nuclear DNA was digested with the restriction enzyme *Hae III,* and hybridised with the minisatellite probe 33.15. Outside lanes on the gel contained molecular weight markers with the scale added down the left margin.

Parentage Analysis

Novel Fragments

Sixteen of the 34 scorable chicks were completely compatible with their social parents. All remaining chicks had at least one novel band present in their fingerprint (Figure 2). Samples extracted from blood showed either just one, or six or more novel bands and those derived from tissue showed either one, three or five novel bands. The frequency distribution describing the number of novel fragments is bimodal for samples derived from blood (Figure 2a), which is expected if both mutation and mis-assignment of parentage has occurred (Westneat 1990; Westneat 1993).

Assuming that the occurrence of a single novel fragment was the outcome of a mutation event, the mutation rate can be calculated. Given that 1192 clearly resolved bands were scored for all chicks and a single novel band was recorded on nine occasions, the mutation rate can be calculated as $9/1192 = 7.55 \times 10^{-3}$. This observed mutation rate lies within the order of 10^{-3} per band per meiotic event calculated for numerous other species (Jeffreys et al. 1985b; Burke & Bruford 1987; Burke et al. 1989; Westneat et al. 1990; Verboven & Mateman 1997). This suggests that it is reasonable to assume that the single novel bands are the result of mutation and not miss-assigned parentage. Twenty-two of the nestlings either had zero or one novel band, and were therefore considered the genetic offspring of their social parents.

The remaining ten nestlings derived from blood samples and two nestlings from tissue samples must be the result of either extra-pair fertilisations or intraspecific brood parasitism. Assignment of paternity was attempted by reconstructing frequency distributions of novel bands between these extra-pair nestlings and all other males run on each gel (Figure 3). As each nestling will be compared to at least nine males on a gel, an element of pseudo-replication is present. However, the importance of the results comes from the trends produced rather than actual frequencies obtained. If the actual genetic father is among the males run on the gel, few or no novel bands should be detected. All other comparisons, between nestlings and non-fathers, should result in more fragments than can be explained by mutation. Frequency distributions of extra-

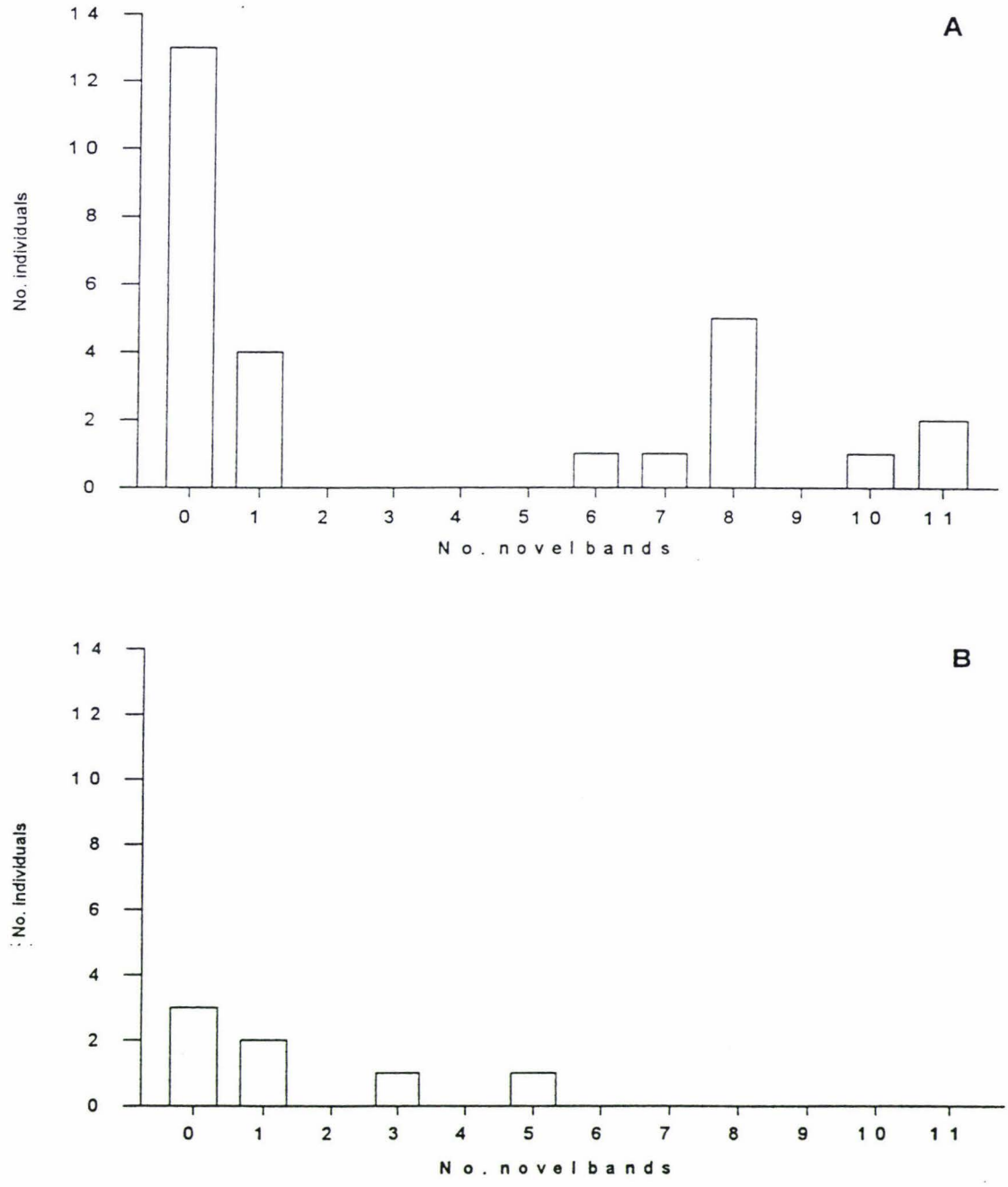


Figure 2. Numbers of offspring with different numbers of novel fragments when compared to both putative parents. A shows offspring samples extracted from blood, and B shows offspring samples extracted from tissue. Individuals with one novel fragment are best explained by mutations, and individuals with more novel fragments are best explained by extra-pair parentage.

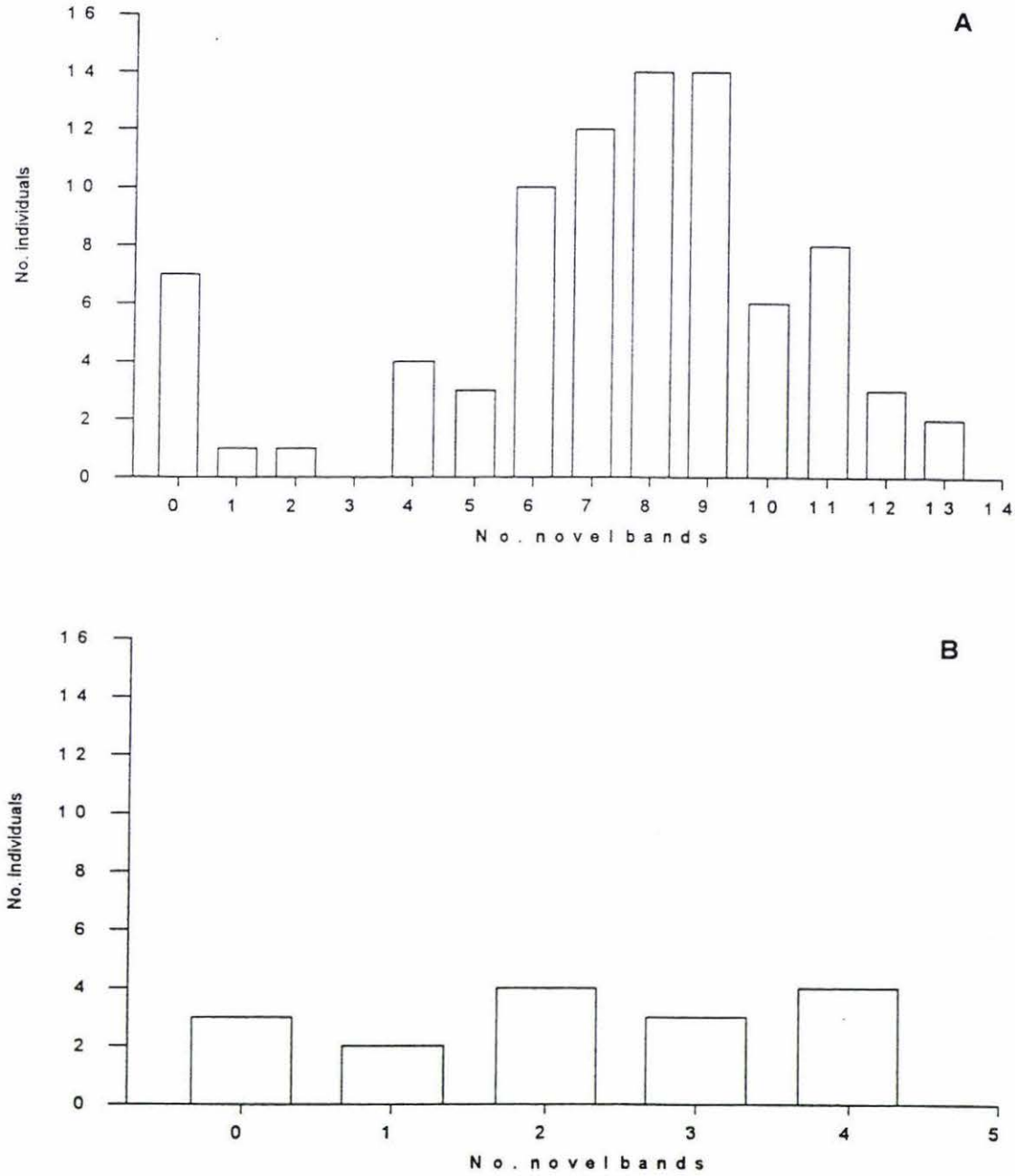


Figure 3. Distribution of extra-pair offspring with different numbers of novel fragments when compared to all other males run on each gel. A shows samples extracted from blood, and B shows samples extracted from tissue. Assuming no instances of intraspecific brood parasitism, paternity can be assigned to those males who explain all fragments, and to individuals with a single novel fragment which is assumed to be due to mutation.

pair nestlings, extracted from blood samples showed a distinct bimodal distribution (Figure 3a).

A single nestling had two novel bands yet grouped into the left-hand portion of this bimodal distribution. Consequently I considered this comparison to represent a parent-offspring relationship. This was later confirmed through comparisons of bandsharing coefficients. Genetic parentage was assigned for nine of the ten nestlings. The unassigned individual contained 8-12 novel bands when compared to all males run on the same gel.

The two nestlings sampled from tissue had all their paternal bands present in an extra-pair male run on the same gel. However, one nestling had its paternal bands present in extra-pair males, and therefore paternity was ambiguous. The small number of bands scored in this nestling were lighter than in all other samples which indicates that the DNA was either underloaded and/or partially degraded. Unfortunately, the majority of these fragments were explained by the female, leaving few potential paternal fragments. Again bandsharing coefficients were able to clarify this problem.

Bandsharing Patterns

The criterion for assigning parentage was supported by comparison of bandsharing coefficients. When compared to males assigned as genetic fathers, through investigation of novel bands, nestlings derived from blood samples had a mean bandsharing coefficient of 0.71 ± 0.08 (SD) for probe 33.15, and 0.70 ± 0.09 (SD) for probe CA_1. This value is similar to that calculated for the mean maternal bandsharing; 0.70 ± 0.06 (SD) for probe 33.15 and 0.73 ± 0.07 (SD) for probe CA. Bandsharing coefficients calculated for nestlings and all non-fathers and non-mothers gave mean values of 0.47 ± 0.1 (SD) for probe 33.15, and 0.49 ± 0.1 (SD) for probe CA. Bandsharing distributions from these samples showed a near distinct bimodal relationship for parent chick dyads and non-parent chick dyads for both probes (Figure 4). For each nestling, the male assigned as the father provided the highest bandsharing coefficient in comparison to other males. This value was always similar to that calculated for the genetic mother, offering support that these values are indicative of first-order relatives.

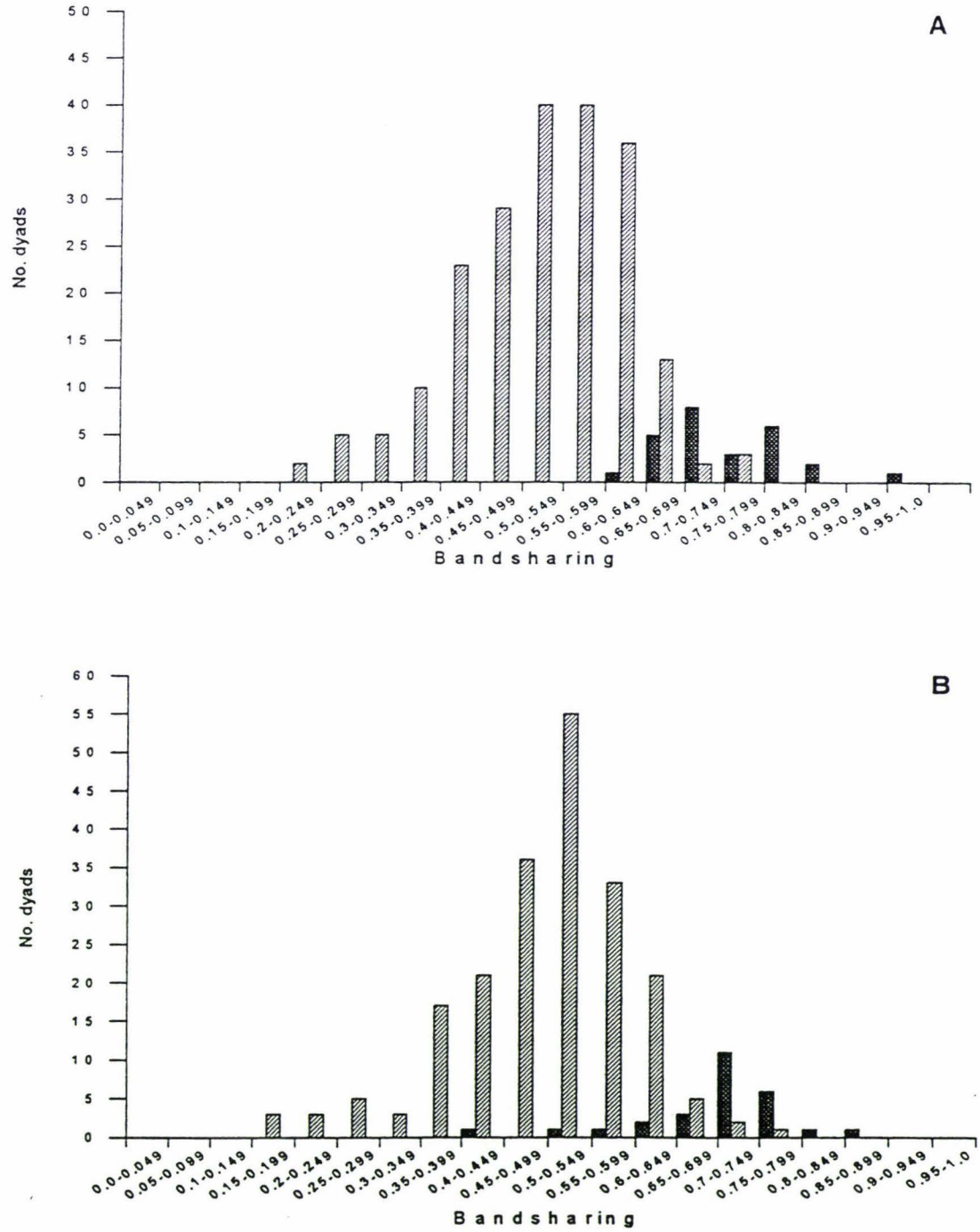


Figure 4. Frequency distributions of band-sharing coefficients among chick-nonparent dyads (light bars) and chick-parent dyads (dark bars) for minisatellite probes 33.15 (A), and CA (B). Parentage is based on assignment of novel bands.

The single nestling whose novel bands were not explained by any male sampled (discussed above) also had bandsharing coefficients which fell among other non-parent chick dyads. This confirms the individual's genetic father was not run on the gel.

Bandsharing distributions for nestling samples derived from tissue showed large overlap between dyads of parent chicks and non-parent chicks. Therefore, assigning parentage was difficult using this method alone. However, these values were useful in determining paternity when analysis of novel fragments was inconclusive. The single case where an extra-pair nestling had all its fragments explained by two males showed clear distinctions in bandsharing coefficients between related versus unrelated dyads. One male provided the highest bandsharing coefficient of 0.774, whereas the second gave a value of 0.667, (among four non-parent chick dyads that lie above 0.6 for this individual).

Assigning Parentage

Genetic parentage was assigned to all but one (33/34) of the scorable nestlings by investigating the presence of novel bands and comparing bandsharing coefficients. All nestlings were assumed to be first-order offspring from the putative female assigned. This was confirmed using bandsharing coefficients, which were similar to paternal relationships and higher than unrelated comparisons. When compared to all males run on the same gel, the single nestling whose genetic father was not assigned contained more novel bands than could be explained by mutation. Also, bandsharing coefficients calculated for this nestling were similar between all chick-male dyads and grouped closely with known, non-parent chick bandsharing values. Paternity for the remaining 33 nestlings was a combination of fertilisations from: males at the nest (67%), paired males that fertilised other females (18%), and unpaired males achieving copulations with paired females (15%)(Figure 5).

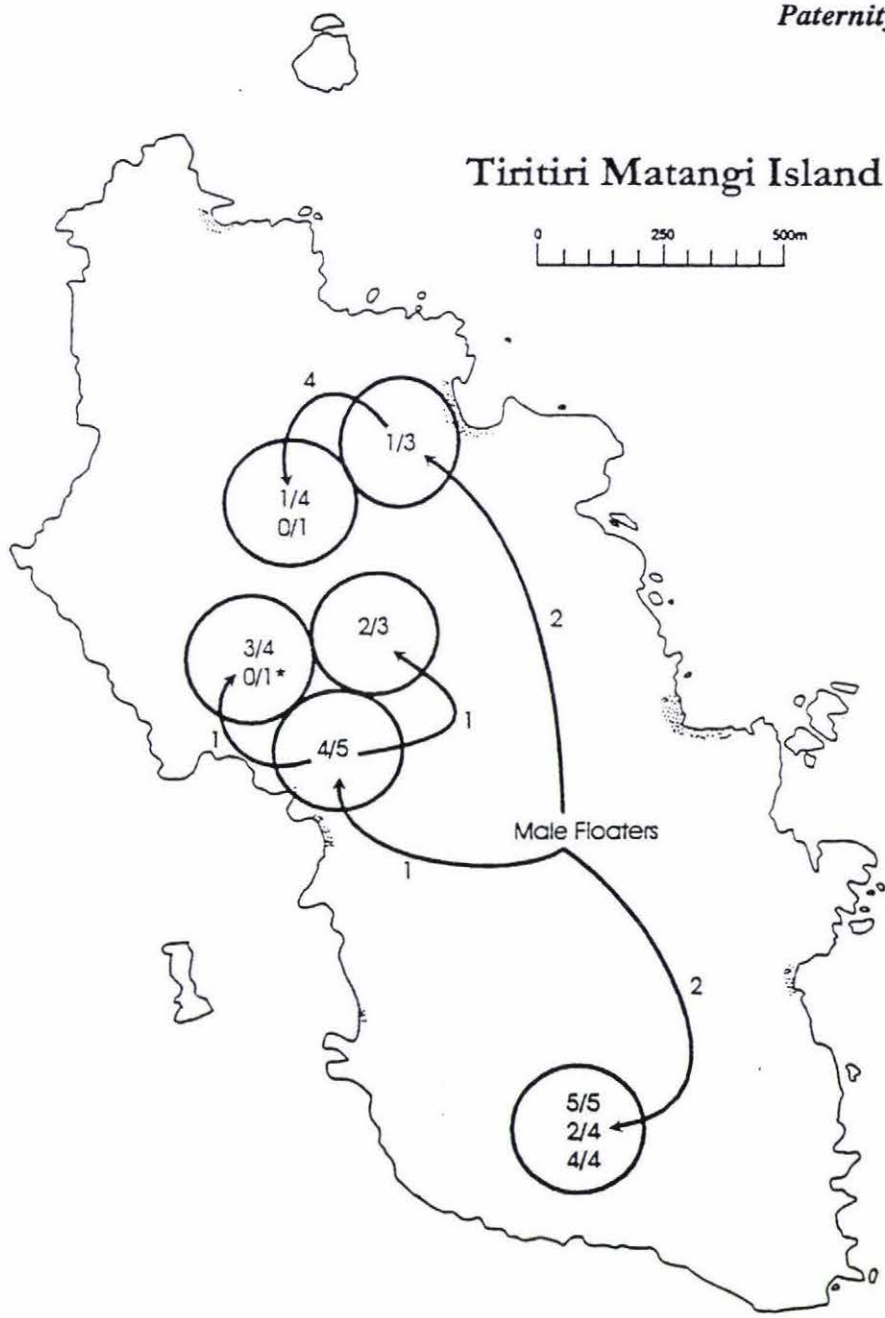


Figure 5. Realised reproductive success of male stitchbirds breeding on Tiritiri Matangi Island. Circles indicate nest sites which were defended by single males. The fractions in each circle show the number of chicks fathered by the resident male over the number sampled from each clutch. Lines with arrows indicate instances of extra-pair fertilisations: lines who's origins start in a circle represent instances of paired males achieving reproductive success with females other than their mates, and lines starting from floating males represent instances of unpaired males achieving reproductive success. Numbers adjacent to lines show number of young fertilised by each extra-pair male. * indicates chick whose paternity could not be determined.

DISCUSSION

Extra-pair paternity was found in stitchbirds breeding on Tiritiri Matangi. DNA fingerprinting revealed that 35% of nestlings (12/34) were the result of extra-pair copulation and that extra-pair young were present in 80% of nests (8/10). The percentage of extra-pair young in nests ranged from 0-75% and within each nest these young were sired by a single extra-pair male. These results group stitchbirds with other species described as having high rates of extra-pair parentage (e.g 35% in indigo buntings, Westneat 1990; 38% in tree swallows, Lifjeld et al. 1993).

Illegitimate nestlings can be the result of: (1) intraspecific brood parasitism (e.g zebra finches, Birkhead et al. 1990), (2) Rapid mate switching and/or brood adoption (e.g swallows, Møller 1985), or (3) extra-pair copulations (review in Birkhead & Møller 1995). I assumed that no instances of intra-specific brood parasitism occurred in the study population (deduced from behavioural observation-see methods), and this was confirmed by high maternal bandsharing coefficients. Mate switching and/or brood adoption did not occur. This is important because such behaviour potentially overestimates the reproductive success resulting from extra-pair copulations (Birkhead et al. 1990).

Where extra-pair paternity is high, the observed reproductive output may differ vastly from the realised reproductive success (Hasselquist et al. 1995). This study is among a few where all possible parents were sampled and complete assignment of parentage was possible (others include Hasselquist et al. 1995 and Gibbs et al. 1990). Assigning paternity to all but one chick has allowed the accurate measurement of reproductive success among individuals. Differences between observed reproductive output and realised reproductive success is exemplified in stitchbird nests where an extra-pair male fathers up to 75% of nestlings. Individual male success is summarized in Figure 5, and shows that substantial reproductive success is achieved through extra-pair copulations. One particular male was paired with a nesting female yet fathered only one nestling from a clutch of three. However, this male fathered a further four chicks through extra-pair copulation with the neighboring female.

Approximately half of the extra-pair nestlings (6/11) were the result of adult males gaining increased reproductive success by adopting a mixed reproductive strategy. Westneat et al. (1990) reviews literature which indicates that among birds most extra-pair young result from such mixed strategies. In contrast, nearly half of the extra-pair stitchbird young were fathered by unpaired males. Three of the six male floaters gained reproductive success from EPCs. To my knowledge, this study is the first to provide evidence that extra-pair fertilisation by unpaired males can be a successful alternative reproductive strategy. Two of the unpaired floaters had more fertilisations than one paired male who had two broods.

The high proportion of stitchbird young fathered by unpaired males may be due to the male bias within this population. Half the adult males in the population were unable to pair and hence their only option, if they wished to breed, was to associate with already paired birds (Chapter 2). Although the male bias in stitchbirds on Tiritiri Matangi may be a direct result of the differential survival of sexes immediately following translocation, the only naturally occurring population is also thought to be male biased (Rasch 1989). Male-biased populations have been reported in numerous bird species (e.g eastern bluebirds, Macdougall-Shackleton et al. 1996; violet green swallows, Beasley 1996; and red-winged blackbirds, Searcy & Yasukawa 1995), and have been implicated in causing both increased male-male competition for females, and increased levels of mate guarding (Beasley 1996). Studies of red-winged blackbirds indicate that even in species with unpaired male floaters (Searcy & Yasukawa 1995) extra-pair paternity is still predominantly the result of EPCs by already paired, neighbouring males (Gibbs et al. 1990; Westneat 1993). Stitchbirds provide an alternative result indicating that unpaired male floaters may gain substantial reproductive success. This provides evidence for increased competition for females when populations become male biased. Further genetic research directed at male-biased populations may reveal other instances of unpaired males gaining reproductive success, and thus provide evidence that such biases cause increased competition for breeding opportunities.

In situations where two or more males copulate with a female during her fertile period, competition between sperm can result (Birkhead & Hunter 1990; Parker 1990). The

high level of extra-pair paternity documented in this study is evidence that intense sperm competition exists among stitchbirds. This confirms predictions from Castro et al.'s (1996) work on social breeding relationships and reproductive anatomy of stitchbirds breeding on Kapiti Island. Although stitchbirds bred in socially monogamous pairs on Tiritiri Matangi, levels of extra-pair paternity indicate that sperm competition is comparable to species where it is documented as intense (Birkhead 1995). Interestingly socially monogamous pairs of dunnocks were also genetically monogamous, suggesting that sperm competition results only from polyandrous relationships and not EPC (Burke et al. 1989). All extra-pair stitchbird young within each nest were fathered by the same extra-pair male. This is in contrast to the aquatic warbler where 44% of broods contained offspring with 3 or 4 different fathers (Birkhead 1993).

Paternity analysis tells us only about the outcome of sperm competition – the end result of a succession of behavioural and physiological processes (Birkhead & Parker 1997). The following chapters deal with behavioural aspects associated with the intense sperm competition found in this population.

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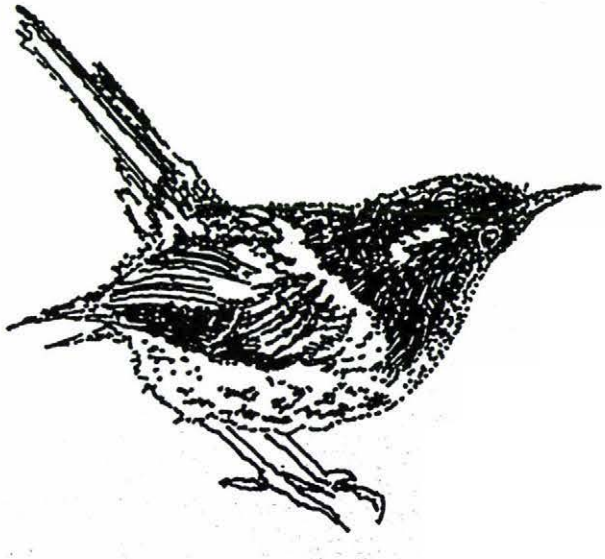
CHAPTER TWO



A behavioural investigation of extra-pair copulation.

ABSTRACT

Extra-pair copulation (EPC) is a frequent occurrence in the Tiritiri Matangi Island stitchbird population and results in extra-pair paternity (Chapter 1). Such copulations occur despite resistance by the female and are therefore assumed to be a male driven reproductive behaviour. Visits made to nest boxes may provide a cue to extra-pair males who concentrate their copulation attempts at peaks in female fertility. Behaviour of extra-pair males suggests they also focus attempted EPCs on females at nest sites. Paired males attempt to defend their paternity by defending an area around the nest site by territorial calling and displacing intruding males. Despite paternity defense of paired males some EPC attempts are still successful. Predicted additional paternity guards, such as frequent copulation do not occur in this population.

INTRODUCTION

Extra-pair copulation (EPC) occurs in many bird species, and has been shown to result in extra-pair paternity (Birkhead & Møller 1995). An EPC occurs when a male mates with a female paired to another male (Parker 1990). Trivers (1972) suggested that a male's reproductive success is limited by his access to females rather than his rate of sperm production. Males may overcome this restriction by gaining extra-pair copulations. In birds, a relatively large time window exists for males to successfully fertilise eggs of a given female. This is because females of most bird species can store viable sperm in specialised sperm storage tubules (Birkhead et al. 1991; Birkhead & Hunter 1990). Because females can store sperm, and males can produce large numbers of sperm, there is strong potential for extra-pair fertilisation.

EPC in stitchbirds has been recorded previously on Kapiti Island (Castro et al. 1996), and multi-male chases of females have also been reported in breeding stitchbird populations (Kapiti Island, Lovegrove 1985; Little Barrier Island, Angehr 1984). These multi-male chases of females and descriptions of observed copulations suggest that

females actively resist EPC. Resisted copulations have been recorded in many bird species (e.g bank swallows, Beecher & Beecher 1979; zebra finch, Birkhead et al. 1988; purple martins, Morton et al. 1990; Smith's longspur, Briskie 1992; white fronted bee-eater, Emlen & Wrege 1986; indigo buntings, Westneat 1987; numerous waterfowl, Afton 1984 & McKinney et al. 1983; also see early review of monogamous colonial birds in Gladstone 1979). Assuming females behave optimally, then apparent resistance suggests they are unlikely to benefit from EPC. However, a female could potentially resist EPC to test a male's ability to overcome her resistance, and therefore use EPC as a form of mate choice (Eberhard 1996). Lack of resistance doesn't necessarily imply the absence of costs to the female – the costs of resistance may simply be greater (Birkhead & Parker 1997). Although a female may be forced to copulate, she may have post-copulatory control over which sperm fertilize her egg (Birkhead et al. 1993; Birkhead & Møller 1993a; Oring et al. 1993).

Various factors have been suggested that may affect the potential for EPC (Westneat et al. 1990). These include: (1) the density of breeding individuals, (2) the inclusive operational sex ratio -i.e. the ratio of the number of sexually active males to fertilizable, but paired, females, (3) the degree of breeding synchrony, and (4) the intensity of mate guarding. Stitchbirds breeding on Tiritiri Matangi have a highly skewed sex ratio biased toward males, and females bred asynchronously (see general introduction). It is therefore predicted that the potential for EPC is high in this population.

The presence of mixed paternity within clutches (Chapter 1) indicates that EPC does occur in this stitchbird population. The success of EPCs in achieving fertilisation will depend on various factors including: (1) the timing of copulation relative to the female's fertile period (Colegrave et al. 1995; Birkhead et al. 1987), (2) the relative amounts of sperm ejaculated (Birkhead 1995; Gomendio & Roldan 1993), and (3) potential post-copulatory control by the female (Birkhead et al. 1993). An extra-pair male's knowledge of a female's fertile period is predicted to influence the effectiveness of EPCs in fertilising eggs -i.e. the male can time copulations to coincide with the peak in female fertility (Birkhead et al. 1987). For many birds this peak in fertility is thought to be 2-3 days before egg laying (Westneat 1987; Colegrave et al. 1995). Extra-pair males

may use various cues to assess the fertility of the female including: (1) state of the nest, (2) guarding behaviour, (3) flight behaviour of the female, and (4) within-pair copulations (Gomendio & Roldan 1993; Birkhead et al. 1987).

Along with the benefits gained by some males through EPC is the concurrent loss of reproductive success suffered by the paired male. In the majority of cases it is accepted that it is in the paired male's best interest to prevent EPCs from occurring (but see Jamieson 1994). Such competition for paternity has led to a range of elaborate paternity defense behaviours in birds (Birkhead 1995; Macdougall-Shackleton et al. 1996). These include: (1) mate guarding (e.g western bluebirds, Dickinson & Leonard 1996), (2) defending resources necessary for females to successfully breed (e.g nest guarding by tree swallows, Beasley 1996, and (3) frequent within-pair copulation (e.g Smith's longspur, Briskie 1992). Males may be faced with the choice of guarding females as they forage, or guarding nest sites. Females may be easiest to find when returning to nests. Increased EPC attempts and social harassment at nest sites is thought to be a result of this in colonial bird species (Alexander 1974; Emlen & Wrege 1986). If most EPC occurs near the nest, then paired males who restrict paternity defense to nest sites will effectively guard the female. Males of many bird species defend nests, and this is recognised as a form of paternity defense (Birkhead 1995). Stitchbirds on Kapiti Island are known to defend areas around the nest by calling and behaving aggressively toward any intruding males (Castro 1995). It is not known whether this defense of nest sites is a form of resource defense, or the guarding of females who stay near the nest site.

This chapter addresses three primary questions. Firstly, are EPCs resisted by females? This has implications concerning the adaptive significance of such behaviours for both males and females. To determine this, behaviours associated with both within-pair and extra-pair copulations are compared.

Secondly, are EPCs timed to maximise chances of fertilisation by coinciding with the peak in female fertility? This is important because knowledge of a female's fertile period is predicted to influence the effectiveness of EPC as a successful reproductive behaviour (Birkhead 1987). In order to determine if extra-pair males attain knowledge

of females' fertile periods it is necessary to observe whether males are altering their behaviour as females progress through their reproductive cycles. Two measures of extra-pair male behaviour were assessed; (1) presence of extra-pair males within 30 m of the nest site, and (2) frequency of EPC attempts. It was predicted that if males attained knowledge of the females' fertile period then there would be an increase in these two parameters as females progress through their reproductive cycle, peaking around 2-3 days prior to laying the first egg.

Visits made by extra-pair males to nest boxes were recorded to investigate whether extra-pair males use the stage of nest completion as a cue to assess the fertility of breeding females. If males visit nests to assess female fertility it is predicted male visits would follow no pattern, and occur throughout the reproductive cycle. The alternative is that males visit nest boxes to find females and obtain EPCs. If males are seeking EPCs, the predicted pattern of nest visits would be similar to the patterns of extra-pair male presence and EPC attempts.

Finally, are paired males responding to the presence of EPC by attempting to defend their paternity? To test whether paired males defend females and/or nest sites, two parameters were assessed: (1) territorial call frequency and, (2) frequency of displacements. Resource defense and mate guarding are regarded as alternative paternity defense behaviours (Beasley 1996). In order to determine whether male stitchbirds defend females or nest sites the percentage of each sample that the male and female stayed within 30 m of the nest site was recorded. It was predicted that if males defended females then the time males are on the territory will closely correspond to when females are there. This will only provide an indirect measure to the extent of mate guarding as the absence of both members of a pair does not indicate that they are together. Alternatively, males may defend nest sites. It was predicted that if males defended nest sites they would spend time near the nest regardless of female presence, and would continue to act defensively. To assess this defensive behaviour territorial calls and displacements were recorded both when the female was present and absent. It was predicted that paired males who defend nest sites would continue to call territorially and displace intruding males when the female was absent.

Additionally, paired males may copulate frequently with their mates to devalue rival males' sperm (Birkhead et al. 1987). It was predicted that the nests with the highest rate of attempted EPC would have the highest rate of within-pair copulation. Paired males would also be predicted to initiate the majority of copulations.

METHODS

Individuals Sampled

The breeding behaviour of stitchbirds was observed over two breeding seasons (1995/96 and 1996/97) on Tiritiri Matangi. In both years the operational sex ratio was heavily skewed toward males, 12:4 in 1995/96 and 12:6 in 1996/97. Data were collected from the single successful breeding female (1 of 4) in the first breeding season and all 6 breeding females in the second. Twelve males were present in both seasons. Four males died in the intervening winter, but a single male chick produced in the first year survived to breed in the second, and three other males were the result of a second translocation of stitchbirds to the island in August 1996. Three of the four females survived to breed in the second season, including the single female represented in the data. One female chick produced in the first year survived to breed in the second, and the other two females came from the second translocation.

All individuals were captured and banded with unique combinations of wrap-around C size plastic bands and a numbered aluminum band for easy identification (see Chapter 1). In addition some males had unique plumage and/or call types which aided quick recognition in the feild.

Sampling Regime

Observations of breeding behaviour were initiated as early in the reproductive cycle as possible (range 15 to 3 days before egg laying). Nest building was used to identify when each female was entering her fertile period. Systematic searches of stitchbird nest boxes and general observation of bird behaviour (as detailed in Chapter 2) usually

enabled early identification of nesting.

Orange flagging tape was used to mark out a circular area of 30 m radius around each nest site. Observations were restricted to within this area allowing direct comparisons between nests. This distance is partially justified by earlier observations of stitchbirds breeding on Kapiti Island (Castro et al. 1996). Castro et al. (1996) revealed that paired males would call and behave aggressively toward any other males within a 30 m radius of their nesting tree. Due to the nature of the habitat, it would be difficult to accurately observe all behavioural interactions in any larger area.

Behavioural observations were conducted at three time intervals each day, from when the nest site was discovered to when egg laying was complete. The three time intervals were two hours in duration (06:30-08:30, 11:00-13:00 and 16:00-18:00), and the observation time was divided equally among nests. Due to nesting asynchrony there were never more than two nests to observe at one time. Therefore, each nest was usually observed for at least 50 minutes per time period, allowing time to move between nest sites within each set time interval.

An all occurrence sampling regime (Martin & Bateson 1993) was used to record stitchbird breeding behaviour. The following behaviours were recorded for analysis;

- All attempted copulations
- Presence of each member of the pair
- Territorial call frequency of the paired male
- Displacements of intruding extra-pair males by the paired male
- Presence of any extra-pair males or females
- Visits to the nest box by extra-pair males

Data were recorded with a microcassette recorder, and later transcribed and categorised for analysis.

Copulation attempts were either within-pair or extra-pair. For each copulation, I described the behaviour of birds involved and any other birds in the area. I also described individuals' behaviour leading directly to copulation attempts. Where the

outcome of a male-female interaction was not known, e.g. when the female was chased from the nesting area, it was also recorded as an attempted copulation. Therefore the data may underestimate the number of successful copulations. Copulations also occurred in nesting boxes. This could be observed in the first breeding season because females nested in boxes with large openings and these openings were positioned near the top of the box, allowing full view of the female while she was sitting on the nest. Such copulations were accompanied by distinctive calls from both birds, and noises made from wings flapping inside the box. In the second breeding season the nest boxes used were designed specifically for stitchbirds. These boxes have a 4 cm × 5 cm entrance hole, hence behaviour of birds could not be directly observed. If noises similar to those described in observed box copulations were heard when a male and female were in a box, I assumed a copulation was occurring.

Copulation data were also quantified with respect to the following parameters;

- Within-pair copulation attempts
- Extra-pair copulation attempts
- Proportions of successful copulations
- Proportions of copulations initiated by different individuals
- Number of males involved

Data Analysis

Since sample periods were at fixed times, and stitchbirds are generally wide ranging, one or both birds in a pair were often absent from the sample area. To account for this, frequencies of certain behaviours were recorded as a proportion of the amount of time each individual spent in the territory. The frequency of copulations and attempted copulations were calculated in terms of female hours. Extra-pair male displacements were calculated from paired male hours. Call frequency was split into calls when the female was present and also when she was absent. The frequency of visits to the nest box, by any individual, were recorded per observation hour. Any frequencies calculated from individuals spending less than 10% of their time in the territory were ignored from any analysis. This was to remove any spurious results that may arise when frequencies

are calculated from very short sample times. A measure of each extra-pair male's presence was obtained by tallying all instances when they were involved in defined behaviours -i.e. I counted every time an individual was seen being displaced, attempting to copulate, calling, visiting the nest box or seen while I searched the area within 30 m radius of the nest. Those individuals counted more times within the nesting area were assumed to spend more time there.

Paired t-tests were used to test: (1) whether paired males and females differed in the amount of time spent on their territories, and (2) whether the male spent more time on the territory when the female was also present than when she was absent, and (3) whether territorial call frequencies of paired males were different when the female was present vs when she was absent.

Regression analysis was used to investigate the relationship between within-pair copulation attempts and EPC attempts. An average rate was calculated for each nest over the period corresponding to that used in the remainder of analyses (7 days before egg laying through till clutch completion). Each nest was observed for an equal number of hours at the different stages (see below), so differences between nests should not be biased by changes in EPC over the reproductive cycle. No transformations were necessary as the residuals conformed closely to normality.

Data were grouped into periods for the remainder of statistical analysis. The period from 7 days before egg laying through to clutch completion was divided into five periods in order to create measurable categories with reasonably even numbers of samples. Periods were distinguished by the day relative to egg laying as follows;

- 5-7 days before laying
- 3-4 days before laying
- 1-2 days before laying
- 1^{st}-2^{nd} day of laying
- 3^{rd}-5^{th} day of laying

Data up to 15 days before egg laying were only available for two females and therefore were omitted from the analysis. Observations from this time are used to describe the recorded range of certain behaviours.

ANOVA was used to test whether presence of extra-pair males, attempted extra-pair copulations, difference in call frequency, total call frequency and rates of displacements by paired males varied according to: (1) female's breeding period, and (2) time of day. I also controlled for differences among individual nests by including the nest as a third factor in the ANOVA. The individual observation sessions were treated as the units of replication. Data on total call frequencies were log transformed in order to normalise the data set. All other behavioural data conformed closely to normality when residuals were investigated. Unless otherwise stated means are expressed as mean ± SE.

RESULTS

Data were recorded from 232 observation sessions with an average length of 3061 ± 25.6 s, totaling 197 h.

Copulation Behaviour

Within-pair copulations

A total of 75 attempted within-pair copulations were observed, of which 22 resulted in copulation. Copulations could be grouped into four different types: (1) female solicited, (2) male solicited, (3) resisted "face-to-face" copulation, and (4) copulation on the nest (Figure 1). Although male solicited copulations were the most common form of copulation (37%), males solicited more often than females, and a larger percentage of male solicitation attempts were unsuccessful (Table 1). Copulation behaviour in the nest box could not be observed, but I assumed a copulation had taken place if the criteria detailed in the methods were met.

Females initiated solicitation by flying to the ground near where the male was perched. Solicitation occasionally began above the ground but the female always quickly moved

onto the ground. Females solicited copulations by tilting forward facing the male, with their tails slightly raised. Their wings would hang slightly open, and lowered, and the female would vibrate them gently. Throughout the solicitation the female would quietly warble while looking up toward the male. All solicitations, and subsequent copulations, occurred on or very near the ground (within 30 cm). Often the female solicited repeatedly in this manner before the male either flew away (75% of female solicitations) or moved to the ground and copulated.

Male solicitations were brief in comparison and the majority were unsuccessful (81%). Solicitations were initiated above the ground near the female. Males would typically warble quietly, in a similar manner to the female, with wings slightly lowered. This was followed shortly after with a display flight to the ground near where the female was perched. Display flights were characterised by a slow flapping, almost gliding flight. A rounded shoulder posture seemed to expand the bird's shoulders and forewings during such flights. This appeared to display, or enhance, the bird's yellow and white shoulder colouration. During such flights males would continue their quiet warble. Such display flights were only seen when soliciting to the female, or when males left nest boxes during a female's fertile period.

Subsequent copulations were similar for both male and female solicitations. Males and females would circle each other on the ground, both warbling and vibrating their wings. Males mounted females from the rear, flapping their wings during mounting. Typically two or three mountings would occur in a copulation bout. Each mounting was only brief, lasting no more than 4-5 s. Occasionally one of these multiple mountings was in the face-to-face position (see below). Mountings were separated by circling and neck rubbing behaviours. Neck rubbing involved birds pushing up against each other chest to chest, and rubbing necks, quickly alternating sides, while continuing wing vibration and warbling. Females were also observed mounting the male in a manner similar to the male-on-female's back position.

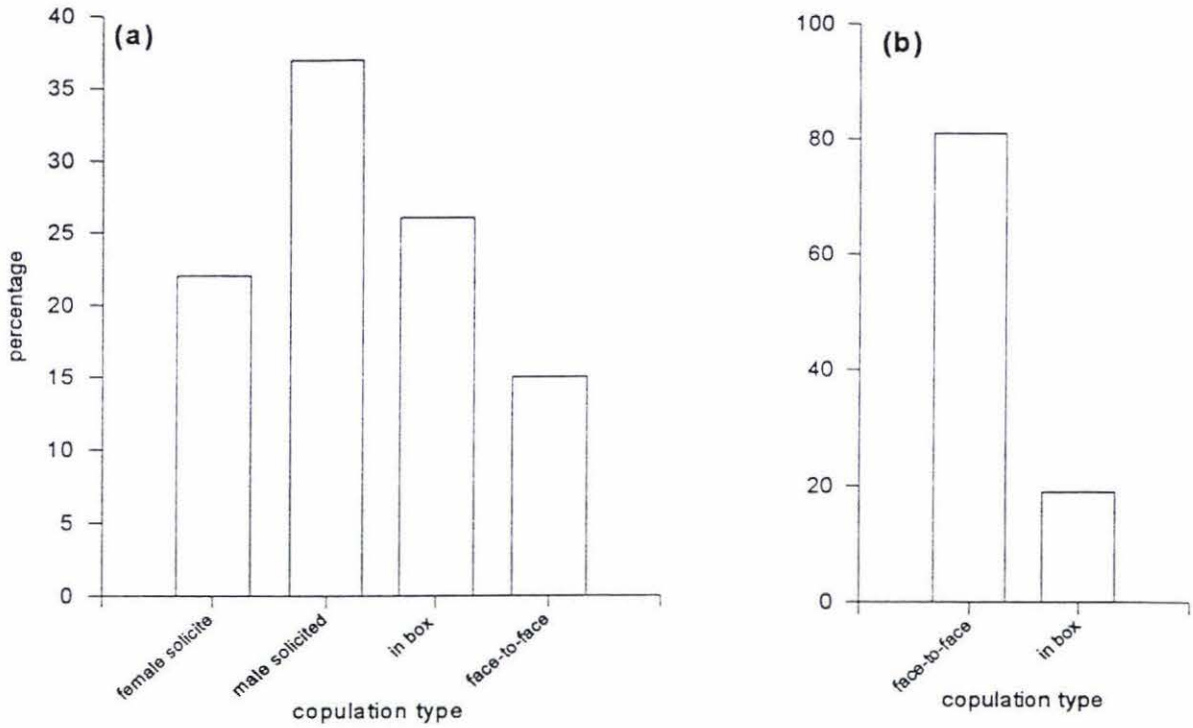


Figure 1. Percentage representation of different types of copulations observed between (a) paired stitchbirds (total 22 observed copulations), and (b) extra-pair males copulating with already paired female stitchbirds (total 36 observed copulations).

Table 1. Proportions of successful copulation attempts relative to the major categories of failed attempts, and a comparison between the mean number of males involved in both successful and failed attempts (mean number calculated from combined fail categories). WPC= within-pair copulations, EPC= extra-pair copulations.

		Total attempts (%)	No. birds involved (mean ±SE)
WPC	Successful	22 (29)	1.07 ± 0.34
	Failed female solicited	19 (25)	
			1.03 ± 0.15
	Failed male solicited	34 (45)	
EPC	Successful	36 (11)	4.59 ± 0.5
	Paired male prevented	203 (61)	
			5.4 ± 0.17
	Failed for other reasons	93 (28)	

Three resisted face-to-face copulations were observed between individuals in a pair. There were no pre-, or post-copulatory displays, although on one occasion the female was soliciting, in bouts, for 8.3 minutes directly preceding the resisted copulation. The paired male would chase the female to the ground and pin her onto her back and copulate as detailed in Castro et al. (1996). Males would clasp the female's legs with his own, and occasionally peck at the female's throat and chest area. During one of these copulations the male stayed clasped to the female for 80 s rolling slowly down a hill. In this copulation the female issued no alarm calls, seeming to lie passively still throughout.

Six copulations were assumed to have occurred inside the nest box. Males either followed the female into the box, or arrived and entered the box while the female was already inside. The birds stayed in the box for an average of 28.7 ± 4.6 seconds during which time characteristic calls and flapping of wings ensued. After this time both birds would leave the nestbox, with the males performing a typical display flight.

Extra-pair copulations

A total of 333 attempted EPCs were observed, of which 36 were successful. These EPCs could be grouped into two copulation types: (1) resisted "face-to-face" copulation, and (2) resisted copulation in the nest box (Figure 1).

All attempted EPCs occurring outside of the nest box were similar. Females would be chased by between 1 and 10 males until the attempt either failed or a single male was successful at pinning the female to the ground and copulating. During such chases the female would constantly alarm call (rapid repeated 'pew') and males would chatter loudly and fight amongst themselves. The majority of such attempts were unsuccessful (89%) largely because the female managed to hide under foliage on or near the ground in a position where her mate could defend her. Also, the constant fighting between males seemed frequently to result in confusion as to the whereabouts of the female. Such confusion, and subsequent failure of copulation attempts, didn't seem to be related to the number of males involved. The mean number of male chasers involved in failed attempts was only slightly higher than the mean number involved in successful attempts

(Table 1). Such pressure on the female would often last an entire observation session, with all extra-pair males constantly fighting amongst themselves and chasing the female whenever she tried moving from her refuge. Females behaved evasively during such encounters, frequently trying to "sneak" away from present males by moving along the ground or by climbing up a tree, not flying until above the canopy. This evasive behaviour was often successful, with extra-pair males and the paired male continuing to fight amongst themselves and look where the female was last seen after she had already left the nest area.

On 29 occasions EPCs resulted from chases of the female. Only a single male would ever mount the female in an EPC. The remaining extra-pair males seemed to fight amongst themselves until the female moved again. Occasionally another male (usually the paired male) would intervene and attempt to displace the copulating extra-pair male. All such copulations were in the face-to-face position and lasted on average 10 s. Throughout such copulations the female constantly alarm called and rapidly flapped her wings. Males behaved in a similar fashion to that described for within-pair, face-to-face copulations. No pre- or post- copulatory displays were seen.

The other seven EPCs occurred in nest boxes. Four of these could be observed because the female was nesting in a box with a large opening. Males typically approached the nest box slowly, while continuously warbling. During this approach the male would display by raising his tail and erectile ear tufts. The male would then enter the box, pin the female to the nest and copulate in the male-on-female's-back position. While copulating, the male would periodically issue loud chatters and continue warbling. Both birds would attempt to flap their wings, making characteristic noises in the confines of a nest box. Females always alarm called continuously throughout the copulation. Copulations lasted 16 ± 2 s on average, after which the male would leave the nest box with the characteristic display flight.

Ad Libitum Observation

Two additional behaviours were observed in male stitchbirds, and these were similar to reproductive behaviours previously discussed. On three occasions recently fledged

juveniles were face-to-face copulated by unrelated adult males. This occurred during the female parent's fertile period before her second clutch. Juveniles are similar in plumage to females and were still being fed by their mothers. Behaviour of males was similar to extra-pair males involved in face-to-face copulations with females, and juveniles behaved in a similar manner to females although appeared not so good at escaping.

On eighteen occasions male stitchbirds were observed neck-rubbing with foliage. The females were never present when these behaviours were observed. Males would typically search in the female's most frequently used hiding refuge while constantly warbling and displaying with raised tail and ear tufts. Neck rubbing would begin as birds moved up to a stick with wings lowered and vibrating. This behaviour would last on average 8.6 ± 1.7 seconds with the male behaving in a similar way to males involved in neck-rubbing behaviours with female stitchbirds (see above).

Extra-pair male behaviour

Extra-pair males enter the nesting area of fertile females both when she is present and absent (Figure 2). The marked increase in male presence when females are in the vicinity of the nest site may be due to the sampling method. Males locating a female are more likely to stay within the sample area and therefore be counted. Such behaviours may be predicted if extra-pair males find it difficult to locate fertile females away from the nesting area (females on average spend only 32% of their time in the territory, see below).

Presence of extra-pair males increased significantly as females approach egg laying, then dropped off toward the end of egg laying ($F_{4,80}$=12.158; p=0.000) (Table 2, Figure 2). This trend suggests males are timing their behaviour in response to female fertility, or some correlated indicator. The presence of extra-pair males also varied significantly among nests (Table 2). Intruding males visit nest boxes throughout the female's fertile period (Figure 3). The frequency of visits was lowest during the period when females were most likely to be fertile, suggesting males use nest visits for purposes other than finding the female.

Table 2 Analysis of variance testing for changes in the relative presence of extra-pair males in relation to stages of the females' fertile period. Stages were (1) 5-7 days before egg laying, (2) 3-4 days before egg laying, (3) 1-2 days before egg laying, (4) 1st and 2nd day of egg laying, and (5) 3rd-5th days of egg laying. Times were (1) AM (06:30-08:30), (2) MID (11:00-13:00), and (3) PM (16:00-18:00).

Source	Sum-of-squares	DF	Mean-square	F-ratio	P
Nest	264.326	8	33.041	46.148	0.000
Time	3.498	2	1.749	2.443	0.093
Stage	34.821	4	8.705	12.158	0.000
Error	57.278	80	0.716		

EPCs were attempted throughout some females' fertile periods. Data from the two females sampled for extended periods showed extra-pair males start attempting to copulate at least twelve days before egg laying. The first extra-pair copulation was also observed twelve days before the first egg was laid. Data for all females suggests the number of attempted copulations peaks about four days before egg laying, and decreases during egg laying (Figure 4). However the difference between stages was not statistically significant at the 5% level ($F_{4,174}$=1.996; p=0.097) (Table 3). Copulation attempts continued throughout egg laying and successful copulations were observed even after some females had completed egg laying.

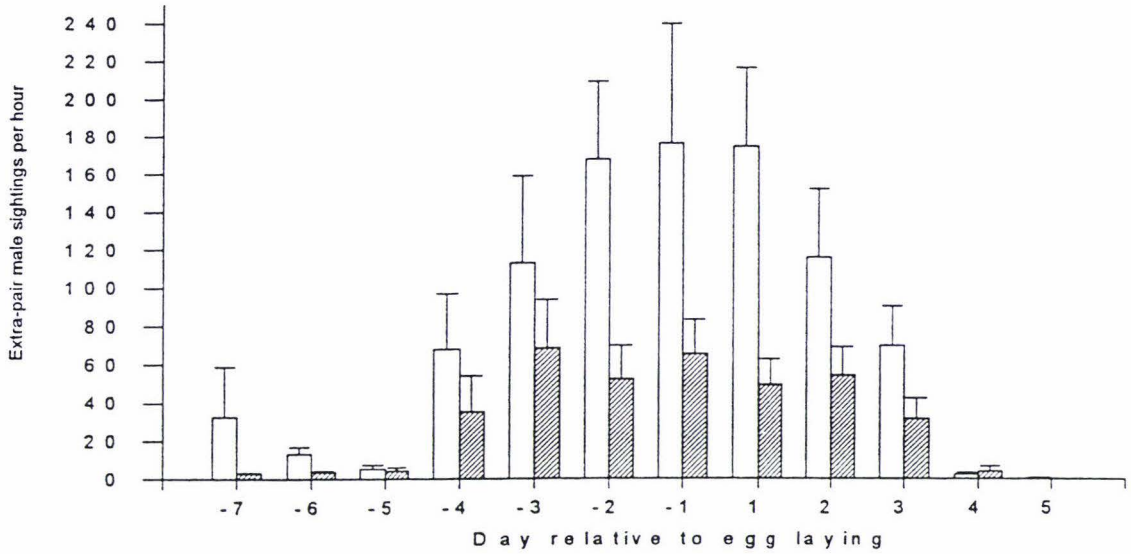


Figure 2. Frequency of extra-pair male sightings within 30 m of the nest box, both when the female is present (clear bars) and when the female is absent (hatched bars), in relation to timing of egg laying. Data are for nine nests with standard error bars fitted.

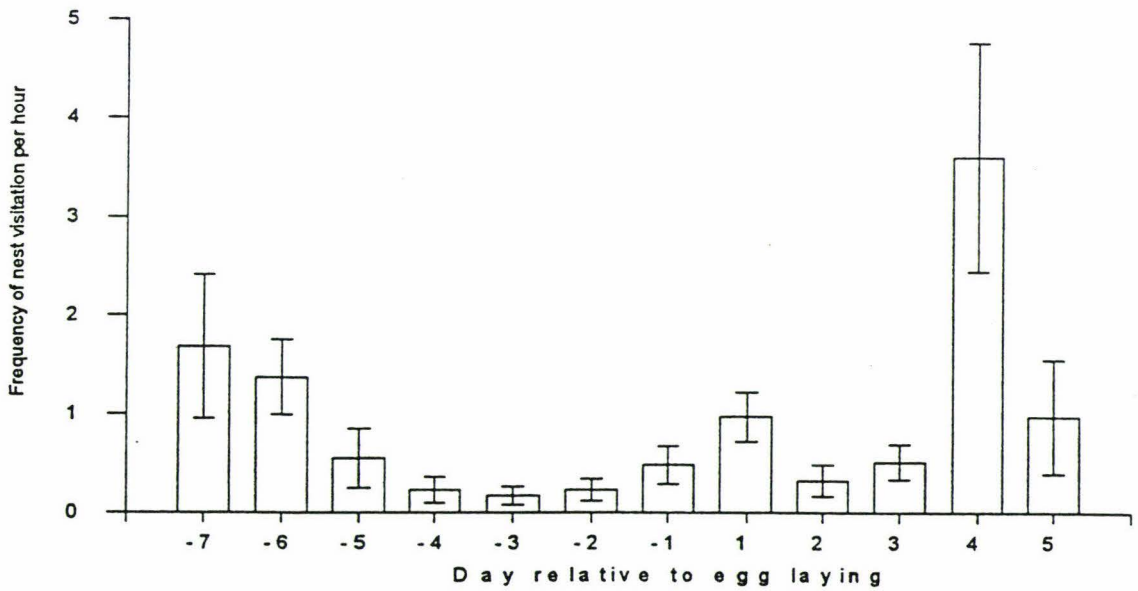


Figure 3 Frequency of nest visits by extra-pair males in relation to timing of egg laying. Data are for nine nests with standard error bars fitted.

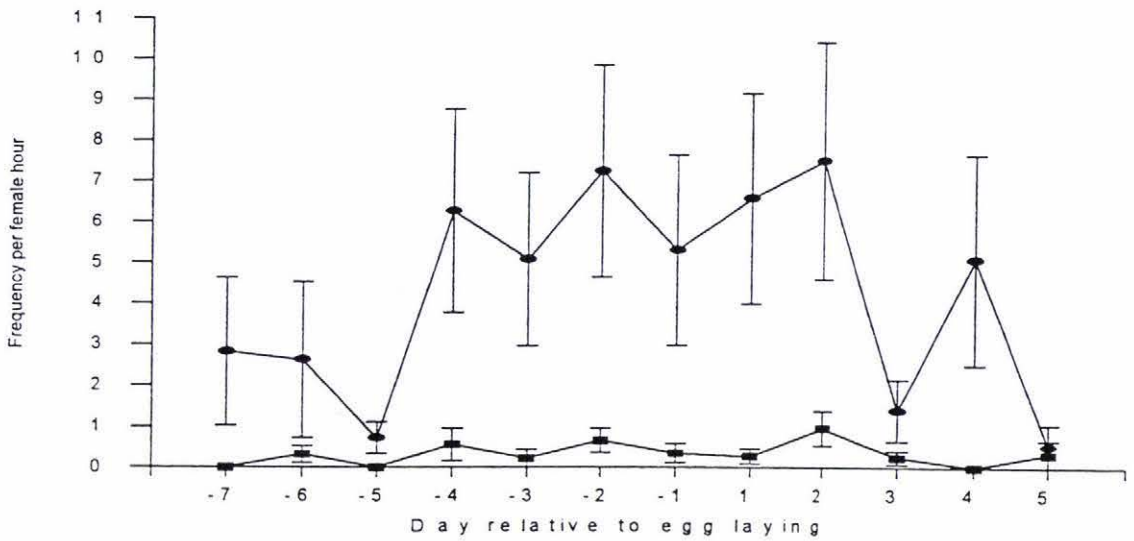


Figure 4. Changes in total attempted extra-pair copulation frequency (circles) and successful extra-pair copulation frequency (squares) in relation to egg laying. Data show the mean total for all extra-pair males. Error bars are standard errors, using each observation session as a unit of replication.

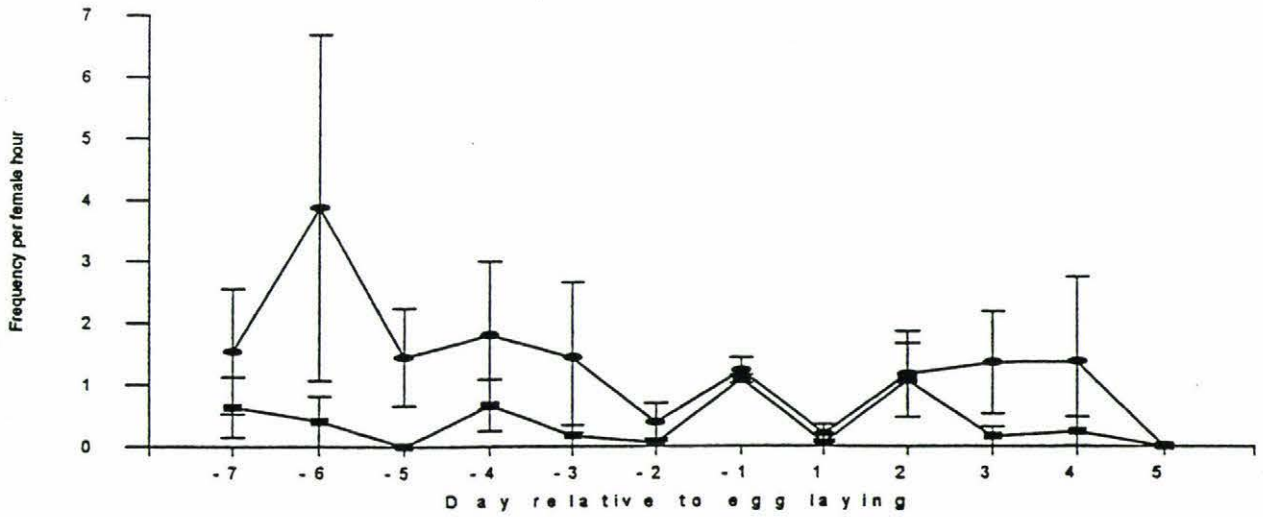


Figure 5. Changes in total within pair copulation attempts (circles) and successful within pair copulations (squares) in relation to egg laying. Data show the mean total for all extra-pair males. Error bars are standard errors, using each observation session as a unit of replication.

Table 3 Analysis of variance testing for changes in frequency of attempted extra-pair copulations in relation to stages of the female's fertile period. Stages were (1) 5-7 days before egg laying, (2) 3-4 days before egg laying, (3) 1-2 days before egg laying, (4) 1st and 2nd day of egg laying, and (5) 3rd-5th days of egg laying. Times were (1) AM (06:30-08:30), (2) MID (11:00-13:00), and (3) PM (16:00-18:00).

Source	Sum-of-squares	DF	Mean-square	F-ratio	P
Nest	57.488	8	7.186	7.189	0.000
Time	11.976	2	5.988	5.99	0.003
Stage	7.982	4	1.995	1.996	0.097
Error	173.928	174	1.000		

Paternity defense of paired males

Paired male stitchbirds spent significantly more time within 30 m of the nest box than the female (paired sample t-test, t_{228}= 20.345, p= 0.000). Males were present 77% of the time on average, and females for 33% of the time, and both male and female presence remained fairly consistent throughout the recorded period (Figure 6). A similar trend is observed from the two females sampled up to 15 days before the first egg was laid. While males spent most of their time near their nests, they spent significantly more time if the female was also present (paired sample t-test, t_{95}=8.504, p=0.000). Male presence drops from on average 94% of time near the nest when the female is present to only 66% when she is absent (Table 4.). Although there is a decrease in male presence when females are absent, males spend more time guarding nests than guarding females- i.e. when females are away from nests, males spend 66% of their time near the nest, leaving a maximum of 34% of time they could be guarding the female.

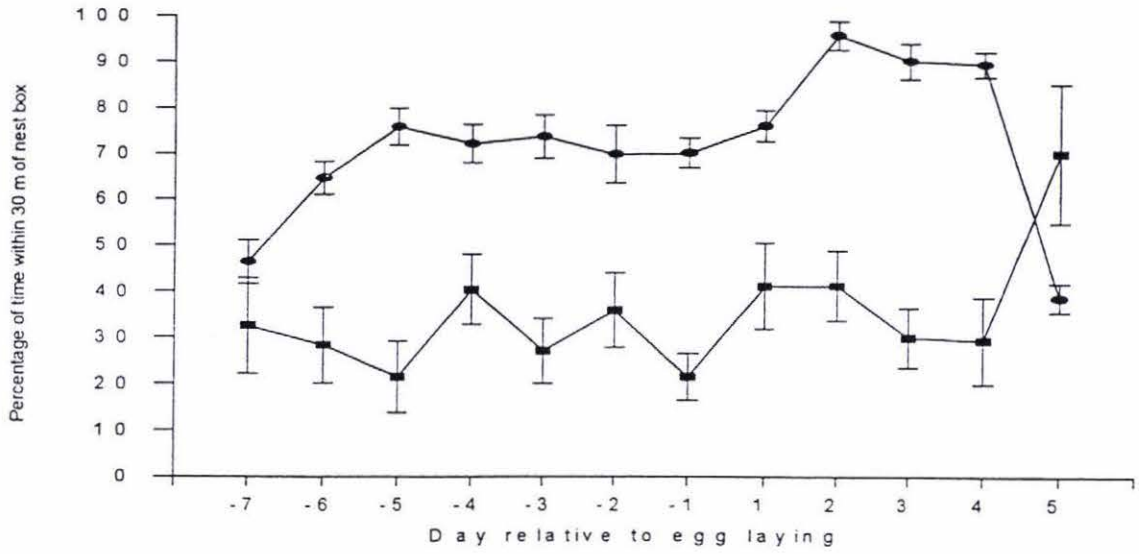


Figure 6. Time spent within 30 m of the nest site by the paired male (circles) and the female (squares) in relation to egg laying. Data are means for nine nests. Error bars are standard errors, using each observation session as a unit of replication..

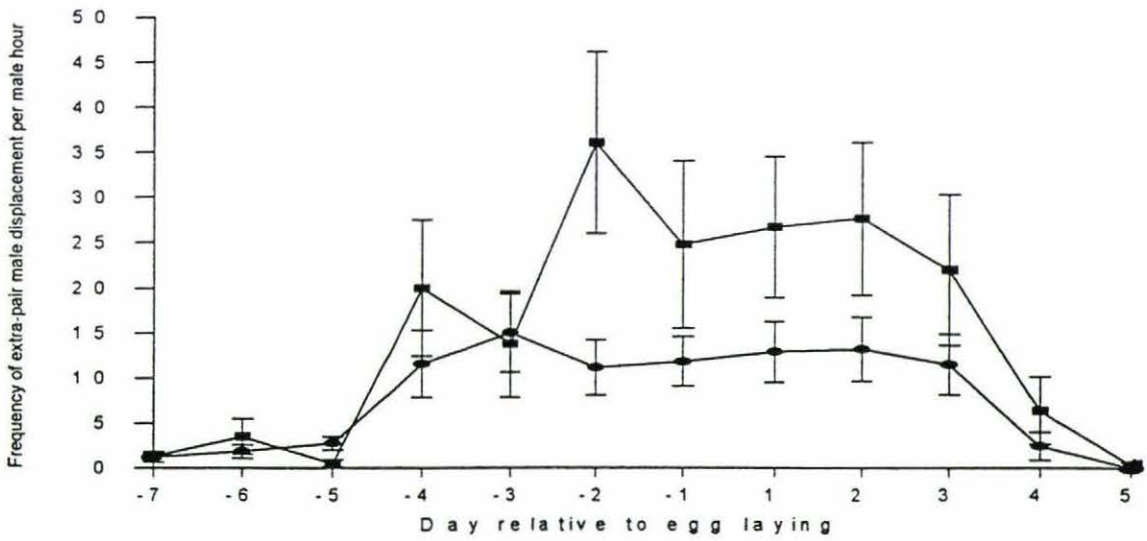


Figure 7. Frequency of extra-pair male displacements by the paired male when the females is present (squares) and absent (circles) relative to timing of egg laying. Data are for seven paired males and nine nests. Error bars are standard errors using each observation session as a unit of replication.

Table 4. Percentage of time paired males spend within 30 m of the nest site when the female is present and is absent. Each sample session is the unit of replication.

	Female present	Female absent
Number	96	96
Mean %	94	66
SE	1.7	3.1
SD	16.7	30.7

Table 5. Frequency of territorial calls by paired males within 30 m of nest sites in relation to time of day.

Time	Mean (n)	SE
AM	3.8 (66)	± 0.39
MID	5.11 (62)	± 0.6
PM	3.34 (62)	± 0.67

Territorial call frequencies showed male stitchbirds defended nest areas both when the female was present and absent. Paired t-tests showed call frequencies increased significantly when the female was present (t_{119}= 6.102, p= 0.000), raising from a mean of 1.32 calls per minute to 3.28 calls per minute. This difference in call frequency remained consistent throughout the measured fertile period ($F_{4,105}$=0.541; p= 0.706) suggesting no change in the relative frequency of calling when females were present vs when they were absent (Table 6). Males also did not significantly change their overall call frequencies with respect to the females' fertile periods ($F_{4,163}$=0.579; p=0.678) (Table 7). However, call frequency did vary significantly among females and time periods ($F_{8,163}$=5.395; p=0.000 & $F_{2,163}$=3.99; p=0.02 respectively) (Table 7). Males on average called more frequently during the midday and least frequently during the morning and evening observation sessions (Table 5).

Table 6. Analysis of variance testing for changes in the difference between call frequencies of paired males in the presence and absence of females in relation to stages of the female's fertile period. Stages were (1) 5-7 days before egg laying, (2) 3-4 days before egg laying, (3) 1-2 days before egg laying, (4) 1st and 2nd days of egg laying, and (5) 3rd-5th days of egg laying. Times were (1) AM (06:30-08:30), (2) MID (11:00-13:00), and (3) PM (16:00-18:00).

Source	Sum-of-squares	DF	Mean-square	F-ratio	P
Nest	85.248	8	10.656	0.827	0.580
Time	14.798	2	7.399	0.574	0.565
Stage	27.882	4	6.971	0.541	0.706
Error	1352.549	105	12.881		

Table 7. Analysis of variance testing for changes in the call frequency of paired-males in relation to stages of the female's fertile period. Stages were (1) 5-7 days before egg laying, (2) 3-4 days before egg laying, (3) 1-2 days before egg laying, (4) 1st and 2nd day of egg laying, and (5) 3rd-5th days of egg laying. Times were (1) AM (06:30-08:30), (2) MID (11:00-13:00), and (3) PM (16:00-18:00).

Source	Sum-of-squares	DF	Mean-square	F-ratio	P
Nest	8.887	8	1.111	5.395	0.000
Time	1.643	2	0.822	3.990	0.020
Stage	0.477	4	0.119	0.579	0.678
Error	33.562	163			

Male stitchbirds also defended their nest areas by attempting to displace extra-pair male intruders both when the female was present and absent (Figure 7). The rate of displacements increased as females progressed through their fertile periods, peaking during egg laying ($F_{4,139}$=11.806; p=0.000) (Table 8). This trend was similar to that measured for extra-pair male presence ($F_{4,80}$=12.158; p=0.000) (Table 2). This suggests males stayed fairly constant in their behaviour toward extra-pair males.

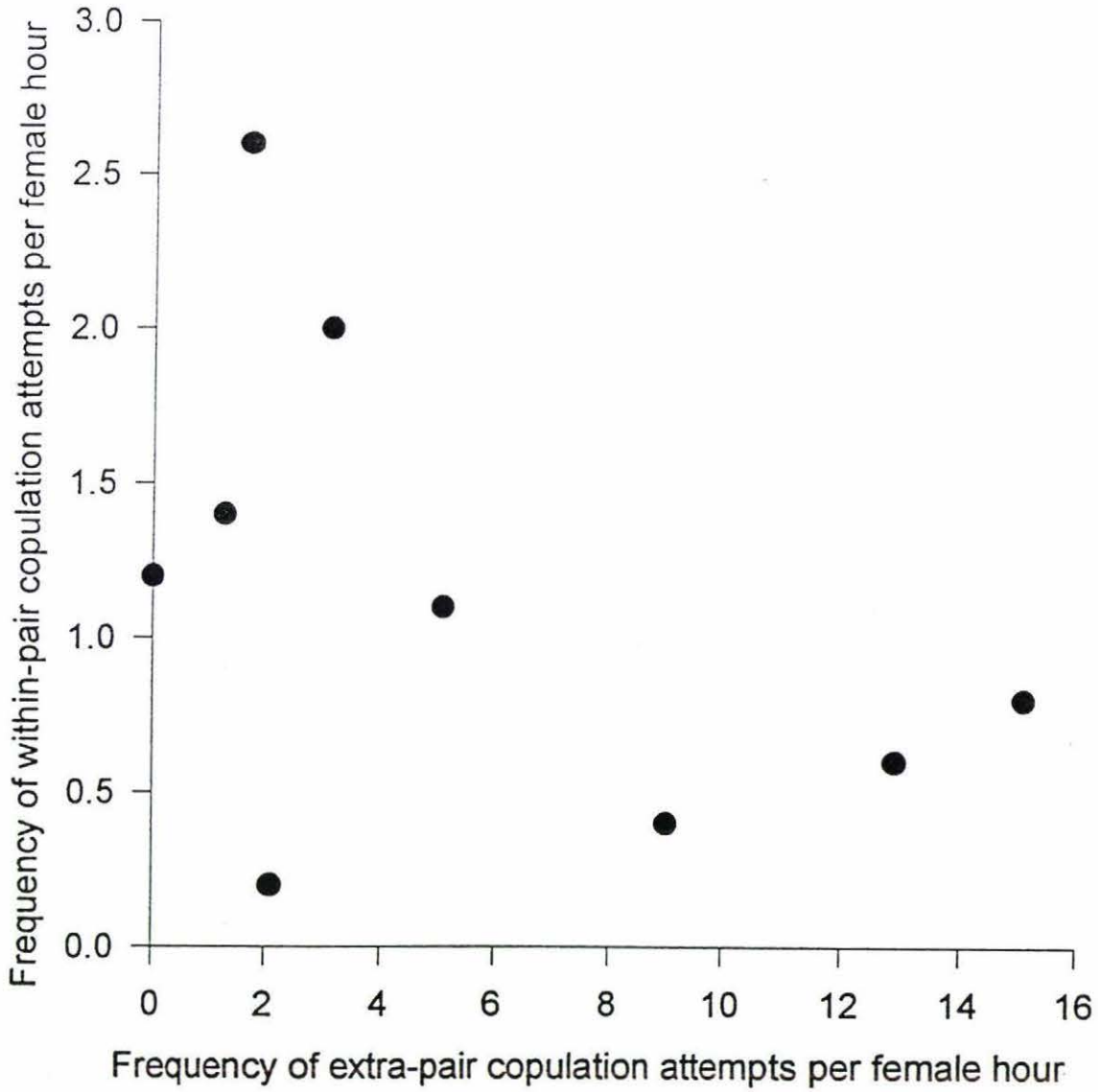


Figure 8. Relationship between the frequency of extra-pair copulation attempts and within-pair copulation attempts. Each point represents an individual nest. Linear regression was carried out on non-transformed data, which conformed to normality.

Table 8 Analysis of variance investigating differences in number of displacements by paired males in relation to the female's fertile period. Stages were (1) 5-7 days before egg laying, (2) 3-4 days before egg laying, (3) 1-2 days before egg laying, (4) 1st and 2nd days of egg laying, and (5) 3rd, 4th, and 5th days of egg laying. Times were (1) AM (06:30-08:30), (2) MID (11:00-13:00), and (3) PM (16:00-18:00).

Source	Sum-of-squares	DF	Mean-square	F-ratio	P
Nest	192.036	8	24.005	25.499	0.000
Time	12.898	2	6.449	6.850	0.001
Stage	44.457	4	11.114	11.806	0.000
Error	130.854	139	0.941		

There was no significant relationship between the frequency of within-pair copulation attempts at a nest and the frequency of EPC attempts ($F_{1,1}$=1.918; p=0.209), suggesting paired males do not increase copulation frequency to devalue sperm of rival males. This result occurs despite great variation in both within-pair and extra-pair copulations- i.e. a situation with good potential to see a trend if it were there (Figure 8). Although data on patterns of copulation frequency lack statistical power, within-pair copulation frequency seemed to remain fairly constant throughout the females fertile period (Figure 5). Data from the two females sampled for extended periods showed attempted within-pair copulations were observed 15 days before the first egg was laid and the first successful copulation was observed 11 days prior to egg laying.

DISCUSSION

Stitchbirds form pairs on Tiritiri Matangi, and paired males pursue a mixed reproductive strategy by seeking EPCs when the pair's fertile period is complete. The remaining unpaired males in the population take advantage of the asynchronous breeding by moving from one female to the next attempting EPCs, coinciding with the fertile period of each. This strategy fits with that suggested by Westneat (1990) in that males have increased opportunities for EPC when females are staggered in their fertilizable periods.

Copulation Behaviour

Copulation behaviour in stitchbirds involved both mutually accepted copulations and resisted copulations. Mutually accepted copulations were preceded by active solicitation by either sex, and often involved copulatory displays (eg. neck rubbing, circling and female mounting behaviours). All observed mutually accepted copulations were between paired individuals. Resisted copulations were not preceded by solicitation, and involved no copulatory displays. In most cases females continuously issued alarm calls. All EPCs and a minority of within-pair copulation attempts were in this second category.

The differences in behaviour between mutually accepted and resisted copulations in stitchbirds are similar to differences described for many bird species. Resisted copulations often differ from mutually accepted copulations by lacking pre-copulatory solicitation displays (eg great egret, Gladstone 1979; waterfowl, McKinney et al. 1983), involving females alarm calling (e.g indigo bunting, Westneat 1987), and in some species involving multi-male chases (e.g bank swallows, Beecher & Beecher 1979; white-fronted bee-eater, Emlen & Wrege 1986). Most recorded resisted copulations occur between extra-pair males and already paired females. However, in some waterfowl (eg. mallard ducks, Barash 1977), in Smith's longspur (Briskie 1992) and in stitchbirds, resisted copulations also occur between paired individuals.

A resisted copulation implies lack of female choice. Males forcing copulations on resisting females suggests that such encounters are disadvantageous to females (Birkhead & Biggins 1987). Birkhead & Biggins (1987) have detailed various hypotheses addressing the costs involved in resisted copulations, including injury, loss of choice and a reduction of parental care from the paired male. Although no females appeared to be injured during resisted copulations, the intensity of such interactions could lead to injury from aggressive males or from attracting predators (eg. attempted morepork predation of copulating stitchbirds, I. Castro pers. obs. Mokoia Island). In addition, females were often harassed by extra-pair males throughout the entire observation session and this may be costly to females even without copulation. Females may be prevented from foraging efficiently and lose condition as a result of this pressure

(Beasley 1996).

Males may force a female to the ground and copulate with her, but they have little control over what happens inside her body. Cryptic female choice involves the processes involved in sperm transport and fertilisation of eggs (Eberhard 1996; Birkhead & Møller 1992). If forced copulations are representative of copulations resulting in offspring, then it may be assumed that the presence of extra-pair fertilisations in this population (see Chapter 1) discounts cryptic female choice as a successful form of female control. However, females may resist copulations from males as a ploy to test their phenotypic and presumably the genotypic quality (Westneat et al. 1990). This has been postulated in stitchbirds by Castro (1995), yet is difficult to test because predicted behaviours are similar to those expected when females resist to avoid costs.

Paternity defense and EPC behaviour

My results support the hypothesis that paired male stitchbirds guard nest sites rather than guarding females directly. Males spent significantly more time within 30 m of the nest site than females although they are more likely to be in this area if the female is also present. They defend this area through territorial calling and displacing extra-pair males, regardless of female presence. Territory defense in stitchbirds is restricted to the breeding season and is centered on the nest site, lasting only as long as such sites are active. Such territoriality, especially centered on the period when a male's partner is fertile, is seen in many bird species and regarded as a form of paternity defense (Birkhead 1995). Female stitchbirds on average spend only 32% of their time within 30 m of the nest, suggesting nest defense may be a poor form of paternity assurance. Males may be facing some form of trade-off between defending the nest site and guarding their mate (Lifjeld & Marstein 1994). This often occurs in colonial species where feeding sites are distant from the breeding colony (e.g common murre, Birkhead et al. 1985; montezuma oropendolas, Webster 1995), and is thought to result in increased opportunities for EPC (Westneat et al. 1990).

From the extra-pair male's perspective, mate guarding restricted to nesting sites creates a potential for EPCs with the female when she is away from the nest (Birkhead et al. 1987). Females may take advantage of this by actively soliciting copulations (e.g eastern bluebirds, Gowarty & Bridges 1991) or may be subject to forced copulations while not protected by her mate (e.g white-fronted bee-eater, Emlen & Wrege 1986). Although this environmental potential for EPC exists in stitchbirds it appears that extra-pair males do not take advantage of it. This is supported by three types of evidence obtained from observing behaviour of extra-pair males near nest sites.

1. Extra-pair males frequently attempt to copulate with fertile females near the nest even though the majority of attempts are unsuccessful
2. Extra-pair males enter the nesting territory both when the female is present and absent. This suggests that these males may attempt to encounter fertile females by visiting nesting sites rather than trying to locate them elsewhere.
3. Behaviour of extra-pair males suggests they use cues at nest sites to determine a female's fertile period.

An ecological basis may exist for such apparently poor mate guarding, and the focusing of EPC attempts at nest sites. Female stitchbirds behave evasively at nest sites when being pursued by extra-pair males, often leaving the territory undetected by both her mate and any extra-pair males. Additionally, female plumage is relatively dull and cryptic (see general introduction). This may make them difficult to find and follow by males. Poor mate guarding, due to the difficulty of following partners, has been suggested previously in indigo buntings (Westneat 1987). Alternatively, males may be achieving both nest defense and mate guarding by staying at the nest site. Extra-pair males may be restricted to attempting copulations at the nest site. If all copulation attempts occur in this area paired males are effectively guarding the female. This is thought to occur in house martins where all copulations occur on the nest (Lifjeld & Marstein 1994). This hypothesis is supported by paired male stitchbirds staying on the territory when the female is present, suggesting some form of compromise rather than purely mate guarding or defending the nest site.

Attempting EPCs near nest sites is also reported in colonial species where one cost of social living is the increased social harassment by conspecifics (Alexander 1974). Emlen and Wrege (1986) investigate this cost by suggesting that breeding female white-fronted bee-eaters constitute a reliable resource- i.e. they are predictable both in space and time. Males take advantage of this by chasing females and attempting forced copulations at the colony. Although stitchbirds are not colonial breeders the data suggest a similar pattern. Nest sites may provide increased chances of encountering females, especially if females are generally wide-ranging and difficult to find.

Extra-pair male presence near the nest increases significantly as females progress through their fertile periods. Their presence peaks around 2 to 3 days before egg laying and starts to decline after the second egg is laid. Additionally, extra-pair copulation attempts seem to increase from around 4 days before egg laying, and remain high through to the laying of the penultimate egg (caution is needed when interpreting copulation frequency trends as they were not statistically significant). Such behaviour suggests that extra-pair males have some knowledge of the females' fertile periods and are timing their copulations to coincide. The egg is fertilized soon after ovulation, which occurs soon after the previous egg is laid in birds (Drachmann et al. 1997). Although copulations occurring during this period (insemination window) can potentially result in fertilisation (Cheng et al. 1983), insemination is thought to be less effective close to egg-laying because sperm retention is low at this time (Birkhead et al. 1996). A peak in female fertility has been reported to occur 2-3 days before the onset of egg laying (Westneat 1987 and references therein; Colegrave et al. 1995). Timing of extra-pair copulation attempts in stitchbirds is similar to this, and copulations made during this time probably coincide with a peak in female fertility.

Four possible cues have been recognised which extra-pair males may use to judge the fertility of females: (1) state of the nest, (2) guarding behaviour, (3) flight behaviour of the female, and (4) within-pair copulations (Gomendio & Roldan 1993; Birkhead et al. 1987). The amount of time paired males spend near the nest remained constant throughout the recorded sample period. Also territorial call frequencies did not change as females peaked in fertility. Guarding behaviour of paired males may therefore not act as a reliable cue to extra-pair males. No statistically clear pattern of within-pair

copulation attempts existed in this population and copulations were observed for extended periods before egg laying. This also suggests that extra-pair males would not be able to use within-pair copulations as a reliable cue. Heavier flight behaviours of females carrying eggs is only likely to act as a cue from one day before egg laying through to completion of the clutch. However, extra-pair males visit the nests of breeding females throughout the recorded period. Patterns of extra-pair males' visitations to nest boxes suggests they judge the fertility of the female from the stage of nest completion.

If extra-pair males use the stage of nest completion to judge female fertility they may not be able to identify fertile females away from nest sites i.e. copulations away from the nest may be wasted on non-fertile females. Evidence for copulations with non-fertile females comes from Castro et al.'s (1996) study of stichbirds breeding on Kapiti Island. Here stitchbirds were observed copulating at artificial feeding stations (another spatially predictable resource) up to 17 days before egg laying, and the majority of females involved were unpaired. In comparison copulations at nest sites were not observed until 5 days before egg laying. Although the range for sperm storage in birds varies widely, from 6–45 days (Birkhead & Møller 1993b), the median time recorded to date is 10 days (Birkhead & Møller 1992). Sperm storage time is known for only two passerines: 10 days in zebra finches (Birkhead et al. 1989) and 8 days in Bengalese finches (Birkhead 1992). Copulations up to 17 days before egg laying possibly have minimal chances of fertilizing eggs, and could occur because males are unable to determine if the female is fertile. The single nest on Tiritiri Matangi where EPCs were observed 12 days before egg laying also had an extended period between the finishing of nest building and the onset of egg laying. Use of nest completion as a cue may also explain why extra-pair males attempt copulations with fledglings at nest sites of fertile females. Extra-pair males may simply associate a "female looking" bird with the nest site, and copulate.

Paired males attempt to prevent other males from forcibly copulating with their partners. Occasionally such copulations are successful despite the efforts of paired males, or occur when the paired male is not near the nest site. Knowledge of EPCs may affect a

paired male's certainty of paternity, and are predicted to result in additional paternity guards (see examples in Parker 1990), and/or a reduction in provisioning investment of the young (see next Chapter). Certainty of paternity is known to alter provisioning of nestlings in dunnocks (Burke et al. 1989; Davies et al. 1992), and reed buntings (Dixon et al. 1994). A paired male's investment in mate guarding has been frequently described as a best-of-a-bad-job strategy, especially where females control EPCs (Birkhead & Møller 1992; Møller & Birkhead 1993; Lifjeld & Marstein 1994). I suggest similar conclusions can be drawn with stitchbirds where forced EPC occurs despite paired males' guarding efforts, and these copulations result in extra-pair paternity (see Chapter 1).

Witnessing both actively solicited and resisted EPCs leads to immediate bouts of within-pair matings in many bird species (e.g mountain bluebird, Barash 1976, also see examples in Birkhead et al. 1987). Observations of copulations in stitchbirds suggest that paired males do not regularly attempt to copulate with females immediately following an EPC. However, paired males do initiate the majority of copulations. This is predicted if frequent copulation were a male-driven paternity guard (Lifjeld & Marstein 1994), and this pattern is not recorded in most species with high copulation frequencies (Hunter et al. 1993). The majority of these solicitations were rejected by the females. This sometimes led to resisted within-pair copulations, which were also recorded by Castro et al. (1996) on Kapiti Island. Although these copulations are not optimally timed to devalue extra-pair male's sperm they could be a form of paternity assurance.

Frequent within-pair copulation has been suggested as a strategy used by paired males to devalue sperm of rival males (Birkhead et al. 1987). This usually occurs as an alternative to mate guarding (Møller & Birkhead 1991), but may be predicted if paired males are unsuccessful at guarding females. There was no correlation between the frequency of within-pair and extra-pair copulations among nests. This suggests stitchbirds do not use frequent copulation as a form of paternity defense. However within-pair copulations occurred more frequently than is assumed necessary to fertilise all eggs in a clutch (Birkhead & Møller 1993b).

Based on my results, EPC is a frequent occurrence in this stitchbird population and is a male driven strategy achieved through forcing copulations of females. Extra-pair males appear to assess the fertility of females by using the stage of nest completion as a cue. Paired males attempt to defend their paternity by defending nesting sites, predominantly when the female is also present. Successful EPCs occur despite these efforts, yet paired males do not appear to increase copulation frequencies as an additional paternity guard. Chapter 3 investigates the effects that observed EPCs has on a paired males certainty of paternity, and his provisioning of nestlings.

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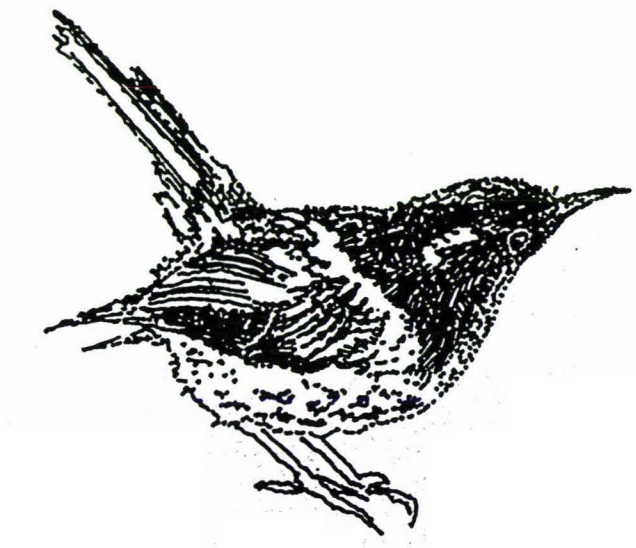
CHAPTER THREE



Actual paternity, certainty of paternity and paternal provisioning of nestlings.

ABSTRACT

Paired male stitchbirds on Tiritiri Matangi alter their care of nestlings by assessing their share of paternity using behavioural cues. The frequency of EPC attempts varied significantly between nests and these attempts were often witnessed by the paired male (Chapter 2). This variation mirrored closely the variation in the percentage of extra-pair paternity. Attempted EPCs was more strongly correlated with paternal care than was actual paternity, suggesting paired males assess their paternity using behavioural cues rather than actually discriminating related from unrelated offspring. Additionally, the strong relationship between EPC attempts and extra-pair paternity indicates that the observed EPCs were representative of those which led to fertilisation.

INTRODUCTION

Behavioural ecologists assume that individuals act in ways that maximise their reproductive success (Maynard Smith 1988). Biparental care is common among socially monogamous birds (Lack 1968), and is thought to constrain birds to such mating systems because two parents are necessary to successfully raise young (Emlen & Oring 1977). Other studies have suggested that biparental care may arise from: (1) males being confident of their paternity in monogamous systems (Trivers 1972), or (2) males feeding young as a by-product of provisioning rules developed in the context of parental care (Jamieson & Craig 1987).

Where biparental care occurs, provisioning young is the most costly investment of reproduction for male birds (Walsberg 1983 cited in Møller & Birkhead 1993). Males providing resources to unrelated offspring not only suffer the cost of provisioning, but also lose opportunities to provide resources to related offspring (Westneat & Clark 1995) and obtain further matings (Westneat et al. 1990). As studies revealing mixed paternity within clutches become increasingly prevalent, questions arise as to the effects

mixed paternity has on paternal provisioning. This conflict is thought to occur in socially monogamous species where EPCs result in fertilisation (Westneat 1988; Møller 1988), and also in polyandrous and polygynandrous species (Burke et al. 1989).

Trivers (1972) was the first to develop a theoretical framework to understand the costs and benefits of parental investment. He suggested that in monogamous species showing promiscuous behaviour, males would be selected to evolve kin recognition and provide parental care only to kin. Many authors have built on this original theory, primarily by altering assumptions of the model, and have predicted that: (1) the evolution of parental care is directly related to certainty of paternity (Winkler 1987), (2) the evolution of parental care is related to certainty of paternity by way of a threshold (Whittingham et al. 1992), (3) the evolution of parental care is related to certainty of paternity only if the mean parentage differs between broods within a pair (Westneat & Sherman 1993) (Maynard Smith 1977) and (4) the evolution of parental care is not related to certainty of paternity (Werren et al. 1980).

Similarly, empirical studies in birds have revealed highly variable patterns of parental care in relation to paternity. Many studies have shown no such relationship (eg. purple martins, Wagner et al. 1996; red-winged blackbirds, Westneat & Clark 1995; tree swallows, Lifjeld et al. 1993; and razorbills, Wagner 1992), and frequently offer alternative predictors of paternal provisioning which better accounts for variation in behaviour (eg. red-winged blackbirds in relation to demand, Yasukawa et al. 1993; 'status hypothesis' in purple martins, Wagner et al. 1996). Other studies have revealed various levels of relationship between paternal provisioning and paternity, from low in European starlings (Wright & Cotton 1994) and indigo bunting (Westneat 1988), to relatively high in alpine accentor (Hartley et al. 1995), reed bunting (Dixon et al. 1994), polyandrous dunnocks (Davies et al. 1992; Burke et al. 1989), and swallows (Møller 1988). Also see (Møller & Birkhead 1993) for more examples. Relating observed patterns to the models is often difficult because patterns of parental investment are related to many other variables that impinge on an individual's ability to vary behaviour solely in relation to paternity.

Data from species which alter provisioning in relation to paternity suggest males lack the ability to actually recognise unrelated offspring (Kempenaers & Sheldon 1996). Instead it appears that these males follow behavioural "rules of thumb" (Burke et al. 1989; Hartley et al. 1995). Such responses require only that reduced parentage be reliably associated with environmental cues (Westneat & Sherman 1993). Investigations of these cues requires a relationship to be found between three variables: (1) the behavioural cue, (2) extra-pair paternity, and (3) paternal provisioning. In species where unrelated offspring are the result of EPC, males may use the rate of observed EPC as a behavioural cue. There are three studies, which have investigated this relationship. Westneat's (1988) study of indigo buntings showed a relationship between rate of EPC, extra-pair paternity, and provisioning, whereas Wagner et al.'s (1996) study of purple martins and Lifjeld et al.'s (1993) study of tree swallows showed none. Other studies of monogamous species, where cuckoldry occurs through EPC, have shown relationships between paternal investment and paternity (Dixon et al. 1994), and EPC attempts and paternal provisioning (Møller 1988).

Stitchbirds breeding on Tiritiri Matangi form socially monogamous pairs. Attempted EPCs are frequently observed at many nests (Chapter 2) and result in extra-pair paternity (Chapter 1). Males are not involved in nest building or incubation (Oliver 1955; Craig 1985). Observations of breeding in stitchbirds on Kapiti Island indicate males are involved with provisioning young albeit at about one-third the rate of females (Castro 1995).

In this chapter I investigate patterns of provisioning in the Tiritiri Matangi population of stitchbirds, and relate this to data on extra-pair paternity and EPC presented in previous chapters. While focusing on male contribution to nestling provisioning, information will be provided on (1) the relative nestling feeding rates by paired males and females and (2) the contribution, if any, of extra-pair males in provisioning young. Establishing relative feeding rates of males and females throughout nestling growth will test assumptions presented by Castro et al. (1995) indicating males feed at a constant rate, approximately 1/3 to that of the female. To understand patterns of male contribution to provisioning, I will firstly investigate if there is a relationship between the intensity of

EPC attempts and levels of extra-pair paternity within each nest. It is important to determine if such behaviours have the potential to act as cues. Additionally, such relationships will justify the conclusions drawn in the previous chapter concerning observed copulations being those representative of copulations resulting in paternity. Secondly, I investigate relationships between paternal provisioning rates and both attempted EPC rates and extra-pair paternity. A comparison with both EPC rates and paternity will allow conclusions to be drawn as to which most accurately predicts provisioning patterns. If males use behavioural 'rules of thumb' then it is predicted that EPC rates will explain the observed patterns of paternal provisioning more accurately than actual paternity.

METHODS

Individuals Sampled

Male provisioning of nestlings was recorded during the first two breeding seasons of stitchbirds on Tiritiri Matangi. Similar to the previous chapter, the single successful female from the 1995/96 season and all six females from the 1996/97 season were observed. Two females attempted second clutches during the second breeding season, and the single female sampled in the 1995/96 is also represented in the data for the 1996/97 breeding season. As noted previously, all individuals were identifiable by unique combinations of coloured plastic leg bands and a numbered aluminum band.

Sampling Regime

Primarily for consistency, behavioural observations were conducted in three time intervals. These time intervals were two hours in duration (06:30-08:30, 11:00-13:00 and 16:00-18:00), and were split evenly between nests. Due to the asynchronous nature of breeding in the population it was possible for two observers to sample two nests each at peak times. As such, observation periods were usually between 50 minutes and one hour in duration.

The sampling regime differed between years. During the 1995/96 breeding season sampling began five days after the last egg had hatched and continued, every fifth day, until all nestlings had fledged. Among other things, feeding of nestlings by extra-pair males was investigated. As no extra-pair males were observed feeding in this year, the sampling regime was increased for the subsequent breeding season. Therefore sampling was initiated three days after the last egg had hatched in the 1996/97 breeding season, and samples were continued every third day through until the last nestling had fledged. No information was collected on fledgling provisioning although *ad libitum* observation showed that both members of a pair fed young for extended periods following fledging.

To measure the relative contribution to nestling feeding, both members of a pair, and any extra-pair male visits to the nest were recorded. It was assumed that nestlings were fed during each visit. This was necessary because the birds nested in boxes with small entrances that made it impossible to directly observe behaviour. Several other behaviours may have occurred during these visits, such as cleaning away nestling excrement. Therefore the sampling technique may overestimate provisioning rates.

Throughout the nestling stages of the reproductive cycle, frequent nest checks were conducted to monitor the survival of nestlings (see Chapter 4). These checks occurred three times each day and coincided with sample periods on days when sampling occurred, and close to these periods when nest sites were not being observed, primarily for consistency.

Two measures were used to describe the contribution of each sex to nestling provisioning (as detailed in Wagner et al. 1996). Relative provisioning was calculated for paired males as the percentage of total visits made to the nest. Absolute provisioning was recorded as the number of visits per nestling, as clutch size varied and mortality was common. Absolute feeding rates are influenced by many factors such as food availability and time of season (Davies 1986; Møller 1988). Relative measures of provisioning should focus solely on paternal contribution.

The proportion of extra-pair young within each clutch is taken from Chapter 1, and attempted EPC rates are calculated from results in Chapter 2. Attempted EPC rate was the average from seven days before the onset of egg laying through to the laying of the penultimate egg. This period corresponds to analyses using EPC attempts in Chapter 2.

Data Analysis

Extra-pair males were observed entering three different nests involving two females during the 1996/97 breeding season. However, these visits were very rare, accounting for less than 0.6% of the total visits made to nests. Due to such low frequencies of extra-pair visitation these data were excluded from analysis.

The remaining data were grouped by nestling age into five categories (0-8 days, 9-14 days, 15 days, 16-23 days, and 24-30 days). These categories were created to provide reasonably even numbers of samples for replication. No transformations were necessary as all data conformed closely to normality when residuals were investigated. ANOVA was used to test whether measures of male feeding varied with: (1) nest, (2) nestling age, and (3) time of day. Additionally, interactions between nestling age and nest were investigated. Regression analyses were used to investigate the relationship between;

- EPC rate and % paternity within each nest
- % paternity and relative male feeding
- % paternity and male visits per chick
- EPC rate and relative male feeding
- EPC rate and male visits per chick

RESULTS

Behavioural data were recorded from 184 observation sessions totaling 160 h. These sessions were on average 3138 ± 415 s (SD), allowing comparisons to be made without problems of spurious results from variable sample lengths.

Comparison of feeding effort

Both the paired male and female always fed young throughout the 27-30 day nestling period. Males' feeding rates per nestling were lower than that for females (Table 1), indicating males invest less in chick provisioning. Relative feeding rates varied among males and varied with nestling age ($F_{6,112}$=9.822; 0.000 & $F_{4,112}$= 5.689; p=0.000) (Table 2). There was an overall trend for males to reduce their relative contribution as the chicks got older (Table 1). However, patterns of provisioning and nestling age were complex with different nests showing different feeding patterns ($F_{24,112}$=1.694 for age class and nest interaction term; p=0.035)(Table 2). Relative feeding rate did not vary significantly with time of day ($F_{2,112}$=1.458; p=0.237)(Table 2).

Table 1. Mean visitation rate per chick per hour by paired male and female stitchbirds. Least squares means were calculated from ANOVA that took nest and time of day into account.

Age class	Female		Male	
	LS Mean	SE	LS Mean	SE
0-8 days	1.909	0.102	0.669	0.064
9-14 days	2.381	0.084	0.640	0.052
15 days	2.205	0.478	0.409	0.066
16-23 days	2.037	0.145	0.489	0.053
24-30 days	1.735	0.577	0.240	0.057

Extra-pair males were seen visiting three nests a total of nine times. Each nest was visited by a different extra-pair male, with one male accounting for seven visits. This male was not the genetic father of any nestlings. Of the other two visits, one male was the genetic father of one chick in the nest he visited. The other male was unrelated to all

chicks in the nest

The female of one nest disappeared six days after five chicks had hatched. The male subsequently did all provisioning to the nest. While two nestlings died just before the female went missing, the remaining three survived and fledged 28 days after hatching. Because the male was sole parent of this nest, it was left out of all analyses.

Table 2. Analysis of variance investigating variation in relative feeding rate between paired males, different age classes of nestlings, and times of day.

Source	Sum-of-squares	DF	Mean-square	F-ratio	P
Nest	12227.555	6	2037.926	9.822	0.000
Age class	4721.329	4	1180.332	5.689	0.000
Time	605.118	2	302.559	1.458	0.237
Age class*Nest	8436.587	24	351.524	1.694	0.035
Error	23237.597	112	207.479		

Certainty of paternity and actual paternity

I measured the certainty of paternity for pair males by comparing observed rates of attempted EPCs. Those males whose partners were subject to high rates of attempted EPC were assumed to be less certain of their paternity than those whose partners were not. There was a significant difference in frequencies of EPC attempts between nests ($F_{8,174}$=7.189; p=0.000) (see Table 3, Chapter 2). Paired males were present within the nesting area 77% of the time on average, and were therefore present for the majority of EPC attempts (see Chapter 2). Extra-pair paternity also varied between nests. Percentage of young resulting from EPC in each clutch range from 0 to 75% (Chapter 1). In some nests, 100% of young that fledged were produced by EPC.

Nests with the highest rate of EPC attempts had a lower percentage of young fathered by paired males ($F_{1,7}$=17.641; p=0.004)(Figure 1). This supports the argument that observed copulations are representative of those leading to fertilisation, and that the rates of EPC attempts have the potential to act as a behavioural cue to paired males.

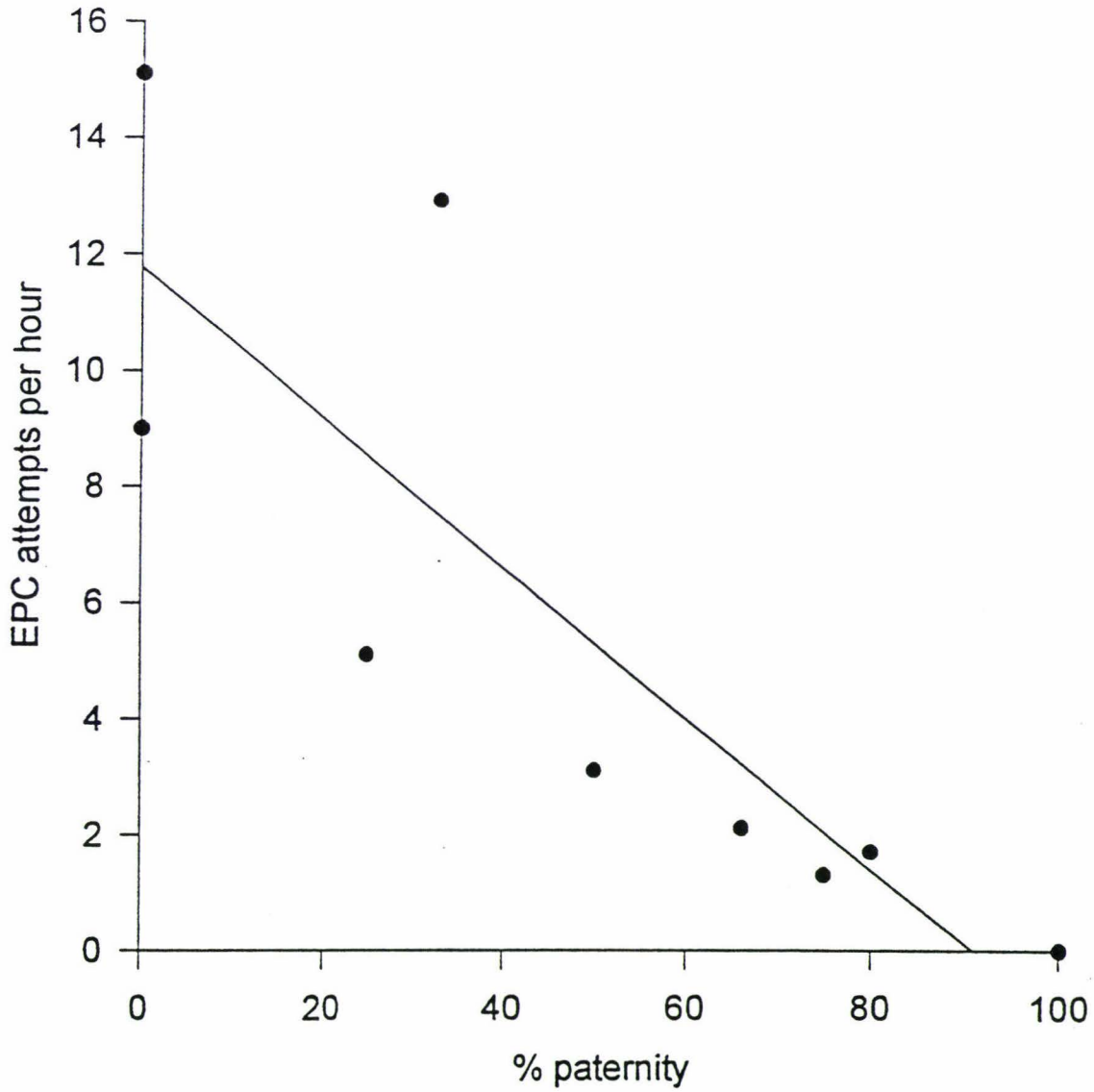


Figure 1. Paired males' percentage share of paternity within broods in relation to attempted EPC rates recorded during the females' fertile period. Linear regression was carried out on non-transformed data, which conformed to normality.

Actual paternity and paternal feeding effort

Analysis of male feeding rates used least squares means calculated from ANOVA (Table 2) which took nestling age and interactions between nest and nestling age into account. The single nest where the female disappeared six days after her eggs hatched was excluded from this analysis. There was no significant trend between absolute male feeding rates and actual paternity ($F_{1,5}$=1.421; p=0.287). Relative feeding rates, which controlled all factors apart from the male's contribution to provisioning, showed an almost significant positive relationship ($F_{1,5}$=5.99; p=0.058)(Figure 2).

Certainty of paternity and paternal feeding effort

Similarly to actual paternity, absolute feeding measures showed no relationship to attempted EPC rates ($F_{1,5}$=1.777; p=0.240). In contrast, relative feeding by paired males did show a significant relationship with attempted EPC rates ($F_{1,5}$=11.029; p=0.021). This suggests that paired males are using rates of attempted EPCs as a cue to assess possible cuckoldry, and reacting by reducing provisioning effort (Figure 3). The observed variation in paternal provisioning is correlated more strongly to EPC attempt frequencies than to actual paternity. This supports the hypothesis that males cannot discriminate kin from non-kin, and instead rely on an environmental cue to assess paternity.

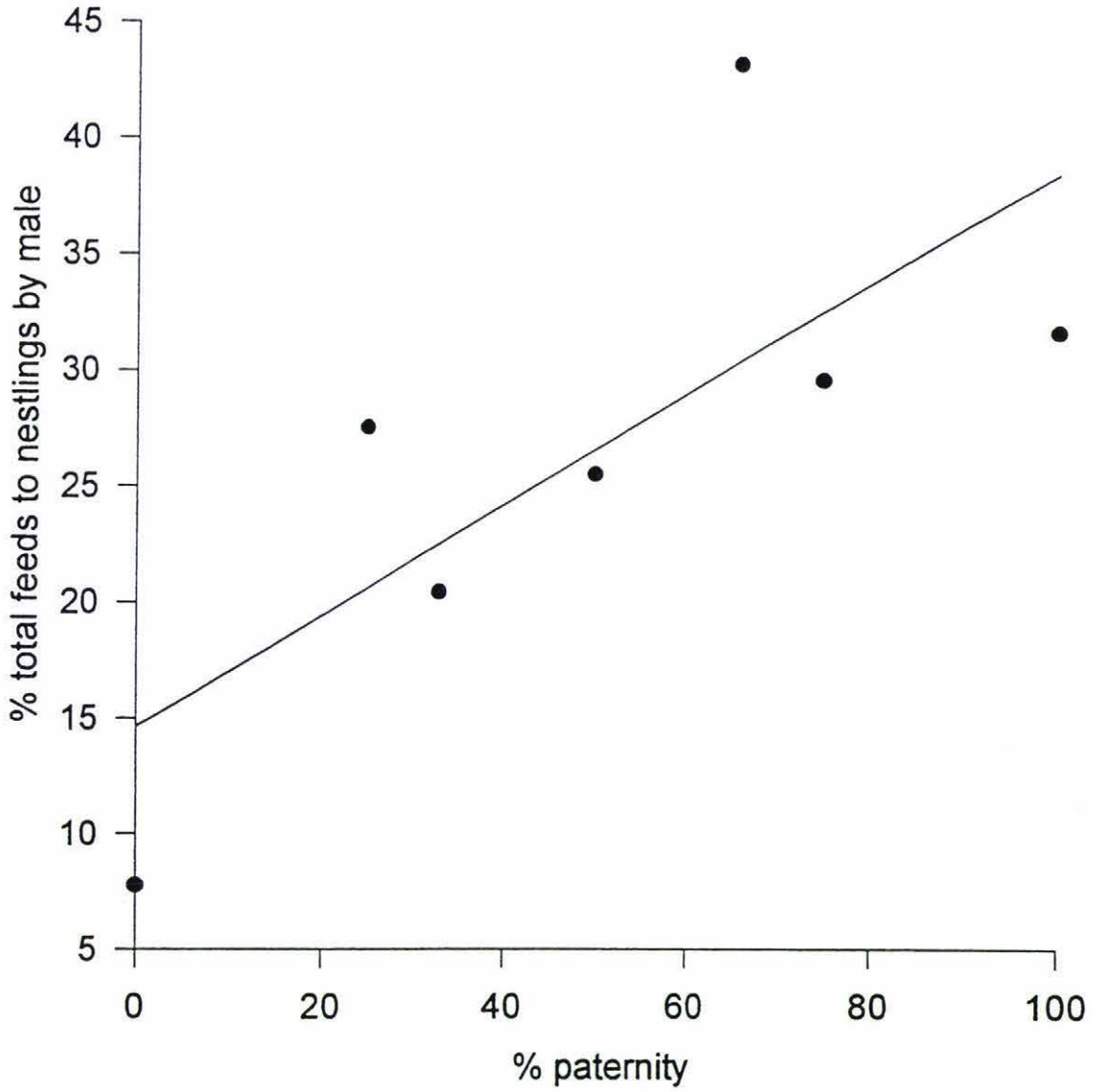


Figure 2 Paired males' relative parental effort in relation to percentage of actual paternity within broods. Linear regression was carried out on non-transformed data, which conformed to normality.

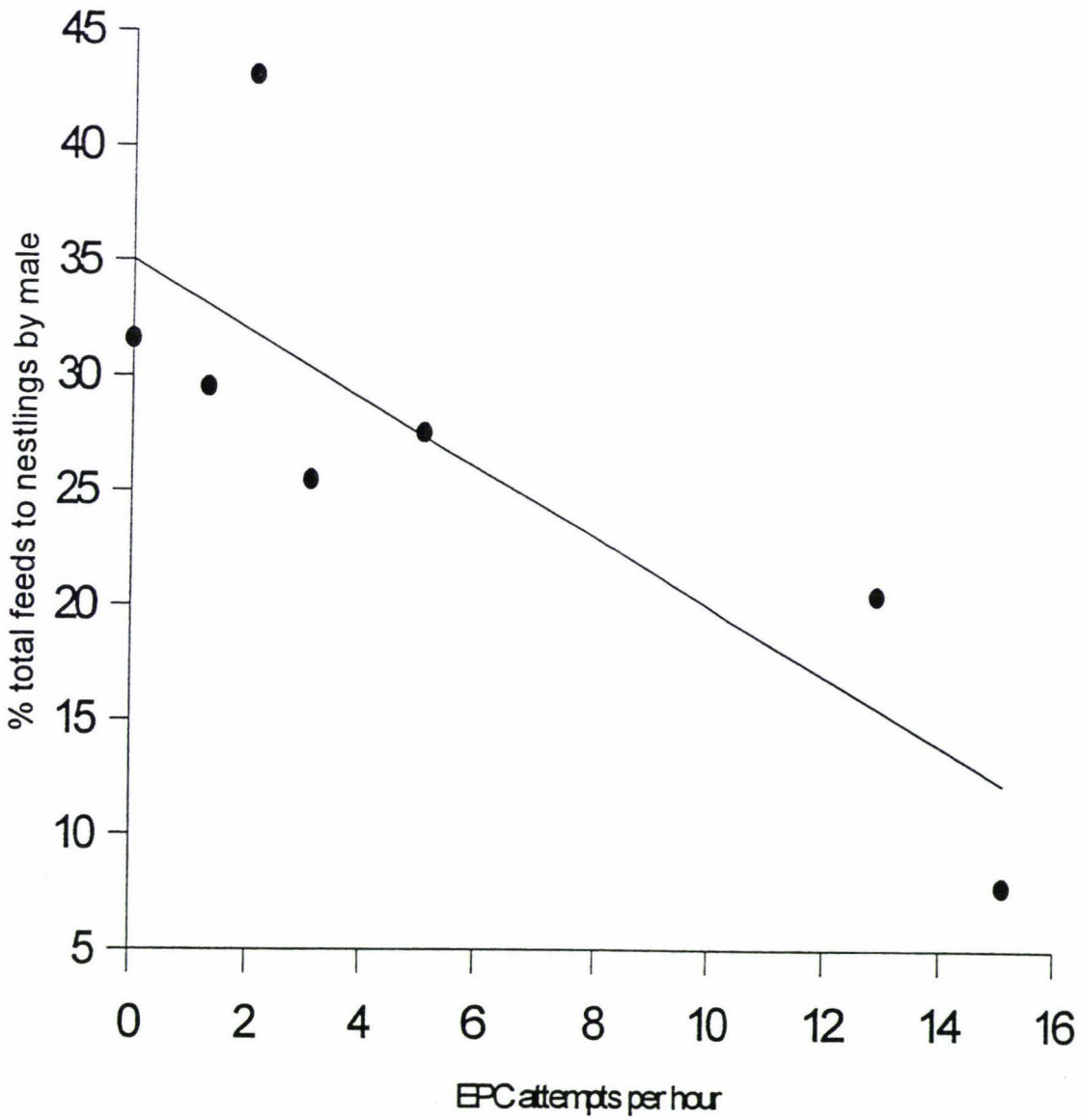


Figure 3. Paired males' relative parental effort in relation to attempted EPC rates recorded during the females' fertile periods. Linear regression was carried out on non-transformed data, which conformed to normality.

DISCUSSION

Frequency of attempted EPCs and actual paternity

The frequency of attempted EPCs is strongly correlated to the actual paternity within each stitchbird nest. Nests where EPC attempts were relatively high also had higher levels of extra-pair paternity. These results are similar to those found through investigations of EPC in indigo buntings (Westneat 1988). Although different parameters were used, studies of both alpine accentors (Hartley et al. 1995) and polyandrous dunnocks (Burke et al. 1989; Davies et al. 1992) have also revealed reliable behavioural indicators of actual paternity.

Observations of copulations do not always give a good measure of paternity. In many species, DNA fingerprinting has revealed the presence of extra-pair paternity even though behavioural observation concluded EPC to be very rare (e.g indigo buntings, Westneat 1990; red-winged blackbirds, Gibbs et al. 1990; Westneat 1992; Westneat 1993; tree swallows, Lifjeld et al. 1993). Various reasoning has been invoked to explain this, including: (1) EPCs may be more difficult to observe (Hartley et al. 1995), (2) EPCs may be more effective than within-pair copulations in fertilising eggs (Lifjeld et al. 1993), and (3) females may have post-copulatory control of fertilisation, and favour sperm from extra-pair males (Birkhead & Møller 1993).

The observed pattern between EPC and paternity detailed here has several implications. Firstly, this study shows that observed rates of attempted EPC mirror closely the actual variation of paternity within clutches. This close agreement between observed EPC attempts and extra-pair paternity indicates accurate field observation of copulation patterns. Secondly, as all observed attempted EPCs were resisted by the female (see Chapter 2), this pattern indicates that resisted copulations result in paternity. Finally, because resisted copulations result in fertilised eggs (see Chapter 1), it is unlikely that females have post-copulatory control of fertilisation.

Certainty of paternity, actual paternity and male parental effort

The relationship between attempted EPC and paternity reveals a potential cue available to paired male stitchbirds to assess their paternity. Presence of such behavioural cues have been suggested to impact on paternal effort in nestling feeding (Møller 1988; Westneat 1988; Burke et al. 1989; Davies et al. 1992; Hartley et al. 1995). Absence of such behavioural cues has also been implicated in the lack of differential feeding effort of males subject to cuckoldry (Lifjeld et al. 1993; Westneat & Clark 1995).

Paired males fed nestlings more when the frequency of EPC attempts on their female was lower. This behavioural cue explained the observed patterns of paternal provisioning better than did actual paternity. This suggests that males are unable to distinguish kin from non-kin, and instead adopt behavioural 'rules of thumb' to determine their certainty of paternity.

Theorists predict a tradeoff between nestling provisioning and the seeking of additional matings through EPC (Westneat et al. 1990). Males may increase their fitness more by attempting EPCs rather than feeding offspring (Werren et al. 1980; Westneat 1988). The asynchronous nature of breeding in this stitchbird population (see Chapter 2) suggests that there is potential for paired males to adopt mixed reproductive strategies by pursuing EPCs. Males appear free to do this while females incubate their clutches (approximately 14 days). During nestling development opportunities may be restricted because the majority of males help feed young. However, average feeding rates for males ranged from 0.24-0.67 feeds per chick per hour. Therefore, males may also have time to seek EPCs, at least with neighboring females.

Provisioning can be costly to both adults and young, in that the energetic costs involved in obtaining food by adults may be high (Walsberg 1983, cited in Møller & Birkhead 1993), and the lack of parental care to offspring may reduce nestling fitness (Davies et al. 1992; Davies et al. 1996). If one parent is almost effective as two in caring for young, and if the prospects of mating again are high it may pay for one parent to desert (Trivers 1972; Maynard Smith 1977). More subtly, when provisioning is costly to adults it is predicted that some form of discrimination will evolve (Westneat & Sherman

1993). However, if costs are high for nestlings, pressure may prevent accurate discrimination of actual paternity, as incorrect decisions would incur large costs (i.e. reduced fitness through potential mortality of related offspring) (Westneat & Clark 1995). Although a single male stitchbird successfully raised three offspring, and other studies show single females can raise entire clutches (Castro 1995), it is not known what effect this has on future reproductive success. The fact that biparental care is typical of stitchbirds, and that males vary effort according to behavioural cues, suggests that such behaviour is adaptive. Assuming that individuals act in ways that maximise their reproductive success, biparental care may have evolved to achieve this.

The observed patterns of paternal provisioning are predicted to occur when a male's paternity differs from one breeding attempt to the next (Westneat & Sherman 1993; Maynard Smith 1977; Maynard Smith 1978). Where paternity does not differ between attempts, and if males cannot accurately discriminate kin (as is generally accepted in birds - see Kempenaers & Sheldon (1996) a situation potentially arises whereby males reduce feeding rates to their own offspring without receiving long-term benefits. Limited information for subsequent clutches in the present study suggests that paternity does vary. The single male represented over two breeding seasons had only 50% paternity in the 1995/96 clutch, and 100% in the 1996/97 clutch. Additionally, the two males, who double clutched, also had variable levels of paternity among living chicks (0% - 75% and 0% - 25%).

In order to accurately discriminate kin from non-kin it is necessary to develop phenotypic markers which can act as signals to males (Kempenaers & Sheldon 1996). Signaling paternity is likely to result in conflict of interests between adult males and females and also between extra-pair and within-pair offspring (Davies 1992). Extra-pair young should hide any paternity signals that may cause the paired male to discriminate against them (Kempenaers & Sheldon 1996). In populations where individuals commonly seek a mixed reproductive strategy, by pairing with one female while also seeking EPCs with other paired females, it seems hard to imagine how such signals could be selected for. Unless males are able to actively pass on signals only to offspring resulting from within-pair copulations, and not to those from EPCs, the advantage

gained on one hand may be lost because extra-pair young may be discriminated against. EPCs in birds are thought to occur predominantly through males adopting mixed reproductive strategies (Westneat et al. 1990), and this may offer another explanation for the lack of paternity discrimination.

This study is one of only two that has found that males of monogamous species appeared to alter paternal provisioning effort by assessing their share of paternity using behavioural cues (also see Westneat 1988). Data presented suggests that males do this by using the frequency of EPC attempts on their female as a behavioural cue, rather than direct kin discrimination. Stitchbird behaviour fits many of the predictions made by theoreticians concerning the evolution of discriminatory behaviour, primarily: (1) differential paternity between clutches, (2) the presence of an accurate behavioural cue, and (3) the tradeoff between EPC and nestling provisioning. Results from this study show stronger trends to those reported in indigo buntings by Westneat (1988). Other studies have partially addressed these patterns and have also found similar results (eg. reed buntings (Dixon et al. 1994) and tree swallows (Møller 1988). However, such trends are not universal, with other studies using similar methodology finding no pattern (Wagner et al. 1996; Lifjeld et al. 1993). Additionally, Kempenaers & Sheldon (1996) detail other experiments, that suggest that birds largely can neither, discriminate between kin and non-kin nor use cues to assess overall paternity within a clutch. These results are not necessarily conflicting. Discrimination is likely to evolve only if there is a net fitness benefit (Kempenaers & Sheldon 1996), and as many of the theoretical models detail, there are situations which may not favour such behavioural patterns.

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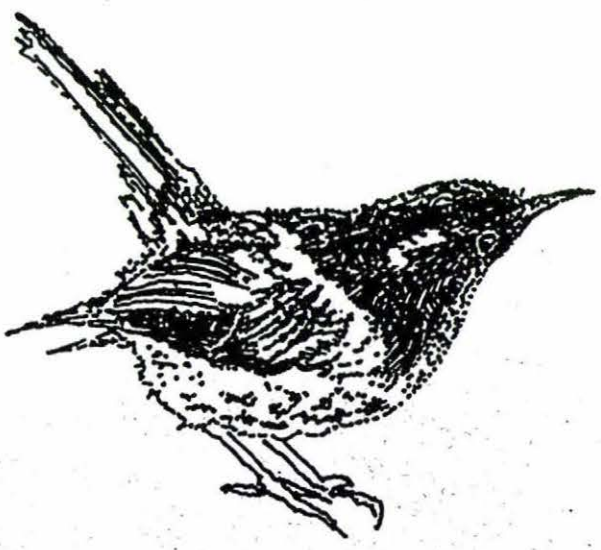
CHAPTER FOUR



Monitoring and management of stitchbirds on Tiritiri Matangi Island.

ABSTRACT

This chapter details management protocols used while monitoring stitchbirds for the first 18 months following translocation to Tiritiri Matangi. It details all management techniques I feel are important for gaining knowledge about the success/failure of this translocation, increasing public participation, and increasing the chances of success in establishing a self sustaining population. Many techniques are adapted from those used on other island stitchbird populations and modifications proposed by Glenfield College.

INTRODUCTION

Translocation is a tool widely used by conservation managers in New Zealand to save endangered species (Armstrong & McLean 1995; Serena & Williams 1995). With over 700 offshore islands, there are unique opportunities to undertake translocations to avert the extinction of both threatened animals and plants (Saunders 1995). For the moment, the fate of the stitchbird is inextricably linked to the fate of the Little Barrier Island population (Rasch et al. 1996). This is because confinement to a single locality increases the chance of extinction through any of the following four stochastic perturbations: (1) demographic stochasticity, (2) environmental stochasticity, (3) natural catastrophes, and (4) genetic stochasticity (Shaffer 1981).

Releases of stitchbirds to Tiritiri Matangi in 1995 & 1996 are the most recent in a series of translocations aimed at establishing at least one other 'self-sustaining' population (long term goal, and short-term objective three of 1996 stitchbird recovery plan, Rasch et al. 1996). Although transfers to Mokoia Island and Tiritiri Matangi are still being assessed, none of the other transfers have resulted in 'self-sustaining' populations (Rasch et al. 1996). A lack of information from these early translocation attempts has resulted in the causes of failure remaining unclear. This general criticism of translocations in New Zealand has led to suggestions concerning improved post-translocation monitoring (Armstrong & McLean 1995), and designing experimental

reintroductions to assess factors affecting their success or failure (Armstrong et al. 1995).

Advocacy, increasing public knowledge and participation, is proposed as a key component in the conservation of New Zealands endangered species (Craig 1997), and has been incorporated into many species' recovery plans (eg. Kakapo, Cresswell 1996). Short-term objective number four and work plan 6.6 both promote the importance of advocacy and public participation in stitchbird conservation (Rasch et al. 1996). Tiritiri Matangi provides an opportunity for both enhancement of public knowledge and direct involvement by the public in monitoring and management (Craig 1990; also see examples with other bird translocations in Galbraith & Hayson 1995).

The primary aim of this chapter is to provide a set of protocols to focus post-translocation monitoring and management. Additionally, the promotion of advocacy is highlighted where I have felt appropriate. As such, this report is written in a form designed for easy reading by the general public. Each section begins with a basic background outlining the importance of such monitoring/management. All management protocols are set out in step-by-step manner for ease of interpretation. At the risk of repetition, sections are clearly separated, and follow a temporal pattern, from the beginning of one breeding season through to the beginning of the next. Copies of this report should be readily available on the island for quick reference.

BREEDING SEASON

Nest Boxes

Justification

Tiritiri Matangi has approximately 6% climax coastal forest (Mitchel 1985). The remainder of the island consists of a mixture of replanted vegetation and naturally regenerating vegetation, leaving 40% in grassland (Cashmore 1995). All regenerating habitat is no older than 40 years, with replanted habitat younger than 15 years. Because there are few old trees, artificial nest boxes need to be provided for successful breeding.

Although some natural cavities may be available, stitchbirds have only nested in boxes (Ewen pers. obs.). This may be for the following reasons;

1. Stitchbirds may actually prefer nest boxes to natural cavities.
2. There may be competition with other cavity nesters. Castro et al. (1994) notes that on Kapiti kakariki were observed fighting for cavities with stitchbirds every year. Tiritiri Matangi also has many kakariki, along with other cavity nesters including saddleback, New Zealand kingfisher and introduced mynas.
3. Stitchbirds may have specific cavity preferences as has been noted with other avian species (Van Balen et al. 1982). Chances of suitable cavities are reduced when choice is restricted.

Stitchbirds readily use boxes when available (Rasch 1989; Castro et al. 1994; Armstrong et al. 1997). Perrott (1997) suggests that provision of nest boxes on Mokoia may be allowing stitchbirds to reside in an otherwise unsuitable habitat. It is likely that this is also the case on Tiritiri Matangi. Due to a design fault in the first breeding season, stitchbirds were unable to use the boxes designed for them. Females nested in boxes designed for saddleback, kaka and robins. All females subsequently used the appropriate boxes once this design fault was corrected.

Current stitchbird boxes are designed to exclude other cavity users. This was achieved by placing a small entrance hole at the bottom of the box. This design may prevent nest predation and competition with the Indian myna, which is becoming a problem with the cavity nesting saddleback on Tiritiri Matangi (B. Walter pers. com.). The design also prevents access by morepork, which sometimes prey on saddleback in nest boxes on Tiritiri Matangi (B. Walter pers. com.).

Monitoring of reproductive success is made easier due to birds nesting in boxes at known positions. Accurate data can be gathered with a minimum of effort. This is essential to improve the knowledge of success/failure of this translocation.

Placement

For ease of monitoring, nest boxes should be placed at about chest level for the average person. Boxes are designed with a lifting lid for access. The observer should be able to stand steadily on the ground while checking nests. This is important, especially when removing eggs or chicks. Although it is not known whether height has any affect on the success of stitchbird nesting, birds have readily used chest height.

Placement of boxes around the island hasn't followed any set protocol. However birds that bred in both the 1995/96 and 1996/97 breeding seasons returned to the same or nearby nesting sites the previous year. Therefore I recommend keeping boxes in the same places if they were used. Over the previous two breeding seasons stitchbirds have restricted their nesting attempts to three bush areas: Bush 1, Bush 22 and Big Wattle Valley (plus a partially built nest at Paa Point in 1996), although boxes were provided in eight of the main bush areas. It is suggested that additional boxes should be provided in these areas as the population increases. Stitchbirds may be restricting their breeding to these sites because;

1. They coincide with placement of supplementary feeding stations
2. The birds like to breed in close proximity to one another
3. These may contain the best habitat for breeding (also preferred habitat for robnins)
4. There is no preference and this result is due to the very small sample size of females on Tiritiri Matangi (4 in 1995/96 and 6 in 1996/97).

It is also important to continue providing boxes in other localities because birds may spread more widely in the future.

Nests should be placed with the following in mind;

- At chest height for easy access to the nest
- North facing or in areas as dry as possible- this is to prevent nests from becoming damp.
- Preferably not above or near streams-*this is because chicks may jump the nest at an early age.*
- In groups of two or three (about 10m apart)- *this is because females are*

likely to initiate nest building in more than one cavity before committing to one. Also if a female attempts multiple clutches she is likely to stay in the same area (Ewen pers. obs.).

Design

Nest boxes were designed as for Mokoia Island, with modifications based on the following disadvantages-problems with the nest boxes (Glenfield College 1995):

1. Heavy mite infestations
2. Entrance becoming fouled with faeces

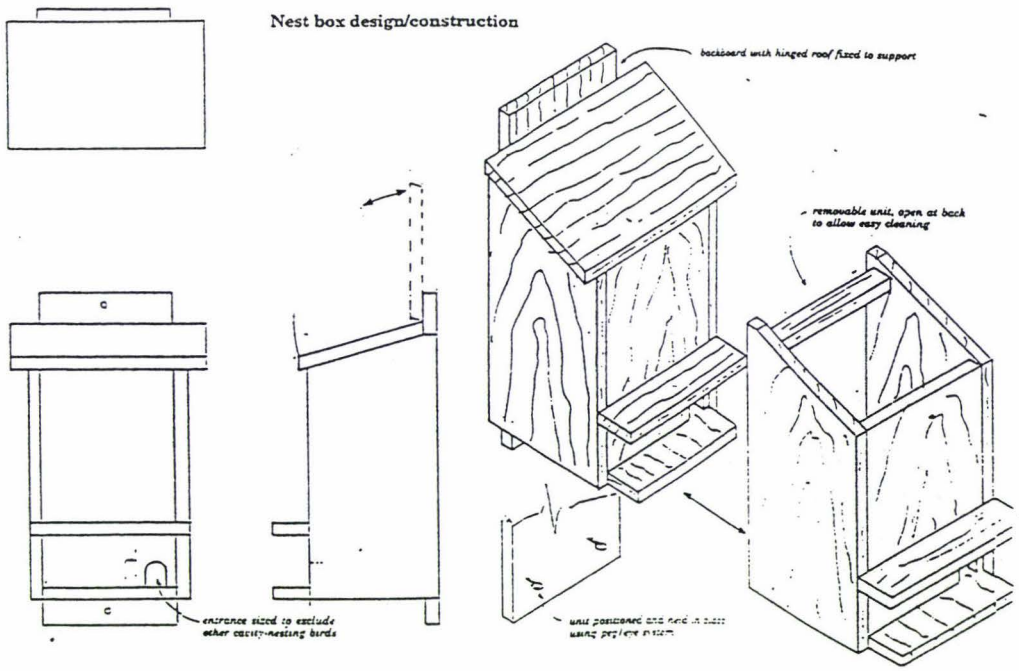


Figure 1. Stitchbird nest box design. Glenfield College 1995.

Cleaning

After fledging or failure of a nest the box needs to be replaced with a clean frontal unit and the backboard needs to be disinfected.

- Backboards need to be scrubbed with a mild disinfectant such as Milton or Johnson & Johnson baby disinfectant.
- Frontal units should be taken to nursery area and hosed under high pressure, then disinfected in a similar fashion to the back units.

- After cleaning frontal units can be left to air in the sun for as long as possible, then either stored or reused (depending on demand for boxes). If there has been a problem with mites in the nest then sprinkle the box with mite powder.
- Ideally once a box is used it can be stored until the following season - *this is to reduce the possibility of bacterial and/or fungal growth causing problems with nestlings and/or adult birds.*
- Once the backboard has dried out, attach a clean frontal unit.

Nest mite management

Justification

Nest mites have been a major problem limiting reproduction in the Mokoia Island stitchbird population. Some broods were killed by nest mites, and many more would have been killed without nests being carefully managed (Armstrong et al. 1997). Mites are a common parasite among birds and usually pose little threat to nestling survival. However in stitchbird boxes, the numbers are reaching densities that are resulting in chicks becoming anaemic and eventually causing death. Most nests on Tiritiri Matangi have had problems with mites. However, because nests have been replaced when mites become abundant, the potential loss is not known.

Nest Checks

Mite checks should be conducted daily at nests involved with brooding chicks. Extreme care must be taken when conducting nest checks and only people directly involved with hihi management should do this. The earliest nest change on Tiritiri Matangi has been at 14 days. A step-wise protocol for checking nests is outlined below;

1. Sit well away from the nest entrance (at least 10m) and watch for the female leaving the nest.
2. Once the female has gone from sight, go to the box and look around the lid for presence of mites. Often I have seen large numbers of mites around this area and few in the nest itself. If mites are detected then replace the nest.
3. Lift the lid and place a hand down into the cup, being careful with the chicks.

When the nest is full of chicks gently place fingers down the sides around the nestlings.

4. After approximately 20 seconds, close box and look closely for any mites, which may have crawled onto your hand. *These may be very small so take time when doing this.*
5. If your hand has any more than 20 mites (personal judgement used) then the nest needs to be replaced. Wipe your hand with a damp cloth or try and squash as many as possible otherwise they'll crawl all over you!
6. Armstrong et al. (1997) identifies nest mites as a key area for further research. Therefore it is advised that all mite checks should be recorded on an appropriate data sheet (see Appendix 1).

NOTE: Mites can bite you. This is generally just a nuisance, but if you are allergic to arthropod bites it may be best to get somebody else to do this work.

Nest Changes

When mite numbers start to rise the nest should be changed for one that is "treated" or mite free. Isabel Castro developed this form of management on Mokoia Island and nest boxes have been changed after 14 days or earlier (Armstrong et al. 1997). The following protocol has been changed slightly from that set out by (Castro & Mason 1995). The principle reasons for this are:

- Nests should only be changed if it is absolutely necessary, as it may stress the birds.
- With the design of boxes on Tiritiri Matangi, box changing includes the removing and replacing of only the front panel, as described above.

The protocol for changing nests can be broken into two categories: the pre-changing regime and the changing regime.

Pre-changing regime

1. Have a large supply of treated empty boxes (unused boxes or boxes that have been disinfected and treated with mite powder). See cleaning section for

protocols on treating used boxes.

2. At the beginning of each breeding season, gather any nests that have been abandoned before hatching. Avoid using nests that had chicks in them as they may transfer disease and/or mites. Stitchbird nests are preferred, but nests can be from any species. I have only used robin or saddleback nests. Barbara Walter is a great source of saddleback nests and is very happy to help in any way with the stitchbirds.
3. Substitute nests should be treated for mites. Sprinkle the entire nest (inside and out) with red mite powder. This powder can be purchased from any aviary bird specialists. Leave over night, then gently shake the nest to remove any surplus powder. Leave nest in a well-aired place for 3-4 days to remove odour.

IMPORTANT: Chicks will jump from the nest if they detect any mite powder. Make sure all items dosed with mite powder are well aired and never apply powder directly onto chicks.

4. Have a dry supply of small sticks, which can be used to create a nest base inside a box. These "propping sticks" (Castro & Mason 1995) help lift the nest cup off the base of the box and simulate a natural nest.
5. Female stitchbirds generally start several nests (Castro et al. 1994; Ewen pers.obs.) before finishing one. This provides a source of ready made nest bases, which can be used (rather than building a base as in 4). It is important to replace the removed frontal unit with a treated one in case the female starts a second clutch.

Changing regime

1. Take the following with you when changing nests;
    - Treated nest box with treated cup and base
    - Hammer
    - Bottle of warm disinfected water & sponge
    - Dry cloth

- As many banding bags as nestlings

2. Wait approximately 10 m from nest until female has left the nest. I usually wait even if female is not there when I arrive. This is because the process takes time and it is possible that the female could return when halfway through changing a nest.
3. Once she has moved well away, remove chicks from the nest and put them in banding bags. Use one bag per chick to reduce the chance of one getting out unintentionally while removing another chick.
4. Remove frontal section of box by taking out the metal peg under the lid and gently pulling box toward yourself. The hammer may be required here, as the metal pegs become oxidized and stick in the holes. Try not to destroy the nest at this stage.
5. Wipe down backboard with the Milton solution. This will remove all mites, and also clean any buildup of faeces etc. Dry off with a clean cloth.
6. Place treated cup on top of the natural nest base or build a base from the dry sticks collected earlier. The nest should be firmly in place by jamming it down amongst the base sticks or by pinning through the outer edge with wooden "pegs".
7. Place treated nest box with nest onto the backboard unit attached to the tree. Replace nail and make any fine adjustments to the nest cup.
8. Sprinkle a little mite powder onto the outer surface of the nest box lid, especially around the edges where mites tend to accumulate (Ewen pers obs).
9. Replace chicks and move 10 m away. Observe the nest to determine if the female has accepted it. Two visits by the female should mean a successful replacement has occurred. It may take a little while for the female to accept the box. Keep the old nest in case she refuses to enter the treated box. In such circumstances I would have tried replacing the old nest. All females have accepted the boxes to date on Tiritiri Matangi.
10. Once the female has been observed entering the nest and feeding the young the old nest can be tipped out and the box cleaned for future use.

Feeding regime

Justification

A form of soft-release can be achieved by providing species with supplementary feeding stations until the birds establish themselves at their release site (Armstrong & McLean 1995). Continued use of feeding stations, as a management tool after this initial period requires further monitoring/research. It is uncertain how important supplementary feeding is to the survival of stitchbirds on Mokoia (Perrott 1997). Tentative evidence is provided below. However a small sample size necessitates continued monitoring to provide definitive answers.

Research on Kapiti Island highlighted strong seasonal use of supplementary feeding stations, with frequent visits during the breeding season especially when laying eggs and fledging first clutches (Castro 1995). On Mokoia Island similar trends were observed with birds using feeders less outside the breeding season (Perrott 1997). Perrott (1997) hypothesizes that stitchbirds may show increased preference for feeding stations because they are time limited rather than energy limited. Thus feeders are allowing birds to rapidly gain their energy requirements, freeing them for other non-foraging behaviour.

Food product information

Stitchbird food is provided in hummingbird feeders designed by Perky Pet Products. They can be ordered through the following address;

Perky-pet products co., 2201 50 Wabash street, Denver, Colorado 80231

During the breeding season the feeders are supplied with Womboroo. This is an Australian nectar feeder mix, designed as a full dietary supplement. Wombaroo can be ordered direct from the distributors by contacting;

Phone/Fax 006183791339 (Australia)

Placement

Four permanent feeding platforms have been established on the island, in Bush 22, Wattle Valley, and two in Bush 1 (Figure 2). Careful monitoring should be maintained to identify possible exclusion of stitchbirds from feeders by bellbirds. This has occurred in Wattle Valley over the last two breeding seasons. When this situation arises remove the feeder immediately. Hopefully this will prevent more bellbirds from "learning" to use these stations. If the Wattle Valley feeder is used by bellbirds again it may be best to remove the station completely.

Food preparation

1. Measure 1 cup (300 g) of Wombaroo for each litre of water used. Because feeders are changed daily judge how much is needed depending on use.
2. Add powder to a bottle, followed by a little warm water. Shake vigorously until most powder is dissolved. Slowly add more water, mixing occasionally until made up to level. All powder should be in solution and the solution should not be too thick.
3. Distribute food between feeder bottles then close each using a coke bottle lid. Make sure there is a feeder base for every bottle with food.

Servicing feeding stations

1. Remove wire-front from feeding station and then take old feeder from cage.
2. While bottle is upside down attach the base by screwing until finger tight. Quickly turn feeder over and allow Wombaroo to fill base. Tilt feeder slightly until a little liquid pours from the "flower" which the birds will use. This will remove any air bubbles still in the base.
3. Hang new feeder onto hook and position perch in front off one of the "flowers". Replace wire netting frontal section to feeding platform.
4. Take old feeder well away from feeding station, preferably out of the bush patch to empty it. This is a protein rich food and will promote the growth of bacteria, which may be detrimental to the hihi.

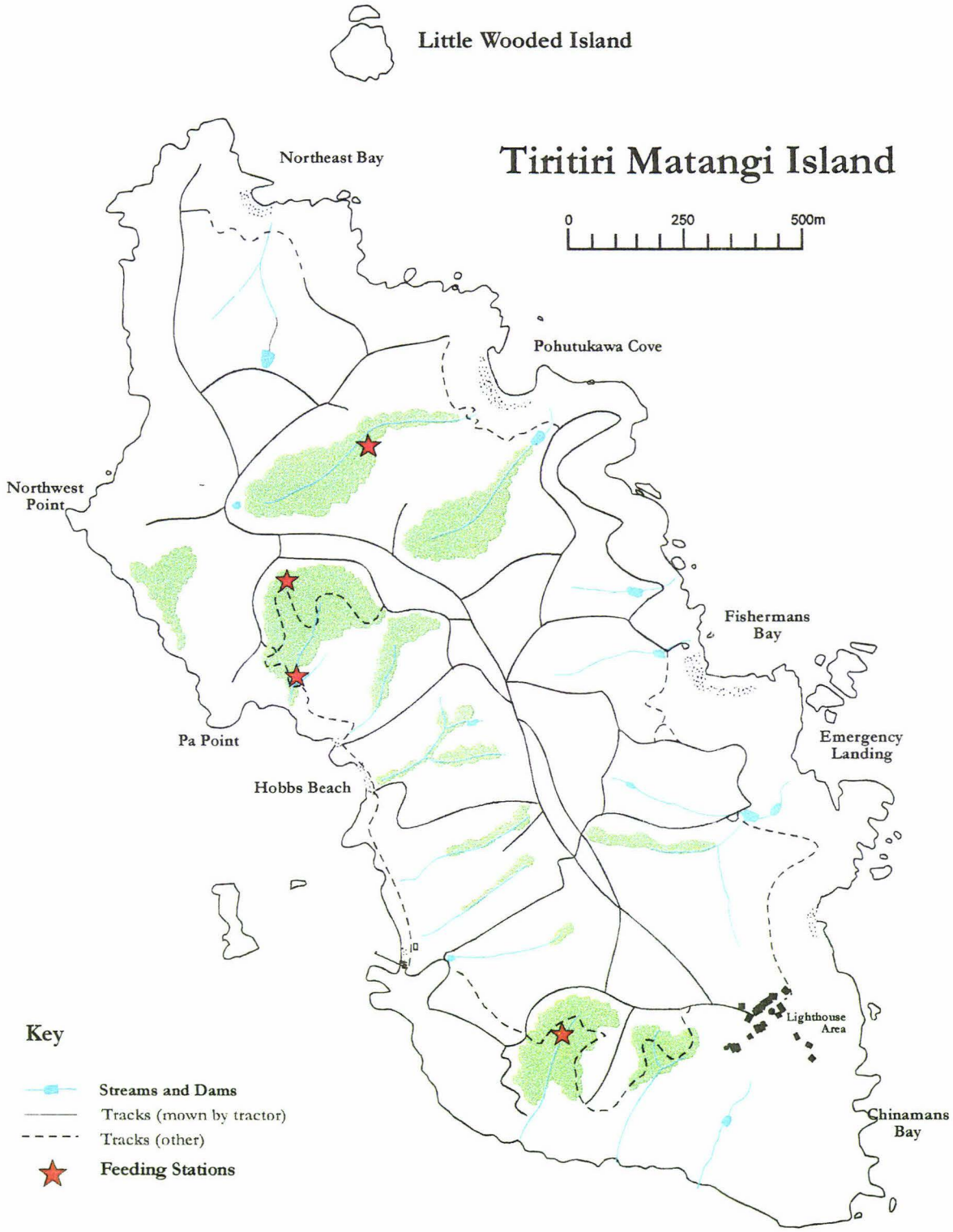


Figure 2. Map of Tiritiri Matangi Island showing placement of permanent feeding stations and mature bush patches.

It is important that feeders be changed every day. Wombaroo goes off very quickly or separates out, forming a gluggy mass in the feeder base. This is not a healthy supplement for the stitchbird.

Cleaning of feeders

Cleanliness is imperative to the survival of stitchbirds on Tiritiri Matangi. Birds will congregate around feeding stations and all will be feeding from a single "flower". The potential for disease transmission is very real. Therefore cleaning feeders may be the most important task required for successful stitchbird management.

1. Use a bottle brush to scrub out both bottles and bases with hot water. Make sure that the coke bottle used for mixing womboroo is also cleaned in this way.
2. Mix up a bucket of mild baby disinfectant in water. Milton or Johnson & Johnson tablets are both good sources for this. Follow instructions on packet for appropriate strength.
3. Soak all feeders, mixing equipment and cleaning gear in this solution for the specified amount of time to ensure successful disinfecting. Make sure all equipment is completely covered in solution and there are no air bubbles in the feeder bases.
4. Rinse all equipment thoroughly with water before re-using.

NOTE- Once a week feeding stations should be cleaned with mild disinfectant to remove any buildup of faeces. Also wipe the perch birds sit on while feeding.

Bird Monitoring

Monitoring adult population and fledgling success

All sightings, including information on when and where the bird was seen, should be recorded in the stitchbird re-sighting book kept by Ray and Barbara Walter. As chicks are banded their combinations should be added to this re-sighting book.

Sighting stitchbirds can be difficult. They are small fast flying passerines, which move over large areas. Below are a few tips;

- Monitor feeding stations as birds may congregate in large numbers at certain times of the year.
- Watch around nest sites when a female is almost ready to lay eggs. Many males should be in the vicinity of the nest at some time.
- Watch any natural concentrated food source e.g flowering Rewarewa trees (about October), flowering Pohutukawa trees (about December/January) and patches of fruiting *Coprosma robusta* (about February).

It is also important to develop a set method of searching the entire island thoroughly for stitchbirds. Keep eyes and ears open the whole time while out on the island, as the birds can turn up in the most unexpected places!

This should be an ongoing process, updating the record sheets daily and specifically searching for birds that have not been seen from one week to the next.

Monitoring nests

All nest boxes need to be checked daily from the beginning of October until all females have nested. Female stitchbirds generally start building more than one nest before finishing one. Keep records of nest box status to avoid confusion with the large numbers of boxes that will have nesting material in them.

Once a nest has been found, intensive monitoring should be conducted throughout incubation and brooding. A stepwise protocol for monitoring active nest sites, from initiation to completion, is presented below;

- Check the nest every second day to determine when the clutch is complete and when incubation has begun. Note females often bury eggs under nest cup material, making eggs difficult to see. Take care when searching for eggs so as not to crush these buried eggs. The nest can then be left until day 12 of incubation.
- Daily checks of nests are recommended from day 12 to determine when eggs

hatch. Un-hatched eggs need to be removed on day 17, or three days after the first eggs have hatched.

- Daily checks of nests should be conducted throughout the nestling stage. This is to monitor nestling survival and nest condition. See appropriate section for protocols on processing of dead chicks and section on nest checks under mite management for nest mite protocols.
- Band and measure chicks 21 to 25 days after hatching. See section below for protocols on this.
- Record day of fledging and replace nest box.
- Throughout the nestling stages of the active nest look for signs of re-nesting nearby.

Banding and measuring chicks

All nestlings need to be banded 21 to 25 days after hatching. This enables monitoring of post-fledging survivorship and the following of lineages. Three measurements are also taken when banding chicks;

1. Weight
2. Tarsus
3. Head-Bill

To date stitchbirds on Tiritiri Matangi have been banded with C size wrap-around bands. Care must be taken with band type and size, as split-type bands caused leg injuries to stitchbirds on Mokoia Island (Armstrong et al. 1997). Band combinations and measurement information should be recorded on a specific data sheet (Appendix 2), and information sent to the banding office in Wellington.

NOTE: A permit is required to band stitchbirds. Banding is a difficult technique should only be attempted by experienced people.

Processing dead birds

Any dead birds found need to be carefully processed so that causes of mortality can be determined. Bodies can be sent to the Veterinary Department at Massey University (contact Maurice Alley) for *post-mortem* analysis. All information should be recorded in the final report of each year (March).

It is important to provide as much information as possible, to be sent with the body to veterinarians. A general information sheet, to be included with each body, can be seen in Appendix 3.

1. Nestlings

Small featherless chicks should be placed whole into 10% formalin solution. Larger chicks near fledging age should be processed using the same methods as for adult birds (see next section).

2. Adults

Adult birds found in relatively good condition should be processed using methods adopted by Castro & Mason (1995) on Mokoia. Badly decomposed bodies will provide little information and may as well be left. However make sure to note the band combination.

- Place the bird on its back, remove the feathers on the breast, and cut it open at the sternum towards the neck and then on the sides following the ribs. Make sure not to damage internal organs by cutting too deep.
- Put the specimen in a jar with 10% formalin solution. Pull open the ribs after the cuts to make sure that formalin can penetrate the bird's body and fix the tissues.

NEVER FREEZE THE BIRD as the tissues forming the organs will be destroyed and a *post-mortem* would be impossible.

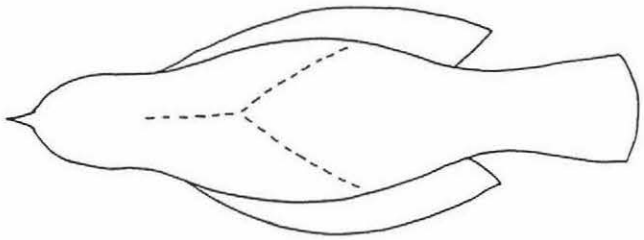


Figure 3. Diagram of dead bird showing recommended incision placements.

NON-BREEDING SEASON

Justification

It is recommended that management and monitoring of stitchbirds be continued throughout the non-breeding season. There is little point investing a lot of time into breeding season management if heavy mortality during the winter undoes all ground gained. The little information gathered from past failures suggests that birds appeared to breed well in their first few seasons then slowly decline (Lovegrove 1985; Castro 1995; Rasch et al. 1996). However this trend could be a product of people assuming that because the birds were breeding, the populations were healthy. There is the possibility that the populations gradually declined from the beginning. Lack of monitoring following stitchbird releases has resulted in a failure to provide adequate information on possible causes. Only continued monitoring/research will provide concrete answers. Tiritiri Matangi has the potential of providing a relatively easy opportunity for long term monitoring of population viability. This is because;

1. Tiritiri Matangi is small (220 ha) and divided into distinct bush patches which cover a total of 60% of the island (Wilson 1997).
2. The island is low lying (88 m) with a well-developed network of tracks for ease of access.
3. There are experienced Department of Conservation staff on the island who have been involved with the stitchbirds since their arrival in 1995.

4. Enthusiastic volunteers, many with experience in monitoring bird populations, are constantly participating with the Tiritiri Matangi project.
5. The Supporters of Tiritiri Matangi Inc. are also available to provide logistical and field support to this project.

Monitoring birds

Low intensity continuous monitoring

Throughout the year all stitchbird sightings should be recorded on data sheets to be included with the final annual breeding season report. Population dynamics can be mapped from this information and used to identify critical periods where management can be targeted.

Any dead stitchbirds discovered on the island need to be processed. *Post-mortems* on 'fresh' bodies will provide more accurate information on cause of mortality compared to those from badly decomposing carcasses. Autopsies of birds from Mokoia Island have shown that Aspergillus infections are the main cause of death (Armstrong et al. 1997). The role of disease in the decline of hihi may be an important area to target. See previous section on bird monitoring for protocols on processing dead birds.

April population check

A population census in April will provide information on adult and juvenile survivorship. Identification of juveniles will also provide information about sex of fledglings as juveniles will have molted into adult plumage.

This population census should include a thorough search of the island to maximize chances of sighting all surviving individuals. The search should include;

- Observations at feeding stations
- Searching in areas of concentrated natural food sources. Spend time in these areas and keep returning to them, as stitchbirds can be very gregarious. For example during the winter flowering of *Albizia* in wattle valley, 12 of the 18

birds could be found in one small patch of this species (Ewen pers obs). This knowledge was only gained through successive visits to this area.

Data should be recorded on re-sighting sheets with the fledglings included as part of the adult population. This will give a total population count and highlight the sex ratio.

September population check

A similar population census should be conducted prior to the breeding season. This will provide accurate information about winter survivorship and allow numbers of potential nesting attempts to be determined. If there are high numbers of female stitchbirds surviving through to their first breeding season it may be necessary to provide additional nest boxes. There should ample nest boxes available to the females to select from.

Winter supplementary feeding

Justification

Winter supplementary feeding on Tiritiri Matangi will serve the dual role of providing a food source to birds and to aid in the advocacy of the species. Section 6.6 in the work plan and short-term objective number four of the stitchbird recovery plan (Rasch et al. 1996) suggests that an advocacy program should aim to educate and inform the public, as well as gain support and cooperation for management activities. Raising the profile of this species will be achieved through;

- Use of volunteers to change feeding stations- a form of direct involvement with the management program.
- A feeding station near a main track on the island will allow the general public to observe birds and to witness a form of management.

Data on the effects of food supplementation on the condition and survival of stitchbirds through the non-breeding season has yet to be analyzed. On Mokoia Island research suggests that feeder access had little influence on birds' weights or survival (Perrott 1997). Supplementary feeding stations may also provide essential resources during unpredictable crunch periods.

Feeding Regime

From March to September a single feeding station could be maintained. It is recommended that this feeder be placed in Bush 1, just above the "podium". The feeder should be supplied with sugar water rather than Wombaroo. Sugar water will last 2-3 days without 'going off'. Changing of a feeder at such low intensity should be an easy task to offer volunteers.

Measure one cup of white table sugar for every litre of water. Fill feeders according to demand by birds.

- See section on feeding regime and servicing for cleaning instructions

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APPENDIX 1.

MITE CHECK DATA SHEET

Year ________

Parents Female ________ Male ________

Clutch Number ________

Box Number ________

Month	Day	Mite Numbers	Change	Comments

APPENDIX 2.

STITCHBIRD BANDING RECORD SHEET

Date	Mother	Band No.	Colours	WT (g)	HB (mm)	Bill (mm)	TARS	WL

APPENDIX 3.

DEAD BIRD INFORMATION SHEET

Species _____ Island _____

Sex _____ Band Combination _____

Date Found _____ Area Found _____

Bird Age (if known) _____

Comments (describe noticable injuries or unusual behaviours before death)

_____

_____

_____

_____

_____

_____